AN INVESTIGATION OF ANTIMICROBIAL COMPOUNDS FOR IMMUNOMODULATING AND ANTI-ADHESION PROPERTIES

THESIS SUBMITTED FOR THE FULFILMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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THIS PIECE OF MY RESEARCH IS

DEDICATED

TO MY NIECES AND NEPHEWS

ALFEYA, FAZL-E-ABBAS, HUSSAIN, ABBAS, SHABBIR, ISMAT AND ATOOFA

SO THAT THEY CAN PRODUCE MASTER PIECES IN THEIR FUTURE LIVES
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SUMMARY
SUMMARY

Global emergence of resistance in bacteria to the antimicrobial agents has made it difficult for the physicians to find effective therapeutic agents for diseases caused by MDR TB, MDR - Salmonella, MRSA, Ps. aeruginosa, Acinetobacter, Candida etc. Since long infectious disease are being cured by traditional practitioners with the help of herbs and higher plants. At present antibiotics, obtained from molds fungus or synthetically prepared have lost their efficacy. The new trend to combat the pathogens is to use water and organic extracts made from different parts of medicinal plants.

In this study, 30 medicinal plants and their different parts were screened for their in-vitro anti-microbial activity, anti-adhesion activity under in-vitro and in-vivo in rat model, their toxicity and immunomodulating properties in BALB/c mice.

Screening for the antimicrobial activity was carried out against twenty different bacterial species that includes ATCC© and clinical strains. The screening of antibacterial activity was performed by the agar well diffusion and micro broth dilution method. Beside water solution of Rosa centifolia (rose) and fruit, leaves and stem solutions of C.fistula was also included. From among eleven plant species, only clove aqueous extract showed broad-spectrum of antibacterial activity against Ps. aerogenosa and S. agalactiae and others. The antibacterial component present in the clove was found to be heat stable as the autoclaving of the solutions did not affect its activity. Aqueous extracts of Rosa centifolia (rose) and C.fistula (Amaltas) commonly used as therapeutic agents showed anti-microbial activity. M. lysoditius, Staph. aureus, C. pseudodipheroid and Sh dysenteri, S. typhi, E. coli and S. pyogenes at significantly higher concentrations. Four fractions of organic extracts i.e. P1-P2, 80MR, EA2 and ALCR from the fruit of C. fistula were also were found to inhibit MDR Salmonella typhi at a Minimal Inhibitory Concentration (MIC) range of 125 - 500 µg/ml.

Methanolic extract of leaf extract of Bombax ceiba was found to be effective against S. epididermis, S. typhi. B. Subtilis. Shemamin - a novel pure compound isolated from the leaf extract of Bombax ceiba showed antimicrobial activity against Listeria, B. subtilis, Ps. aeruginosa, Sh dysenteri, C. pseudo dipheroid and E.colae. Third group of plant products including Persea americana mill (avocado) peel, pulp, Beta vulgaris (beet) root peel and pulp, Porphyra tenera (nori, Sea vegetable) and Solanum tuberosu (potato) Peel, sub peel and core were screened against several pathogens.

The all plant solutions and extracts had MIC value >25 mg/ml except for Methanol extract of Persea americana peel that had MIC value for Klebsiella 6.25 mg/ml, Proteus 15 mg/ml, Candida 1.5 mg/ml, Pseudomonas 1.5 mg/ml, E coli type 1 12.5 mg/ml. Staph aureus 6.25 mg/ml, Strep sanguis, Strep sobrinus and Ecoli >25 mg/ml.

Formulation of synergistic compound

Since it takes a long time to introduce new anti-microbial compounds, a short cut strategy is to prepare synergistic formulations. In this study, synergistic preparations were developed by combining fruit solution of C.fistula that is an important medicine of Ayurvedic and traditional medicine with amoxicillin a broad-spectrum antibiotic that has lost its efficacy. The new formulation developed was named as Amoxy-cassia. It has already been patented by the Government of Pakistan (Patent
serial number 137124). We preferred the use of water solution as both amoxicillin and C.fistula fruit have proven efficacy in oral dosage in aqueous form. Amoxy-cassia was formulated first by screening with disc diffusion method and further confirmed by the checkerboard titration method and time kill kinetics.

