PHARMACOLOGICAL STUDIES OF SELECTED PLANTS USED TRADITIONALLY IN PAIN AND INFLAMMATION

ARSHAD IQBAL

DEPARTMENT OF BOTANY
ISLAMIA COLLEGE PESHAWAR
2015

PHARMACOLOGICAL STUDIES OF SELECTED PLANTS USED TRADITIONALLY IN PAIN AND INFLAMMATION

Thesis submitted in partial fulfillment of the requirements for the award of degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

ARSHAD IQBAL

DEPARTMENT OF BOTANY
ISLAMIA COLLEGE PESHAWAR
2015

APPROVAL CERTIFICATE

This dissertation, submitted by **Mr. Arshad Iqbal**, is accepted in its present form by the Department of Botany, Islamia College Peshawar as satisfying the thesis requirements for the Degree of Doctor of Philosophy (Ph.D).

Supervisor:

Prof. Dr. Siraj Ud Din
Chairman
Department of Botany
University of Peshawar

Co-Supervisor:

Dr. Inamullah Khan Marwat
Assistant Professor
Department of Pharmacy,
University of Peshawar

External Examiner:

Prof. Dr. Jehan Bakht
Institute of Biotechnology & Genetic Engineering,
University of Agriculture Peshawar

Chairman:

Dr. Samin Jan
Department of Botany
Islamia College Peshawar

Department of Botany
Islamia College Peshawar
2015

Dedicated to

My Mother

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Detail
WHO	World Health Organization
T.A	*Tamarix aphylla*
T.D	*Tamarix dioica*
A.C	*Acacia cyanophylla*
A.S	*Acacia stenophylla*
A. drepanolobium,	*Acacia. drepanolobium,*
A. hockii	*Acacia. Hockii*
A. tortilis	*Acacia. Tortilis*
A. seyal	*Acacia. Seyal*
T. boveana	*Tamarixboveana*
T.hispida	*Tamarix hispida*
T. ramosissima	*Tamarix ramosissima*
MS	Mass Spectroscopy
UV	Ultra violet
TLC	Thin Layer Chromatography
GC-MS	Gas chromatography-mass spectrometry
CC	Column chromatography
GC	Gas chromatography
Rf	Relative flow
R. time	Real time
LD50	Lethal Dose 50
MIC	Minimum inhibitory concentration
ppm	Parts Per Million
IC50	Inhibitory concentration 50
$FeCl_3$	Ferric chloride
CHCl3	Chloroform

EtOAc	Ethyl-Acetate
BuOH	*n- Butanol*
DMSO	Dimethyl Sulfoxide
GIT	Gastro-intestinal tract
COX	Cyclo-oxygenase
LOX	Lipo-oxygenase
H_2SO_4	Sulphuric acid
NH_4OH	Ammonium hydroxide
RSA	Radical Scavenging Activity
S.D	Standarddeviation
p	Significance
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
E. coli	*Escherichia coli*
S. typhi	*Salmonella typhi*
B. subtilis	*Bacillus subtilis*
B. atropheus	*Bacillus atropheus*
E. carotovora	*Erwiniacarotovora*
K. pneumonia	*Klebsiella pneumonia*
M. canis	*Mycosporumcanis*
C. albicans	*Candida albicans*
TNF- α	Tissue necrosis factor-alpha
PGE2	Prostaglandin-E2

ACKNOWLEDGEMENTS

Thanks to ALMIGHTY ALLAH for giving me patience and vehemence to accomplish my PhD project, which is certainly a landmark in my life.

I am extremely thankful to my teacher and research supervisor Prof. Dr. Siraj-ud-Din, Chairman Department of Botany, University of Peshawar for his generous help and encouragement during the course of study.

It is a great honor and pleasant feeling to express my in-depth gratitude to incomparable and cherished research co-supervisor Dr. Inamullah Khan Marwat, Assistant Professor, Department of Pharmacy, University of Peshawar. Throughout the research project, his keen and incessant participation, precious supervision and untiring efforts enabled me to achieve this task effectively. His guidance and knowledge that he has bestowed on me is an unending source of inspiration, not only for this study but will also enlighten my ways in the days to come.

I deeply owe thanks to Dr. Samin Jan, Chairman Department of Botany, Islamia College Peshawar for his keen interest in my research activities. Similarly, I would like to appreciate the cooperation of all the teaching faculty of the department.

Indeed it is my privilege to acknowledge the senior scientific officers and lab staff of PCSIR, Complex Peshawar. Special thanks to senior scientific officers Dr. Mushtaq, Naeem-ud-Din, Miss Farah Gul and lab staff Muhammad Anwar.

My thanks are due to Mr. Fazal Rabi, Lecturer, Department of Pharmacy, Abasyn University Peshawar and Dr. Mehboob Alam, Assistant Professor, Department of Pharmacy, Comsat Institute, Abbottabad.

Sincere expression of deep regards for my beloved Parents and my entire family and all friends.

Arshad Iqbal

ABSTRACT

Natural products based drug discovery is still a challenging area for the exploration of new lead compounds. The present research project aims to scientifically validate the folkloric use of selected plants (*Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla*) in pain and inflammation. Phytochemical screening showed the presence of alkaloids, carbohydrates, saponins, terpenes, flavonoids, tannins, anthraquinones and amino Acids in crude methanolic extract of *Tamarix aphylla, Tamarix dioica*and *Acacia stenophylla*, while *Tamarix aphylla* showed negative result for steroids, glycosides and sterols. The crude extract of*Acacia cyanophylla* showed positive test results for alkaloids, carbohydrates, saponins, cholesterol, flavonoids, steroids, anthraquinones, terpenes, sterols and tannins.

The crude extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem bark were screened for fatty Acids**.** Experimental data showed that all of the four plants contained different concentrations of various fatty Acids. Major fatty Acid in all the studied plant samples was Linoleic Acid; its concentration was 0.11%, 0.41%, 0.22% and 012% in *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* respectively. Linoleic Acid was followed by Palmitic Acid (0.04%) in *Tamarix aphylla*, Octadecadienoic Acid in *Tamarix dioica, Acacia cyanophylla* (0.12% and 0.10% respectively) and gamma-linolenic Acid (0.08%) in *Acacia stenophylla*.

The crude methanolic extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* obtained from stem bark were screened for various biological/ pharmacological activities. Our results declared that all the four plants demostrated good antimicrobial activity at the concentration of 2mg $disc^{-1}$ against the tested microbes. Among the tested plants, highest zone of inhibition was shown by *Tamarix aphylla* (81.25 %) against *Bacillus atrophus* (gram positive). Similarly, highest antifungal activity was shown by *Acacia cyanophylla* against *Candida albican* (72.22%).

In case of antioxidant activity, the crude extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* possessed good antioxidant activity of 831, 976, 1153 and 1467 (IC_{50}) respectively. The plant extracts also showed enzyme inhibitory activity. Acetylcholinesterase was inhibited by *Acacia cyanophylla* and *Acacia stenophylla* only. Maximum inhibitory activity was demonstrated by *Acacia stenophylla* (37.11µg/ml) in Ethyl acetate fraction, and minimum inhibition was shown by aqueous fractions (91.46µg/ml) of *Acacia cyanophylla*. The results revealed that maximum Lipoxygenase inhibitory activity was shown by Ethyl acetate extracted sample of *Tamarix aphylla* which was 27.3µg/ml, minimum was recorded for aqueous fraction of *Acacia stenophylla* (142.3µg/ml).

Similarly, the crude methanolic extracts of all the four plant species possessed significant anti-inflammatory, analgesic and antipyretic activity at all doses. The results revealed that the analgesic activity of the crude methanolic extract of different plant species when measured by acetic Acid model was dose dependent and the increasing concentration of the extract increased its activity. Maximum activity of 66.19% was shown by *Acacia stenophylla* at the dose of 400 mg/kg followed by *Tamarix dioica* (64.33%) at the same dose. The analgesic activity conducted by Hot Plate method indicated that the same activity was dose and time dependent. Maximum activity of 54.49% was achieved by

Tamarix aphylla, when the mice were exposed to 90 minutes at higher dose of 400 mg/kg. Similarly, minimum analgesic activity by the same assay was measured in case of *Acacia stenophylla* (11.22%) at a dose of 200 mg/kg. Our data also indicated similar pattern for antipyretic activity as was observed for analgesic activity. *Tamarix aphylla* revealed maximum inhibitory activity at the higher dose of 300 mg kg^{-1} during the 3^{rd} hr, while minimum activity was noted for *Acacia stenophylla* (0.02%) at lower dose of 100 mg/kg exposed to the 1^{st} hour.

Anti-inflammatory activity evaluated by Carageenan-Induced Paw Edema and Xylene-Induced Ear Edema Model revealed that anti-inflammatory activities were dose and time dependent. Maximum anti-inflammatory activity (54.12%) was shown by crude methanolic extract of *Tamarix dioica* at a dose of 200 mg kg^{-1} after 5 hours on Carrageenan-Induced Paw Edema Model. *Tamarix dioica* was followed by *Tamarix aphylla* (51.84%). Similarly, anti-inflammatory effect assessed via Xylene-Induced Ear Edema Model revealed that maximum effect of 68.80% was demonstrated by *Acacia cyanophylla* at the dose of 200 mg/kg after 60 minutes. It was followed by *Tamarix aphylla* (68.59%). Minimum inhibitory effect was shown by *Tamarix dioica* which was 32.29% at lower concentration of 50 mg/kg when the tested animals were exposed for 15 minutes. All plants were screened for acute in-vivo toxicity using albino mice and no considerable toxicity was observed up to the dose of 2000 mg/kg.

This study explicitly validated folk uses of the selected plants (*Tamarix aphylla, Tamarix dioica, Acacia cyanophylla, Acacia stenophylla*) in various diseases. Furthermore, this data indicate the strong potential of all these plants for isolation and identification of new bioactive compounds for better management of respective diseases.

CHAPTER 1

INTRODUCTION

1.1 SIGNIFICANCE AND BACKGROUND:

Since the dawn of life on planet earth the human plant nexus has been of the fundamental importance. Man is and has been dependent on plant resources for curing and ailment of various diseases and infections in addition to augmenting its other livelihood requirements (Ballabh *et al.*, 2007). Human dependencies on plant resources increased manifold with the passage of time due to increase in population, needs and requirements. (Ali and Qaiser, 2009). It has been observed that human civilizations were more dependent on plants than animals.They experimented on a wide variety of plants and discovered that plants can be used as medicine (Babekov *et al.*, 1999). Plants are traditionally used as medicines in varous cultures (Shrestha *et al*., 2003). The utilization of many plants in the curing and management of different diseases was first reported by the subcontinent of India. "Rig Veda" a book, written by Aitareya Aranyaka, in between 4500 and 1600 B.C. was probably the oldest book containing information regarding medicinally important plants (Chopra *et al.*, 1956).

The Egyptians, in 1550 B.C. highlighted the significance of plants as remedies for several diseases in *Ebers Papyrus*. A book "*Susruta Samhita"* written in 1000 B.C. also has information on important medicinal plants (Raju and Vadrevu, 2003). A sizeable work on herbal medicines was performed during Buddhist Era. They cultivated medicinal plants and experts in the field of herbal medicine supervised them (Yao and Jama, 1990). The close ties among Indians, Greeks, Persians, Arabs and Romans further promoted Indian Materia Medica. *Shen Nong Ben Cao Jing* (22-250 A.D) period is known for the development of medicine in China. Li Shizhen, a famous naturalist and great physician wrote *Ben Cao Gang Mu* (a pharmacopeia), wherein he discussed 1894 plants used as medicine. Later on the book was published and is still used as an authentic source in China. The contributions of Arab scientists in this field are no less important. "Ibn-Al-Baitar", a prominent plant scientist and druggist, has written in the book "*Kitab al-jami*

fi-al-Mufradat" about the worth of several medicinal plants. Al-Idrisi (1099-1166 A.D.), another well known Muslim scientist also authored a book named "*Kitab al-jami lisifat ashtatal-nabata"* in six different languages about medicinal plants (Soll *et al.*, 2007). Mohammad Ibn -e- Zakariaya Al-Razi (864-930 A.D.) authored "*Kitab-al-Mansoori*" of 10 volumes. In his book he talked about Greco-Arab remedies and he used opium as an anesthetic for the first time (Chopra *et al.*, 1956). Al-Tabari (883-870 A.D.) in the sixth part of his book *"Firdous al-Hikma"* discusses poisons and drugs while Ibn -e- Sina (980-1037) indicated more than six hundreds (679) medicinal plants in the book "*Qanun fi al-Tibb*" (Chopra *et al.*, 1956).

The first pure chemical agent was benzoic Acid that was obtained from plant in 1560 (Patankar *et al.*, 2008). Some simple chemical substances like citric, lactic, tartaric and oxalic Acid were isolated by a German chemist, Karl W. Scheele (1742-1786) from organisms (Zenk and Meinhart, 2007). Morphine was the first valuable substance of known structures that was obtained from opium (*Papaver somniferum* L.).This research was conducted by F.W. Serturner (1783-1841) in 1806 whose efforts are duly creditable. Later on, Caventou and Pelletier got caffeine, brucine, strychnine, quinine and cinchonine from plants. In this regard, conine was the first established and synthesized alkaloid (Farber and Eduard., 1950). Active agents like atropine, aspirin, tubocurarine, reserpine and morphine are also obtained from plants (Gilani, 2005).

Medicinal plants are used in headache and stomatic conditions. They are also used for cuts and wounds (Bhardwaj and Gakhar, 2005). World Health Organization (WHO) recommends medicinal plants as remedies for various diseseas in South Asia. Nineteen (19%) out of the approximately 8,000 of these plants are found in Pakistan (Akerele, 1984). Based on the knowledge of plants as traditional remedies, approximately 119 plant givenactive medicines were introduced in the open market (Farnsworth *et al.*, 1985). In accord with WHO, more than 20, 000 plants are used as traditional medicines; the number of such plants has now enhanced to 70, 000 throughout the world (Olsen and Ambio, 2005). The knowledge of local people lead more than 89 plants derived products to be used in modern medicine (Rawat, 2006). The clinical results of herbal medicines show that they are as effective as currently available agents against various diseases.

Pakistan has nearly 1572 genera of which 5521 species have medicinal importance being found mostly in the hilly areas (Ali and Qaiser, 2009). Major chunk of population in developing countries depends largely on medicinal flora for the management of large number of diseases. Indigenous herbal treatment is still being practiced to varying degrees by rural population due to its efficacy, unavailability of modern drugs and their traditional belief (Shrestha *et al.*, 2003). The indigenous knowledge with regards to medicinal plants in Pakistan is centuries old, and being passed generation to generation via verbal communication (Shinwari and Gilani, 2010). Keeping in view the tremendous research work being conducted in the recent and distant past and the huge treasures of indigenous knowledge being transferred from generations over the years, it was felt seriously to find out plants being used traditionally as antipyretic, analgesic and anti-inflammatory through ethnobotanical literature survey and then further scientifically validate the folkloric knowledge and use of selected medicinal plants in pain and inflammation. Though studied in bits and pieces, yet detailed investigations with regards to the extent and magnitude of chemical constituents and activities of the subject plants in Pakistan were found either missing or grossly insufficient. The present study aims to identify & verify the general families of natural products of selected plants, identify various classes of phytochemicals found, scientifically validate the ethnomedicinal uses of selected plants in conditions associated with inflammation, pain and fever, study the pharmacological mechanisms involved in anti-inflammatory, analgesic and antipyretic effects and identify acute toxicity associated with these plants. The current study shall not only scientifically validate the folkloric use of the selected medicinal plants species but shall also contribute significantly to plant scientists to further enlarge their research canvas to screen these plants for active metabolite and further explore biosynthetic pathways. This in turn shall result in translating folkloric knowledge to modern pharmaceutical practices and shall be of greater help and benefits to society at large in the near future.

1.2. GENUS *TAMARIX:*

The genus *Tamarix* belongs to family Tamaricaceae (Salah *et al.*, 2010). It has 125 species, documented from various parts of Asia, Africa, Europe and USA (Sultanova *et*

al., 2001). The genus *Tamarix* is native to Asia, Africa, and Europe (Carpenter *et al.*, 1998; Robinson, 1965). The Western Asia subregion of Irano-Turanian Region comprised of 30 species including 13 endemic species. The C. Asia subregion of Irano-Turanian Region comprised of 20 species including 6 endemic species while the Mediterranea Region comprised of 12 species including 6 endemic species (Zhang *et al.*, 2003). *Tamarix* has about 10 species in Algeria (Quezel and Santa, 1963).

The members of genus *Tamarix* are useful in leucodermic condition. Some species are used in the treatment of eye and spleen diseases (Sharma and Parmar, 1998). The members of *Tamarix* are found to have biologically active constituents like tannins, phenolic Acids, flavonoids and coumarins (Mahmood *et al.*, 1994; Djurdjevic *et al.*, 2006). Some biological activities have also been repored by investigators such as antimicrobial and antioxidant activities from *Tamarix* species like *Tamarix ramosissima,* (Sultanova *et al*., 2001) and *Tamarix hispida* (Sultanova *et al.,* 2004).

1.2.1. Genus *Tamarix* reported work:

1.2.1.1. Ethnobotany:

Tamarix is a tall woody tree or long-lived shrub, living between 50-100 years (Tesky, 1992), used traditionally in rheumatism, wound, abscesses, eye inflammation, fever, toothache (dental pain), wound healing and inflammation e.g. *Tamarix aphylla* (Marwat *et al.,* 2011; Azaizeh *et al.*, 2006; Kamal *et al.,* 2009; Abbas *et al.*, 2002). *Tamarix dioica* is used ethnobotanically in splenic inflammation (Samejo *et al.,* 2013a; Qadri *et al.*,1987; Samejo *et al.*, 2013b). *Tamarix aphylla* is used as a good shelter hedge in coastal gardens. The use of wood of the *Tamarix* spp is many folds. It is used for timber, fuel and in preparation of agricultural tools. The leaves of *Tamarix aphylla* are eaten by camels (Marwat, 2008)**.** The extracts of the needles (leaves) of *Tamarix aphylla* is applied in tetanus. The outer bark (ground part) is applied for healing wounds (Azaizeh *et al.*, 2006; Shahidullah, 2000; Marwat, 2008). *Tamarix aphylla* is used traditionally as phytotherapy for jaundice. The seeds of this specie are placed on ash (burnt) of the wood of *Tamarix* which produces somke. Inhalation of this smoke is used for evils (Marwat, 2008).

1.2.1.2. Phytochemistry:

Tamarix species have been screened for their pharmaceutically active phytochemicals like phenolic compounds such as tannins, flavonoids, phenolic Acids and coumarins (Mahmood *et al.,* 1994; Djurdjevic *et al.,* 2006). The aerial parts of some members like *Tamarix gallica* were studied for their chemical constituents. Pharmaceutically active compounds have been iosalted from different solvent extracted samples of *Tamarix gallica.* These were; 3,3,5- tri hydroxy 4,7- diméthoxy flavone, isorhamnetine and 5-hydroxy 4,3,7-trimethoxyflavone (Salah *et al.,* 2010)**.** *Tamarix indica* and *Tamarix passernioides* contain alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, Gums, mucilage, anthraquinones and terpenoids (Naz *et al.,* 2013; Habiba *et al.,* 2010). *Tamarix boveana* contains volatile oils, Hexadecanoic Acid, docosane, germacrene, fenchyl acetate, Benzyl benzoate and 2.4 Nonadienal (Saidana *et al.,* 2008). *Tamarix nilotica* contains ferulic Acid ester, kaempferol 4'-methyl ether, coniferyl alcohol 4-O-sulphate, quercetin 3-O-beta-D-glucupyranuronide and tamarixetin (AbouZid and Sleem, 2011).*Tamarix ericoides* contains alkaloids, flavonoids, phenolic, tannins, glycosides, steroids and saponins (Bhadange and Jadhao, 2013). *Tamarix dioica* contains steroids, phlobatannins, phenols, tannins, terpenoids, flavonoids and saponins (Samejo *et al.,* 2013a). *Tamarix hispida* contains phenolic compunds (Sultanova *et al.,* 2001)**.** *Tamarix hispida* also contains quercetin , isorhamnetin, tamarixetin, and potassium tamarixetin 3-O-sulfate (Umbetova *et al.,* 2004). *Tamarix ramosissima* contains Di (2-ethylhexyl) phthalate, methylvanillate, methylparaben, 7, 4′-dimethoxykaemferol, Methyl syringate, eugenol, vanillin and Ferulic Acid. It also contains 4-sulfate of coniferylic alcohol (Sultanova *et al.,* 2001). *Tamarix ramosissima* leaves contain *p*-coumaric Acid and coumarins. The bark of *Tamarix ramosissima* possesses coumarins, flavonoids, and anthocyans while flowers contain kaempferol, tamarixetin, isoquercitrin, quercetin, tamarixin etc. Fruits contain flavonoids, anthocyans and coumarins, while green branches possess flavonoids & coumarins (Bikbulatova and Korulkina, 2001).

Tamarix elongata and *Tamarix laxa* contain oxidized forms of flavonoids, hydrolyzed tanning agents, phenolic, carbohydrates, amino Acids and tamarixetin 3 -O- b- D-glucoside. This tamarixetin 3-O-b-D- glucoside is also present in *Tamarix nilotica*

(Umbetova *et al.*, 2004). *Tamarix indica* contains reducing sugars, tannins, gums, flavonoids and saponins (Sarker and Sarker, 2009). *Tamarix pauciovulata* contains phenolic compounds, such as syringic Acid, quercetin kaempferol, isorhamnetin isoquercetin, catechin, epicatechin (Mohammedi and Atik, 2012). *Tamarix macrocarpa* contains Phenolic and Polyphenolic compounds, Gallic Acid, Isoferulic Acid, Kampferol and Quercetin (Ali *et al.*, 1979). *Tamarix pakistanica* contains hydrolysable tannins and tamarixinins (Yoshida and Takashi *et al*, 1993). The flower of *Tamarix hampeana* yielded new derivative of long chain alcohol, laserine and sitosterol (Aykac and Akgül, 2010).

The green twigs of *Tamarix hokenakeri* possess 9.2 % tanning agents (Bikbulatova and Korulkina, 2001). The leaves of *Tamarix africana* are rich in polyphenols like phenolic Acids, flavonoids & tannins (Benabdullah *et al.*, 2014; Khennouf *et al.*, 2003). About ten compounds were isolated from the twigs and leaves of *Tamarix chinensis*, these are tamarixinol, β-sitosterol, tamarixone, tamarixol, daucosterol, 3', 4'-di-O-methyl-quercetin and four long chain compounds (Jiang and Zuo, 1988), while *Tamarix aucheriana* possess syringic Acid (Abaza *et al.*, 2013). Table 1.3 shows previous work on *Tamarix*

Table 1.1: Phytochemistry of different species of genus *Tamarix*.

Genus Tamarix	Part used	Compounds	Reference
Tamarix boveana	Plant extract	volatile oils, Hexadecanoic Acid, docosane, germacrene, fenchyl acetate, Benzyl benzoate, 2.4 Nonadienal	(Saidana *et al.*, 2008)
	Plant extract	Naringenin	(Naija *et al.*, 2014b)
Tamarix nilotica	Plant extract	tamarixetin (4),ferulic Acid ester, quercetin 3-O-beta-D-glucupyranuronide , coniferyl alcohol 4-O-sulphate , and kaempferol 4'-methyl ether ,	(Abouzid and Sameh Saleem, 2009)
	Whole plant	tamarixetin 3-O-b-D-glucoside	(Umbetova *et al.*, 2004)
Tamarix ericoides	Whole plant	alkaloids, flavonoids, phenolic, tannins, glycosides, steroids and saponin	(Bhadange and A.B. Jadhao, 2013; Bhadange and Jadhao, 2013)
Tamarix indica	Leaves	alkaloids,flavonoids, carbohydrates, Gums, mucilage, anthraquinones and terpenoids	(Naz *et al.*, 2013)
	Leaves	Alkaloides, glycosides, flavonoids, saponins, tannins.	(Habiba *et al.*, 2010)
	Bark	Reducing sugars, tannins, gums, flavonoids and saponin.	(Sarker and Sarker, 2009)
Tamarix passernioides	Leaves	alkaloids,flavonoids, carbohydrates, Gums, mucilage, anthraquinones and	(Naz *et al.*, 2013)

		terpenoids	
Tamarix dioica	Stem bark	Steroids, phlobatannins, phenols, tannins, terpenoids, flavonoids and saponins	(Samejo *et al.*, 2013a)
T. hispida	Plant extract	Phenolic compounds	(Sultanova *et al.*, 2002)
	Plant extract	quercetin , isorhamnetin, tamarixetin, and potassium tamarixetin 3-O-sulfate	(Umbetova *et al.*, 2004)
Tamarix pakistamica	Plant extract	Hydrolysable tannins, tamarixinins	(Yoshida and Takashi et al, 1993)
T. ramosissima	Plant extract	4-sulfate of coniferylic alcohol	(Sultanova *et al.*, 2001)
	Plant extract	Di (2-ethylhexyl) phthalate, methylvanillate, methylparaben, 7, 4′-dimethoxykaemferol, Methyl syringate, eugenol, vanillin, Ferulic Acid.	(ZJ Mao *et al.*, 2011)
	Leaves	*P*-coumaric Acid and coumarins.	(Bikbulatova and Korulkina, 2001)
	Bark	Coumarins, flavonoids, and anthocyans	(Bikbulatova and Korulkina, 2001)
	Flowers	Quercetin, kaempferol, isoquercitrin, tamarixetin, and tamarixin	(Bikbulatova and Korulkina, 2001)
	Fruits	Coumarins, flavonoids and anthocyans	(Bikbulatova and Korulkina, 2001)
	Green twigs	Coumarins and flavonoids	(Bikbulatova and Korulkina, 2001)
Tamarix pauciovulata	Leaves	Phenolic compounds, e.g. syringic Acid, quercetin kaempferol, isorhamnetin isoquercetin, catechin, epicatechin	(Mohammedi and Atik, 2012)
Tamarix macrocarpa	Arial part	Phenolic and Polyphenolic compounds, Gallic Acid, Isoferulic Acid, Kampferol and Quercetin.	(Ali *et al.*, 1979)
Tamarix gallica	Plant extract	Flavonoids, 3,5,7-trihydroxy-4'-methoxyflavone (2) & 5-Hydroxy-3,7, 4' -trimethoxyflavone (1) and	(Mostafa, 2010)
Tamarix hampeana	Flowers	New derivative of long chain secondary alcohols, sitosterol and laserine.	(Aykac and Akgül, 2010)
Tamarix hokenakeri	Green twigs	9.2% tanning agent	(Bikbulatova and Korulkina, 2001)
Tamarix elongata	Whole plant	Oxidized forms of flavonoids, hydrolyzed tanning agents, phenolic, carbohydrates, amino Acids and tamarixetin 3-O-b-D-glucoside	(Umbetova *et al.*, 2004)
Tamarix laxa	Whole plant	Oxidized forms of flavonoids, hydrolyzed tanning agents, phenolic, carbohydrates, amino Acids and tamarixetin 3-O-b-D-glucoside	(Umbetova *et al.*, 2004)
Tamarix africana	Leaves	Polyphenols (phenolic Acids, flavonoids and tannins)	(Benabdullah *et al.*, 2014)
	Leaves	Polyphenols (phenolic Acids, flavonoids and tannins)	(Khennouf *et al.*, 2003)
Tamarix chinesis	Twigs, leaves	Tamarixinol, β-sitosterol, tamarixone, tamarixol, daucosterol, 3', 4'-di-O-methyl-quercetin and four long chain compounds.	(Jiang and Zuo, 1988)
Tamarix aucheriana	Plant extract	Syringic Acid	(Abaza *et al.*, 2013)

1.2.1.3. Pharmocological studies on genus *Tamarix*:

Various pharmacologically active compounds have been reported from *Tamarix*. *Tamarix aphylla* has antioxidant activity (Auribie, 2011). The aerial parts of *Tamarix indica* and *Tamarix passernioides* are given as ailment in chronic diseases such as diarrhea and dysentery, also used as an astringent tonic (Naz *et al.*, 2013). A decoction of these both plants are observed to have antinociceptive activity (Sarker and Sarker, 2009). Roots of these plants also have antinociceptive, cytotoxic and diuretic activity (Naz *et al.*, 2013).

Tamarix indica leaves possess antinociceptive, antidiarrhoeal, cytotoxic activities (Habiba *et al.*, 2010; Sarker and Sarker, 2009). *Tamarix dioica* possess antiinflammatory activity of Jigrine (Karunakar *et al.*, 1997). *Tamarix ramosissima* possess antioxidant, antibacterial, antifungal anti-inflammatory, analgesic and DNA damaging activities as well as wound healing in mice skin (Sultanova *et al.*, 2001). *Tamarix gallica* is reported to have analgesic and anti-inflammatory effects (Chaturvedi *et al.*, 2012). It is also antioxidant and antimicrobial action (Mostafa, 2010; Riadh *et al.*, 2009; Drabu *et al.*, 2012). *Tamarix hohenackeri* arial parts act as ACE and platelet aggregation inhibitors (Xing *et al.*, 2014).

Tamarix pauciovulata is used as an anti-oxidant (Mohammedi and Atik, 2012), while *Tamarix macrocarpa* is an antimicrobial (Rahman *et al.*, 2005). *Tamarix boveana* is an anti-oxidant and antimicrobial in action, also has Free Radical Scavenging activity (Saidana *et al.*, 2008). One another specie *Tamarix hispida* is used as an antioxidant, antimicrobial, and fungicidal (Sultanova *et al.*, 2001).

Tamarix africana has remarkable biochemical and pharmacological activities such as used for GIT disturbances. It also possesses antibacterial, antiviral and anti-inflammatory activities (Benabdullah *et al.*, 2014). *Tamarix aucheriana* has antimitogenic and chemo-sensitizing activities in human coleo-ractal cancer cells (Abaza *et al.*, 2013).

Table 1.2: Pharmacological activities of different species of Genus *Tamarix*.

Genus tamarix	Part used	Activity	Reference
Tamarix ramosissima	Plant extract	Antioxidant, DNA damaging antibacterial & antifungal activities	(Sultanova et al., 2001)
	Plant extract	Anti-inflammatory, Wound Healing in Mice Skin, anti-oxidant and Analgesic,	(Mao *et al.*, 2011)
Tamarix gallica	Plant extract	anti-inflammatory & analgesic	(Chaturvedi *et al.*, 2012)
	Leaves	Anti-microbial activity	(Mostafa, 2010)
	Leaves	antioxidant and antimicrobial activities	(Riadh *et al.*, 2009)
T. aphylla	Plant extract	antioxidant activity	(Auribie, 2011)
T. dioica	Plant extract	antiinflammatory activity of *Jigrine*	(Karunakar *et al.*, 1997)
	Leaves	Antimicrobial Activity	(Khan, 2013)
Tamarix pauciovulata	Leaves	antioxidant activity	(Mohammedi and Atik, 2012)
Tamarix macrocarpa	Arial part	antimicrobial activity	(Rahman *et al.*, 2005)
Tamarix indica	Leaves	antinociceptive, antidiarrhoeal, cytotoxic activities	(Habiba *et al.*, 2010)
	Whole plant	antinociceptive activity	(Sarker and Sarker, 2009)
	Arial part	chronic diseases e.g. diarrhea and dysentery	(Naz *et al.*, 2013)
	Bark	astringent tonic	(Naz *et al.*, 2013)
	Root	antinociceptive, cytotoxic and diuretic properties	(Naz *et al.*, 2013)
Tamarix passernioides	Whole plant	antinociceptive activity	(Sarker and Sarker, 2009)
	Arial part	chronic diseases e.g. diarrhea and dysentery	(Naz *et al.*, 2013)
	Bark	astringent tonic	(Naz *et al.*, 2013)
	Root	antinociceptive, cytotoxic and diuretic properties	(Naz *et al.*, 2013)
Tamarix hokenakeri	Arial part	ACE and platelet aggregation inhibitors	(Xing *et al.*, 2014)
T. boveana	Plant extract	Antimicrobial activity	(Saidana *et al.*, 2008)
	Plant extract	Anti-oxidant, Free radical scavenging activity	(Naija *et al.*, 2014a)
T. hispida	Plant extract	antioxidant, antimicrobial, and fungicidal	(Sultanova *et al.*, 2001)
Tamarix aucheriana	Plant extract	Antimitogenic and chemo-sensitizing activities in human coleo-ractal cancer cells	(Abaza *et al.*, 2013)

1.3. GENUS *ACACIA*:

This genus belongs to family Leguminosae and sub-family Mimosaceae. (Hemamalini *et al.*, 2014; Lorenzo *et al.*, 2010). The genus has about 1380 species around world, among this 1000 are found in continent of Australia. African continent has about 144 species. North and South America have 185 species that is more than Asia, which has about 89 species (Lorenzo *et al.*, 2010).

Leaves of genus *Acacia* are bipinnate. It has stipular spines. Colporate pollen grains with smooth exines. (Miller *et al.*, 2003). Some members of *Acacia* genus have leaves, used as poultice (This preparation is moist, used to improve pain and circulation). Due to the presence of tannins, leaves & pods (young) of some species are quite astringent in action (Mbatchou *et al.*, 2011). The exudates of stem-bark are in the form of gums which is also used medicinally. This bark is used for cough (Van, 2008). The members of Genus *Acacia* are reported to contain l-arabinose, catechol, galactoaraban, galactose, galactan, tannin, sulphoxides pentosan, N-acetyl jenkolic Acid and saponin (Pande *et al.*, 1981). Thus members of this genus are used in diarrhoea, cancer, hemorrhoid, inflammation, ophthalmia, leprosy, bleeding piles, and leucoderma problems (Khare and Chandrama, 2007).

1.3.1 Genus *Acacia* reported work:

1.3.1.1 Ethnobotany:

Accacia leucophloea is traditionally used in diarrhea, cancer, inflammation, ophthalmia, hemmorrhide, leprosy, bleeding piles, and leucodermic problems (Ziaulhaq *et al.*, 2013)**.** The said plant has traditional use to cure cancer, pharyngitis, high level cholesterol, gingivitis, mouth sores, diabetes and indigestion in pead (Ziaulhaq *et al.*, 2013)**.**

Its arerial parts are used by cattles. Its stem bark smells foul, using bark fibers for making nets of fish. The constituents of bark are used for dyes, in treatments of snake bite (Imran et al., 2011). *Accacia leucophloea* seeds are used as vegetable in Indonesia. It is believed that the leaves of *Accacia leucophloea* contain CNS-depressent, antisyphilitic and

antimicrobial principles. The bark gum contains demulscent properties (Khare and Chandrama, 2007).

Acacia stenophylla produces very good fuel (Ventura *et al.*, 2004). The wood of this tree is used for furniture and fenceposts (Khanzada *et al.*, 1998). This beautiful plant is used for ornamental pupose in inland areas. It is also used for shade as well as for windbreak. Due to the suckering propensity, this plant is used for soil stabilization (DR Michael *et al.*, 2011). Australian indigenous people used the pods and seeds for food purpose (Lim, 2014). *Acacia saligna (cyanophylla)* is cultivated for variety of purposes (Midgley and Turnbull, 2003). *Acacia cynophylla* is used for fuel-wood or charcoal (Marchante *et al.*, 2003). The said plant is used for vine stakes and some agricultural equipments (Michaelides, 1979). *Acacia saligna* is a fodder plant (Anon, 1955; Michaelides, 1979; Dumancic and Le-Houerou, 1980). The gum of *Acacia stenophylla* is used in certain foodstuffs (Michaelides, 1979). This species is used by dyers to dye wool to lemon colour (yellow) using an alum mordant (Martin, 1974a). It is traditionally used for hypoglycemic (Zeweil and Saber, 2008), antibacterial (Sotohy *et al.*, 1997), and anti-inflammatory (Dafallah *et al.*, 1996) effects. *Acacia arabica* is effective against various types of diseases including cancer and skin diseses. Parts are also used as antimicrobial, demulcent, anti-diarrhoeal and aphrodisiac (Rajvaidhya *et al.*, 2012). *Acacia nilotica* is traditionally used for bleeding diseases, prolapse and leucorrhoea (Farzana *et al.*, 2014).The bark, leaves, seeds, pods and gum of *Acacia arabica* are used for medical purposes. The plant is used traditionally internally as well as externally (Chandra *et al.*, 2008; Farzana *et al.*, 2014).

1.3.1.2. Phytochemistry:

The memebers of *Acacia* have been studied for a huge number of their bioactive agents like tannins, saponins, polyphenols, oil and polysaacharides etc (Okoro *et al.*, 2012; Hameed *et al.*, 2011; Mohammad *et al.*, 1997; Meena *et al.*, 2006; Nadkarni, 1979; Freire *et al.*, 2007). The stem bark of *Acacia senegal* contain tannins sterols and saponins (Okoro *et al.*, 2012). *Acacia nilotica* contains alkaloids, tannins, saponins, carbohydrates, flavonoids, anthraquinone and cardiac glycosides (Deshpande, 2013; Mbatchou *et al.*,

2011). It also contains 1-acetyl beta carboline, Hydroxycitronellal, 3-picoline-2-nitro,Trans decalone, Lavandulyl acetate, D-Glucoronic Acid and Propionic Acid-2-chloro,ethyl ester (Hemamalini *et al.*, 2014). *Acacia nilotica* leaves and fruits contain tannin 32% and flowers contain stearic Acid, kaempferol-3- glucoside, isoquercetin, leucocyanidin (Chopra *et al.*, 1956; Chatterjee & Chatterjee, 2000). The bark contains 20% of tannins. There are some polyphenolic compounds that have been reported and identified as (+) dicatechin, quercetin, gallic Acid (Said, 1997; Freire *et al.*, 2007). The bark also contains ∞ - amyrin, ß-sitosterol and sucrose (Asolkar *et al.*, 2005; Chatterjee and Chatterjee, 2000; Narayan and Kumar, 2005). The pods of babul contain tannin, 12-19% in the whole pod and after removal of seeds is about18-27% (Nadkarni, 1979; Freire *et al.*, 2007; Gulco, 2001; Hakim, 2002). The gum of *Acacia nilotica* contains 1.8 % moistures. *Acacia nilotica* posses L-arabinose, galactose, L-rhamnose and four aldobiouronic Acids. 3-O-ß-L-arabinopyranosyl, arabinose and L-arabinose % were also found (Nadkarni, 2005; Chatterjee and Chatterjee, 2000; Freire *et al.*, 2007; Gulco, 2001). Further it contains calcium, polysaccharides, magnesium salts, potassium, sugar, moisture, ash and malic Acid and oxidative enzymes (Said, 1997; Chatterjee and Chatterjee, 2000). Wood contains chlorides (Dymock *et al.*, 2005).

Acacia catechu contains terpenes (Negi and Dave., 2010). *Acacia confusa* contains okanin, melacAcidin (flavonoids) (Lin and Chang, 2013; Sachin *et al.*, 2012). *Acacia leucophloea* contains alkaloids, terpenes, flavanoids and tannins (Sachin *et al.*, 2012). Oils obtain from flowers of *Acacia aroma* flower contain eugenol and methyl salicylate (Lamarque *et al.*, 1998). *Acacia auriculiformis* is rich in tannins, saponins, methylglucuronic Acid, glucuronic Acid, galactose, arabinose and rhamnose (Rajbir *et al.*, 2007; Anderson, 1978). *Acacia drepanolobium*, *Acacia hockii*, *Acacia polyacantha*, *Acacia tortilis*, and *Acacia seyal* contain tannins and phenolic contents (Rubanza *et al.*, 2005). *Acacia pennata* contains flavonoids (Dongmo *et al.*, 2005). *Acacia salicina* leaf and bark contain tannins and pods possess saponins in rich amount. (Bouhlel *et al.*, 2007; Everist, 1969; Hall, 1972). *Acacia victoria* contains triterpenoid saponins (Valsala *et al.*, 2001). *Acacia aneura* leaves are rich in condensed tannins and oxalates (Gartner and Hurwood, 1976) while *Acacia cambagei* leaf contains cyanogenic glycosides and oxalates (Cunningham *et al.*, 1981). The leaves and stems of *Acacia doratoxylon* and

Acacia georgina contain Cyanogenic glycosides and cyanogenic hydrolase respectively (Cunningham *et al.*, 1981).

Table 1.3: Phytochemistry of different species of genus *Acacia.*

Genus Acacia	Part used	Compounds	Reference
Acacia senegal	Stem bark	Sterols, tannins and saponins.	(Okoro *et al.*, 2012)
Acacia nilotica	Plant extract	Anthraquinone, alkaloids, saponins, tannins, carbohydrates, cardiac glycosides and flavonoids,	(Deshpande, 2013)
	Plant extract	anthraquinones,alkaloids, flavanoids, amino Acids, general glycosides, steroids, tannins, saponins and terpenoids in stem	(Mbatchou *et al.*, 2011)
	Plant extract	3-picoline-2-nitro, Trans decalone, 1-acetyl beta carboline, Hydroxy citronellal,Propionic Acid-2-chloro,ethyl ester, D-Glucoronic Acid and Lavandulyl acetate	*(Hemamalini et al., 2014)*
	Leaves	tannin 32%,	(Chopra *et al.*, 1956)
	Fruit	stearic Acid, kaempferol-3-glucoside, isoquercetin, leucocyanidin	(Chatterjee and Chatterjee, 2000)
	Bark	20% of tannins.	(Said, 1997)
	Bark	polyphenolic compounds e.g. (+) dicatechin, quercetin, gallic Acid,	(Freire *et al.*, 2007; Gulco, 2001)
	Bark	∞ - amyrin, ß-sitosterol and sucrose.	(Asolkar *et al.*, 2005)
	Pods	tannin, 12-19%	(Nadkarni, 2005)
	Gums	1.8% moistures,	(Nadkarni, 2005)
	Gums	galactose, aldobiouronic Acids, L-arabinose arabinose, L-rhamnose,3-O-ß-L-arabinopyranosyl, L-arabinose.	(Chatterjee and Chatterjee, 2000)
	Gums	calcium, polysaccharides, magnesium salts, potassium, sugar, moisture, ash and malic Acid and oxidative enzymes	(Said, 1997)
	Wood	Chlorides	(Dymock *et al.*, 2005)

Acacia catechu	Plant extract	Terpene	(Negi and Dave., 2010)
Acacia confusa	Plant extract	okanin (**9**), melacAcidin (**3**) (flavonoids)	(Lin and Chang, 2013)
Acacia leucophloea	Plant extract	alkaloids, terpenes, flavanoids and tannins	(Sachin *et al.*, 2012)
Acacia aroma	Flower	volatile oils contain eugenol & methyl salicylate	(Lamarque *et al.*, 1998)
Acacia auriculiformis	Plant extract	tannins, saponins,	(Anderson, 1978)
	Plant extract	methylglucuronic Acid, glucuronic Acid, galactose, arabinose and rhamnose	(Rajbir *et al.*, 2007)
Acacia drepanolobium	Plant extract	Tannins and phenolic contents,	(Rubanza *et al.*, 2005)
Acacia hockii	Plant extract	tannins and phenolic contents,	(Rubanza *et al.*, 2005)
Acacia polyacantha,	Plant extract	tannins and phenolic contents,	(Rubanza *et al.*, 2005)
Acacia tortilis	Plant extract	tannins and phenolic contents,	(Rubanza *et al.*, 2005)
Acacia seyal	Plant extract	tannins and phenolic contents,	(Rubanza *et al.*, 2005)
Acacia pennata	Plant extract	Flavonoids	(Dongmo *et al.*, 2005)
Acacia salicina	Leaf, bark	Tannins	(Bouhlel *et al.*, 2007)
	Pods	Saponins	(Hall, 1972)
Acacia victoria	Plant extract	Triterpenoid saponins	(Valsala *et al.*, 2001)
Acacia aneura	Leaves	Condensed tannins and oxalates	(Gartner and Hurwood, 1976)
Acacia cambagei	Leaves	Cyanogenic glycosides and oxalates	(Cunningham *et al.*, 1981)
Acacia doratoxylon	Leaves, stems	Cyanogenic glycosides	(Cunningham *et al.*, 1981)
Acacia georgina	Leaves, stems	cyanogenic hydrolase	(Cunningham *et al.*, 1981)

1.3.1.3 Biological studies on genus *Acacia:*

Various pharmacological activities have been reported from *Acacia*. Genus *Acacia* different parts are used in hair-fall, ear-ache, cholera, dysentery, syphilis and leprosy (Asolkar *et al.*, 2005). The parts (leaves) of *Acacia nilotica* are used as garglein (spongy gums), in sore throat and as wash in wounds and hemorrhagic ulcers. It has also an astringent effect. The leaves of *Acacia nilotica* are tonic to liver and brain (Nadkarni, 2005; Said, 1997; Narayan and Kumar, 2005). It also strengthens vision and cure eye diseases (Hakim, 2002; Narayan and Kumar, 2005). The leaves are also antipyretic and enriches the blood (Antaki., 1998). The bark (stem) of *Acacia nilotica* is used as an astringent agent. It is also used in gonorrhoea, cystitis, vaginitis, leucorrhoea, prolapsed of the uterus and piles (Nadkarni, 1979; Said, 1997; Kritikar and Basu, 2003). The bark extract of this specie may block the body's pain triggers (Ameh, 2010) andgums are used in diarrhea, dysentery and diabetes mellitus (Nadkarni, 1979; Said, 1997; Kritikar and Basu, 2003; Chopra *et al.*, 1956; Rushd, 1987), also used in antipyretic, expectorant and cure lung troubles (Kritikar and Basu, 2003). The pods of *Acacia nilotica* are use for urino-genital disorder, impotency and in dry cough (Mohanty *et al.*, 1996)**.** The fruits of *Acacia nilotica* are useful in diarrhoea, dysentery and diabetes and roots are useful in leucorrhoea, wound healing and burning sensation (Gilani, 1999; Rao and Santhanam., 1967).

Acacia senegal stem bark is used as antibacterial and toxicological in action (Okoro *et al.*, 2012). *Acacia catechu* stem bark is used as anti-microbial in action, such as anti-bacterial and anti-fungal (Negi and Dave., 2010). The roots of *Acacia confusa* has antioxidant activity (Lin and Chang, 2013). *Acacia leucophloea* is used in diarrhoea, hemorrhoid, cancer, bleeding piles, inflammation, ophthalmia, leprosy, and leucoderma problems. The pods and young leaves are used as astringents. The green leaves of this specie is believed to possess hypotensive, antisyphilitic, CNS-depressent and antimicrobial principles and gums possesses demulscent properties (Ziaulhaq *et al.*, 2013).

Acacia aroma is used as antiseptic, in wound healing and in the management of gastrointestinal disorders. The leaves and barks of this plant are used as diuretic, cicatrizant and anti-inflammatory agents (Arias *et al.*, 2004).

Two other species such as *Acacia pennatula* and *Acacia angustissima* have cytotoxic activities (Popoca *et al.*, 1998). The extracts of *Acacia ancistrocarpa* contain potent cyclooxygenase-1 (COX-I) inhibitory activity (Li *et al.*, 2003). It has been reported that *Acacia auriculiformis* has anti-oxidant, central nervous system-depressant, spermicidal and filaricidal activities. (Rajbir *et al.*, 2007; Mahato and Garai, 1997; Ghosh *et al.*, 1996). *Acacia adsurgens*, *Acacia nilotica*, *Acacia ancistrocarpa* and *Acacia catechu* possess anti-inflammatory activity by specifically inhabiting COX-1 except *Acacia nilotica* which inhabits COX-2 (Dongmo *et al.*, 2005; Trivedi *et al.*, 1986; Li *et al.*, 2003). The extracts from leaves of *Acacia pennata* are used to manage pain, cough, inflammatory troubles like rheumatism, headaches and fever (Dongmo *et al.*, 2005). *Acacia longifolia* has significantly basal respiration, higher microbial biomass and b-glucosaminidase activity (Marchante *et al.*, 2008). *Acacia dealbata* possess anti-fungal and anti-bacterial activity (Lorenzo *et al.*, 2010).

Acacia salicina possess hypoglycaemic effect (Wadood *et al.*, 1989), cestocidal activity (Ghosh *et al*., 1996), anti-inflammatory potetial (Dafallah and Al-Mustafa, 1996), antibacterial effects (Sotohy *et al.*, 1997), anti-platelet aggregatory effect (Shah, 1997), antihypertensive and antispasmodic activities (Gilani, 1999). This plant extract also has spasmogenic as well as vasoconstrictor actions (Amos *et al.*, 1999), antioxidant effect (Bouhlel *et al.*, 2007; Chang *et al.*, 2001) and also has anti-viral activity (hepatitis C virus) (Hussein *et al.*, 2000). The growth of tumor cell and induction of apoptosis is also inhibited by *Acacia victoria* (Valsala *et al.*, 2001).

Table 1.4: Pharmacological activities of different species of genus *Acacia.*

Genus Acacia	Part used	Activity	Reference
Acacia senegal	Stem bark	Antibacterial and toxicological	(Okoro *et al.*, 2012)
Acacia nilotica	Stem bark	Antimicrobial	(Deshpande, 2013)
	Stem bark	typhoid fever	(Mbatchou *et al.*, 2011)
	Leaves, bark	Antibacterial activity, antimalarial activity, antifungal activity, antibiotic activity, anti-diarrhea activity, molluscidal activity, anti hypertensive activity, anthelmentic activity, anti denaturation property, antioxidant and anticancer property	*(Hemamalini et al., 2014)*
	Gum	Diarrhea, dysentery and diabetes mellitus	(Kritikar and Basu, 2003)
	Pods	Urino-genital disorder, impotency and in dry cough.	(Mohanty *et al.*, 1996)
	Fruit	Diarrhoea, dysentery and diabetes	(Gilani*et al.*, 1999)
	Root	Leucorrhoea, wound healing and burning sensation	(Rao et al., 1967)
Acacia catechu	Stem bark	Antimibacterial and antifungal	(Negi and Dave., 2010)
Acacia confusa	Root	Antioxidant	(Lin and Chang, 2013)
Acacia leucophloea	Leaves, pods	diarrhoea,hemorrhoid, cancer, bleeding piles, inflammation, ophthalmia, leprosy, leucoderma problems, hypotensive, antisyphilitic, CNS-depressent and antimicrobial activity	(Ziaulhaq *et al.*, 2013)
Acacia aroma	Whole plant	Wound healing, antiseptic, gastrointestinal disorders treatment.	(Arias *et al.*, 2004)
	Leaves and barks	Diuretic, anti-inflammatory and cicatrizant	(Arias *et al.*, 2004)
Acacia angustissima	Plant extract	Cytotoxic activities	(Popoca et al., 1998)
Acacia pennatula	Plant extract	Cytotoxic activities	(Popoca et al., 1998)
Acacia ancistrocarpa	Plant extract	Cyclooxygenase-1 inhibition activity	(Li et al., 2003)

Acacia auriculiformis	Plant extract	Anti-oxidant, central nervous system-depressant, spermicidal and filaricidal activities.	(Rajbir et al., 2006)
Acacia adsurgens	Plant extract	anti-inflammatory activity by inhabiting COX-1	(Dongmo et al., 2005)
Acacia ancistrocarpa	Plant extract	anti-inflammatory activity	(Dongmo et al., 2005)
Acacia pennata	Leaves extract	Pain, cough, inflammatory disorders such as rheumatism, headaches and fever.	(Dongmo et al., 2005)
Acacia longifolia	Plant extract	Microbial biomass, b-glucosaminidase and basal respiration activity.	(Marchante et al., 2008)
Acacia dealbata	Leaves	anti-fungal and anti-bacterial activity	(Lorenzo et al., 2010)
Acacia salicina	Plant extract	hypoglycaemic effect	(Wadood et al., 1989)
	Plant extract	cestocidal activity	(Ghosh et al., 1996)
	Plant extract	anti-inflammatory potential	(Dafallah and Al-Mustafa, 1996)
	Plant extract	Anti-microbial effects	(Sotohy et al., 1997)
	Plant extract	anti-platelet aggregatory activity	(Shah et al., 1997)
	Plant extract	antihypertensive & antispasmodic effects	(Gilani et al., 1999)
	Plant extract	Spasmogenic, vasoconstrictor actions.	(Amos et al., 1999)
	Plant extract	Anti-viral activity (hepatitis C virus)	(Hussein et al., 2000)
	Plant extract	Antioxidant effects	(Chang et al., 2001)
Acacia victoria	Plant extract	tumor cell growth ihabitor, induce apoptosis	(Valsala et al., 2001)

1.4. TAXONOMICAL POSITION OF *TAMARIX APHYLLA:*

Kingdom: Plantae

Subkingdom: Vascular plants - Tracheophytes

Superdivision: Seed plants - Spermatophytes

Division: Flowering plants - Magnoliophyta

Class: Dicotyledonae - Magnoliopsida

Order: Caryophyllales

Family: Tamaricaceae

Genus: *Tamarix*

Species: *Tamarix aphylla*

1.4.1. Plant morphology:

Tamarix aphylla is an evergreen, perennial tree. It produces from seeds. It is a spreading tree of about 15 meters in height. It has pendulous jointed branches. The younger tree of this specie has light grey trunk and stem. In older trees the stem is grey brown and bark is dark grey to black which is thick and rough. The diameter of stem of mature tree can be upto 1 meter. The small needles like leaves which are dull green are like those found in pines. The leaves are sessile, alternately positioned, entire and minutely cuspidate. They ae about less than 0.5 to 2 mm in length and are pointed abruptly. The branchlets are gray-green and distinctly articulate. However this is a flower bearing plant. The flowers are stalkless, small in size, having pinkish-white colour. It grows on long spike of about 30–40 mm. The fruit of *Tamarix aphylla* is bell shaped with a hairy bunch. It has many small and cylindrical shaped seeds. It is coriaceous capsule like of about 0.1-0.2 inch (2-4 mm) long. The fine hairs found on seeds help in seed dispersal through wind. The roots of this tree are strong and woody. They penetrate and spread deeply in the soil.

(Carpenter *et al.*, 1998; Felger, 2000; Hickman, 1993; Shreve and Wiggins., 1964; United States Department of Agriculture, 2001).

1.4.2. Distribution:

Tamarix aphylla is considered the most common species cultivated as roadside tree across the country (Pakistan) (Marwat, 2008). It has worldwide distribution. It is found in Africa (Sudan, Tunisia, Morocco, Algeria, Abyssinia, Kenya, Eagypt, Libya, Somalia, Eriterea and Senegal), Midle East (Yemen, Jordan, Kuwait, Saudi Arabia, Iran, Israel, Iraq), Pakistan, Afghanistan, India. Also found in South West United States, from Texas to California (Marwat, 2008; Hickman, 1993; United States Department of Agriculture, 2001). *Tamarix aphylla* is commonly found on saline habitats like salt flats, springs, along the streams and rivers (Powell, 1988; Tesky, 1992). It has also been found along the Salton Sea Basin and Coloradoand Gila Rivers (Turner and Brown, 1982). *Tamarix aphylla* is also distributed with irrigation ditches in the bottomlands (Benson and Darrow., 1981).

1.4.3. Ethnobotanical uses of *Tamarix aphylla:*

Tamarix aphylla has antioxidant activity (Auribie, 2011). It is also used in jaundice, rheumatism, bad evils, wound and abscesses (Marwat *et al.*, 2011). In coastal gardens, *Tamarix aphylla* serves as a good shelter hedge. Its wood is used for timber and fuel. Also used for making agricultural appliances. The green leaves of *Tamarix aphylla* are consumed by camels as food (Marwat, 2008). Therapeutic uses of *Tamarix aphylla* reveal that the gall and bark of the tree are used as astringent, tonic and aphrodisiac. It is also used in the management of syphilis, eczema, skin diseases, hepatitis and scaly skin diseseases (Rao, 2001). The leaves of the plant have been reported to have germicidal potential. It is also used in eye inflammation, fever, toothache (dental pain), wound healing, inflammation (Kamal *et al.*, 2009; Abbas *et al.*, 2002), in cold & in flu. The extracts from the leaves are applied in treatment of tetanus (Marwat *et al.*, 2011). The bark (ground part) is applied on wounds for healing (Shahidullah, 2000; Azaizeh *et al.*, 2006; Marwat, 2008).

1.4.4. Reported isolated compounds of *Tamarix aphylla*:

Tamarix aphylla leaves contains alkaloids, flavonoids, steroides, cardiac glycosides, terpenoides, tannins and saponins (Auribie, 2011). The bark of *Tamarix aphylla* reported to possess bioactive agents such as triterpene D-friedoolean-14-en-3α, 3-ketone, 3 β isomer (myricadiol) and 28-diol (isomyricadiol) (Souliman *et al.*, 1991). From the stem bark of *Tamarix aphylla* many other active compounds have benn isolated and characterized including diaryloxy furanofuran lignan, isoferulylglyceryl ester, polyphenolic and dehydrodigallic Acid etc. (Souliman *et al.*, 1991).

Figure 1.1: Tree of *Tamarix aphylla*

1.5. TAXONOMICAL POSITION OF *TAMARIX DIOICA:*

Kingdom: Plantae

Subkingdom: Vascular plants - Tracheophytes

Superdivision: Seed plants - Spermatophytes

Division: Flowering plants - Magnoliophyta

Class: Dicotyledons - Magnoliopsida

Order: Caryophyllales

Family: Tamaricaceae

Genus: *Tamarix*

Species: *Tamarix dioica*

1.5.1. Plant morphology of *Tamarix dioica:*

Tamarix dioica is locally known as ghaz or jhau or khagal. It is an evergreen shrub or small tree about 6 meters having reddish bark. It has vaginate leaves, and purple flowers. Flowers are about 3 mm in diameter. These flowers are unisexual, pink or purple colored. The spikes are cylindrical in shape and closely or compactly aranged. Fruit is a capsule shaped about upto 5 mm in lenght, cone like in shape and consists of 3 valves (Khan *et al.*, 2013).

1.5.2. Distribution:

This plant is native to Pakistan, Afghanistan, Iran, India, Kashmir, Nepal, Bangladesh, Bhutan, Kashmir, Nepal, and Myanmar. In Pakistan, it grows throughout the country and is mostly exist in the provinces of Khyber Pukhtunkhwa (KPK) and Sindh (Khan *et al.*, 2013). *Tamarix dioica* has been reported from Sindh, Balochistan, Khyber Pukhtunkhwa and Punjab (Samejo *et al.*, 2013a).

1.5.3. Ethnobotanical uses of *Tamarix dioica:*

Tamarix dioica is locally used in management of splenic and hepatic inflammation, also used as diuretic and carminative (Samejo *et al.*, 2013a). The leaves of *Tamarix dioica* possess cytotoxic, antimicrobial and antifungal activity in their crude extracts (Khan *et al.*, 2013). The plant of this specie is also used as an astringent for symptoms such as leucorrhea (Samejo *et al.*, 2013a; Qadri *et al.*, 1987; Khan *et al.*, 2004). *Tamarix dioica* also possess anti-inflammatory activity of Jigrine (Karunakar *et al.*, 1997).

1.5.4. Reported isolated compounds of *Tamarix dioica:*

Different parts of the plant have been investigated for chemical constituents analysis of the data indicated that various phytochemicals like tannins, phlobatannins, flavonoids, phenols, steroids, saponins and terpenoids were found present, while glycosides, alkaloids, amino Acide and protein were absent (Samejo *et al.*, 2013a). From the leaves, many other agents have also been obtained such as D-mannitol, Kaempferide and tamarixetin (Rastogi and Mehrotra., 1990).

Table 1.5: Phytochemicals in variou parts of *Tamarix dioica.*

S.No	Constituents	Stem	Flowers	Leaves	Roots
01	Steroids	+	+	+	+
02	Phlobatannins	-	+	+	+
03	Phenols	-	-	-	-
04	Tannins	+	+	+	+
05	Terpenoids	-	-	-	-
06	Flavonoid	-	+	+	-
07	Saponin	+	+	+	-
08	Proteins	+	+	+	+
09	Alkaloid	-	-	-	-
10	Glycosides	+	+	+	-
11	Amino Acids	-	-	-	-

Figure 1.2: Tree of *Tamarix dioica*

1.6. TAXONOMICAL POSITION OF *ACACIA CYANOPHYLLA:*

Kingdom: Plantae

Subkingdom: Vascular plants - Tracheophytes

Superdivision: Seed plants - Spermatophytes

Division: Flowering plants - Magnoliophyta

Class: Dicotyledons - Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Legumenoseae

Genus: *Acacia*

Species: *Acacia cyanophylla*

1.6.1. Plant morphology:

Acacia cyanophylla is a variable shrubs or trees, is about 2–10 m tall. The trunk is either single- or multi-stemmed, about 5–40 cm and is straight to rather crook. It is often suckering and sometimes forming thickets. The root of this plant may grow to 16 m deep in sand (Knight *et al*. 2002). There is also development of sub- surface lateral roots (Messines *et al.*, 1952).

1.6.2. Distribution:

Acacia cyanophylla is native to Australia (Akkari *et al.*, 2008). It was introduced in Tunisia in 1930 (Asma *et al.*, 2013). It is cultivated in Iran, Morocco, Algeria and Egypt. It is cultivated in Pakistan, especially in Rawalpindi and Peshawar etc.

1.6.3. Ethnobotanical uses of *Acacia cyanophylla:*

Acacia cyanophylla has an antinematode activity against gastrointestinal nematode parasitism in sheep (Akkari *et al.*, 2008; Asma *et al.*, 2013)**.** This species has been extensivey planted (within Australia & abroad both) for ornamental purposes (Martin, 1974b).

