In the Name of Allah,

The Most Beneficent The Most Merciful
EVALUATION OF SOME INDIGENOUS HERBAL

PLANT EXTRACTS FOR THEIR ANTIEMETIC

ACTIVITY

Thesis submitted in the fulfillment of the requirements for the award of the degree of Doctor of Philosophy

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With love and respect,
I dedicate this work to my Parents,
my Husband and my Children
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ABSTRACT

Emesis is a common problem especially in females during pregnancy (motion sickness). The market available allopathic drugs are costly and a number of adverse effects are reported for them. It was, therefore, considered worthwhile to look for some cheap herbal medicine, capable of preventing or inhibiting emesis and which may be easily procurable in both rural and urban areas of Pakistan. Keeping this objective in view, Cymbopogon citraus and Prunus domestica were selected to evaluate its anti-emetic action. Both the herbs are indigenous, cost effective and safe for use.

Cymbopogon citratus

On phytochemical analysis it was observed that alcoholic extract of C. citratus leaves possess most important vital chemical constituents like triterpenes, alkaloids, tannins, saponins, flavonoids, sterols, protein and carbohydrates. A dose dependant antioxidant activity is present in crude extract of the leaves of C. citratus and its ethyl acetate fraction, while in the case of chloroform and n-butanol fractions a non-significant activity was found which is increasing very little as the concentration is increased. Significant antibacterial activity is found against Salmonella typhi. A moderate activity was found against Bacillus cereus and Proteus mirabilis. According to acute oral toxicity test results, the test drug C. citratus was found safe up to the dose of 7.5g/kg body weight. It was found that the test drug has slight CNS depressant effects. C. citratus extract possesses significant analgesic activity persisting for 1 hour and then the effect started diminishing up to 4 hours at 300mg/kg dose. When the dose is increased to 500mg/kg body weight the values are slightly increased. The results of anti-emetic assay revealed that C.
*Citratus* possesses very low activity against emesis at lower dose (26.45% inhibition) and high activity against emesis at high dose (48.23% inhibition).

**Prunus domestica**

The phytochemical screening reveals that the alcoholic extract of *P. domestica* contains tannins, saponins, flavonoids, sterols, protein and carbohydrates which were also confirmed by other research studies. Significant antioxidant activity at all concentrations in ethyl acetate fraction and at 5% concentration in crude extract and chloroform fractions was found. A highly significant antibacterial activity is present against *E. coli, S. typhi, K. pneumoniae* and *P. mirabilis*. The ethanol extract of *P. domestica* did not show any untoward effect up to the dose of 5g/kg body weight. The animals were found active and alert after the administration of the test drug at both doses. They were found to have a strong grip up to 4 hour period of observation. Their pain, sound and touch responses were found to be normal. Pinna reflex was also found to be accurate. The extract of *P. domestica* has a CNS stimulant effect up to 3 hours of administration of drug.

The results of analgesic activity revealed that at 500mg/kg dose, possesses highly significant and prolonged activity in dose dependent manner. In anti-emetic assay the results are expressing mild significant activity at 300 mg/kg dose and highly significant activity at the dose of 500mg/kg body weight, which is almost equivalent to that of standard anti-emetic drug i.e. Motilium.

In short after pharmacological screening of ethanol extracts of *C. citratus* and *P. domestica*, it was found that both the plants extracts possess significant activities against different disease related with nausea and vomiting. Further research can be carried out to find the mode of action and the chemical constituents responsible for these pharmacological actions.
1. INTRODUCTION

1.1 EMESIS

Emesis is a defense mechanism gifted to human body to expel out the poisonous or hazardous substances from the human body which enter by mistake (Mitchelson F, 1992). Sometimes emetic stimuli emerge as a result of movement, surgical operations, pregnancy, drug treatment and nuclear radiation. Disliked odors and unpleasant sights are also responsible for emesis. Whatever may be the cause of nausea and vomiting, these are distressing to the patient. Emesis or vomiting process can be comprised into the following three phases.

*Nausea*

Nausea is a disgusting feeling which is further converted into vomiting. The symptoms of nausea are cold sweat, salivation, disturbance in gastrointestinal tone and contraction of duodenum. As a result the intestinal contents are refluxed into stomach, and a feeling of emesis is developed.

*Retching*

Retching is a different term in which all symptoms are of vomiting except that the glottis is closed and no expulsion of gastric contents. There is labored spasmodic respiratory movements, contractions of abdominal and chest wall muscles but no evacuation of gastric contents in retching.

*Vomiting*

Vomiting is after a powerful contraction of abdominal and chest wall muscles, as a result the diaphragm is ascended and the gastric cardia is opened. This reflux activity is not under the control of individual. The stomach is evacuated with a force and the contents are expelled out from the individual’s mouth (Koch, 1995).
Receptors and Transmitters involved in emesis

A number of receptors and neurotransmitters comprising of various types are present in the brain area which play role in emesis and its control. In the gastrointestinal tract there are peripheral receptors which are also involved in emesis. The neurotransmitters involved in emesis are histamine, acetylcholine, dopamine, nor adrenaline and 5-Hydroxytryptamine. The antagonists of these receptors may produce anti-emetic activity (Leslie and Gwynn, 1984; Schwartz et al., 1986; Koch, 1995, Quingley et al., 2001).

Causes of emesis

Nausea and vomiting are caused mainly as a result of particular drug treatment like the drug therapy in cancer and surgery, labyrinth disorders, meniere's disease, endocrine causes including pregnancy, infections of gastrointestinal tract, haemorrhage, meningitis, different drugs used after operation including anaesthetics and analgesics and central nervous system problems like migraine and bulimia nervosa.

Treatment of emesis

The patients of nausea and vomiting are managed firstly through the identification and elimination of the underlying cause, then controlling the symptoms and finally the correction of electrolyte, fluid or nutritional deficiencies.

Anti-emetic Drugs

The anti-emetic drugs used are anticholinergics like scopolamine, anti-histamines like cinnarizine, cyclizine, promethazine, etc., cannabinoid like nabilone, corticosteroid like dexamethasone, histamine analogues e.g. betahistine, dopamine antagonists including metoclopramide, domperidone and haloperidol, and antagonists of 5HT3 receptor comprising of granisetron, ondansetron and tropisetron (British National Formulary, 2002). An approach of combination of
drugs is often used in clinical practice for the management of different types of nausea and vomiting.

Emesis is a common problem especially in females during pregnancy (motion sickness). All of the above mentioned allopathic drugs are costly and a number of adverse effects are reported for them. It was, therefore, considered worthwhile to look for some cheap herbal medicine, capable of preventing or inhibiting emesis and which may be easily procurable in both rural and urban areas of Pakistan. Keeping this objective in view, *Cymbopogon citraus* and *Prunus domestica* were selected to evaluate its anti-emetic action. Both the herbs are indigenous, cost effective and safe for use.
1.2 **CYMBOPOGON CITRATUS (LEMON GRASS)**

![Image of Cymbopogon citratus](image)

**Figure 1:** Whole plant and fresh leaves of *C. citratus*

<table>
<thead>
<tr>
<th>KINGDOM</th>
<th>Plantae</th>
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<tr>
<td>SUBKINGDOM</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>SUPERDIVISION</td>
<td>Spermatophyta</td>
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<tr>
<td>DIVISION</td>
<td>Mangoliophyta</td>
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<tr>
<td>CLASS</td>
<td>Liliopsida</td>
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<tr>
<td>SUBCLASS</td>
<td>Commelinidae</td>
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<tr>
<td>ORDER</td>
<td>Cyperales</td>
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<tr>
<td>FAMILY</td>
<td>Poaceae</td>
</tr>
<tr>
<td>GENUS</td>
<td>Cymbopogon</td>
</tr>
<tr>
<td>SPECIES</td>
<td>citratus</td>
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(Wikipedia, 2010)
Chemical composition of *C. citratus* leaves and oil is studied by many researchers. Kulkarni (2000) described the commercial importance of oil which has citral as the main constituent. Significant natural variability also exists in lemongrass at the chemotypic level. De Vasconcelos *et al.*, (2000) quantitatively analyzed leaves of *Cymbopogon citratus* for chloride ions and found that it varied from 11.0 mg/100 g. Kasali *et al.*, (2001) reported after detecting the compounds of the oil of *C. citratus* extracted from the lemon grass leaves growing in Lagos State University campus. Twenty three constituents were identified. The main constituents were geranial, neral and myrcene. Small amounts of neomenthol, linalyl acetate, Z-β-ocimene and E-β-ocimene were also present. Dudai *et al.*, (2001) studied that extraction efficacy of lemon grass oil was increased by treating with cell wall hydrolyzing enzyme, but it was still smaller in quantity than the oil extracted through distillation. The major components found were neral and geranial. Schaneberg and Khan (2002) reported a gas chromatography flame ionization detection method for the quantification compounds like neral, geranial, limonene, citronellal, and β-myrcene in the lemon grass (*Cymbopogon citratus*) oil. The authors reported different procedures including solvent extraction, steam distillation extraction by sonication with nonpolar solvents for the extraction of essential oils from *C. citratus* and compared those with each other. All the other methods showed comparable results to the steam distillation method. Almeida *et al.*, (2002) determined the levels of sodium, potassium, calcium, magnesium, iron, aluminum, manganese, and zinc in samples of *C. citratus* along with some other plants. The plants were dried and then extracted in boiling water. Atomic Absorption Spectrophotometer was used for the detection of calcium, magnesium, manganese, and zinc, while Molecular Absorption Spectrophotometer was used for the detection of aluminum and iron, and Flame Emission Photometry was used for the detection of potassium and sodium. The
data obtained were compared with the daily recommended values (RDA). No fruitful results were obtained in terms of *C. citratus*. Carlson *et al.*, (2001) extracted *C. citratus* oil with dense carbon dioxide at a 23°C and 50°C temperatures with 85 and 120 bar pressure respectively. The samples were collected in between initial and final hours of the extraction period. Gas chromatography-mass spectrometry was the technique used for analyzing the samples. The processing condition of 120 bars pressure and 40°C temperature was thought to be ideal for the extraction of *C. citratus* oil. The experiment is useful for obtaining good-quality product with an improved rate of extraction and high yield. Shahi (2005) reported that from some accessions of *Cymbopogon citratus* found in India contained citral as the major constituent i.e. approximately about 80% which is widely used in the flavor and scent production. The essential oil quality index can be predicted through a multiple regression equation. The given equation can be used for obtaining a better quality of essential oil. The validation of the essential oil quality index model is carried out by the correlation of the predicted and calculated values of citral (%) which exhibited significant r-value. Description of essential oil quality through a mathematical model would be useful in evaluating the growth processes and crop management strategies. Chahal *et al.*, (2007) reported that thin layer chromatographic analysis of lemon grass oil which showed five compounds having Rf values of 0.13, 0.15, 0.29, 0.58, and 0.76 respectively. The efficacy of *C. citratus* oil and along with its fractions was tested against the adults of *Tribolium castaneum*. The oil was found to be effective at all the concentrations tested i.e. 50-2000 mg/g. The polar fraction was purified and characterized which showed more activity than the non-polar fractions. Shah *et al.*, (2011) identified compounds including terpenes, alcohols, aldehyde, ketones and esters in *Cymbopogon citratus*. The other reported phytoconstituents are citral a, citral b, citronellal, flavonoids, geraniol, geranyl acetate,
myrecene, nerol, phenolic compounds (luteolin, isoorientin 2’-O-rhamnoside, quercetin, kaempferol and apiginin), terpinolene and terpinol methylheptenone.