This formulation is found effective against MDR Salmonella typhi, MRSA and E.coli. MDR S typhi causes Typhoid Fever that is endemic in developing countries like Pakistan whereas MRSA is a common cause of nosocomial infections in the hospitalized patients. It has become the threat to hospital personnel, as it mostly invades the patients with knee and hip implantation. Urinogenital infections are very common especially among the women. Another synergistic combination, which was developed during this study, was between Shaminin and Amoxicillin named as Shamoxicillin. This preparation is found to be effective against S. typhi and E.coli.

**In- vitro anti-adhesion Activity of the Plant Products**

Infectious diseases can also be controlled by the inhibition of the attachment of bacterial strains to the host tissue. Because once pathogens are able to attain foothold on to the eukaryotic cell with the help of adhesins present in their pili, they survive there by enveloping themselves with the capsule that is recognized with difficulty by the human immune system. They then produce toxins and harmful products, which damage the tissues or interfere with the metabolic processes.

Most important step in the pathogenesis of the disease is the adhesion of the pathogens to the host surface through the adhesin present in their pili. Periodontal diseases and dental caries are due to the adhesion of bacteria to the salivary pellicle like S.sanguinis. Aggregation to the polymer dextran e.g. S sobrinus and coaggregation of A.naeothiil to S.sanguinis. They may enhance subsequent colonization of Porphyra gingivalis that is associated with adult periodontitis. Organism was treated with the extracts for one hour. Three models were set up for the study of adhesion. First one was attachment of S.sanguinis to human 'O' group blood, second was aggregation of S.sobrinus with dextran and third was coaggregation of A.naeothiil T14V1 with S.sanguinis 35. Importance of controlling the dental plaque lies in the fact that most of the pathogens like Streptococcal species present in the dental plaque is responsible for causing pulmonary disease and bacterial endocarditis. The pathogens invade the lung and heart through oral cavity.

All four plants studied including - Beta vulgaris (beet) root, Persea americana mill (avocado), Solanum tuberosum (potato) and Porphyra tenera (sea vegetable -Nori) has the potential of interfering with the adhesion of bacteria to host epithelial surfaces. The most significant feature of this finding is that the water solutions of all the plants are playing an important role in the anti-adhesion activity of these plants. From this we can say that when avocado, Beetroot, Potato and sea vegetable is eaten, the components of the plant material get in contact with the bacteria present in plaque and thereby result in inhibition of colonization of bacteria on the teeth surface. Urinary infections caused mainly by E.coli are among the most common infectious diseases. Most of the uropathogenic E.coli can express type P. fimbriae and 1 that contain adhesin, which recognizes cell receptors present on the host cells. In our assays, we found that water extracts of P. tenera (sea vegetable) and B. vulgaris (beet) root inhibited the adhesion of E. coli to guinea pig RBC thus inhibiting infection of oral surfaces. Most significant inhibition of adhesion to RBCs was observation with P.
americana (avocado) extract. It would suggest that just like cranberry juice, avocado juice can also be consumed to avoid urinary tract infections with E.coli Type - A. The result of our study reveals that all the four plants studied Beta vulgaris (beet) root, Persea americana mill (avocado), Solanum tuberosum (potato) and Porphyra tenera (sea vegetable) has the potential to interfere with the adhesion of all the oral bacteria.