1.6.4. Reported isolated compounds of *Acacia cyanophylla:*

Acacia cyanophylla possess sufficient amount of crude proteins and tannins (Maslin and Mcdonald, 2004)**.** A compound Iso-salipurposide has been isolated from flowers (Ghouila *et al.*, 2012).

Figure 1.3: Tree of *Acacia cyanophylla*

1.7. TAXONOMICAL POSITION OF *ACACIA STENOPHYLLA:*

Kingdom: Plantae

Subkingdom: Vascular plants - Tracheophytes

Superdivision: Seed plants - Spermatophytes

Division: Flowering plants - Magnoliophyta

Class: Dicotyledons - Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Legumenoseae

Genus: *Acacia*

Species: *Acacia stenophylla*

1.7.1. Plant morphology:

Acacia stenophylla is a bushy tall shrub or tree of naerly 4 to 12 meter in height. Under favourable conditions, its height may reach upto 20 meters (Hall *et al.*, 1972). It is either one stemmed or may be bracnhed into many stems, which are above the ground at a height of about 1 meter or more. The trunk of the tree becomes mis-shapen. It has pale grayish green leaves which are usually straight, some times twisted or slightly curved. Mature plant has dark brown, rough and deeply textured bark.The stem bark is in sharp contrast with its leaves. This plant has showy and creamy yellow colored flowers which are produced from winter (late) to spring in smaller rounded clusters (Cunningham *et al.*, 1981).

1.7.2. Distribution:

It is native to Australia and has a diverse distribution especially in the arid parts of Australia. In distribution, it extends from North Eastern-Western Australia, East through Northern Territory to Queenlsand and South to the Murray-Lachlan-Darling River system in South Australia, Victoria and New South Wales (Cowan and Maslin, 2001). In Victoria and South Australia, *Acacia stenophylla* usually occupies the drier habitats. This plant has not been extensively introduced outside Australia (Doran *et al.*, 1997).

1.7.3. Ethnobotanical uses of *Acacia stenophylla:*

Acacia stenophylla is used as fuel (Hall *et al.*, 1972). Its wood is used for fencepost (Marcar *et al.*, 1995). In inland areas, this beautiful tree is used for ornamental purpose. It is also used for shade as well as for windbreak. Due to the suckering propensity, this plant is used for soil stabilization (Michael *et al.*, 2011)

1.7.4. Reported isolated compounds of *Acacia stenophylla:*

This specie possesses a marked amount of alkaloids. But there is no authentic research article is available regarding chemical constituents of *Acacia stenophylla*.

Figure 1.4: Tree of *Acacia stenophylla*

1.8. NATURAL PRODUCTS:

Plants are used as drugs to treat various diseases; it is a centuries old practice and has made a long history. Plants as traditional medicines date back to Mesopotamia (2600 B.C.), and still are used as natural remedies (Koehn and Carter, 2005). Approximately, 35,000 to 70,000 plants have been studied for their medicinal value uptill now (Farnsworth *et al.*, 1991; Dev, 1999).

It has been investigated that flavonoids have a key role in various pharmacological activities such as anti-inflammatory, anti-allergic, antioxidant, anti-viral, anti-cancer, anti-thrombotic and hepatoprotective (Najafi *et al.*, 2010). Tannins have use in various preparations like homeostatic, anti-diarrheal and anti-hemorrhoidal. Saponins, which are steroid alkaloids, present in plants particularly in their skins as waxy coating, have important role in various activities like lowering cholesterol level, also used as anti-inflammatory & anti-oxidant entities. Saponins can also be used to coagulate and precipitate red blood cells (Najafi *et al.*, 2010). Terpenoids, the most important natural

products found in almost all types of organisms. Terpenoids, a class of wide variety of compounds exhibit various pharmacological properties like antibacterial (Selvan *et al.*, 2012). They also have a key role in healing of wound, skin strengthening and restoration of inflamed tissue by increasing supply of blood (Krishnaiah *et al.*, 2009). Another class of compounds is Phenolic compounds. These chemicals also possess different biological potentials including anti-inflammatory, cardiovascular protection, anti-aging, antiapoptosis, anti-cancer, anti-atherosclerosis, improvement of endothelial function. They also have the ability of inhibition of angiogenesis and cell proliferation activities. Steroids have diverse range of biological activities. Sex hormones are steroids in nature. Steroids have been found to possess antibacterial activities (Yadav and Agarwala, 2011).

1.9. ESSENTIAL OILS:

Essential oils are the compounds which are odorous as well as volatile and are found in 10% of the plant kingdom. These are also known as ethereal or volatile oils. There are various prts in plants which serve as storage parts for these oils. These parts include glands, secretory cavities, secretory ducts, resin ducts and secretory hairs. They are different from fixed oils as they evaporate easily on heating. (Ahmadi *et al.*, 2002; Imelouane *et al.*, 2009; Ciccarelli *et al.*, 2008; McConkey *et al.*, 2000; Liolios *et al.*, 2010; Morone-Fortunato *et al.*, 2010; Sangwan *et al.*, 2001)**.** Volatile oils are found in plants in quantity of about 1% (Bowles, 2003). In some cases, these oils reah upto 10 % as present in *Syzygium aromaticum* (clove) and *Myristica fragrans* (nutmeg). They are of hydrophobic nature and water insoluble, rather soluble in organic solvents of non polar or weakly polar nature such as alcohols and waxes etc**.** They are mostly colourless; some are light yellow colored and blue colored (exceptional) oils of chamomile (*Matricaria chamomilla*) are also found. These oils are mostly liquid in nature. Essential oils are less dense than water with few exceptions like cinnamon, clove, vetiver ad sassafras etc. Due to unsaturation (double bonds) and functional groups, these oils oxidize in air, light and heat (Skold *et al.*, 2006; Skold *et al.*, 2008; Cavar *et al.*, 2008; Martín *et al.*, 2010; Gupta *et al.*, 2010).

1.9.1. Significance of Essential Oils:

It is given in the following table in detail along with types of compounds.

Table 1.6: Types of Essential Oil and their significance.

S.No	Classes of compound	Types of compounds	Significance	References
1	**Hydrocarbons**	sabinene,myrcene, Pinene, Phellandrene, Cymene, Limonene.	antiviral, Decongestant, Antibacterial, Stimulant, Hepatoprotective, Antitumour.	(Baser and Buchbauer, 2010, Ozbek *et al.*, 2003; Edris, 2007; Bowles, 2003; Svoboda *et al.*, 1999; Deans *et al.*, 1992; Pengelly, 2004; Griffin *et al.*, 1999).
2	**Esters**	Bornyl acetate,eugenol acetate,Linalyl acetate, geraniol Acetate	Spasmolytic, antifungal, Anaesthetic, anti-inflammatory, Sedative.	(Pengelly, 2004; Peana *et al.*, 2002; Ghelardini *et al.*, 1999; De Sousa *et al.*, 2011; De Sousa, 2011, Sugawara *et al.*, 1998)
3	**Oxides**	sclareol oxide,Bisabolone oxide, linalool Oxide,Ascaridole	Anti-inflammatory, Expectorant, Stimulant	(Ghelardini *et al.*, 2001; De Sousa, 2011, Pengelly, 2004)
4	**Lactones**	bergaptene, Costuslactone, Dihydronepetalactone, Alantrolactone, Nepetalactone.	Antiviral; antipyretic, Sedative, Hypotensive; Anti-microbial; Analgesic	(De Sousa, 2011; Gomes *et al.*, 2009; Miceli *et al.*, 2005, Pengelly, 2004.)
5	**Alcohols**	Santalol, Linalol, borneol, nerol, citronellol, menthol, Geraniol.	Antimicrobial, Antiseptic, tonifying, Balancing, Spasmolytic, Anaesthetic; antiinflammatory	(De Sousa, 2011; Pengelly, 2004; Ghelardini *et al.*, 1999; Sugawara *et al.*, 1998; Peana *et al.*, 2002). .
6	**Phenols**	eugenol, Carvacrol, chavicol, Thymol.	spasmo Lytic, anaesthetic, Irritant, Anti-microbial, immune Stimulating.	(De Sousa, 2011; Pengelly, 2004; Ghelardini *et al.*, 1999).
7	**Aldehydes**	myrtenal, Citronellal, Cinnamaldehyde, Citral, Benzaldehyde, Cuminaldehyde.	Spasmolytic, Antimicrobial, Hypotensive, tonic, Calming, antiviral, antipyretic, Sedative, Vasodilators.	(Dorman and Deans, 2000; Pengelly, 2004).
8	**Ketones**	thujone,carvone, pulegone, camphor, menthone, Verbenone, fenchone.	cell regenerating, mucolytic,antiviral, sedative,neurotoxic, digestive, Spasmolytic, analgesic.	(Pengelly, 2004; De Sousa *et al.*, 2008).

1.10. FIXED OILS:

Fixed oils are also referred to as vegetable or base oils. Fixed oils are tri-glycerides, small amounts of phospholipids, free sterols, glycolipids and sterol esters. Fixed oils consumed in diet are obtained from plants along with animal's origin. Fixed oils are energetically very efficient. On the basis of weight, fixed oils are the richest source of energy.

Fixed oils contain important fatty Acids and form a rich source of bioactive compounds. These Acids are needed by the animals. Theses Acids are obtained from natural sources such as plants. These chemical constituents are the carriers of many other compounds such as nutrients, sterols and fat soluble vitamins. The fixed oils provide palatability and taste to our food.

1.10.1. Significance of Fixed Oils:

Fixed oils are essential source of food and are linked to good health and pathological conditions. Some of the various important diseases are connected with fixed oils. These are vascular diseases (both cardiovascular and cerebrovascular). (Bhattacharya and Haldar, 2012), cancers (Vartak *et al.*, 1997), diabetes (Gunstone and & Ismail, 1967), obesity (Gunstone, 2000), immune dysfunction (Zurier, 1993) and mental illness like schizophrenia and depression (Bhattacharya and Haldar, 2012).

1.11. PHARMACOLOGICAL ACTIVITIES:

A. *In vivo* activities:

1. Anti-inflammatory activity

2. Analgesic activity

3. Antipyretic activity

4. Acute toxicity activity

B. *In vitro* activities:

1. Antimicrobial (antibacterial and antifungal) activity.

2. Antioxidant activity

3. In vitro enzyme inhibition activity

1.11.1. Anti-inflammatory activity:

Inflammation is the body response to various harmful stimuli like disease causing agents (pathogens) which result into cell and tissue damage (Ferrero-Miliani *et al.*, 2007). In such conditions, organisms take protetive measures and remove such deleterious stimuli which resul in healing process. There are two types of inflammation depending upon the response. In acute inflammation, the response is short and in chronic inflammation it is prolonged (Watari *et al.*, 2008). In acute inflammation, the response starts by the infiltration of leukocytes and plasma to the sites where injury or infection has caused (Medzhitov, 2008). This happens when receptors of immune system (innate) are activated i.e, TLRs (Toll like Receptors) (Barton, 2008). In this inflammation, there is earlier production of certain mediators of inflammation released by mast cells and macrophages such as interferons (IFN-γ), tumor necrosis factors (TNF-α & TNF-β), cytokines (interleukin-1β (IL-1β), IL-6, IL-12, chemokine IL-8), vasoactive amines (histamine) and eicosanoids (prostaglandins & leukotrienes) (Medzhitov, 2008). In inflammation, inflammatory mediators released in acute inflammation have complex regulatory roles in order to restore tissue homeostasis. Chronic inflammation is caused in the response of persistent and toxic stimuli which results tissue malfunctioning. Such prolonged and persistent inflammatory conditions are associated with a wide variety of chronic disorders in human such as allergy, cancer, arthritis, autoimmune diseases and atherosclerosis (Medzhitov, 2008).

In inflammation, arachidonic Acid is released by mediators like lipoxygenase and cyclooxygenase which results inflammatory responses. Chemotactic compounds, formed by lipoxygenase in neutrophils which in turn release cytokines. Various chemical constituents such as phenolic compounds inhibit the pathway of formation lipoxygenase

and cycloxygenase enzymes. Due to inhibition of this pathway, it lowers the production of arachidonic Acid (FerrÃ¡ndiz *et al.*, 1996; Yoshimoto *et al.*, 1983).

Various responses (immunogenic) are triggered by autacoids synthesis e.g. eicosanoids (like prostaglandins), the end products of cycloxygenase and lipoxygenase pathways. Certain chemical constituents such as flavonoids inhibit this biosynthesis and thus also inhabit inflammatory responses (Moroney *et al.*, 1988)**.**

A cell membrane bound protein; Tyrosine kinase performs various functions like enzyme catalysis, transport across the membrane, energy transfer in synthesis of ATP and signals transduction which functions as growth and hormones receptors. The inhibition of uncontrollable cell growth and spread occurs when these proteins are inhibited. Degranulation of WBCs like neutrophils relases arachidonic Acid as a result inflammation is caused. Inflammatory responses are decreased by inhibiting arachidonic Acid release. Various chemical constituents like flavonoids are used to inhibit the production of arachidonic Acid (Hoult *et al.*, 1994).

1.11.2. Analgesic activity:

The undesirable sensory and emotional experience which is linked with actual or potential tissue damage is known as pain (Elisabetsky and Castilhos, 1990). Different conditions are involved in pain such as sensory-discriminative, cognitive aspects, emotional, affective and motivational aspects (Mersky, 1986). To sense the pain and to respond pain killers are the complex processes which involve many biochemical pathways. Genetic variables greatly influence these biochemical pathways which change the perception of pain and/or response to pain killers. A wide range of variability exists in pain sensation and analgesic drugs providing relief. Numerous plants which are traditionally used in pain have been investigated to have agents possessing analgesic activity with no or less side effects (Malairajan *et al.*, 2006).

Analgesic compounds are of two types; these are opioid and non-opioid drugs (analgesics).

i) Opioid Analgesics:

Opioids agonist produce pain by binding to specific receptors (G protein-coupled), which are located largely in the brain and spinal cord involved in the modulation and transmission and of pain.

There have been identified three major classes of opioid receptors (μ, κ and δ) in various sites of nervous system other tissues. All these receptors belong to G protein-coupled receptors family and show significant homologies in amino Acid sequence.

Molecular study reveals that these opioid receptors produce proteins which interact to G-proteins physically. Such protein-protein interaction affect ion channel gating, modulate intracellular Ca^{2+} disposition, and change phosphorylation of protein (Pomonis *et al.*, 2003).

There are two well-established direct actions of opioids on neurons;

(1) Pre-synapticaly: On pre-synaptic nerve terminals, voltage-gated Ca^{2+} channels are closed as a result transmitter releases are reduced like glutamate, which is the major excitatory amino Acid released from nociceptive nerve terminals, also serotonin, acetylcholine, substance P and norepinephrine.
(2) Post-synaptically: They hyperpolarize and thus inhibit post-synaptic neurons by opening K^{+} channels result in no pain transmission.

The majority of currently available opioid analgesics act primarily at the μ opioid receptor. Analgesia, as well as the euphoriant, respiratory depressant, and physical dependence properties of morphine result principally from actions at μ receptors.

ii). Non-opioid analgesics:

The most important mechanism of action of Non-opioid analgesics is the inhibition of cyclo-oxygenase (COX) enzymes which are responsible for the production of prostaglandins. These are;

COX-I: It is constitutive (already present), having cytoprotective function.

COX-II: It is inducible at site of inflammation by cytokines, Leukotriens and TNFα. It has role in inflammation.

COX-III: It present in the brains and is inhibited by aniline derivatives.

1.11.3. Anti-Pyretic activity:

Fever is also known as pyrexia. It is the sign of infection and inflammation (Chattopadhyay *et al.*, 2005). Normally, the body creates such an internal environment where disease causing agents are not possible survive (Chattopadhyay *et al.*, 2005). The damaged tissue started the formation of pro-inflammatory mediators e.g. interleukin 1β, α, β, and TNF- α. These elevate the production of prostaglandin E2 (PgE2) close to hypothalamic area. This result in increase in body temperature (Spacer and Breder, 1994). Temperature of the body when increases blood vessels dilation occurs due to nervous feedback mechanism. There is also increase in sweating from the body. The temperature is regulated by hypothalamus by vasoconstriction mechanism. Elevation in fever increases the chances of disease development (progression) which is due to increase dehydration, tissue catabolism, and existing complaints, as found in HIV.

Majority of the antipyretic drugs reduce the high body temperature by inhibiting COX-2 expression which in turn inhibit the biosysnthesis of PgE2 (Cheng *et al.*, 2005; Duraisankar and Ravichandran, 2012). Drugs inhibit COX-2 irreversibly with a high selectivity and are very toxic to cardiac muscles, glomeruli of kidneys, liver cells and cortex of brain. Natural COX-2 blockers have lower specificity with less untoward effects (Cheng *et al.*, 2005). Natural antipyretic drugs with low or no toxicity is therefore very important. Many plants leaves are used in fever and become cost effective alternative antipyretic agents (Ghani, 1998; Alamkhan *et al.*, 2008; Gomathi *et al.*, 2011).

1.11.4. Acute toxicity:

Toxicity, a sign of being poisonous, in which a state of untoward effects is produced by the interaction of cells and toxic agents. This interaction varies depending on the chemical features of the toxicants (toxic agents) and cell membrane. This interaction occurs at various places, it may occur on the cell surface, within the cell body, in the

tissues beneath or in the extracellular matrix. The toxic agents bind to vital organs like kidneys and liver and produce toxic effects. This becomes very important to evaluate the toxic properties of an agent in respect to public health protection, because exposure to such chemical substances can be hazardous and leads to harmful effects on human beings. This evaluation of toxic agents usually includes acute, sub-chronic, chronic, reproductive and carcinogenic effects (Asante-Duah, 2002; Jothy *et al.*, 2011).

1.11.5. Antimicrobial (Antibacterial and Antifungal) activity:

Since the discovery of penicillin, which is the first antibiotic, the need for antimicrobial substances is yet to be satisfied. This discovery also leads to the emergence of resistant strains of micro-organisms (Davies, 1994). Resistance of bacteria to antibiotics is nowadays a public health concern issue (Monroe and Polk, 2000). Resistant bacterial strains show strong resistance to availale antibiotis. New antimicrobial agents are prepared for this reason (Bhavnani and Ballow, 2000). Plant based drugs are thought to have low or no side effects as compared to synthetic pharmaceuticals which make them a revival of interest (Chariandy *et al.*, 1999). Due to less cost of plant preparations, and low side effects, this makes an attractive option (Palombo and Semple, 2001; Shah *et al.*, 2004). The plants are used alone or in combination for the management of microbial infections (Gotep *et al.*, 2010).

1.11.6. Anti-oxidant activity:

Human beings have highly complicated antioxidant systems (enzymic and non-enzymic), which work synergistically. Antioxidants are the agents which protect the body (cells and organ systems) from free radical damage. These antioxidants can be produced endogenous or taken from outside. Some food substances that do not neutralize free radicals but increase endogenous activity, can also be categorized under antioxidants (Safa *et al.*, 2010).

Plants form the major source of antioxidants (natural) that serve as leads for the development of bioactive agents. Various antiinflammatory, hepatoprotective, digestive, anti-necrotic and neuroprotective substances have presently been accepted to possess an

anti-radical scavenging and/or antioxidant mechanism of action as part of their activity (Huang *et al.*, 2004; Repetto and Llesuy, 2002). Natural antioxidants and agents with radical scavenging potential have been investigated like echinacoside in *Echinaceae* root (Kitts *et al.*, 2000), phenolic compounds (Rice-Evans *et al.*, 1997), anthocyanin (Espin *et al.*, 2000), extracts of roasted *Cassia tora* (Yen *et al.*, 2000), thioredoxin protein from sweet potato (Larkindale and Huang, 2004) and whey proteins (Tong *et al.*, 2000; Chang *et al.*, 2007).

1.11.7. Enzyme inhibition:

Many pharmacological activities cab be determined by studying te inhibition of enzymes involved in such activities. Enzyme inhibition activities are measured *in-vitro* (laboratory), under the conditions those oftenly do not simulate *in-vivo*. Enzyme inhibition activity is determined by measuring the amount of enzymes under defined conditions which are selected usually at the optimum pH, temperature that is convenient to maintain, and at 'saturating' substrate concentrations. The activity is determined in opposite direction to that of the enzyme's natural function. Such enzyme activity can be compared between one sample and other and between one laboratory to the other (Rossomando, 1990).

1.11.8 Aims and Objetives:

Following were the specific objectives of the present study:

1. To identify various classes of phytochemicals found in selected plants.
2. To scientifically validate the ethnomedicinal uses of selected plants in conditions associated with inflammation, pain and fever.
3. To study the pharmacological mechanisms involved in anti-inflammatory, analgesic and antipyretic effects of selected plants.
4. To identify acute toxicity associated with selected medicinal plants.

CHAPTER 2

MATERIALS AND METHODS

2.1. REAGENTS USED:

Reagents of commercial grade used in different experiments have ben given in the **Table 2.1**.

Dose of crude methanolic extract was prepared in DMSO (Dimethyl Sulfo Oxide) and normal saline for different activities. Both DMSO and normal saline were used as negative control.

Table 2.1: Chemicals and drugs used.

S.No.	**Drug/Chemicals**
1	Silica gel
2	Carrageenan
3	Diclofenac Sodium
4	Paracetamol
5	Dragendorff's reagent
6	Molish's reagent
7	$CHCl_3$ (chloroform).
8	Mayer's reagent
9	NH_4OH
10	Ferric chloride ($FeCl_3$)
11	Ninhydrin reagent
12	Acetic anhydride
13	Glacial acetic Acid
14	Sodium chloride
15	McFarland standard
16	n-hexane,
17	Chloroform,
18	Butanol
19	Ethyl acetate
20	Azithromysin
21	Ciprofloxacin
22	Clotrimazole
23	DMSO
24	DPPH
25	Aspirin
26	Xylene
27	Dexamethasone
28	Fatty Acid Methyl Esters

2.2. GENERAL EXPERIMENTAL CONDITIONS:

The current research work was carried out in Department of Pharmacy, University of Peshawar and PCSIR-Complex, Peshawar, Pakistan.

2.3. PLANT MATERIALS:

Selected plants were collected from Pakistan Forest Institute, Peshawar (*Tamarix aphylla, Acacia cyanophylla* and *Acacia stenophylla)* and Takht Bhai, Mardan (*Tamarix dioica.).* Following identification process, plants were given voucher numbers and placed in Herbarium, Department of Botany, Islamia College Peshawar.

2.4. EXTRACTION:

Stem bark of the collected plants were dried (in shade) for three weeks at ordinary room temperature and processed with electric grinder for powder formation. The powdered material of *Acacia cyanophylla*, *Acacia stenophylla*, *Tamarix dioica* and *Tamarix aphylla* (600 g each) was soaked in commercial grade methanol (6.3, 4.3, 5 and 4.35 L respectively) for three weeks at room temperature with occasional shaking. Three weeks after, they were filtered and the filterates obtained were concentrated with the help of rotary evaporator at 50 ^{o}C and were further dried on water bath at 50 ^{o}C. The crude extracts obtained were preserved for various phytochemical and pharmacological activities (*in-vivo* & *in-vitro*).

2.5. PHYTOCHEMICAL TESTS:

2.5.1. Screening for different groups of compounds:

A total of 2 gm extract was taken and dissolved in 30 ml ethanol first. Following filtration, added 20 ml ethanol and re-filtered. Thus 50 ml filtrate was obtained. As per procedure of (Ayoola *et al.*, 2008), filtrate of *Tamarix (aphylla, dioica) and Acacia (cyanophylla, stenophylla)* were separately screened for different classes of compounds**.** All these different qualitative analysis are given below:

1) Carbohydrates:

Two ml ethanolic extract was taken, then added 2 drops of molish's reagent (10% α-nephthol/ethanol) and well shaken and subsequently added H_2SO_4/2ml. Observed the color till it was turned reddish brown that revealed presence of carbohydrates.

2) Cholesterol:

For cholesterol determination taken 2 ml ethanolic extract and added 2 ml $CHCl_3$ (chloroform) followed by addition of 10 drops of acetic anhydride + 2-3 drops of Conc. H_2SO_4. Subsequently the appearance of blue color precipitates confirmed presence of cholesterol.

3) Saponins:

Plant material (0.1 gm) was extracted with boiling water (10 ml) in test tube. This mixture was allowed to cool at room temperature and shaken vigorously till froth (foam) was produced. The samples were then left for 15 minutes and following observations were made:

Negative sign stands for no froth (foam), one plus sign indicates froth less than 1 cm, 2 plus sign means more than 2 cm froth and 3 plus sign shows strong positivity.

4) Alkaloids:

An ethanolic extract of each material was made separately by pouring 50 ml of ethanol (70 %) to 1gm of each powdered material of each plant collected. To 2 ml portions of each alcoholic extract in separate test tubes was added Draggendorff's reagent. Following treatment with Draggendorff's reagent reddish brown precipitation was observed that confirmed the presence of alkaloids (Ameyaw and Duker-eshun, 2009).

5) Flavonoids:

One gram of the powdered plant material was taken in ethanol (5 ml) to determine flavonoids. Few drops of concentrated HCl and 5 g of Magnesium were added to the

above mixture. The colored apperence (formation of pink color) confirmed that flavonoids were present.

6) Terpenes:

For determination of terpenes, five ml of crude extract was mixed with chloroform (2 ml). Then added concentrated H_2SO_4 (few drops) carefully. A layer was formed. The appearance of ring (blue/green) showed the presence of terpenes.

7) Tannins:

Taken a half gram (0.5 g) of plant material in a test tube, added 10 ml of hot distilled water, filtered & poured 2 ml of gelatin solution (1%). Tannins form precipitate upon adding gelatin solution that confirmed the presence of tannins. The presence of tannin can also be confirmed by formation of precipitate when Ferric Chloride (2 ml) solution is added to test tube.

8) Steroids:

In a test tube, 0.25 g of crude extract was taken.Then added acetic anhydride (1 ml). The formation of blue or green colour showed the presence of steroidal compounds in the sample.

9) Amino Acids:

Ninhydrin (5-6 drops) was added to 2 ml of methanolic extract followed by heating on water bath at boiling point for 5 minutes. The formation of purple color confirmed that amino Acids were present.

10) Glycosides (Kellere Kiliani test):

In this screening procedure, glacial acetic Acid (1 ml), $FeCl_3$ and concentrated H_2SO_4 (few drops) added to 2 ml extract (ethanolic). Glycosides were confirmed when precipitate of green/blue formed.

11) Phlobatannins:

Boiled two ml of plant extract with two ml 1% HCl. The presence of phlobatannins was indicated by the appearance of red color.

2.6. OIL ANALYSIS:

2.6.1. Extraction:

Taken 7 gm material of each plant in 250 ml flask separately. It was then mixed in 2:1 of water and hexane respectively. Shaken vigorously for 2-3 minutes. Taken the upper layer (hexane) in another tube and added 1 ml more hexane. Shaken and separated the hexane layer. Then heated on water bath, till oil from each of plant material obtained in different quantities.

2.6.2. Preparation of Fatty Acid Methyl Esters (Appelqvist and Lars-ake, 1968):

Methyl esters of Fatty Acid were used because of their low boiling point. It had many steps to prepare Fatty Acid Methyl Esters. These were;

1. Taken 25 to 40 mg sample in a tube and added 1.5 ml of 0.5 N NaOH methanolic.
2. Boiled for 5 minutes and then cooled.
3. Then added 2.5 ml of 10% BF_3 and boiled for 30 minutes and cooled.
4. Added 5 ml of saturated NaCl and 1 ml hexane and shaken vigorously for 2-3 minutes.
5. Took the upper layer (hexane) in another tube and added 1 ml hexane.
6. Shaken and separated the hexane layer
7. Took 2 ml hexane extract and filtered through membrane and injected 1µl to GC-MS (GCMS-QP-2010 PLUS (Shimadzu, japan)
8. Diluted hexane to 2 ml.
9. Filtered through membrane filter
10. At the end inject 1µl to Gas Chromatography (GC).

2.7. *IN-VITRO* BIOLOGICAL ACTIVITIES:

Crude methanolic extract and various fractions of *Acacia cyanophylla, Acacia stenophylla, Tamarix aphylla* and *Tamarix dioica* were screened for various *in-vitro* pharmacological activities. The following were the different activities performed.

2.7.1. Anti-microbial activity (Anti-bacterial and Anti-fungal activity):

Culture media:

Different growth media were used in this experiment. For culturing microorganisms, used nutrients agar medium, while for incubations & standardizations of microbes, nutrient broth was used. Composition of both the culture media have been given in the following tables.

Table 2.2: Composition of nutrient agar.

Nutrient agar modified	
Composition	**g/L**
01 Agar medium	15
02 Gelatin extract	05
03 Sodium chloride	05
04 Beef extract	01
05 Yeast extract	02
Total	**28**

Table 2.3: Composition of nutrient broth.

Nutrient Broth modified	
Compositions.	**g/L**
01 Sodium chloride	05
02 Gelatin peptone	05
03 Yeast extract	02
04 Beef extract	01
Total	**13**

Preparation of culture media:

Distilled water was to prepare both the culture media in required amount i.e, nutrient and nutrient broth. They were then taken into flasks (conical). In test tubes, some additional nutrient broth was also taken. All the test tubes and flasks were plugged and sterilized at pressure of 1.6 pound and temperature 120˚C-121˚C in an autoclave for about 20 minutes. When sterilization of media completed, poured nutrient agar media particularly into sterilized petri plates near 6 inches flame of laminar flow hood. To avoid contamination, all the steps were protectively performed in sterilized environment. Then media were then left to to be solidified for some timein petri plates. For avoiding evaporation of water, the petri plates were then kept in incubator in inverted position at 36 ˚C for 24 hours. About 24 hours after, microbes' free petri plates were utilized for growthof the tested microorganisms.

Microorganisms used:

Inhibitory activity of the selected plants was measured against the following microbes given in Table: 2.4

Table 2.4: Types of microbes used.

Microbes	**Type**	**Source**
Staphylococcus aureus	Gram positive	Isolation of clinical microbe from micro-lab PCSIR, Peshawar, Pakistan
Bacillus subtilis	Gram positive	do
Bacillus atropheus	Gram positive	do
Pseudomonas aeruginosa	Gram negative	do
Escherichia Coli	Gram negative	do
Salmonella typhi	Gram negative	do
Erwinia carotovora	Gram negative	do
Klebsiella pneumonia	Gram negative	do
Candida albicans	Fungus	do

Stock solution of extract used:

Methanolic extracted samples of *Acacia cyanophylla*, *Acacia stenophylla*, *Acacia dioica* and *Acacia aphylla* stem bark were examined for antimicrobial potentials. The dried crude extract (0.5 g) of each plant was dissolved in 3000 µl Dimethyl Sulfoxide (DMSO) and adjusted to 1 mg $6\mu l^{-1}$ and 2 mg $12\mu l^{-1}$.

McFarland turbidity standard:

McFarland of 0.5 ml (half ml) was made by adding 0.5 ml of Barium Chloride dehydrate (1.176 %) solution to 1% Sulfuric Acid (98.8 ml). The turbidity standard was liquated into test tube and was sealed with Para film. Shake the test tube before every use; untill mixing of the white precipitate of barium sulfate. Its clearness and efficiency was measured by spectrometer. For McFarland standards (0.5), the suspension adjusted, should give a count of 10^8 colony forming units/ ml.

Disc Diffusion Susceptibility Method (Zaidan *et al.*, 2005):

Disc Diffusion Method was applied to measure the antimicrobial potential of selected plant samples. Bacterial cultures adjusted to McFarland turbidity standards were inoculated on to sterilized nutrient agar medium after 24 hours. Sterile filter paper discs impregnated with crude extract in concentrations of 6 & 12 µl volumes were applied. Bacterial and fungal cultures were allowed to incubate for 18 hrs at 38 ˚C.

Antimicrobial activities bioassay:

The methods and procedures for the evaluating antimbacterial and antifungal activities are given below:

1st day: - In distilled water, the known amount of nutrient broth and nutrient agar media were prepared in distilled water in flask. 7-9 ml of nutrient broth was taken per test tube. Both the media in flasks and test tubes were sterilized for 21 minuutes at 120-121˚C and pressure of 17 pounds. Alongwith media, all the apparatus, petri plates, micropeppites tips, Whatman filter paper discs were also sterilized. Nutrient agar media was then poured into petri plates after sterilization in biosafety cabinet (cell culture hood), let them

to solidify.Then they were kept in incubator at 37 ˚C as to avoid contamination during experiment.

2nd day: - The stock of microbial culture was freshened at two different concentrations of 6µl & 12µl by streaking (called first streak) on nutrient agar medium in plates using steriled inoculation loops in laminar flow hood. This step should be done sensitively. They were then incubated for 24 hoursat 37˚C.

3rd day: - Microbes from the so called first streaked culture were streaked on fresh agar media (petri plates) again, called as second streak and again incubated at for 24 hours 37˚C.

4th day: - Inoculums were made by taking the streaked cultures into autoclaved nutrient broth of 20 to 27 ml $flask^{-1}$and incubated in shaking incubator for standardization at 37˚C for 18 hrs.

5th day: - The incubated materials (cultures) were diluted in nutrient broth (sterilized) taken in test tubes for standardization which was made by comparing the test tubes with 0.5 McFarland solution. The microbial cultures (standardized) were spread using glass made spreader on each plate containing nutrient agar. These inoculated plates were then refrigerated for 15 mnts for absorption.

Applying test for Disc Diffusion Method:

After absorption, the the inoculated petri plates were taken from refrigerator and brought them again into laminar flow hood. Sterilized discs of filter paper (Whitman) were placed on nutrient agar media in petri plates using forceps (sterilized). The crude extracts of selected plants were subjected in concentration of 6 µl and 12 µl volumes on discs. All the standards (antibiotics) were subjected in concentration of 6µl/disc as positive control on separates plates. Azithromysin was used against gram-positive bacteria, ciprofloxacin against gram-negative bacteria and clotrimazole was for fungal species. DMSO was used as negative control in 6 µl/disc. All the plates were then kept in incubator for 24 hours at 37˚C.

At day 6th: Antimicrobial activity of tested samples was recorded by measuring zone of inhibition in mm. Pictures were captured with the help of a digital camera.

2.7.2. Acetylcholinesterase inhibition assay:

This bioassay was carried out according to the procedure of Lopez et al., with slight modification (Ingkaninan *et al.*, 2003). The reaction mixture contained Tris-HCl (50 mM) with pH 8.0, (200 µl), BSA buffer of 1%, 100 µl of the test sample, made the final concentration to 100 µg/mL. Prior to the addition of acetylthiocholine iodide (ATCI) substrate (100µl, 15mM), 5, 5 V- dithiobis [2-nitrobenzoic Acid] 500 µl (DTNB 3 mM) and incubated for 2 minutes at 25 ˚C. The yellow color obtained was measured at 405 nm after 4 minutes. Galathamine, as positive control was used in final conc. 100 µg /ml. Percent inhibition of AChE was measured by the following formula;

$$\text{Percent Ach.E inhibition} = [(A-B) \times 100] / A$$

In which A is for change in absorbance without test sample, and B indicates change in the absorbance with test sample.

2.7.3. Lipoxygenase inhibitory activity:

This enzyme inhibitory activity was measured by the method Tappel, 1962 with slight modifications (Wallace J.M and E.L., 1975). The total volume of assay mixture was 200 µl. It contained, 160 µl sodium phosphate buffer (100 mM, pH 8.0), 10 µl test extract (25 to 100 µg extracted material in 100 mM Tris buffer pH 7.4) and 20 µl lipoxygenase enzyme. All these contents were incubated at a temperature of 25°C for 10 minutes. The substrate, Linoleic Acid solution (10 µl) was added to initiate the reaction. At the wave length of 234 nm, change in absorbance was noted after 6 minutes. All these reactions were done in triplicates in 96-well microplate reader Spectra Max 384 plus (Molecular Devices, USA). The assay included the negative and positive controls. The following given formula was used to calculate the percentage inhibition.

$$\text{Percent inhibition (\%)} = \{(\text{Abs. of control} - \text{Abs. of test sample}) / \text{Abs. of control}\} \times 100$$

IC50 values (concentration at which enzyme inhibition 50 %) of test samples were measured with the help of software "EZ–Fit Enzyme kinetics" (Perella Scientific Inc. Amherest, USA).

2.7.4. Antioxidant assay:

The crude extracts of selected plants were separately screened for their antioxidant potential according to the reported procedure of (Shaheen *et al.*, 2005) (DPPH Radical Scavenging Bioassay). DMSO was use to dissolve the test samples. To make reaction mixture, 95 µl of DPPH and 5 µl of test samples were added in ethanol. Reaction mixture was then put into 96-well micro plate & incubated for 30 minutes (at 37^{o}C). The absorbance (515nm) of test samples was recorded with Multiple Reader's Spectrophotometer (Spectra-Max 3400). Positive control used was Ascorbic Acid. The radical scavenging activity (percent) of tested samples was determined in comparison to positive control.

Percent Radical Scavenging Activity (RSA) = 100 (Optical Density of test well /Optical Density of control) ×100

2.8. *IN-VIVO* BIOLOGICAL ACTIVITIES:

The crude methanolic extracts of selected plants screened for various *in-vivo* biological activities.

2.8.1. Experimental animals:

During pharmacological experiments, mice of either sex were obtained from Department of Pharmacy, University of Peshawar. To keep these animals healthy, recommended guidelines were followed throughout the experiments (Muhammad *et al.*, 2008).

2.8.2. Anti-inflammatory activity:

Crude methanolic extract of each plant was subjected for evaluating anti-inflammatory activity by two different methods i.e Carrageenan Induced Edema and Xylene Method.

2.8.2.1. Carrageenan Induced Paw Edema Model:

Tested animals (mice) of either sex (25-30 g body weights) were chosen for this purpose. The animals were distributed into 5 different groups. In every group, there were 6 animals (n = 6). Two groups (I & II) were selected as negative and positive control respectively. Group I was administered with normal saline by the dose of 10 ml kg^{-1} (body weight). Indomethacin (standard drug) was administered at a dose of 10 mg/kg (body weight) to another group (group II). Remaining groups such as group III, IV & group V were subjected with crude extract with doses of 50, 100 and 200 mg/kg (body weight). Carrageenan of 1% was administered in injected in subplanter tissue of the hind paw (right) of every animal (mouse), 30 minutes after, of the above mentioned treatments. Anti-inflammatory potential was recorded for 5 hrs (0, 1, 2, 3, 4 and 5 hours by using plethysmometer (LE 7500 plan lab S.L) after the administration of Carrageenan (Khan *et al.*, 2009). The % inhibition of edema was measured with the help of formula given below.

$$\% \text{ Inhibition } = \frac{A - B}{B} X\ 100$$

In which;

A is for edema volume in negative control

B for paw edema in tested groups.

2.8.2.2. Xylene-Induced Ear Edema:

All the tested organsims consisted of five groups (six mice in each group) were administered orally with distilled water (10 ml/kg), plant extracts (50–200 mg/kg) and Dexamethasone (.5 mg/kg) in Xylene induced ear edema study. After 30 min, xylene (0.03 ml) was applied to the inner surface of the right ear for the induction of edema. The left ear was used as control. Then after 15 min, kill the mice with the help of ether anaesthesia. Cut off both ears and then weighed. For each group, the mean of the difference between the left & right ears was calculated (Nunez Guillen *et al.*, 1997).

The percent inhibitory effect was measured with the help of formula given below.

% inhibition = 100 (Vc – Vt / Vc)

In which;

Vc is for difference in weight of ear in control

Vt is for difference in weight of ear in group treated with standard & extract.

2.8.3. Antipyretic activity:

2.8.3.1. Pyrexia induction with Brewer's Yeast:

Antipyretic effect of crude methanolic extract of each plant was screened by mice (30–35 g) of either sex. Before screening, mice were provided with water only for 12 hours. The tested animals were separated into 5 different groups (n = 6). The group I was called negative and group II was treated as positive control. The animals of group I were administered with normal saline at a dose of 10 ml/kg (body weight). Group II was treated with paracetamol at 150 mg/kg dose (body weight).Groups III, IV & V were subjected with crude extracts at doses of 100, 200 and 300 mg/kg (body weight). Normal body temperature of every animal was recorded with the help of digital thermometer. To induce pyrexia, aqueous suspension of Brewer's (15%) was subcutaneously injected with a dose of 10 ml/kg to mice. Digital thermometer was used to note the rise in body temperature 24 hours after. The mice were selected for further study that showed at least 0.5 ^{o}C increase in body temperature while those animals were excluded who showed rise in body temperature less than 0.5 ^{o}C (Khan *et al.*, 2008). Through i.p route all doses were injected to all groups. The temperature of rectum was noted regularly at 1st, 2nd, 3rd, 4th & 5th hour of each mouse in all treated groups. Reduction in body temperature (%) was measured by the formula given below:

$$\% \text{ Reduction in body temperature} = 100 - \frac{B - Cn}{B - A} \text{ X } 100$$

In which;

A is for normal body temperature;

B indicates body temperature after 24 hrs

C is for temperature at 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} hr of treatment.

2.8.4. Analgesic activity:

Following protocols were used to ascertain analgesic potential of the plants crude methanolic extracts.

2.8.4.1. Acetic Acid Induced Writhing:

The tested samples (crude methanolic extract) of each plant were applied to evaluate the analgesic potential. The tested animals (mice) of either sex (18-22 g body weights) were taken in this experiment. The mice were separated into 5 different groups (n = 6). Among these groups, group I was called as negative control while group II was treated as positive control. The normal saline at a dose of 10 ml/kg (body weight) was administered group I (negative control), while group II was subjected with Diclofenac Sodium (standard drug) at the dose of 10 mg/kg (body weight). They were fed according to the guidelines recommended. Two hrs before the start of activity, the food supply was stopped (Muhammad *et al.*, 2008). Groups III, IV & V (remaining groups) were subjected with crude extracts at 100, 200 & 400 mg/kg doses (body weight) respectively.

After administration of extracts, waited for 30 minutes. After this, acetic Acid (1%) was injected to all tested groups through intra-peritoneal route. The abdominal writhing (constrictions) were started 5 minutes after acetic Acid injection, which were counted for next 10 minutes. The analgesic potential (percent) was calculated with the help of the following given formula:

$$\% \text{ Analgesic effect } = 100 - \frac{\text{No of writhing in tested animals}}{\text{No of writhing in control animals}} \text{ X } 100$$

2.8.4.2. Analgesic effect by Hot Plate Method:

In this experiment, analgesic effect was measured by using Eddy's Hot Plate Method (Turner, 1965). For this purpose, Hot Plate was maintained at a temperature of 55±1^{0}C and tested animals (mice) were placed on Hot Plate individually. Every mouse was observed for reaction on Hot Plate in the form of licking or jumping. The animals (of both sexes) used in this experiment were randomly selected. These selected mice were separated into five groups, group I, group II, group III, group IV and group V. Each group was consisted of six mice for both control and test samples. All of the above mentioned groups were given a particular treatment i.e. positive control with Tramadol in 20 mg/kg, p.o and the test sample (plants extract) of 200 and 400 mg/kg respectively.

The animals were placed on Eddy's Hot Plate maintained at a temperature of 55±1^{0}C. The time of 15 seconds was a cut off period to oberve animals on Hot Plate. After the treatment, the reaction time in treated and control animals was recorded at 0, 30, 60, 90 and 120 minutes (Eddy and Nathan, 1959). Percent analgesia was calculated with the help of following formula.

$$\% \text{ Analgesic effect} = \frac{\text{latency time of test} - \text{latency time of control}}{\text{cut} - \text{off time} - \text{latency time of contol}} \text{ X 100}$$

2.8.5. Acute toxicity:

This was measured by subjecting the crude methanolic extracts of selected plants at different doses ranging from 500 mg/kg (body weight of mouse) up to 2 gm/kg. The tested animals (mice) were equally divided in various groups & given with different doses of selected plants crude extracts. One group named as negative control group that was treated with distilled water at the dose of 10 ml/kg. After treatment, the tested animals were taken in observation for 24 hours (one day whole). Acute toxicity effects were observed in animals for the first 4 hours. After 24 hours, percent death was calculated (Muhammad *et al.*, 2008).

CHAPTER 3

RESULTS

3.1. PHYTOCHEMICAL SCREENING (QUALITATIVE):

3.1.1 Phytochemistry of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla:*

The crude methanolic extract of each plant stem bark was studied for detecting different groups of phytochemicals (**Table 3.1).** The extract (crude) of all plants gave positive results for Alkaloids, Carbohydrates, Saponins, Cholesterols, Flavonoides and Anthraquinones, while negative result for Glycosides. Steroids and Terpenes were present in all of the plants stem bark extracts except *Tamarix aphylla.* Amino Acids were the only phytochemicals that were present in the *Tamarix aphylla* stem bark extract.

Table 3.1: Qualitative phytochemical screening of crude methanolic extract of selected plants.

S.NO.	Phytochemicals	*Tamarix aphylla*	*Tamarix dioica*	*Acacia cyanophylla*	*Acacia stenophylla*
1	**Carbohydrates**	Positive	Positive	Positive	Positive
2	**Alkaloides**	Positive	Positive	Positive	Positive
3	**Saponins**	Positive	Positive	Positive	Positive
4	**Cholesterols**	Positive	Positive	Positive	Positive
5	**Flavonoides**	Positive	Positive	Positive	Positive
6	**Steroids**	Negative	Positive	Positive	Positive
7	**Anthraquinones**	Positive	Positive	Positive	Positive
8	**Glycossides**	Negative	Negative	Negative	Negative
9	**Terpenes and Sterols**	Negative	Positive	Positive	Positive
10	**Tannins**	Positive	Negative	Positive	Positive
11	**Amino Acides**	Positive	Negative	Negative	Negative

3.2. OIL ANALYSIS:

The crude extracts of *Tamarix aphylla*, *Tamarix dioica*, *Acacia cyanophylla* and *Acacia stenophylla* stem bark were screened for fatty Acids profile (**Table 3.2-3.5**).

3.2.1. Oil analysis of *Tamarix aphylla*:

Data shown in **Table 3.2** reveals various Fatty Acids quantitetively, such as C16:0; Palmitic Acid (0.04%), C18:2c; Linoleic Acid (0.11%) and C18:2t; Octadecadienoic Acid (0.02%).

Table 3.2: Quantitative analysis of stem bark oil.

S.No	Name of Fatty Acid	R. Time	Area	Conc. (%)
01	C16:0; Palmitic Acid	14.619	18271	0.04
02	C18:2c; Linoleic Acid	21.341	10033	0.11
03	C18:2t; Octadecadienoic Acid	21.732	1673	0.02

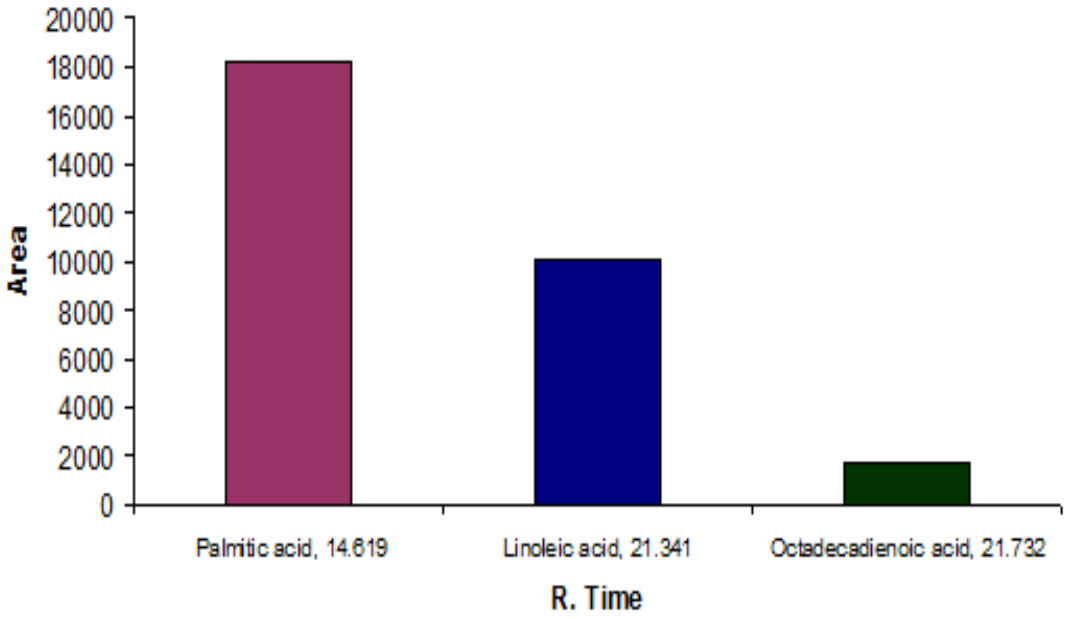

Figure 3.1: Quantitative analysis of stem bark oil.

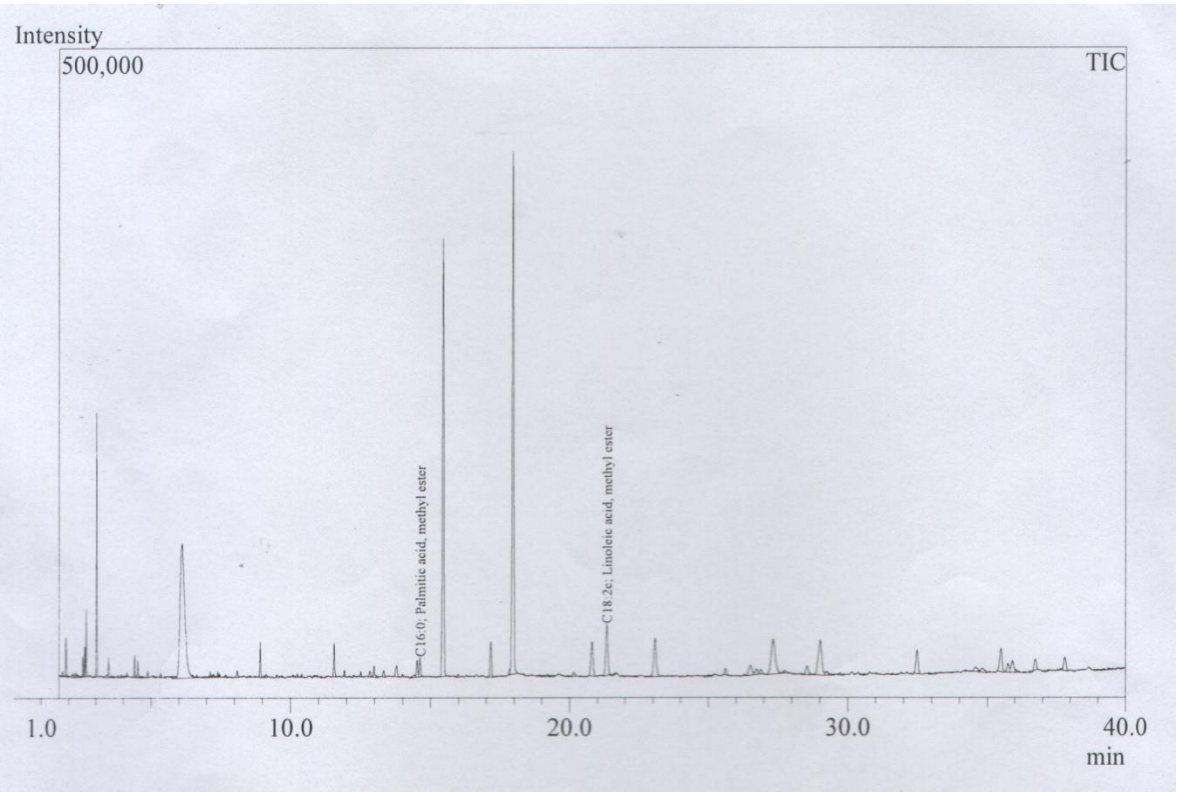

Figure 3.2: Quantitative analysis of stem bark oil (GCMS Report).

3.2.2. Oil analysis of *Tamarix dioica*:

Tamarix dioica stem bark extract was screened for fatty Acid presence (**Table 3.3**). These were; C8:0; Caprylic Acid (0.01%), C12:0; Lauric Acid (0.01%), C14:0; Myristic Acid (0.01%), C16:0; Palmitic Acid (0.08%), C18:1c; Oleic Acid (0.07%), C18:2c; Linoleic Acid (0.41%), C18:2t; Octadecadienoic Acid (0.12%) and C18:3n6; g-Linolenic Acid (0.08%) respectively.

Table 3.3: Quantitative analysis of stem bark oil.

S.No	Name of fatty Acid	R.Time	Area	Conc. (%)
1.	C8:0; Caprylic Acid	5.062	1707	0.01
2.	C12:0; Lauric Acid	8.521	2073	0.01
3.	C14:0; Myristic Acid	10.951	2268	0.01
4.	C16:0; Palmitic Acid	14.616	35978	0.08
5.	C18:1c; Oleic Acid	20.147	7537	0.07
6.	C18:2c; Linoleic Acid	21.343	41216	0.41
7.	C18:2t; Octadecadienoic Acid	21.718	12620	0.12
8.	C18:3n6; g-Linolenic Acid	23.084	5571	0.08

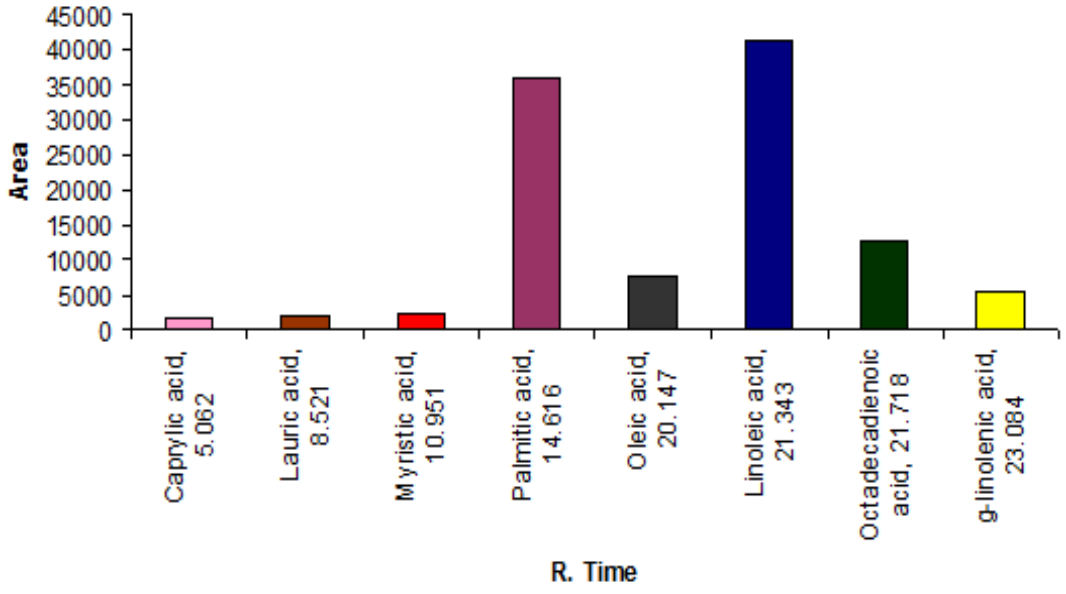

Figure 3.3: Quantitative analysis of stem bark oil.

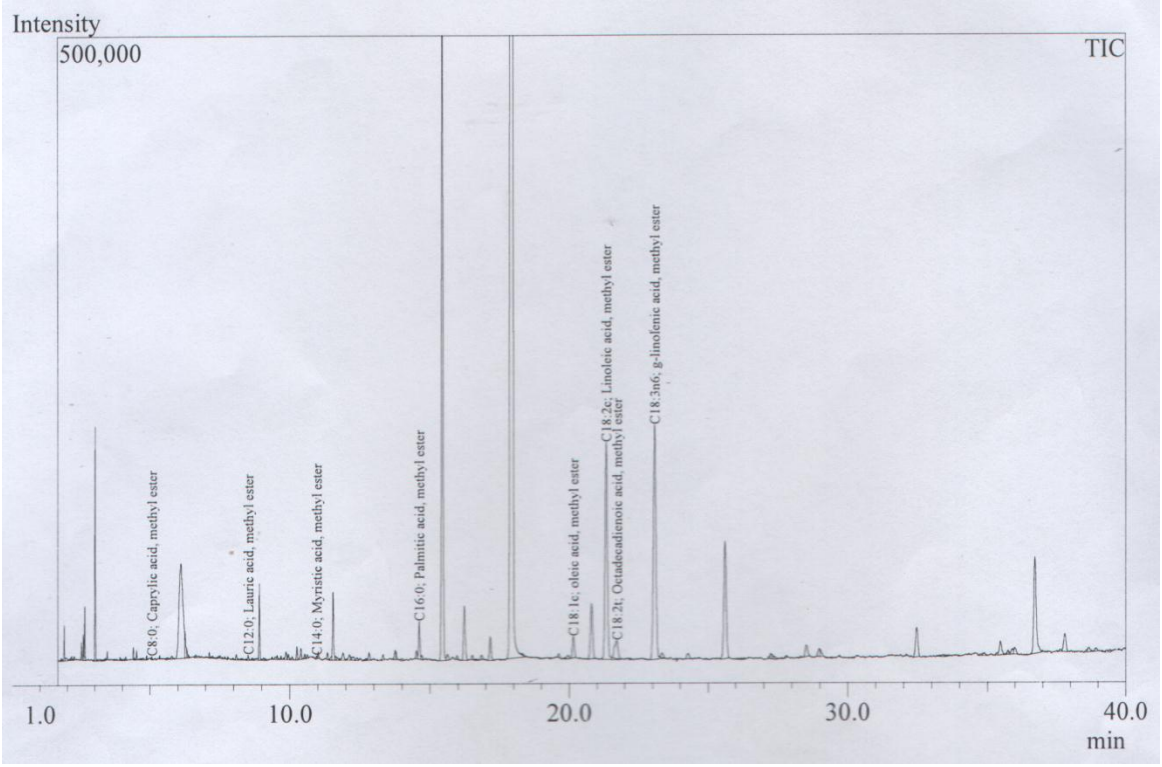

Figure 3.4: Quantitative analysis of stem bark oil (GCMS Report).

3.2.3. Oil analysis of *Acacia cyanophylla*:

The data about Fatty Acid profile of *Acacia cyanophylla* has been shown in **Table 3.4.** The fatty Acid found were; C8:0; Caprylic Acid Methyl Ester (0.00%), C12:0; Lauric Acid Methyl Ester (0.01%), C14:0; Myristic Acid Methyl Ester (0.01%), C14:1c; Myristoleic Acid Methyl Ester (0.04%), C16:0; Palmitic Acid Methyl Ester (0.05%), C18:1c; Oleic Acid Methyl Ester (0.03%), C18:2c; Linoleic Acid Methyl Ester (0.22%), and C18:2t; Octadecadienoic Acid Methyl Ester (0.10%).

Table 3.4: Quantitative analysis of stem bark oil.

S.No	Name of Fatty Acid	R.Time	Area	Conc. (%)
1.	C8:0; Caprylic Acid	5.063	1115	0.00
2.	C12:0; Lauric Acid	8.522	3686	0.01
3.	C14:0; Myristic Acid	10.951	4355	0.01
4.	C14:1c; Myristoleic Acid	11.547	3629	0.04
5.	C16:0; Palmitic Acid	14.614	23382	0.05
6.	C18:1c; Oleic Acid	20.163	2912	0.03
7.	C18:2c; Linoleic Acid	21.337	21987	0.22
8.	C18:2t; Octadecadienoic Acid	21.724	10095	0.10

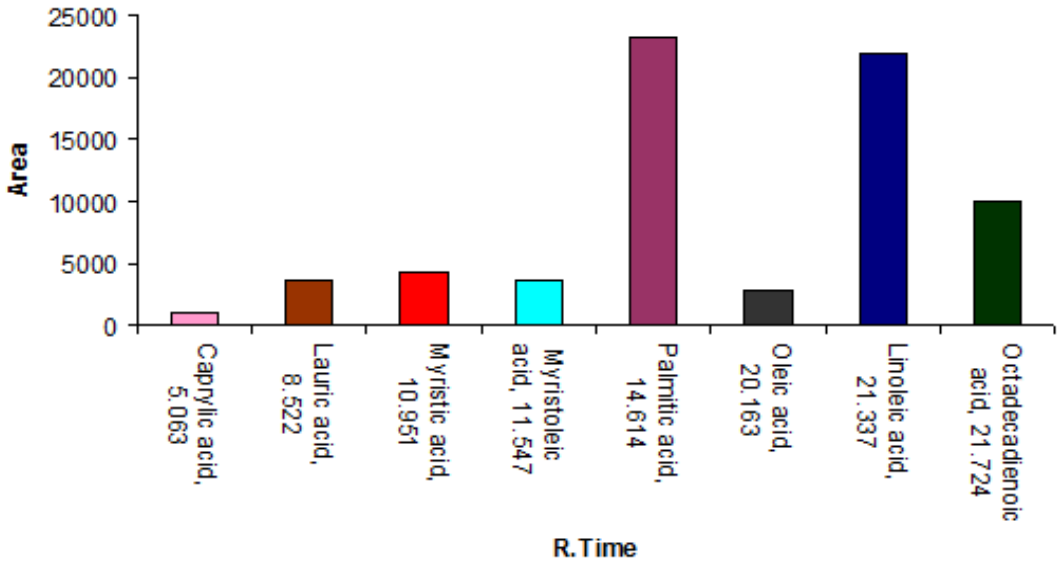

Figure 3.5: Quantitative analysis of stem bark oil.