Antimicrobial activity is reported by different scientists. Singatwadia and Katewa (2001) studied the essential oil obtained from Cymbopogon citratus against some fungal strains including Aspergillus fumigates, A. niger, Candida sp., Cladosporium sp., Mucor, Trichophyton rubrum, and Trichophyton violaceum, which are pathogenic for human beings. The oils of both plants were found toxic against the above mentioned strains. The essential oil of C. citratus was more effective against Cladosporium sp., Aspergillus inger and Mucor in low concentration, while the oil of C. martini was effective against Candida sp., Aspergillus fumigates, and Trichophyton rubrum. The oils of C. citratus and C. martini suppressed the fungal growth at similar concentrations in Trichophyton violaceum, the concentration of 0.1% of both the oils was found to be lethal for all the above strains. Rauber et al., (2002) reported that essential oil of Cymbopogon citratus can be used in emulsions and nanocapsules for the treatment of fungal infections of the skin caused by Candida, Epidermophyton flaccosum, Microsporum canis, and Trichophyton rubrum. Abe et al., (2003) reported the effect of lemon grass essential oil, which is used as antifungal in aromatherapy treatment. The effect was studied on the growth of Candida albicans. Mycelial growth of C. albicans is responsible for the invasion of mucosal tissue by the fungus. This was inhibited by 100 µg/mL of lemongrass oil in the medium. The lemongrass oil and its major component citral were found to be effective. Paranagama et al., (2003) studied and developed a natural fungicide against fungal strain Aspergillus flavus, which produces aflatoxins in the stored rice, with the oil of lemon grass. C. citratus oil activity was determined against Aspergillus flavus and it was found both fungistatic at 0.6 mg/ml and fungicidal at 1.0 mg/ml concentration. It completely suppressed
the aflatoxin production at 0.1 mg/ml concentration. The oil was further analyzed by thin layer and
gas chromatographic assays and it was found that “citral a” and “citral b” are the major antifungal
constituents in the test compound. Then fumigant toxicity assay of the oil was performed and it
was found that the spore formation of *A. flavus* was suppressed at 2.80 mg/ml and the mycelial
growth was inhibited at and 3.46 mg/ml concentrations. These results suggest that oil of lemon
grass can be used in the management of aflatoxin problem during rice storage. Shin (2005) tested
both *C. citratus* oil and citral for antimicrobial activity against the antibiotic acceptable and
resistant strains of *Salmonella enteritidis* and *S. typhimurium*. The oil significantly inhibited both
the strains with a minimum inhibiting concentration indexes in the range of 0.28 to 1.00 which was
evaluated through checkerboard microtiter assay. Significant synergistic or additive effects were
obtained with the essential oil fractions of *C. citratus* and citral with streptomycin or kanamycin
against *S. typhimurium* strains. In short, a combination of lemongrass oil or citral and streptomycin
can be used for reducing the minimum effective dose of antibiotic for the treatment of resistant *S.
typhimurium* infections. Wannissorn *et al.*, (2005) conducted the microbiological evaluation of
indigenous lemon grass oil of Thailand by using disk diffusion assay. The activity was evaluated
against different pathogenic organisms including *Salmonella species, Escherichia coli, Campylobacter jejuni*, and *Clostridium perfringens* which play a vital role in export of poultry.*Cymbopogon citratus* showed significant micro organism inhibiting activity against all the test
strains. Mahanta *et al.*, (2007) carried out microbiological evaluation of essential oil of *C. citratus*
and reported its activity against seven pleurotous species spawn-contaminating fungi, *Aspergillus
flavus, Aspergillus fumigates, Aspergillus niger, Alternaria alternate, Penicillium rinum, Curvularia lunata* and *Trichoderma harzianum*. The test compound was found to be significantly
active in the reduction of pathogen growth and inhibited fungal sporulation up to eighty percent of
all the strains except for *C. lunata*, which was inhibited up to 30% only in the highest concentration i.e. 1500 p.p.m. The oil of lemon grass decreased the spore germination in *A. flavus, A. fumigatus, A. alternata, P. citrinum* and *T. harzianum*, in a dose dependant manner and at 250 p.p.m. enhanced spore germination for *A. niger* and *P. citrinum*. At higher concentrations the test compound showed total inhibition. The test results showed *C. lunata* as the most resistant strain and *A. niger* as the least resistant strain against the test drug. Oussalah *et al.*, (2007) evaluated various essential oils for their antimicrobial activity against *Escherichia coli, Listeria monocytogenes, Salmonella typhimurium* and *Staphylococcus aureus* which are pathogenic strains. Brain Heart Infusion agar (BHI 15mL) was used in which essential oils were added at a concentration ranging from 0.003% to 0.8% (volume/volume) and the minimum inhibitory concentration and the maximal tolerated concentration was found for all the pathogens tested. Results indicated *Corydthymus capitatus* and *Cinnamomum verum* were the most significant. Minimum inhibitory concentration was found to be $\leq 0.05\%$ and maximal tolerated concentration except *S. Typhimurium, Cinnamomum verum* and *Cinnamomum cassia, $\leq 0.013\%$ was observed for all other tested bacterial strains. The oils of *Satureja hortensis, Thymus vulgaris carvacroliferum, Origanum compactum* exhibited a minimum inhibitory concentration $\leq 0.01\%$ against the organisms tested. The oils of *Thymus vulgaris thymoliferum, Thymus serpyllum, Thymus satureioides, Cymbopogon martini, Pimenta dioica, Cinnamomum verum*, *Eugenia caryophyllus* exhibited low antibacterial activity with a minimum inhibitory concentration $\leq 0.04\%$ against four bacterial strains. The remaining thirteen aromatic oils were least effective exerting a minimum inhibitory concentration $\geq 0.8\%$ against at least one bacterium. Josphat *et al.*, (2011) also evaluated the antifungal activity of essential oil of *Cymbopogon citratus* against five mycotoxigenic fungal strains of the genus *Aspergillus* i.e. *A. flavus, A. parasiticus, A. ochraceus, A. niger* and *A.
*fumigatus* isolated from maize samples. The oil was found active against all the tested strains in a dose dependant manner. The activity of the oil against the mycotoxigenic fungi had minimum inhibitory concentration values ranging from 15 to 118 mg/ml. The result indicates that the *C. citratus* essential oil has significant antifungal activities against poisonous mycotoxins producing fungi found in foods and this oil can be used for food preservation.

Biological Activities were also studied by number of research scientists. Saleem *et al.*, (2003) extracted oils of some herbs including *Cymbopogon citratus* by steam distillation and analyzed the samples by gas chromatography-mass spectrometry. In all species of genus *Citrus* citral b is a common constituent, which is found as a good inhibitor of β-glucuronidase. Saleem *et al.*, (2003) further tested the essential oil of lemon grass against some human, plant and animal pathogenic micro organisms and a positive response was achieved. Nakamura *et al.*, (2003) developed a simple system for glutathione S-tranferase activity determination for the detoxification of poly cyclic hydrocarbons. The authors used cultured rat normal liver epithelial cells. Citral (3, 7-dimethyl-2, 6-octadienal) was isolated from the *C. citratus* extract, which is a newly identified inducer of glutathione S-tranferase. Citral is a mixture of two stereo isomers neral and geranial, induced the total and pi-class-specific activities of glutathione S-tranferase in dose and time dependant manner. The results of the work carried out suggests that an E-isomer, geranial, is mainly responsible for the inducing the glutathione S-tranferase activity. The data was found constant in *in vitro* studies. When the cells were pretreated with di-ethanol maleate the basal activity was significantly enhanced and the citral-stimulated activity of glutathione S-tranferase was also increased, but when it was pretreated with N-acetyl-cysteine, the activity of glutathione S-tranferase was inhibited. Moreover, when RL 34 cells were pretreated with geranial for thirty
minutes, the intracellular glutathione level was attenuated significantly. When the application time was increased to eighteen hours, the level was enhanced significantly. The study also shows the antioxidant role of glutathione S-transferase induction by citral in mouse skin, providing a new approach for the prevention of skin cancer. Rabbani et al., (2005) reported the anti-clastogenic effect of citral in a dose of 20 mg/kg was tested in albino mice against the known mutagens cyclophosphamide, mitomycin-C and nickel metal. The research work included the evaluation of micronucleus frequency in bone marrow as well as in peripheral blood erythrocytes. The samples were collected at first, second and third day of clastogen treatment. The results showed that citral had significantly decreased the frequency of nickel induced by the three clastogens in bone marrow and peripheral blood erythrocytes. The level of significance was found to be \( p < 0.01 \).

Cini Paul (2005) patented a remedy of lemon grass for the treatment of a skin condition. The remedy comprised of an aqueous extract of *Cymbopogon* with some excipients. In this patent, a method for the treatment of a skin condition in an individual is described by giving the effective quantity of the plant extract to the patients suffering from acne. Shine et al., (2005) carried out a study to investigate the skin irritation potential of lemon grass essential oil. A volume of 0.5 ml of *C. citratus* oil was applied to intact and abraded skins of six healthy male albino rabbits, for one day. Different parameters like weight, illness, skin rash or irritation and death were measured for three days observation period. A severe edema was noticed in all rabbits at one day and three days after the application of test material. Slight to severe erythema and (or) slight eschar formation was also noticed in the rabbits. All the animals were recovered on seventh day except one, which recovered on eleventh day, but the eschar formation was not recovered. There were no treatment-related adverse effects which mean that when the undiluted lemon grass essential oil is applied on the skin once, it can cause inflammation and skin irritation in rabbits. The primary irritation index
score of 5.63 was found which indicated the severe irritation. As a result of this study, it is suggested that lemon grass essential oil should be used with great care and in diluted form especially when it is applied directly to the skin.

Antioxidant activity is reported by Shan et al., (2005). The investigation includes analysis of major phenolics in the spice extracts by standard chromatographic method. A significant positive linear relationship ($R^2 = 0.95$) was found between the two parameters measured i.e. the antioxidant capacity and phenolic content. This shows that these compounds in the tested spices exhibited the antioxidant activity. The types of phenolic compounds were identified as phenolic acids, phenolic diterpenes, flavonoids, and volatile oils (e.g., aromatic compounds). Tchoumbougnang et al., (2005) reported about the analysis of the essential oil of Cymbopogon citratus, obtained by hydro distillation from fresh leaves of the two plants growing in Cameroon through gas chromatography and gas chromatography-mass spectrometry. The major constituents found from test compounds of the plant were $\gamma$-terpinene, myrcene and $\beta$-pinene. The anti malarial effects of both the test drugs were evaluated against Plasmodium berghei growth which showed high anti malarial activity in experimental animals. The dose given was 200, 300 and 500 mg/kg of mouse in 24 hours and it resulted in the significant activity with 62.1%, 81.7% and 86.6%, 75.2% and 77.8% percentages of inhibition respectively by the essential oil of C. citratus. Chloromum (10 mg/kg /mouse) was used as a standard drug which gave 100% suppressive activity. Behr et al., (2006) describes an invention including lemon grass extract that can suppress extra cellular proteases chosen from the matrix metalloprotease-1, matrix metalloprotease-2, matrix metalloprotease-3, matrix metalloprotease-9 and human leukocyte elastase. This patent describes process for evaluating plant extract having the protease inhibition property which can be incorporated into different dermatological formulations. The patent also mentioned that different herbal extracts can be used in skin treatment and
prevention from skin diseases like wrinkles, skin and hair damage from sunlight, skin line deepening, elastotic changes in the skin and regular skin, hair and nail care. Tognolini et al., (2006) reported screening of herbal extracts including *C. citratus* for their anti-platelet activity and inhibition of clot retraction in experimental laboratory animals (Guinea pigs and albino rats). The chemical testing of these oils was carried out and the effect on hemostasis was noticed. A significant correlation exists between anti-platelet suggesting and in the prevention of clot formation for these oils. The present study provides the basis to consider the anti-platelet activity an important factor in the pharmacological evaluation of herbs and phytochemicals containing Phenyl-propanoids. Valero et al., (2006) reported that biocide activity is present in a large number of essential oils including lemon grass oil against various pathogenic agents. The studies were carried out to find a treatment for an emergent parastiosis, anisakisis, caused by L3 larvae of *Anisakis simplex*, with essential oils and or their major constituents. For this purpose, *in vivo* study was carried out to test the biocide activity of geraniol and it was compared to the standard drug Perillaldehyde. The experiments also included the histological study of larval damage in which a dose-response curve for geraniol and perellaldehyde was established and tisular myeloperoxidase activity was measured in the gastrointestinal tract of rat after administration of two monoterpenic derivatives. Zhang et al., (2007) patented a remedy comprising of *Cymbopogon citratus* as an ingredient. This invention can enhance immunity, prevent diseases and promote the growth of aquatic animals. The above mentioned formula could be used as a feed additive in aquatic animals resulting in high efficacy, rapid action, reduction of drug resistance, free from drug residue and environmental pollution. Yoo et al., (2008) evaluated antioxidant property and cytoprotective actions. A high relation exists in total phenolic and total flavonoids with antioxidant property. Almost all the herbs increased cell viability and indicated protection from oxidative stress
produced by hydrogen peroxide in test animals’ lung fibrablast cells. The plants used in this research work also exhibited high protection in gap-junction intercellular communication in comparison with gallic acid and catechin, as well as dose-dependently increased the activity of the antioxidative enzymes including superoxide dismutase and catalase. Figueirinha et al., (2010) reported about the anti-edematic activity of edible parts of Cymbopogon citratus or lemongrass. The effect of C. citratus (dehydrated grass) was studied and its polyphenolic, flavonoid, tannin, and phenolic acid-rich fractions were evaluated on the nitric oxide production through the induction of lipopolysaccharide in a skin-derived dendritic cell line. Lemon grass infusion extensively suppressed the lipopolysaccharide-induced nitric oxide production and inducible nitric oxide synthase protein expression. The polyphenolic fractions decreased the inducible nitric oxide synthase protein levels and nitric oxide production, without affecting cell viability, with very strong effects for the fractions with mono- and polymeric flavonoids (polyphenolic fractions-flavonoid-, tannin-fractions, respectively). The findings also showed that the anti-edematic activities of polyphenolic fractions-flavonoid, were primarily due to luteolin glycosides. In short, it can be concluded that lemon grass has nitric oxide scavenging property and it suppresses inducible nitric oxide synthase expression which is useful in the treatment of edematic diseases, especially of the alimentary canal. In another study Cymbopogon citratus was screened for various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties and positive results were achieved. Various other effects like antimalarial, antimutagenicity, antimycobacterial, antioxidants, hypoglycemic and neurobehavioral were also evaluated. The results are encouraging and indicate that this C. citratus needs further studies for the confirmation of these results (Shah et al., 2011).
Mosquito repellent activity is also reported by Paranagama et al., (2004) that the essential oils of the leaves of *Cymbopogon citratus* grown in Sri Lanka was tested for repellent activity, fumigant toxicity, and contact toxicity against *S. oryzae*. The repellency test results showed that *C. citratus* oil was found to be the most toxic oil against the test strain i.e. *S. oryzae* in fumigant toxicity test with an LC50 value of 0.035 g/L. Trongtokit et al., (2005) reported the mosquito repellent activity of essential oil in various concentrations which were tested against the mosquito *Aedes aegypti* under lab conditions using human volunteers. The test oil in the dose of 0.1 ml was applied on a volunteer’s forearm per 30 cm² of exposed skin. No significant activity was reported.