Those combinations in which treated S.sanguis and A.naeschlundii which showed lower haemagglutination titer as compared to the titer of untreated bacteria were further checked by the flow cytometry technique. The flow cytometry result confirmed our first result obtained. Polyphenol Oxidase (PPO) and asparaginase are the two plants enzymes that show anti-adhesion activity in vitro. These compound were tested for their in-vivo anti-adhesion activity against S. agalactiae (GBS) that normally resides in the female vagina and cause neonatal meningitis. GBS infects neonates during their passage through the vagina birth canal. It is one of the leading causes of due to neonatal meningitis. From our rat infection model it was concluded that treatment of animals post infection with plant enzymes like polyphenol oxidase, inhibited adhesion of bacteria. Another enzyme - Aparaginase helped in the adhesion of GBS to vagina of the female rats. Thus PPO – plant enzyme can be used instead of antibiotics to avoid GBS infection.

Immune system plays an important role in the control infectious diseases. Enhancement of immune system is another important mode of controlling the infectious disease. In this study of Amoxy cassia and C. fistula were found to be effective immunomodulator. In this study effect of Amoxy cassia, fruit solution of C. fistula amoxicillin on immune system specific humoral immune system of BALB/c mice employing sheep RBC as the antigen. Uncountable antibodies producing cell were formed in the spleens of the mice treated with Amoxy cassia similarly the haemagglutination titer was highest too C. fistula fruit solution also exhibited immune enhancing property. Thus novel synergistic preparation Amoxy cassia has posses dual activity antimicrobial and immune enhancing to combat with the pathogens. Our newly formed synergistic compound - Amoxy-cassia as well as fruit extract of C.fistula exhibited a very significant immunoenhancing effect in BALB/c mice. This was observed as high titer of anti-SRBC antibody as well as uncountable anti-SRBC antibody producing plasma cells in spleens of mice treated with Amoxy-cassia. Control mice treated with saline showed fewer plaques /spleen as compared to mice receiving only C. fistula or amoxicillin alone. This study shows that amoxy cassia and C. fistula to be an immune enhancing compound. They have the potential to be an excellent drug for the patient having impaired immune system like AID patients, person suffering from malignanissies and immunocompromised host. It we look at the efficacy of the amoxy cassia, it in vitro inhibits the nosocomially acquired MRSA infection.

This is the first report about the immunomodulatory properties of the C. fistula fruit extract Thus the novel synergistic formulation Amoxy-cassia is not only inhibiting the growth of difficult to treat organism including MDR Salmonella typhii strains, MRSA and, E.coli but it also enhances host immune response to specific antigens. Amoxy cassia has the potential of being developed into an effective therapeutic agent for treatment of bacterial infections as well as an effective immunoenhancing substance to provide protection against infections.
INTRODUCTION
INTRODUCTION

A. INFECTIOUS DISEASES AND ANTIBIOTIC RESISTANCE

Infectious diseases are the world leading cause of premature deaths, killing more than 50,000 people every day. In recent years drug resistance in human pathogenic bacteria has been reported from all over the world and the situation is quite alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The emerging antibiotic resistance in bacterial and fungal pathogens has further complicated the treatment of infectious disease specially in immuno compromised, AIDS and cancer patients. The emergence of multiple drug resistance in human pathogens like MDR Salmonella typhi, Methicillin Resistant Staph aureus (MRSA), Mycobacterium tuberculosis and Ps. aeruginosa has necessitated renewed search for new antimicrobial substances from alternative sources like marine and terrestrial plants (93).

Herbal remedies used in the traditional folk medicine provides an interesting and still largely unexplored source for development of potentially new drugs for chemotherapy which might help to overcome the growing problem of resistance and also the toxicity of currently available commercial antibiotics. The traditional medicinal plants play a vital role to cover basic health needs in the developing countries. Medicinal plants, according to the world health organization (WHO) is defined as any plant which contain substances that can be used for therapeutic purposes or which are precursor of chemo pharmaceutical semi synthetic new drugs (49,105.236).
B. ANTIMICROBIAL PRODUCTS OF PLANT ORIGIN

Exploration of medicinal properties in the plants used by traditional healers is very important to reveal the active principal by isolation and characterization of the constituents, to develop better drugs against cancer as well as viral and microbial infections. It is necessary that documentation of medicinal plants be treated as a matter of extreme urgency as changes in land use due to urbanization destroys much of the habitat of useful plant and this is irreversible loss of plant species. Therefore, efforts should be made to document the medicinal use of plant before much of this is eliminated. There is alike feeling between natural products chemists and microbiologists that multitude of potentially useful phytochemical structure, which could be synthesized chemically, is at risk of being lost irretrievably. There is a scientific discipline known as ethno-botany (or ethno-pharmacology) whose goal is to utilize the impressive array of knowledge assembled by indigenous people about the plant and animal products they have used to maintain health (30.186).