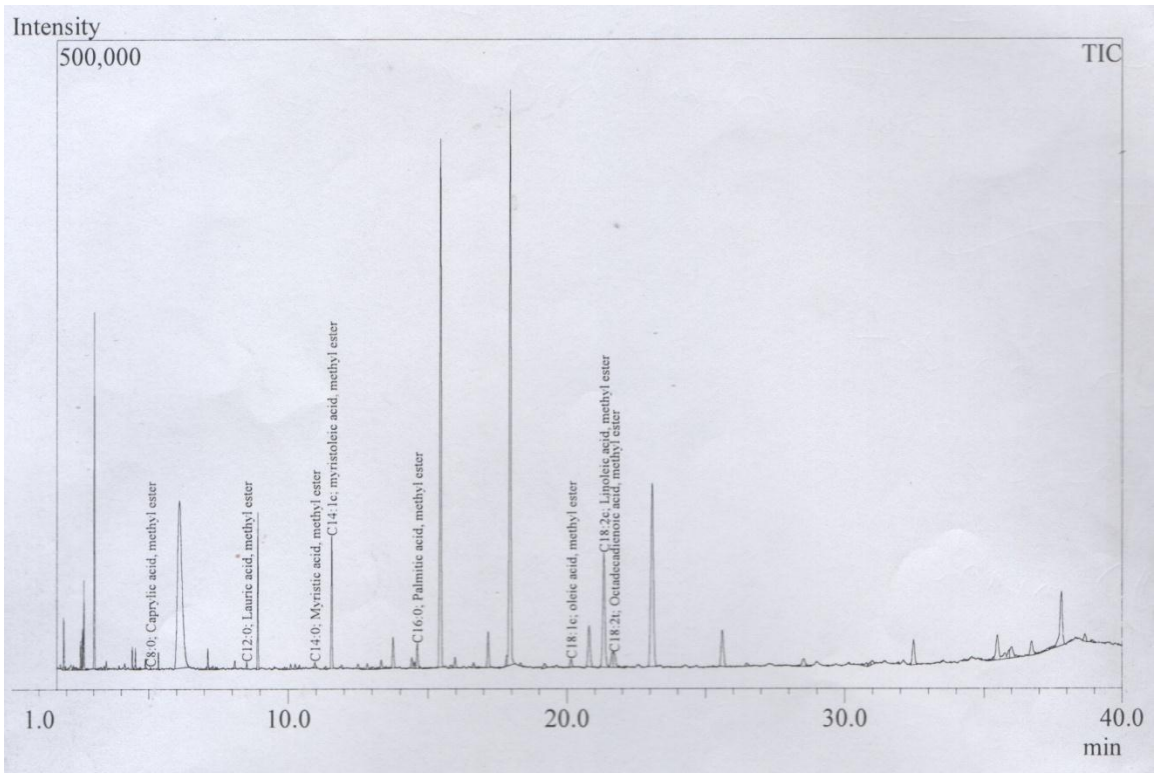

Figure 3.6: Quantitative analysis of stem bark oil (GCMS Report).

3.2.4. Oil analysis of *Acacia stenophylla*:

Fatty Acid profile of *Acacia stenophylla* showed various fatty Acids, such as; C12:0; Lauric Acid, Methyl Ester (0.00%), C14:0; Myristic Acid Methyl Ester (0.00%), C14:1c; Myristoleic Acid Methyl Ester (0.02%), C16:0; Palmitic Acid Methyl Ester (0.04%), C18:1c; Oleic Acid Methyl Ester (0.02%), C18:2c; Linoleic Acid Methyl Ester (0.12%), C18:2t; Octadecadienoic Acid methyl ester (0.07%) and C18:3n6; g-linolenic Acid, Methyl Ester (0.08%) **(Table 3.5)**

Table 3.5: Quantitative analysis of stem bark oil.

S.No	Name of fatty Acid	R.Time	Area	Conc. (%)
1	C12:0; Lauric Acid	8.522	1552	0.00
2	C14:0; Myristic Acid	10.951	1931	0.00
3	C14:1c; Myristoleic Acid	11.549	1702	0.02
4	C16:0; Palmitic Acid	14.614	18864	0.04
5	C18:1c; Oleic Acid	20.146	1814	0.02
6	C18:2c; Linoleic Acid	21.334	13080	0.12
7	C18:2t; Octadecadienoic Acid	21.722	8339	0.07
8	C18:3n6; g-Linolenic Acid	23.080	5582	0.08

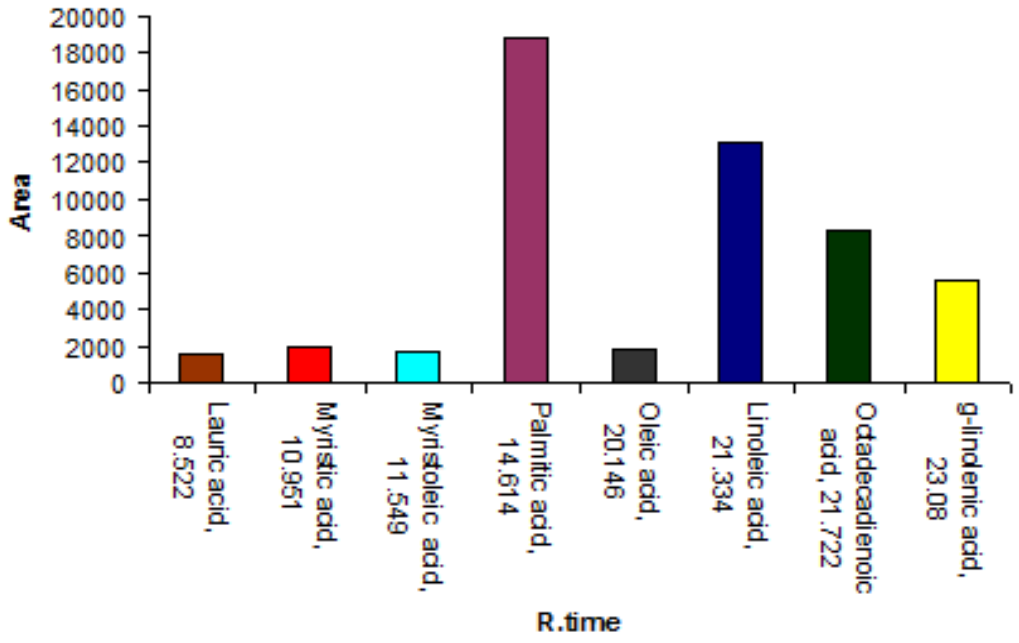

Figure 3.7: Quantitative analysis of stem bark oil.

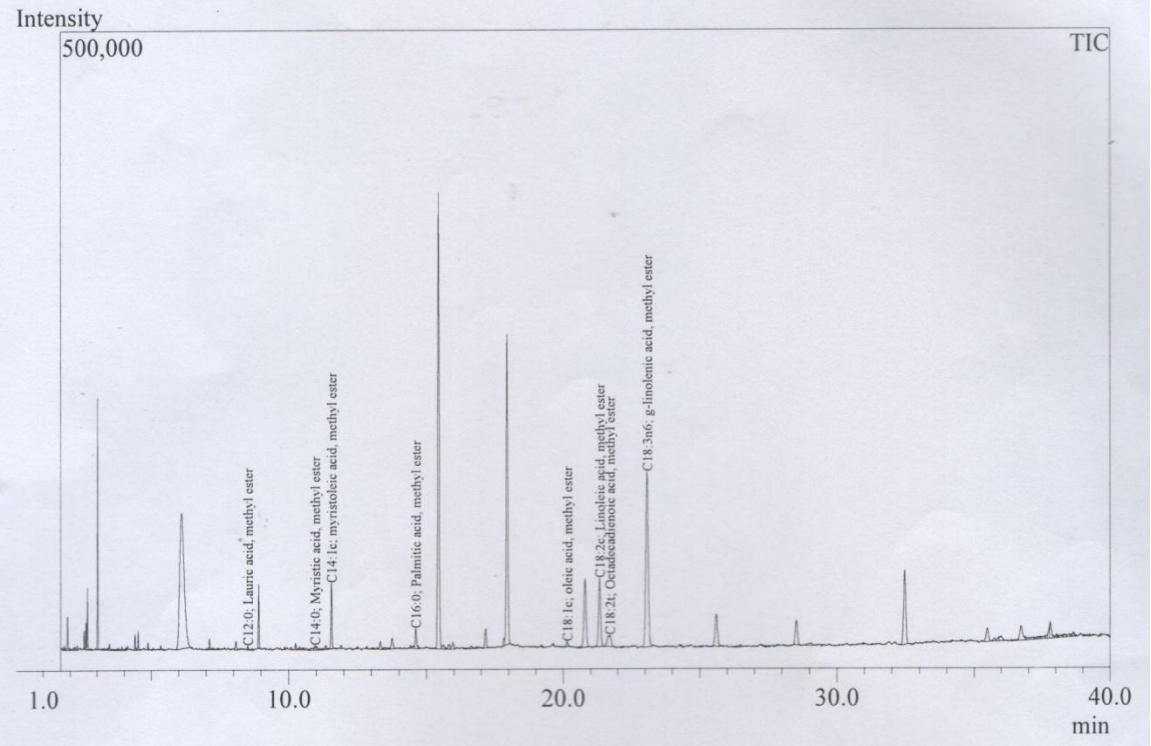

Figure 3.8: Quantitative analysis of stem bark oil (GCMS Report).

3.3. *IN-VITRO* BIOLOGICAL ACTIVITIES:

3.3.1. Anti-Bacterial Activiity:

Data shown in **Table 3.6** indicated the anti-bacterial activity of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem bark extract against *Escherichia coli*. Analysis of the data revealed that extracted samples of *Tamarix aphylla* inhibited the growth by 44.11% and 51.47% at concentration of 1 mg/disc and 2 mg/disc respectively, for *Tamarix dioica* the maximum activity was recorded for high concentration i.e. *Tamarix dioica* reduced the growth by 42.64% at concentration of 1 mg/disc and reduced the growth by 58.82% at 2 mg/disc. *Acacia cyanophylla* inhibited the growth by 50.00% and 52.94% at concentrations of 1 and and 2 mg $disc^{-1}$ respectively, while in case of *Acacia stenophylla*, it reduced the growth by 41.17% at concentration of 1 mg/disc and 47.05% at 2 mg/disc.

Table 3.6: Anti-bacterial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *E. coli.*

Escherichia coli					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Ciprofloxacin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	15	44.11	34	-
	2	17.5	51.47		
Tamarix dioica	1	14.5	42.64	34	-
	2	20	58.82		
Acacia cyanophylla	1	17	50.00	34	-
	2	18	52.94		
Acacia stenophylla	1	14	41.17	34	-
	2	16	47.05		

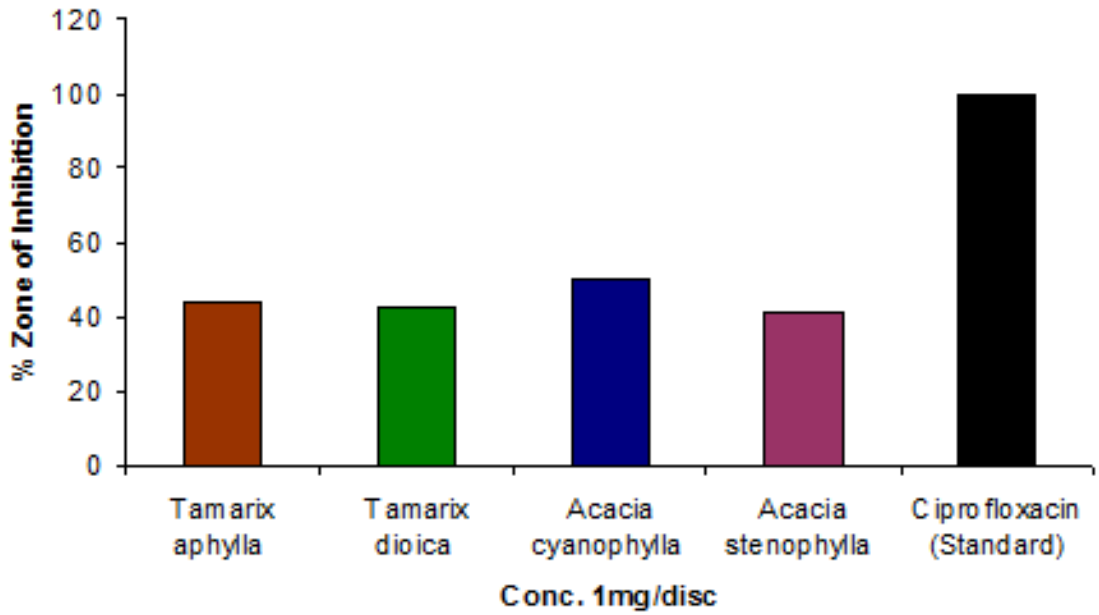

Figure 3.9: Antibacterial activity of crude methanolic extracts (1mg/disc conc.) against *Escherichia coli*

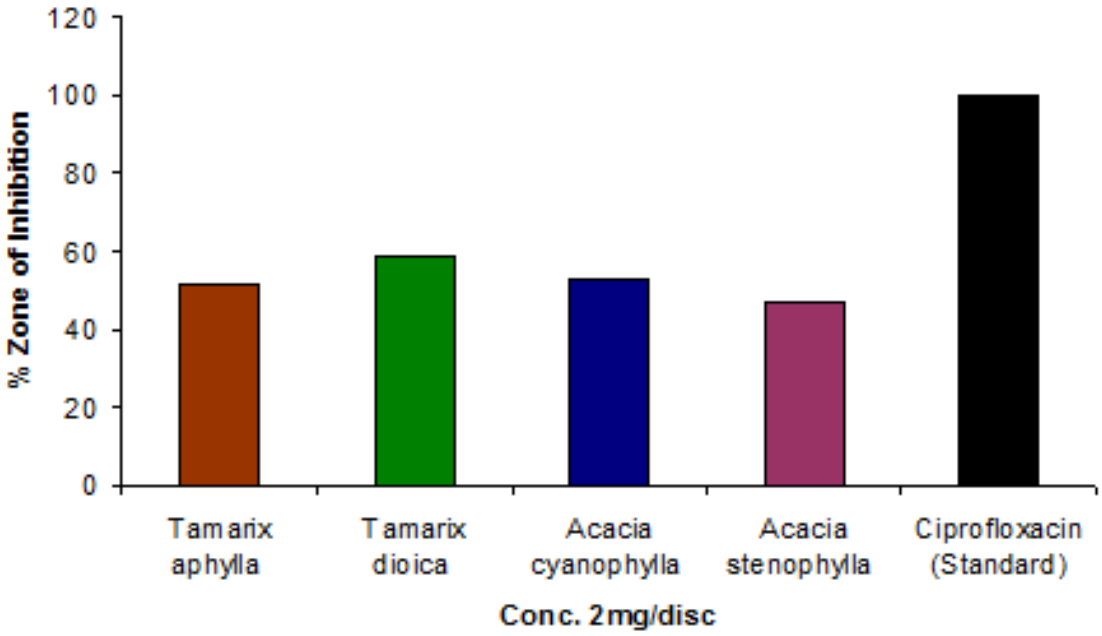

Figure 3.10: Antibacterial activity of crude methanolic extracts (2mg/disc conc.) against *Escherichia coli*

The stem bark extract of *Tamarix aphylla*, *Tamarix dioica*, *Acacia cyanophylla* and *Acacia stenophylla* indicated good anti-bacterial activity against *Salmonella typhii*. *Tamarix aphylla* inhibited the growth by 67.30% and 71.15% at concentration of 1 mg $disc^{-1}$ and 2 mg $disc^{-1}$ respectively. **Table 3.7** shows that maximum activity was recorded for *Tamarix dioica* at higher concentration. It reduced the growth by 63.46% at concentration of 1 mg $disc^{-1}$ and 75.00% at 2 mg/disc. The inhibitory potential of *Acacia cyanophylla* was 57.69% and 63.46% at concentration of 1 mg/disc and 2 mg/disc respectively, while in *Acacia stenophylla*, the growth reduced by 65.38% at concentration of 1 mg/disc and 69.23% at 2 mg/disc.

Table 3.7: Antimicrobial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *Salmonella typhii.*

Salmonella typhii					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Ciprofloxacin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	17.5	67.30	26	-
	2	18.5	71.15		
Tamarix dioica	1	16.5	63.46	26	-
	2	19.5	75.00		
Acacia cyanophylla	1	15	57.69	26	-
	2	16.5	63.46		
Acacia stenophylla	1	17	65.38	26	-
	2	18	69.23		

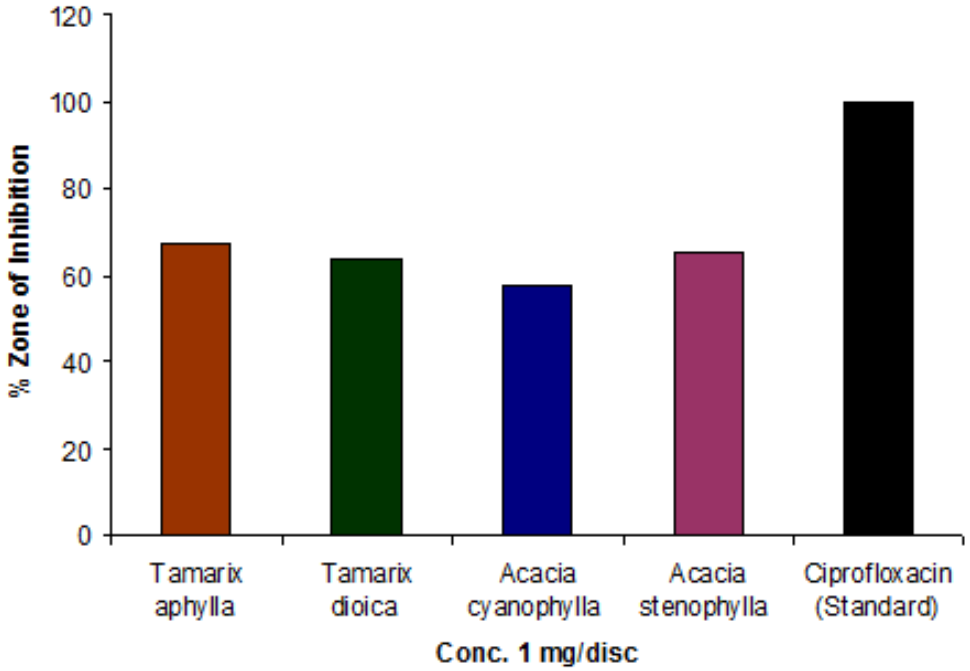

Figure 3.11: Antibacterial activity of crude methanolic extracts (1mg/disc conc.) against *S. typhii*

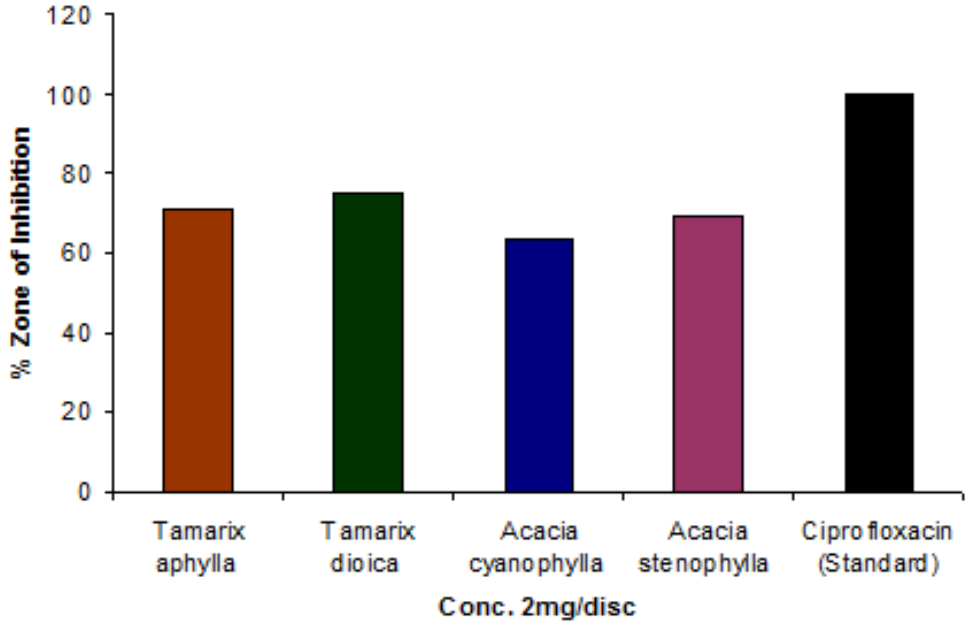

Figure 3.12: Antibacterial activity of crude methanolic extracts (2mg/disc conc.) against *S. typhii*.

The stem bark of all the selected four plants showed good anti-bacterial activity against *Klebsella pneumonia*. *Tamarix aphylla* inhibited the growth by 46.66% and 58.33% at concentrations of 1 mg/ disc and 2 mg/ disc respectively. The results revealed that maximum zone of inhibition were shown by *Tamarix dioica*. It reduced the growth by 55.00% at concentration of 1 mg/ discand 60.00% at 2 mg/disc. Similarly, *Acacia cyanophylla* inhibited the growth by 51.66% and 53.33% at concentration of 1 mg/disc and 2 mg/disc respectively. For *Acacia stenophylla*, the zone of inhibition was 48.33% at 1 mg/disc and 56.66% at 2 mg/disc.

Table 3.8. Antimicrobial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *Klebsella pneumonia*.

Klebsiella pneumonia					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Ciprofloxacin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	14	46.66	30	-
	2	17.5	58.33		
Tamarix dioica	1	16.5	55.00	30	-
	2	18	60.00		
Acacia cyanophylla	1	15.5	51.66	30	-
	2	16	53.33		
Acacia stenophylla	1	14.5	48.33	30	-
	2	17	56.66		

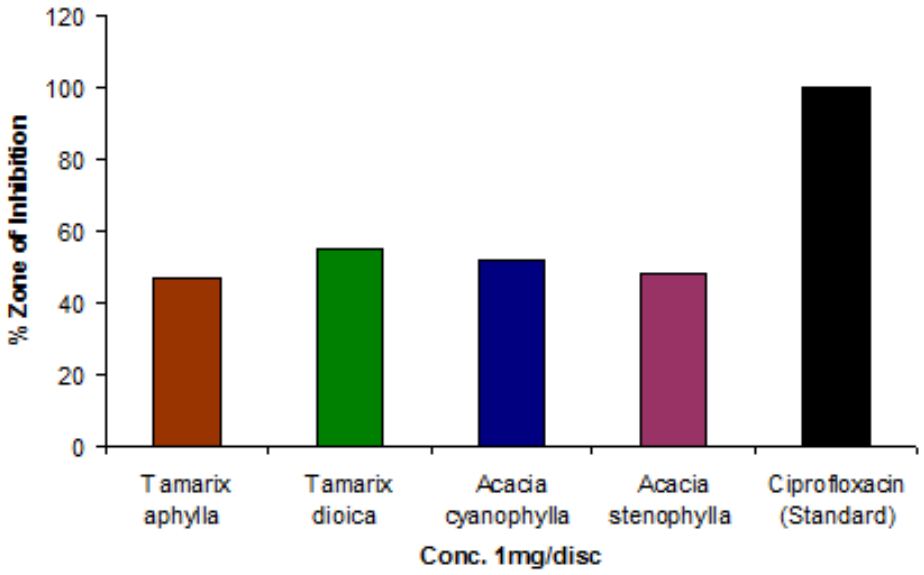

Figure 3.13: Antibacterial activity of crude methanolic extracts (1mg/disc conc.) against *K. pneumonia.*

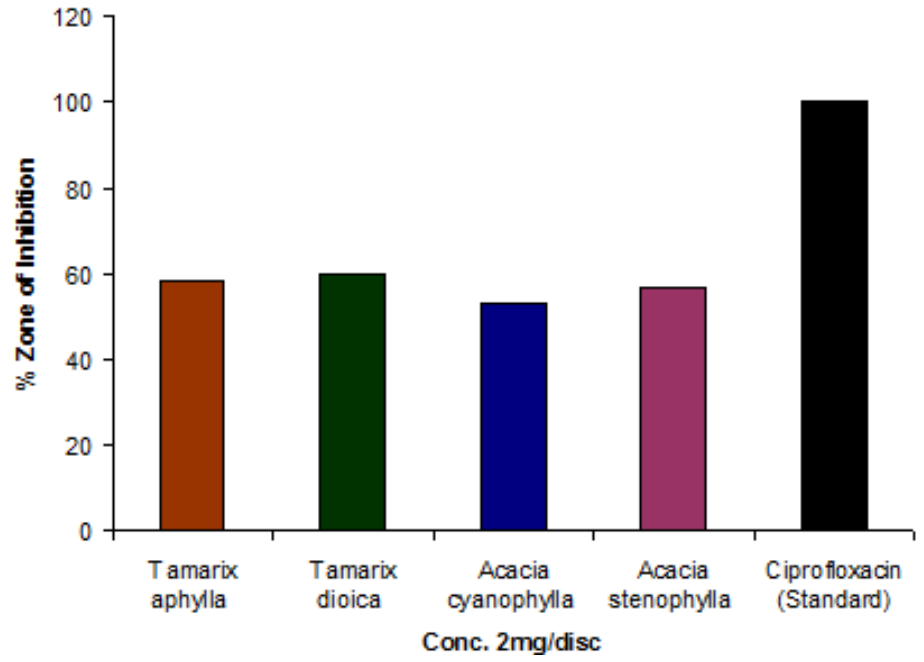

Figure 3.14: Antibacterial activity of crude methanolic extracts (2mg/disc conc.) against *K. pneumonia*

Data shown in **Table 3.9** indicates the anti-bacterial activity of selected plants against *Erwinia carotovora*. The results revealed that maximum inhibitory activity was shown by *Tamarix dioica* at both concentrations (69.69% at concentration of 1 mg/disc and 72.72% at concentration of 2 mg disc^{-1}), followed by *Acacia stenophylla* which showed 60.60% and 66.66% zone of inhibition at 1 mg/ disc and 2 mg/disc respectively. *Tamarix aphylla* inhibited the growth by 56.06% and 63.63% at concentration of 1 mg disc and 2 mg/disc respectively, while *Acacia cyanophylla* reduced the growth by 57.57% and 63.63% 1 mg discand 2 mg/disc respectively.

Table 3.9: Anti-bacterial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *E. carotovora.*

Erwinia carotovora					
Plants	**Conc. Mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Ciprofloxacin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	18.5	56.06	33	-
	2	21	63.63		
Tamarix dioica	1	23	69.69	33	-
	2	24	72.72		
Acacia cyanophylla	1	19	57.57	33	-
	2	21	63.63		
Acacia stenophylla	1	20	60.60	33	-
	2	22	66.66		

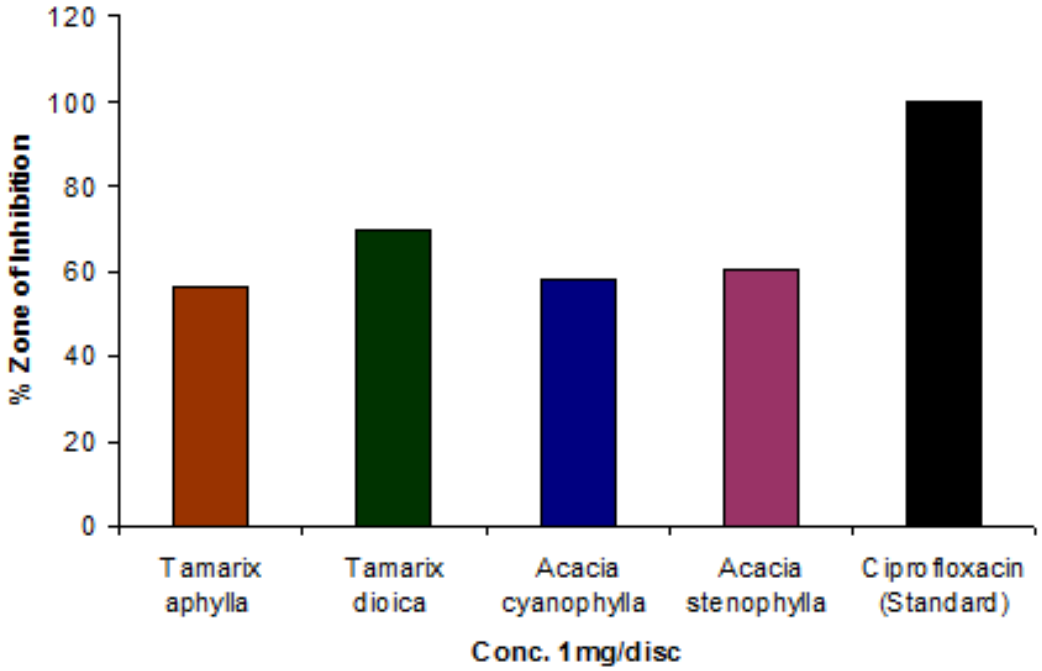

Figure 3.15: Antibacterial activity of crude methanolicextracts (1mg/disc conc.) against *E. carotovora.*

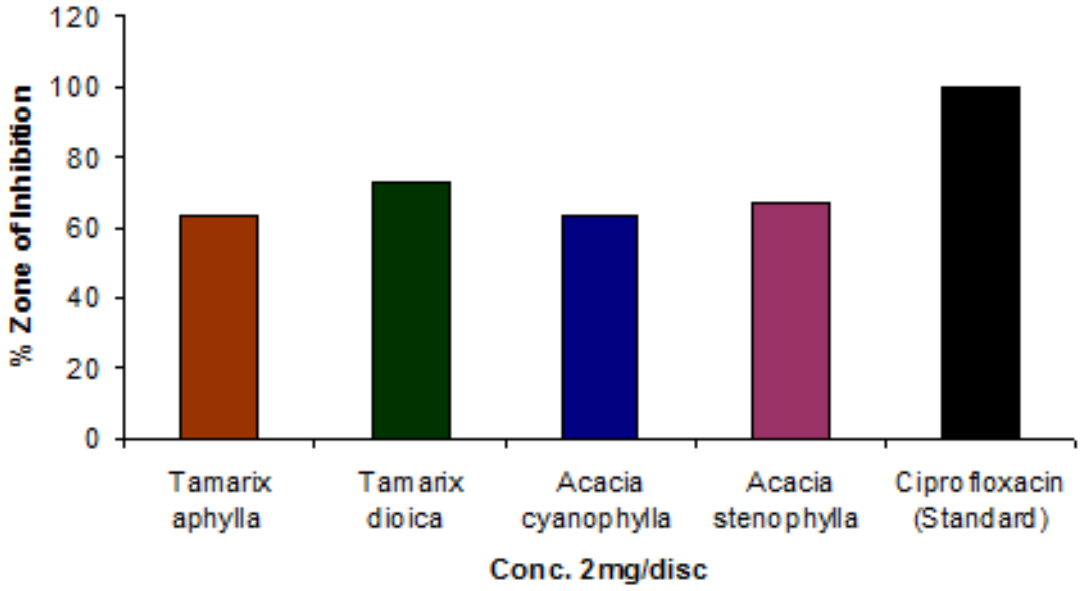

Figure 3.16: Antibacterial activity of crude methanolic extracts (2 mg/disc conc.) against *E. carotovora.*

The crude methanolic extracts of selected plants indicated good anti-bacterial activity against *Bacillus subtilis* (**Table 3.10).** Maximum zone of inhibition was measured for *Tamarix aphylla* at subjected concentrations i.e, 69.35% and 80.64% at1 mg/disc & 2 mg disc respectively. *Tamarix dioica* reduced the growth by 46.77% at concentration of 1 mg/disc and 64.51% at concentration of 2 mg disc^{-1}. Similarly, *Acacia cyanophylla* inhibited the growth by 51.61% and 61.29% at 1 mg/disc and 2 mg/disc respectively, while *Acacia stenophylla* inhibited it by 61.29% at 1 mg disc^{-1} and 70.96% at 2 mg/disc respectively.

Table 3.10. Anti-bacterial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *Bacillus subtilis.*

Bacillus subtilis					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Azithromycin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	21.5	69.35	31	-
	2	25	80.64		
Tamarix dioica	1	14.5	46.77	31	-
	2	20	64.51		
Acacia cyanophylla	1	16	51.61	31	-
	2	19	61.29		
Acacia stenophylla	1	19	61.29	31	-
	2	22	70.96		

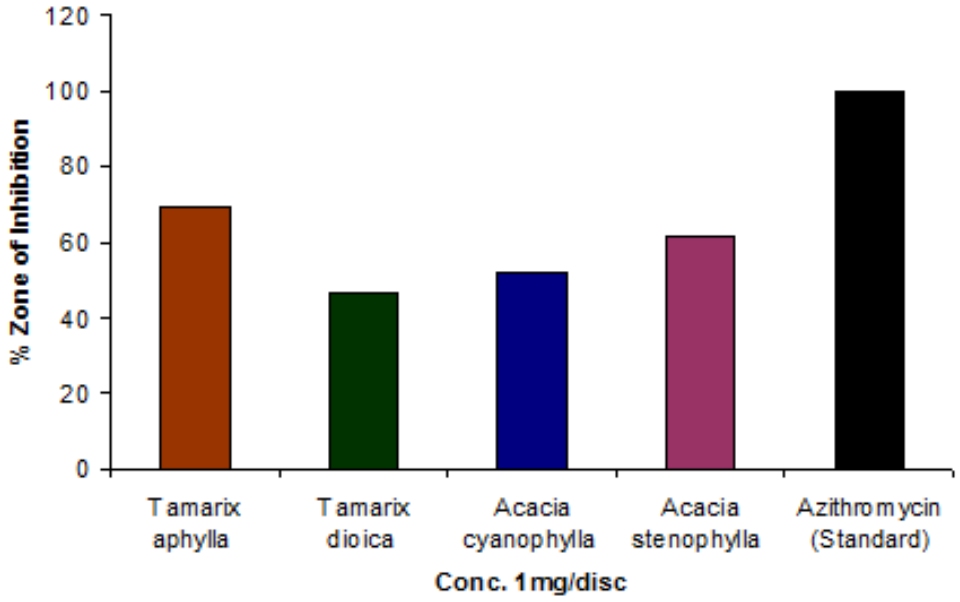

Figure 3.17: Antibacterial activity of crude methanolic extracts (1 mg/disc conc.) against *B. subtilis.*

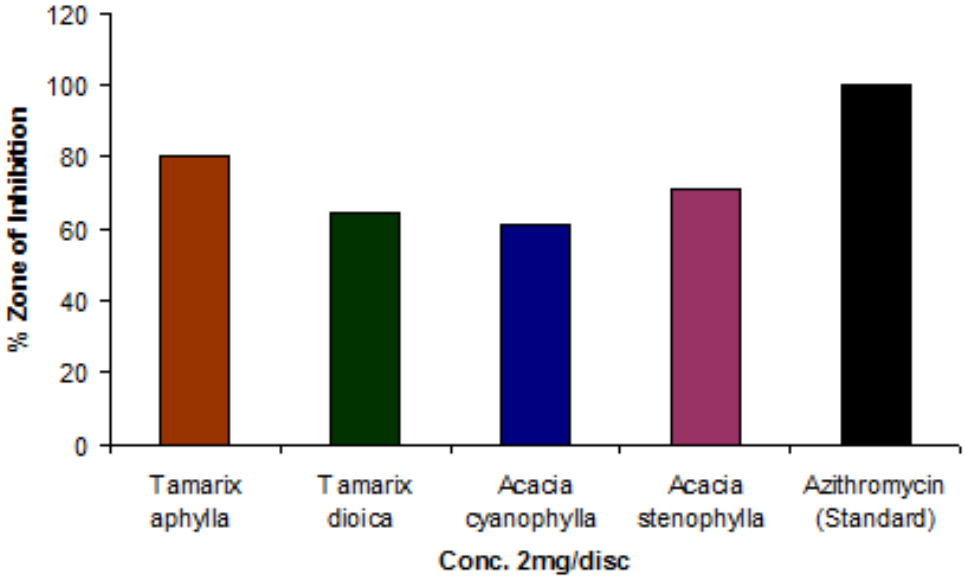

Figure 3.18: Antibacterial activity of crude methanolic extracts (2 mg/disc conc.) against *B. subtilis.*

The anti-bacterial activity of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* against *Bacillus atrophous* has been shown in **Table 3.11**. Among the tested plants, *Tamarix aphylla* was found more effective against the tested bacterium. It inhibited the growth by 70.31% and 81.25% at concentration of 1 mg/discand 2 mg/disc respectively which is the maximum inhibition. *Tamarix dioica* reduced the growth by 50.00% at concentration of 1 mg/disc and inhibited growth by 56.25% at concentration of 2 mg/disc. Similarly, *Acacia cyanophylla* showed good results as compared to *Acacia stenophylla.* The zone of inhibition measured by *Acacia cyanophylla* was 62.50% and 68.75% at concentration of 1 mg/discand 2 mg/disc respectively, in *Acacia stenophylla*, the inhibitory effect was 53.12% at 1 mg/disc and 59.37% at 2 mg/disc.

Table 3.11: Antimicrobial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *Bacillus atrophous.*

Bacillus atrophous					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Azithromycin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	22.5	70.31	32	-
	2	26	81.25		
Tamarix dioica	1	16	50.00	32	-
	2	18	56.25		
Acacia cyanophylla	1	20	62.5	32	-
	2	22	68.75		
Acacia stenophylla	1	17	53.12	32	-
	2	19	59.37		

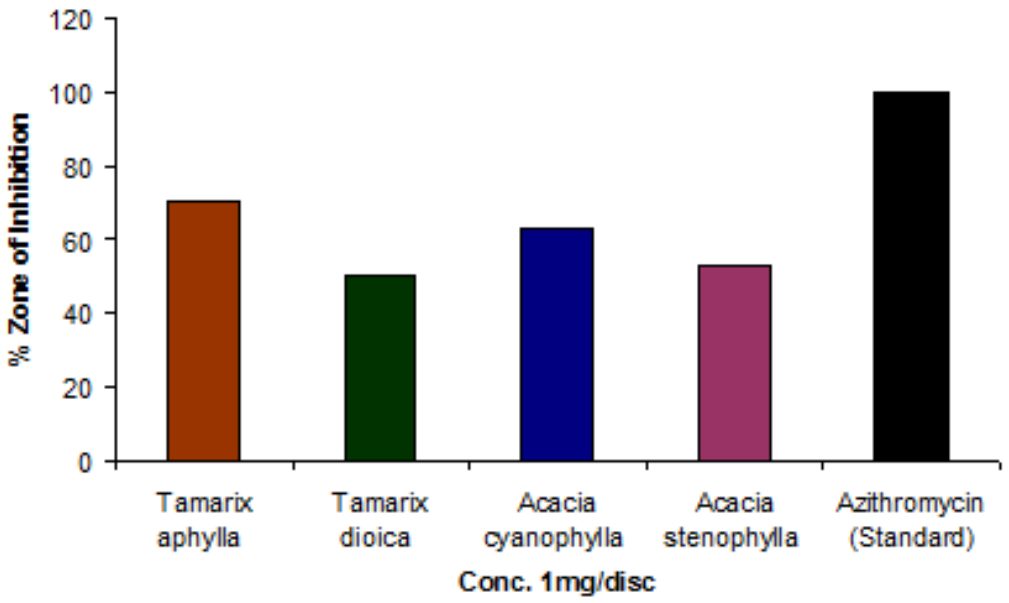

Figure 3.19: Antibacterial activity of crude methanolic extracts (1 mg/disc conc.) against *B. atrophous.*

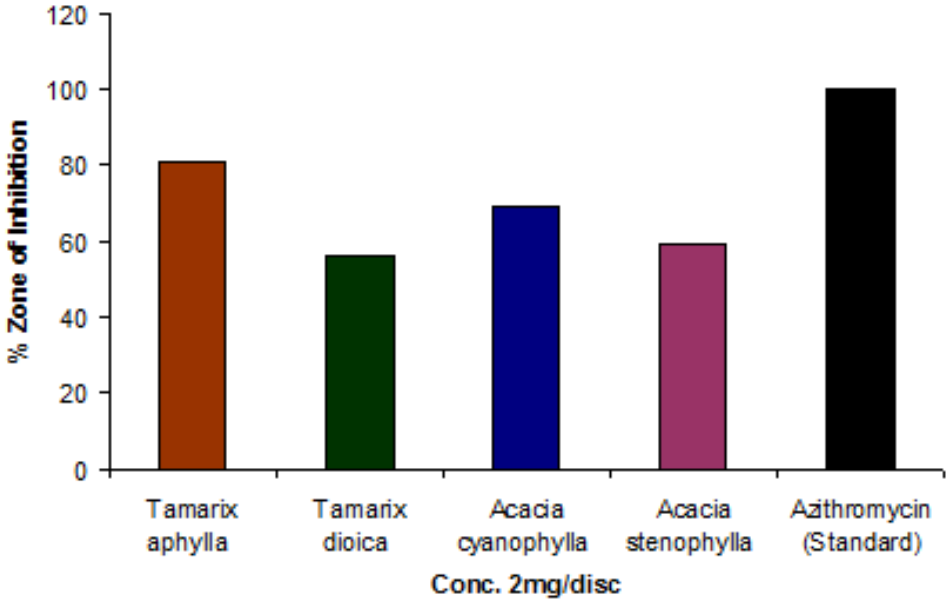

Figure 3.20: Antibacterial activity of crude methanolic extracts (2 mg/disc conc.) against *B. atrophous.*

Data shown in **Table 3.12** indicates the zone of inhibition of selected plants against *Staphylcoccus aureus*. All the plants showed good activity. Data analysis revealed that maximum activity was recorded for *Tamarix dioica* at the concentration of 2 mg disc^{-1} which was 72.41%, followed by *Acacia stenophylla* (68.96%) at the same concentration. Similarly, *Tamarix aphylla* inhibited the growth by 56.89% and 60.34% at concentration of 1 mg/disc & 2 mg disc respectively, while *Acacia cyanophylla* inhibited it by 55.17% and 58.62% at 1 mg/disc & 2 mg/disc respectively.

Table 3.12: Anti-bacterial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *Staphylcoccus aureus.*

Staphylcoccus aureus					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Azithromycin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	16.5	56.89	29	-
	2	17.5	60.34		
Tamarix dioica	1	16.5	56.89	29	-
	2	21	72.41		
Acacia cyanophylla	1	16	55.17	29	-
	2	17	58.62		
Acacia stenophylla	1	16	55.17	29	-
	2	20	68.96		

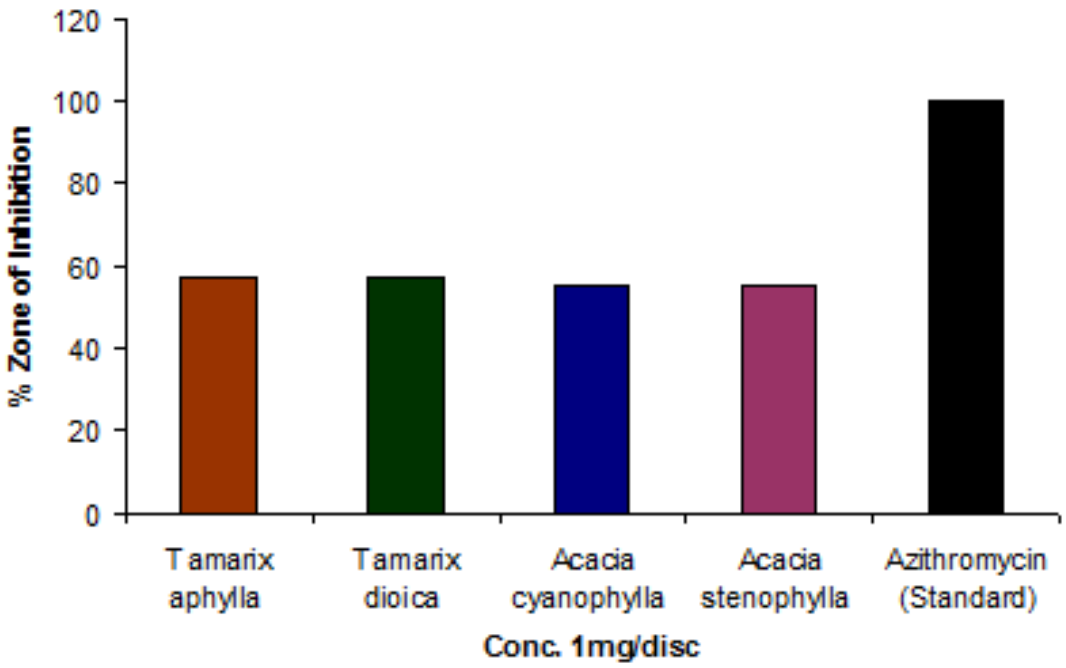

Figure 3.21: Antibacterial activity of crude methanolic extracts (1 mg/disc conc.) against *S. aureus.*

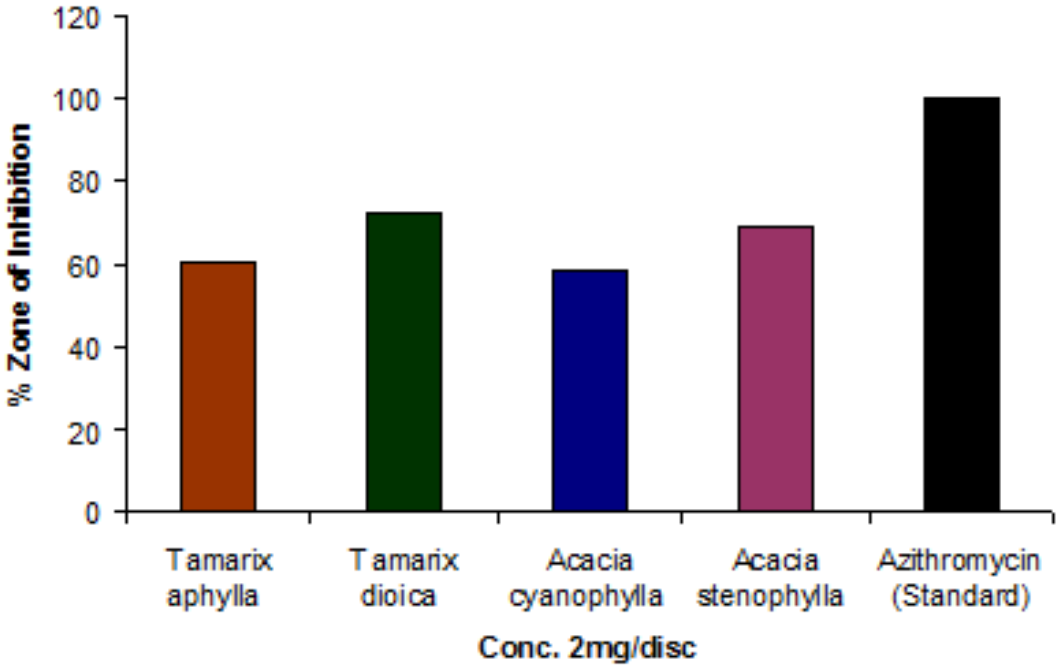

Figure 3.22: Antibacterial activity of crude methanolic extracts (2 mg/disc conc.) against *S. aureus.*

The growth of *Pseudomonas aeruginosa* was almost equally reduced by all tested plants (**Table 3.13**). All the selected plants were found equally effective against *Pseudomonas aeruginosa.* The extracted samples of *Tamarix aphylla* inhibited the growth by 53.12% and 57.81% at concentration of 1 mg/disc & 2 mg/disc respectively. *Tamarix dioica* showed reduction in growth by 51.56% at concentration of 1 mg/disc & inhibited growth by 59.37% at concentration of 2 mg/ disc. The inhibitory potential of *Acacia cyanophylla* was about 51.56% and 56.25% at dose of 1 mg/discand 2 mg/disc. Similarly, *Acacia stenophylla* inhibited the growth by 53.12% at concentration of 1 mg/disc & 60.93% at concentration of 2 mg/disc.

Table 3.13: Anti-bacterial activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *P. aeruginosa.*

P. aeruginosa					
Plants	**Conc. mg disc^{-1}**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Ciprofloxacin (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	17	53.12	32	-
	2	18.5	57.81		
Tamarix dioica	1	16.5	51.56	32	-
	2	19	59.37		
Acacia cyanophylla	1	16.5	51.56	32	-
	2	18	56.25		
Acacia stenophylla	1	17	53.12	32	-
	2	19.5	60.93		

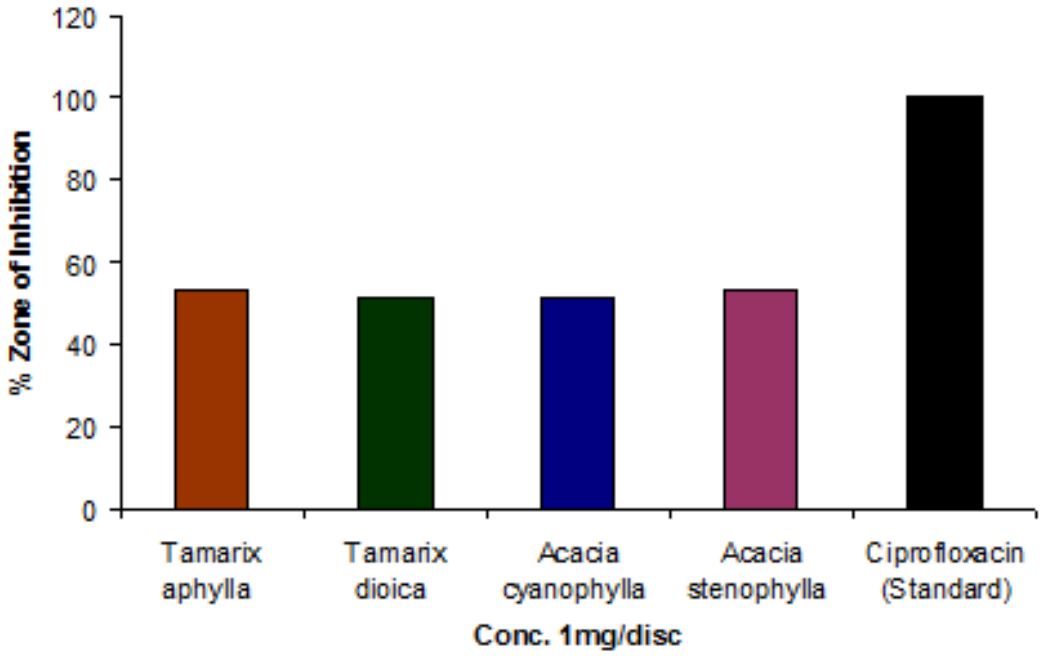

Figure 3.23: Antibacterial activity of crude methanolic extracts (1 mg/disc conc.) against *P. aeruginosa.*

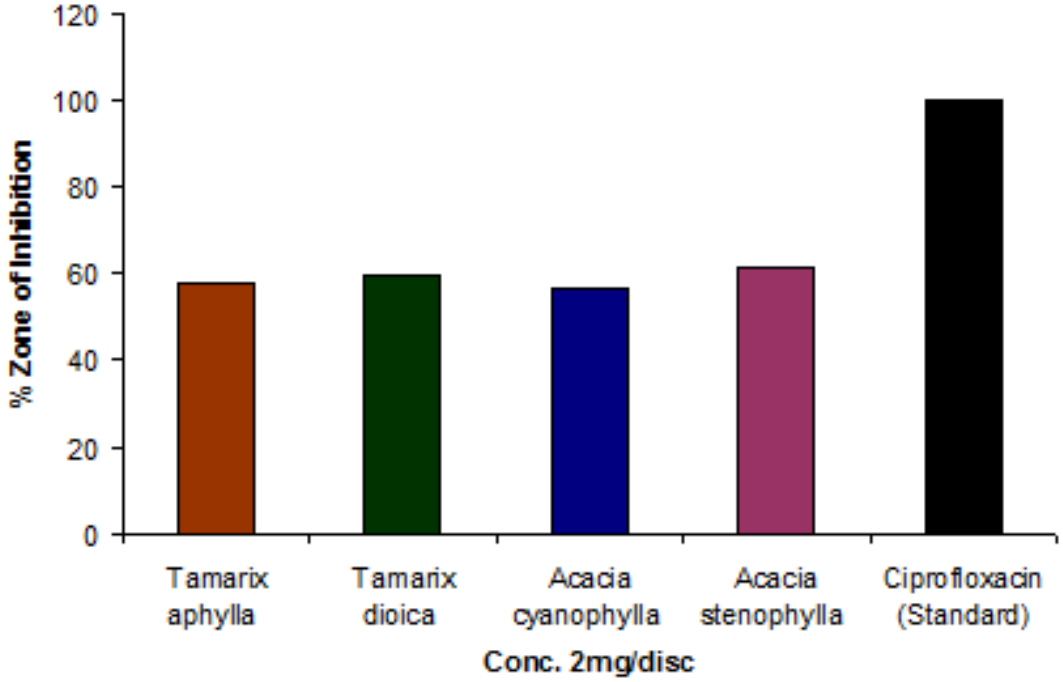

Figure 3.24: Antibacterial activity of crude methanolic extracts (2 mg/disc conc.) against *P. aeruginosa.*

3.3.2. Anti-Fungal Activity:

The antifungal activity of selected plants has been shown in **Table 3.14**. Analysis of the data revealed that maximum antifungal activity was measured for *Acacia cyanophylla* at subjected concentrations i.e, 66.66% and 72.22% at concentration of 1 mg/disc & 2 mg/disc respectively. Extracted samples of *Tamarix aphylla* inhibited the growth by 64.81% and 70.37% at concentration of 1 mg/disc and 2 mg/disc respectively while *Tamarix dioica* reduced the growth by 62.96% at concentration of 1 mg/disc and 64.81% at concentration of 2 mg/disc. Similarly, *Acacia stenophylla* reduced the growth by 59.25% and 62.96% at concentration of 1 mg $disc^{-1}$ and 2 mg $disc^{-1}$ respectively.

Table 3.14: Anti-fungal activity of methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem against *Candida albicans.*

Candida albicans					
Plants	**Conc. mg $disc^{-1}$**	**Zone of Inhibition (mm)**	**Zone of Inhibition (%)**	**Positive control. Clotrimazole (mm)**	**Negative control. DMSO (mm)**
Tamarix aphylla	1	17.5	64.81	27	-
	2	19	70.37		
Tamarix dioica	1	17	62.96	27	-
	2	17.5	64.81		
Acacia cyanophylla	1	18	66.66	27	-
	2	19.5	72.22		
Acacia stenophylla	1	16	59.25	27	-
	2	17	62.96		

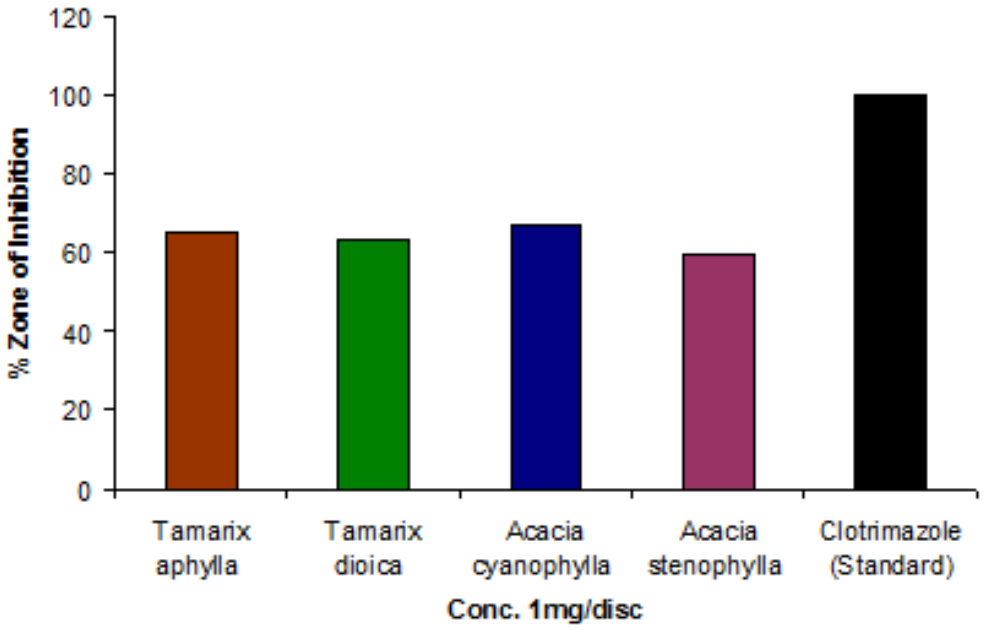

Figure 3.25: Anti-fungal activity of crude methanolic extracts (1 mg/disc conc.) against *C. albicans.*

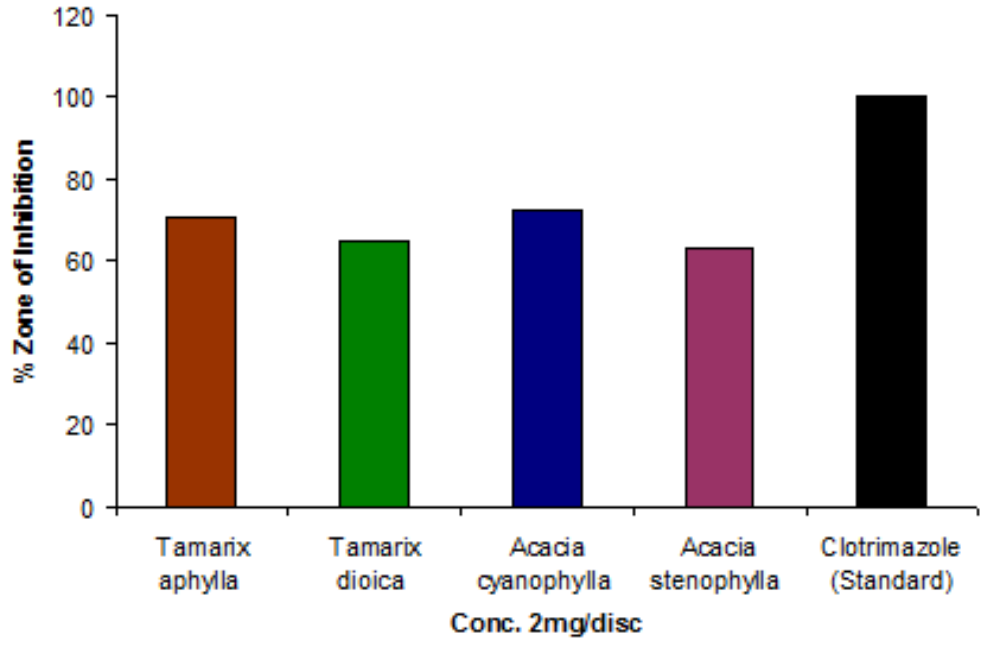

Figure 3.26: Anti-fungal activity of crude methanolic extracts (2 mg/disc conc.) against *C. albicans.*

3.3.3. Acetylcholinesterase Inhibitory Activities:

3.3.3.1. Acetylcholinesterase inhibitory activity of *Tamarix aphylla*:

The crude methanolic extract of *Tamarix aphylla* was screened for Acetylcholinesterase inhibitory activity. Results presented by IC_{50} value, given in **Table 3.15**. Same type activities were also studied for *n*-hexane, EtOAc, $CHCl_3$ and aqueous fractions. The Acetylcholinesterase inhibitory activity of standard drug (Galathamine) was 0.5 µM. The tested plant showed no inhibitory activity in any fraction.

Table 3.15: Acetylcholinesterase inhibitory activity of *Tamarix aphylla*.

Samples	**IC_{50} (µgml^{-1})**
	Acetylcholinesterase
Crude extract	N/A
n - Hexane fraction	N/A
Chloroform fraction	N/A
Ethyl acetate fraction	N/A
Aqueous fraction	N/A
Galathamine	0.5±0.07µM

3.3.3.2. Acetylcholinesterase inhibitory activity of *Tamarix dioica*:

Inhibitory activity of *Tamarix dioica* using various fractions against Acetylcholinesterase has been shown in **Table 3.16**. All the subjected fractions of *Tamarix dioica* were found ineffective and showed no activity against the tested enzyme. The Acetylcholinesterase inhibitory activity of standard drug (Galathamine) was 0.5 µM (IC_{50}).

Table 3.16: Acetylcholinesterase inhibitory activity of *Tamarix dioica.*

Samples	**IC_{50} (μgml^{-1})**
	Acetylcholinesterase
Crude extract	N/A
N-Hexane fraction	N/A
Chloroform fraction	N/A
Ethyl acetate fraction	N/A
Aqueous fraction	N/A
Galathamine	0.5±0.07µM

3.3.3.3. Acetylcholinesterase inhibitory activity of *Acacia cyanophylla*:

Acetylecholinesterase inhibitory activity of *Acacia cyanophylla* was tested by subjecting crude methanolic extract and other fractions (**Table 3.17**). The maximum inhibitory activity (IC_{50}) was observed for EtOAc (52.62µg/ml), followed by CHCl3 (67.29µg/ml). Similarly, crude methanolic extract showed 71.93µg/ml inhibition, while minimum activity was shown by aqueous fraction that was 134.2µg/ml as shown in **Figure 3.27**. The Acetylcholinesterase inhibitory activity of standard drug (Galathamine) was 0.5 µM.