Plant pathology and botanical studies conducted include the study of Martins et al., (2004) who accomplished an anatomical and ultra structural study of leaves of *Cymbopogon citratus* belonging to family Poaceae. As a result of anatomical studies, it was found that the mesophyll faces are distinct, the ad axial surface of the leaf is occupied by the bulliform cells and the biggest vascular bundles are situated in the medium region which involve sclerenchymatous hem, the extensions of which reaches up to both epidermis. Three to five small vascular bundles are observed between the biggest vascular bundles. Some pointed and unicellular structures, known as prickle-hair, having a long basal cell and an oval distal cell are found in the regions of vascular bundles. Li et al., (2005) studied the allelopathic effects of lemon grass on the growth of corn or barnyard grass seeds. The seedling growth of the above mentioned plant was also monitored. The oil of *C. citratus* grown with corn or barnyard grass was studied through gas chromatography-mass spectrometry to find out the chemical constituents of the test compound. The results of showed that the germination rate of the seeds had no significant difference from the control, but the seedling growth was significantly suppressed. Ten components were found in the volatile oil of *C. Citratus* roots. The main component was found to be ongifolene- (V4), comprising of 56.67 % of the total, the other
component was selina-6-en-4-ol consisting of 20.03%, while the rest were less than 10%. In the volatile oil obtained from *C. citratus* shoots twelve components were found. The major component was citral comprising of 53.98%, the other was *z*-citral consisting of 34.40%, and rest were less than 4%. There were two monoterpenes and nine sesquiterpenes in the test oil of shoot, and all sesquiterpenes from the root portion. So the allelopathy of lemon grass should be taken in account when grown with other crops. Yi *et al.*, (2006) reported a tissue culture method comprising of a sterilizing tender tissue such as leaf of *Cymbopogon citratus* and making small pieces of the tissue of about one square centimeter, culturing on callus inducing medium (MS+6-BA 0.5 mg/L +2,4-D 1.5 mg/L+NNA 1.0 mg/L) to induce formation of callus, transferring callus on differentiation inducing medium (MS+KT 1.0-2.0 mg/L+NAA 0.05 mg/L), for a period of twenty days, then culturing the young plants of about 2-3 centimeters on root inducing medium for a period of twenty days, and acclimating plants. The tissue culture method is of low cost, easy to carry out with rapid propagation rate, and is suitable for large scale production.
### 1.3 PRUNUS DOMESTICA (PLUM)

![Whole Plant and Fresh Fruit of P. domestica](image)

**Figure 2: Whole plant and fresh fruit of P. domestica**

<table>
<thead>
<tr>
<th>KINGDOM</th>
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(Wikipedia, 2010)
LITERATURE CITED

Studies related to chemistry of *P. domestica* includes the work of Parmar *et al.*, (1992) who has isolated two new natural products from the heartwood of *P. domestica* i.e. 5,7,4′-trihydroxy-3-methoxyflavanone and 3,5,7,6,4′-dimethoxyflavanone and five other compounds were identified as isosakuranetin, prudomestin, dihydrokaempferide, naringenin and 3, 5, 7,8,4′-dimethoxyflavanone. The compound mentioned in the end is detected for the first time from the genus *Prunus*. Natakani *et al.*, (2000) reported that neochlorogenic acid and cryptochlorogenic acid, isolated from *Prunus domestica* and analyzed by nuclear magnetic resonance and mass spectrometry. Further, the amounts of chlorogenic acid isomers in *prunus domestica* were evaluated by high performance liquid chromatography. The isomers i.e. neochlorogenic acid, cryptochlorogenic acid and chlorogenic acid were present in the ratio of 78.7, 18.4, and 3.9, respectively. Among these cryptochlorogenic acid was detected in *P. domestica* for the first time and its quantitative analysis was also carried out which showed the presence of high amounts of it in prunes. Anti-oxidative assay of these isomers were carried out by scavenging activity on superoxide anion radicals and methyl linoleate oxidation suppressing activity was also studied. All the isomers showed anti-oxidative activities in a similar manner. Stacewicz-Sapuntzakis *et al.*, (2001) summarized a wide-ranging literature review providing the existing information of phytochemistry of *Prunus domestica* and the pharmacological activities of these constituents with a special reference to person’s health. The fruit of *P. domestica* is good in flavor and have anti-constipation activity. Dried fruits contain almost 6.1 % of dietary fiber, whereas its juice is freed from fiber by filtration before packing in bottles. Both prune fruit and prune juice possess laxative activity because of excessive sorbitol amount i.e. 14.7 % and 6.1 %, respectively. Kayano *et al.*, (2002) reported new compound, 4-amino-4-carboxychroman-2-one, which was isolated along with
four previously reported compounds i.e. *p*-coumaric acid, vanillic acid β-glucoside, protocatechuic acid, and caffeic acid. Their structures were determined by nuclear magnetic resonance and mass spectrometry. These isolated compounds were tested for oxygen radical absorbance capacity which exhibited 0.15-1.43 units / micromol, and the newly identified compound exhibited a noteworthy synergistic response on caffeoylquinic acid isomers. Kikuzaki *et al.*, (2004) reported some novel abscisic acid related constituents, along with (+)-abscisic acid, (+)-β-D-glucopyranosyl abscisate, (6S,9R)-roseoside, and two lignan glucosides i.e. (+)-pinoresinol mono-beta-D-glucopyranoside and 3-(β-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2R,3S)-dihydrobenzofuran from the prune ethanolic extract. The structures of four novel compounds were elucidated through nuclear magnetic resonance and mass spectrometric data and were reported as rel-5-(3S,8S-dihydroxy-1R,5S-dimethyl-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2Z,4E-pentadienoic acid (1), rel-5-(3S,8S-dihydroxy-1R,5S-dimethyl-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2Z,4E-pentadienoic acid 3’-O-beta-d-glucopyranoside (2), rel-5-(1R,5S-dimethyl-3R,4R,8S-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2Z,4E-pentadienoic acid (3), and rel-5-(1R,5S-dimethyl - 3R, 4R, 8S - trihydroxy - 7-oxabicyclo [3,2,1] – oct – 8 - yl) – 3 – methyl -2Z, 4E - pentadienoic acid (4). The antioxidant properties of these newly and previously known isolated compounds were evaluated by finding out oxygen radical absorbance capacity. The results of abscisic acid related compounds i.e. first seven compounds were not significant. Two lignans were found to be more effective antioxidants having oxygen radical absorbance capacity values of 1.09 and 2.33 micromol of Trolox equiv/micromol, respectively. Kosar *et al.*, (2009) prepared *n*-butanol and ethyl acetate soluble fractions of *Prunus domestica* and isolated purunusides A, purunusides B, purunusides C, new homoisoflavone glucosides along with the known compounds β-sitosterol and 6,7-methylenedioxy-8-
methoxycoumarin. Their structures were elucidated with the help of spectral studies. The compounds purunusides A, purunusides B and purunusides C exhibited strong inhibitory activity against the enzyme α-glucoronidase. Slimestad et al., (2009) studied six prune cultivars (Prunus domestica) grown in Norway with respect to phenolic composition. Neochlorogenic acid was detected as the dominant phenolic acid in total tested cultivars, and there was a significant difference in quantities between all the cultivars. Cyanidin 3-rutinoside was calculated less than 60% of the total anthocyanin content. A small amount of flavonols (rutin and quercetin 3-glucoside) were found in all cultivars. Mehmood et al., (2010) reported about the isolation of prunusins A, prunusins B and a novel C-alkylated flavonoid from the seed kernels of prune fruit. The structures were elucidated through nuclear magnetic resonating spectra and by correlation spectroscopy. Two compounds i.e. 3, 5, 7, 4'-Tetrahydroxyflavone and 3, 5, 7-trihydroxy-8, 4'-dimethoxyflavone have also been reported from this species. The constituents, prunusins A and prunusins B, exhibited high activity against pathogenic fungal strain Trichophyton simmi.

Biological studies conducted by Stacewicz-Sapuntzakis et al., (2001) on the P. domestica fruit. It contains high quantity of phenolic compounds mainly comprising of neochlorogenic and chlorogenic acids, which supports in anti constipation activity and interrupt sugar absorption. Phenolic compounds of P. domestica fruit had been found to reduce human LDL oxidation, and thus prevent chronic diseases like heart disease and cancer. The increased amount of potassium content is beneficial for heart and blood vessels. Dried fruit is a vital source of boron, which can prevent of osteoporosis. One hundred gram of prunes provides the amount of boron needed per day i.e. 2 to 3 mg/day. Kayano et al., (2002) reported antioxidant activity of isolated compounds which was tested for oxygen radical absorbance capacity which exhibited 0.15-1.43 units / micromol, and the newly identified compound exhibited a noteworthy synergistic response on
caffeoylquinic acid isomers. The antioxidant activity of the methanol eluate was due to the major components of *P. domestica*, caffeoylquinic acid isomers and the novel isolated compound.

Kayano *et al.*, (2003) conducted a study to search the possible role of caffeoylquinic acid isomers in the oxygen radical absorbance capacity of prunes, and to screen out the existence of other antioxidant components. Ethanol extracts of *P. domestica* was prepared and caffeoylquinic acid isomers were isolated from the extract, which were then quantified by high performance liquid chromatography. The degree of contribution of these isomers to the oxygen radical absorbance capacity was found to be 28.4%. This shows that the remaining oxygen radical absorbance capacity is due to other antioxidant compounds present in the extract. Kayano *et al.*, (2004) in another study tested *P. domestica* for its pythochemicals, their structure identification and its antioxidant activity. It was detected that it contains quite elevated levels of 4-O-caffeoylquinic acid. After the detection of 28.4% role of caffeoylquinic acid isomers to the antioxidant activity it was assumed that residual oxygen radical absorbance capacity is reliant on some other unidentified antioxidant components. Twenty eight constituents were detected from *P. domestica* and their structures were identified by Nuclear Magnetic Resonance and Mass Spectrometry. Among four abscisic acid related compounds, a chromanon, and a bipyrrole were the new compounds. The isomers of caffeoylquinic acid isolated from *P. domestica* exhibited significant antioxidant activities when it was tested by the oil stability index method, oxygen ion scavenging activity, and oxygen radical absorbance capacity. The other identified constituents were hydroxycinnamic acids, benzoic acids, coumarins, lignans, and flavonoid which exhibited significant oxygen radical absorbance capacity values. In addition, a newly identified constituent chromanon exhibited a noteworthy synergistic outcome on oxygen radical absorbance capacity of caffeoylquinic acid isomers. Deyhim *et al.*, (2005) reported earlier that prunes in dried form were tested in animal
models of osteoporosis against bone loss. This research work was to evaluate the ability of above mentioned test compound to recover from bone loss in osteopenic ovariectomized Sprague-Dawley rats. The test drug in very low dose (5%) was found to be significant in recovering femoral and tibial bone density. It also improved lumbar bone density, with high dose. An enhancement in femoral bone density of rats fed with the test drug was found which shows the improvement in bone quality from 6.0% and 6.9%. Different doses of dried prune were also found effective in improving trabecular microarchitectural properties as compared to the ovariectomized controls. The improvement in biomechanical parameters of long bones due to dried prunes, might be as a result of favorable micro structural change which can be observed by increase in tibial bone volume and connectivity. Bone volume decrease followed by trabecular connectivity decrease is normally considered an irreparable process, but the above mentioned observations recommend that dried prune improves trabecular microstructure of tibia after previously developed losses. Fujii et al., (2006) separated ethanol fraction from concentrated juice of *P. domestica* and evaluated the anticancer effects on two types of cancer cells *in vitro*. The extract decreased the possible cell number of Caco-2, KATO III in a dose-dependent manner, but did not decrease the possible cell number of normal colon fibroblast cells (CCD-18Co) of man used as a normal cell model. *Prunus* extract treatment for one day led to apoptotic changes in Caco-2 like cell contraction and blabbed surfaces because of the convolutions of nuclear and plasma membranes and chromatin condensation, but this was not observed in CCD-18Co. *Prunus* extract induced nucleosomal DNA fragmentation typical of apoptosis in Caco-2 after one day treatment. It can be concluded from the results that *prunus* extract induced apoptosis in Caco-2. In addition, by Caco-2 treatment with Hydrogen per oxide chelator catalase and calcium ion-chelator, the *prunus* extract-induced cytotoxic pathway was totally blocked, and the viable cell number of Caco-2 was not changed.
Bouayed *et al.*, (2007) reported that oxidative stress has a link with neurodegenerative diseases, cardiovascular diseases, cancer, anxiety and depression. Chlorogenic acid is a dietary antioxidant present in fruits including prunes. Its anxiolytic effect was investigated in mouse models of anxiety including the light/dark test, the free exploratory test and the elevated plus maze was investigated. Further, the antioxidative effect of chlorogenic acid on peripheral blood granulocytes was also evaluated. Chlorogenic acid in a dose of 20 mg/kg reduced the anxiety-related behaviors showing an anxiolytic-like effect of this polyphenol. The anti-anxiety effect was blocked by flumazenil suggesting that a decrease in anxiety is due to the activation of the benzodiazepine receptor. In vitro, chlorogenic acid protected granulocytes from oxidative stress. Chlorogenic acid anxiolytic effects coupled with antioxidant activity was demonstrated *in vivo* and *in vitro* for the first time.