There are five different approaches through which a microbiologist and phyto-chemist selects the plant for pharmacological screening. Random approach which involves the collection of all plants found in the study area. Phytochemical targeting which entails the collection of all members of plant family known to be rich in bioactive compounds. Ethno-directed sampling approach based on traditional medicinal use of plant. Chemotaxonomic approach. A method based on specific plant parts, such as seeds (105).
Health care in underdeveloped countries is far from adequate. Malaria is the leading cause of mortality, followed closely by AIDS related diseases such as diarrhea and acute respiratory infections. Basic pharmaceuticals are not reliably available. When available, they are often prohibitively expensive. Traditional herbalism is relied upon to meet day-to-day healthcare needs (80). In a study conducted by Hamill et al. (2000) in which plants belonging to the family Asteraceae were screened for bioactivity, twenty-six species were found to be good for ethnomedical use. In another study, Rwandan medicinal plants were screened for bioactivity. All of them were found to be active against Gram-positive bacteria and yeast (199). Mexican medicinal plants used by traditional healers to treat respiratory and infectious diseases were investigated for their bioactivity. Out of 18 plants studied, 16 showed broad-spectrum antibacterial activity (176).

In Jordan, herbal medicine is a major part of the heritage of herbal materia medica of the Mediterranean region. Mahasneh et al. studied antimicrobial properties of crude petroleum, ether, ethanol, butane and water extracts of the aerial parts of *Stellaria media*, *Salvia syriaca*, *Cardaria draba*, *Euphorbia prostrata*, *Rubia unctoria* and *Arbutus androechin* and found variable antibacterial and antifungal activity. *Cyclamen persiitum*, *Ononis spinosa* and *Bryonia syriaca* exhibited strong broad-spectrum antibacterial activity. The methanol and hexane extract did not show antimicrobial activity (4.126).

The different systems of medicine practiced in India - Ayurveda, Siddha, Unani, Amchi and local health tradition utilize a large number of plants for the treatment of
human diseases. Most of them have been identified and their uses are well documented (Nadkarni, 1976; Dastur 1985, Saradamma 1990, Jain 1991, Kirtikar and Basu 1991, Ambasta 1992) but the efficacy of many of these plants is yet to be verified. Valsaraj et al (1997) studied seventy medicinal plant used to treat fever, bronchitis, ulcer, diarrhea and dysentery. Ethanol extract of plant was were active against Staphylococcus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger. The most active extracts were of Ahungium salvifolium, Tuchyspermum ammi, Terminalia bellerica, Acacia catechu and Plumbago indica (225).


Srinivasanet al (2001). studied the antimicrobial activity of Indian medicinal plants belonging to 26 different families. Among the 50 plants tested, 79% exhibited antimicrobial activity. The extract which exhibited the maximum spectra of activity were of Albizia lebbeck Allium cepa, Allium sativum, Cucumis longa, Datura metal, Dodonoe viscosa, Eucalyptus globulus, Tamarpho glandulifera, Leucas aspera, Tamarindus indica, Tectona grandis, Zingiber officinale. Plants mainly used in Jaundice, Phyllanthus amarus and Salolium serritense also exhibited antibacterial activity (204).
Ibrak et al (2000) studied the Sinai plant on random versus Ethno-directed research. They investigated hexane, ethyl acetate and ethanol extract of sixty plant species growing wild in Sinai Egypt. Out of sixty plants, 36 species were collected randomly and 24 collected on the basis of their medicinal use. Half of the plant species were found to be active (4,105).