Table 3.17: Acetylcholinesterase inhibitory activity of *Acacia cyanophylla*.

Samples	**IC_{50} (μgml^{-1})**
	Acetylcholinesterase
Crude extract	71.93±0.13
n-Hexane fraction	N/A
Chloroform fraction	67.29±0.24
Ethyl acetate fraction	52.62±0.17
Aqueous fraction	134.27±0.14
Galathamine	0.5±0.07µM

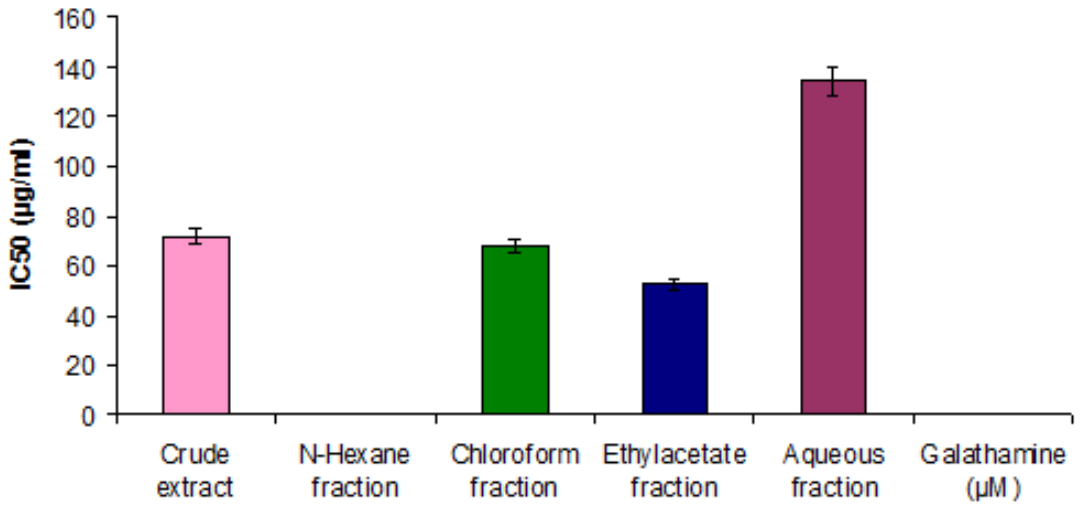

Figure 3.27: Acetylcholinesterase inhibitory activity of *Acacia cyanophylla*. (Bar repsresents ± SEM)

3.3.3.4. Acetylcholinesterase inhibitory activity of *Acacia stenophylla*:

The stem bark of *Acacia stenophylla* was tested for Acetylcholinesterase inhibitory activity by subjecting various solvent extracted samples as shown in **Table 3.18**. The maximum and minimum inhibitory activity was observed for EtOAc (37.11µg/ml) and aqueous fraction (91.46µg/ml) respectively. Chloroform fraction showed 41.89µg/ml inhibition while inhibitory activity measured for methanolic fraction was 57.23µg/ml. No inhibitory activity was observed for n-hexane fraction. The Acetylcholinesterase inhibitory activity of standard drug (Galathamine) was 0.5 µM (IC_{50}).

Table 3.18: Acetylcholinesterase inhibitory activity of *Acacia stenophylla.*

Samples	**IC_{50} (μgml^{-1})**
	Acetylcholinesterase
Crude extract	57.23±0.12
N-Hexane fraction	N/A
Chloroform fraction	41.89±0.16
Ethyl acetate fraction	37.11±0.08
Aqueous fraction	91.46±0.17
Galathamine	0.5±0.07µM

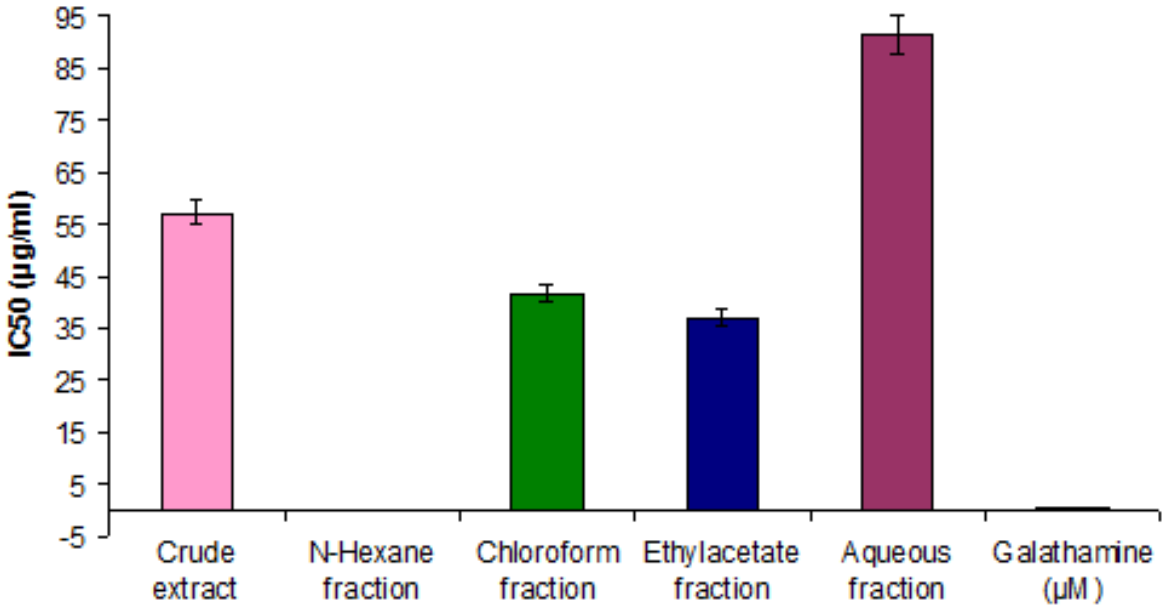

Figure 3.28: Acetylcholinesterase inhibitory activity of *Acacia stenophylla.* (Bar repsresents ± SEM)

3.3.4. Lipoxygenase Inhibitory Activity:

3.3.4.1. Lipoxygenase inhibitory activity of *Tamarix aphylla*:

Table 3.19 indicates the inhibitory activity of *Tamarix aphylla* in various solvents extracted samples against lipoxygenase. Results showed that maximum inhibitory activity was observed for EtOAc which was 27.3µg/ml, followed by methanolic fraction (49.2µg/ml). Chloroform extracted sample showed inhibition of 63.2µg/ml, aqueous fraction (92.6µg/ml) and minimum enzyme inhibition was recorded for *n*-hexane fraction (127.7µg/ml) as shown in **Figure 3.29**. The Lipoxygenase inhibitory activity of standard drug (Baicalein) was 22.0 µM.

Table 3.19: Lipoxygenase inhibitory activity of *Tamarix aphylla.*

Samples	**IC_{50} (μgml^{-1})**
Crude extract	49.2±0.09
N-Hexane fraction	127.7±0.13
Chloroform fraction	63.2±0.19
Ethyl acetate fraction	27.3±0.14
Aqueous fraction	92.6±0.41
Baicalein	22.0±0.05 (µM)

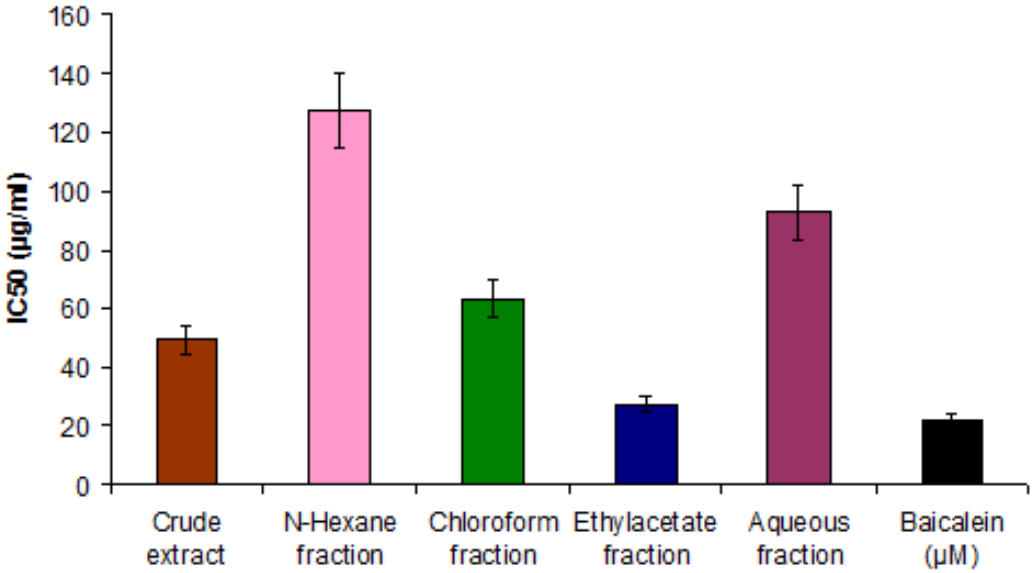

Figure 3.29: Lipoxygenase inhibitory activity of *Tamarix aphylla*. (Bar repsresents ± SEM)

3.3.4.2. Lipoxygenase inhibitory activity of *Tamarix dioica*:

Tamarix dioica inhibited the lipoxygenase enzyme using various solvent extracted samples of the stem bark (**Table 3.20**). The maximum activity was observed for EtOAc (34.1µg/ml) followed by methanolic extract (56.9µg/ml). Similarly, minimum enzyme inhibition was observed for *n*-hexane (103.5µg/ml) followed by aqueous fraction (87.4µg/ml). Chloroform extracted sample inhibited the enzyme by 64.2µg/ml. The Lipoxygenase inhibitory activity of standard drug (Baicalein) was 22.0 µM.

Table 3.20: Lipoxygenase inhibitory activity of *Tamarix dioica.*

Samples	**IC_{50} (μgml^{-1})**
Crude methanolic extract	56.9±0.23
N-Hexane fraction	103.5±0..43
Chloroform fraction	64.21±0.17
Ethyl acetate fraction	34.1±0.12
Aqueous fraction	87.4±0.27
Baicalein	22.0±0.05 (µM)

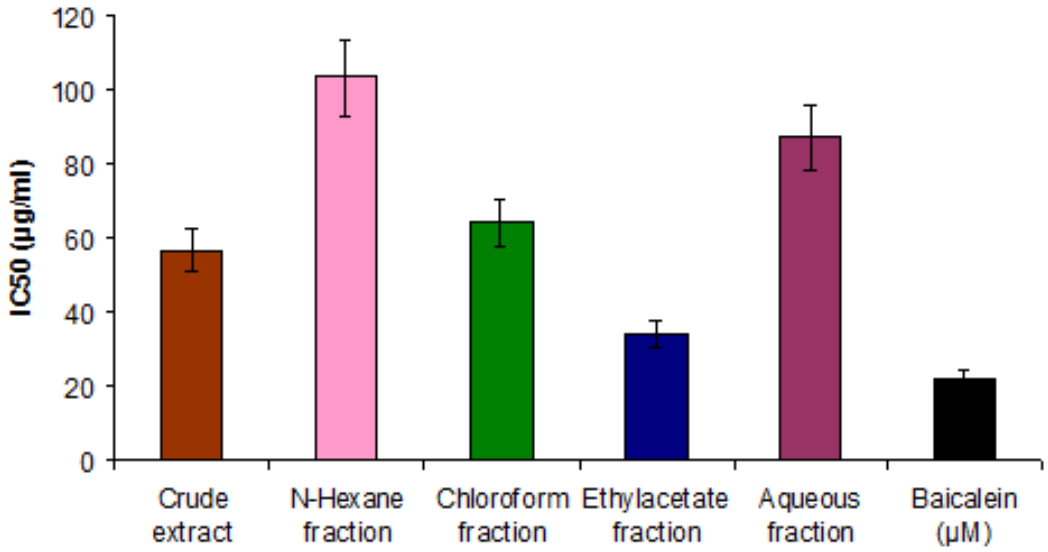

Figure 3.30: Lipoxygenase inhibitory activity of *Tamarix dioica.* (Bar repsresents ± SEM)

3.3.4.3. Lipoxygenase inhibitory activity of *Acacia cyanophylla*:

The stem bark of *Acacia cyanophylla* was screened for Lipoxygenase inhibitory activity using various solvent extracted samples.**Table 3.21** indicates that the maximum activity was meaured for EtOAc (37.3µg/ml) followed by CHCl3 (51.8µg/ml) and minimum inhibitory activity was shown by *n*-hexane (124.7µg/ml) followed by aqueous fraction (117.6µg/ml). Methanolic extract inhibited the enzyme by 82.3µg/ml as shown in **Figure 3.31**. The Lipoxygenase inhibitory activity of standard drug (Baicalein) was 22.0 µM.

Table 3.21: Lipoxygenase inhibitory activity of *Acacia cyanophylla*.

Samples	**IC_{50} (μgml^{-1})**
Crude extract	82.3±0.17
N-Hexane fraction	124.7±0.37
Chloroform fraction	51.8±0.16
Ethyl acetate fraction	37.3±0.42
Aqueous fraction	117.6±0.34
Baicalein	22.0±0.05 (µM)

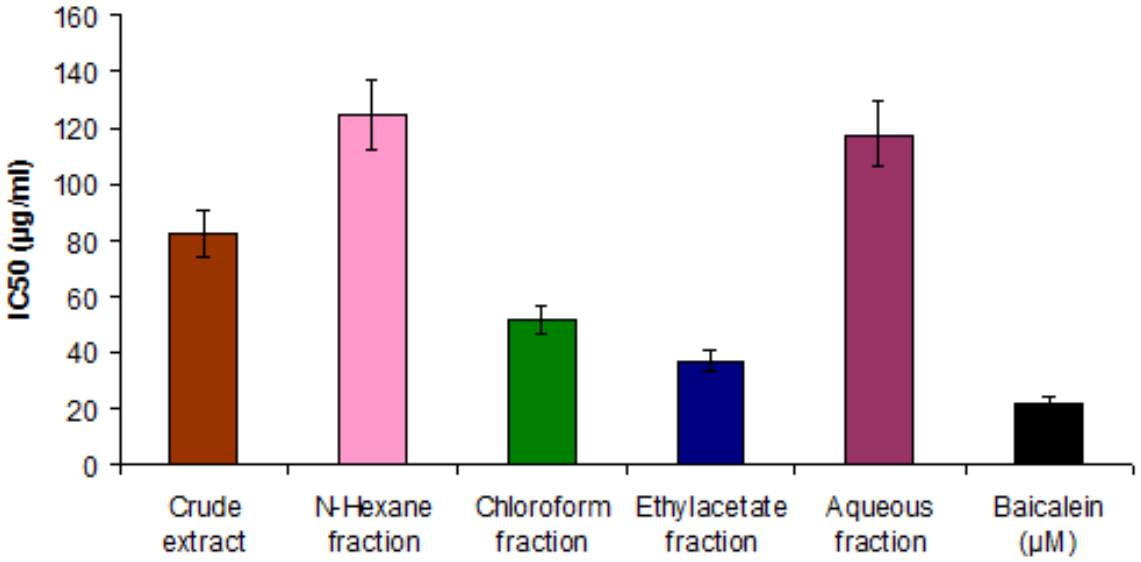

Figure 3.31: Lipoxygenase inhibitory activity of *Acacia cyanophylla*. (Bar repsresents ± SEM)

3.3.4.4. Lipoxygenase Inhibitory activity of *Acacia stenophylla*:

The Lipoxygenase inhibitory activity of *Acacia stenophylla* has been shown in **Table 3.22**. Ethyl acetate extracted sample showed maximum inhibitory activity of 39.9µg/ml followed by chloroform (47.1µg/ml). Methanolic extract showed 68.2µg/ml inhibition and *n*-hexane (92.4µg/ml).The minimum enzyme inhibitory activity was measured for aqueous fraction which was 142.3µg/ml. The Lipoxygenase inhibitory activity of standard drug (Baicalein) was 22.0 µM.

Table 3.22: Lipoxygenase inhibitory activity of *Acacia stenophylla*.

Samples	**IC_{50} (μgml^{-1})**
Crude extract	68.2±0.14
N-Hexane fraction	92.4±0..11
Chloroform fraction	47.1±0.27
Ethyl acetate fraction	39.9±0.28
Aqueous fraction	142.3±0.37
Baicalein	22.0±0.05 (µM)

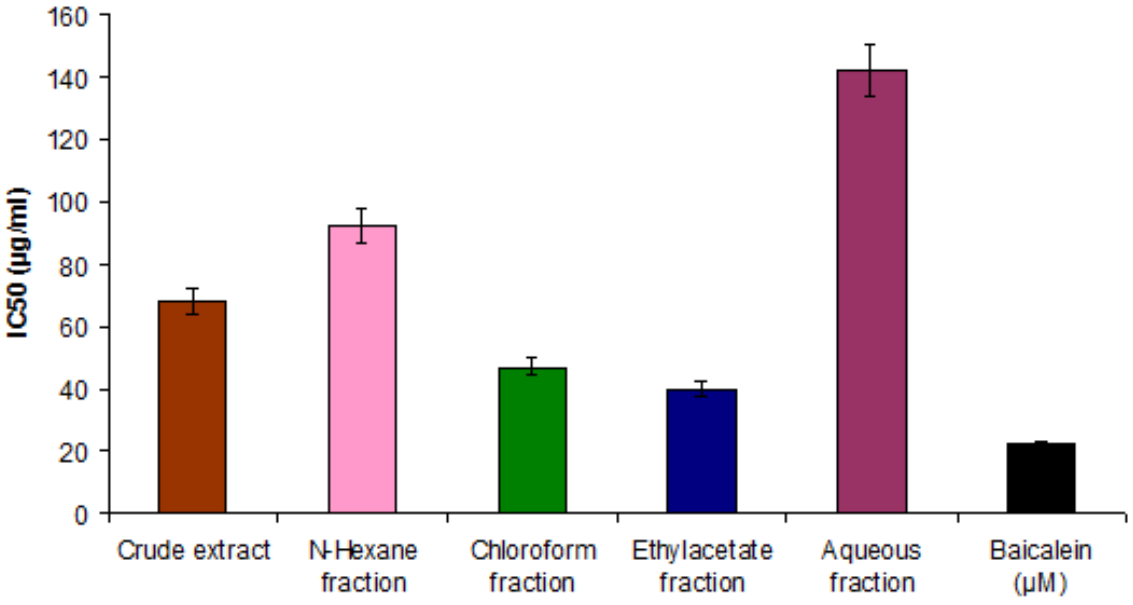

Figure 3.32: Lipoxygenase inhibitory activity of *Acacia stenophylla.* (Bar repsresents ± SEM)

3.3.5. Antioxidant Activity (Dpph Radical Scavenging Assay):

The crude methanolic extracts of selected plants were screened for antioxidant potential. All the tested plants showed good antioxidant activity as given in given in **Table 3.23.** Maximum activity (IC_{50}) was measured for *Tamarix aphylla* which was 831 followed by *Tamarix dioica* (976). Similarly, antioxidant activity recorded for *Acacia cyanophylla* and *Acacia stenophylla* was 1153 and 1467 respectively.

Table 3.23: DPPH Radical Assay of *Tamarix aphylla, Tamarix dioica, Aacia cyanophylla* and *Acacia stenophylla.*

Samples	**IC_{50} (µg/ml)**
Tamarix aphylla	831 ± 0.02
Tamarix dioica	976 ± 0.01
Acacia cyanophylla	1153 ± 0.03
Acacia stenophylla	1467 ± 0.01
Ascorbic Acid	8.451 ± 0.17 µg/ml

3.4. *IN-VIVO* BIOLOGICAL ACTIVITIES:

3.4.1. Anti-Inflammatory Activities:

3.4.1.1. Carrageenan Induced Paw Edema Model:

The crude extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* in different doses of 50 mg/kg, 100 mg/kg & 200 mg/kg were subjected for anti-inflammatory effect.The results have been shown in **Tables 3.24-3.27** and **Figures 3.34-3.49.** Plants have been shown by abbreviation (TA: *Tamarix aphylla*, TD: *Tamarix dioica*, AC: *Acacia cyanophylla*, and AS: *Acacia stenophylla*).

1) Effect of *Tamarix aphylla* on Carrageenan Induced Paw Edema Model:

Anti-inflammatory effect of the crude methanolic extract of *Tamarix aphylla* has been shown in **Table3.24.** Analysis of the data indicated that the anti-inflammatory activity of the tested plant was time and dose dependent. After 5 hours, maximum anti-inflammatory activity of 51.84% was measured by the dose of 200 mg/kg, followed by 100 mg/kg that showed 36.00% inhibition. At the dose of 50 mg/kg, the inhibitory activity was 30.84% after 5 hours. Similarly, at 3 hours, maximum inhibition was shown by 200 mg/kg which was 42.23% followed by 100 mg/kg (23.82%). The dose of 50 mg/kg, showed anit-inflammatory activity of 17.66% at 3 hours.

Table 3.24: Anti-inflammatory effects of *Tamarix aphylla* on Carrageenan-Induced Paw Edema Model.

Treatments	**Dose (mg/kg)**	**3 hours**		**5 hours**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	5.92 ± 0.61	-	6.00 ± 0.63	-
Tamarix aphylla	50	5.23 ± 0.62	17.66	4.15 ± 0.63*	30.84
	100	4.51 ± 0.58*	23.82	3.84 ± 0.60**	36.00
	200	3.42 ± 0.61**	42.23	2.89 ± 0.61**	51.84
Indomethacin	10	3.17 ±0.60**	46.46	1.92 ± 0.61**	68.00

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

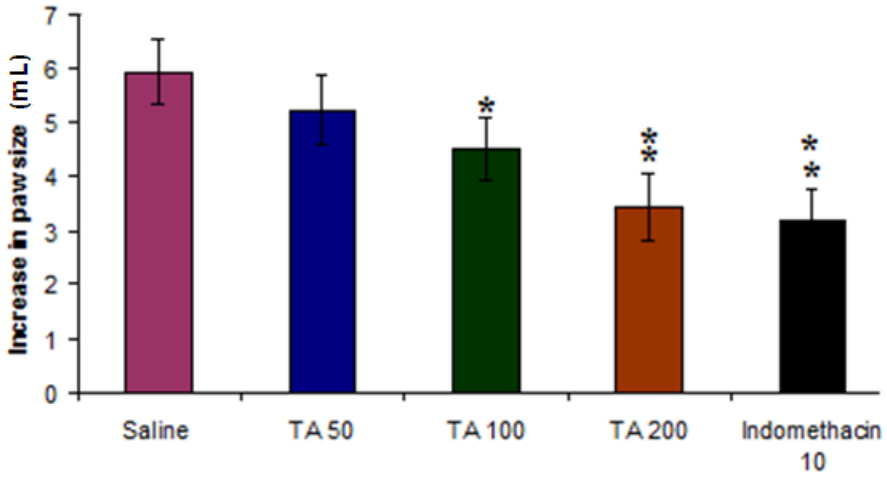

Figure 3.33: Effect of TA (3 hour) on Carrageenan-Induced Paw Edema Model.* $p < 0.05$, ** = $p < 0.01$.

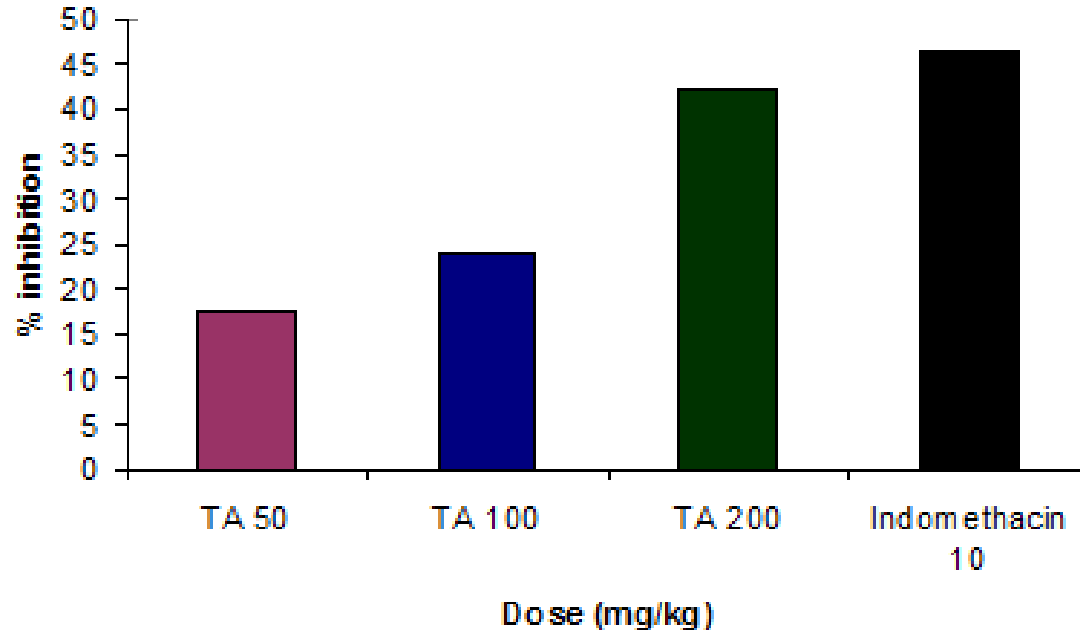

Figure 3.34: Percent inhibition of TA (3 hour) on Carrageenan-Induced Paw Edema Model.* $p < 0.05$, ** = $p < 0.01$.

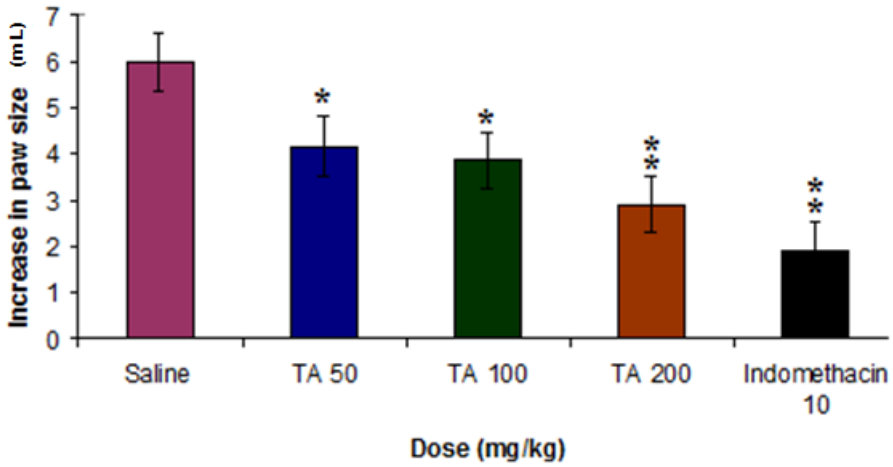

Figure 3.35: Effect of TA (5 hours) on Carrageenan-induced edema model.* $p < 0.05$, ** = $p < 0.01$.

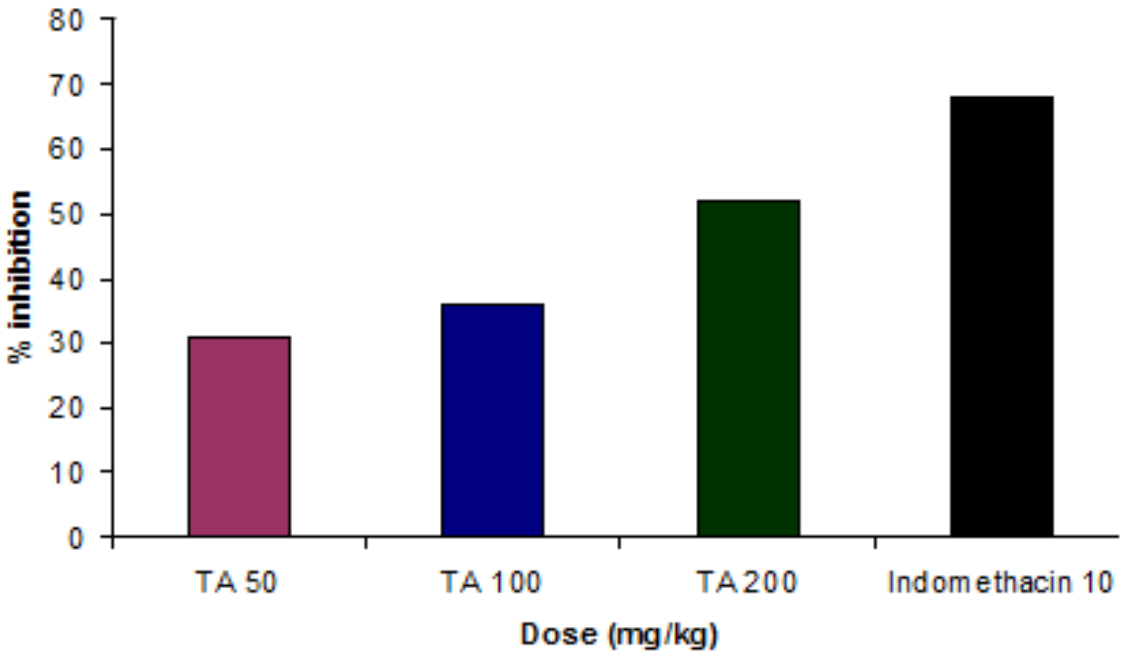

Figure 3.36: Percent inhibition of TA (5 hours) on Carrageenan-Induced Paw Edema Model.* $p < 0.05$, ** = $p < 0.01$.

2. Effect of *Tamarix dioica* on Carrageenan Induced Paw Edema Model:

Table 3.25 indicates the anti-inflammatory poential of *Tamarix dioica*.Analysis of the data reveals that the tested plnat showed the activity at both phases, i.e, 3 hours and 5 hours. The inhibitory activity was time and dose dependent. Maximum anti-inflammatory activity was shown by 200 mg/kg. It inhibited the inflammation by 54.12 and 34.98% after 5 and 3 hours respectively. Anti-inflammatory activity shown by 100 mg/kg after 3 and 5 hours was 18.34 and 33.89% respectively. Similarly, at the dose of 50 mg/kg, the inhibitory activity measured was 6.46% at 3 hours and 28.12% at 5 hours.

Table3.25: Anti-inflammatory effects of *Tamarix dioica* on Carrageenan-Induced Edema Model.

Treatments	**Dose (mg/kg)**	**3 hours**		**5 hours**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	5.89 ± 0.54	-	6.08 ± 0.52	-
Tamarix dioica	50	5.51 ± 0.54	6.46	4.37 ± 0.54*	28.12
	100	4.81 ± 0.55*	18.34	4.02 ± 0.53*	33.89
	200	3.83 ± 0.51**	34.98	2.79 ± 0.52**	54.1
Indomethacin	10	3.07 ±0.49**	47.88	1.91 ± 0.50**	68.59

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

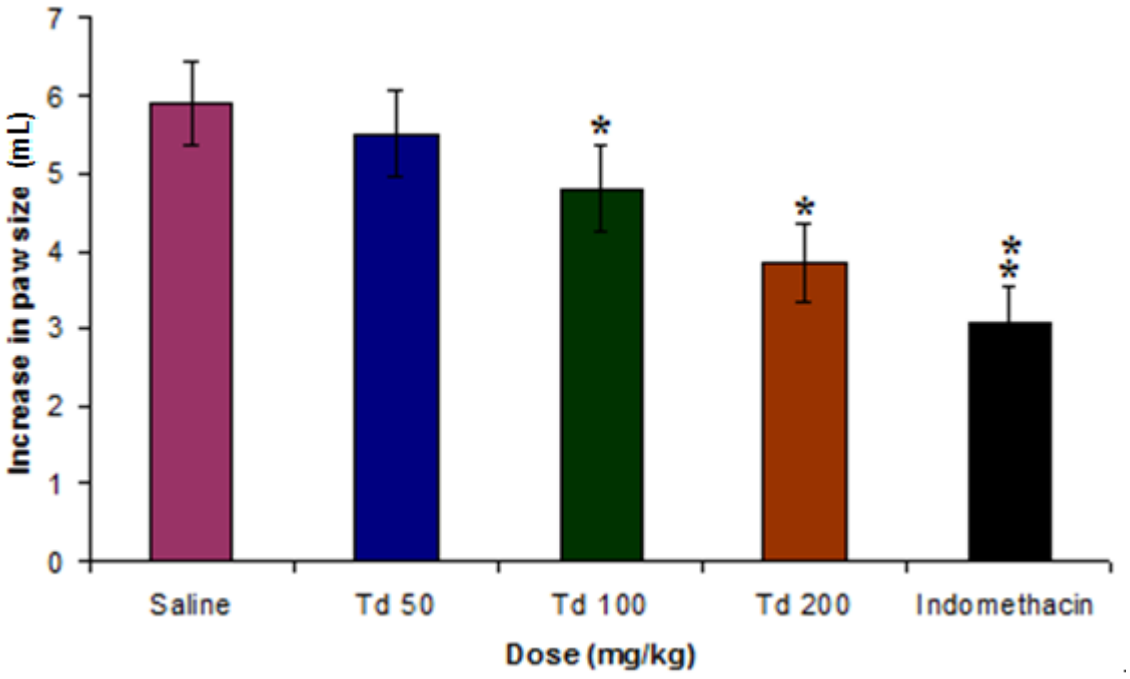

Figure 3.37: Effect of TD (3 hours) on Carrageenan-induced edema model.* $p < 0.05$, ** = $p < 0.01$.

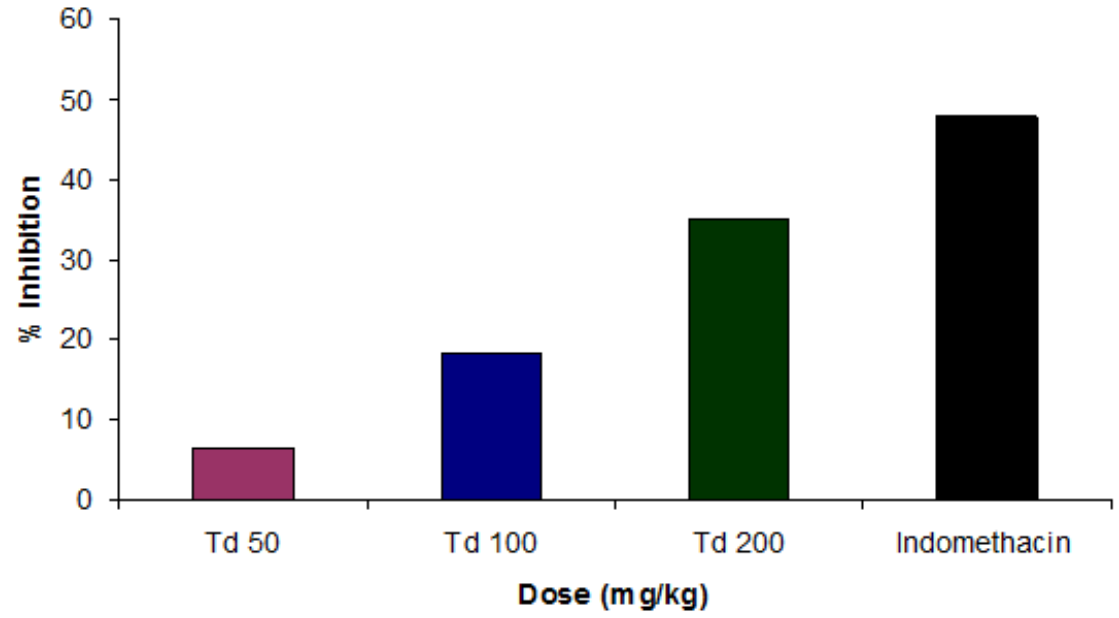

Figure 3.38: Percent inhibition of TD (3 hour) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

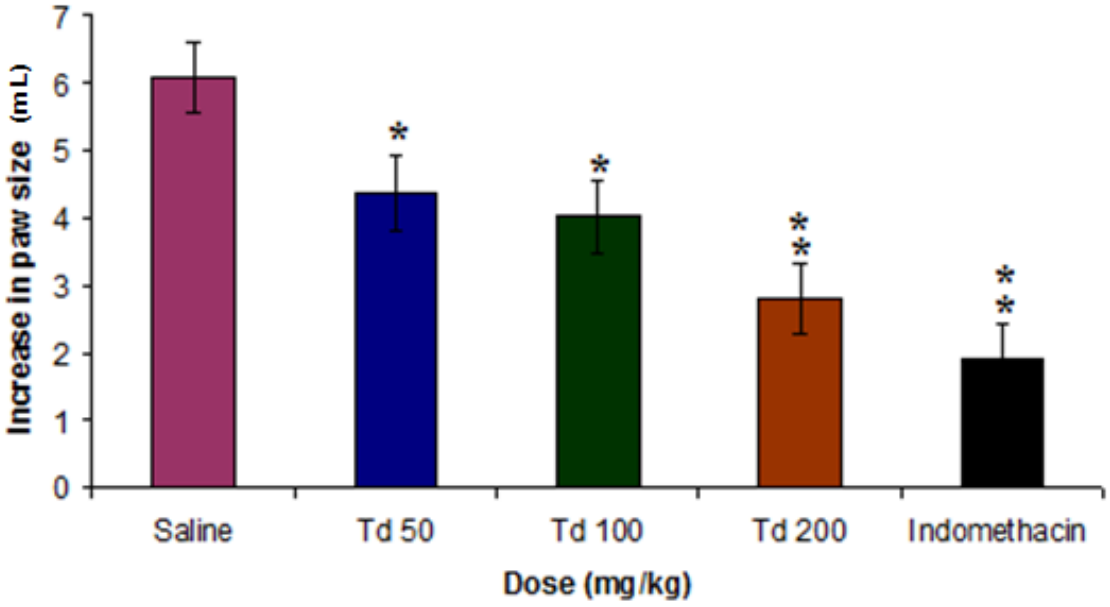

Figure 3.39: Effect of TD (5 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

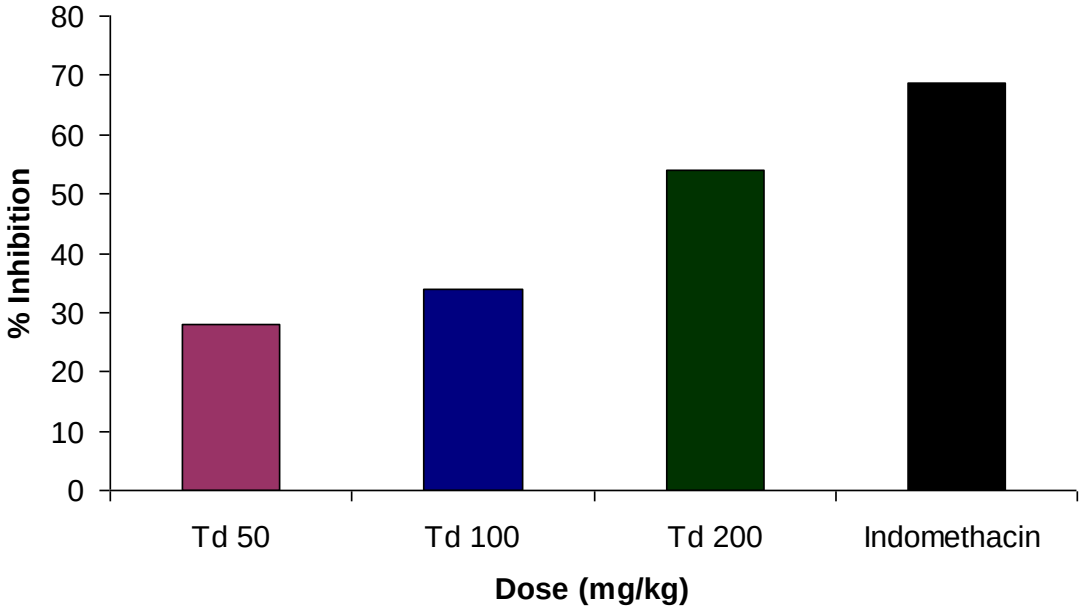

Figure 3.40: Percent inhibition of TD (5 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

2) Effect of *Acacia cyanophylla* on Carrageenan Induced Paw Edema Model:

Anti-inflammatory effect of *Acacia cyanophylla* was tested by Carrageenan Induced Paw Edema Model.**Table3.26** indicates that maximum anti-inflammatory effect was recorded for 200 mg/kg both at 3 and 5 hours which was 27.59 and 43.32% respectively. It was followed by 100 mg/kg which inhibited the inflammation by 18.45% at 3 hours and 28.27% at 5 hours. The anti-inflammatory activity demonstrated by dose of 50 mg/kg was 13.38 and 6.60% at 5 and 3 hours respectively.

Table 3.26. Anti-inflammatory effects of *Acacia cyanophylla* on Carrageenan-Induced Edema Model.

Treatments	**Dose (mg/kg)**	**3 hours**		**5 hours**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	5.91 ± 0.47	-	5.98 ± 0.49	-
Acacia cyanophylla	50	5.52 ± 0.49	6.60	5.18 ± 0.48	13.38
	100	4.82 ± 0.49*	18.45	4.29 ± 0.48*	28.27
	200	4.28 ± 0.46*	27.59	3.39 ± 0.49**	43.32
Indomethacin	10	3.01 ±0.49**	49.07	1.92 ± 0.47**	67.90

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

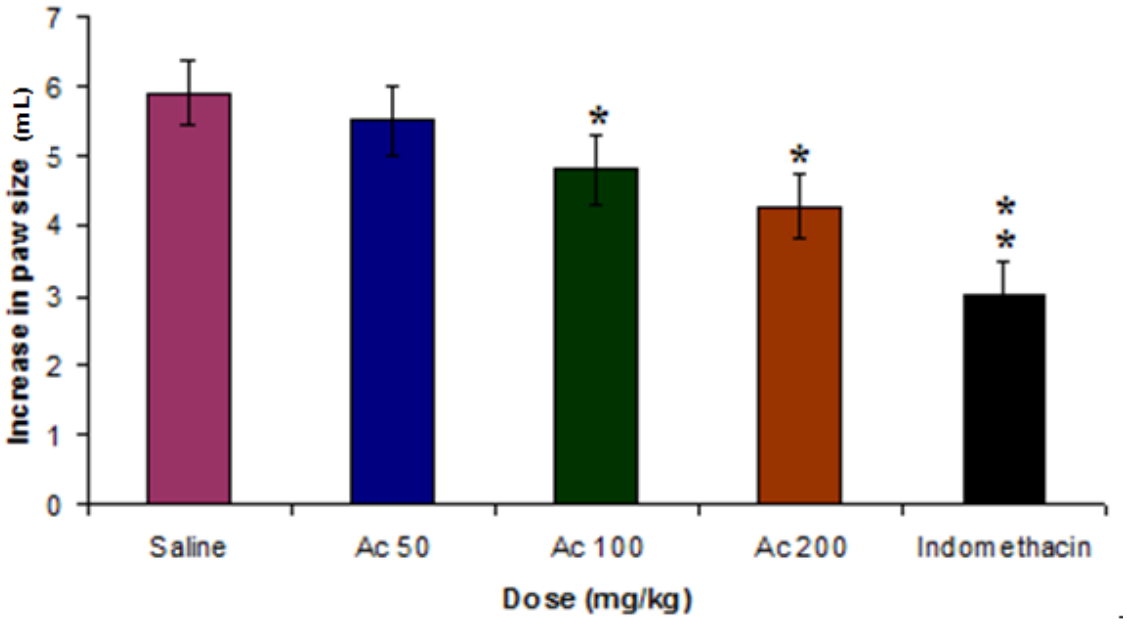

Figure 3.41: Effect of AC (3 hours) on Carrageenan-induced edema model.* $p < 0.05$, ** = $p < 0.01$.

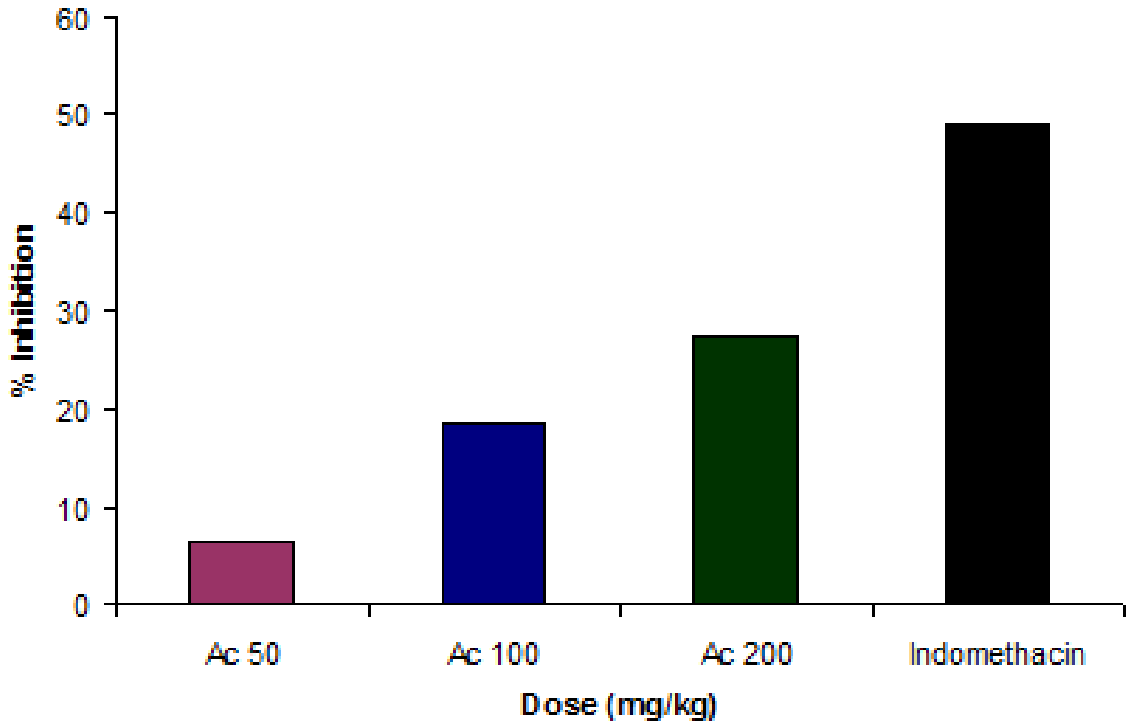

Figure 3.42: Percent inhibition of AC (3 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

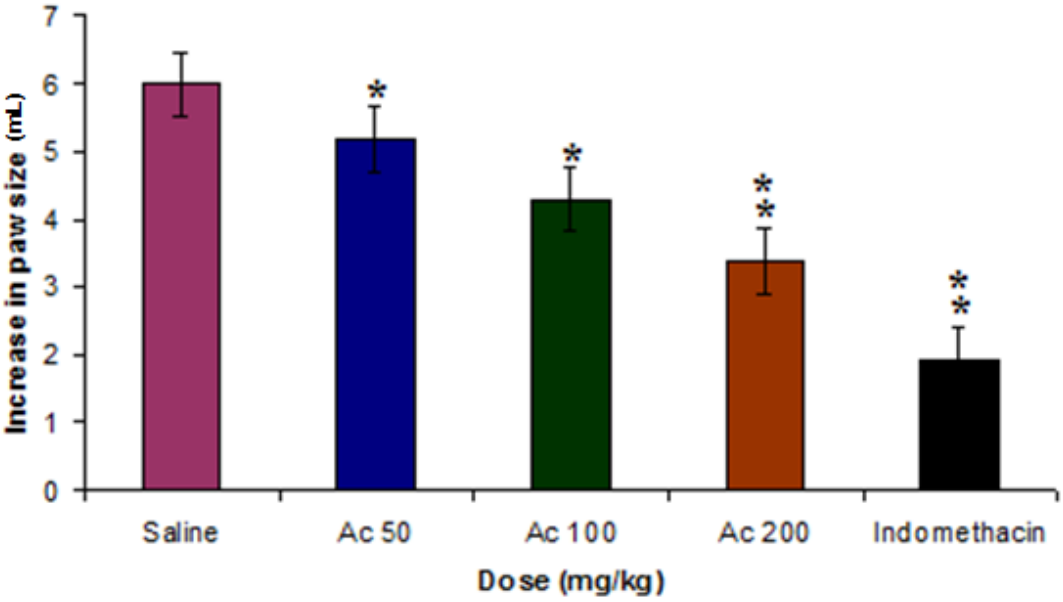

Figure 3.43: Effect of AC (5 hours) on Carrageenan-induced edema model.* $p < 0.05$, ** = $p < 0.01$.

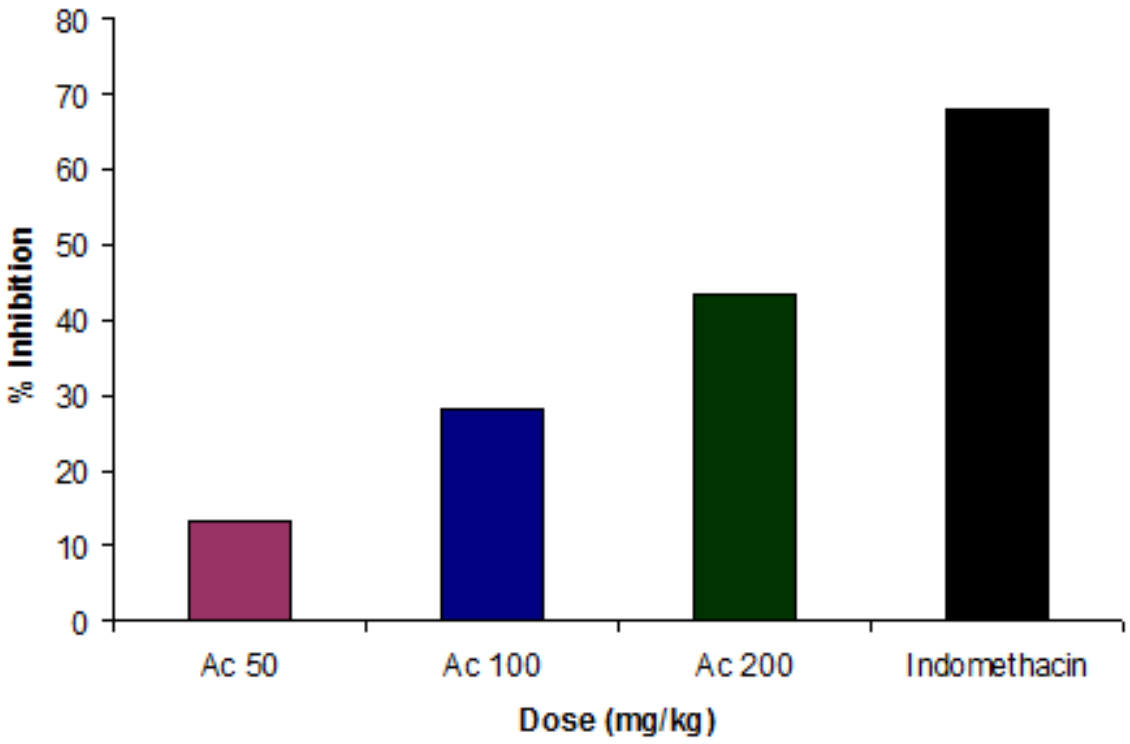

Figure 3.44: Percent inhibition of AC (5 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

3) Effect of *Acacia stenophylla* on Carrageenan Induced Paw Edema Model:

Acacia stenophylla showed significant anti-inflammatory activity at higher doses (**Table3.27).**The plant was analysed at 3 different doses of 200, 100 and 50 mg/kg. Maximum anti-inflammatory effect was demonstrated by 200 mg/kg at both phases (3 and 5 hours). The subjected dose (200 mg/kg) inhibited the inflammation by 46.96 and 28.82% at 5 and 3 hours respectively. At 5 and 3 hours, the dose of 100 mg/kg showed the inhibition by 34.42 and 17.85% respectively. Similarly, 11.41 and 8.74% inhibitory activity was measured for 50 mg/kg after 5 and 3 hours respectively.

Table 3.27. Anti-inflammatory effects of *Acacia stenophylla* on Carrageenan-Induced Edema Model.

Treatments	**Dose (mg/kg)**	**3 hours**		**5 hours**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	5.38 ± 0.50	-	5.26 ± 0.49	-
Acacia stenophylla	50	4.91 ± 0.49*	8.74	4.66 ± 0.51*	11.41
	100	4.42 ± 0.49*	17.85	3.45 ± 0.49**	34.42
	200	3.83 ± 0.49**	28.82	2.79 ± 0.48**	46.96
Indomethacin	10	3.04 ±0.50**	43.50	1.88 ± 0.50**	64.26

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

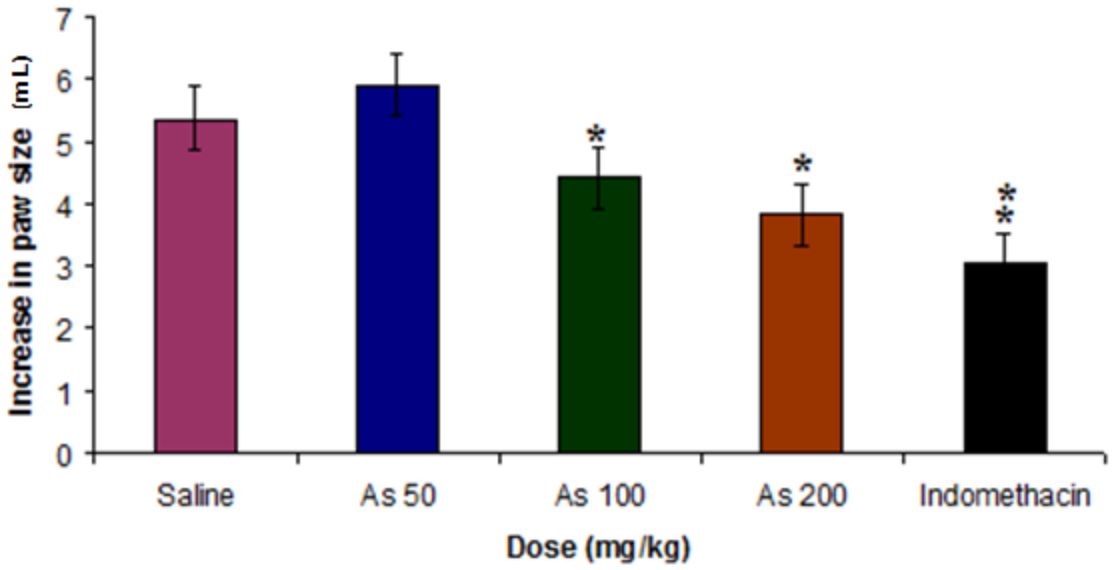

Figure 3.45: Effect of AS (3 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

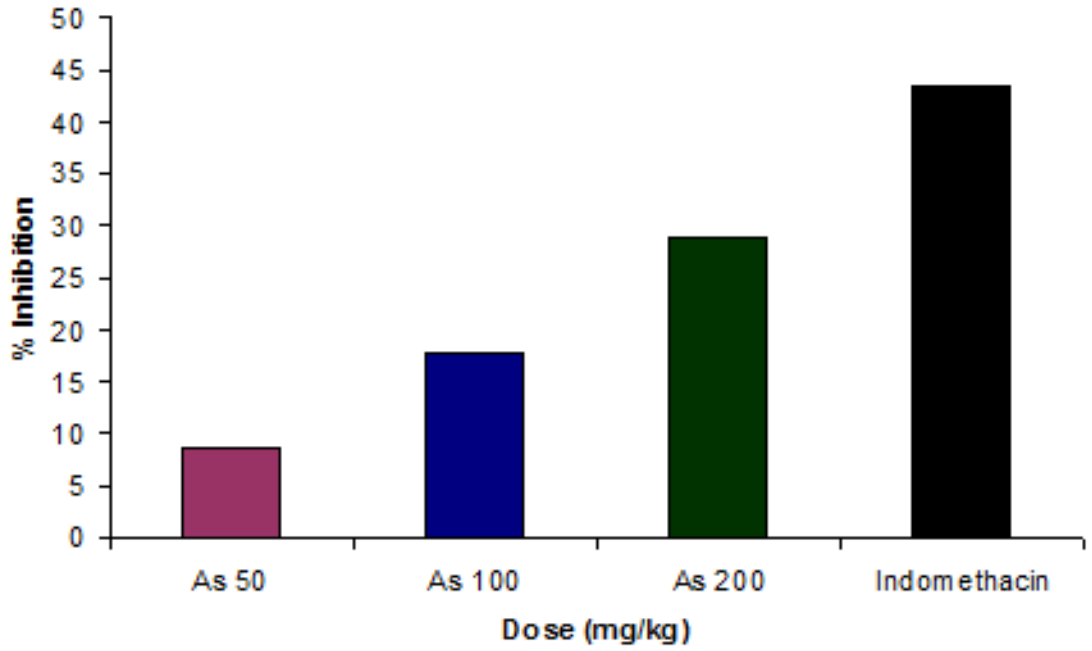

Figure 3.46: Percent inhibition of AS (3 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

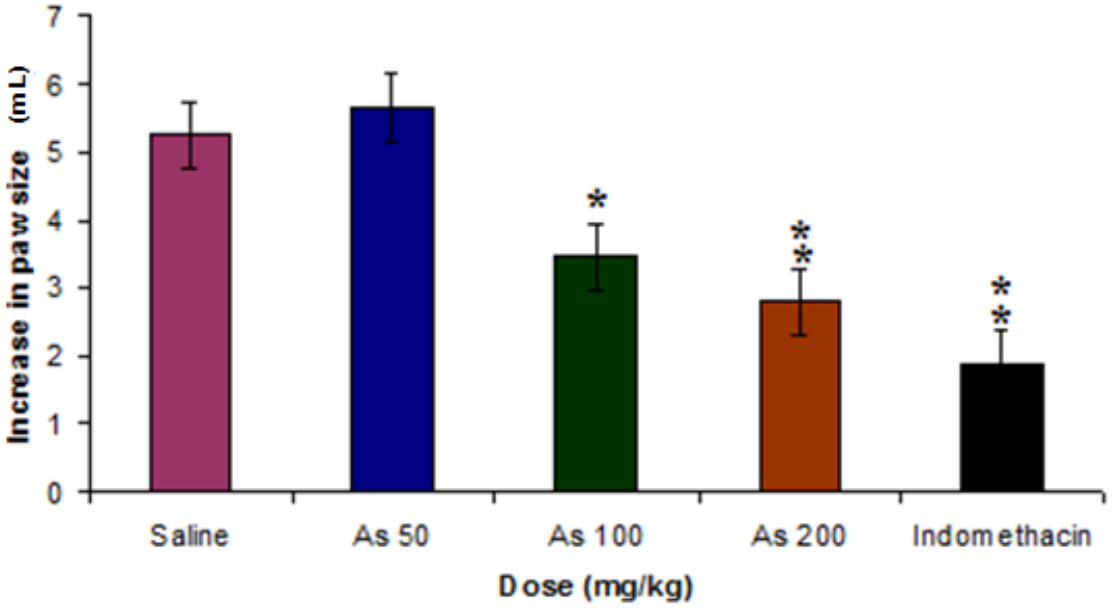

Figure 3.47: Effect of AS (5 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

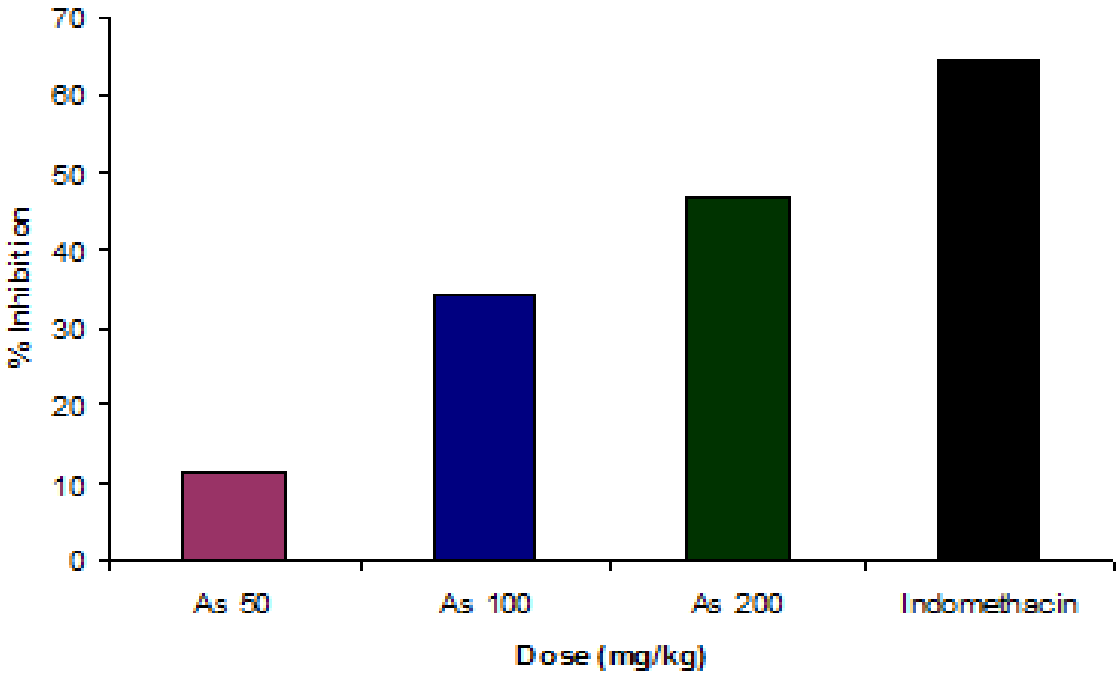

Figure 3.48: Percent inhibition of AS (5 hours) on Carrageenan-Induced Edema Model.* $p < 0.05$, ** = $p < 0.01$.