Kimura *et al.*, (2008) obtained an oligomeric proanthocyanidin fraction from prune extract by an antioxidant-guided assay. The antioxidative oligomer was found to be composed of epicatechin and catechin units and it was characterized as a procyanidin oligomer with an average polymerization degree of five. The oligomer showed greater antioxidative activity than chlorogenic acid, whose antioxidative activity is already studied in prunes. Yoon *et al.*, (2008) reported the effect of plum wine on lipid metabolism and lipid peroxidation in Sprague-Dawley albino rats with chronic ethanol consumption. Animals treated with low concentration plum wine resulted in a significant decrease in liver weight per 100 g body weight, total cholesterol level and atherogenic index in plasma while the ratio of high density lipid-cholesterol to total cholesterol was significantly enhanced in comparison with the animals treated with alcohol. Both low and high concentration plum wine groups showed a significant decrease in total lipid, total cholesterol in liver tissue when compared to the alcohol treated group. Both plum wine treated groups (low and high dose) showed a significant decrease in the level of plasma lipid per-oxidation products. Low
concentration plum wine group also showed a significant decrease in the level of liver lipid per-oxidation products as compared with that of alcohol treated group. The results of this study suggests pure alcohol beverage can be replaced by low concentration plum wine for exerting beneficial effects on lipid metabolism and lipid per-oxidation products by improving lipid profiles in plasma and liver tissues and decreasing plasma and hepatic lipid per-oxidation product. Hiramoto et al., (2008) reported that the seed extract of Prunus domestica is anti-stress agent and immunostimulant. Bu et al., (2008) conducted a study with the objective to find out that the dried prune polyphenols apply extra ordinary direct effects on osteoclasts and their precursors or not. RAW 264.7 macrophages were used as a model for studying osteoclast precursors and osteoclast differentiation and activity. Lipopolysaccharide was used to develop inflammation, polyphenols extracted from dried plum, in dose of 10, 20, and 30 µg/ml was used which as a result down regulated the osteoclast precursor cyclooxygenase expression and nitric oxide. Tumor necrosis factor (TNF)-α was also decreased by the polyphenols and (TNF)-α production in response to oxidative stress was increased. These inhibitory effects on osteoclastogenesis were confirmed in primary bone marrow cultures. It suggests that dried plum polyphenols primarily affect osteoclast differentiation; the data demonstrate that these polyphenols directly inhibit osteoclastogenesis, which in turn leads to a decrease in osteoclact activity, by down regulating NFATc1 and inflammatory mediators. Bouayed et al., (2008) reported that after the feeding of seven varieties of plums, murine granulocyte cells were protected from oxidative stress in vitro as they have a potential to inhibit intracellular reactive oxygen species accumulation. Consumption of 100 g fresh plums can have antioxidants equivalent of 613.98-2137.59 mg vitamin C. As a result of this study, it can be concluded that plum is a good dietary source of polyphenols with antioxidant and anxiolytic effects and can provide health-promoting advantages to the human. Hooshmand et al.,
(2009) reported that osteoporosis affects both female and male but it occurs in a greater extent in female than male. The studies conducted in animals showed that dried prune prevented the ovariectomy-induced bone mineral density decrease of the femur and lumbar vertebra. Another important achievement was that the test drug inverted the loss of trabecular architectural properties including trabecular number, connectivity density, and trabecular separation. The effectiveness of test drug in inversion of bone loss due to skeletal unloading was also found. Bone mineral density and trabecular bone structure was analyzed through microcomputed tomography which showed that prune increased bone recovery during reambulation following skeletal unloading and was found equivalent to the effects of parathyroid hormone. A three month clinical trial was also carried out which indicated that the daily consumption of *P. domestica* in dried form by postmenopausal females significantly increased serum markers of bone formation, total alkaline phosphatase, bone-specific alkaline phosphatase and insulin-like growth factor-I up to 12, 6, and 17 percent respectively. Shukitt-Hale *et al.*, (2009) reported that normal aging in both animals and mans is due to a turn down in cognitive performance which may be as a result of the long-term effects of oxidative stress and edema on neurologic processes. This study was carried out to investigate supplementation with *Prunus domestica* in mitigating age-related deficits in cognitive function. The supplementation with 2% *P. domestica* (dried plum powder), or 100% plum juice for eight weeks, in mitigating age-related deficits in cognitive function in 19 to 21 months aged Fischer 344 rats was studied. The group of rats that were given plum juice improved working memory in the Morris water maze, while rats fed with dried plum powder were similar to that of the control group. The possible reason might be that smaller quantity of phenolics was consumed in the powder group than that of the juice group. The results are discussed in relation to the amount and type of phenolics present in the prune products and in relation to other dietary intervention studies.
in which cognitive benefits have also been reported. Ahmed et al., (2010) designed a clinical trial study to investigate the effects of prunes on hepatic function as prunes are used for the treatment of hepatitis in folk remedies. In this study, 166 human volunteers of good health were distributed randomly in three groups. Prunes (68-69g) were soaked in 250ml water for 12 hours. Two test groups’ volunteers were given prune juice and left fibre of the fruit in single and double doses at fasting in the morning daily for two months; while the control group volunteers were given only one glass of water. Blood samples were collected baseline, weekly and after eight weeks for biochemical analysis. There was noticeable decrease of serum alanine transaminase and serum alkaline phosphatase in low dose group of prunes. No change was observed in serum aspartate transaminase and bilirubin. The results suggest that the alteration in hepatic function by use of prunes may have clinical relevance in appropriate cases and prunes may be beneficial in hepatic disease. Ahmed et al., (2010) further reported the effects of P. domestica on blood pressure. This clinical trial study was carried out to observe the effects of prunes fruit on blood pressure in 259 pre-hypertensive patients having systolic blood pressure in between 120-139 mmHg and diastolic blood pressure in between 80-89 mmHg. Treated groups drank prune juice and ate the whole fruit (dried plums) while either 3 or 6 prunes soaked overnight in a glass of water, while the control group drank only a glass of water early in the morning in empty stomach. Blood pressure was recorded fortnightly for a period of eight weeks, and blood samples were taken at baseline and after eight weeks. There was significant decrease in blood pressure in case of single dose of prunes group and the controls. In case of double dose group, only systolic blood pressure was decreased significantly. In control group, a significant increase in serum high density lipids was also observed while the test groups had a significant decrease in serum cholesterol and low density lipids. The data was analyzed by paired-sample t-test with 95% confidence interval. The results of
this study predict cardiovascular protective effects of \textit{P. domestica}. Soni \textit{et al.}, (2011) reported that the \textit{Prunus domestica} extract methanol:ethanol in a ratio of 70:30 of was made and its hepatoprotective effect was studied. Alteration in the levels of biochemical markers of liver disfunction like serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase, Total bilirubin, direct bilirubin and tissue LPO, growth stimulating hormone, catalase and SOD were tested in both treated and untreated groups. Paracetamol in a dose of 2g/kg and carbon tetra chloride in a dose of 1.5 ml/kg significantly increased serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase, total bilirubin and direct bilirubin and tissue level of growth stimulating hormone. Treatment with extract of \textit{P. domestica} fruits in two doses i.e. 150mg/kg and 300mg/kg returned the disturbed levels of biochemical markers almost near to the original levels dose dependently. Jabeen and Aslam \textit{et al.}, (2011) claim that \textit{P. domestica} has anticancer, antihyperglycemic, anti-hyperlipidemic, antihypertensive, anti-osteoporosis, antioxidant, hepatoprotective and laxative activities. The fruit of \textit{P. domestica} contain amino acids, carbohydrates, dietary fibers, minerals, vitamins and antioxidant poly-phenolic phyto-constituents. Therapeutically active constituents are investigated and their possible mode of actions is also discussed thoroughly.

Plant pathology and botanical studies were reported by Dirlewanger \textit{et al.}, (2002). In this study the sequence of forty one primer pairs of microsatellites from a CT-enriched genomic record of the peach cultivar 'Merrill O'Henry'. The sequences of ten microsatellite-containing clones were similar to plant coding sequences in databases which can be used as markers for known functions. Cross-species amplification was analyzed in six different \textit{Prunus} species including \textit{Prunus domestica}. 75.6\% gave amplification in all the six \textit{Prunus} species tested. Al-caraz-Lopez \textit{et al.}, (2003) performed an experiment in which Ti$^{4+}$-ascorbate was sprayed onto prune plants in
various combinations with some other commercial compounds containing calcium and magnesium ions and then the effects on the commercial quality of fruits was studied, with particular consideration on improving the resistance against post harvest handling damage. All the treatments having titanium enhanced the plant performance i.e. branch elongation, flowering and fruit setting intensity and fruit size. Fruits of the Ti-treated trees at harvesting time resulted in better results to compression and penetration, while a decrease in weight-loss was also noticed during post harvest storage. Similarly the external color was also persistent, but the apparent ripening status of all the treatments seemed to be delayed. However, the fruits from Ti-treated trees exhibited a better behavior in the evolution of the color parameters throughout the storage than did the non-treated fruits. Titanium application increased the calcium, iron, copper and zinc concentrations significantly in whole fruit. This increase in the calcium absorption may be due the beneficial effect of titanium on the absorption, translocation and assimilation procedures.

Lombardi-Boccia et al., (2004) selected Prunus domestica normally and organically grown in farms for finding out the effect of various agronomic practices on antioxidant vitamins like vitamin C, E, β-carotene and phenolics like total polyphenols, phenolic acids, flavonols, etc. concentration. Usual prunes were planted on tilled soil. Three organic plantations were carried out including tilled soil, trifolium covered soil and natural meadow covered soil. Macronutrients variations were negligible, while the concentration of antioxidant vitamins and phenolic compounds markedly varied in different cultivations. Vitamin C, α- and γ-tocopherols, and β-carotene were in high content in organic prunes planted on natural meadow covered soil. The maximum phenolic acids were found in prunes planted on trifolium covered soil. Total polyphenols content was more in usual prunes. Quercetin was higher in usual prunes, but myrecitin and kaempferol were higher in
organic prunes. In the similar cultivar and climatic conditions, the type of soil management changes of prime significance in influencing the concentration of health-promoting compounds.

Harzallah et al., (2004) conducted a survey of bacterial infections due to *Pseudomonas* on fruit trees of *rosaceae* family in forty two orchards of Constantine region (East Algeria). *Pseudomonas* were isolated and identified on the basis of their biochemical and cultural characteristics. A total number of fifty nine phyto pathogenic bacteria were isolated from the infected pome and stone fruit trees. The strains isolated from cherry (*Prunus avium*), plum (*P. domestica*), apricot (*P. armeniaca*), almond (*P. dulcis*) and pear trees (*Pirus communis*) were comparable to *Pseudomonas syringae* and were thirty one in number; sixteen strains comparable to *Pseudomonas syringae* were obtained from samples of cherry and plum. The strains of *Pseudomonas viridiflava* isolated from cherry, apricot and peach (*Prunus persica*) were twelve in number. Hily et al., (2004) reported about the major upsetting diseases of *Prunus* species as plum pox virus. A little work is done to find out the sources of resistance to plum pox virus, among which transgene-based resistance offers a complementary approach to developing plum pox virus-resistant stone fruit cultivars. C5 is a transgenic clone of *Prunus domestica* L. which contains the plum pox virus coat protein gene. In greenhouse tests, it has been proved to possess antiviral effect against the test virus. The test plants exposed to natural aphid vectors in the field remained safe from the infection up to a period of 48 months where as the control plants were found infected within 12 months. The test plants inoculated by chip budding exhibited slight infection and plum pox virus was found in these plants. The plum pox virus-coat protein transgene in C5 was specifically hyper-methylated with no detectable expression. The result of this study indicated the stability and efficiency of post-transcriptional gene silencing-based plum pox virus resistance in *P. domestica* under field conditions. Bouras and Papadoulis (2005) reported that *Euseius stipulatus* (Athias-Henriot) is a
predatory mite which is widely spread in the Mediterranean region. It plays a role in the controlling the citrus plants against spider mites. Growth, survival and fertility of this strain feeding on seven market available pollens were studied at room temperature, 65±5% relative humidity and a photoperiod of 16 light: 8 dark hours. Mites were kept individually at rearing units with sufficient number of almond, apple, apricot, cherry, pear, prune and walnut pollen as nutritive source. When several pollens were tested, it was found that the growth time of mite varied between eight to nine days. Female stability varied from eleven to fifty one days, while fertility ranged from twenty two to forty three eggs in each female. The mite was not able to reproduce when feeding on walnut pollens. The rate of increase ranged from 0.079 to 0.146 daily. Results point out that almond, plum, cherry and apricot pollen possess higher nutritional value for *E. stipulatus* than pear and apple pollen and which may contribute in sustaining and increasing the predator population in field conditions. Walnut pollen can be utilized by the predator only to survive during short periods of time when principal or alternative food sources are limited. Elkereamy *et al.*, (2011) cloned and characterized a new PR-5 cDNA named PdPR5-1 from the European plum or *Prunus domestica* in the present study. Expression of PdPR5-1 was studied in different cultivars varying in resistance to the brown rot disease caused by the necrotrophic fungus *Monilinia fructicola*. Moreover, transgenic Arabidopsis, ectopically expressing PdPR5-1 was used to study the possible role of it in other plant defense responses after fungal infection. The resistant cultivars resulted in much higher levels of transcripts than the susceptible cultivars at the stage of fruit ripening. On the other hand, significant increase in the transcript levels after infection with *M. fructicola* was observed in the susceptible cultivars. Transgenic Arabidopsis plants exhibited more resistance to *Alternaria brassicicola*. In addition, a significant increase in the transcripts of genes involved in the phenylpropanoid biosynthesis pathway such as phenylalanine ammonia-lyase and phytoalexin
(camalexin) pathway was observed which led to an increase in camalexin content after fungal infection. These results suggest that PdPR5-1 gene, along with its anti-fungal properties, has a possible role in activating other defensive pathways, including phytoalexin production.
2. MATERIALS & METHODS

2.1 MATERIALS

The following materials were used during experiments.

- The plant material *Prunus domestica* and *Cymbopogon citratus* were purchased from local market of Karachi, Pakistan. The plant material was identified Dr. Beena Naqvi, Plant taxonomist, Food and Marine Research Center, PCSIR Labs Complex, Karachi. Plant specimens were submitted in Herbarium bearing voucher no. LGK-089-2010. and PDK-090-2010.

- Wistar albino rats and Swiss albino mice of either sex were taken from the Animal House of PCSIR Labs Complex, Karachi, and A-1 Grade chicks were purchased from K&N Farms, Karachi.

- American Type Culture Collection (ATCC) cultures of bacterial Strains of *E. coli*, *S. aureus*, *K. pneumoniae*, *S. typhi*, *B. cereus*, *P. aurogenosa* and *P. melaborus* were purchased from Musajee sons, a supplier of Oxoid company.

- Drugs used were Aspirin (Standard analgesic drug); Diazapam (Standard CNS depressant drug); Indomethacine (Standard anti-inflammatory drug); Motilium (Standard antiemetic drug) and Di Picryl Phenyl Hydrazine (Standard antioxidant drug).

- Microbiological medium including Nutrient agar and Nutrient broth of Merck (Germany)

- Chemicals including sodium dihydrogen phosphate; sodium monohydrogen phosphate; sodium chloride; iodine; sodium hydroxide; sodium nitroprusside; vanillin; NaOCl, lead acetate, Copper sulfate, 1,1-diphenyl-2-picryl-hydrazil were purchased from Merck (Germany) or Sigma Chemical Co. (USA).
a) **SOLVENTS**

1. Methanol (Merck, Germany)
2. Ethanol (Merck, Germany)
3. Ethyl acetate (Merck, Germany)
4. Chloroform (Merck, Germany)
5. Ether (Merck, Germany)
6. Sulfuric acid (Merck, Germany)
7. Hydrochloric acid (Merck, Germany)
8. Acetic anhydride (Merck, Germany)
9. \(n\)-Butanol (Merck, Germany)
10. Carbon tetrachloride (Merck, Germany)
11. Aniline (BDH, England)
12. DMSO (Merck, Germany)
13. Formalin (Fluka, Switzerland)

b) **INSTRUMENTATION**

- Rotary evaporator, Eyela (Japan)
- Sonicator (UK)
- Autoclave (Pakistan)
- Incubator, Sieco (China)
- Lyophilizer (Freezedrier model FD1, Eyela Tokyo Rikakikai Co. Ltd., Japan)
- Shaker SS-80 (Japan)
- Hot box oven (Gallenkamp, UK)
- Jester or micropipette (Gilson, France)
- Spectramax plus 384 Molecular Device (USA)
- UV lamp (original Hanan 254nm, Floutest, London)
- Physical balance (Libror AEG-120 & EB-3200D, Shimadzu, Japan)
- Filter units, Sartorius (Minisart NML, SM16534, USA)
2.2 METHODS

Extraction of Plant Material:

Five kg of plant material was extracted with ten folds of methanol at 40°C for six consecutive days by the traditional method. The crude extracts were lyophilized under 5μm-Hg pressure. All experiments were carried out by using an appropriate amount of material mentioned in procedure.

Scheme no. 1: Extraction procedure of *Cymbopogon citratus*

- **Leaves of Cymbopogon citratus (5 kg) + 70% ethanol (10 litres)**
  - Percolated for 15 days at room temp.
  - Filtered through Whatmann filter paper

- **Residue**
  - Evaporated ethanol under reduced pressure at 40°C and lyophilized

- Crude Extract (66.23g)
  - 30 g separated
  - 36.23 g separated for fractionation

- (E.A. Fr.) (CHCl₃ Fr.) (n-But. Fr.) (Aq. Fr.) were isolated.
Scheme no. 2: Extraction procedure of *Prunus domestica*

Dried Fruit of *Prunus domestica* (5 kg) + 70% Ethanol (10 litres)

Percolated for 15 days at room temp.

↓

Filtered through Whatmann filter paper

↓

Residue

Evaporated ethanol under reduced pressure at 40°C and lyophilized

↓

Crude Extract (84.21g)

↓

34 g separated

↓

50.21g separated for fractionation

↓ ↓ ↓ ↓

(E.A. Fr.) (CHCl₃ Fr.) (n-But. Fr.) (Aq. Fr.) were isolated.
3. EXPERIMENTATION

3.1 Color Reactions for identification of chemical constituents

The following tests were carried out for the identification of chemical constituents:

(a) Liebermann-Burchard Test

This is the test for the presence of triterpenes. A small quantity of methanol extract was taken in a test tube and 5ml of chloroform was added and then 2ml of acetic anhydride was also added in it. Then 2 to 3 drops of concentrated sulfuric acid was added carefully by gentle mixing of solution. Pink color indicates the presence of triterpenes.

(b) Dragendorff Test

A small quantity of methanol extract and water was taken which was acidified with HCl and then few drops of Dragendorff reagent were added. Orange precipitate indicated the presence of alkaloid components.

(c) Lead acetate Test: This is the test for identification of tannins. The plant powder was boiled in distilled water and filtered. Then lead acetate was added in filtrate. If precipitates are formed then this is the sign of presence of tannins.

(d) Phenazone Test: This is another test for identification of tannins. Half gram of sodium acid phosphate was added to 5ml aqueous extract of plant material. It was slightly warmed, cooled and filtered. Then 2% solution of phenazone was added to the filtered material. If the precipitates are formed it indicates the presence of tannins.

(e) Froth Test: This is the test for identification of saponins. A small quantity of dried extract powder was taken in water and shaken vigorously. If the froth is formed it indicates the presence of saponins.
(f) **Ether Test:** This is another test for the identification of saponins. A small quantity of ether in a little quantity of methanol extract of plants was added and shaken. If the precipitates are formed it indicates the presence of saponins.

(g) **Molisch’s Test:** This is the test for identification of carbohydrates. A small quantity of methanol extract was taken and treated with $\alpha$-naphthol and concentrated sulfuric acid. Purple color indicates the presence of carbohydrates.

(h) **Salwaski Test**

This is the test for confirmation of sterols. Only 2-3 drops of sulfuric acid was directly added into a small quantity of methanol extract. If purple ring is formed at the upper surface of the solution, it indicates the presence of sterols.

(i) **Test for Proteins**

A small quantity of powdered extract was taken and mixed with ethanol. If the powder is insoluble then it indicates the presence of proteins which are insoluble in organic solvents.

Proteins are precipitated with solution of picric and tannic acids.

The constituents present or absent are listed in Table 1.

(WHO Guidelines, 1993; Ali, 1994)


3.2 ANTIOXIDANT ASSAY

Determination of Diphenyl Picryl Hydrazil Radical Scavenging Activity

The free radical scavenging effect was detected by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the reported procedure (Glucin et al., 2005). An amount of 0.3 mM of DPPH was dissolved in ethyl alcohol. Five microlitres of the test compound in different concentrations ranging from 62.5µg-500µg were mixed with 95 µl of DPPH solution. The mixed liquid was poured in 96 well plate and kept at 37°C for 30 min in incubator. The absorbance was recorded at 515 nm by microtitre plate reader and percent radical scavenging effect was calculated with respect to that of control by the following formula:

\[
\text{DPPH scavenging effect} \% = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100
\]

where “Ac” is the absorbance of Control (DMSO treated) and “At” is the absorbance of Test sample.

3.3 Acute Oral Toxicity Test

Selection of animals: Before proceeding for toxicity studies, animals (rats of both sexes) housed separately were kept under strict observation for a period of 3 weeks with free access to food and water. Any animal showing sluggish movement or any sign of illness was rejected.

Prunus domestica and Cymbopogon citratus dissolved in physiological saline was administered in a single dose by means of a gavage in graded doses to different groups of animals up to the dose of 5g/kg. Each group comprised of 10 animals (5 male and 5 female). Observations with reference to physico-behavioral changes and mortality rate within 24 hours noted. Animals were further observed for a period of 72 hours for changes in behavioral pattern and delayed mortalities. Macroscopic findings were made on autopsied animals and recorded (Loomis, 1978; USP, 2008).
3.4 Behavior studies:

General behavior of animals was observed by the method Ramanathan et al., (2008). A total number of 60 albino rats of Wister strain (weighing between 150g to 200g) of both sexes were used for the experiment. The animals obtained from the Animal House of PCSIR Labs Complex, were healthy, active and alert kept under observation for three weeks before conducting the experiment. Animals were provided with standard rodent pellet diet and the food and were kept under pathogen free conditions at 26±2°C temperature and 44-56% relative humidity.

For this study animals were divided into ten groups comprising of ten animals in each group. Group-1 and Group-II was given lyophilized Prunus domestica at 300 and 500mg/kg dose. Group- III and Group-IV was given Cymbopogon citratus at 300 and 500mg/kg dose Group V was given Diazepam (5mg/kg) as standard while group VI was given normal saline and served as control group. The behavioral changes were observed if any at 30min, 1 hour, 2 hour, 3 hour, 4 hour and 06 hours after the drug administration. The parameters observed are listed in Table 3, 4, 5 and 6 in the result section of this dissertation.

3.5 CNS ACTIVITY ASSESSMENT

CNS activity was studied by Rota rod and Grip strength test. Both tests were performed on Panlab S.I. and UGO Basile biological research apparatus (Italy) respectively.

In each test animals were divided into six groups comprising of five animals each. Group-1 and Group-II was given lyophilized Prunus domestica at 300 and 500mg/kg dose. Group- III and Group-IV was given Cymbopogon citratus at 300 and 500mg/kg
dose. Group V was given Diazepam (10mg/kg) as standard while group VI was given normal saline and serve as control group.

The test drugs and standard drug were diluted in normal saline and administered orally. In all the tests observations were made for 3 hour after the administration of drug.

a) Rota rod Test

The effect of test and standard drugs on motor coordination or fatigue resistance was assessed on mice weighing between 20-25g (male and female). The motor coordination and performance of each mouse was evaluated 30 min after drug administration by placed mouse on 2.5 cm diameter rotating bar of rota rod divided into five parts at the speed of 5 rpm. The animals which remained on the rotating bars for 3min or more in three successive trials were chosen for the experiment. Observation was to determine the time taken by the animal to move on rod. Firstly mice were trained to make them able to move on rod then the mice of each group were placed on the rod at the time period of 30, 60, 90, 120, and 180 minutes. Any increase or decrease in time taken by the drug treated animals and the number of fall from the rod as compared to the standard group, describes the sedative or stimulant activity of the drug respectively and the animals remain on rota rod for 3min were considered as passed the test (Ramanathan et al., 2008). The results are listed in Table 6.

b) Grip strength Test

Rats weighing between 150-180g (male and female) were used as the test animals in this method. The effect of drug on muscle strength was assessed by using the grip strength meter (UGO Basile biological research apparatus Italy). Assessment of muscles strength
was started at the interval of 15, 45, 75 and 105 min after the last oral and intra peritoneal application of tested and standard drugs.

The animal is placed over a base plate in front of a grasping bar which is the arm of force transducer connected to peak amplifier. When rat pulled by the tail it grasps the bar and after the animal loses its grip on the grasping bar, a peak preamplifier automatically stores the peak pull force achieved by forelimbs and shows it on the liquid crystal display. The apparatus measures the pull force of rats and expressed in grams. Control values for each group of animals were obtained measuring the grip force before treatments with the tested and standard drugs (Lidiya et al., 2005). Results can be seen in Table 7.

### 3.6 ANALGESIC ACTIVITY

**a) Tail flick Test**

Analgesic activity was assessed by Tail flick method on analgesiometer (UGO Basile Italy). Albino rats weighing between 150-180g were used for this study. The animals were obtained from the animal house of PCSIR Labs Complex, Karachi of both sexes, maintained at room temperature with light/dark cycle of 12 hour search and with diet and water *ad libitum*.

For analgesic assessment test animals were divided into six groups comprising of five animals each. Group-1 and Group-II was given lyophilized *Prunus domestica* at 300 and 500mg/kg dose Group- III and Group-IV was given *Cymbopogon citratus* at 300 and 500mg/kg dose Group V was given Aspirin (300mg/kg) as standard drug, while group VI was given normal saline which served as control group.
The results were noted initially and at the intervals of 0.25, 0.5, 1, 2, 3 and 4 hours after the last oral and intra peritoneal application of test and standard drugs. Results are present in Table 8.

### 3.7 ANTI EMETIC ASSAY

Young male chicks of 20-25g were used to assess antiemetic activity was done according to Shin *et al.*, (2002). Animals were divided into 05 groups comprising of six animals each. Group-1 and Group-II was given lyophilized *Prunus domestica* at 300 and 500mg/kg dose Group- III and Group-IV was given *Cymbopogon citratus* at 300 and 500mg/kg dose Group V was given Motilium as standard drug while Group VI was given normal saline as control drug. The chicks were kept in large beakers at room temperature and were rested for ten minutes to stabilize. The test and standard drugs were administered intraperitoneally at a dose of 10ml/kg body weight. After 10 minutes, emesis producing agent i.e. Copper sulfate anhydride was administered orally at a dose of 50mg/kg body weight. Then the number of retching (an emetic action without concomitant vomiting) was recorded during the next ten minutes. The results are presented in Table 9. The inhibition % was calculated as follows:

\[
\text{Inhibition }\% = \left(\frac{A-B}{A}\right) \times 100
\]

Where A is the frequency of retching in control group, and B is the frequency of retching in treated group.

### 3.8 ANTIBACTERIAL ACTIVITY TESTS

**Test organisms**

Antibacterial activity was carried out against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, *K. pneumoniae*, *Salmonella typhi*, *B. cereus*, and *P. melaborus* strains. The culture of organisms was maintained on stock culture agar and
from the stock culture; a loop full of the culture was inoculated in nutrient broth. The broth seeds were incubated at 37°±1°C for twenty four hours. Inocula were prepared by diluting twenty-four hours old cultures in saline. A dilution of 1:100 was used in all the tests.