3.4.1.2. Xylene Induced Ear Edema:

The crude methanolic extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* was subjected at various doses of 50, 100 and 200 mg/kg to evaluate anti-inflammatory potential. The results noted have been given in **Tables 3.28-3.31** and **Figures 3.50-3.65.**

1) Effect of *Tamarix aphylla* on Xylene Induced Ear Edema Model:

Anti-inflammatory activity of *Tamarix aphylla* determined by Xylene Induced Ear Edema Model has been given in **Table 3.28**. Analysis of the data reveals that inhibitory effect was time and dose dependent. With the increasing dose of the plant extract, the inhibitory effect was also increased. The tested plant showed anti-inflammatory activity significantly at all subjected dose but more significantly at higher doeses of 100 and 200 mg/kg. Maximum anti-inflammatory activity of 68.59% was demonstrated by dose of 200 mg/kg after 60 minutes. The effect for the same dose was 62.53%, after 15 minutes. The dose of 100 mg/kg also demonstrated more significant anti-inflammatory effect at 60

minutes which was was 61.17%. It was 53.84% after 15 minutes for the same dose. Similarly, inhibitory activity measured for 50 mg/kg was 53.16 and 39.56% at 60 and 15 minutes respectively.

Table 3.28: Anti-inflammatory effect of *Tamarix aphylla* on Xylene-Induced Ear Edema.

Treatment	**Dose (mg/kg)**	**15 min**		**60 min**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	31.78± 2.91	-	32.21 ± 3.41	-
Tamarix aphylla	50	19.21 ± 3.11*	39.56	15.09 ± 2.140*	53.16
	100	14.67 ± 2.94*	53.84	12.51 ± 3.316**	61.17
	200	11.91 ± 2.47*	62.53	10.12± 3.316**	68.59
Dexamethasone	0.5	9.61±2.77**	69.77	8.12 ± 4.061**	74.80

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

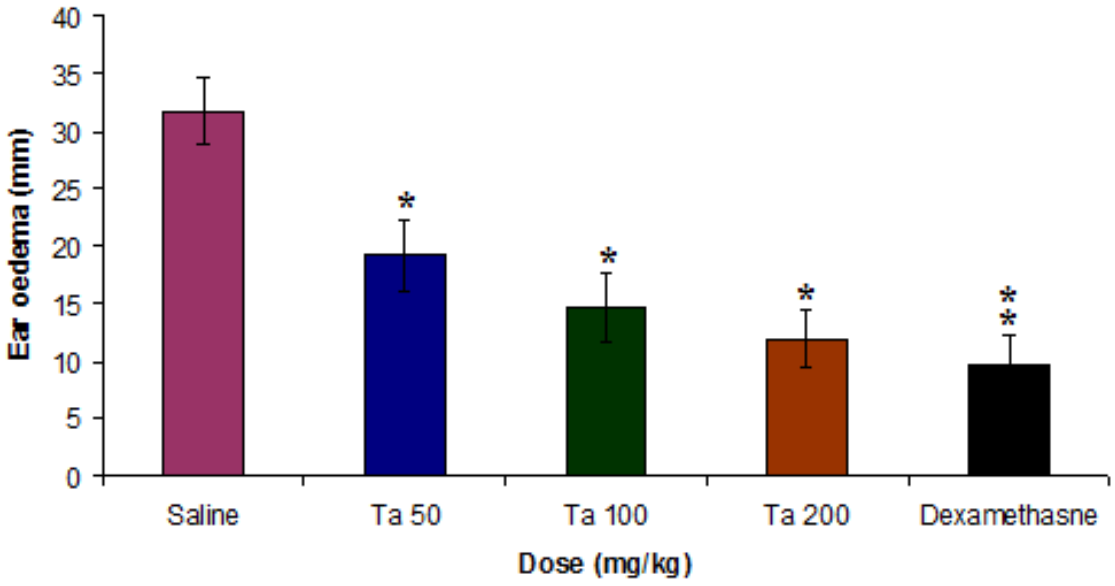

Figure 3.49: Effect of TA (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

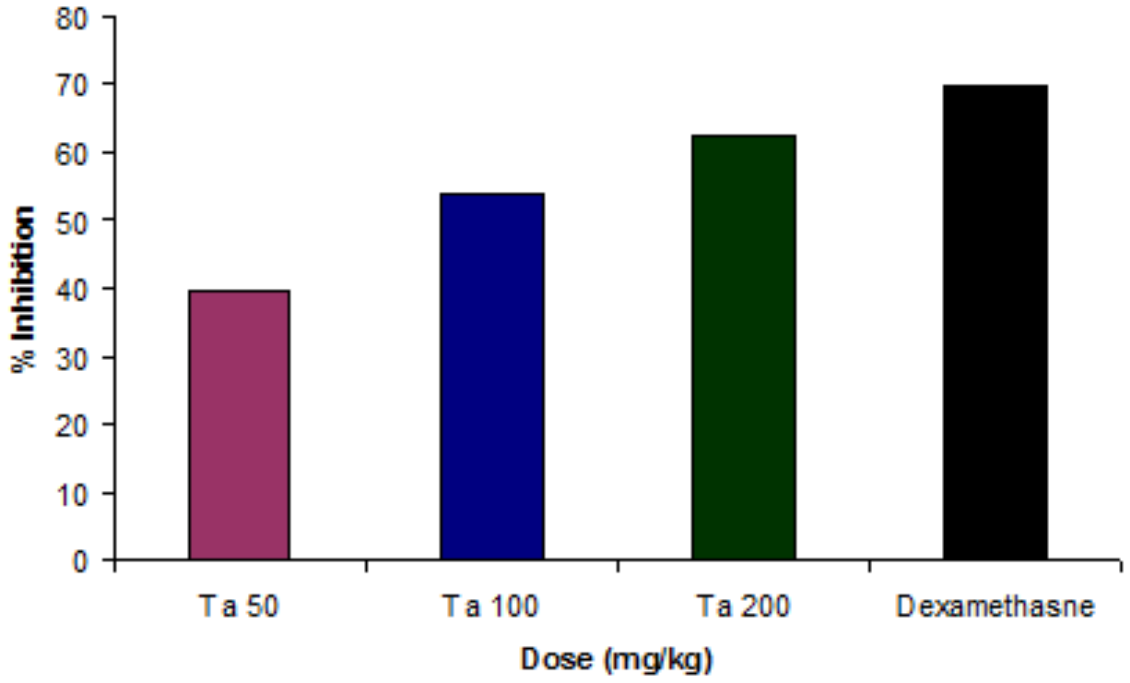

Figure 3.50: Percent inhibition of TA (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

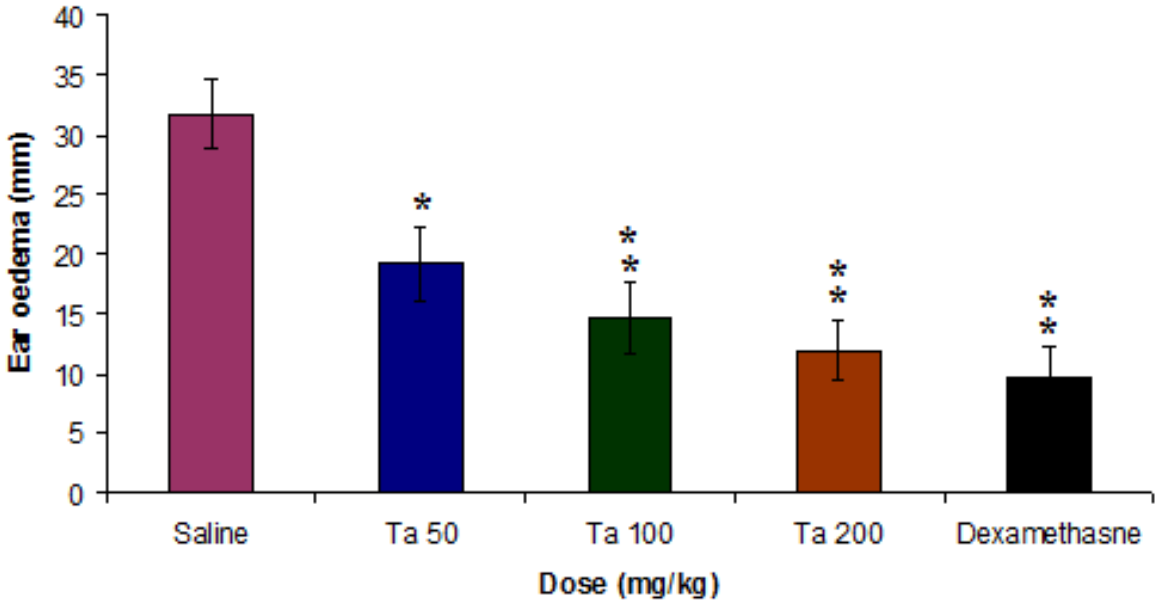

Figure 3.51: Effect of TA (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

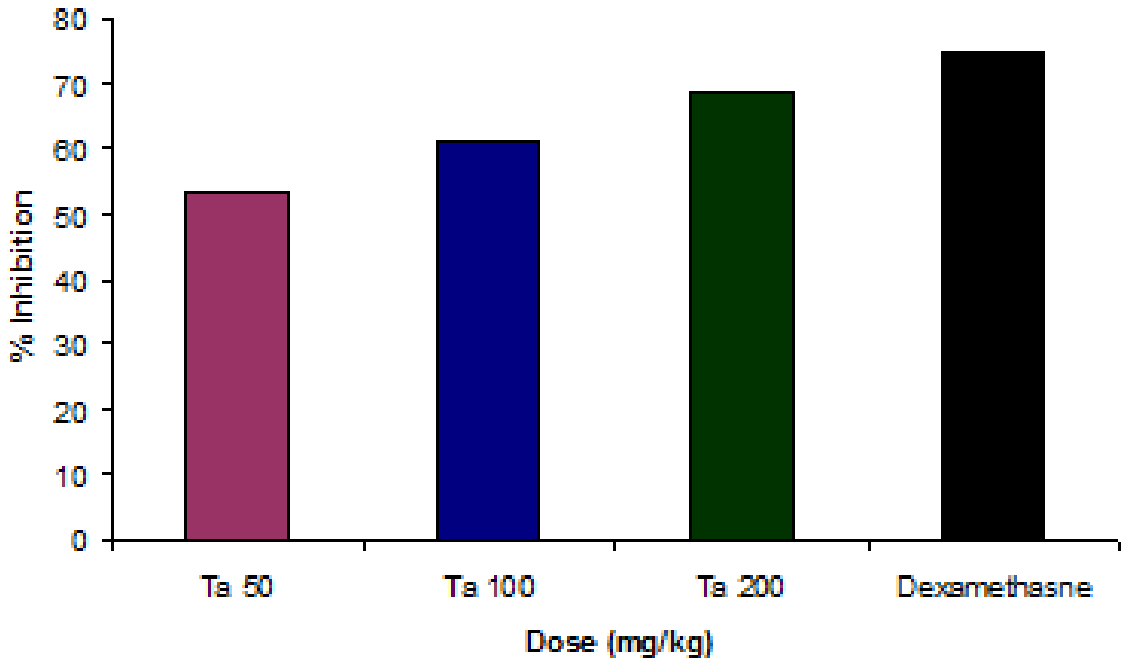

Figure 3.52: Percent inhibition of TA (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

2) Effect of *Tamarix dioica* on Xylene-Induced Ear Edema Model:

Significant inhibitory effect was demonstrated by *Tamarix dioica* at all tested doses (**Table 3.29).** The activity reported was dose and time dependent and was statistically significant and more significant. Maximum anti-inflammatory activity was shown by 200 mg/kg both at 15 and 60 minutes. The inhibitory effect of the said dose was 60.98 and 56.99% at 60 and 15 minutes respectively. Similarly, the inhibitory effect of dose of 100 mg/kg was also more significant at 60 minutes. Its effect at 60 minutes was 53.65 % and at 15 minutes it was 45.29%. The tested plant also demostrated good inhibitory activity at low dose of 50 mg/kg which was 32.29 and 45.92% at 15 and 60 minutes respectively which were also statistically significant.

Table 3.29: Anti-inflammatory effect of *Tamarix dioica* on Xylene-Induced Ear Edema.

Treatment	**Dose (mg/kg)**	**15 min**		**60 min**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	31.78± 2.91	-	32.21 ± 3.41	-
Tamarix dioica	50	21.52 ± 2.92*	32.29	17.42 ± 2.28*	45.92
	100	17.39 ± 2.43*	45.29	14.93 ± 2.97**	53.65
	200	13.67 ± 2.71*	56.99	12.57± 2.49**	60.98
Dexamethasone	0.5	9.61±2.77**	69.77	8.12 ± 4.061**	74.80

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

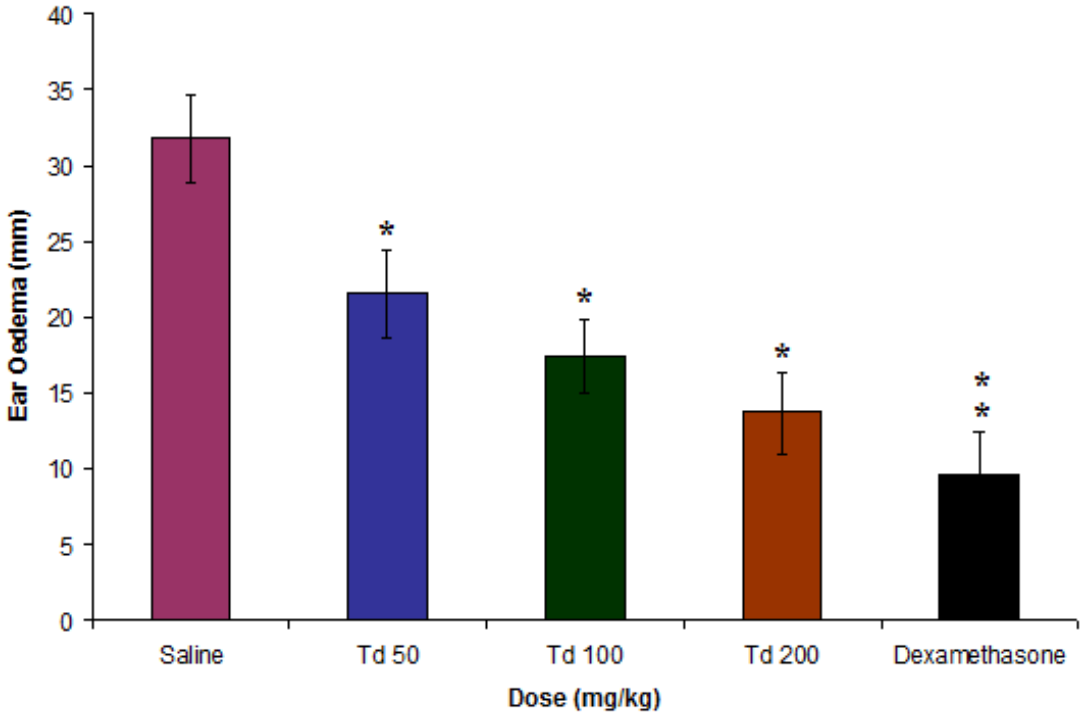

Figure 3.53: Effect of TD (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

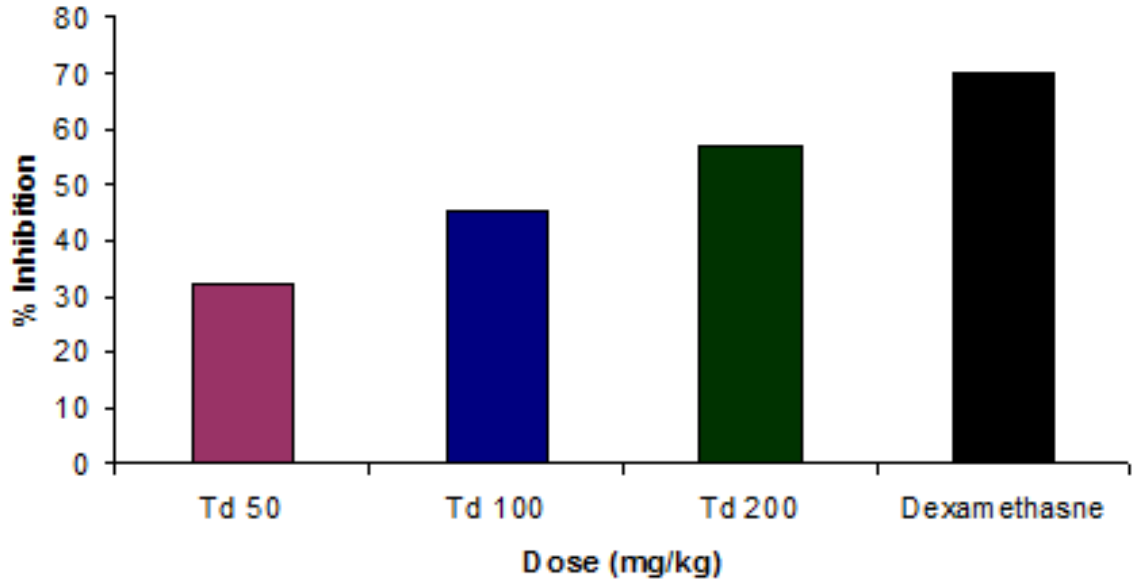

Figure 3.54: Percent inhibition of TD (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

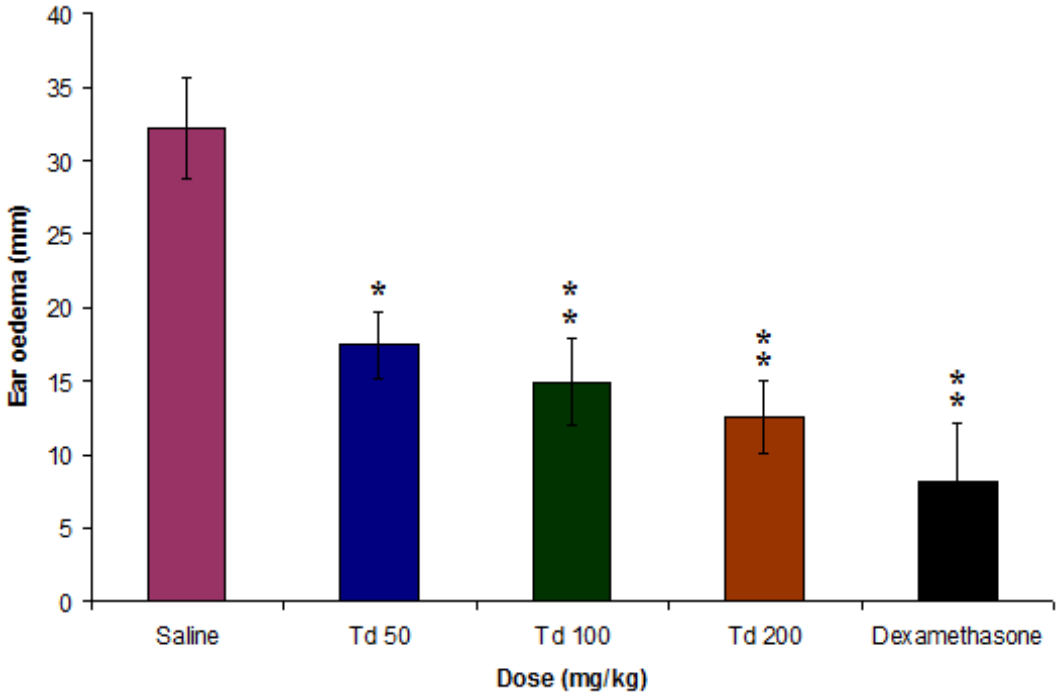

Figure 3.55: Effect of TD (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

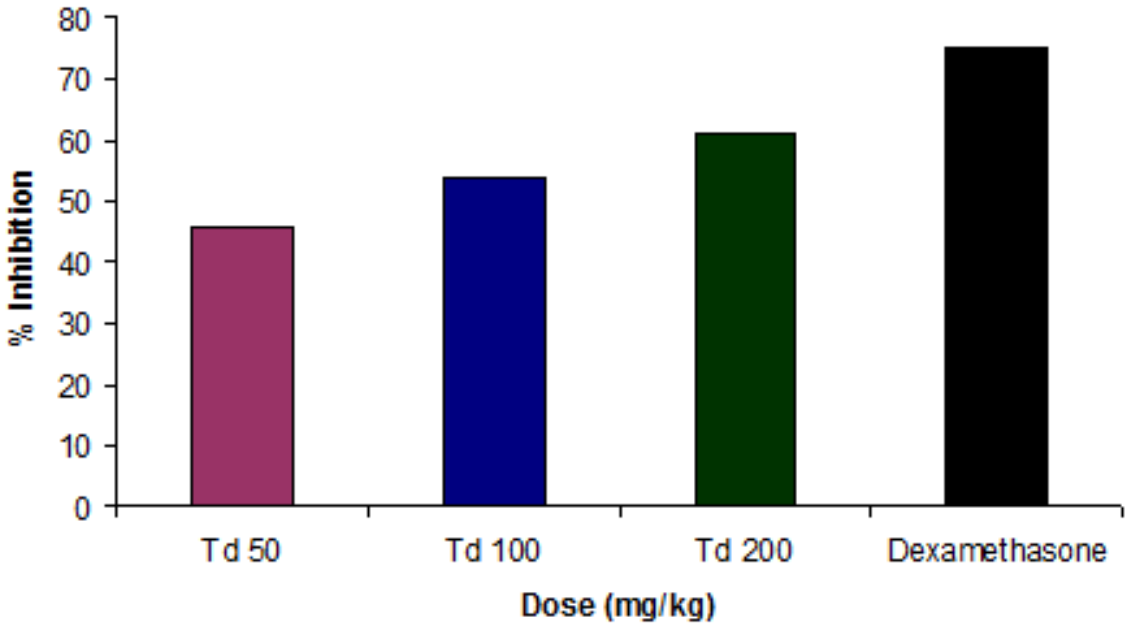

Figure 3.56: Percent inhibition of TD (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

3) Effect of *Acacia cyanophylla* on Xylene-Induced Ear Edema Model:

Acacia cyanophylla showed tremendous anti-inflammatory effects. The inhibitory effect of the tested plant was significant at all doses as shown in **Table3.30.** Maximum activity was demonstrated by 200 mg/kg followed by 100 mg/kg. The activity measured for 200 mg/kg was 68.80% after 1 hour (more significant) and 65.68 % at 15 minutes. The inhibitory effect shown by 100 mg/kg was statistically significant (57.34%) and more significant (61.17%) at 15 and 60 minutes respectively. The low dose (50 mg/kg) reported the activity of 44.84% at 15 minutes and 49.96% at 1 hour.

Table 3.30. Anti-inflammatory effect of *Acacia cyanophylla* on Xylene-Induced Ear Edema.

Treatments	**Dose (mg/kg)**	**15 min**		**60 min**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	31.78± 2.91	-	32.21 ± 3.41	-
Acacia cyanophylla	50	17.53 ± 2.94*	44.84	16.12 ± 2.66*	49.96
	100	13.56 ± 2.36*	57.34	12.51 ± 2.24**	61.17
	200	10.91 ± 2.19*	65.68	10.05± 2.57**	68.80
Dexamethasone	0.5	9.61±2.77**	69.77	8.12 ± 4.061**	74.80

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

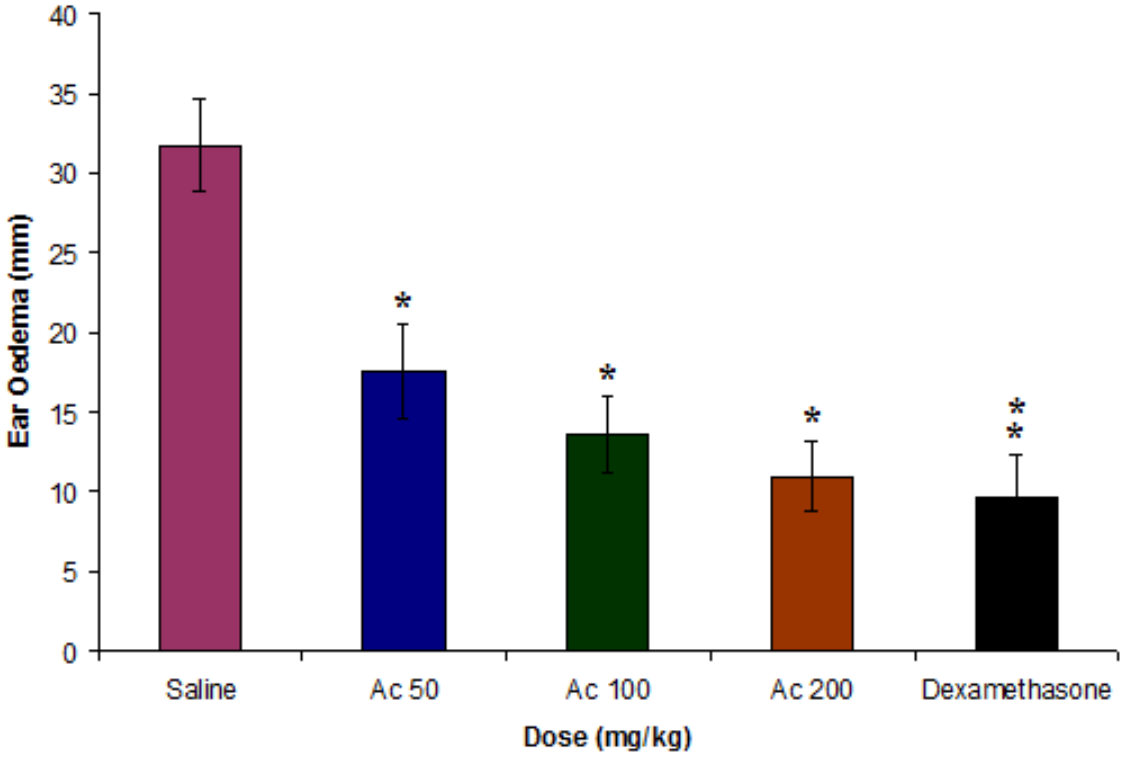

Figure 3.57: Effect of AC (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

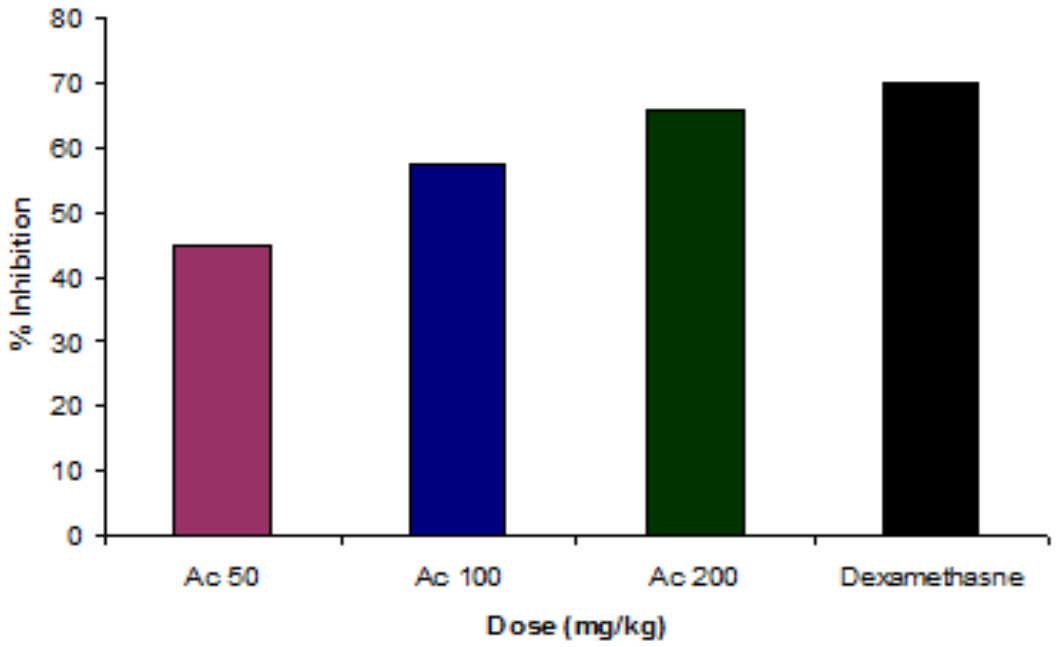

Figure 3.58: Percent inhibition of AC (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

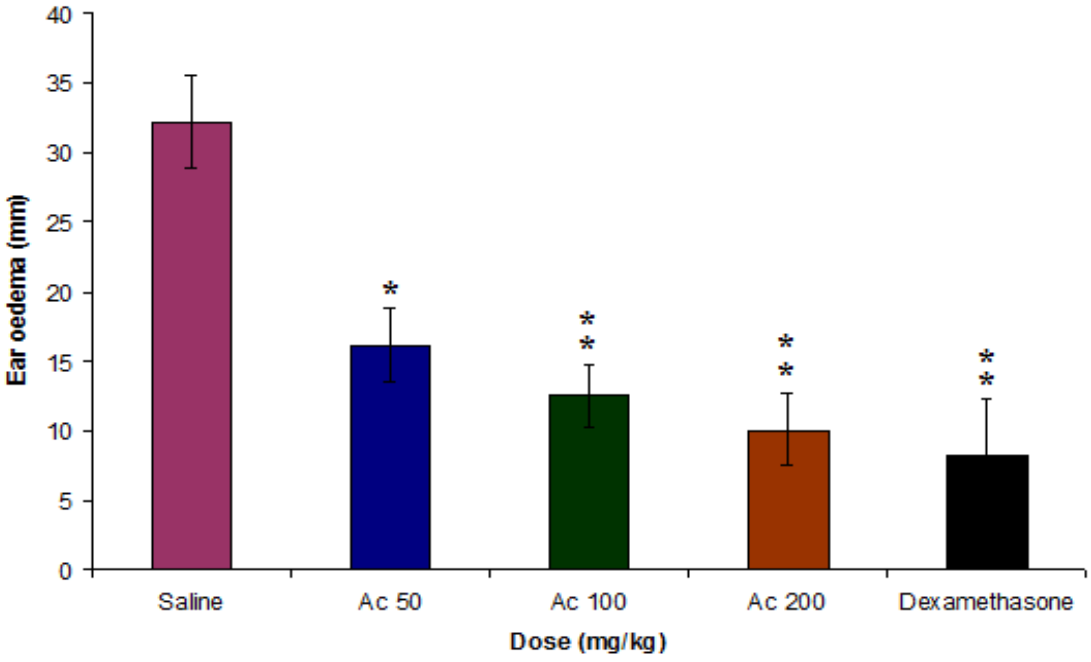

Figure 3.59: Effect of AC (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

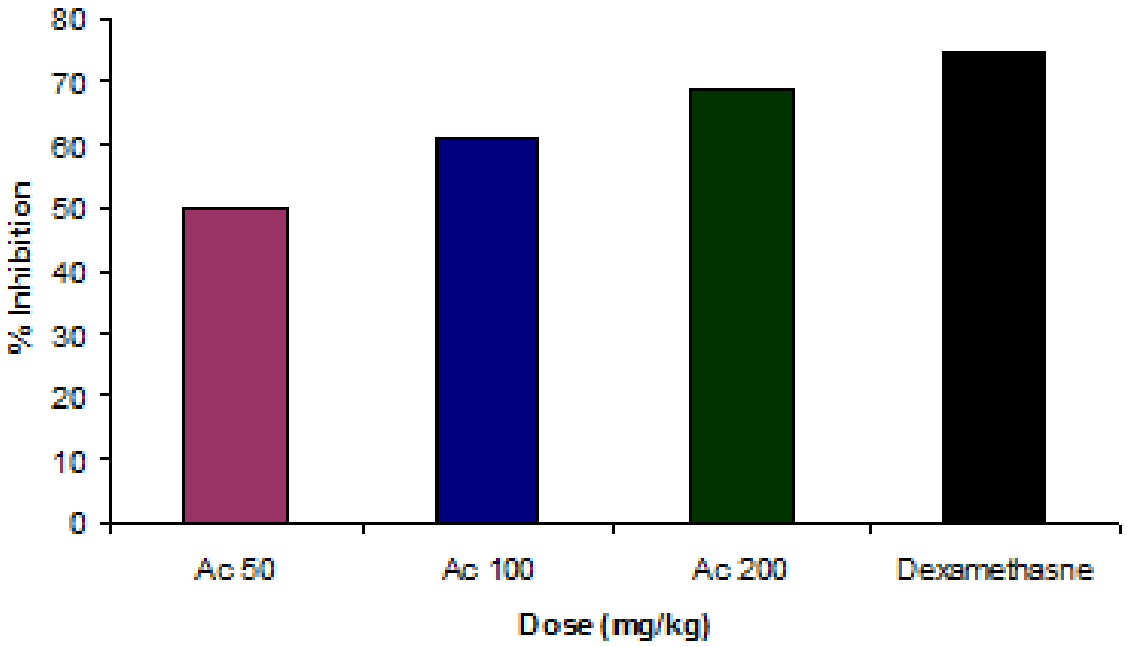

Figure 3.60: Percent inhibition of AC (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

4) Effect of *Acacia stenophylla* on Xylene-Induced Ear Edema Model:

Table 3.31 indicates anti-inflammatory activity of *Acacia stenophylla*. The plant showed statistically significant activity. It was dose and time dependent. Higher doses of 100 and 200 mg/kg revealed statistically more significant effect at 60 minutes. Maximum activity of 63.28 and 59.85% was demonstrated by 200 mg/kg at 60 and 15 minutes respectively. It was followed by 100 mg/kg which inhibited the inflammation by 59.78% at 60 minutes and 48.46% after 15 minutes. Similarly, at the dose of 50 mg/kg, the effect produced was 37.98 and 42.97% after 15 and 60 minutes respectively.

Table 3.31: Anti-inflammatory effect of *Acacia stenophylla* on Xylene-Induced Ear Edema.

Treatments	**Dose (mg/kg)**	**15 min**		**60 min**	
		Difference	**% Inhibition**	**Difference**	**% Inhibition**
Saline	-	31.78± 2.91	-	32.21 ± 3.41	-
Acacia stenophylla	50	19.71 ± 2.83*	37.98	18.37 ± 1.88*	42.97
	100	16.38 ± 1.97*	48.46	14.89 ± 2.16**	59.78
	200	12.76 ± 2.96*	59.85	11.83± 1.87**	63.28
Dexamethasone	0.5	9.61±2.77**	69.77	8.12 ± 4.061**	74.80

Values are reported as standard error mean ± (SEM.), n=5, *p<0.05, **p<0.01, compared to saline group. Data analysed by t-test.

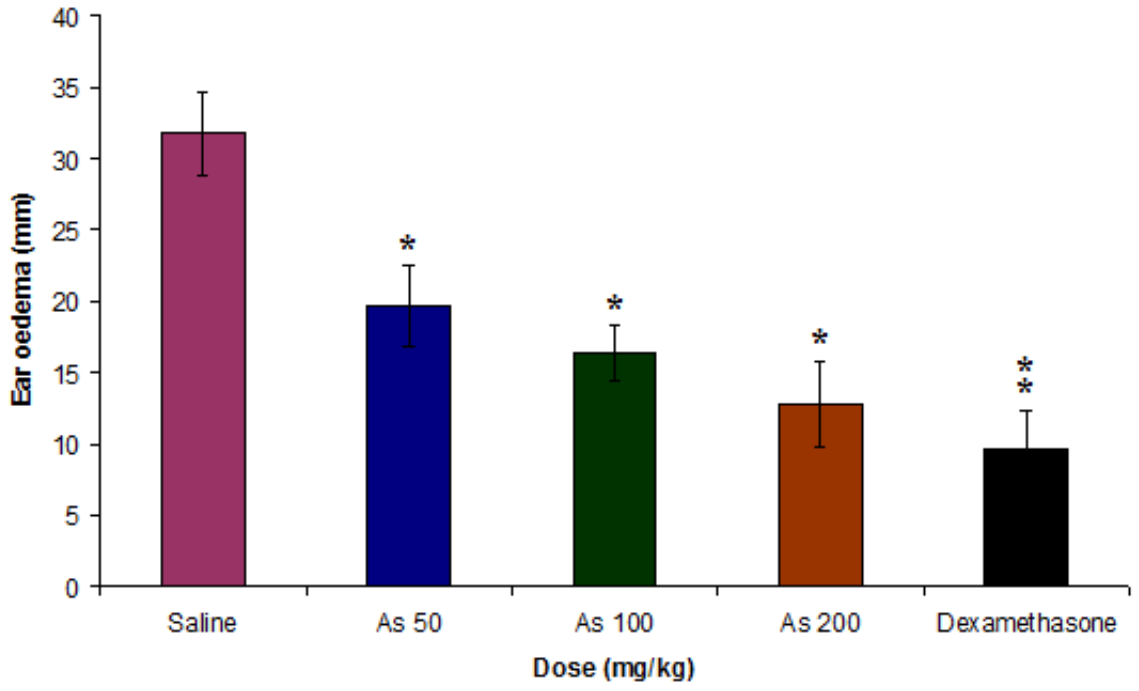

Figure 3.61: Effect of AS (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

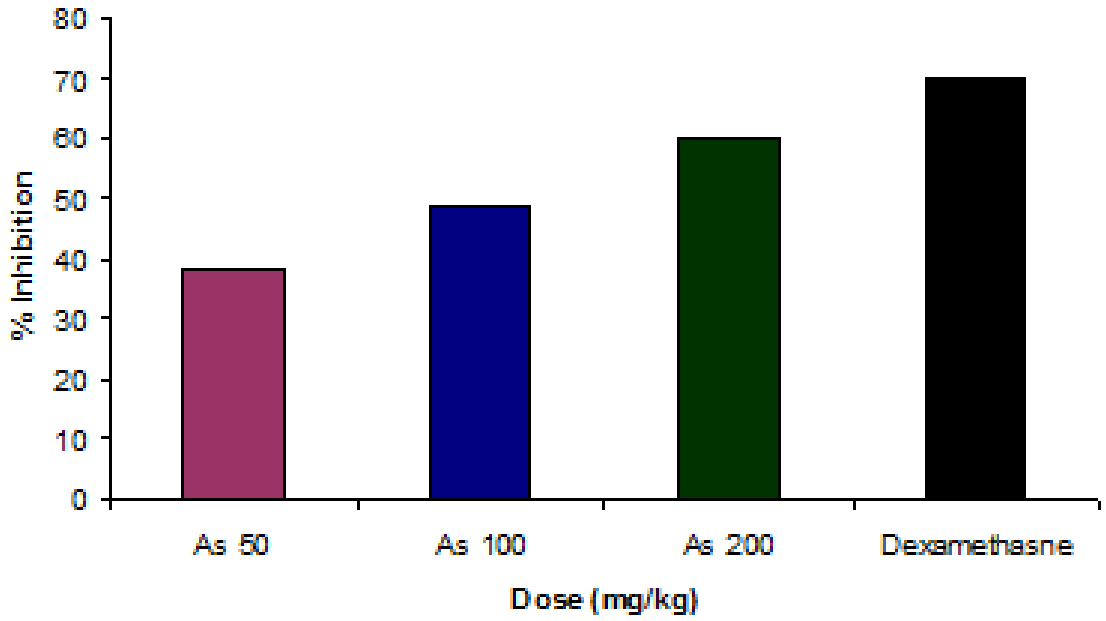

Figure 3.62: Percent inhibition of AS (15 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

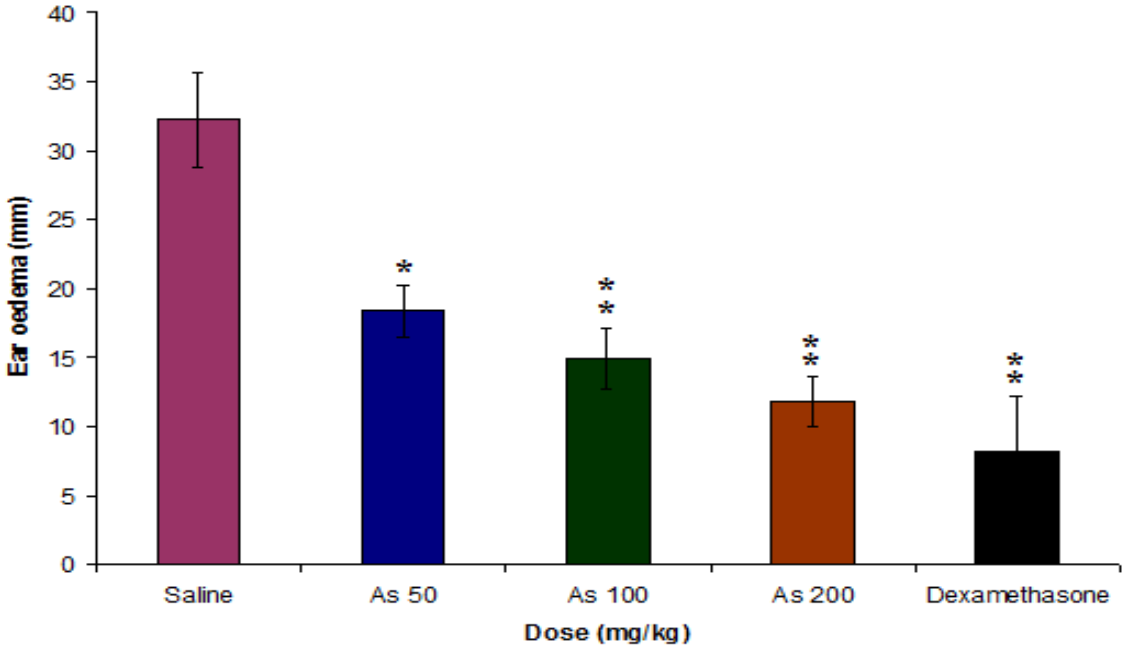

Figure 3.63: Effect of AS (60 minutes) on Xylene-Induced Ear Edema Model.* $p < 0.05$, ** = $p < 0.01$.

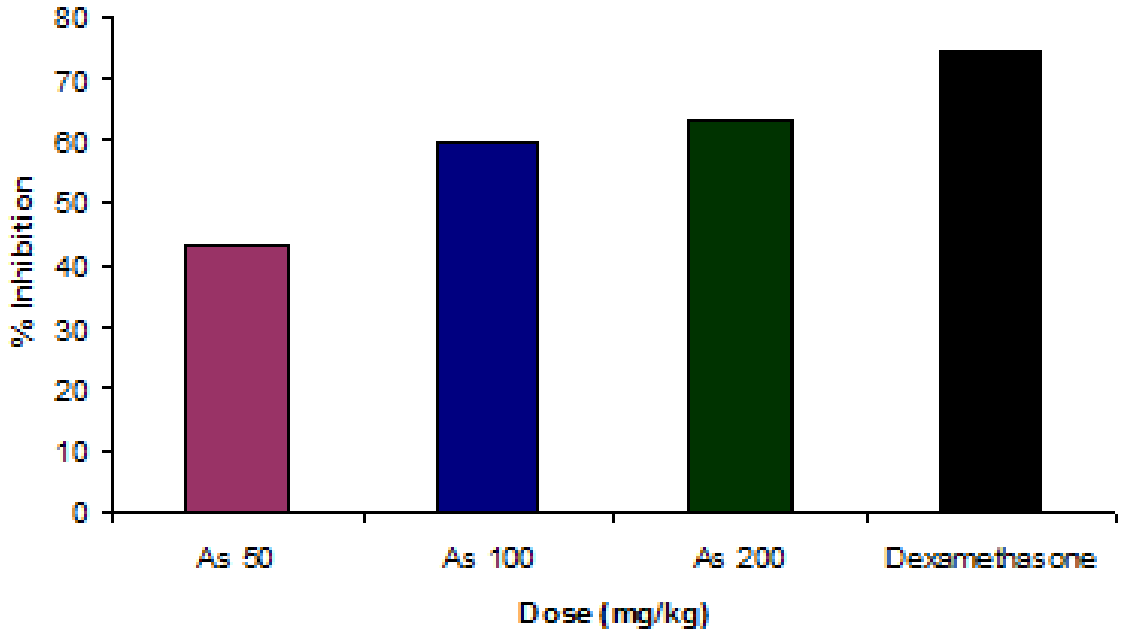

Figure 3.64: Percent inhibition of AS (60 minutes) on Xylene-Induced Ear Edema model.* $p < 0.05$, ** = $p < 0.01$.

3.4.2. Antipyretic Activity:

1) Antipyretic activity of *Tamarix aphylla*:

The antipyretic effect produced by crude methanolic extract has beenshown in **Table 3.32.** The tested plant showed significant antipyretic effect at all doses during whole experiment (1 to 5 hrs) except the dose of 100 mg which did not show any significant result at 1 hr. The % antipyretic potential of all the subjected doses in the first hour was 0.16, 0.52 and 1.14% at 100, 200 and 300 mg/kg respectively. The reduction (percent) in temperature in 2nd hr of the treatment,for 100, 200 and 300mg/kg was 0.47, 1.38 and 1.82% respectively. The percent inhibition measured by all the applied doses at 3rd hour but more significantly at higher dose of 200 & 300 mg/kg which was 2.08 and 2.72% respectively. Antipyretic activity of 1.55, 2.25 and 2.64% was demonstrated by 100, 200 and 300 mg/kg doses respectively in 4th hr of the treatment. At 5th hr, the inhibition was 1.76, 2.40 and 3.07% respectively for 100, 200 and 300 mg/kg. The antipyretic potential (percent) has been shown in **Figure 3.66-70**.

Table 3.32: Antipyretic activity of crude methanolic extract of *Tamarix aphylla.*

Treatments	Dose mg/kg	Rectal temperature (°C)						
		Normal	after 24h	After administration of drug				
				1h	2h	3h	4h	5h
Saline	10 mL	36.69 ± 0.52	39.71 ± 0.26	38.67 ± 0.31	38.62 ± 044	38.61 ± 0.21	38. 71 ± 0.33	38.77 ± 0.33
Paracetamol	150 mg	37.05 ± 0.32	39.42 ± 0.32	38.18** ± 0.26	37.78** ± 0.36	37.33** ± 0.39	37.41** ± 0.44	37.47** ± 0.47
Extract	100	37.07 ± 0.38	39.53 ± 0.38	38.61 ± 0.24	38.44* ± 0.41	38.07* ± 0.71	38.11* ± 0.49	38.09* ± 0.37
	200	37.21 ± 0.21	39.44 ± 0.33	38.47* ± 0.64	38.09* ± 0.43	37.81** ± 0.49	37.84** ± 0.36	37.84** ± 0.47
	300	37.04 ± 0.03	39.59 ± 0.27	38.23* ± 0.56	37.92** ± 0.47	37.56** ± 0.54	37.69** ± 0.51	37.58** ± 0.38

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. *$P < 0.05$, **$P < 0.01$ in comparison to control.

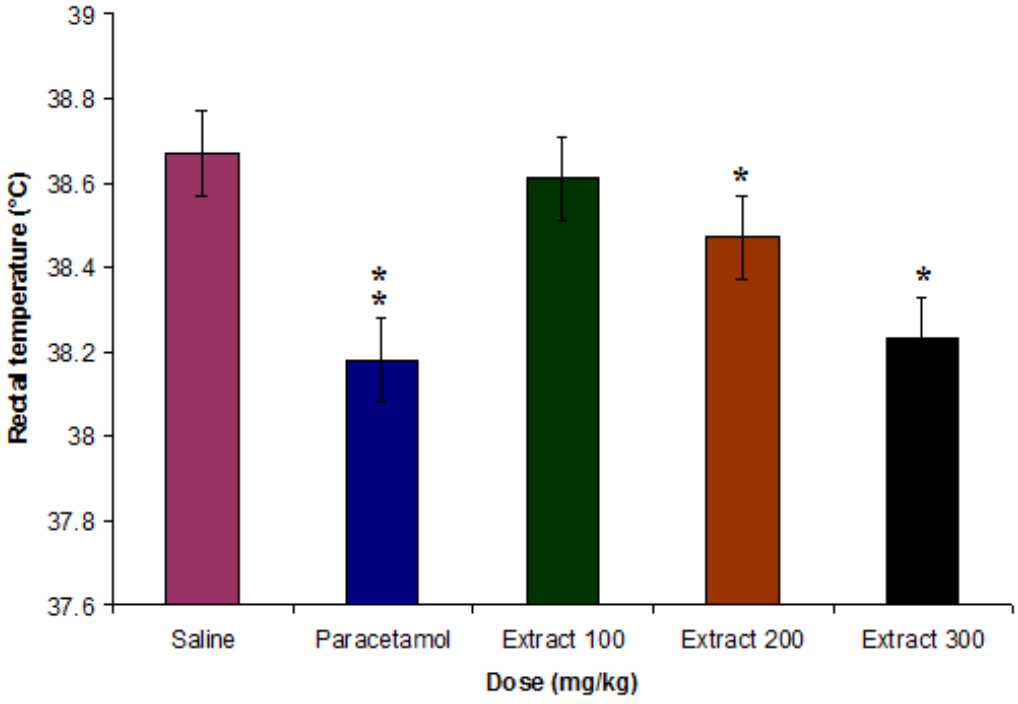

Figure 3.65: Effect of TA (1 hour) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

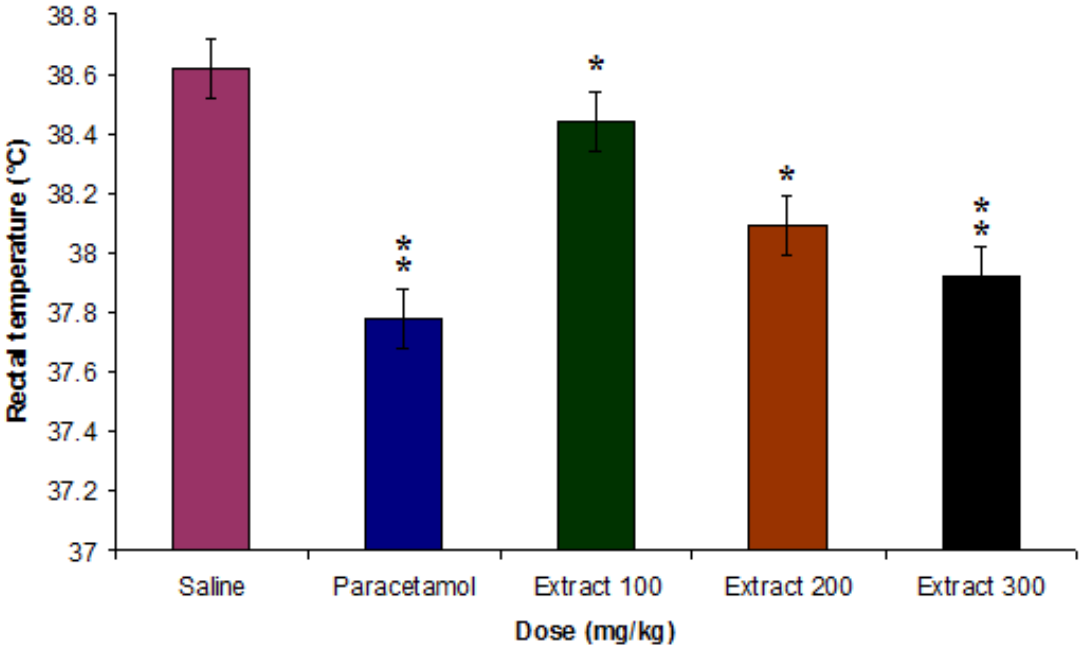

Figure 3.66: Effect of TA (2 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

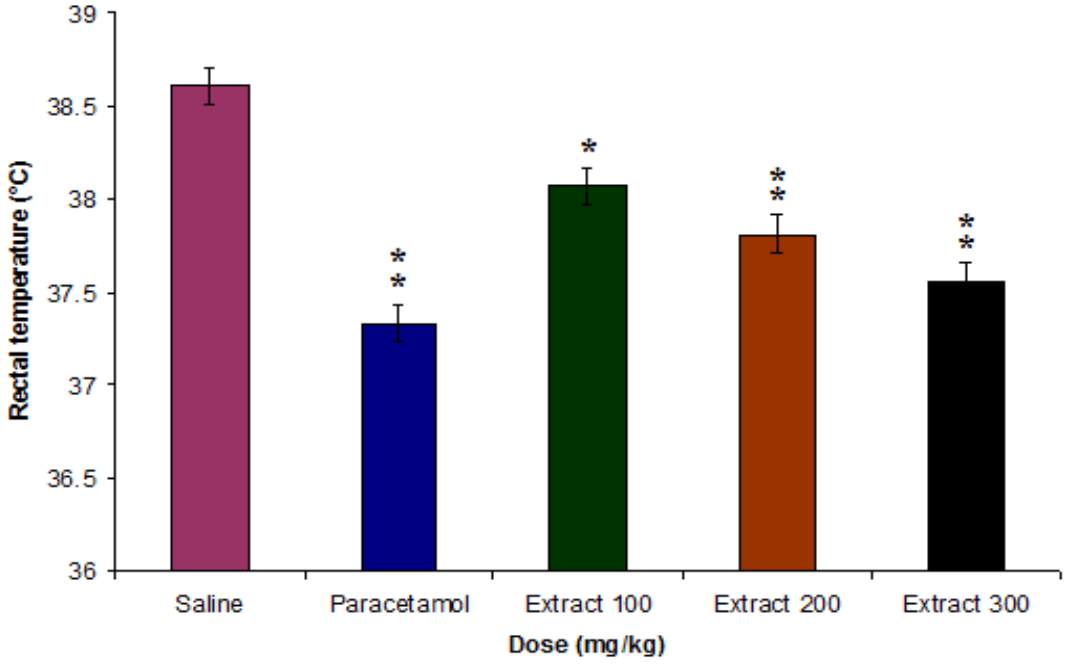

Figure 3.67: Effect of TA (3 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

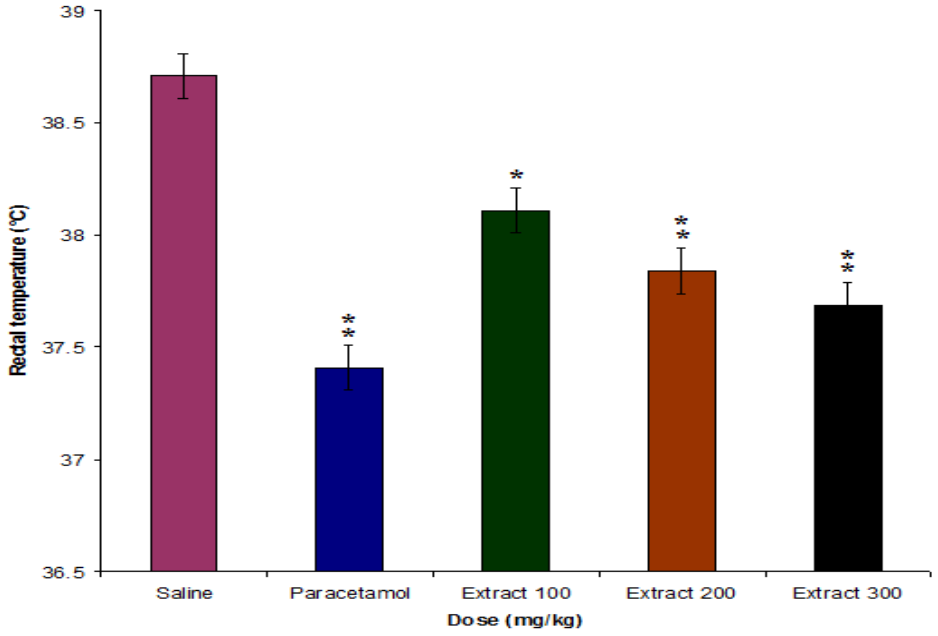

Figure 3.68: Effect of TA (4 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

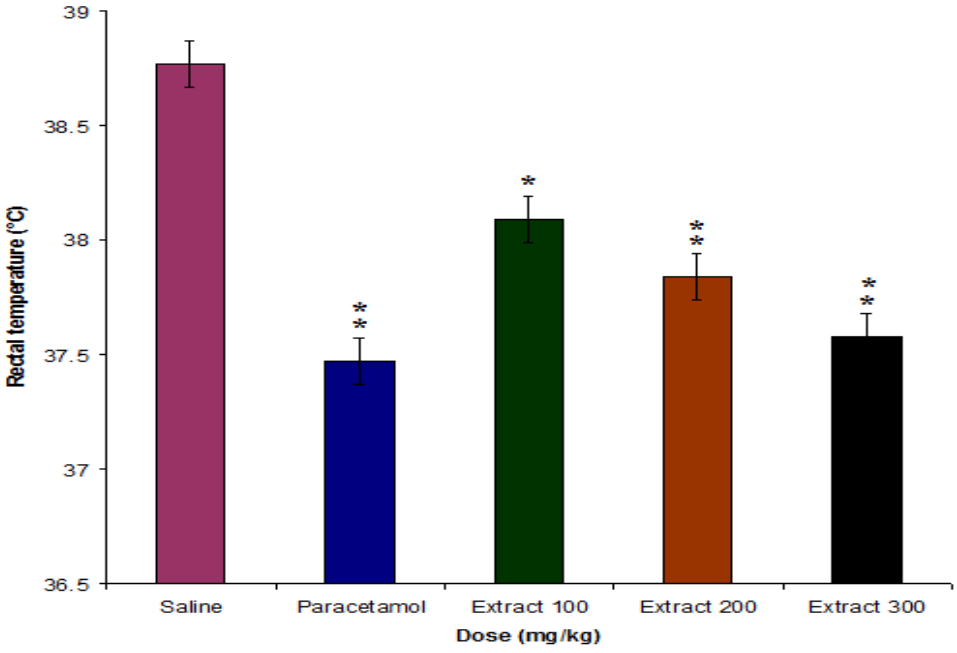

Figure 3.69: Effect of TA (5 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

2) Antipyretic activity of *Tamarix dioica*:

Significant and more significant results have been shown by *Tamarix dioica* at tested doses.**Table 3.33** indicates the percent antipyretic activity of the tested plant.In the 1st hour of the treatment, the percent antipyretic effect produced was was 0.88, 0.37 and 1.14% at 100, 200 and 300 mg/kg respectively. In 2nd hr,100, 200 and 300 mg/kg reduced the body temperature by 0.29, 1.20 and 1.64% respectively. At 2nd hour, the antipyretic effect measured was statistically significant (*P<0.05) for all test doses but more significant for the dose of 300 mg/kg (**P<0.01). Percent inhibition demonstrated by all test doses at 3rd hr, but more significantly at the doses of 200 and 300 mg/kg which was 1.87 and 2.44% respectively (**P<0.01). 1.43, 2.07 and 2.41% antipyretic activity was recorded for 100, 200 and 300 mg/kg doses respectively at 4th hr. Reduction in temperature at 5th hr was 1.42, 2.07 and 2.43% respectively for test doses.