**Media for other bacterial strains**

**NUTRIENT AGAR II (3/158)**

**FORMULA**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>8.0</td>
</tr>
<tr>
<td>Agar No. 2</td>
<td>12.0</td>
</tr>
</tbody>
</table>

**Method for reconstitution:**

Twenty eight g of powder was weighed and dispensed in 1 litre of deionized water. It was allowed to soak for 10 minutes and swirled to mix, then sterilized by autoclaving for 15 minutes at 121°C. Cooled to 47°C. It was mixed well and poured on plates.

**Appearance:** Buff, opalescent gel.

**pH:** 7.3±0.2

**NUTRIENT BROTH No. 2 (3/160)**

**FORMULA**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Extract</td>
<td>10.0</td>
</tr>
<tr>
<td>Balanced Peptone No. 1</td>
<td>10.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Method for reconstitution:**

Exactly 25g of powder was weighed and dispensed in 1 litre distilled water. It was allowed to soak for 10 minutes and then swirled to mix, then dispensed into tubes or bottles, and sterilized at 121°C for 15 minutes.

**Appearance:** Pale Straw, clear.

**pH:** 7.3±0.2  (Microbiology Manual, 2000).
ANTIBACTERIAL ASSAY:
Nutrient agar petri plates were prepared after Kavanagh, (1963), for testing the antibacterial activity of the extracts and 0.1 ml of the diluted culture was poured on each plate and the plates were dried for 30 minutes at 37°C. Plant extract discs were prepared containing 100µl of extract in each disc. The plant extract discs were placed along with the standard antibiotic discs (amoxicillin) marking the sample code properly. The plates were incubated for 24 hours at 37°C. at the end of incubation period, the inhibition zones were measured.

4. STATISTICAL ANALYSIS
Values for observations were expressed as mean after drug administration ± SEM. The significance of difference between means was determined by One Way ANOVA and $f$ distribution and then $p$ values were calculated. All statistical procedure was performed according to the reported method (Walpole, 1998).
5. RESULTS

5.1) Color Reaction for identification of chemical constituents

Chemical Tests

The following tests were carried out for the identification of chemical constituents:

(c) Liebermann-Burchardt Test for Triterpenes  (b) Dragendorff Test for Alkaloids
(c) Test for Tannins  (d) Test for Saponins
(e) Test for Carbohydrates  (f) Salwaski Test for Sterols
(g) Test for Proteins

Procedure is mentioned in the experimental section and the result is presented in following Table1.

TABLE 1: Table showing chemical constituents of C. citratus and P.domestica

<table>
<thead>
<tr>
<th>S#</th>
<th>Plant Name</th>
<th>Chemical Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Triterpenes</td>
</tr>
<tr>
<td>1</td>
<td>C. citratus</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>P.domestica</td>
<td>-</td>
</tr>
</tbody>
</table>

The overview of chemical constituent identified by reactions with various chemical reagents
5.2) Antioxidant Activity

DPPH Radical scavenging Assay

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH). The detail is given in experimental section of this dissertation. Percent radical scavenging activity was determined in comparison with the methanol treated control. Results of *C. citratus* are illustrated in Table 2, and those of *P. domestica* are presented in Table 3.

**Table 2: Antioxidant Activity of *C. citratus***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Concentration</th>
<th>DPPH Scavenging Activity (%) of <em>C. citratus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude Extract</td>
</tr>
<tr>
<td>1</td>
<td>1.25 %</td>
<td>05</td>
</tr>
<tr>
<td>2</td>
<td>2.5 %</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>5 %</td>
<td>82</td>
</tr>
</tbody>
</table>

Graphical representation of Table 2
Table 3: Antioxidant Activity of *P. domestica*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Concentration</th>
<th>DPPH Scavenging Activity (%) of <em>P. domestica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Crude Extract</strong></td>
</tr>
<tr>
<td>1</td>
<td>1.25%</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>2.5%</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>5%</td>
<td>85</td>
</tr>
</tbody>
</table>

Graphical representation of Table 3
### 5.3) Antibacterial Activity

#### Table 4: Antibacterial Activity of *C. citratus* and *P. domestica* showing zones of inhibition in mm

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of organism</th>
<th><em>P. domestica</em> 10mg/ml</th>
<th><em>P. domestica</em> 20mg/ml</th>
<th><em>P. domestica</em> 40mg/ml</th>
<th><em>C. citratus</em> 20mg/ml</th>
<th><em>C. citratus</em> 40mg/ml</th>
<th>Ampicillin 10mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>6±0.173</td>
<td>10±0.264</td>
<td>14±0.173</td>
<td>10±0.15</td>
<td>14±0.156</td>
<td>23.2±0.100</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>9±0.0866</td>
<td>16±0.132</td>
<td>22±0.200</td>
<td>8±0.055</td>
<td>12±0.026</td>
<td>25.5±0.050</td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella typhi</em></td>
<td>13±0.032</td>
<td>15±0.131</td>
<td>19±0.095</td>
<td>13±0.173</td>
<td>18±0.017</td>
<td>20.5±0.173</td>
</tr>
<tr>
<td>4</td>
<td><em>Klebsiella pneumonia</em></td>
<td>7±0.132</td>
<td>13±0.096</td>
<td>17±0.036</td>
<td>8±0.086</td>
<td>15±0.173</td>
<td>22.2±0.100</td>
</tr>
<tr>
<td>5</td>
<td><em>Bacillus cereus</em></td>
<td>8±0.085</td>
<td>10±0.026</td>
<td>12±0.05</td>
<td>9±0.105</td>
<td>14±0.08</td>
<td>20.5±0.173</td>
</tr>
<tr>
<td>6</td>
<td><em>Proteus mirabilis</em></td>
<td>7±0.20</td>
<td>15±0.01</td>
<td>18±0.105</td>
<td>12±0.01</td>
<td>16±0.065</td>
<td>21.5±0.03</td>
</tr>
</tbody>
</table>

All data is represented as Mean ± Standard deviation

*P. domestica, C. citratus and standard drug concentrations*

**Graphical representation of Table 4**
5.4) **Acute Oral Toxicity Test**

*Prunus domestica* and *Cymbopogon citratus* given in two different doses i.e. 2.5 and 5.0 g/kg body weight to healthy and active laboratory animals. No animal showed any sign of untoward effects during 72 hours of observation period. No mortality was observed. Autopsy done revealed no gross pathological and physical changes (Figure 3). All vital organs i.e. heart, liver lungs and kidneys were found to be normal. No mortality was observed during the claimed 72 hours observation period.

![Autopsy images](image)

**Figure 3:** Showing autopsy of male and female groups of test drug
5.5) Behavior Studies

General behavior of animals was observed by the reported method. *Cymbopogon citratus* and *Prunus domestica* at 300 and 500mg/kg dose was given to experimental animals detail of which is already mentioned in experimental section. Diazepam (5mg/kg) served as standard while normal saline and served as control group. The behavioral changes were observed if any at 30min, 1 hour, 2 hour, 3 hour, 4 hour and 06 hours after the drug administration. The parameters observed are listed in Table 5. The results show that both the test drugs did not bring any noticeable change in the behavior of animals during the period of observation as compared to the animals treated with Diazepam which showed depression effects in all the parameters i.e. grip strength, alertness/awareness, pinna reflex and the responses of sound, touch and pain.

![Behavior Studies of C. citratus and P. domestica in Animal Activity Meter](image)

Fig 4: Behavior Studies of *C. citratus* and *P. domestica* in Animal Activity Meter
Table 5: Behavior Studies of *C. citratus* and *P. domestica* Extracts on Albino mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>Behavior type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grip strength</td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I 300mg/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II 500mg/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Prunus domestica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III 300mg/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group IV 500mg/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V 10mg/kg</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>N. saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group VI</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

No effect (-), Slight depression (+), Moderate depression (++), Strong depression (+++), Very strong depression (++++)
5.6) **Neuro pharmacological Studies**

*Rota rod Test*

It describes the motor coordination or fatigue resistance activity of the drug. The effect of extracts of *Cymbopogon citratus* and *Prunus domestica* on motor coordination was determined by using UGO Basile Rota rod apparatus on Swiss albino mice at a dose of 500mg/kg body weight. Results are listed in Table 6. A detail is given in the experimental section. Results indicate that both the extracts of *C. citratus* and *P. domestica* have enhancing effects on muscle coordination of mice at all the test doses, as all the animals maintained their balance on rota rod bar by increase in the time on the bar and decrease in no. of falls. while the results of standard drug diazepam indicate that it possesses significantly higher sedative effects than that produce by extract as the animals was unable to maintain their balance on bar and there was shortening the time of animals spend on rota rod bar. Figure 5 shows the animals on rotating rods.

![Fig 5: Motor co-ordination Activity on Rota Rod Apparatus](image)

*Fig 5: Motor co-ordination Activity on Rota Rod Apparatus*
### TABLE 6: Motor coordination Activity of *C. citratus* and *P. domestica* (in seconds)

<table>
<thead>
<tr>
<th></th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>90 minutes</th>
<th>120 minutes</th>
<th>180 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. citratus</em></td>
<td>120.1 ± 0.12</td>
<td>113.6±0.1</td>
<td>105.9±0.1</td>
<td>130.3 ± 0.1</td>
<td>140.4±0.1</td>
</tr>
<tr>
<td><em>P. domestica</em></td>
<td>180.3 ± 0.39</td>
<td>180.4±0.1</td>
<td>180.4±0.1</td>
<td>180.4±0.1</td>
<td>180.4±0.1</td>
</tr>
<tr>
<td>Diazepam</td>
<td>53.4 ± 71.24</td>
<td>68.8±70.5</td>
<td>77.2±61.4</td>
<td>125.6±75.8</td>
<td>143.8±54.6</td>
</tr>
<tr>
<td>N. Saline</td>
<td>180.4 ± 0.1</td>
<td>180.4±0.1</td>
<td>180.3±0.1</td>
<td>180.4±0.1</td>
<td>180.3±0.1</td>
</tr>
<tr>
<td>F/H value</td>
<td>-</td>
<td>10.266</td>
<td>2.596</td>
<td>7.875</td>
<td>3.074</td>
</tr>
<tr>
<td><em>p</em> value</td>
<td>-</td>
<td>0.016</td>
<td>0.008</td>
<td>0.049</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Statistical calculation by One way ANOVA all values were found to be significant $p \geq 0.05$

Graphical representation of Table 6
**Grip Strength Test**

It describes the effect of test and standard drugs on muscle strength. Rats weighing between 140-180g were used in this experiment. An increase or decrease in the grip strength of the rats indicates the CNS activity of animal. After administration of the extract of *P. domestica* grip strength increases, this shows that it has a CNS stimulatory effect. While in case of *C. citratus* the grip strength was decreased, this shows that it is CNS depressant. Result is shown in Table 7.

**Table 7: Grip Strength test of *C. citratus* and *P. domestica* (in grams)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>0 minutes</th>
<th>15 minutes</th>
<th>45 minutes</th>
<th>75 minutes</th>
<th>105 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. citratus</em></td>
<td>38.0 ± 10.61</td>
<td>33.1 ± 09.28</td>
<td>32.7 ± 06.22</td>
<td>32.4 ± 09.77</td>
<td>30.2 ± 11.14</td>
</tr>
<tr>
<td><em>P. domestica</em></td>
<td>37.0 ± 08.72</td>
<td>38.6 ± 05.84</td>
<td>45.2 ± 10.23</td>
<td>46.3 ± 08.18</td>
<td>40.4 ± 07.34</td>
</tr>
<tr>
<td>Diazepam</td>
<td>36.2 ± 09.06</td>
<td>30.6 ± 11.93</td>
<td>27.8 ± 09.22</td>
<td>26.2 ± 10.21</td>
<td>24.0 ± 8.19</td>
</tr>
<tr>
<td>N. Saline</td>
<td>37.6 ± 07.69</td>
<td>37.2 ± 09.13</td>
<td>37.8 ± 07.28</td>
<td>38.3 ± 08.01</td>
<td>38.0 ± 10.02</td>
</tr>
</tbody>
</table>

Statistical calculation by One way ANOVA; F value = 0.2251 and p value = 0.9201

**Graphical representation of Table 7**

---

54
5.7) Analgesic Activity

Tail Flick Test

In the present study, analgesia was assessed on rats by standard reported procedure on UGO Basile Tail Flick Instrument. The detail of experiment is given in experimental section. The result shows that significant analgesic activity is found in *C. citratus* at both doses i.e. 300mg/kg and 500mg/kg body weight. While in case of *P. domestica* it was found that moderate activity is present at 300mg/kg dose and highly significant activity is present at 500mg/kg dose. A detail is given in Table 8 which is further illustrated graphically.