Table 3.33: Antipyretic activity of crude methanolic extract of *Tamarix dioica.*

Treatments	Dose mg/kg	Rectal temperature (°C)						
		Normal	after 24h	After administration of drug				
				1h	2h	3h	4h	5h
Saline	10 mL	36.69 ± 0.52	39.71 ± 0.26	38.67 ± 0.31	38.62 ± 0 44	38.61 ± 0 .21	38. 71 ± 0.33	38.77 ± 0 .33
Paracetamol	150 mg	37.05 ± 0.32	39.42 ± 0.32	38.18** ± 0.26	37.78** ± 0.36	37.33** ± 0.39	37.41** ± 0.44	37.47** ± 0.47
Extract	100	37.11 ± 0.27	39.56 ± 0.35	38.72 ± 0 .44	38.51* ± 0.41	38.13* ± 0.54	38.16* ± 0.57	38.22* ± 0.46
	200	37.21 ± 0.21	39.44 ± 0.33	38.53* ± 0.51	38.16* ± 0.56	37.89** ± 0.38	37.91** ± 0.34	37.97** ± 0.52
	300	37.04 ± 0.03	39.59 ± 0.27	38.23* ± 0.56	37.99** ± 0.39	37.67** ± 0.43	37.78** ± 0.62	37.83** ± 0.49

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. **P* < 0.05, ***P* < 0.01 in comparison to control

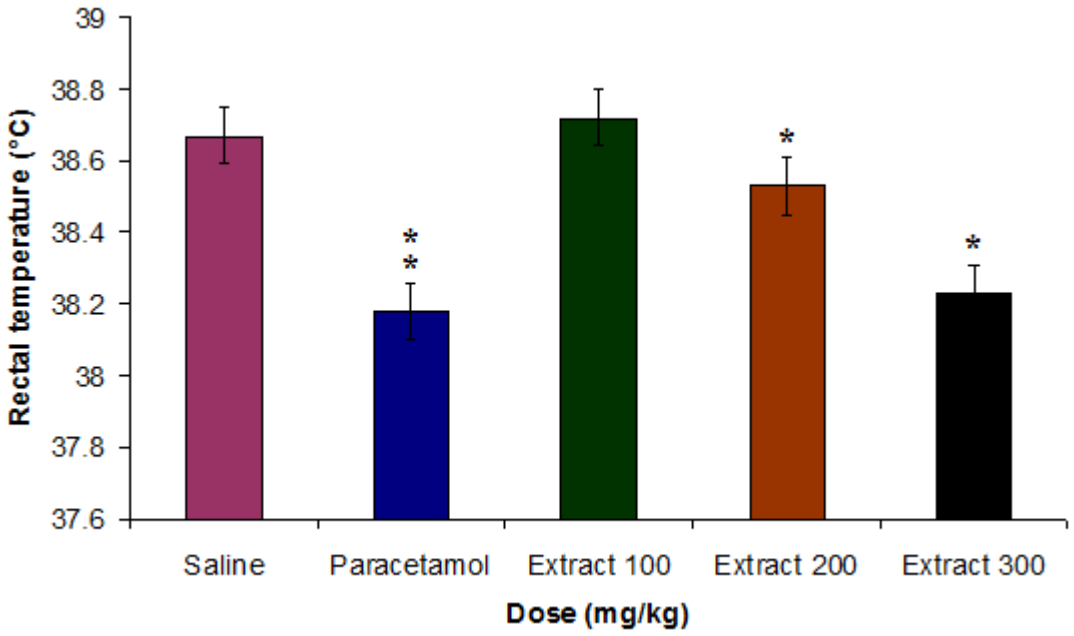

Figure 3.70: Effect of TD (1 hour) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

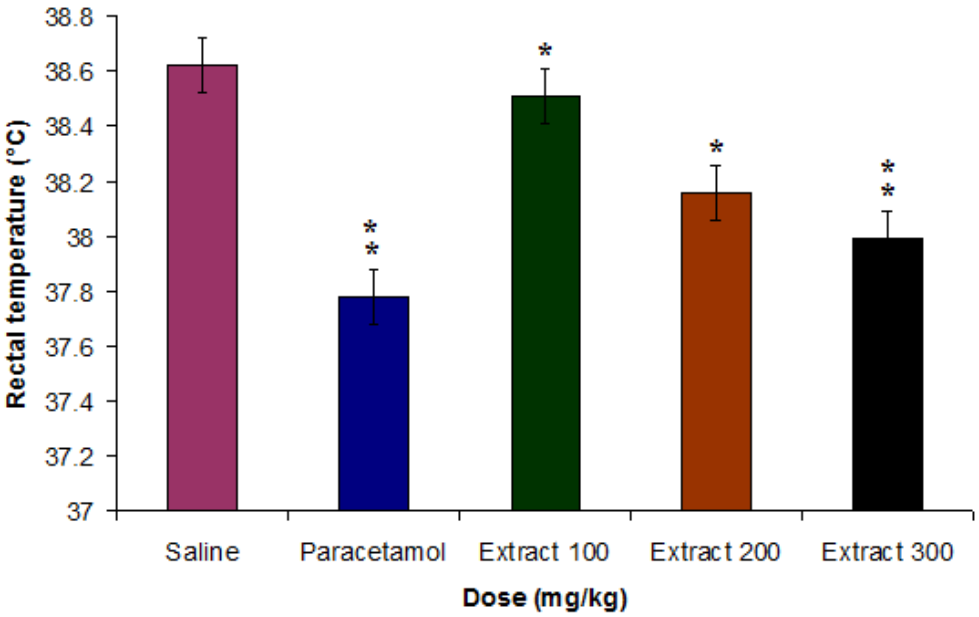

Figure 3.71: Effect of TD (2 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

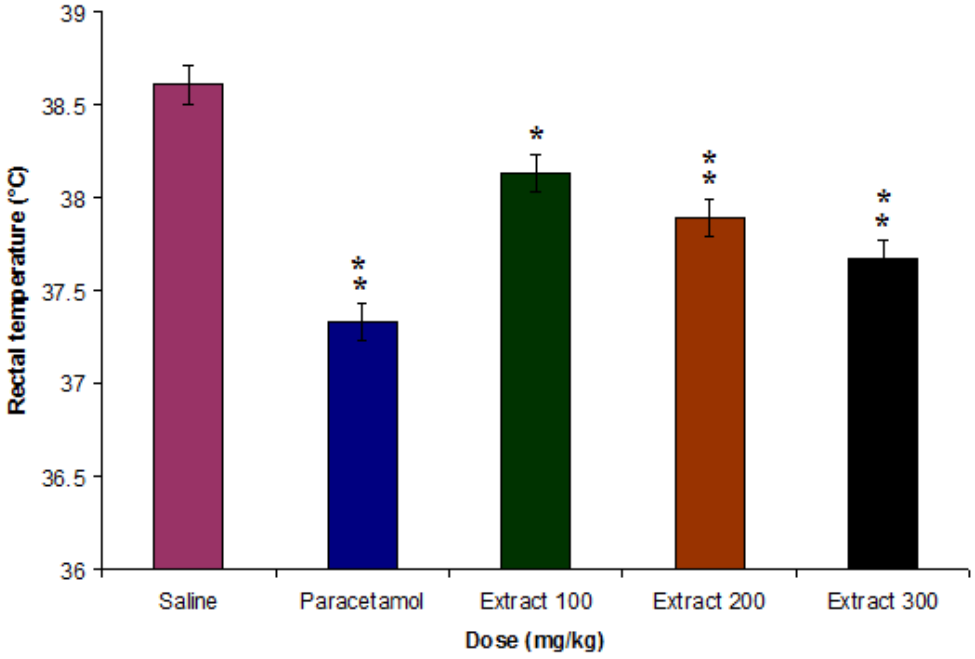

Figure 3.72: Effect of TD (3 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

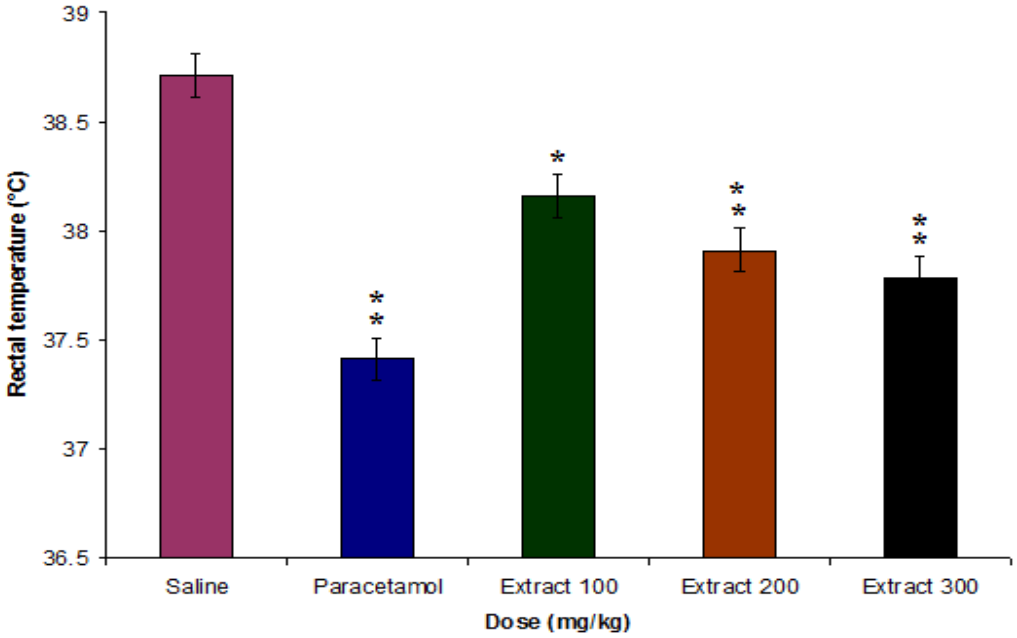

Figure 3.73: Effect of TD (4 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

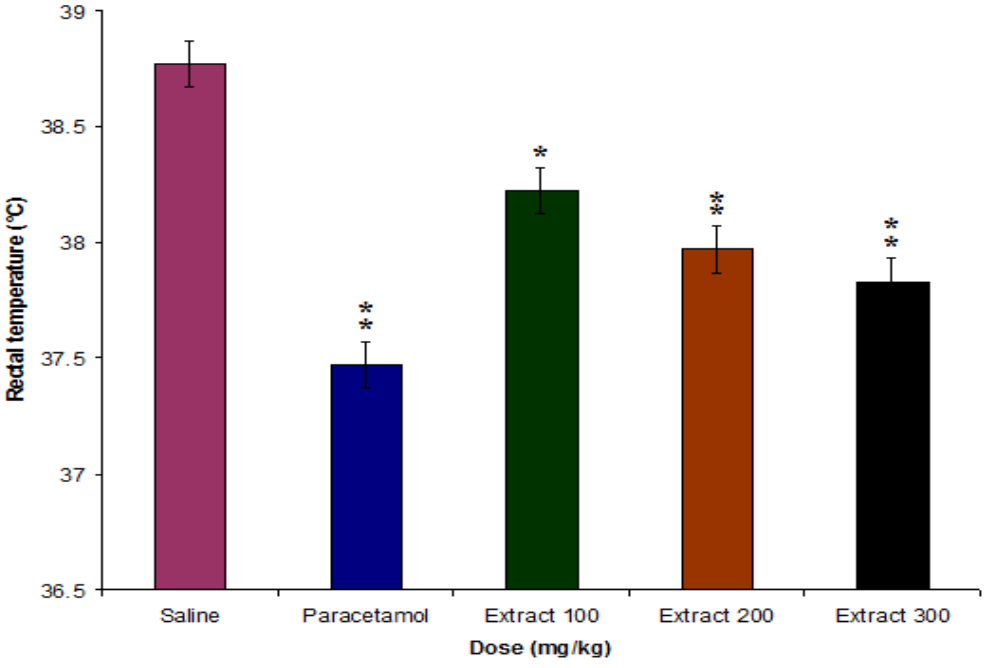

Figure 3.74: Effect of TD (5 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

3) Antipyretic activity of *Acacia cyanophylla*:

The crude extract (methanolic) of *Acacia cyanophylla* was applied to evaluate antipyretic activity and showed statistically significant and more significant effects at different doses (**Table 3.34).** At 1st hr of the treatment, no dose showed significant inhibitory effect. The percent antipyretic effect was 0.12, 0.29 and 0.63% respectively at 100, 200 and 300 mg/kg dose of crude extract. Body temperature was reduced by 0.03, 0.91 and 1.56% at 100, 200 & 300 mg/kg respectively at 2nd hr. All the subjected doses showed statistically significant (*P<0.05) antipyretic activity at 2nd hour. More significant inhibition was measured at 200 and 300 mg/kg doses which was 1.79 and 2.26% respectively (**P<0.01). Antipyretic activity observed after 4th hr was 0.91, 1.99 and 2.33% respectively for 100, 200 and 300 mg/kg dose. At 5th hr, temperature reduction was 1.24, 2.07 and 2.53% respectively for the test doses.

Table 3.34: Antipyretic activity of crude methanolic extract of *Acacia cyanophylla.*

Treatments	**Dose mg/kg**	**Rectal temperature (°C)**						
		Normal	**after 24h**	**After administration of drug**				
				1h	**2h**	**3h**	**4h**	**5h**
Saline	10mL	36.69 ± 0.52	39.71 ± 0.26	38.67 ± 0.31	38.62 ± 0 44	38.61 ± 0 .21	38. 71 ± 0.33	38.77 ± 0 .33
Paracetamol	150mg	37.05 ± 0.32	39.42 ± 0.32	38.18** ± 0.26	37.78** ± 0.36	37.33** ± 0.39	37.41** ± 0.44	37.47** ± 0.47
Extract	100	37.05 ± 0.37	39.46 ± 0.38	38.72 ± 0 .33	38.61* ± 0.39	38.19* ± 0.71	38.36* ± 0.32	38.29* ± 0.13
	200	37.07 ± 0.52	39.73 ± 0.41	38.56 ± 0 .52	38.27* ± 0.87	37.92** ± 0.47	37.94** ± 0.53	37.97** ± 0.31
	300	37.04 ± 0.36	39.59 ± 0.27	38.43 ± 0 .45	38.02* ± 0.62	37.74** ± 0.68	37.81** ± 0.71	37.79** ± 0.67

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. *$P < 0.05$, **$P < 0.01$ in comparison to control.

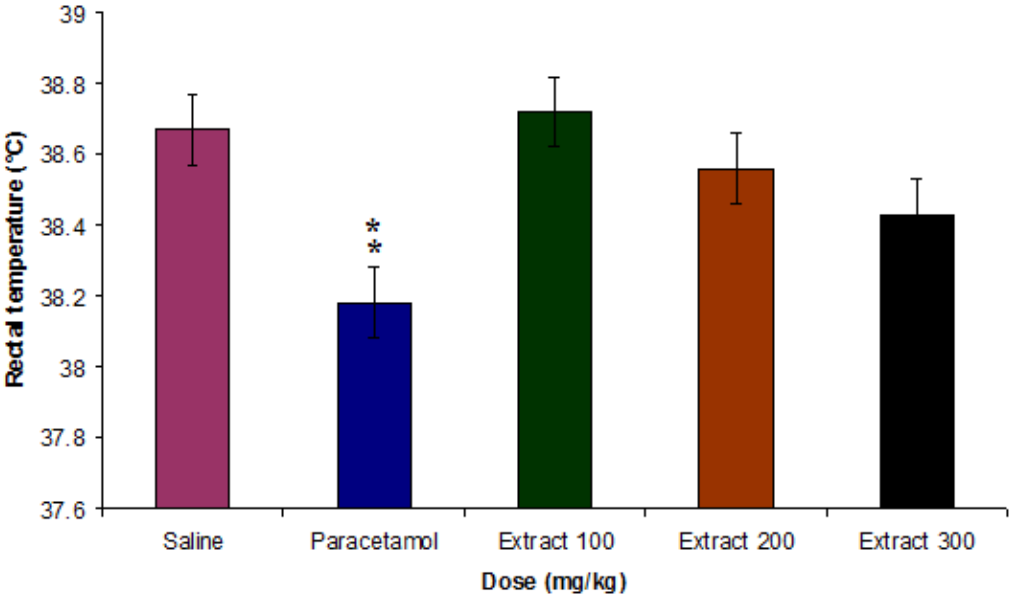

Figure 3.75: Effect of AC (1 hour) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

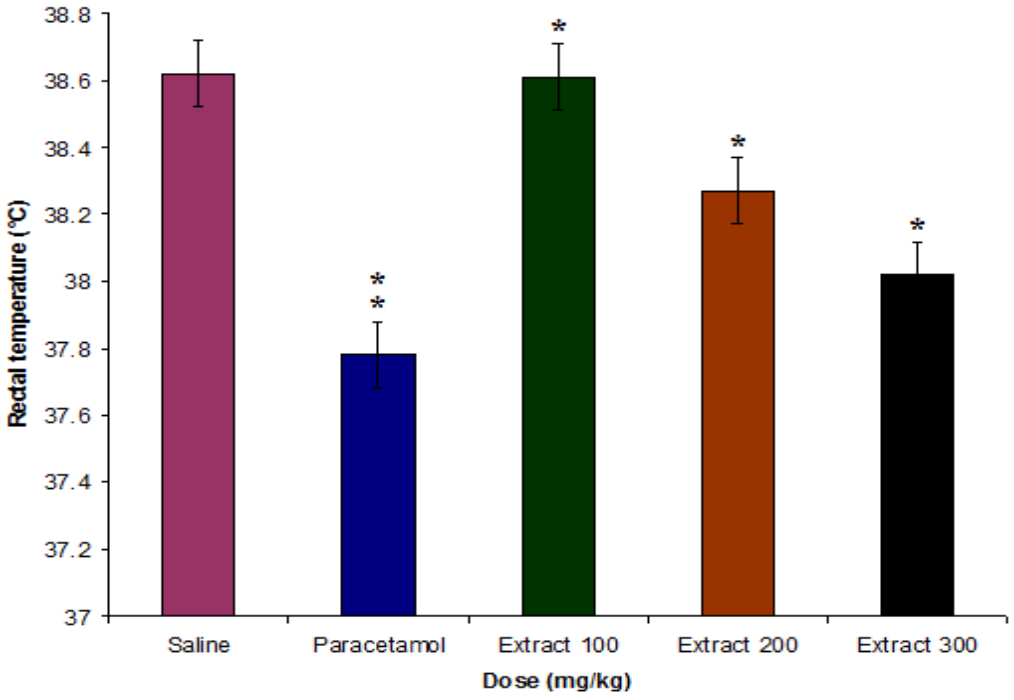

Figure 3.76: Effect of AC (2 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

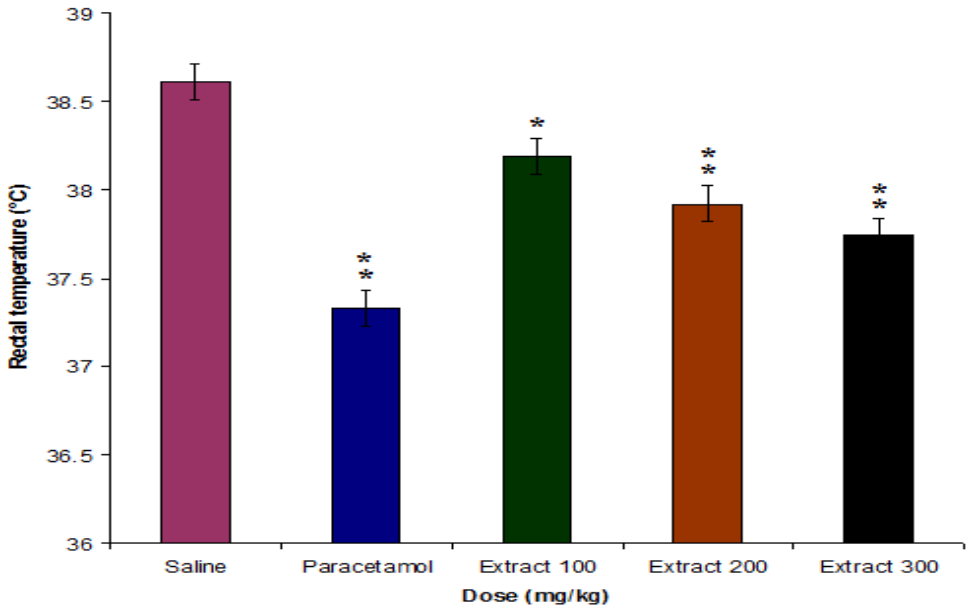

Figure 3.77: Effect of AC (3 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

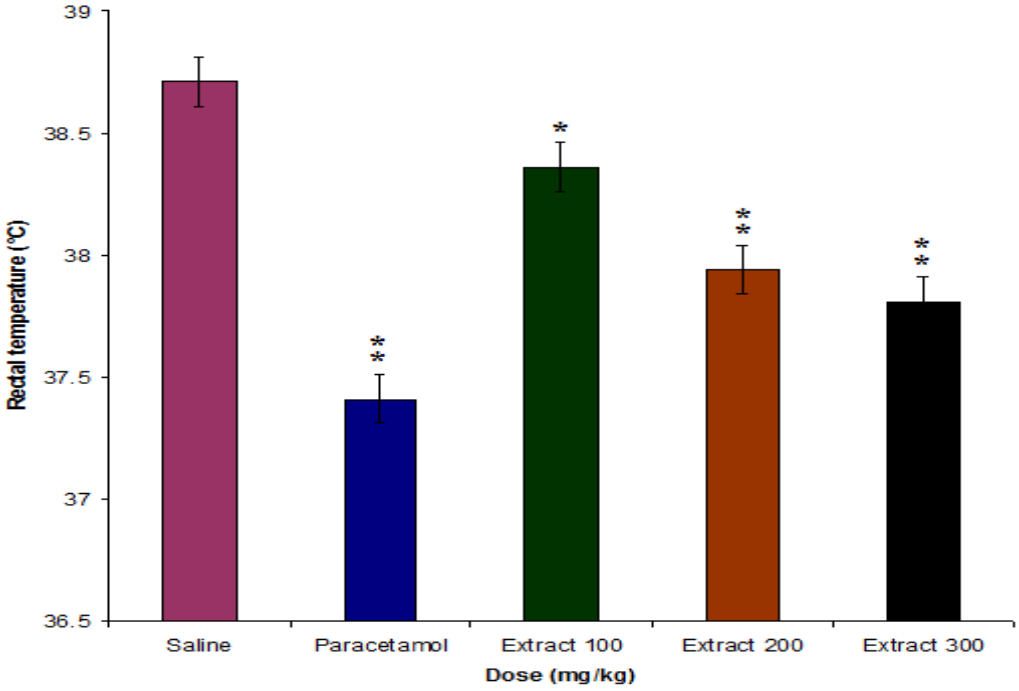

Figure 3.78: Effect of AC (4 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

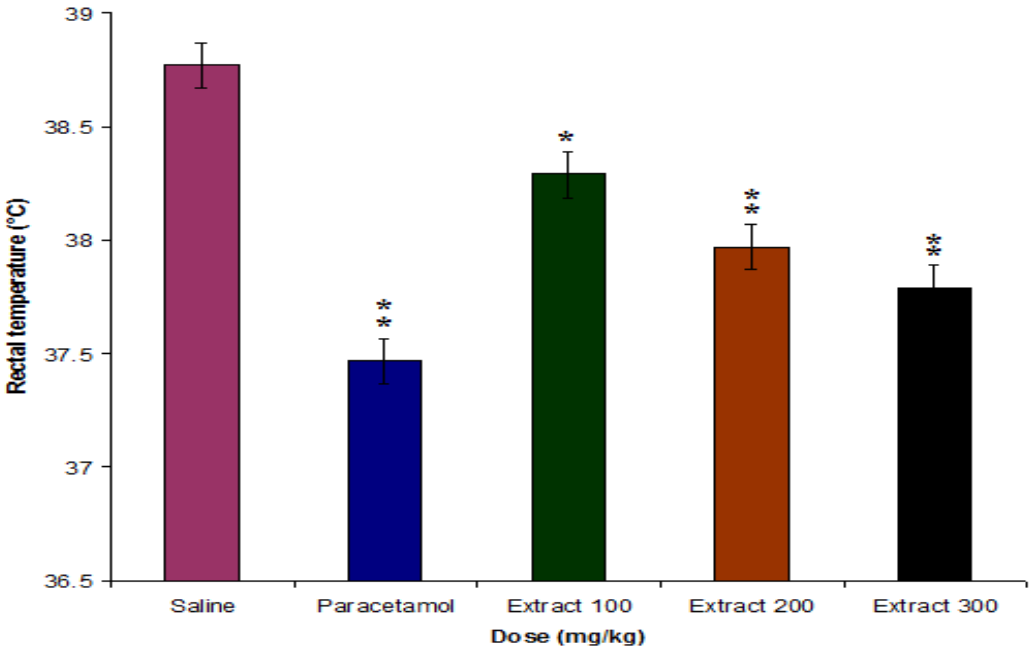

Figure 3.79: Effect of AC (5 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

4) Antipyretic activity of *Acacia stenophylla*:

Acacia stenophylla showed the antipyretic effects in the same pattern as shown by *Acacia cyanophylla*. At 1st hr, no statistically significant results were showed by any dose. After 1st hour, the results were found significant and more significant by different doses till 5th hr as shown in **Table 3.35.** In the 1st hr of the treatment, the percent reduction of temperature was 0.02, 0.37 and 0.68 % at 100, 200 and 300 mg/kg doses respectively. Percent reduction in body temperature at 100, 200 and 300 mg/kg was 0.34, 1.07 and 1.79% respectively in 2nd hr ofthe treatment. This effect was significant (statistically) (*P<0.05). All the applied doses produced percent antipyretic effect at 3rd, 4th & 5th hours, but it was more significant at 200 and 300 mg/kg doses (**P<0.01). In 3rd hr, it was 2.18 and 2.60% for 200 & 300 mg/kg respectively. 1.22, 2.28 and 2.68% antipyretic activity was measured for 100, 200 and 300 mg/kg respectively. Reduction in the body temperature at 5th hr was 1.24, 2.27 and 2.40% for the test doses respectively. The percent antipyretic effect has been shown in **Figure 3.81-85**.

Table 3.35: Antipyretic activity of crude methanolic extract of *Acacia stenophylla.*

Treatments	Dose mg/kg	Rectal temperature (°C)						
		Normal	After 24h	After administration of drug				
				1h	2h	3h	4h	5h
Saline	10 mL	36.69 ± 0.52	39.71 ± 0.26	38.67 ± 0.31	38.62 ± 0 44	38.61 ± 0 .21	38. 71 ± 0.33	38.77 ± 0 .33
Paracetamol	150 mg	37.05 ± 0.32	39.42 ± 0.32	38.18** ± 0.26	37.78** ± 0.36	37.33** ± 0.39	37.41** ± 0.44	37.47** ± 0.47
Extract	100	37.02 ± 0.41	39.77 ± 0.31	38.68 ± 0 .29	38.49* ± 0.36	38.11* ± 0.24	38.24*± 0.19	38.29* ± 0.13
	200	37.03 ± 0.56	39.23 ± 0.24	38.53 ± 0 .34	38.21* ± 0.14	37.77** ± 0.37	37.83** ± 0.28	37.89** ± 0.23
	300	37.02 ± 0.46	39.84 ± 0.19	38.41 ± 0 .28	37.93* ± 0.17	37.61** ± 0.37	37.79** ± 0.26	37.84** ± 0.31

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. **P* < 0.05, ***P* < 0.01 in comparison to control.

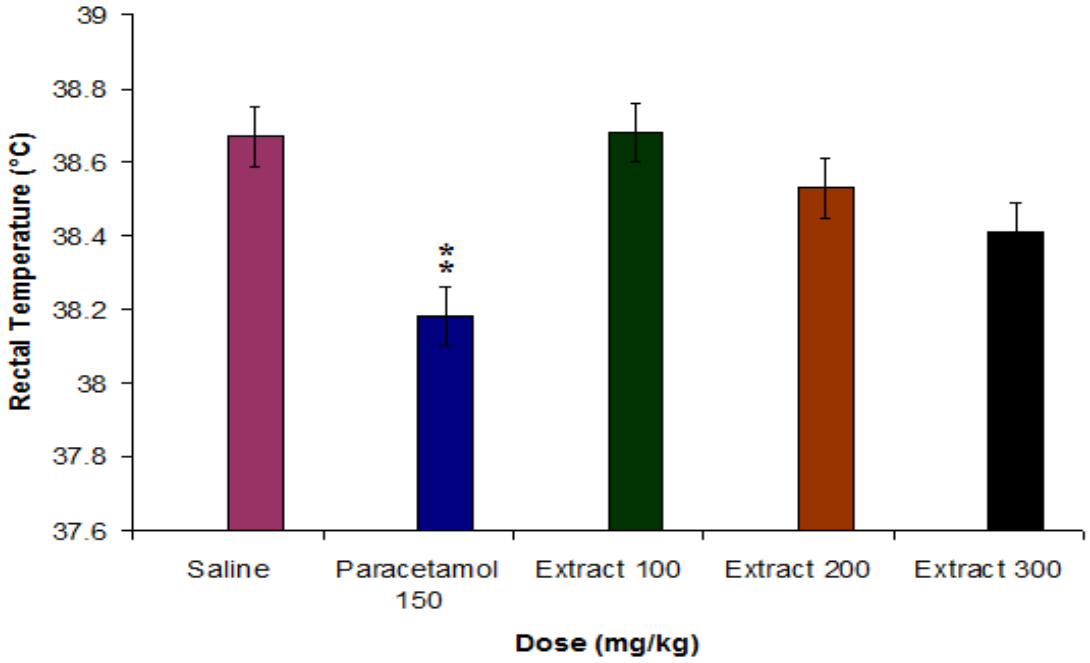

Figure 3.80: Effect of AS (1 hour) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

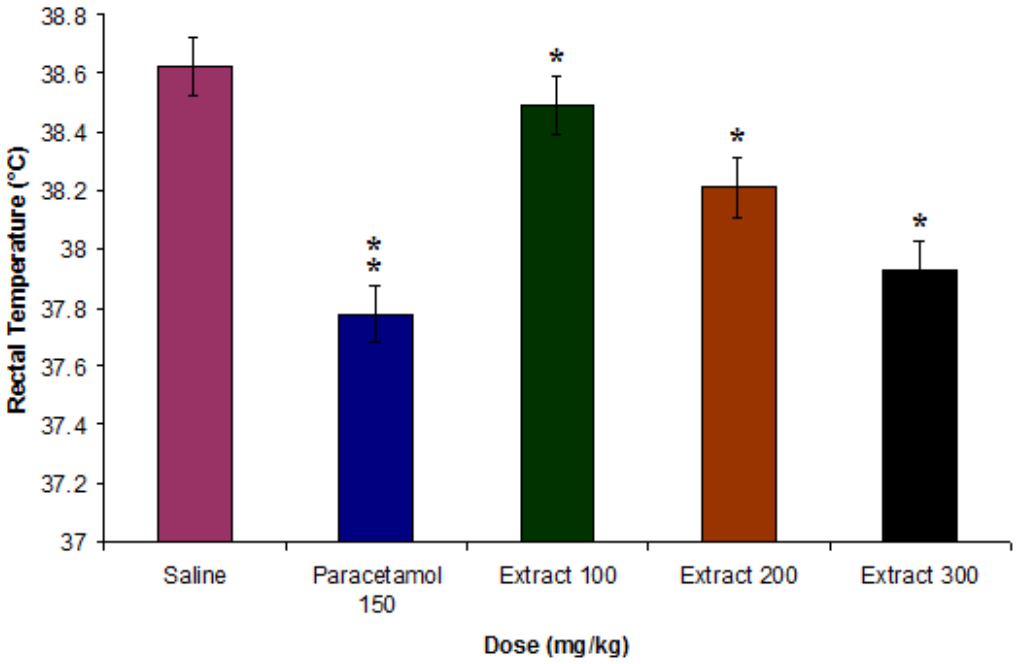

Figure 3.81: Effect of AS (2 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

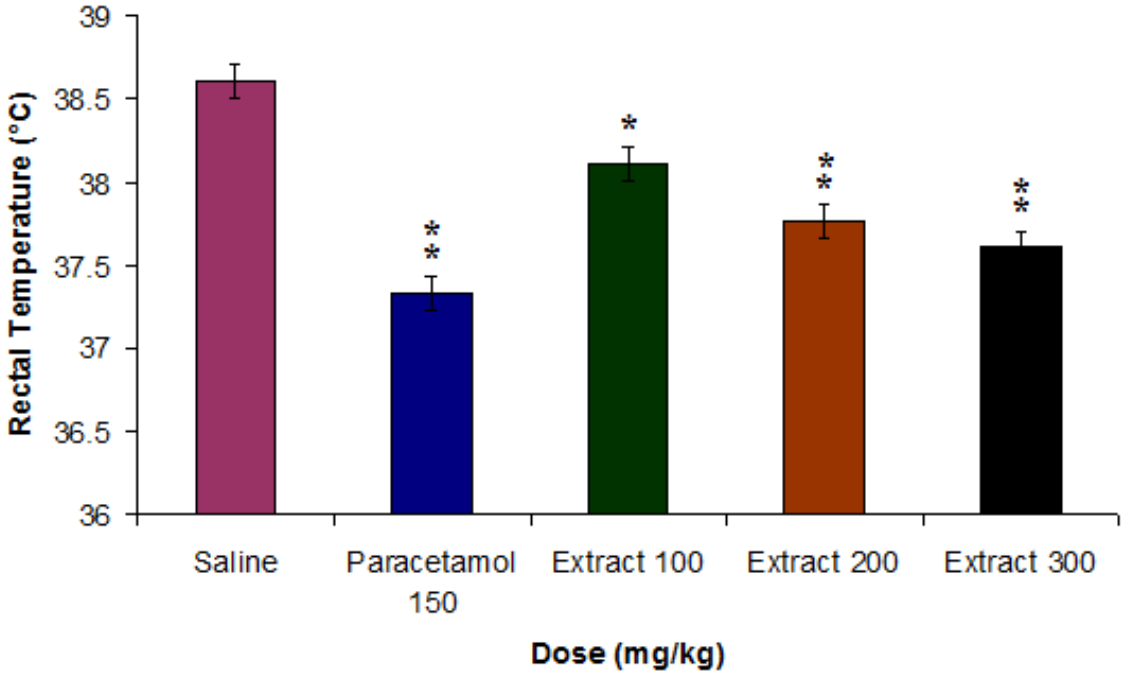

Figure 3.82: Effect of AS (3 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

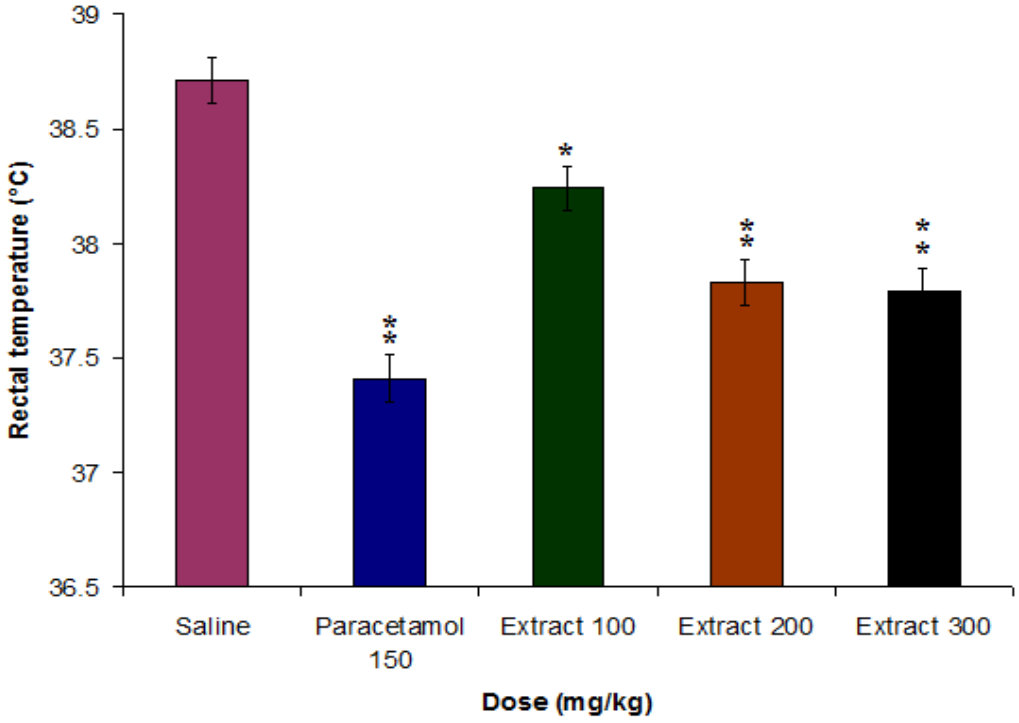

Figure 3.83: Effect of AS (4 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

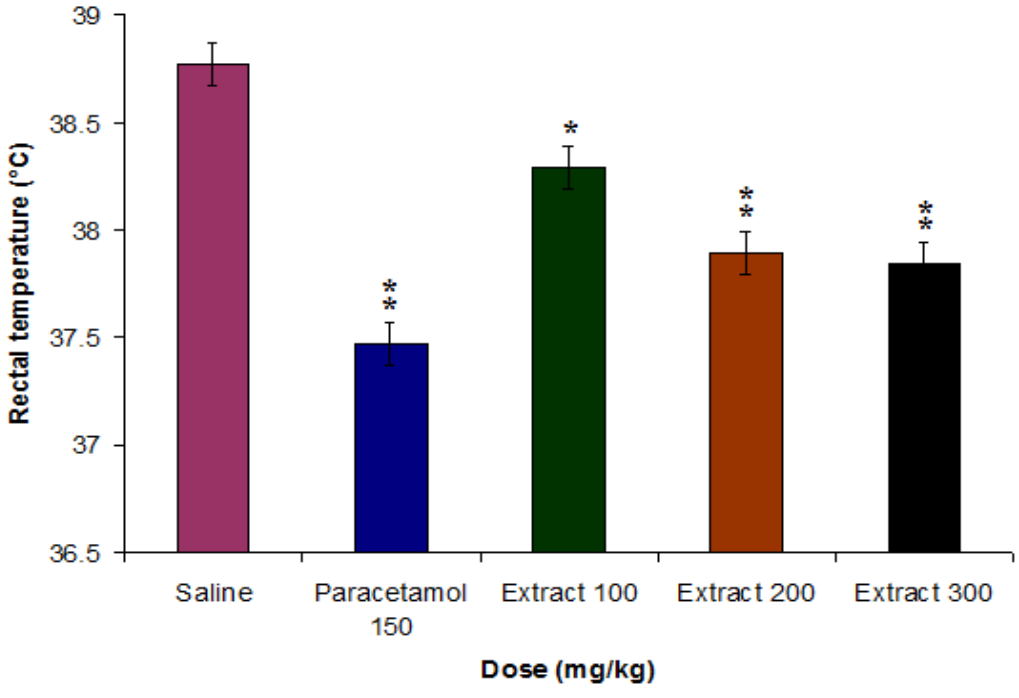

Figure 3.84: Effect of AS (5 hours) to study antipyretic activity.* $p < 0.05$, ** = $p < 0.01$.

3.4.3. Analgesic Activity:

3.4.3.1. Acetic Acid Induced Writhing:

1) Acetic Acid Induced Writhing of *Tamarix aphylla*:

Table 3.36 describes the analgesic activity of crude methanolic extract of *Tamarix aphylla* at various doses (100, 200 & 400 mg/kg). The inhibitory effect of the tested plant was dose dependent. The maximum percent inhibitory activity was demonstrated by dose of 400 mg/kg which was 50.40% (This inhibition was more significant) followed by 200 mg/kg (37.26%). Similarly, the percent writing inhibitory effect measured for 100 mg/kg was 13.68%.

Table 3.36: Analgesic activity of crude methanolic extract of *Tamarix aphylla.*

Treatments	**Dose (mg/kgi.p.)**	**No. of writhing (10min)**	**% Analgesia**
Saline	10 ml/kg	63.38 ± 2.79	-
Extract	100	54.71 ± 2.44	13.68
	200	39.77 ± 2.26*	37.26
	400	31.44 ± 2.11**	50.40
Diclofenac Sod.	10	10.17 ± 1.27**	83.96

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by t-test. *$P < 0.05$, **$P < 0.01$ in comparison to control

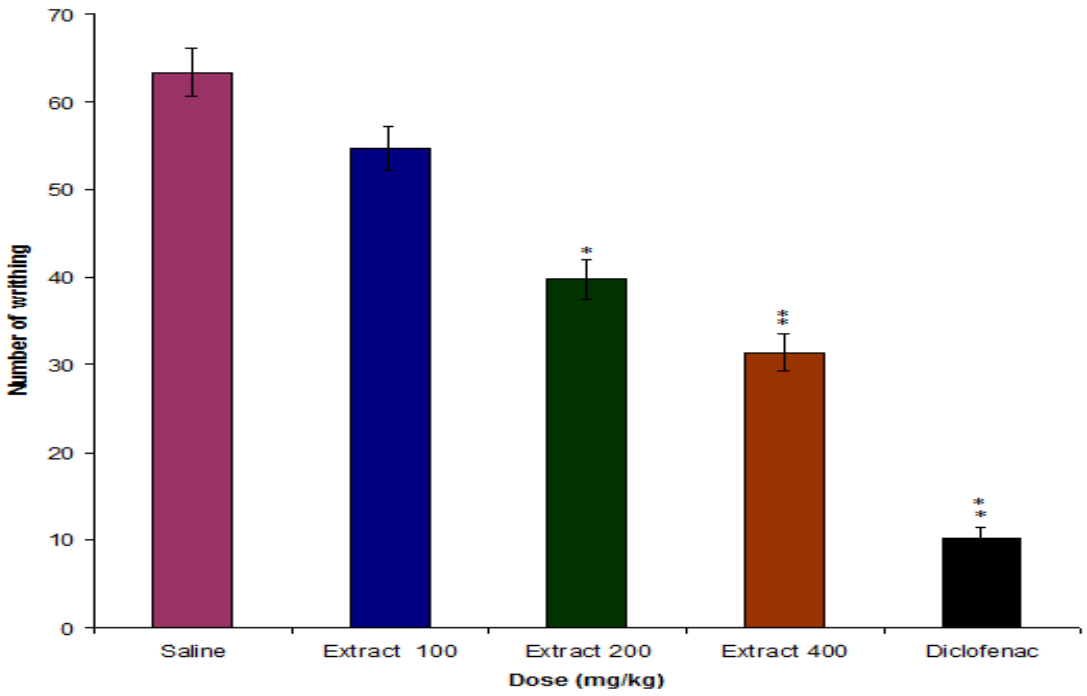

Figure 3.85: Number of writhing decrease by crude methanolic extract of *Tamarix aphylla*

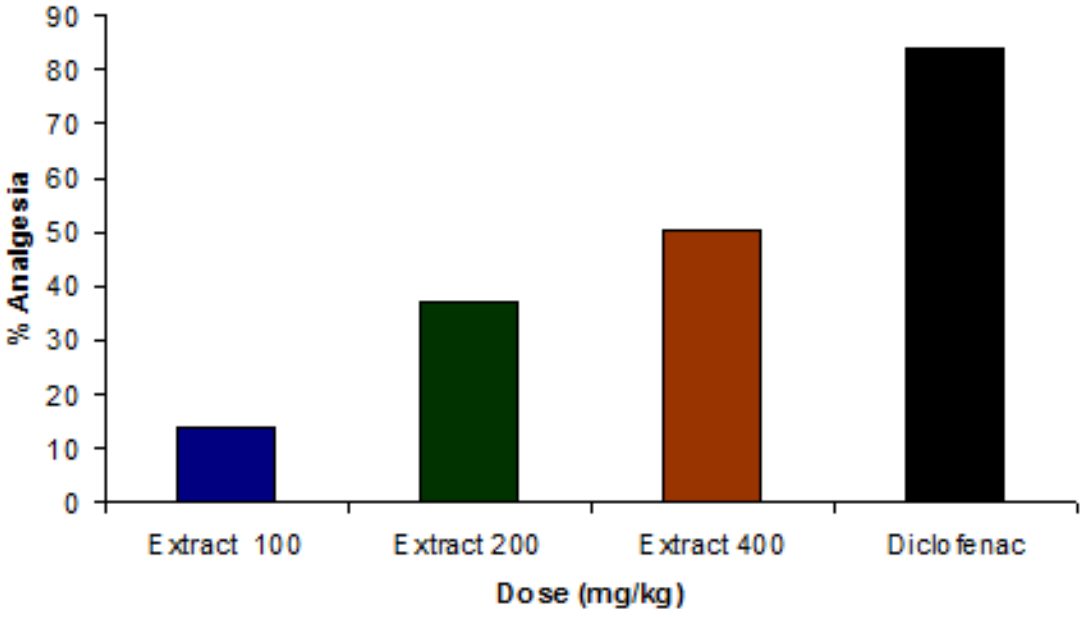

Figure 3.86: Percent analgesia by crude methanolic extract of *Tamarix aphylla*

2) Acetic Acid Induced Writhing of *Tamarix dioica*:

Stem bark extracted sample of *Tamarix dioica* showed significant analgesic effect at all subjected doses (100, 200 and 400 mg/kg). Analysis of the data revealed that the percent writhing inhibitory result was dose dependent (**Table 3.37)**. Maximum analgesic activity was demonstrated by 400 mg/kg (64.33%) which was more significant statistically. Similarly, the dose of 200 mg/kg also inhibited the writhings more significantly which was 56.60%. At a dose of 100 mg/kg the percent writing inhibition was 19.18%.

Table 3.37: Analgesic Activity of crude methanolic extract of *Tamarix dioica.*

Treatments	**Dose (mg/kg i.p.)**	**No. of writhing (10min)**	**% Analgesia**
Control (saline)	10 ml/kg	63.38 ± 2.79	-
Extract	100	51.23 ± 2.77*	19.18
	200	27.51 ± 1.83**	56.60
	400	22.61 ± 1.46**	64.33
Diclofenac Sod.	10	10.17 ± 1.27**	83.96

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by t-test. *$P < 0.05$, **$P < 0.01$ in comparison to control

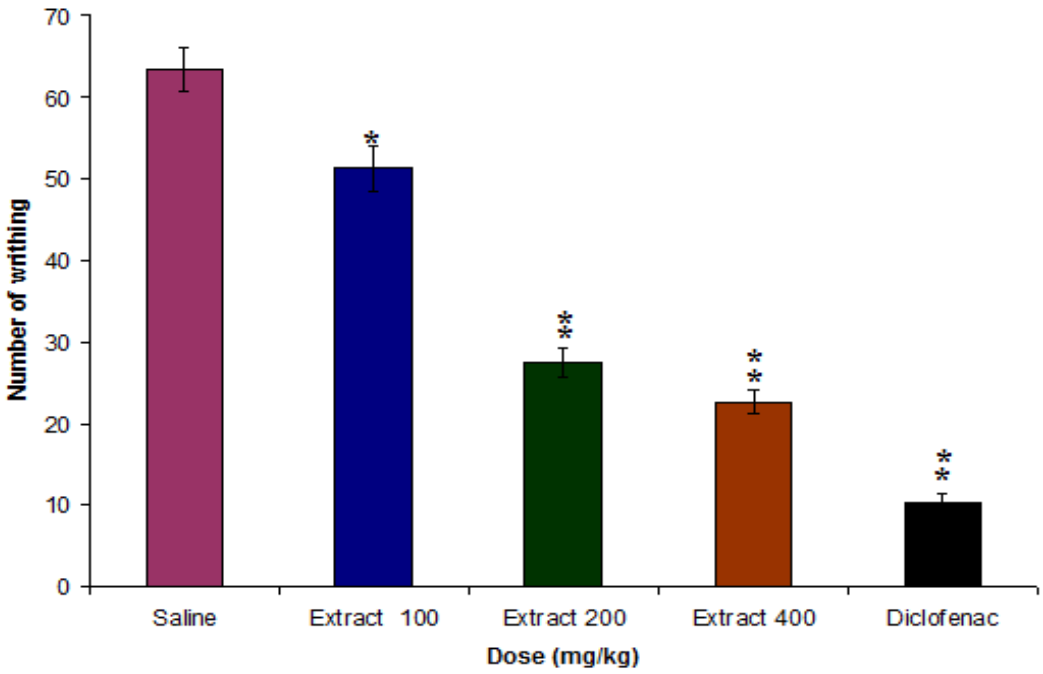

Figure 3.87: Number of writhings decrease by crude methanolic extract of *Tamarix dioica*

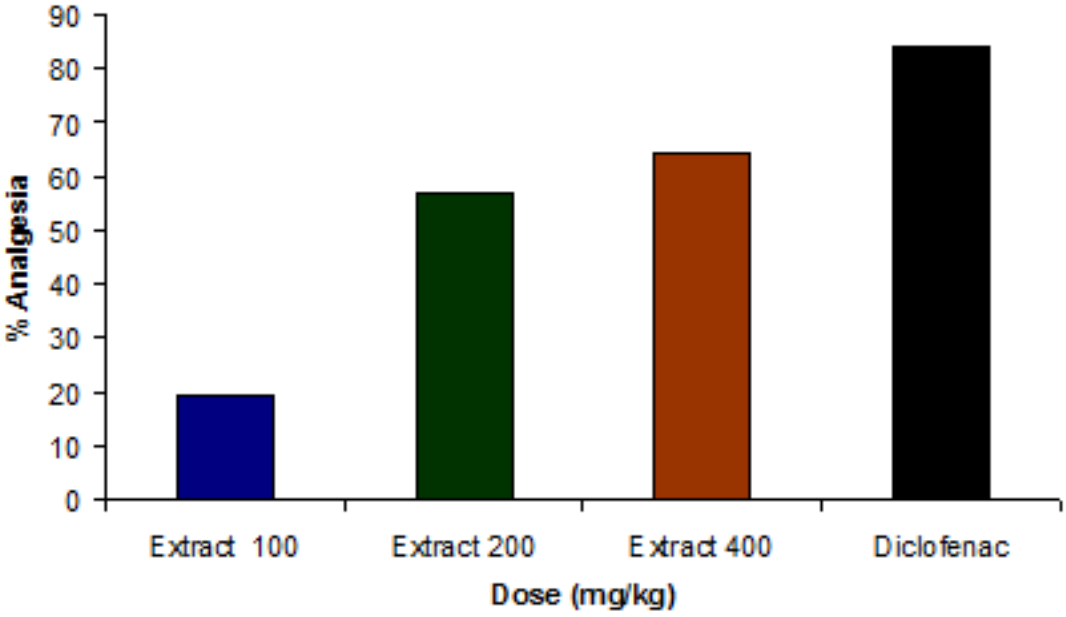

Figure 3.88: Percent analgesia by crude methanolic extract of *Tamarix dioica.*

3) Acetic Acid Induced Writhing of *Acacia cyanophylla*:

Crude methanolic extract of *Acacia cyanophylla* showed decrease in number of writings (mean) at various doses (100, 200 and 400 mg/kg).**Table 3.38** reveals that the inhibitory effect produced by 200 & 400 mg/kg was more significant while that of 100 mg/kg was significant. The inhibitory effect observed for *Acacia cyanophylla* was found dose dependent. Maximum inhibition (percent) was produced at a dose of 400 mg/kg which was 61.60% followed by 200 mg/kg (49.09%). Similarly, 21.87% inhibitory effect was measured for 100 mg/kg.

Table 3.38: Analgesic Activity of crude methanolic extract of *Acacia cyanophylla.*

Treatments	**Dose (mg/kgi.p.)**	**No. of writhing (10min)**	**% Analgesia**
Saline	10 ml/kg	63.38 ± 2.79	-
Extract	100	49.52 ± 2.67*	21.87
	200	32.27 ± 2.03**	49.09
	400	24.34 ± 1.91**	61.60
Diclofenac Sod.	10	10.17 ± 1.27**	83.96

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by t-test. *$P < 0.05$, **$P < 0.01$ in comparison to control.

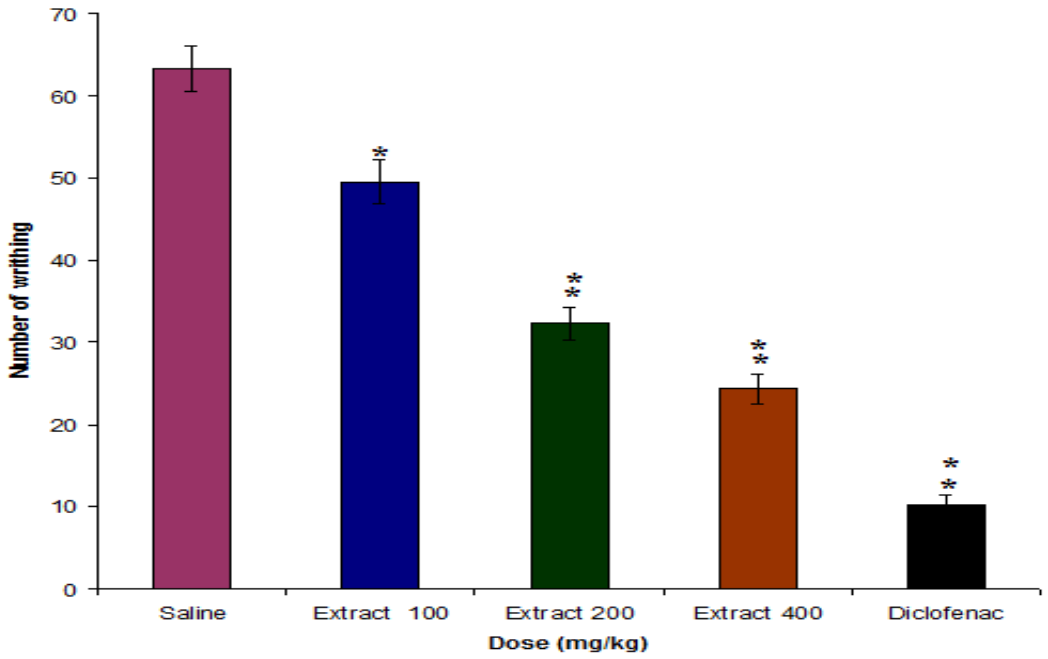

Figure 3.89: Number of writhing decrease by crude methanolic extract of *Acacia cyanophylla*

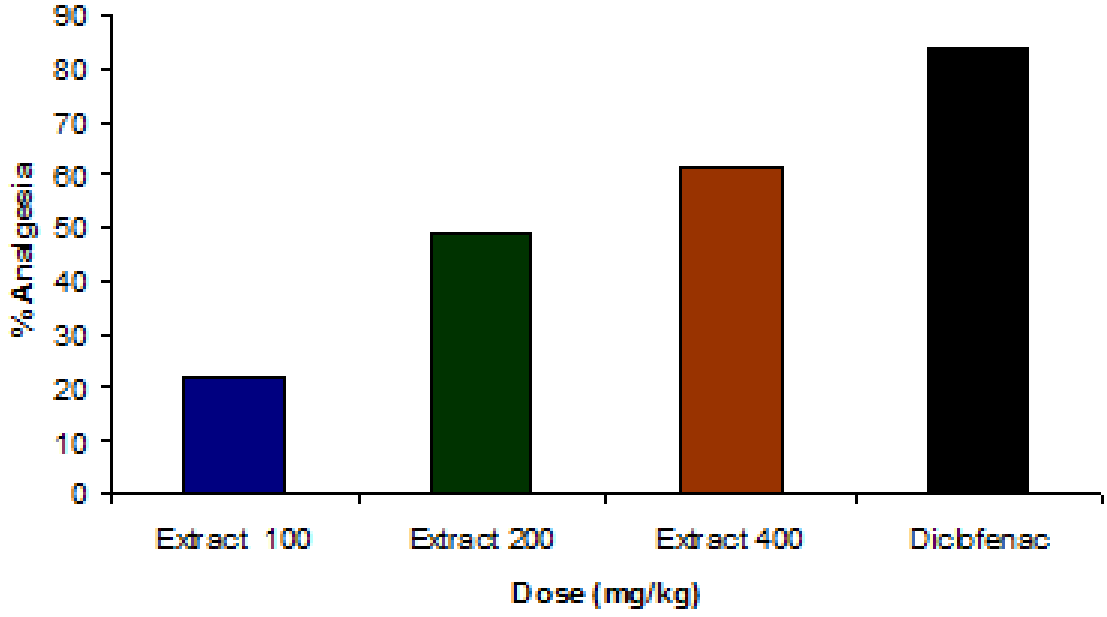

Figure 3.90: Percent analgesia by crude methanolic extract of *Acacia cyanophylla*

4) Acetic Acid Induced Writhing of *Acacia stenophylla*:

Percent decrease in number of writings shown by *Acacia stenophylla*has been indicated in **Table 3.39**. The tested plant was screened at various doses (100, 200 and 400 mg/kg). Analysis of the data revealed that the inhibitory activity dose dependent. With the increase in concentration of the plant extract, the analgesic effect was also increased. The extract showed significant effect at all tested doses but more signicant at doses of 200 and 400 mg/kg. Maximum activity was measured for 400 mg/kg which was 66.19% followed by 200 mg/kg (53.40%). Similarly, 34.20% activity was demonstrated by 100 mg kg^{-1}. Figures 3.92-3.93 indicate the % inhibition in number of writhings by the crude extracts and standard.

Table 3.39: Analgesic activity of crude extract of *Acacia stenophylla.*

Treatments	**Dose (mg/kgi.p.)**	**No. of writhing (10min)**	% Analgesia
Saline	10ml/kg	63.38 ± 2.79	-
Extract	100	41.71 ± 2.14*	34.20
	200	29.54 ± 1.98**	53.40
	400	21.43 ± 1.56**	66.19
Diclofenac Sod.	10	10.17 ± 1.27**	83.96

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by t-test. *$P < 0.05$, **$P < 0.01$ in comparison to control

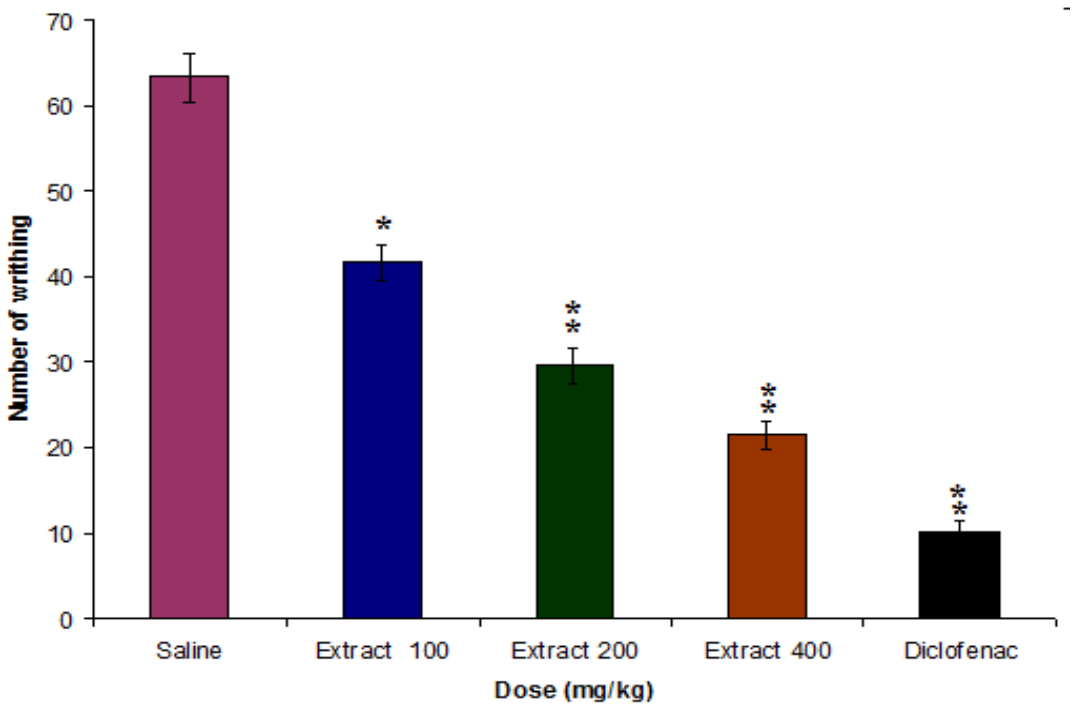

Figure 3.91: Number of writhing decrease by crude methanolic extract of *Acacia stenophylla*

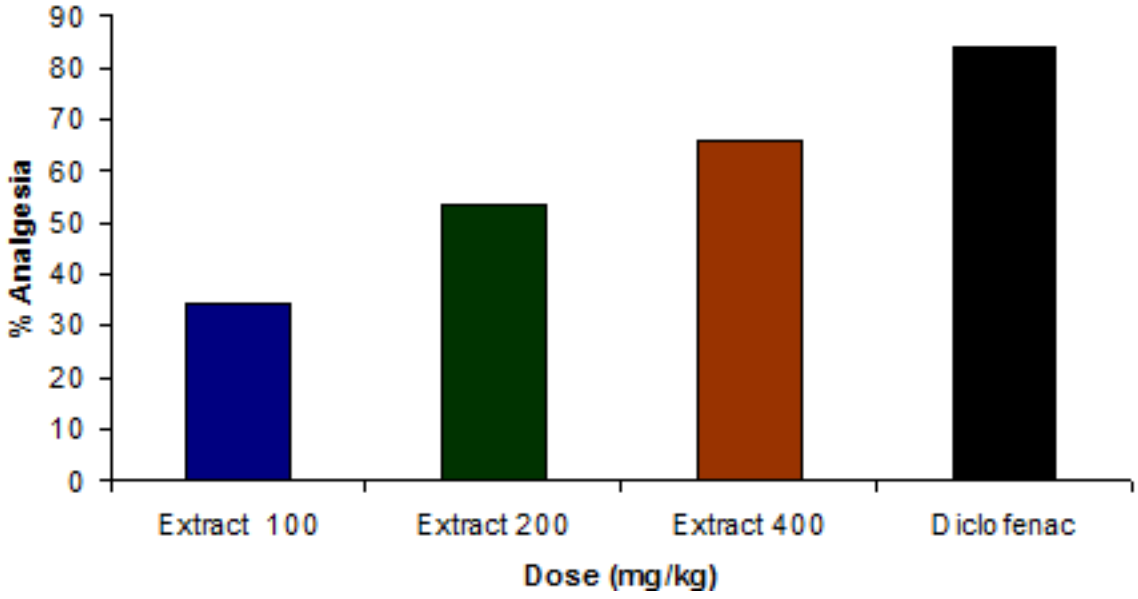

Figure 3.92: Percent analgesia by crude methanolic extract of *Acacia stenophylla*

3.4.3.2. Hot Plate Method:

The crude methanolic extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* was screened for nociceptive response. The analgesic effect produced by crude extract (200 and 400 mg/kg) has been observed at different times (30, 60, 90 and 120 mniutes) as shown in **Table 3.40.**The activity showed by all samples was dose and time dependent.

The percent nociceptive response of the subjected doses of *Tamarix aphylla* in the 30 minutes was 16.62 and 38.08% respectively at 200 and 400 mg/kg. In 1hr of the treatment, the inhibitory effect of 200 mg/kg was significant (36.00%) but was more significant at 400 mg/kg (49.70%). Simlarly, the analgesic activity measured for 200 and 400 mg/kg was significant and more significant respectively at 90 and 120 mniutes. The effect at 90 minutes for the doses of 200 and 400 mg/kg was 37.55 and 54.49%. At 2 hours, percent inhibitory effect was produced by the all the tested doses but it was found more significant at the dose of 400 mg kg^{-1} which was 46.56 % (**P < 0.01). The percent nociceptive response is presented in **Figure 3.94-3.95**.