Fig 6: Assessment of Analgesic activity on UGO Basile Tail Flick Unit
Table 8: Analgesic activity of test and standard drugs by Tail Flick test (in seconds)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tail Flick Time (Sec.)</th>
<th>0</th>
<th>30 min</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. citrates</td>
<td>Group I 300mg/kg</td>
<td>1.42±0.327</td>
<td>1.92±0.363</td>
<td>3.18±0.414</td>
<td>2.46±0.194</td>
<td>3.48±0.496</td>
<td>2.52±0.342</td>
</tr>
<tr>
<td></td>
<td>Group II 500mg/kg</td>
<td>1.50±0.353</td>
<td>2.44±0.207</td>
<td>4.34±0.709</td>
<td>4.7±0.681</td>
<td>3.58±0.396</td>
<td>2.44±0.114</td>
</tr>
<tr>
<td>P. domestica</td>
<td>Group III 300mg/kg</td>
<td>1.48±0.30</td>
<td>2.16±0.56</td>
<td>2.62±0.70</td>
<td>3.70±1.00</td>
<td>3.98±1.11</td>
<td>3.88±0.76</td>
</tr>
<tr>
<td></td>
<td>Group IV 500mg/kg</td>
<td>1.28±0.31</td>
<td>2.74±0.79</td>
<td>3.86±0.42</td>
<td>4.34±0.30</td>
<td>4.82±0.19</td>
<td>5.22±0.67</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Group V 300mg/kg</td>
<td>1.5±0.33</td>
<td>2.08±0.75</td>
<td>3.06±1.58</td>
<td>3.12±0.31</td>
<td>3.68±0.71</td>
<td>3.34±0.75</td>
</tr>
<tr>
<td>N. saline</td>
<td>Group VI</td>
<td>1.3±0.48</td>
<td>1.2±0.30</td>
<td>1.52±0.77</td>
<td>1.54±0.70</td>
<td>1.46±0.27</td>
<td>1.48±0.21</td>
</tr>
</tbody>
</table>

F/H value    | 4.614                  | 18.033     | 17.754     | 16.368     | 24.549     |

p value      | 0.004                  | 0.003      | 0.001      | 0.001      | 0.001      |

Statistical calculation by One way ANOVA all values were found to be significant $p > 0.004$

Graphical representation of Table 8
5.8) Anti-emetic Activity

The results of anti-emetic assay revealed that *C. citratus* possesses non-significant anti-emetic activity at dose of 300mg/kg and significant anti-emetic activity at 500mg/kg dose and *P. domestica* possesses mild significant activity at 300 mg/kg dose and highly significant activity at the dose of 500mg/kg body weight, which is almost equivalent to that of standard anti-emetic drug i.e. Motilium (Table 9).

**Table 9: Showing Anti-emetic activity of extracts *C. citratus* and *P. domestica* (No. of retches)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group</th>
<th>Avg. weight</th>
<th>Dose</th>
<th>No. of retching</th>
<th>Reflex % Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>C. citratus</em></td>
<td>60.66 ± 2.422</td>
<td>300mg/kg</td>
<td>63.00 ± 4.147</td>
<td>26.45 %</td>
</tr>
<tr>
<td>2</td>
<td><em>C. citratus</em></td>
<td>70.13 ± 4.471</td>
<td>500mg/kg</td>
<td>44.00 ± 5.550</td>
<td>48.23 %</td>
</tr>
<tr>
<td>3</td>
<td><em>P. domestica</em></td>
<td>80.66 ± 9.5847</td>
<td>300mg/kg</td>
<td>57.167 ± 3.869</td>
<td>33.20 %</td>
</tr>
<tr>
<td>4</td>
<td><em>P. domestica</em></td>
<td>93.45 ± 8.666</td>
<td>500mg/kg</td>
<td>33.167 ± 4.309</td>
<td>61.29 %</td>
</tr>
<tr>
<td>5</td>
<td>Motilium</td>
<td>93.83 ± 9.621</td>
<td>0.15mg/kg</td>
<td>32.167 ± 5.269</td>
<td>62.45 %</td>
</tr>
<tr>
<td>6</td>
<td>Distilled Water</td>
<td>82.50 ± 9.0719</td>
<td>1 ml</td>
<td>85.667 ± 2.944</td>
<td>0 %</td>
</tr>
</tbody>
</table>

Statistical calculation by One way ANOVA, F value = 127.6544 and *p* value = 0.00

**Graphical representation of Table 9**
6. DISCUSSION

Humans believe that herbal plants have God gifted properties against variety of diseases and many research studies confirm the historical use of plants in treatment of different diseases. Plant had vast pharmacological activities because of the presence of certain phytochemical constituents.

Emesis is a common problem especially in females during pregnancy (motion sickness). The allopathic drugs used for its control are costly and a number of adverse effects are reported, and during the period of pregnancy it becomes more severe. It was, therefore, considered worth while to look for some cheap herbal medicine, capable of preventing or inhibiting emesis and which may be easily procurable in both rural and urban areas of Pakistan and having nil or least side effects. Keeping this objective in view, *Cymbopogon citratus* and *Prunus domestica* were selected to evaluate the anti-emetic action, because of their use in folk medicine. Both the herbs are indigenous, cost effective and safe for use.

The main objective of this research work was to investigate the anti-emetic action, but some other related chemical and pharmacological activities were also conducted which had a direct or indirect relation with nausea and vomiting. Both the plants were screened for their phytochemical constituents to search out that which of the chemical constituents may be responsible for different pharmacological activities with a special reference to anti-emetic activity.
Sometimes with the feeling of nausea and vomiting, the intestinal microbial flora is also disturbed. For this purpose, antimicrobial studies were also conducted.

A wide variety of receptor types and neurotransmitters are found in areas of the brain thought to play a role in emesis and its control. This is why CNS activities were also conducted including Grip strength and Rota rod Test.

Analgesic activity was also investigated because very little allopathic analgesic medicines are allowed during pregnancy. A very significant activity was detected in one of the test drug. Anti-oxidant activity was carried out as a fact that the plants having anti-oxidant activity usually possess anti-emetic activity.

In literature search it was found that prunes increases hemoglobin levels and is effective in correction of electrolyte, fluid or nutritional deficiencies of the body which is necessary in the management of patients with nausea and vomiting.

6.1 *Cymbopogon citratus*

On phytochemical analysis it was observed that alcoholic extract of *Cymbopogon citratus* leaves possess most important vital chemical constituents like triterpenes, alkaloids, tannins, saponins, flavonoids, sterols, protein and carbohydrates (Table 1). These constituents are accountable for various pharmacological actions. The main chemical components of lemongrass which are reported earlier includes citrolo as the major constituent (78-85.5%), flavanoids, saponins, alkaloids, myrcene, limonene, methyl heptenor, linaloal, geraniol, neroal and citronellal. Lemon grass of Africa contains camphorene, bicyclic camphorine, monocyclic terpene, methyl heptinol, linalool, terpeneol, gerniol, nerol, farnesolvaleraldehyde, heptenone, citronellol, decyaldehyde.
etc. While lemon grass from Japan contains 60-70% citrol, barneol but heptenone, terpenes and sesquiterpenes are scarcely present (Crowford et al., 1975; Fedinand, 1966; Kasumov and Babaev, 1983; Hassan et al., 2007).

The compounds identified in *Cymbopogon citratus* by some other researchers include mainly terpenes, alcohols, ketones, aldehyde and esters. Some of the reported phytoconstituents are essential oils that contain Citral a, Citral b, Nerol Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and Terpinol Methylheptenone. The plant also contains reported phytoconstituents such as flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2’-O-rhamnoside, quercetin, kaempferol and apiginin. Newly isolated and identified triterpenoids from leaf wax are cymbopogone and cymbopogonol (Hanson et al., 1976; Shah et al., 2011).

Studies indicate that *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties. Various other effects like antimalarial, antimutagenicity, antimycobacterial, antioxidants, hypoglycemic and neurobehaviorial have also been studied. These results are very encouraging and indicate that this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects (Shah et al., 2011). Major study is carried out on lemon grass oil, and the ethanol extract of *C. citratus* is still empty to be searched out. For this purpose it was selected and remarkable results were found in some fields.

Antioxidant activity results showed non-significant activity at 1.25% concentration in crude extract and all other fractions of the test drug. At 2.5% concentration, the results were mild significant in crude extract and ethyl acetate fraction, while non-significant in
chloroform and \( n \)-butanol fractions of the extract. A highly significant antioxidant activity was found in crude extract and ethyl acetate fraction at 5% concentration and again the results were non-significant in chloroform and \( n \)-butanol fractions. This shows that a dose dependant antioxidant activity is present in crude extract of the leaves of \textit{C. citratus} and its ethyl acetate fraction, while in case of chloroform and \( n \)-butanol fractions a non-significant activity was found which is increasing very little as the concentration is increased (Table 2). Antioxidant activity of lemon grass is reported by some other researchers also (Cheel \textit{et al.}, 2005; Rabbani \textit{et al.}, 2006).

In antibacterial activity, against \textit{Staphylococcus aureus} the test drug was found to possess non-significant activity i.e. 10±0.15 at 20mg/ml and mild significant activity i.e. 14±0.156 at 40mg/ml, as compared to the standard drug “Ampicillin”, which showed 23.2±0.10 mm zones of inhibition at 10mg/ml concentration. In case of \textit{Escherichia coli} again it was found to be non-significant at both 20 and 40mg/ml concentrations i.e. 8±0.055 and 12±0.026 respectively while the standard drug showed 25.5±0.050 mm zones of inhibition.

A highly significant activity (almost 90%) was found at 40mg/ml concentration i.e. 18±0.017, while that of standard was 20.5±0.173 against \textit{Salmonella typhi}. At 20mg/ml concentration moderate significant activity was recorded i.e. 13±0.173 against the same organism. In case of \textit{Klebsiella pneumoniae}, a non-significant activity i.e. 8±0.086 at 20mg/ml and mild significant activity, i.e. 15±0.173 at 40mg/ml was found, as compared to the standard drug “Ampicillin”, which showed 22.2±0.10 mm zones of inhibition. \textit{Bacillus cereus} and \textit{Proteus mirabilis}, both the organisms showed nearly similar results. The activity was non-significant at low concentration and moderate at high concentration.
(Table 4). The results at 20mg/ml were 9±0.105 and 12±0.01, while at 40mg/ml were 14±0.08 and 16±0.065, respectively. The standard drug showed 20.5±0.173 and 21.5±0.03 results against Bacillus cereus and Proteus mirabilis organisms. Antibacterial activity of C. citratus is also reported by some other researchers (Syed et al., 1990; Sumita et al., 2004).

According to acute oral toxicity test results the test drug C. citratus was found safe up to the dose of 7.5g/kg body weight. Autopsy was carried out after observation period showed no effect of drug on of all vital organs it can also be confirmed by studies reported the use of C. citratus in daily life in various form. General behavior of animals was observed by the reported method. Cymbopogon citratus at 300 and 500mg/kg dose was given to experimental animals, detail of which is already mentioned in experimental section. Diazepam (5mg/kg) served as standard while normal saline and served as control group. The behavioral changes were observed if any at 30min, 1 hour, 2 hour, 3 hour, 4 hour and 06 hours after the drug administration. The animals were found active and alert after the administration of C. citratus extract at both doses i.e. 300 and 500mg/kg body weight. They were found to have a strong grip up to 4 hour period of observation. Their pain, sound and touch responses were found to be normal. Pinna reflex was also found to be accurate. In case of standard drug Diazepam at 10mg/kg dose the animals lost their grip just after administration of drug. There was a moderate depression in sound response, strong depression in pain response, pinna reflex and alertness, while a very strong depression in touch response and grip strength. The parameters observed and results noted are listed in Table 5.
Motor coordination Activity of *C. citratus* was analyzed on Rota rod apparatus and it was found that the test drug has slight CNS depressant effects. The animals fed with 500mg/kg *C. citratus* extract fall from the rotating rods at 120.1 ± 0.12 seconds after 30 minutes of drug administration. After 1 hour it was noticed that the animals fell at 113.6±0.1 seconds, after 1.5 hours at 105.9±0.1 seconds. Then there was a rise in falling time after 2 hours i.e. 130.3 ± 0.1 seconds and after 3 hours 140.4±0.1 seconds. This shows that the extract of *C. citratus* has a CNS depressant effect in early phase of administration of drug while the effect started reducing after 2 hours regularly. Normal saline, which was used as negative control showed no effect i.e. almost 180.3±0.1 during the whole period of observation. Diazepam, which was used as positive control showed highly significant CNS depressant effect i.e. 53.4 ± 71.24 seconds at 30 minutes, 68.8±70.5 at 60 minutes, 77.2±61.4 at 90 minutes, 125.6±75.8 at 2 hour and 143.8±54.6 at 3 hours. Statistical calculation by Student *t* test and the values are found to be significant at the level *P* ≤ 0.05 (Table 6).

Another test by which CNS stimulation or depressant effect can be judged was Grip strength (test performed on UGO Basile Grip Strength Meter). The animals administered 500mg/kg of *C. citratus* extract showed CNS depressant effect as they lost their grip after administration of the test drug. The baseline reading was 38.0 ± 10.61g which was reduced to 33.1 ± 09.28g after 15 minutes, 32.7±06.22g after 45 minutes, 32.4 ± 09.77g after 75 minutes and 30.2 ± 11.14g after 105 minutes. Diazepam showed more depressant effect i.e. 36.2 ± 09.06g at 0 minutes, , 30.6 ±11.93g after 15 minutes, 27.8 ± 09.22 after 45 minutes, 26.2 ± 10.21 after 75 minutes and 24.0 ± 8.19 after 105 minutes. The readings of negative control group were 37.6 ± 07.69, 37.2 ± 09.13, 37.8 ±7.28, 38.3 ±
08.01 and 38.0 ±10.02 after 0, 15, 45, 75 and 105 minutes respectively (Table 7). The values are found to be significant at $P \leq 0.05$.