Analgesic activity of *Tamarix dioica* at the dose of 200 was found significant only at 120 minutes, while the dose of 400 mg/kg, the analgesic effect was significant at 30 and 60 minutes but more significant at 90 and 120 minutes. In the treatment of 30 minutes, the activity observed for 200 and 400 mg/kg was 13.32 and 39.50% respectively.After 60 minutes, the percent reduction in analgesia for 200 and 400mg/kg was 38.09 and 39.02% respectively. At 90 minutes, the nociceptive response observed for 200 and 400 mg/kg was 36.52 and 52.64%. At 2 hours,the inhibitory effect demonstrated by 400 mg/kgwas 48.05 %. The percent nociceptive response is presented in **Figure 3.96-3.97**.

The analgesic effect produced by *Acacia cyanophylla* was found significant at the dose of 400 mg/kg only. It showed 11.32 and 30.07% analgesic effect at 200 & 400 mg/kg respectively in 30 minutes treatment. The inhibitory effect at 60 minutes was 20.09 and 33.68% respectively for 200 and 400 mg/kg dose. The nociceptive response observed at 90 minutes for 200 and 400 mg/kg was 20.62 and 36.98%. After 2 hours, percent response demonstrated by 400 mg/kg was 34.21 %. The percent nociceptive response is presented in **Figure 3.98-3.99**.

The analgesic effect of *Acacia stenophylla* was also screened. The dose of 200 mg/kg demonstrated no significant effect, while 400 mg/kg dose indicated significant analgesic effect at 30 and 60 mniutes and more significant at 90 and 120 minutes. In the 30 minutes of the treatment, the response was 12.26 and 31.36% respectively at 200 and 400 mg/kg. In 1hr of the treatment the percent reduction in pain for 200 and 400 mg/kg was 21.13 and 35.07% respectively. The analgesic response noted at 90 minutes for 200 and 400 mg/kg was 22.46 and 38.82%. In 2 hours of the treatment, the percent nociceptive response observed for 400 mg/kg was 35.81%.

Table 3.40: Analgesic activity of crude methanolic extract of selected plants.

Treatments	**Dose (mg/kg)**	**Latency of nociceptive response in min (mean ± SEM)**				
		0	30	60	90	120
Vehicle	-	8.22±0.23	8.48±0.18	8.61±0.43	8.68±0.29	8.74±0.51
Acacia stenophylla	200	8.36±0.36	9.52±0.27	10.43±0.22	10.63±0.54	9.94±0.37
	400	8.32±0.53	11.14±0.39*	11.63±0.43*	12.05±0.62**	11.87±0.68**
Acacia cyanophylla	200	8.31±0.27	9.44±0.51	10.34±0.42	10.47±0.78	9.81±0.29
	400	8.26±0.42	11.03±0.57*	11.51±0.39*	11.89±0.41*	11.73±0.38*
Tamarix aphylla	200	8.45±0.31	9.89±0.52	11.71±0.67*	11.94±0.41*	11.73±0.53*
	400	8.49±0.58	11.71±0.64*	12.89±0.63**	13.41±0.47**	12.81±0.47**
Tamarix dioica	200	8.36±0.36	9.61±0.42	11.89±0.69	11.85±0.47	11.96±0.64*
	400	8.49±0.46	11.83±0.41*	11.97±0.73*	13.25±0.56**	12.94±0.58**
Tramadol	20	8.51±0.37	12.59±0.74**	15.89±0.52**	16.24±0.23**	15.62±0.23**

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. *$P < 0.05$, **$P < 0.01$ in comparison to control.

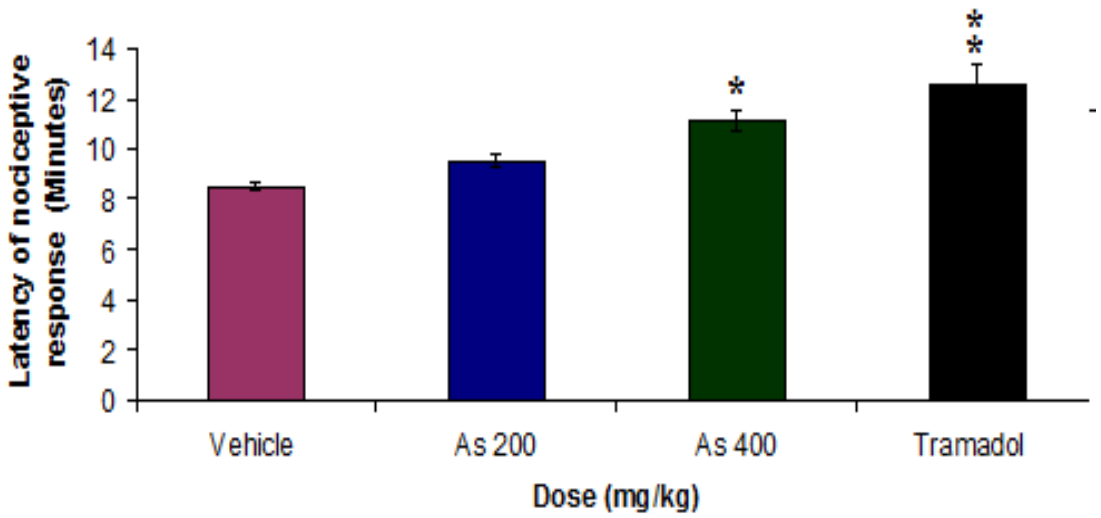

Figure 3.93: Effect of AS (30 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

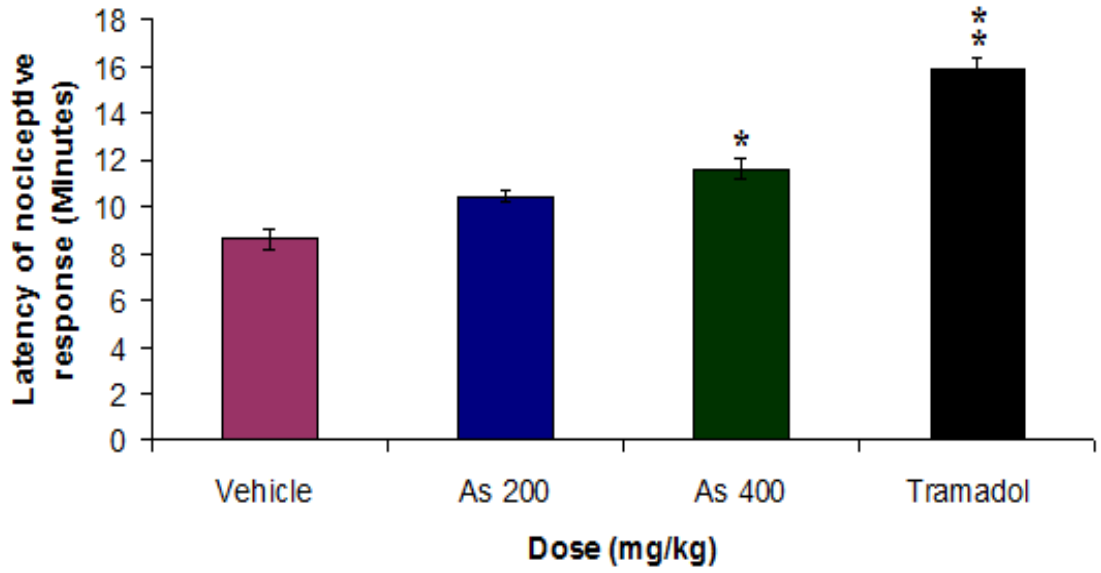

Figure 3.94: Effect of AS (60 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

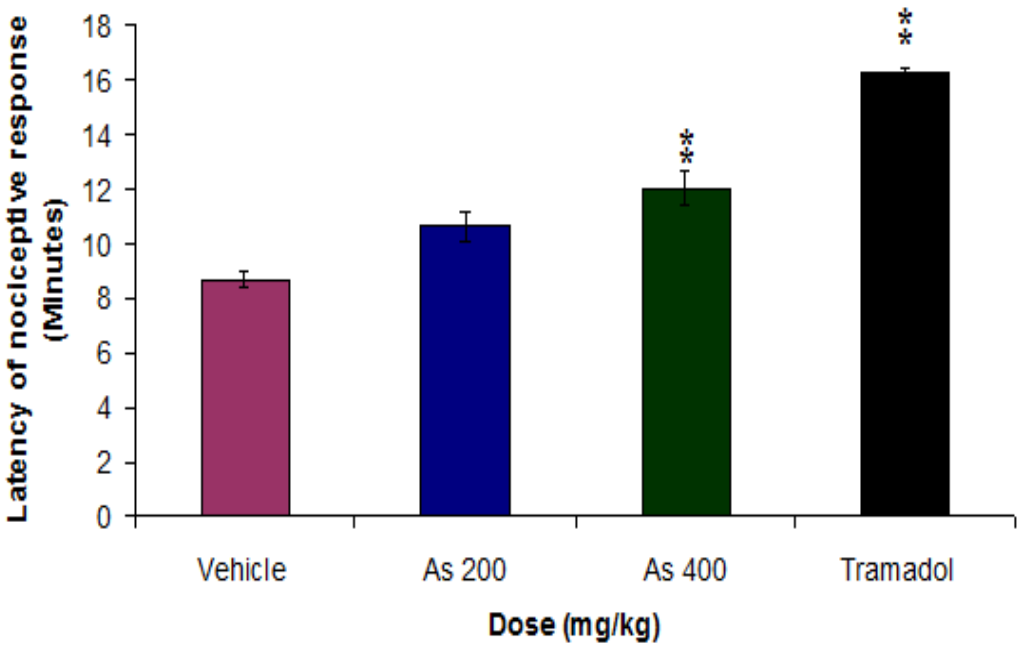

Figure 3.95: Effect of As (90 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

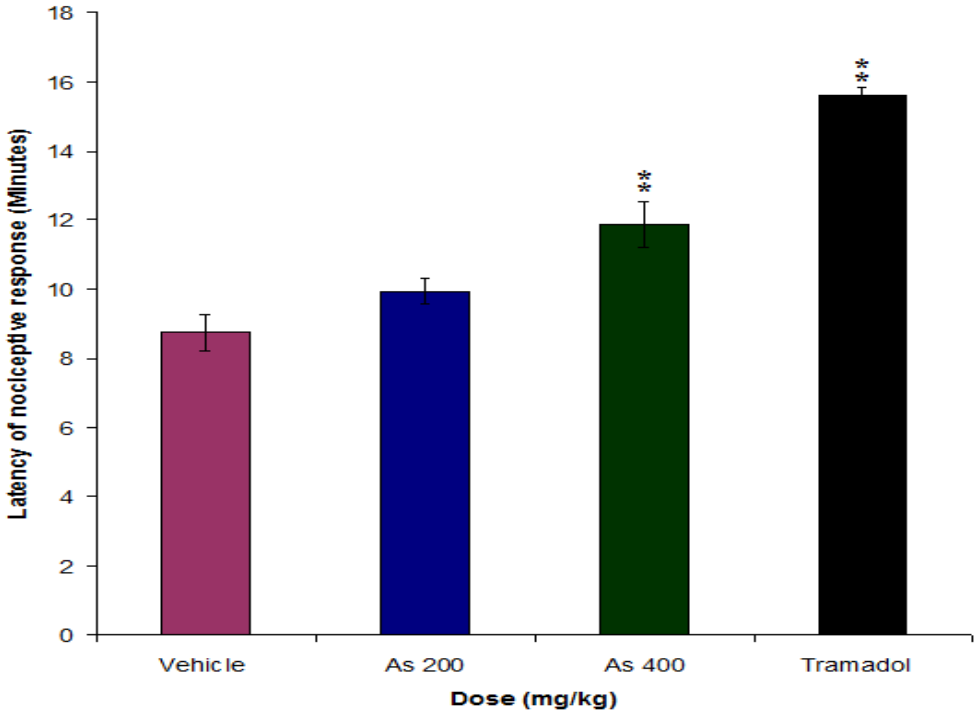

Figure 3.96: Effect of AS (120 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

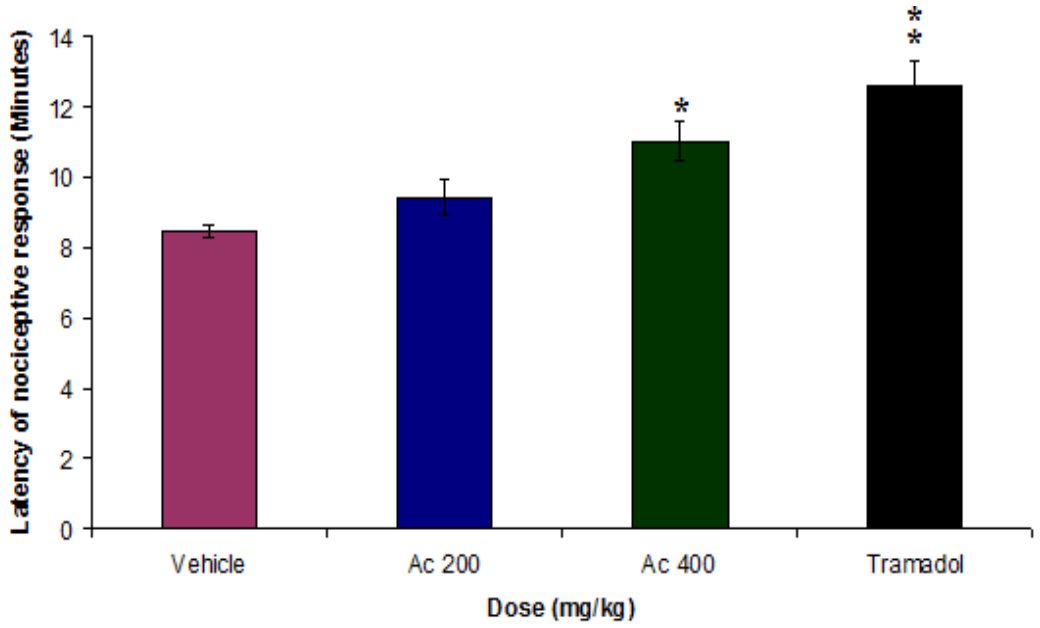

Figure 3.97: Effect of AC (30 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

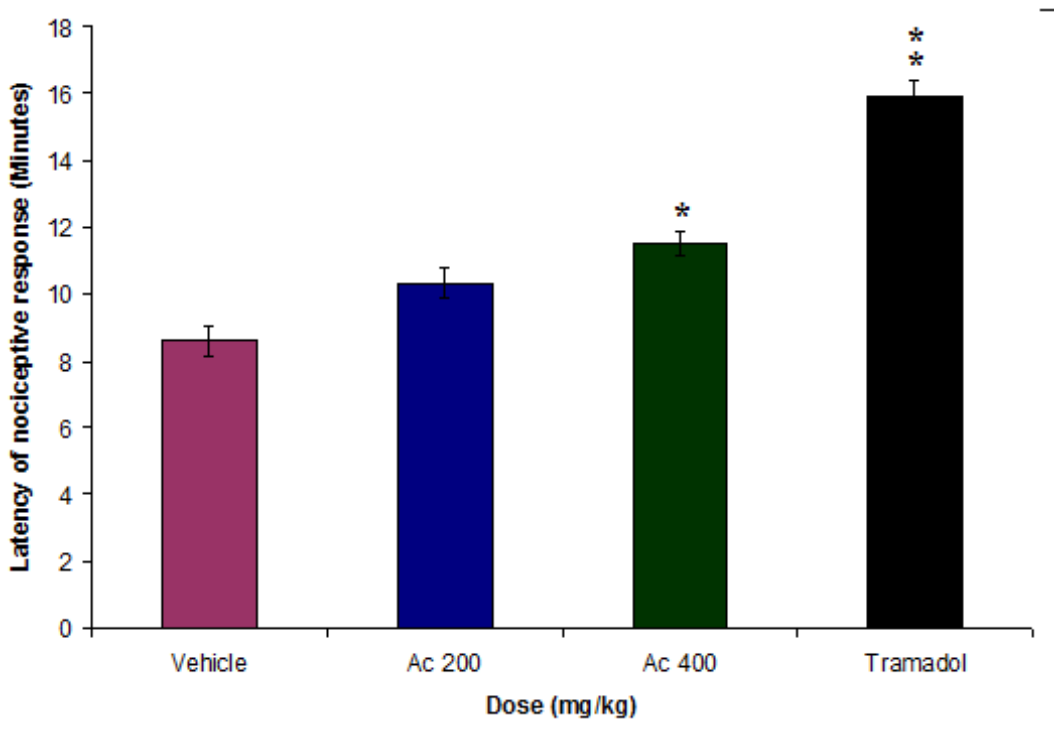

Figure 3.98: Effect of AC (60 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

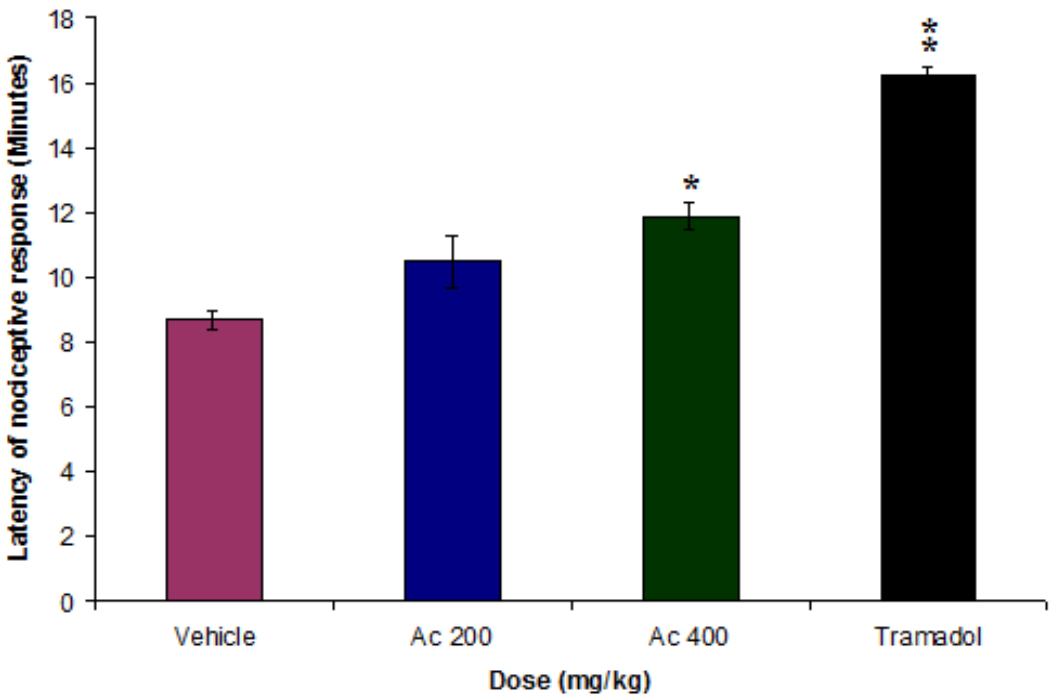

Figure 3.99: Effect of AC (90 minutes) on Hot Plate Method. * $p < 0.05$, ** = $p < 0.01$.

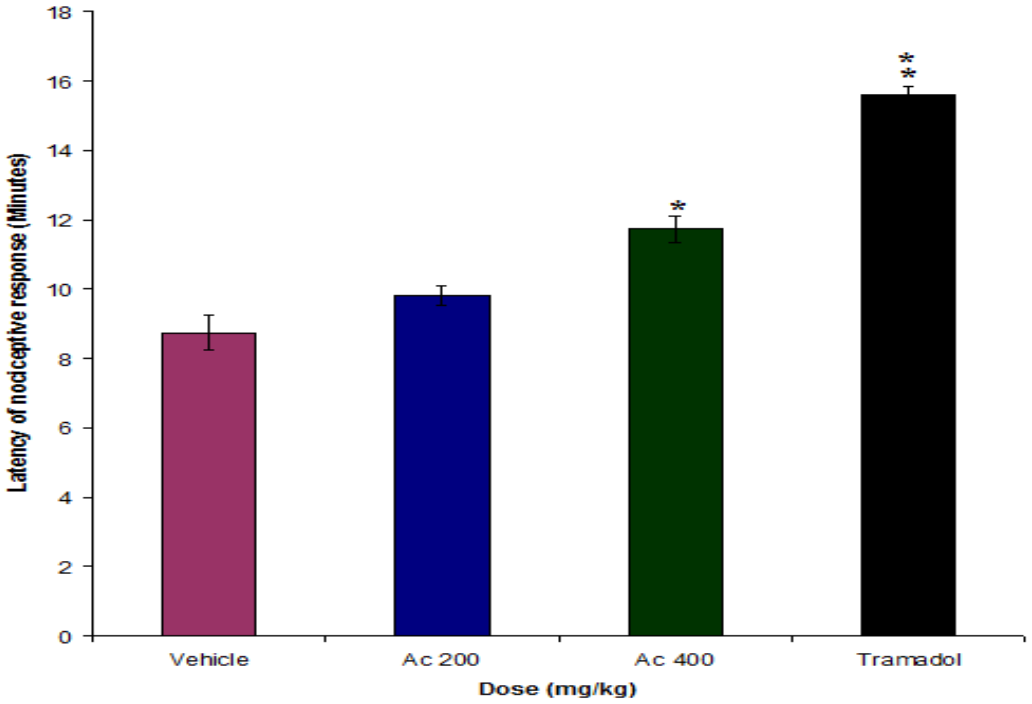

Figure 3.100: Effect of AC (120 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

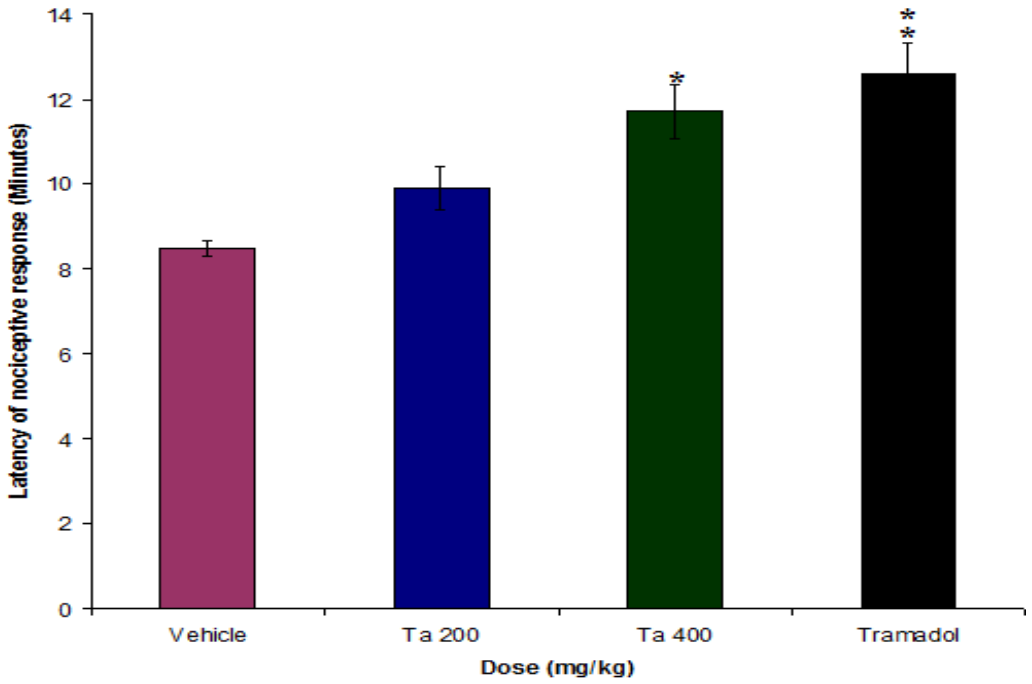

Figure 3.101: Effect of TA (30 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

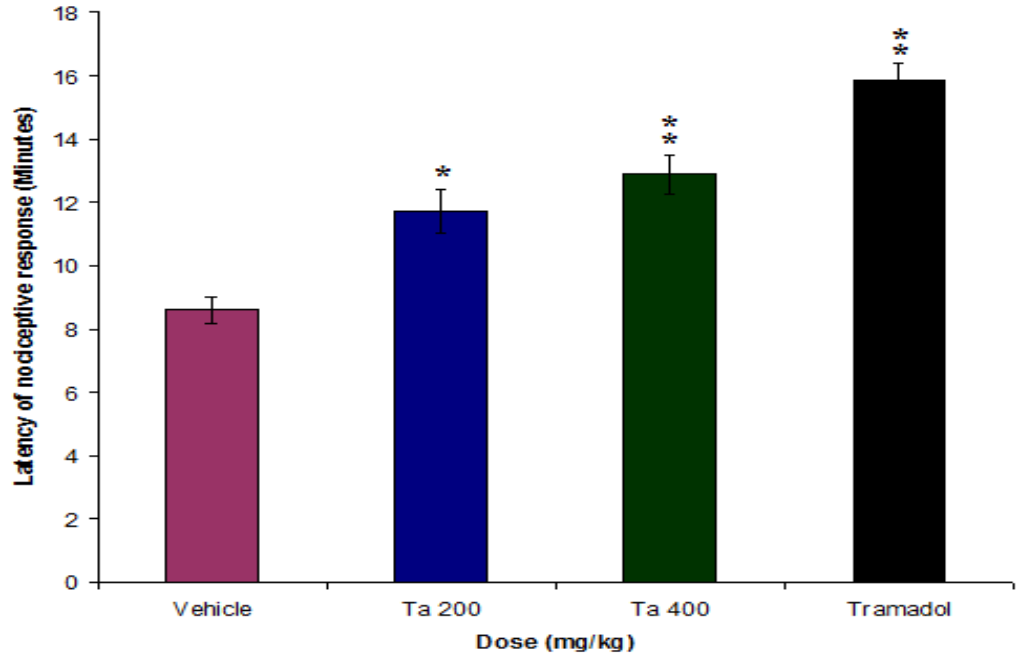

Figure 3.102: Effect of TA (60 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

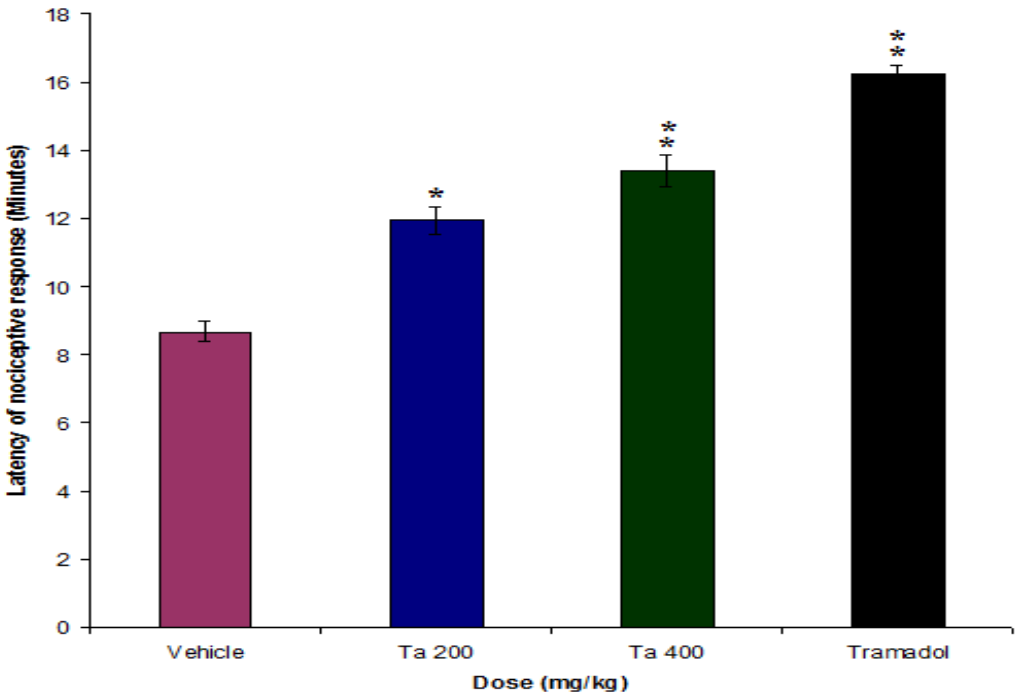

Figure 3.103: Effect of TA (90 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

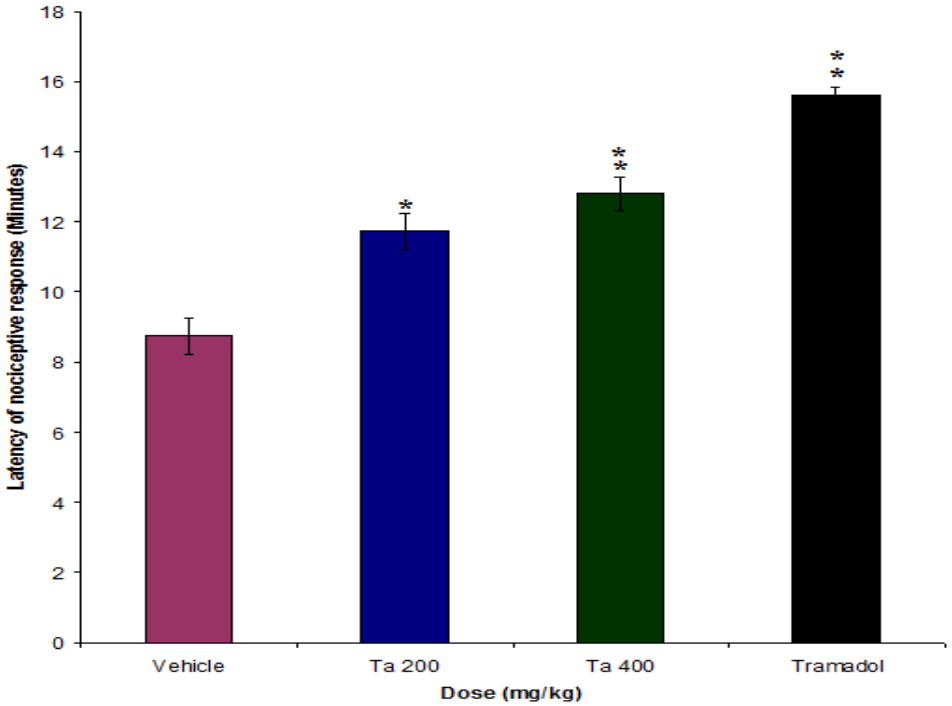

Figure 3.104: Effect of TA (120 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

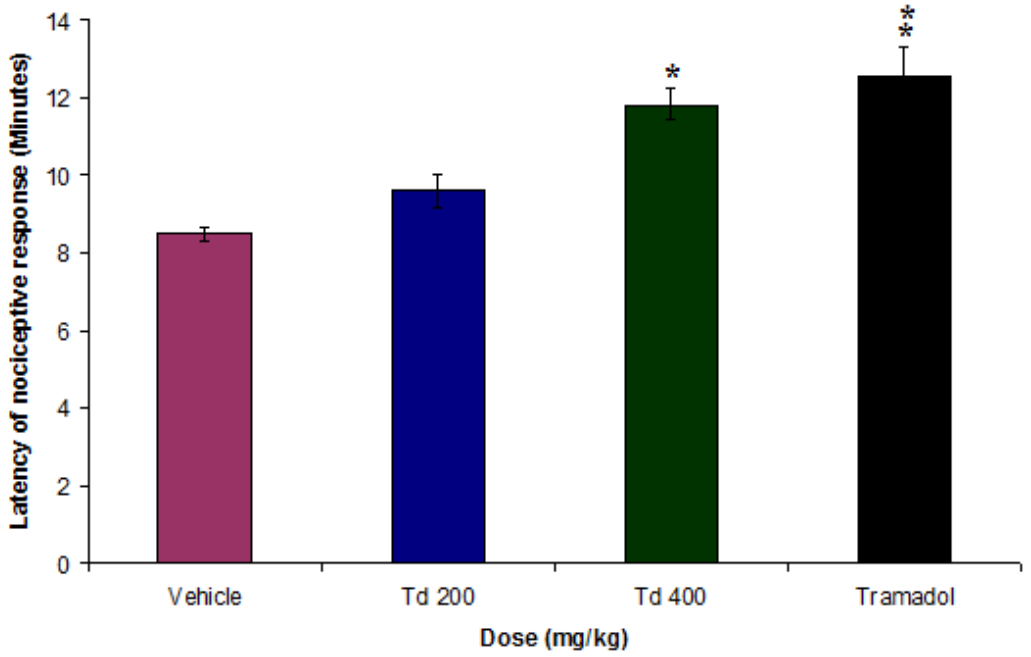

Figure 3.105: Effect of TD (30 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

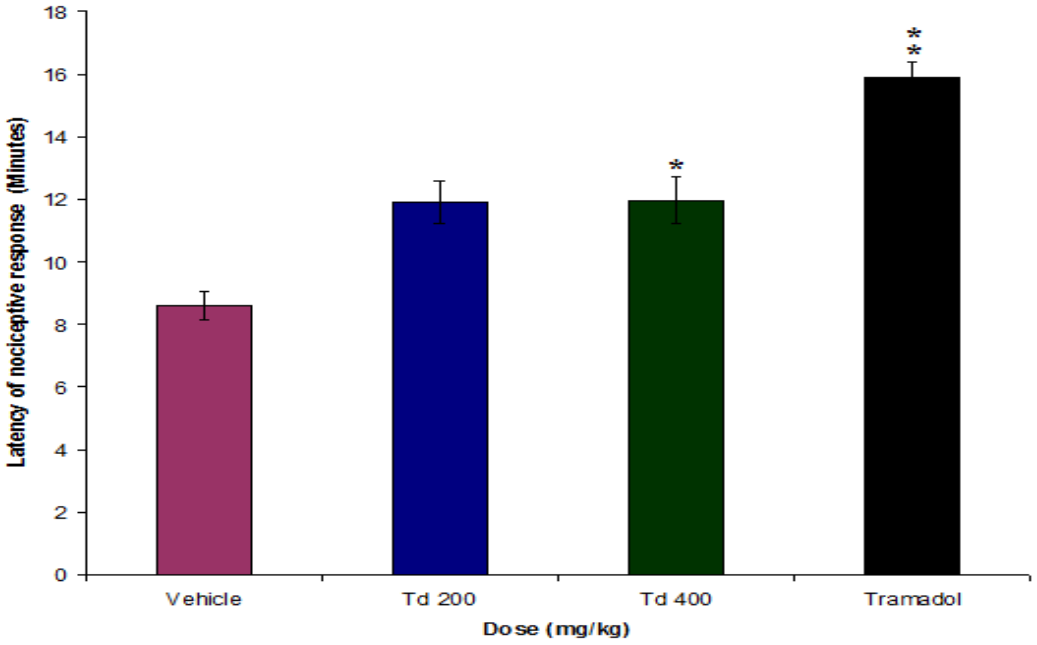

Figure 3.106: Effect of TD (60 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

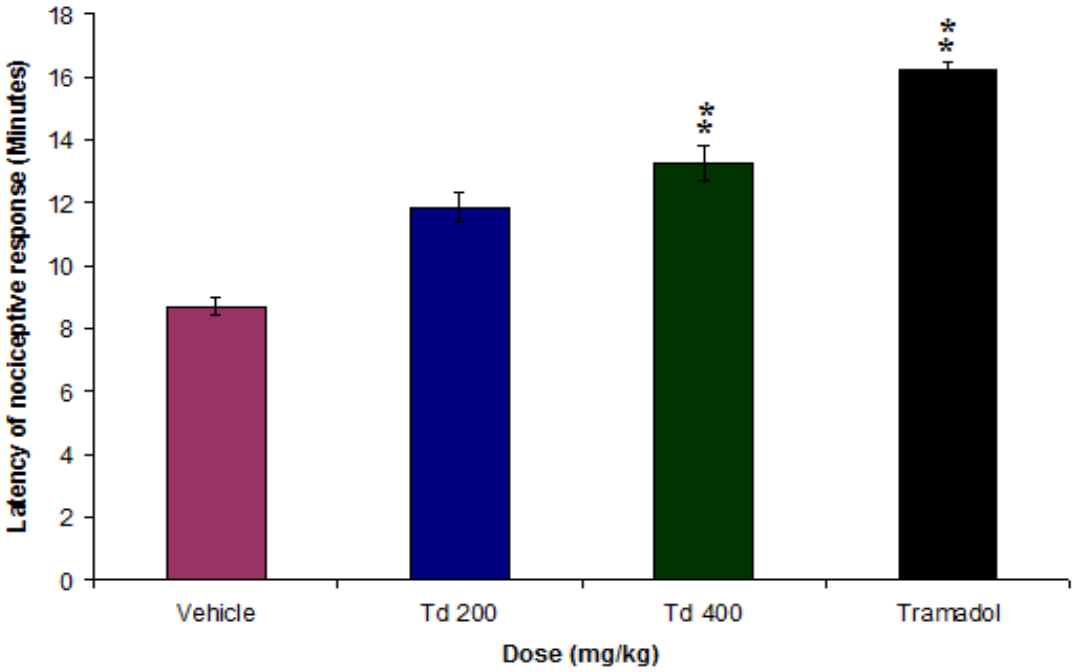

Figure 3.107: Effect of TD (90 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$.

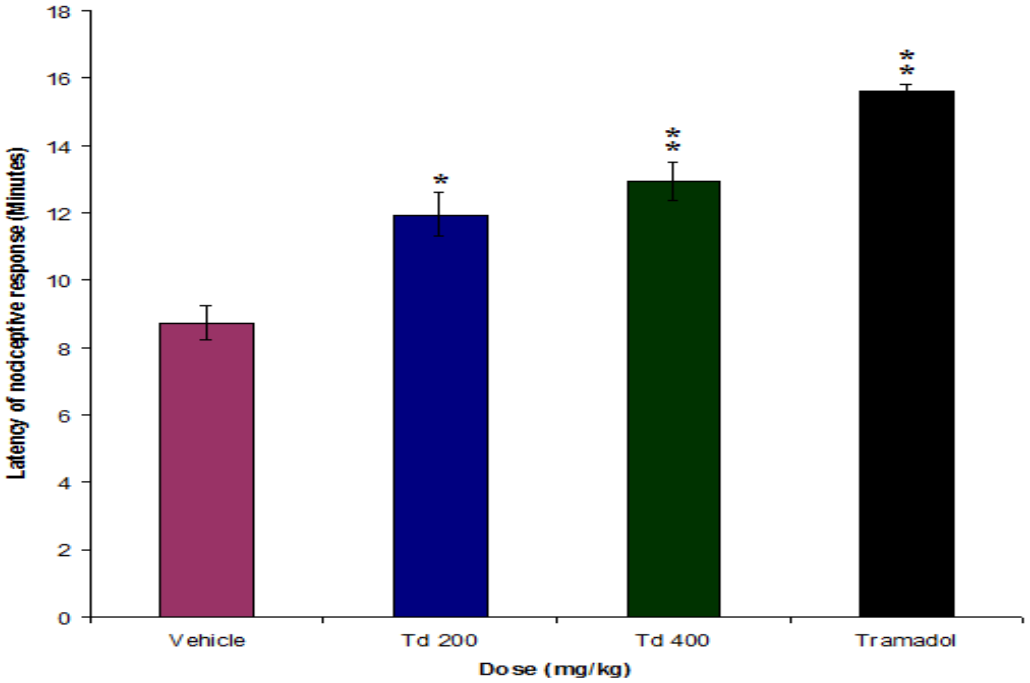

Figure 3.108: Effect of TD (120 minutes) on Hot Plate Method.* $p < 0.05$, ** = $p < 0.01$

3.4.4. Acute toxicity:

All the extracts of selected plants were observed safe at all the subjected doses of 500, 1000 & 2000 mg/kg i.p. In 24 hrs evaluation period, all the tested animals were found normal. No considerable difference was found between saline group and test groups in moving, eating, respiration and others behaviors.

CHAPTER 4

DISCUSSION

4.1. PHYTOCHEMISTRY:

The presence of different phytochemicals, which have been given in Table 3.1-3.4, may show therapeutic activities of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla*. Previous studies on plants indicate that plant derived natural products have extensively been used in various biological activities. Flavonoids have been studied for their pharmacological activities such as anti-inflammatory, anti-allergic, hepatoprotective, anti-oxidant, anti-viral, anti-carcinogenic and anti-thrombotic activities (Najafi *et al.*, 2010). Similarly, tannins are used as hemostatic, anti-hemorrhoidal and anti-diarrheal agents. (Samejo *et al.*, 2013b)

Saponins, which are chemically glycosides of steroids, are found in plants as waxy prtective layer. These compounds have been reported to have various pharmaceutical actions i.e., lowering the cholesterol contents, anti-inflammatory and anti-oxidants activities (Najafi *et al.*, 2010). Moreover, saponins have also been found to possess the property of coagulating and precipitating red blood cells. Some other properties of saponins include hemolytic activity, bitterness, cholesterol binding properties and foam formation in aqueous solutions (Samejo *et al.*, 2013b).

Terpenoids, a group of important naturally occuring substances found in almost evry kind of organism. Terpenoids have been investigated for their biochemical and pharmacological actions. They have been reported to possess antibacterial properties (Selvan *et al.*, 2012). Terpenoids have also been found to play an important role in the concentration of antioxidants in wounds, wound healing, restore inflamed tissues and strengthen the skin (Krishnaiah *et al.*, 2009).

Another diverse class of phytochemicals, Phenolic compounds have been studies against various biological activities including antiinflammatry, antiaging, anticarcers, cardiovascular protection, anti-atherosclerosis, anti-apoptosis, improvement of

endothelial function, inhibition of angiogenesis and cell proliferation activities (Samejo *et al.*, 2013b).

Steroids, a class of important organic compounds have relationship with sex and growth hormones. Various pharmaocological activies like antimicrobial have been reported for steroids (Yadav and Agarwala, 2011).

Thus each class of compounds identified in stem bark extract of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophyll* has its own biological importance and further study of these plants phytochemicals by *in-vivo* and *in-vitro* methods can prove their further medicinal importance in future and can be an effective and efficient drug source in cheaper rate as they have higher biomass availability.

4.2. OIL ANALYSIS:

The stem bark of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* was screened for fatty Acid profile. Data shown in Table 3.4-3.8 confirmed the unsaturated fatty Acids of omega class. Such unsaturated fatty Acids are useful for cardiac diseases. For example, n-3 fatty Acids help in preventing cardiac arrhythmia and thus preventing sudden cardiac death in human. (Leaf and JX., 1996).

There are some poly-unsaturated faty Acids which can not be synthesized by body. Such faty Acids are called essential fatty Acids and must be taken in food for normal functioning of body. Like omega-6 fatty Acids, omega-3 fatty Acids are also essential poly-unsaturated faty Acids. These fatty Acids must obtain from food like canola, sunflower, nut and fish oils.

Fat consumption patterns have been shifted by technological development over the last 100-150 years. The use of omega-6 fatty Acids has specifically been increased due to consumption of various types of oils like corn, soybean and sunflower oils, margarines made from these oils and animal products derived from grain-fed livestocks.

Essential poly-unsaturated fatty Acids have two series of n-6 series and n-3 series.

Fatty Acids of both the series can be synthesized by plants but animals lack this ability and such fatty Acids must be takn in food. Deficiency of such fatty Acids leads to abnormalities. Deficiency of linoleic Acid (n-6 fatty Acid) leads to skin lesions, poor growth, reproductive failure and fatty liver (Connor *et al.*, 1992). n-3 fatty Acids like a-linolenic Acid on other hand produces deficiency symptoms which are very unclear and can only be well demarcated in human infants and experimental animals. Deficiency symptoms produced by n-3 fatty Acids include impaired cognition and behavior, abnormal electro-retinogram results and reduced vision (Neuringer *et al.*, 1984).

Caprylic Acid, which is present in coconut oil, has been found to have antifungal activity. These fatty aci that are found in coconut oil. It is an active antifungal agent that kills *Candida* cells. It also restores stomach Acidity to its normal level. This fatty Acid prevents *Candida* cells alone and in combination also.

All such fatty Acids as Palmitic Acid, Caprylic Acid, Myristic Acid, Lauric Acid, Oleic Acid, g-linolenic Acid, Linoleic Acid and Octadecadienoic Acid, etc. are found in the stem bark extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla.* It is, further, suggested that if oils are extracted from the seeds of the selected plants instead, plenty of omega oil will be screened out.

4.3. *IN-VITRO* BIOLOGICAL ACTIVITIES:

4.3.1. Anti-microbial activity:

Plants form the natural source of bioactive compounds which are directly or their derivatives used as chemotherapeutic agent. For achieving this goal *in-vitro* antimicrobial activities are assayed (Tona *et al.*, 1998). In the current study *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem bark were used for evaluating antimicrobial activity. For this purpose, methanolic extracts of selectedplants stem bark were used. The selected microorganisms used for the present study were five Gram negative bacterial strains i.e. *E. coli, E. carotovora, K. pneumonia. S. typhi, P. aeroginosa* and three Gram positive bacterial strains i.e. *B. subtilis, B. atrophous, S. aureus*. The fungal specie tested was *Candida albicans.*

Results presented in the previous chapter showed that *Tamarix aphylla*, *Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem bark extracts were significantly effective against all the tested microorganisms. Results also indicated that *Klebsella pneumonia, Erwinia carotovora* and *Pseudomonas aeroginosa* were susceptible to all plants. The data further revealed that the extracted samples of selected plants species were equally effective against the tested bacterial strains (both Gram positive and Gram negative) and fungal specie. Results regarding antibacterial activity of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem bark extracted sample showed that it inhibited the growth of *E. coli* (Gram negative bacterium), *Staphylococcus aureus* (Gram positive bacterium) and *Bacillus subtilis* (Gram positive bacterium), such kind of results were also reported by (El-astal *et al.*, 2005; Adams *et al.*, 2011).

All selected plants showed better activity against the tested microbes at concentration of 2 mg $disc^{-1}$, as compared to the concentration of about 1 mg $disc^{-1}$. Antibacterial activity showed that *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem barks can be considered as an important source of antibacterial drugs and they can be used in different diseases. Our results support the folkloric use of tested plants in several ailments such as leprosy (Khare and Chandrama, 2007).

Fungal strains produce various diseases in human beings specially *Candida albicans*. *Candida albicans* produces skin, bronchial and ear candidiasis in human being (Ginter-Hanselmayer *et al.*, 2004; Truss, 1981). In plants, there are various types of compounds which show anti-fungal activities. These may be used in various diseased conditions, which support the traditional use of the plants (Harvey, 2008).

From investigations it is concluded that *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* stem bark extracts exhibited strong antibacterial and antifungal activity. Furthermore, these investigations form the gateway for new bioactive antimicrobial compounds and treating various infectious diseases.

4.3.2. Enzyme Inhibition Activities:

Tamarix aphylla, Tamarix dioica, Acacia cyanophylla and *Acacia stenophylla* were screened for Acetylcholinesterase (AchE) and lipoxygenase inhibitory activities. Acetylcholinesterase has a key role in biological system. This enzyme carries out the breakdown of acetylcholine (ACh) to acetate and choline. Acetylcholinesterase has a central role in the transfer of impulses in the cholinergic system (Collerton, 1986). There are various chemical agents (drugs)generated to target the said enzymespecifically (Davies, 1979). By inhibiting this enzyme, the amount of acetylcholine increases in cholinergic synapses and hence may be used in the therapy of Alzheimer's Disease (AD) patients (Inestrosa *et al.*, 1996).

Tamarix aphylla, *Tamarix dioica*, *Acacia cyanophylla* and *Acacia stenophylla* stem barks were screened for Lipoxygenase inhibitory activity and they showed good activity against this enzyme. Lipoxygenase, a class of enzymes consisting of non-heme iron, catalyzing reactions which are involved in the metabolism of xenobiotic. Fatty Acids are metabolized; which then trigger inflammatory reactions. This enzyme has a magnificent role in the growth of cancer cells, invasiveness, metastasis, cell survival & induces Tumor Necrosis Factor (TNF) (Khan. *et al.*, 2009; Khan *et al.*, 2011a). the active site (Catalytic site) of lipoxygenase (LOX) is consisted of three amino Acid residues which are called catalytic triad (His 523, His 518, Ile 875) as well as iron atom. Many compounds, having the ability of interactions with the active sites may be developed as therapeutically active Lipoxygenase inhibitors. Surprisingly, *Tamarix aphylla*, *Tamarix dioica, Aacia cyanophylla* and *Acacia stenophylla* revealed strong molecular interactions with amino Acid residues inside active site of lipooxygenase particularly the catalytic triad.

The significant enzyme inhibitory effect of *Tamarix aphylla, Tamarix dioica, Aacia cyanophylla* and *Acacia stenophylla* shows that the plant extracts act as LOX inhibitors. These plants may be used for the management of inflammatory conditions and some other related abnormal conditions that are linked with leukotrienes.

4.3.3. Anti-oxidant Activities:

The results revealed that extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* has good antioxidant activity.

Highly reactive radicals are produced under oxidative stress conditions like peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$), superoxide anion (O_2^{-}) and hydroxyl ($OH^{\cdot}$) (Blokhina *et al.*, 2003). Pathological and degenerative conditions are caused by these reactive radicals such as Alzheimer's Disease, cancer, hear disease (coronary) and aging (Diaz *et al.*, 1997), inflammation, neurodegenerative disorders and cataracts and atherosclerosis (Aruoma, 1998). Plants always serve as a natural source for remedies. Plants have been and still are used extensively as a rich source for development of new bioactive antioxidant drugs (Huang *et al.*, 2002). For the past few years, a large number of plants have been investigated for searching novel natural anti-oxidants and radical scavenging agents (Hu and Kitts, 2000). These anti-oxidants provide further protection against oxidative damage caused by free radicals (Brown and Adam., 1998). A large number of natural anti-oxidants have been studied in herbs, vegetables and fruits (Hu and Kitts, 2000). Humans take numerous antioxidants in the form of food items. Antioxidants introduced into humans in the form of foods include tocopherols, ascorbic Acid, terpenoids, carotenoids, phenolic compounds like tannins and flavonoids (Atoui *et al.*, 2005).

Polyphenolic compounds like tannins are likely invloved in lipids stabilization and have role as anti-oxidant agents (Duch *et al.*, 1993). Anti-oxidative actions are directly contributed by these compounds (Duh *et al.*, 1999). Different phenolic compounds have different mechansims of anti-oxidative actions, both alone and in combinations. Metal chelation, reactive oxygen species scavenging and inhibition of generation of free radicals and chain-breaking activity are the different functional properties possessed by these anti-oxidants (Chen *et al.*, 2005). It has been reported that reducing power of the extracts and anti-oxidant capacity have direct correlation. The anti-oxidant potential of compounds is due to their reducing property (by hydrogen atom) as a result breaking of the free radicals chain occurs (Finkel and Holbrook, 2000). Due to these free radical scavenging activities, diseases caused by free radicals and oxidants are treated and

prevented by ant-oxidants which is of great significance (Almeida *et al.*, 1997). In the present study, it was found that all the tested plants have tannins. So, it is suggested to investigate the tannin extracts for isolation and identification of chemical agents which might be used as potential anti-oxidants to prevent or slow the progress of various oxidative stress conditions.

4.4. *IN-VIVO* BIOLOGICAL STUDIES:

4.4.1. Anti-inflammatory activities:

4.4.1.1. Carrageenan-Induced Paw Edema Model:

The antiinflammatory potential of crude methanolic extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla*revealed dose dependent effects at different doses. It is evident from the results shown in previous that the selected plants demonstrated percent antiinflammatory effect. The dose of 200 mg kg^{-1} of the crude methanolic extract measured more significant results particularly at 3 & 5 hours (maximum effect) in contrast with the control drug. The doses of 50 and100 mg kg-1also revealed statistically significant results. Our results support the traditional use of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* in inflammation.

The *in-vivo* anti-inflammatory activity of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* revealed that the carrageenan produced biphasic inflammatory events had been controlled significantly ($p<0.05$) by plant extracted samples and hence might be used as active anti-inflammatory agents. Certain chemical substances such as serotin, histamines and some other related compounds in the first phase (90–180 minutes) of inflammation. Increase in the volume of hind paw characterisez the second phase of inflammation (270-360 minutes). This increase in volume is due to presence of certain inflammatory mediators (Khan *et al.*, 2011b). No significant difference in terms of morbidity and mortality was observed between the animals of treatment and those of negative control.

The data conclude that the anti-inflammatory mechanism of all the selected plants and Indomethacin might be same. It is evident from the present anti-inflammatory activities

that *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* need further research work to isolate new compounds for the treatment of inflammation.

4.4.1.2. Xylene Induced Ear Edema Model:

The inhibitory (anti-inflammatory) potential assayed by Xylene Induced Ear Edema Model of crude extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* at various subjected doses indicated dose dependent action. All the applied doses demonstrated significant inhibitory effect. The inhibitory effect was found statistically more significant at the dose of 200 mg kg^{-1} both at 15 and 60 minutes. Xylene induced ear paw edema model support the presence of anti-inflammatory potential in *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla*.

All the tested plants exerted a significant ($P<0.05$) inhibition at highest dose (200 mg/kg),which might be due to phospholipase A_2 inhibition. An enzyme caled Phospholipase A_2 has a key role in xylene induced inflammation (Lin *et al.*, 1992). All the selected plants were found effective at all the subjected doses (50, 100 and 200 mg/kg) but better effect was observed after one hour (late phase)**.** Stadard drug, Dexamethasone which is a steroid anti-inflammatory agent indicated significant reduction in the mean right ear weight of the tested animals (positive control) due to inhibition of phospholipase A_2 (PL-A2). The results showed that mechanism of action of selected plants extracted samples resembles those of NSAID group of the anti-inflammatory drugs. These drugs have anti-inflammatory activities both in central and peripheral tissue. The significant anti-inflammatory activity of all the tested plants is considered to be due to the fact that these are of typical falvonoid type (Okokon, 2011).

4.4.2. Antipyretic Activity:

4.4.2.1. Yeast Induced Pyrexia:

Several types of antifever drugs are openly available which are frequently taken because of their effectiveness, but these medicines have some limitations. These medicines are associated with the issue of interactions and their side effects. That's why plants are

continuously investigated for pharmaceutically active natural products with less harmful side effects.

The crude methanolic extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* possessed statistically significant antipyretic effect. The reduction in temperature was dose dependent. The antipyretic effect was highest at 3rd hr for all the test doses.

Anti-pyretic activity showed dose dependent decrease in the temperature of mice, treated with the tested samples of selected plants. These results showed that all the subjected extracts of selected plants act both centrally and peripherally like aspirin (Ferreira *et al.*, 1978). Aspirin reduces the fever by decreasing prostaglandin E_2 brain concentration, especially through its action on COX-3 in the hypothalamus too (Vane, 1971).

To test the antipyretic effect of various chemicals isolated from various plants or those made synthetically, Brewer's yeast model is extensively used all around the world (Zakaria *et al.*, 2008). Brewer's yeast administration (S.C route) induces the increase production of prostaglandins, which inturn rise the body temperature (Perianayagam *et al.*, 2004). The available antipyretic drugs in the market (Paracetamol) reduce the body temperature by inhibiting the synthesis of prostaglandins through inhibition of cyclo-oxygenase pathway. Various substances have been identified which act as mediators for hyperthermia in human. These mediators when blocked by different chemicals, antipyretic effect is produced (Lamien *et al.*, 2006; Shukla *et al.*, 2010).

The crude extracts of all selected plants decreased the rectal temperature of tested animals (mice) significantly. This indicates that some compounds are present, which might be involved in the inhibition of prostaglandins. Our research work about antipyretic activity supports the etnobotanical use of selected plants by the local people in different diseases (Abbas *et al.*, 2002).

4.4.3. Analgesic Activities:

4.4.3.1. Acetic Acid-Induced Abdominal Constriction:

Acetic Acid-Induced Abdominal Constriction assay is not a specific test. There are various mechanisms which are responsible for reduction of the muscular constrictions such as sympathetic system through the production of chemical substances called cyclooxygenases (COX) and inhibition of their metabolites, biogenic amines and through mechansims of opioid receptors.

Crude methanolic extracts of *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* showed significant analgesic effects at higher doses. Acetic Acid-Induced Abdominal Constriction Assay is commonly used model. It is sensitive, easy and rapid technique used to measure peripheral analgesic effect. (Atta and Alkofahi, 1998; Gupta *et al.,* 2005).

The constriction of abdominal smooth muscles coupled with extension of the fore limbs and elongation of the body, all these result in writhing. The local receptors (peritoneal) are involved in constriction of abdomin in mice (Honore *et al.*, 2002). The production of prostaglandins is due to the increased sensitization of peritoneal receptors. This is generally considered that when analgesic activity assayed with acetic Acid induced model, release of prostanoids such as PGE2, PGF2α and lipooxygenase derivatives is enhanced in the peritoneal fluids serving as pain mediators. Prostanoids e.g. PGF2α and PGE2 and lipooxygenase derivatives are synthesized by the pathway of cyclo-oxygenase (COX) that is resulted from arachidonic Acid. These are produced from phospholipids of inflammed abdominal tissues (Cipollone *et al.*, 2004; Funk *et al.*, 2013). These chemical substances which liberate in the peritoneal fluids become the causal agents for pain that appears in the form of constrictions (abdominal). The inhibition in number of writhings by different agents is due to inhibition or decreased synthesis of prostanoids. This is considered for inhibition of pain through the peripheral nervous system (Marchand *et al.*, 2005).

The results showed that the extracts of selected plants exerted analgesic effect peripherally by inhibiting abdominal nociceptive receptors. This occurs due to decreased production or inhibition of prostanoids synthesis. Analgesic potential measured by crude methanolic extracts of selected plants in sense of decreased number of writhings was due to biologically active components present in their crude extracts. This suggests that the mechanism of action of plant extracts is purely associated with pain mediators.

4.4.3.2. Hot Plate Method:

Analgesic (anti-nociceptive) activity of isolated compounds from plants has been studied by many research groups. The pseudo akuammigine have opioids like effect (central) that synergize by its peripheral effect and purine alkaloid named acrine, showed good analgesic effect assayed by Hot Plate method (Malairajan *et al.*, 2006).

It is evident from the previous chapter that the crude methanolic extracts of all the tested plants identified to be active analgesic agents assayed by Hot Plate method at the doses of 200 and 400 mg/kg.

The production of endogenous substances and some other pain mediators such as arachidonic Acid by cyclo-oxygenase, and biosynthesis of prostaglandin initiates analgesia with ion in the physiological system (Utar *et al.*, 2011). Supra-spinal analgesia is determined by Hot Plate method, which is a type of spinal reflex test. This test is specifically applied to understand the analgesic effect of drugs and chemicals that act centrally such as morphine and its analogues. Anti-nociceptive drugs that act peripherally are found to be inactive on temperature induced hyper algesia (Coutaux *et al.*, 2005).

It is clear from the above discussion that all the tested plants possess pain killing action through central nevous system like opoids analgesic drugs.

CONCLUSION AND RECOMMENDATIONS

The current research work was comprised of two major areas i. e. phytochemical screening and pharmacological activities. For this purpose, four plants named *Tamarix aphylla, Tamarix dioica, Acacia cyanophylla* and *Acacia stenophylla* had been selected. From our results it can be concluded that:

1. In phytochemical screening, major classes of natural products detected were alkaloids, flavonoids, tannins, terpenes, saponins, carbohydrates etc. Each class of compounds has its own pharmacological potential.
2. The selected plants were also screened for fatty Acids and found oil of emga classes which have important role in health and nutrition.
3. The antimicrobial activities of the selected plants revealed promising activity against fungi, Gram positive and Gram negative bacteria tested.
4. The crude plant extracts showed good antioxidant potential.
5. The selected plants also demonstrated good enzyme inhibitory activity.
6. All the tested plants revealed more significant analgesic, antipyretic and anti-inflammatory activity especially at high doses.

On the basis of the above conclusion, it is recommended that:

1. The studied plants can be used as folk medicines as evident scientifically from our results.
2. Further, the tested plants should be investigated for the isolation and characterization of novel bioactive compounds for use in pharmaceutical industries.

REFERENCES

Abaza, M. Salah, Al-Attiyah, Raja'a, Bhardwaj, Radhika, Abbadi, Ghaneim, Koyippally, Mathew, Afzal, and Mohammad. 2013. Syringic Acid from Tamarix aucheriana possesses antimitogenic and chemo-sensitizing activities in human colorectal cancer cells. *Pharma Biol.,* 51 (9):1110-1124.

Abbas, B., A. Al-Qarawi, and A. Al-Hawas. 2002. The ethnoveterinary knowledge and practice of traditional healers in Qassim Region, Saudi Arabia. *J Arid Envir.,* 50 (3):367-379.

AbouZid, S., and A. Sleem. 2011. Hepatoprotective and antioxidant activities of Tamarix nilotica flowers. *Pharma Biol.,* 49 (4):392-395.

Abouzid, S. F., and Sameh Saleem. 2009. A new ferulic Acid ester and other constituents from Tamarix nilotica leaves. *Chem Pharm Bull.,* 57 (7):740-742.

Adams K.N, Takaki K, Connolly L.E, Wiedenhoft H, Winglee K, Humbert O, Edelstein P.H, Cosma C.L, and R. L. 2011. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. *J.cell.,* 145:39-53.