Analgesic activity of the test (C. citratus extract) and standard (Aspirin) drugs was assessed by UGO Basile Tail Flick Unit. Present results show that C. citratus extract possesses significant analgesic activity persisting for 1 hour and then the effect started diminishing up to 4 hours at 300mg/kg dose. The values are 1.42±0.327 at 0 minutes, 1.92±0.363 at 30 minutes, 3.18±0.414 at 1 hour, 2.46±0.194 at 2 hours, 3.48±0.496 at 3 hours and 2.52±0.342 at 4 hours. The activity is dose dependant i.e. it is slightly increased with the increase in dose. When the dose is increased to 500mg/kg body weight the values are slightly increased i.e. 1.50±0.353 at 0 minutes, 2.44±0.207 at 30 minutes, 4.34±0.709 at 1 hour, 4.7±0.681 at 2 hours, 3.58±0.396 at 3 hours and 2.44±0.114 at 4 hours. The standard drug Aspirin at 300mg dose showed high analgesic effects i.e. 1.5±0.33 at 0 minutes, 2.08±0.75 at 30 minutes, 3.06±1.58 at 1 hour, 3.3±0.51 at 2 hours, 3.68±0.71 at 3 hours and 3.34±0.75 at 4 hours. Normal saline values are 1.3±0.48, 1.2±0.30, 1.52±0.77, 1.54±0.70, 1.46±0.27 and 1.48±0.21 at 0 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and 4 hours of drug administration respectively (Table 9). Comparing the values of test and standard drug, it was found that C. citratus extract possesses significant analgesic activity at both the doses. Statistical calculation were carried out by Student $t$ test; values are found to be significant at $P \leq 0.01$ level (Table 8).

Previously, research of some scientists show that the essential oil of Cymbopogon citratus possesses a significant antinociceptive activity both at the peripheral and at the central levels (Viana et al., 2000).
Anti-emetic assay was performed on chicks of 60-90g using copper sulfate as emesis inducing agent. The results of anti-emetic assay revealed that C. citratus possesses non-significant anti-emetic activity at dose of 300mg/kg i.e. number of retching reflexes were recorded as 63.00 ± 4.122 which showed 26.45% inhibition and significant anti-emetic activity at 500mg/kg dose i.e. number of retching reflexes were recorded as 44.00 ± 5.688 which showed 48.23% inhibition. When these results are compared to the standard drug Motilium, it was found to be 32.16 ± 9.267 number of reflex with 62.45% inhibition. The number of reflex recorded for normal saline was 85.66 ± 2.961. Statistical calculation by Student t test; values are found to be significant at $P \leq 0.05$ (Table 9).

C. citratus has demonstrated anti-emetic activity in experimental models and it is used to reduce nausea and vomiting in human traditionally, the exact mechanism of which is still unknown. It appears that several key constituents and several different mechanisms are responsible. There is an assumption that both citrol and citronellols may be responsible for its anti-emetic action because both compounds are reported to have anti-oxidant action and usually the compounds having anti-oxidant action bear anti-emetic activity (Rabbani et al., 2006).

### 6.2 Prunus domestica

It is well known that herbal material have variety of pharmacological activities because of its numerous active chemical constituents. The phytochemical screening of P. domestica showed that it possesses active chemical constituents which were also confirmed by other research studies (Qiaser and Naveed, 2011). The phytochemical
screening reveals that the alcoholic extract of *P. domestica* contains tannins, saponins, flavonoids, sterols, protein and carbohydrates (Table 1).

Antioxidant activity results showed significant activity at 1.25% concentration in ethyl acetate fraction, mild significant activity in crude extract and chloroform fraction and non-significant activity in *n*-butanol fraction of *P. domestica*. At 2.5% concentration, the results were non-significant in and *n*-butanol fraction, mild significant in crude extract and chloroform fraction, while highly significant activity in ethyl acetate fraction of the *P. domestica* extract. A highly significant antioxidant activity was found in crude extract, ethyl acetate and chloroform fractions at 5% concentration and again the results were non-significant in *n*-butanol fraction. This shows that a dose dependant antioxidant activity is present in crude extract of the dried fruit of *P. domestica* in all the fractions i.e. the activity is increased as the concentration is increased (Table 3).

In antibacterial activity, against *Staphylococcus aureus* *P. domestica* was found to possess non-significant activity i.e. 6±0.173 at 10mg/ml concentration, 10±0.264 at 20mg/ml and 14±0.173 at 40mg/ml, as compared to the standard drug “Ampicillin”, which showed 23.2±0.10 mm zones of inhibition at 10mg/ml concentration.

In case of *Eschrichia coli* again it was found to be non-significant at 10mg/ml, mild significant at 20mg/ml and highly significant at 40mg/ml concentrations i.e. 9±0.0866, 16±0.132 and 22±0.200 respectively while the standard drug showed 25.5±0.050 mm zones of inhibition.

A highly significant activity (almost 90%) was found at 40mg/ml concentration i.e. 19±0.095, while that of standard was 20.5±0.173 against *Salmonella typhi*. At 10mg/ml
and 20mg/ml concentration moderate significant activity was recorded i.e. 13±0.032 and 15±0.131 against the same organism.

In case of *Klebsiella pneumoniae*, a non-significant activity i.e. 7±0.132 at 10mg/ml, mild significant activity, i.e. 13±0.096 at 20mg/ml and highly significant activity i.e. 17±0.036 at 40mg/ml was found, as compared to the standard drug “Ampicillin”, which showed 22.2±0.10 mm zones of inhibition.

*Bacillus cereus* and *Proteus mirabilis*, both the organisms showed nearly similar results at 10mg/ml concentration i.e. 8±0.085 and 7±0.20 mm zones, respectively. The results at 20mg/ml were 10±0.026 and 15±0.01, while at 40mg/ml were 12±0.05 and 18±0.105, respectively. The standard drug showed 20.5±0.173 and 21.5±0.03 results against *Bacillus cereus* and *Proteus mirabilis* organisms. The activity was non-significant at low concentration and moderate at little high concentration. At highest concentration mild significant activity was achieved against *Bacillus cereus*, while highly significant activity was achieved against *Proteus mirabilis*. The details can be seen in result section of this dissertation (Table 4).

Acute oral toxicity test was carried out according to the reported method (Loomis, 1978). The ethanol extract of *P. domestica* did not show any untoward effect up to the dose of 5g/kg body weight. No mortality was observed. Autopsy done revealed no gross pathological and physical changes. All vital organs i.e. heart, liver lungs and kidneys were found to be normal (Figure 3). No mortality was observed during the claimed 72 hours observation period.

General behavior of animals was observed by the reported method. *P. domestica* at 300 and 500mg/kg dose was given to experimental animals in a similar manner as that of *C.*
citrus. Diazepam (5mg/kg) served as standard while normal saline and served as control group. The behavioral changes were observed if any at 30min, 1 hour, 2 hour, 3 hour, 4 hour and 06 hours after the drug administration. The animals were found active and alert after the administration of P. domestica extract at both doses i.e. 300 and 500mg/kg body weight. They were found to have a strong grip up to 4 hour period of observation. Their pain, sound and touch responses were found to be normal. Pinna reflex was also found to be accurate. In case of standard drug Diazepam at 10mg/kg dose the animals lost their grip just after administration of drug. There was a moderate depression in sound response, strong depression in pain response, pinna reflex and alertness, while a very strong depression in touch response and grip strength. The parameters observed and results noted are listed in Table 5.

Motor coordination Activity of P. domestica was analyzed on Rota rod apparatus and it was found that the test drug has slight CNS stimulant effects. The animals fed with 500mg/kg P. domestica extract remain stuck on the rotating rods at 180.3 ± 0.39 seconds after 30 minutes of drug administration. After 1 hour, 1.5 hours, 2 hours and 3 hours it was noticed that the animals remained stuck up to 180.4±0.1 seconds. This shows that the extract of P. domestica has a CNS stimulant effect up to 3 hours of administration of drug. Normal saline, which was used as negative control showed no effect i.e. almost 180.3±0.1 during the whole period of observation. Diazepam, which was used as positive control showed highly significant CNS depressant effect i.e. 53.4 ± 71.24 seconds at 30 minutes, 68.8±70.5 at 60 minutes, 77.2±61.4 at 90 minutes, 125.6±75.8 at 2 hour and 143.8±54.6 at 3 hours. Statistical calculation by Student t test and the values are found to be significant at the level $P \leq 0.05$ (Table 6).
Another test by which CNS stimulation or depressant effect can be judged was Grip strength Test performed on UGO Basile Grip Strength Meter. The animals administered 500mg/kg of *P. domestica* extract showed highly significant CNS stimulant effect as the grip strength was increased gradually up to 75 minutes of administration of the test drug which persists up to 105 minutes of drug administration. The baseline reading was 37.0 ± 08.72g which was increased to 38.6 ± 05.84g after 15 minutes, 45.2±10.23g after 45 minutes, 46.3 ± 08.18g after 75 minutes and 40.4 ± 07.34g after 105 minutes. Diazepam showed more depressant effect i.e. 36.2 ± 09.06g at 0 minutes, 30.6 ±11.93g after 15 minutes, 27.8 ± 09.22 after 45 minutes, 26.2 ± 10.21 after 75 minutes and 24.0 ± 8.19 after 105 minutes. The readings of negative control group were 37.6 ± 07.69, 37.2 ± 09.13, 37.8 ±7.28, 38.3 ± 08.01 and 38.0 ±10.02 after 0, 15, 45, 75 and 105 minutes respectively (Table 7). The values are found to be significant at \( P \leq 0.05 \).

The results of analgesic activity using tail flick instrument revealed that all test and standard drugs possesses analgesic activity by boost in reaction time on analgesiometer but *P. domestica* extract at 500mg/kg dose possesses highest significant and prolonged activity in dose dependent manner as compared to the standard drug (Table 8). The presence of analgesic activity in *P. domestica* may be due to the presence of flavonoids, tannins, carbohydrates, and sterols which have been reported to have the ability to inhibit pain sensitivity (Rao *et al.*, 1998; Okwu and Josiah, 2006; Argal and Pathak, 2006; Malairajan *et al.*, 2006; Vanu *et al.*, 2006; Meena *et al.*, 2009; Zulfiker *et al.*, 2010). This statement was also confirmed by Hajare *et al.*, in 2000 (Hajare *et al.*, 2000), according to them enzyme prostaglandins are involved in pain perception and its synthetase is
inhibited by flavonoids so it might be possible that the reduce availability of prostaglandins produce analgesic effects.

The results of anti-emetic assay revealed that *P. domestica* possesses mild significant activity at 300 mg/kg dose and highly significant activity at the dose of 500mg/kg body weight, which is almost equivalent to that of standard anti-emetic drug i.e. Motilium. At dose of 300mg/kg the number of retching reflexes were recorded as 57.22 ± 3.903 which showed 33.20% inhibition and at 500mg/kg dose the number of retching reflexes were recorded as 33.16 ± 4.301 which showed 61.29 % inhibition. When these results are compared to the standard drug Motilium, it was found to be 32.16 ± 9.267 number of reflex with 62.45 % inhibition. The number of reflex recorded for normal saline was 85.66 ± 2.961. Statistical calculation by Student t test; values are found to be significant at *P* ≤ 0.05(Table 9).

A wide variety of receptor types and neurotransmitters are found in areas of the brain thought to play a role in emesis and its control. In the gastrointestinal tract there are peripheral receptors which are also involved in emesis. The neurotransmitters involved in emesis are histamine, acetylcholine, dopamine, nor adrenaline and 5-Hydroxytryptamine. The antagonists of receptors for these transmitters have anti-emetic effect (Leslie and Gwynn, 1984; Schwartz et al., 1986; Koch, 1995, Quingley et al., 2001).

Anti-emetic activity of *P. domestica* is reported earlier (Qureshi et al., 1988). Many chemical constituents of *P. domestica* and *C. citratus* are same including flavonoids and sterols. It is reported that flavonoids and sterols act as anti-emetic principles (Shin et al., 2002). Most probably these compounds serve as antagonist of any of the above
mentioned receptors, as a result of which this plant shows anti-emetic activity. But further research study is needed to isolate specific flavonoid or sterol having anti-emetic property.

A comparison of *C. citratus* and *P. domestica* shows that both plants possess constituents like tannins, saponins, flavonoids, sterols, protein and carbohydrates while triterpenes and alkaloids are present in *C. citratus* only. A very significant antioxidant activity was found in both the plants. The microbiological analysis showed that almost similar antimicrobial activity was found in both plants against all the organisms tested except the activity of *P. domestica* against *E. coli* was more significant than *C. citratus*. The pharmacological activities showed a depressant effect in animals treated with *C. citratus* as compared to those treated with *P. domestica* which exhibited CNS stimulant effect. The analgesic activity results are different i.e. mild analgesic effect in *C. citratus* while highly significant effect was found in *P. domestica*. Similar was the case in anti-emetic activity i.e. mild anti-emetic effect in *C. citratus* while highly significant effect was found in *P. domestica*. Both analgesic and antiemetic activities exhibited dose dependant effect in *C. citratus* and *P. domestica*. 
7. CONCLUSION

From the present research work, it is concluded that the two plants i.e. *C. citratus* (leaves) and *P. domestica* (Fruit) selected for pharmacological screening with a special reference to anti-emetic activity possess a number of activities like analgesic, antibacterial, antioxidant, anti-emetic etc. Both plants are safe and could be used as a medicine in different herbal preparations.

*C. citratus* exhibited significant anti-emetic and antioxidant activities at higher dose and milder at lower dose. It has slight CNS depressant effect with mild analgesic activity. A strong antibacterial action was found against *Salmonella typhi* and moderate antibacterial action was found against other tested organisms.

*P. domestica* exhibited strong analgesic and anti-emetic activity. Further it possesses Central Nervous System stimulating effect. So it could be useful during pregnancy because women feel lethargic and dull during the period and they often have a feeling of nausea and vomiting, in addition to the fact that allopathic analgesics are avoided during pregnancy. *P. domestica* along with inhibiting emesis action also provides necessary vitamins and nutritional elements which help in maintaining healthy condition of the patient.
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