Adams, K. N., K. Takaki, L. E. Connolly, H. Wiedenhoft, K. Winglee, O. Humbert, P. H. Edelstein, C. L. Cosma, and L. Ramakrishnan. 2011. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. *Cell.,* 145 (1):39-53.

Ahmadi, L., Mirza M, and S. F. 2002. The volatile constituents of Artemisia marschaliana Sprengel and its secretory elements. *Flav Frag J.,* 17:141-143.

Akerele, O. 1984. WHO's traditional medicine programme: progress and perspectives. *WHO chronicle.,* 38 (76):20-25.

Akkari, H., Ben Salem H., Gharbi M., A. S., and Darghouth M.A. 2008. Feeding Acacia cyanophylla Lindl. foliage to Barbarine lambs with or without PEG: Effect on the excretion of gastro-intestinal nematode eggs. *Ani Feed Sci and Technol.,* 147:182-192.

Alamkhan, M. Rahman, and S. Islam. 2008. Antipyretic activity of Peperomia pellucida leaves in rabbit. *Turk J Biol.,* 32 (1):37-41.

Ali, A., Al-Shamma, Enas J. Kadhum, M. M.A., and Al-Hiti. 1979. Antimicrobial Activity And Phytochemical Investigation Of Tamarix Macrocarpa (Ehrenb.)Bge Wildly Grown In Iraq. *J. of Nat. prod.,* 42 (6):633-642.

Ali, H., and M. Qaiser. 2009. The ethnobotany of Chitral valley, Pakistan with particular reference to medicinal plants. *Pak J Bot.,* 41 (4):2009-2041.

Almeida, L. M., A.P. Cunha, T.C. Dinis, and a. J. R. Soares. 1997. Antioxidant activities of some extract of Thymus Zygis. *Free Rad. Res.,* 26:469-478.

Ameh, J. 2010. Pods and roots of acacia nilotica have demulcent, styptic and astringent properties. The stem bark can be ingested for leucorrhoea, diarrhea, dysentery, cough and diabetes mellitus type II. *Tropic J Pharma Res.,* (2):119-125.

Ameyaw, Y., and G. Duker-eshun. 2009. The alkaloid contents of the ethno-plant organs of three antimalarial medicinal plant species in the eastern region of Ghana. *Int. J. Chem. Sci.,* 7 (1):48-58.

Amos, S., Akah P.A, Odukwe C.J, Gamaniel K.S, and W. C. 1999. The pharmacological effects of an aqueous extract from Acacia nilotica seeds. *Phytother. Res.,* 13:683-685.

Anderson, D. M. W. 1978. Chemotaxonomic aspects of the chemistry of acacia gum exudates. *Kew Bull.,* 32 (3):529-536.

Anon. 1955. Acacia cyanophylla as a forage species *Rep Fores Depart Cypr.,* 66.

Antaki., A. 1998. Tazkiradu Ulil Albab. *Beirut: Dar Alkutub Al Ilmiyah* 1:1-3.

Appelqvist, and Lars-ake. 1968. Rapid methods of lipid extraction and fatty Acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. *Arkiv for kemi.,* 28 (36):551-570.

Arias, M. E., J. D. Gomez, N. M. Cudmani, M. A., Vattuone, and M. I. Isla. 2004. Antibacterial activity of ethanolic and aqueous extracts of Acacia aroma Gill. ex Hook et Arn. *Life Sci.,* 75:191-202.

Aruoma, O. I. 1998. Free radicals, oxidative stress and antioxidants in human and disease. *JAOCS.,* 75:199-212.

Asante-Duah, K. 2002. *Public health risk assessment for human exposure to chemicals*. Vol. 6: Springer Science & Business Media.

Asma, E. A., Hichem Ben Jannet, and F. Harzallah-Skhiri. 2013 Effects of Acacia cyanophylla Lindl. extracts on seed germination and seedling growth of four crop and weed plants. *Turk J Biol.,* 37 (3):329-335.

Asolkar, L. V., Kakkar K.K, and C. O.J. 2005. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Part I. *NISCAIR, CSIR.,*:P.p 362.

Atoui, A.K., G. Boskou, P. Kefalos, and a. A. Mansouri. 2005. Tea and herbals infusions: Their antioxidative activity and phenolic profile. Food Chem 89 :27-34. *Food and Chemical Toxicology*.

Atta, A., and A. Alkofahi. 1998. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. *Journal of ethnopharmacology* 60 (2):117-124.

Auribie, M. A. 2011. Antioxidant activity of tannin fromTamarix aphylla L. leaves. *Basra J.Agric.Sci.,* 24 (1):33-37.

Aykac, A., and Y. Akgül. 2010. A new analogue of fatty alcohol from Tamarix hampeana L. *Natur prod res.,* 24 (1):34-39.

Ayoola, G., H. Coker, S. Adesegun, A. Adepoju-Bello, K. Obaweya, E. Ezennia, and T. Atangbayila. 2008. Phytochemical screening and anti-oxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Trop. J. Pharm. Res.,* 7:1019.

Azaizeh, H., N. Salhani, Z. Sebesvari, S. Shardendu, and H. Emons. 2006. Phytoremediation of selenium using subsurface-flow constructed wetland. *Int. J. Phytorem.,* 8:187-198.

Babekov, A. U., A. I. Saidkhodzhaev, and B. M. Keneshov. 1999. Terpenoids of ferula litwinowiana. *Chem. Nat. Compd.,* 135:365.

Ballabh, Basant, and O. P. Chaurasia. 2007. Traditional medicinal plants of cold desert Ladakh-used in treatment of cold, cough and fever. *J. ethnopharmacol.,* 112 (2):341-349.

Barton, G. M. 2008. A calculated response: control of inflammation by the innate immune system. *J. clinical investig.,* 118 (2):413.

Baser, K. H. C., and G. Buchbauer. 2010. Handbook of essential oils: Science, Technology, and Applications. *CRC Press NW.*

Benabdullah, H., K. Gharzouli, S. Khennouf, S. Amira, and S. Soufane. 2014. Phytochemical analysis and anti-lipid peroxidation activity of Tamarix africana L. extracts. *Global J Res. Med.,* 3 (7):278-285.

Benson, L., and R. A. Darrow. 1981. The trees and shrubs of the Southwestern deserts. The University of Arizona Press, Tucson, Arizona.

Bhadange, D. G., and A.B. Jadhao. 2013. *The SIJ Transactions on Advances in Space Research & Earth Exploration (ASREE),* 1 (1).

Bhadange, D. G., and A. B. Jadhao. 2013. Phytochemical Analysis of Tamarix Ericoides Rotti (Tamaricaceae)-A Medicinally Important Plant of West Vidarbha Region.

Bhardwaj, S., and S. Gakhar. 2005. Uses of local plant biodiversity among the tribal communities of pangi valley of district chamba in cold desert himalaya, India. *Indian J. Tradit. Know.,* 75 (4).

Bhattacharya, S., and P. K. Haldar. 2012. Protective role of the triterpenoid-enriched extract of Trichosanthes dioica root against experimentally induced pain and inflammation in rodents. *J Agri & Food Chem.,* 26 (24):2348-2352.

Bhavnani, S. M., and C. H. Ballow. 2000. New agents for Gram-positive bacteria. *Current O. micro.,* 3 (5):528-534.

Bikbulatova, T. N., and L. M. Korulkina. 2001. Composition of Tamarix hokenakeri and T. ramosissima. *Chemistry N. Comp.,* 37:216-218.

Blokhina, O., E. Virolainen, and K. V. Fagerstedt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of bot.,* 91 (2):179-194.

Bouhlel, I., H. Ben Mansour, I. Limem, M. Ben Sghaier, A. Mahmoud, J. Ben Chibani, K. Ghedira, and L. Chekir-Ghedira. 2007. Screening of antimutagenicity via antioxidant activity in different extracts from the leaves of Acacia salicina from the center of Tunisia. *Environ. Toxicol. Pharmacol.,* 23:56-63.

Bowles, E. J. 2003. *The Chemistry of Aromatherapeutic Oils; 3rd Edition Griffin Press.*

Brown, J. E., and C. Adam. 1998. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radic Res.,* 29:247-255.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applica.,* 8:559-568.

Cavar, S., M. Maksimovic, M. E. Solic, A. Jerkovic-Mujkic, and R. Besta. 2008. Chemical composition and antioxidant and antimicrobial activity of two Satureja essential oils. *Food Chem.,* 111 (3):648-653.

Chandra, Anu Mahdi, Abbas Ali Singh, Raj Kumar Mahdi, Farzana Chander, and Ramesh. 2008. Effect of Indian herbal hypoglycemic agents on antioxidant capacity and trace elements content in diabetic rats. *J. med. food.,* 11 (3):506-512.

Chang, H. Y., Y. L. Ho, M. J. Sheu, Y. H. Lin, M. C. Tseng, S. H. Wu, G. J. Huang, and Y. S. Chang. 2007. Antioxidant and free radical scavenging activities of Phellinus merrillii extracts. *Botanical stud.,* 48 (4):407-417.

Chang, S. T., J. H. Wu, S. Y. Wang, P. L. Kang, N. S. Yang, and L. F. Shyur. 2001. Antioxidant activity of extracts from Acacia confusa bark and heartwood. *J. Agric. Food Chem.,* 49:3420-3424.

Chariandy, C. M., C. E. Seaforth, R. H. Phelps, G. V. Pollard, and B. P. S. Khambay. 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. ethnopharmacol.,* 64 (3):265-270.

Chatterjee, J., and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ. Pollut.,* 109:69-74.

Chattopadhyay, D., Arunachalam G, and G. L. 2005. Antipyretic activity of Alstonia macrophylla Wall ex A. DC: An ethnomedicine of Andaman Islands. *J. Pharmacy and Pharma. Sci.,* 8:558-564.

Chaturvedi, S., S. Drabu, and M. Sharma. 2012. Anti-Inflammatory and Analgesic activity of Tamarix gallica. *Int J Pharm Pharm Sci.,* 4 (3):653-658.

Chen, H.J., D.J. Huang, C. D. Lin, and a. Y. H. Lin. 2005. Antioxidant and antiproliferative activities of water spinach (Ipomoea aquatica Forsk) constituents. Bot. Bull., 46: 99-106.

Cheng, L., M.-l. H, and Lars B. 2005. Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial. *Acta Pharm. Sin.,* 26:926-933.

Chopra, R. N., S. L. Nayar, and I. C. Chopra. 1956. Indian medicinal plants. *Glossary of med. plants,* 198.

Ciccarelli, D., F. Garbari, and A. M. Pagni. 2008. The flower of Myrtus communis (Myrtaceae): Secretory structures, unicellular papillae, and their ecological role. *Flora Morphal.,* 203 (1):85-93.

Cipollone, F., B. Rocca, and C. Patrono. 2004. Cyclooxygenase-2 expression and inhibition in atherothrombosis. *Arterioscler, thromb, and vascul biol.,* 24 (2):246-255.

Collerton, D. 1986. Cholinergic function and intellectual decline in Alzheimer's disease. *Neurosci.,* 19 (1):1-28.

Connor, W. E., G. Weidner, S. L. Connor, and J. F. Hollis. 1992. Improvements in hostility and depression in relation to dietary change and cholesterol lowering: the Family Heart Study. *Annals Inter. Med.,* 117 (10):820-823.

Coutaux, A., F. Adam, J.-C. Willer, and D. Le Bars. 2005. Hyperalgesia and allodynia: peripheral mechanisms. *Joint Bone Spine,* 72 (5):359-371.

Cowan, R. S., and B. R. Maslin. 2001. Acacia stenophylla. In Flora of Australia 11B. ABRS and CSIRO Publishing, Canberra and Melbourne,.102-103.

Cunningham, G.M., W. E. Mulham, P. L. Milthorpe, and J. H. Leigh. 1981. Plants of Western New South Wales. Soil Conservation Service of N.S.W. and N.S.W. Government Printing Office.

Dafallah, A. Abdullah, and Z. Al-Mustafa. 1996. Investigation of the anti-inflammatory activity of Acacia nilotica and Hibiscus sabdariffa. *American j. Chinese medi.,* 24 (3):263-269.

Davies, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Sci.,* 264 (5157):375-382.

Davies, P. 1979. Neurotransmitter-related enzymes in senile dementia of the Alzheimer type. *Brain res.,* 171 (2):319-327.

De Sousa, D. P. 2011. Analgesic-like Activity of Essential Oils Constituents. *Molecules,* 16:2233-2252.

De Sousa, D. P., G. A. Júnior, L. N. Andrade, F. R. Calasans, X. P. Nunes, J. M. Barbosa-Filho, and J. S. Batista. 2008. Structure and spasmolytic activity relationships of analogues found in many aromatic plants. *Z. Naturforsch.,* 63:808-812.

De Sousa, D. P., G. A. S. Junior, L. N. Andrade, and J. S. Batista. 2011. Spasmolytic Activity of Chiral Monoterpene Esters. *Rec. Nat. Prod.,* 5:117-122.

Deans, S. G., K. P. Svoboda, M. Gundidza, and E. Y. & Brechany. 1992. Essential oil profiles of several temperate and tropical aromatic plants: their antimicrobial and antioxidant activities. *Acta Hortic.,* 306:229-232.

Deshpande, S. N. 2013. Preliminary Phytochemical Analysis and In Vitro Investigation of Antibacterial Activity of Acacia nilotica against Clinical Isolates. *J Pharmacog and Phytochem.,* 3 (1):84-90.

Dev, S. 1999. Ancient-modern concordance in ayurvedic plants: Some examples. *Environmental Health Perspect.,* 107:783-789.

Diaz, M. N., B. Frei, J. F. Keaney, and J. A. Vita. 1997. Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.,* 337:408-416.

Djurdjevic, L., M. Mitrovic, P. Avlovic, G. Gajic, and O. Ostic. 2006. ecophysiological and biochemical trait of three herbaceous plants growing on the disposed coal combustion fly ash of different weathering stage. *Arch Environ Contam Toxicol.* 50(4): 488-495. .

Dongmo, A. B., T. Nguelefack, and M. A. L. Dubois. 2005. Antinociceptive and anti-inflammatory activities of Acacia pennata wild (Mimosaceae). *J Ethnopharmacol.,* 98 (1):201-206.

Doran, J. C., J. W. Turnbull, P. N. Martensz, L. A. J. Thomson, and N. Hall. 1997. Acacia auriculiformis, in J.C.Doran & J.W.Turnbull (eds), Australian Trees and Shrubs: Species for Land Rehabilitation and Farm Planting in the Tropics. *Aus Centre Inter Agri Res.,*:112-115.

Dorman, H. J. D., and S. G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Applied Microbiol.,* 88:308-316.

Drabu, S., S. Chaturvedi, and M. Sharma. 2012. Antioxidant Activity, Total Phenolic, And Flavonoid Content Of Aerial Parts Of Tamarix Gallica. *Inter. J Phytomed.,* 4 (2):174-180.

Duch, P. D., C. L. Tsai, and G. C. Yen. 1993. The relationship between antioxidant activity and maturity of peanut hulls. *J Agric Food Chem.,* 41:67-70.

Duh, P. D., Y. Y. Tu, and G. C. Yen. 1999. Antioxidant activity of water extract of Harng Jyur (Chyrsanthemum morifolium Ramat). *Lebensmittel-wissenschaft and Technol.,* 32:269-277.

Dumancic, D., and H. N. Le-Houerou. 1980. Acacia cyanophylla Lindl. as supplementary feed for small stock in Libya. In *Browse in Africa.,*: International Livestock Centre for Africa Addis Ababa, 321-325.

Duraisankar, M., and V. Ravichandran. 2012. Antipyretic Potential of Polyherbal Ayurvedic Products. *Asian J Pharm and Clinical Res.,* 5 (2):146-150.

Dymock, W., Warden C.J.H, and H. D. 2005. Pharmacographica Indica. A History of the principal drugs of vegetable origin. New Delhi Shrishti book distributors. 1:556.

Eddy, and B. Nathan. 1959. Compounds Related to Pethidine--IV. New Genaral Chemical Methods of Increading the Analgesic Activity of Pethidine. *J Medicinal Chem.,* 2 (1):31-45.

Edris, A. E. 2007. Pharmaceutical and Therapeutic Potentials of Essential Oils and Their Individual Volatile Constituents. *Phytotheray Res.,* 21:308-323.

El-astal, Z. Y., A. Aera, and A. Aam. 2005. Antimicrobial activity of some medicinal plant extracts in Palestine. *Pak. J. Med. Sci.* 21 (2):P.p.187.

Elisabetsky, E., and Z. Castilhos. 1990. Plants used as analgesics by Amazonian caboclos as a basis for selecting plants for investigation. *Pharmaceut. Bio.* 28 (4):309-320.

Espin, J. C., C. Soler-Rivas, H. J. Wichers, and C. García-Viguera. 2000. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. *J Agri. Food Chem.,* 48 (5):1588-1592.

Everist, S. L. 1969. Use of Fodder Trees and Shrubs. Queensland Department of Primary Industries. *Advisory Leaflet no. 1024.*

Farber, and Eduard. 1950. Bio-active substances in the Nineteenth Century. *Chymia.,*:63-76.

Farnsworth, N. R., O. Akerele, and A. S. Bingel. 1985. *Bull. World Health Organiz.,* 63:965.

Farnsworth, N. R., O. Akerele, A. S. Bingel, D. D. Soejarto, and Z. Guo. 1991. Global importance of medicinal plants, in The Conservation of Medicinal Plants.

Farzana, M. U. Z. N., I.Al Tharique, and Arshiya sultana. 2014. A review of ethnomedicine, phytochemical and pharmacological activities of Acacia nilotica (Linn) willd. *J Pharmacog Phytochem.,* 3 (1):84-90.

Felger, R. S. 2000. Flora of the Gran Desierto and Río Colorado of Northwestern Mexico. University of Arizona Press, Tucson.

FerrÃ¡ndiz, M. L., B. Gil, M. J. Sanz, A. Ubeda, S. Erazo, E. GonzÃ¡lez, R. Negrete, S. Pacheco, M. PayÃ¡a, and M. J. Alcaraz. 1996. Effect of bakuchiol on leukocyte functions and some inflammatory responses in mice. *Journal of pharmacy and pharmacology* 48 (9):975-980.

Ferreira, S. H., B. B. Lorenzetti, and F. M. Corrêa. 1978. Central and peripheral antialgesic action of aspirin-like drugs. *Euro. J pharmacol.,* 53 (1):39-48.

Ferrero-Miliani, L., O.H. Nielsen, P. S. Andersen, and a. S. E. Girardin. 2007. Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin. Exp. Immunol.,* 147:227-235.

Finkel, T., and N. J. Holbrook. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature.,* 808:239-247.

Freire, Carmen SR Silvestre, Armando JD Neto, and C. Pascoal. 2007. Demonstration of long chain n-alkyl caffeates and Δ7- steryl glucosides in the bark of Acacia species by gas chromatography–mass spectrometry. *Phytochem. Anal.,* 18 (2):151-156.

Funk, C. D., W.-C. Song, and G. A. FitzGerald. 2013. Biosynthesis of Eicosanoids. *Yen & Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clin. Manag.,*:108.

Gartner, R. J. W., and I. S. Hurwood. 1976. The tannin and oxalic Acid content of Acacia aneura (mulga) and their possible effects on suphur and calcium availability. *Aus. Veter. J.,* 52:194-195.

Ghani, A. 1998. Medicinal Plants of Bangladesh. *Asiatic Soc.,* 1:p.p.37.

Ghelardini, C., N. Galeotti, and G. Mazzanti. 1999. Local anaesthetic activity of the essential oil of Lavandula angustifolia. *Planta Medica.,* 65:Pp. 700–703.

———. 2001. Local anaesthetic activity of monoterpenes and phenylpropanes of essential oils. *Planta Medica.,* 67:P.p. 564-566.

Ghosh, N. K., B.S.P. Sinha, N.C. Sukul, and I. Ito. 1996. Cestocidal activity of Acacia auriculiformis. *J Helminthol.,* 70:171-172.

Ghouila, H., N. Meksi, W. Hadder, M.F. Mhenni, and H. B. Jannet. 2012. Extraction, identification and dyeing studies of isosalipurposide a natural chalcone dye from Acacia cyanophylla flowers on wool. *Ind Crops Prod.,* 35:31-36.

Gilani. 2005. Trends in ethnopharmacology. *J Ethnopharmacol* 100:43-49.

Gilani, A. H. 1999. Studies on antihypertensive and antispasmodic activities of methanol extract of Acacia nilotica pods. *Phytother. Res.,* 13:665-669.

Ginter-Hanselmayer, G., J. Smolle, and A. Gupta. 2004. Itraconazole in the treatment of tinea capitis caused by Microsporum canis: experience in a large cohort. *Pediatric dermatol.,* 21 (4):499-502.

Gomathi, P., U. K. Mazumder, and M. Gupta. 2011. Anti-pyretic potential of galega purpurea root. *Inter Res J Pharm.,* 2:151-152.

Gomes, N. G. M., M. G. Campos, J. M. C. Orfao, and C. A. F. Ribeiro. 2009. Plants with neurobiological activity as potential targets for drug discovery. *Progress in Neuro-Psychopharmacol & Bio Psychiat.,* 33:1372-1389.

Gotep, J., G. Agada, D. Gbise, and S. Chollom. 2010. Antibacterial activity of ethanolic extract of Acalypha wilkesiana leaves growing in Jos, Plateau State, Nigeria. *Mala J Microbiol* 6 (2):69-74.

Griffin, S. G., S. G. Wyllie, J. L. Markham, and D. N. Leach. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour and Frag J.,* 14:322-332.

Gulco, P. 2001. Medicinal Plants in Folklores of Bihar and Orissa. *CCRUM New Delhi.* 25.

Gunstone, F. D. 2000. Composition and properties of edible oils. *Edible oil processing*:1-33.

Gunstone, F. D., and I. A. & Ismail. 1967. Fatty Acids. Part 13. The synthesis of all the cis n-octadecenoic Acids. Chemistry and Physics of Lipids, 1(3), 209-224.

Gupta, M., U. Mazumder, R. S. Kumar, P. Gomathi, Y. Rajeshwar, B. Kakoti, and V. T. Selven. 2005. Anti-inflammatory, analgesic and antipyretic effects of methanol

extract from Bauhinia racemosa stem bark in animal models. *J Ethnopharmacol.*, 98 (3):267-273.

Gupta, V., P. Mittal, P. Bansal, S. L. Khokra, and D. Kaushik. 2010. Pharmacological potential of Matricaria recutita. *Inter J Pharmaceut Sci & Drug Res.*, 2:12-16.

Habiba, U., U. Bose, and A. Rahman. 2010. Antinociceptive, antidiarrhoeal and cytotoxic activity of Tamarix indica leaf. *Pharmacol online* 1:275-283.

Hakim, A. 2002. Bustanul Mufredat. Part I. New Delhi: Idarae Kitabul Shifa. 64:65.

Hall, N. 1972. The Use of Trees and Shrubs in the Dry Country of Australia. Australian Government Printer, Canberra.

Hall, N. H., R. W. Boden, C. S. Christian, R. W. Condon, F. A. Dale, A. J. Hart, J. H. Leigh, J. K. Marshall, A. G. McArthur, V. Russell, and J. W. Turnbull. 1972. The Use of Trees and Shrubs in the Dry Country of Australia,. *AGPS, Canberra.*:P.p.352.

Hameed, Mansoor Ashraf, Muhammad Al-Quriany, F Nawaz, Tahira Ahmad, Muhammad Sajid Aqeel Younis, Adnan Naz, and Nargis. 2011. Medicinal flora of the cholistan desert: a review. *Pak J Bot.*, 43:39-50.

Harvey, A. L. 2008. Natural products in drug discovery. *Drug discovery today.*, 13 (19):894-901.

Hemamalini, G., P. Jithesh, and P. Nirmala. 2014. in silico screening of phytochemicals identified from Acacia nilotica by GCMS method for its anticancer activity. *Inter J Pharm Sci & Res.*, 5 (6):150-156.

Hickman, J. C. 1993. The Jepson manual: higher plants of California. University of California Press. Berkeley and Los Angeles, CA.:1400.

Honore, P., J. Mikusa, B. Bianchi, H. McDonald, J. Cartmell, C. Faltynek, and M. F. Jarvis. 2002. TNP-ATP, a potent P2X 3 receptor antagonist, blocks acetic Acid-induced abdominal constriction in mice: comparison with reference analgesics. *Pain.*, 96 (1):99-105.

Hoult, J. R. S., M. A. Moroney, and M. PayÃ¡. 1994. [44] Actions of flavonoids and coumarins on lipoxygenase and cyclooxygenase. *Methods in enzymology* 234:443-454.

Hu, C., and D. D. Kitts. 2000. Studies on the antioxidant activity of Echinacea root extract. *J Agric Food Chem.,* 48:1466-1472.

Huang, P.C., and C. C. Lin. 2002. Antioxidant and hepatoprotective effects of Acathaponay Senticosus. *Phytother. Res.,* 14:489-494.

Huang, D.-J., L. Chun-Der, C. Hsien-Jung, and L. Yaw-Huei. 2004. Antioxidant and antiproliferative activities of sweet potato (Ipomoea batatas [L.] LamTainong 57') constituents. *Bot Bull Aca Sin.,* 45.

Hussein, G., H. Miyashiro, N. Nakamura, M. Hattori, and N. Kakiuchi. 2000. Inhibitory effects of sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. *Phytother. Res.,* 14:510-516.

Imelouane, B., H. Amhamdi, J.-P. Wathelet, M. Ankit, K. Khedid, and A. El Bachiri. 2009. Chemical composition and antimicrobial activity of essential oil of thyme (Thymus vulgaris) from Eastern Morocco. *Int. J. Agric. Biol.,* 11 (2):205-208.

Imran, I., L. Hussain, M. Zia-Ul-Haq, K. H. Janbaz, A. H. Gilani, and V. De Feo. 2011. Gastrointestial and respiratory activities of Acacia leucophloea. *J Ethnopharmacol.,* 138 (3):676-682.

Inestrosa, N. C., A. Alvarez, C. A. Perez, R. D. Moreno, M. Vicente, C. Linker, O. I. Casanueva, C. Soto, and J. Garrido. 1996. Acetylcholinesterase accelerates assembly of amyloid-β-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron.,* 16 (4):881-891.

Ingkaninan, K., P. Temkitthawon, K. Chuenchom, T. Yuyaem, and W. Thongnoi. 2003. *J. ethnopharmacol.,* 89:261.

Jiang, Y., and C. Zuo. 1988. Studies of chemical constituents from Tamarix chinesis L. *Acta Pharma Sin.,* 10:004.

Jothy, S. L., Z. Zuraini, and S. Sasidharan. 2011. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of Cassia fistula seeds extract. *J Med Plants Res.,* 5:1941-1947.

Kamal, M., M. Sultan, H. Muhammad, M. Subhan, U. Saad, M. Asim, and T. Shaheen. 2009. Ethnobotanically important plants of district Bannu, Pakistan. *Pak J Plant Sci.,* 15:87-93.

Karunakar, N., K. K. Pillai, S. Z. Husain, and M. Rao. 1997. Investigations of anti-inflammatory activity of Jigrine. *Ind J physio & pharmacol.,* 41:134-138.

Khan, I., M. Nisar, M.R. Shah, H. Shah, S.N. Gilani, F. Gul, S.M. Abdullah, M. Ismail, N. Khan, and W.A. Kaleem. 2011a. Anti-inflammatory activities of Taxusabietane A isolated from Taxus wallichiana Zucc. *Fitoterapia.,* 82 (7):1003-1007.

Khan, I., M. Nisar, F. Ebad, S. Nadeem, M. Saeed, H. Khan, F. Khuda, N. Karim, and Z. Ahmad. 2009. Anti-inflammatory activities of Sieboldogenin from Smilax china Linn.: experimental and computational studies. *J Ethnopharmacol.,* 121 (1):175-177.

Khan, I., M. Nisar, M. R. Shah, H. Shah, S. N. Gilani, F. Gul, S. M. Abdullah, M. Ismail, N. Khan, and W. A. Kaleem. 2011b. Anti-inflammatory activities of Taxusabietane A isolated from Taxus wallichiana Zucc. *Fitoterapia*.

Khan, M., J. Kang, N. Park, J. Cho, M. Lee, H. Fujii, and Y. Hong. 2008. Antipyretic, analgesic, and anti-inflammatory activities of the seaweed Sargassum fulvellum and Sargassum thunbergii in mice. *J Ethnopharmacol.,* 116 (1):187-190.

Khan, S. 2013. In vitro antimicrobial and cytotoxic activity of Tamarix dioica Roxb. leaves. *Turk J Biol.,* 37:329-335.

Khan, S., F. Ullah, and T. Mahmood. 2013. In vitro antimicrobial and cytotoxic activity of Tamarix dioica Roxb. leaves. *Tur J Biol.,* 37 (3):329-335.

Khan, S. U., Khan G.M, and M. S.U.K. 2004. Antifungal activities of Tamarix dioica - An in vitro study. *G J Med Sci.,* 2:40-42.

Khan., I., M. Nisar, F. Ebad, S. Nadeem, M. Saeed, H. Khan, F. Khuda, N. Karim, and Z. Ahmad. 2009. Anti-inflammatory activities of Sieboldogenin from Smilax china Linn.: Experimental and computational studies. *J Ethnopharmacol.,* 121:175-177.

Khanzada, A. N., J. Morris, R. Ansari, P. Slavich, and J. Collopy. 1998. Groundwater uptake and sustainability of Acacia and Prosopis plantations in Southern Pakistan. *Agri Water Manag.,* 36 (2):121-139.

Khare, and P. Chandrama. 2007. *Indian medicinal plants: an illustrated dictionary*: Springer Science & Business Media.

Khennouf, S., Benabdallah H, Gharzouli K, Amira S, Ito H, Kim TH, Yoshida T, and G. A. 2003. Effect of tannins Quercus suber and Quercus coccifera leaves on ethanol-induced gastric lesions in mice. *Agric. Food. Chem.,* 51:1469-1473.

Kitts, D. D., A. N. Wijewickreme, and C. Hu. 2000. Antioxidant properties of a North American ginseng extract. *Molecular and cellular biochem.,* 203 (1-2):1-10.

Koehn, F. E., and G. T. Carter. 2005. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov.,* 4:206-220.

Krishnaiah, D., Devi T, Bono A, and S. R. 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plants Res., 3(2): 67-72.

Kritikar, K. R., and B. D. Basu. 2003. Indian Medicinal plants with illustrations. *Uttaranchal Oriental Press,* 4 (2):1289-1292.

Lamarque, A., D. Maestri, J. Zygadlo, and N. Grosso. 1998. Volatile constituents from flowers of Acacia caven(Mol.) Mol. var. caven, Acacia aroma Gill. ex Hook., Erythrina crista-galli L. and Calliandra tweedii. *Benth. Flav.. Frag. J.* 13:266-268.

Lamien, C. E., I. P. Guissou, and O. G. Nacoulma. 2006. Anti-inflammatory, Analgesie and Antipyretic Activities of Dicliptera verticillata. *Inter J Pharmacol.,* 2 (4):435-438.

Larkindale, J., and B. Huang. 2004. Thermotolerance and antioxidant systems in Agrostis stolonifera: involvement of salicylic Acid, abscisic Acid, calcium, hydrogen peroxide, and ethylene. *J Plant Physiol.,* 161 (4):405-413.

Leaf, A., and K. JX. 1996. Prevention of cardiac sudden death by N-3 fatty Acids: a review of the evidence. *J internal med.,* 240:5-12.

Li, R. W., M. S.P., Leach D.N. Leach, G.D.Lin, and G.Leach. 2003. A cross-cultural study: anti-infl ammatory activity of Australian and Chinese plants. *J Ethnopharmacol.,* 85:25-32.

Lim, T. K. 2014. Acacia cultriformis. In *Edible Medicinal And Non-Medicinal Plants*: Springer, 735-737.

Lin, C., S.C. Lin, C.P. Chang, and M.G. Rosenfeld. 1992. Pit-1-dependent expression of the receptor for growth hormone releasing factor mediates pituitary cell growth.

Lin, H. Y., and S. T. Chang. 2013. Industrial Crops and Products. 49:871-878.

Liolios, C. C., K. Graikou, E. Skaltsa, and I. Chinou. 2010. Dittany of Crete: a botanical and ethnopharmacological review. *J Ethnopharmacol.,* 131 (2):229-241.

Lorenzo, P. Rodriguez-Echeverria, S. Gonzalez, L. Freitas, and Helena. 2010. Effect of invasive Acacia dealbata Link on soil microorganisms as determined by PCR-DGGE. *Appli. Soil Ecol.,* 44 (3):245-251.

Mahato, S. B., and S. Garai. 1997. Advances in microbial steroids biotransformation. *Steroids.,* 62:332-345.

Mahmoud, A., M. Nawwar, A. Sahar, and M. Hussein. 1994. Gall polyphenolics of Tamarix aphylla. *Phytochem.,* 36 (4):1035-1037.

Malairajan, P., G. Gopalakrishnan, S. Narasimhan, and K. J. K. Veni. 2006. Analgesic activity of some Indian medicinal plants. *J Ethnopharmacol.,* 106 (3):425-428.

Mao, Guifeng L, Yingjie H, Yucheng W, and Y. C. 2011. Construction of Forward and Reverse Subtracted cDNA Libraries from the Leaves of Tamarix ramosissima under Drought Stress [J]. *J Northeast Forestry Uni.,* 3 (2):58-63.

Mao, Z. J., L. Guifeng, H. Yingjie, W. Yucheng, and C. Yanguang. 2011. Construction of Forward and Reverse Subtracted cDNA Libraries from the Leaves of Tamarix ramosissima under Drought Stress [J]. *J Northeast Forest Uni.,* 5:52-55.

Marcar, N. E., D. F. Crawford, P. Leppert, T. Jovanovic, R. Floyd, and R. & Farrow. 1995. Trees for Saltland: a Guide to Selecting Species for Australia. CSIRO Division of Forestry, Canberra. .

Marchand, F., M. Perretti, and S. B. McMahon. 2005. Role of the immune system in chronic pain. *Nat Rev Neurosci.,* 6 (7):521-532.

Marchante, Helia Marchante, Elizabete Freitas, and Helena. 2003. Invasion of the Portuguese dune ecosystems by the exotic species Acacia longifolia (Andrews) Willd.: effects at the community level. *Plant Invasions: Ecological Threats and Management Solutions. Leiden, The Netherlands: Backhuys*:75-85.

Marchante, E., A. Kjøller, S. Struwe, and H. Freitas. 2008. Short and long-term impacts of Acacia longifolia invasion on the belowground processes of a Mediterranean coastal dune ecosystem. *Appli Soil Ecol.,* 40:210-217.

Martín, A., S. Varona, A. Navarrete, and M. J. Cocero. 2010. Encapsulation and Co-Precipitation Processes with Supercritical Fluids: Applications with Essential Oils. *Open Chem Eng J.*, 4:31-41.

Martin, V. 1974a. Dyemaking with Australian flora. (Rigby: Australia.).

———. 1974b. Dyemaking with Australian Flora. Rigby: Adelaide, Australia.

Marwat, S. K. 2008. Salvadora persica, Tamarix aphylla and Zizyphus mauritiana: Three Woody Plant Species Mentioned in Holy Quran and Ahadith, and their Ethnobotanical Uses in North Western Part (DI Khan) of Pakistan. *Ethnobotanical Leaflets* 2008 (1):135.

Marwat, S. K., Fazal-ur-Rehman, M. A. Khan, M. Ahmad, M. Zafar, and S. Ghulam. 2011. Medicinal folk recipes used as traditional phytotherapies in district Dera Ismail Khan, KPK, Pakistan. *Pak. J. Bot.*, 43 (3):1453-1462.

Maslin, B. R., and M. W. Mcdonald. 2004. AcaciaSearch - Evaluation of Acacia as a woody crop option for southern Australia: Acacia saligna (Labill.) H. Wendl. Rural Industries Research and Development Corporation.204-214.

Mbatchou, V C. Ayebila, and O. A J. Apea. 2011. Salmonella typhi. *J Animal & Plant Sci.*, 10 (1):1248-1258.

McConkey, M. E., J. Gershenzon, and R. B. Croteau. 2000. Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. *Plant Physiol.*, 122 (1):215-224.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. *Nature.*, 454 (7203):428-435.

Meena, Punar Dutt Kaushik, Pallavi Shukla, Shalini Soni, Anil Kumar Kumar, Manish Kumar, and Ashok. 2006. Anticancer and antimutagenic properties of Acacia nilotica (Linn.) on 7, 12-dimethylbenz (a) anthracene-induced skin papillomagenesis in Swiss albino mice. *Asian Pac J Cancer Prev.*, 7 (4):627-632.

Mersky, Y. H. 1986. Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. *Pain suppl.*, 3:1-226.

Miceli, N., M. F. Taviano, G. D.;, A. Trovato, O. Tzakou, and G. E.M. 2005. Antiinflammatory activity of extract and fractions from Nepeta sibthorpii Bentham. *J Ethnopharmacol.,* 97:261-266.

Michael, R. B. Cunningham, and D. B. Lindenmayer. 2011. Regrowth and revegetation in temperate Australia presents a conservation challenge for reptile fauna in agricultural landscapes. *Biol conserv.,* 144 (1):407-415.

Michael, D. R., Cunningham, Ross B. Lindenmayer, and D. B. 2011. Regrowth and revegetation in temperate Australia presents a conservation challenge for reptile fauna in agricultural landscapes. *Biol conserv.,* 144 (1):407-415.

Michaelides, E. D. 1979. *Mini-monograph on Acacia cyanophylla. Invited paper to technical consultation on fast-growing plantation broadleaved trees for mediterranean and temperate zones held in Lisbon, Portugal 16-20 October. FO:FGB-79-6/1. (FAO: Rome.).*

Midgley, S., and J. Turnbull. 2003. Domestication and use of Australian acacias: case studies of five important species. *Aus Sys Bot.,* 16 (1):89-102.

Miller, J. T, Bayer, and R. J. 2003. Molecular phylogenetics of Acacia subgenera Acacia and Aculeiferum (Fabaceae: Mimosoideae), based on the chloroplast matK coding sequence and flanking trnK intron spacer regions. *Aus Sys Bot.,* 16 (1):27-33.

Mohammad, R. S., Shamsi Roohi, Zaman Malik, and Itrat. 1997. Bark of Acacia Arabica–A Nature's Gift: An Overview.

Mohammedi, Z., and F. Atik. 2012. A review on plants. *J Life Sci.,* 6:883-891.

Mohanty, R. V., Padhy S.N, and D. S.K. 1996. Traditional phytotherapy for diarrhoeal disease in Ganjan and phulbani District of South Orissa. *India Ethanobot.,* 8:60-65.

Monroe, S., and R. Polk. 2000. Antimicrobial use and bacterial resistance. *Current opinion in microbiol.,* 3 (5):496-501.

Morone-Fortunato, I., C. Montemurro, C. Ruta, R. Perrini, W. Sabetta, A. Blanco, E. Lorusso, and P. Avato. 2010. Essential oils, genetic relationships and in vitro establishment of Helichrysum italicum (Roth) G. Don ssp. italicum from wild Mediterranean germplasm. *Indust Crops and Products* 32 (3):639-649.

Moroney, M. A., Alcaraz M, Forder R, Carey F, and H. J. 1988. Selectivity of Neutrophil 5-Lipoxygenase and Cyclo-oxygenase Inhibition by an Anti-inflammatory Flavonoid Glycoside and Related Aglycone Flavonoids. *J Pharm and pharmacol.,* 40:787-792.

Mostafa, L. 2010. Anti-microbial activity of tamarix gallica L. *Adv Natural and Appli Sci.,* 4 (3):289-292.

Muhammad, N. Khan, Inamullah, S. U. Simjee, A. H. Gilani, and H. Perveen. 2008. Anticonvulsant, analgesic and antipyretic activities of Taxus wallichiana Zucc. *J Ethnopharmacol.,* 116 (3):490-494.

Nadkarni, A. K. C. 1979. *Indian Materia Medica, Popular publication Pvt. Ltd, Mumbai,*.

Nadkarni, K. M. 2005. The Indian Plants and Drugs. New Delhi. *Shrishti Book Distributors.,* 4:P.p.5.

Naija, D. S., A. Bouzidi, O. Boussaada, A. N. Helal, M. A. Mahjoub, N. Echafai, and Z. Mighri. 2014a. The anti-oxidant and free-radical Scavenging activities of tamarix boveana and Suaeda fruticosa fractions and related active compound. *Euro Sci J.,* 10 (18).

———. 2014b. The anti-oxidant and free-radical Scavenging activities of tamarix boveana and Suaeda fruticosa fractions and related active compound. *Euro Sci J.,* 10 (18):32-33.

Najafi, S., Sanadgol N, Nejad BS, Beiragi MA, and S. E. 2010. Phytochemical screening and antibacterial activity of Citrullus colocynthis (Linn.) Schrad against Staphylococcus aureus. *J Med Plants Res.,* 4 (22):2321-2325.

Narayan, D. P., and U. Kumar. 2005. Agro's Dictionary of Medicinal Plants. India (Jodhpur): Updesh Purohit for Agrobios. 3.

Naz, F., S. Qamarunnisa, Z. K. Shinwari, A. Azhar, and S. I. Ali. 2013. Phytochemical investigation of Tamarix Indica Willd. and Tamarix passernioides Del Ex Desv. leaves from Pakistan. *Pak. J. Bot.,* 45 (5):1503-1507.

Negi, B. S., and B. P. Dave. 2010. In Vitro Antimicrobial Activity of Acacia catechu and Its Phytochemical Analysis. *Ind J Microbiol.,* 50 (4):369-374.

Neuringer, M., W. E. Connor, C. Van Petten, and L. Barstad. 1984. Dietary omega-3 fatty Acid deficiency and visual loss in infant rhesus monkeys. *J Clin Invest.,* 73 (1):272.

Nunez Guillen, M. Elena, da Silva Emim, J. Artur, C. Souccar, and A. J. Lapa. 1997. Analgesic and Anti-inflammatory Activities of the Aqueous Extract of Plantago major L. *Pharm Biol.,* 35 (2):99-104.

Okokon, J. E. 2011. ANTIINFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF PANICUM MAXIMUM. *African Journal of Biomedical Research* 14 (2):125-130.

Okoro, S., A. Kawo, and A. Arzai. 2012. Phytochemical screening, antibacterial and toxicological activities of Acacia senegal extracts. *Bayero J Pure and appli sci.,* 5 (1):163-170.

Olsen, and C. S. Ambio. 2005. Phytochemical and pharmacological investigations of Ferula narthex. *J. Hum. Environ.,* 34:607.

Ozbek, H., S. Ugras, H. Dulger, I. Bayram, I. Tuncer, G. Ozturk, and A. & Öztürk. 2003. Hepatoprotective effect of F. vulgare essential oil. *Fitoterapia.,* 74:317-319.

Palombo, E. A., and S. J. Semple. 2001. Antibacterial activity of traditional Australian medicinal plants. *J Ethnopharmacol.,* 77 (2):151-157.

Pande, M. B., T. P.M., P. J.S., and S. PC. 1981. Note on the nutritive value of babul (Acacia nilotica L.) seeds (extracted). *Indian J. Anim. Sci.,* 51 (1):107-108.

Patankar, S., S. Dobhada, M. Bhansali, S. Khaladkar, and J. Modi. 2008. Quality of Reporting of Randomised Controlled Trials of Herbal Interventions in ASEAN Plus Six Countries: A Systematic Review. *J Altern Complment Med.,* 14 (10):1287-1290.

Peana, A. T., P. S. D'Aquila, F. Panin, S. G.;, P. Pippia, and M. D. L. Moretti. 2002. Antiinflammatory activity of linalool and linalyl acetate constituents of essential oils. *phytomedi.,* 9:721-726.

Pengelly, A. 2004. The Constituents of Medicinal Plants; Allen & Unwin.

Perianayagam, J. B., S. Sharma, A. Joseph, and A. Christina. 2004. Evaluation of anti-pyretic and analgesic activity of Emblica officinalis Gaertn. *J Ethnopharmacol.,* 95 (1):83-85.

Pomonis, J. D., J. E. Harrison, L. Mark, D. R. Bristol, K. J. Valenzano, and K. Walker. 2003. N-(4-Tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl) tetrahydropyrazine-1 (2H)-carbox-amide (BCTC), a novel, orally effective vanilloid receptor antagonist with analgesic properties: II. in vivo characterization in rat models of inflammatory and neuropathic pain. *J Pharmacol and Experi Ther.*, 306 (1):387-393.

Popoca, J., A. Aguilar, D. Alonso, and M. L. Villarreal. 1998. Cytotoxic activity of selected plants used as antitumorals in Mexican traditional medicine. *J Ethnopharmacol.,* 59:173-177.

Powell, A. M. 1988. Trees and shrubs of Trans-Pecos Texas including Big Bend and Guadalupe Mountains National Parks. Big Bend Natural History Association, Big Bend National Park, Texas.536.

Qadri, S. S. H., K. Jabeen, M. Mahboob, and M. Mustafa. 1987. Haemotoxicity to chicken (Gallus gallus-domesticus) by technical and formulation grades of some phosphoric and synthetic pyrethroid esters. *J. appl. Toxicol.,* 7:367-371.

Quezel, P., and S. Santa. 1963. Nouvelle flore d'Algerie et des regions desertique meridionales. Paris. CNRS, 2

Rahman, A., Al-Shamma, Enas J. Kadhum, and M. M. A. Al-Hiti. 2005. Antimicrobial Activity And Phytochemical Investigation Of Tamarix Macrocarpa (Ehrenb.)Bge Wildly Grown In Iraq. *J. of Nat. prod.* 42 (6):633-642.

Rajbir, S., S. R.R., and T. S.K. 2007. Prehavest foliar application of calcium and boron influences physiological disorders, fruit yield and quality of strawberry (Fragaria ananasa). *Scin Horticul.,* 2 (2):215-220.

Raju, and K. Vadrevu. 2003. Susruta of ancient India. *Indian journal of ophthalmology* 51.2 (119).

Rajvaidhya, S., B. Nagori, G. Singh, B. Dubey, P. Desai, and S. Jain. 2012. A review on Acacia arabica-An indian medicinal plant. *Inter J Pharm Sci Res* 3:1995-2000.

Rao, A. M. a. 2001. *Medicinal Plants of Cholistan Desert In: Medicinal Plants of Pakistan. P.1.*

Rao, G., and Santhanam. 1967. Folklore practices in Orissa. *Health sci.,* 14:161.

Rastogi, K., and M. Mehrotra. 1990. Compendium of Indian medicinal plants. *PID Delhi*, 1.

Rawat, R. B. S. 2006. Approaches towards Evaluation of Medicinal Plants prior to Clinical Trials. *J Ethnopharmacol.,* 14:14-23.

Repetto, M., and S. Llesuy. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz J med and biol res.,* 35 (5):523-534.

Riadh, K., Hanen Falleh, Wided Megdiche, Najla Trabelsi, Baya Mhamdi, Kamel Chaieb, Amina Bakrouf, Christian Magne, and C. Abdelly. 2009. Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. *Food and Chem Toxicol.,* 47:2083-2091.

Rice-Evans, C., N. Miller, and G. Paganga. 1997. Antioxidant properties of phenolic compounds. *Trends in plant sci.,* 2 (4):152-159.

Robinson, T. W. 1965. Introduction, Spread, and Areal Extent of Saltcedar Tamarix in the Western States. *Geological Survey Professional Paper 491-A.*

Rossomando, E. 1990. Measurement of enzyme activity. *Method enzymol* 182:38-49.

Rubanza, C. D. K., M. N. Shem, R. Otsyina, S. S. Bakengesa, T. Ichinohe, and T. Fujihara. 2005. Polyphenolics and tannins effect on in vitro digestibility of selected Acacia species leaves. *Anim. Feed Sci. Technol.,* 119 (1 & 2):129-142.

Rushd, I. 1987. *Kitabul Kulliyat. New Delhi: CRUM.,* 2 (56):114-117.

Sachin, J., Dinesh Kumar Jain, and N. Balekar. 2012. In-Vivo Antioxidant activity of ethanolic extract of Mentha pulegium leaf against CCl4 induced toxicity in rats. *Asian Pacific J Tropic Biomed.,* 3 (2):737-740.

Safa, A., G. B. Nair, and R. Y. Kong. 2010. Evolution of new variants of Vibrio cholerae. *Trends microbiol* 18 (1):46-54.

Said, H. M. 1997. Hamdard pharmacopeia of Eastern Medicine. *Sri Satguru publication, A Division of Indian Book Centre.,* 2 (2):353.

Saidana, D., M. Mahjoub, O. Boussaada, J. Chriaa, I. Chéraif, M. Daami, Z. Mighri, and A. Helal. 2008. Chemical composition and antimicrobial activity of volatile compounds of Tamarix boveana (Tamaricaceae). *Microbiol res.,* 163 (4):445-455.

Salah, A., M. Lefahal, M. Benahmed, S. Louaar, S. Louaar, A. Zallagui, H. Duddeck, and K. Medjroubi. 2010. Antimicrobial Activity of Tamarix gallica L. Extracts and Isolated Flavonoids. *Adv Natural and Applied Sci.,* 4:289-292.

Samejo, M. Q., S. Memon, M. I. Bhanger, and K. M. Khan. 2013a. Comparison of chemical composition of Aerva javanica seed essential oils obtained by different extraction methods. *Pak. J. Pharm. Sci.,* Vol.26, (No.4):757-760.

Samejo, M. Q., A. Sumbul, S. Shah, S. B. Memon, and S. Chundrigar. 2013b. Phytochemical screening of Tamarix dioica Roxb. ex Roch. *J Pharm Res.,* 7 (2):181-183.

Sangwan, N., A. Farooqi, F. Shabih, and R. Sangwan. 2001. Regulation of essential oil production in plants. *Plant growth regul.,* 34 (1):3-21.

Sarker, M. A. M., and M. D. A. M. Sarker. 2009. Antinociceptive activity of methanol extract of Tamarix indica *Bang J Physiol Pharmacol.* 25 (1 & 2):1-3.

Selvan, R., M. A.K.S, S. M.A, and A. N.Md. 2012. Phytochemical screening of Acalypha indica L. leaf extract. *Int journel. Appl. Biol. Pharm. Technol.* 3(2):158-161.

Shah, A., R. F. Cross, and E. A. Palombo. 2004. Identification of the antibacterial component of an ethanolic extract of the Australian medicinal plant, Eremophila duttonii. *Phytother Res.,* 18 (8):615-618.

Shah, B. H., Safdar, B., Virani, S.S., Nawaz, Z., Saeed, S.A. and Gilani, A.H. . 1997. Antiplatelet aggregatory activity of Acacia nilotica is due to blockade of calcium influx through membrane calcium channels. *Gen. Pharmacol. 29(2): 251-5.*

Shaheen, F., M. Ahmad, M. T. H. Khan, S. Jalil, A. Ejaz, M. N. Sultankhodjaev, M. Arfan, and M. I. Choudhary. 2005. Alkaloids of Aconitum laeve and their anti-inflammatory, antioxidant and tyrosinase inhibition activities. *Phytochem.,* 66 (8):935-940.

Shahidullah. 2000. Ethnobotanical Studies of district Bannu, N.W.F.P., Pakistan. *Ethnobot Leaf.,* 12:111-113.

Sharma, S. K., and V. S. Parmar. 1998. New natural products and their natural studies. *J Sci and Indus Res.,* 57:873-890.

Shinwari, Z. K., and S. A. Gilani. 2010. Phytochemical studies of medical plant species of Pakistan. *J. Med. Plant. Res.,* 4:161.

Shrestha, P. M, Dhillion, and S. S. 2003. Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. *J Ethnopharmacol.,* 86 (1):81-96.

Shreve, F., and I. L. Wiggins. 1964. Vegetation and flora of the Sonoran Desert: Vols. I and II. *Stanford University Press. Stanford, California.,*:P.p.1740.

Shukla, S., A. Mehta, P. Mehta, S. P. Vyas, S. Shukla, and V. K. Bajpai. 2010. Studies on anti-inflammatory, antipyretic and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. *Food and Chem.Toxicol.,* 48 (1):61-64.

Skold, M., L. Hagvall, and A. T. Karlberg. 2008. Autoxidation of linalyl acetate, the main compound of lavender oil, creates potent contact allergens. *Contact Dermat.,* 58:P.p.9-14.

Skold, M., A. T. Karlberg, M. Matura, and A. Borje. 2006. The fragrance chemical-caryophyllene air oxidation and skin sensitization. *Food and Chem Toxicol.,* 44:538-545.

Soll, J., P. Burke, and R. P.-C. Hsia. 2007. Cultural Translation in Early Modern Europe. New York: Cambridge University Press and European Social Science Foundation. *Amer Histor Rev.,* 113 (3):893-894.

Sotohy, S. A., Sayed A.N, and A. M.M. 1997. Effect of tannin-rich plant (Acacia nilotica) on some nutritional and bacteriological parameters in goats. *Dtsch Tierarztl Wochenschr.,* 104:432-435.

Souliman, A. M., H. H. Barakat, A. M. El-Mousallamy, M. S. Marzouk, and M. A. Nawwar. 1991. Phenolics from the bark of Tamarix aphylla. *Phytochem.,* 30 (11):3763-3766.

Spacer, C. B., and C. D. Breder. 1994. The neurologic basis of fever. . *New England J. Med.,* 330:1880-1886.

Sugawara, Y., C. Hara, K. Tamura, Fujii, T.; , K. Nakamura, T. Masujima, and T. Aoki. 1998. Sedative effect on humans of inhalation of essential oil of linalool: Sensory evaluation and physiological measurements using optically active linalools. *Anal Chim Acta.,* 365:293-299.

Sultanova, N., T. Makhmoor, A. Yasin, Z.A. Abilov, V.B. Omurkamzinova, Atta-ur Rahman, and M.I. Choudhary. 2004. *Planta Med.,* 70 (1):65-67.

Sultanova, N., T. Makhmoor, Z.A. Abilov, Z. Parween, V.B. Omurkamzinova, and A. Rahman;. 2001. Traditional indian medicines used for the management of diabetes. *J Ethnopharmacol.,* 78:201-205.

Sultanova, N. T., Z. A. Makhmoor, Z. Parween, V.B. Omurkamzinova, and A. Rahman. 2002. Anti-emetic effects of bioactive natural products. *Chem Natural Comp.,* 38 (1):32-35.

Svoboda, K., J. Hampson, and T. Hunter. 1999. Secretory tissues: Storage and chemical variation of essential oils in secretory tissues of higher plants and their bioactivity, Vol.9, pp. 124-131. *Inter J Aromather.,* 9:124-131.

Tappel, A. 1962. Anti-lipoxygenase activity of some indigenous medicinal plants. *Method enzymol.,* 5:539-541.

Tesky, J. L. 1992. Tamarix ramosissima. In: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Fire effects information system. Website: http://www.fs.fed.us/database/feis/plants/tree/tamram/all.html.

Tona, L., K. Kambu, N. Ngimbi, K. Cimanga, and A. Vlietinck. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. *J Ethnopharmacol.,* 61 (1):57-65.

Tong, L. M., S. Sasaki, D. J. McClements, and E. A. Decker. 2000. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J Agri & Food Chem.,* 48 (5):1473-1478.

Trivedi, J. K., L. M. Bahuguna, S. C. Tewari, and N. Lal. 1986. Bronchodilator and anti-inflammatory effect of glycossidal fraction of Acacia farnesiana. *Indian J Physio Pharmacol.,* 89 (4):685-691.

Truss, C. O. 1981. The role of Candida albicans in human illness. *J Orthomol Psychiat.,* 10 (4):228-238.

Turner, R. 1965. Screening Methods in Pharmacology. *Acad press.,* 1:105-108.

Turner, R. M., and D. E. Brown. 1982. Sonoran desertscrub. In: Brown, D.E. (ed.). Biotic communities of the American Southwest - United States and Mexico. Desert Plants 4 (1&4):181-221.

Umbetova, A. K., S. Z. Esirkegenova, M. I. Chaudry, V. B. Omurkamzinova, and Z. A. and Abilov. 2004. Flavonoids of plants from the genus Tamarix. *Chem Natural Comp.,* 40:297-298.

United States Department of Agriculture, N. R. C. S. 2001. The PLANTS database, Version 3.1 (http://plants.usda.gov/plants/). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Utar, Z., M. I. A. Majid, M. I. Adenan, M. F. A. Jamil, and T. M. Lan. 2011. Mitragynine inhibits the COX-2 mRNA expression and prostaglandin E2 production induced by lipopolysaccharide in RAW264. 7 macrophage cells. *Journal of ethnopharmacology*.

Valsala, H., C. J., Arntzen, and J. U. Gutterman. 2001. Avicins, a family of triterpenoid saponins from Acacia victoriae (Bentham), inhibit activation of nuclear factor-kB by inhibiting both its nuclear localization and ability to bind DNA. *PNAS* 98 (20):11557-11562.

Van, W. B.-E. 2008. A review of Khoi-San and Cape Dutch medical ethnobotany. *J Ethnopharmacol.,* 119 (3):331-341.

Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature: New biology* (231):232-235.

Vartak, S., M. E. Robbins, and A. A. Spector. 1997. Polyunsaturated fatty Acids increase the sensitivity of 36B10 rat astrocytoma cells to radiation-induced cell kill. *Lipids.,* 32 (3):283-292.

Ventura, M. R., J. I. R. Castanon, M. C. Pieltain, and M. P. Flores. 2004. Nutritive value of forage shrubs: Bituminaria bituminosa, Rumex lunaria, Acacia salicina, Cassia sturtii and Adenocorpus foliosus. *Small Ruminant Res.,* 52 (1):13-18.

Verma, Sheetal, and S. P. Singh. 2008. Current and future status of herbal medicines. *Veter world* 1 (11):347-350.

Wadood, A., Wadood N, and S. S.A. 1989. Effects of Acacia arabica and Caralluma edulis on blood glucose levels of normal and alloxan diabetic rabbits. *J. Pak. Med. Assoc.,* 39 (8):208-212.

Wallace J.M, and W. E.L. 1975. Lipoxygenase from wheat an examination of its reaction characteristics. *J. Agric. Food Chem.,* 23:146-150.

Watari, K., S. Nakao, A. Fotovati, Y. Basaki, F. Hosoi, B. Bereczky, R. Higuchi, T. Miyamoto, M. Kuwano, and M. Ono. 2008. Role of macrophages in inflammatory lymphangiogenesis: enhanced production of vascular endothelial growth factor C and D through NF-κB activation. *Biochem and biophys res communica.,* 377 (3):826-831.

Xing, Y., J. Liao, Y. Tang, P. Zhang, C. Tan, H. Ni, X. Wu, N. Li, and X. Jia. 2014. ACE and platelet aggregation inhibitors from Tamarix hohenackeri Bunge (host plant of Herba Cistanches) growing in Xinjiang. *Pharmacog magazine.,* 10 (38):111.

Yadav, R. N. S., and M. Agarwala. 2011. Phytochemical analysis of some medicinal plants. *J Phytol.,* 3 (12):10-14.

Yao, J. S., and Jama. 1990. Health data in the information age. *J. Am. Med. Assoc.,* 264:3075.

Yen, G. C., P. D. Duh, and D. Y. Chuang. 2000. Antioxidant activity of anthraquinones and anthrone. *Food Chem.,* 70 (4):437-441.

Yoshida, and Takashi et al. 1993. Tannins and related polyphenols of Tamaricaceous plants Part 4-Dimeric hydrolysable tannins from tamarix Pakistamica phytochemistry. *Phytochem.,* 33 (1):197-202.

Yoshimoto, T., M. Furukawa, S. Yamamoto, T. Horie, and S. Watanabe-Kohno. 1983. Flavonoids: potent inhibitors of arachidonate 5-lipoxygenase. *Biochemical and biophysical research communications* 116 (2):612-618.

Zaidan, M., A. Noor Rain, A. Badrul, A. Adlin, A. Norazah, and I. Zakiah. 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop Biomed.,* 22 (2):165-170.

Zakaria, Z., A. Ghani, Z. D. F. Abdul Nor, R. Nur, S. R. Mohd, Gopalan, H. Kumar, Sulaiman, Mohd Roslan Jais, A. M. Mat, M. N. Somchit, Kader, Arifah Abdul Ripin, and Johari. 2008. Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of Dicranopteris linearis leaves in experimental animal models. *J natural med.,* 62 (2):179-187.

Zenk, and H. Meinhart. 2007. Evolution and current status of the phytochemistry of nitrogenous compounds. *Melanie Juenger Phytochem.,* 68 (22):2757-2772.

Zeweil, and H. Saber. 2008. Advisor Committee, Alexandria University.

Zhang, Y., D. Zhang, and Y. Chen. 2003. Plant communities and their interrelations with environmental factors in the middle reaches of the Tarim River. *Acta Geographica Sin.,* 58 (1):109-118.

Ziaulhaq, M., Sanjajavar, M. Qayum, I. Khan, and S. Ahmad. 2013. Chemical composition and antioxidant potential of Acacia leucophloea Roxb. *Acta Bot. Croat.,* 72 (1):133-144.

Zurier, R. 1993. Fatty Acids, inflammation and immune responses. *Prostaglandins, leukotrienes and essential fatty Acids* 48 (1):57-62.