From the plants collected on the ethno directed basis, 83.3% of the plant species were found to be active (105). *Apocynum wilkesiana* is used as an ornamental plant in the gardens of southern Nigeria; some of its species have been cited in the literature as being used for medication. Water extract from the leaves of plant possess activity against *Staph. aureus* and *P. aeruginosa*. Photochemical analyses of these plant reveals the presence of phenol and saponins. Oil and crude alkaloid of *Aphananthis polystachyos* seeds showed promising anti fungal activity.

The genus *Warburgia*, which belongs to the family *Canellaceae* consists of nine species all of which are trees producing aromatic oil, the bark of *Warburgia solanifolia* is used in traditional medicine as an expopant and smoked for cough and cold including a tropical application for sores and inflammation (210).

Healing properties of spices, derived from aromatic plants are attributed to the volatile oil present within them. It is reported that hexane extract of Cinnamon, clove and rosemary have broad-spectrum antimicrobial activity. (79). Traditional plants used for the treatment of Gonorrhea in Guatemala were screened for their antibacterial activity against five strains of *N. gonorrhoea*. Bioactivity was mostly found in bark of *Bixa orellana*, fruits of *Pimenta racemosa*, leaf of *Diphysa rubioides*, *Eupatorium*
Glycyrrhizae, Gilricidia sepium, Physalis angulata, Piper aduncum and Prosopis jujiflora root of Casimiroa edulis and whole Clematis vitalba (36).

Toudes et al. presented detailed bioactive studies of plant Tridax procumbens and found that n-hexane and ethyl extract of the flower and aerial parts showed activity against Escherichia coli, Mycobacterium smegmatis, Staphylococcus, Bacillus, Klebsiella and Salmonella species. While the aqueous extract showed no antimicrobial activity.

Hexane and methanol extract of sixteen medicinal plants collected around Karachi, Pakistan, namely Bivinca, Caesalpinia Pulcherima Cassva alata, Cassia angustifolia, Cassetia occidentalis, Cassetia fistula, Cassia occidentalis, Cassia siamea and Delon vegra were screened for their antimicrobial activity. As compared to hexane extract, the methanol extract of all examined plants exhibited greater activity against both bacterial and fungal.

Plants have almost limit less ability to synthesize aromatic compounds most of which are phenols or their oxygen substituted derivatives (70) or they are secondary metabolites of which at least 12,000 have been isolated (19). In many cases, these substances serve as a plant defense mechanism against predation by microorganisms, insects and herbivores. For instance, terpenoids give plant their odors; others (quinones and tannins) are responsible for plant pigments. Many compounds are responsible for plant flavor (e.g. terpenoids that from chili powder) and some of the same herbs and spices used by us to season food also yield useful medicinal compounds (188,189).
Some of the simplest bioactive phyto-chemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are the common representatives of a wide group of phenyl propane derived compounds. Catechol and Pyrogallol both are hydroxylated phenols shown to be toxic to microorganisms. Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds being colored are responsible in the browning reaction in cut or injured fruits and are intermediate in the melanin synthesis pathway in human skin (185). Their presence in henna gives that material its dyeing properties. Kazmi et al. (1994) described an anthraquinone from Cassia italicae, a tree found commonly in Pakistan, which is bacteriostatic for Bacillus anthracis. Corynebacterium pseudotuberculosis and Pseudomonas aeruginosa and bactericidal for Pseudomonas pseudomallei. Hypericin, an anthraquinone from St. Johns wort (Hypericum perforatum) has been found to have antidepressant and antimicrobial activity (101).

Flavones are phenolic structure containing one carbonyl group (as opposed to two carbonyl in quinones) (Fig. 1). The addition of a 3-hydroxyl group yields flavonoids, which are hydroxylated phenolic substance but occur as a C-C unit linked to an aromatic ring since they are known to be synthesized by plant in response to microbial infection (55). So it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide range of microorganism. Catechins, the most reduced form of C3 unit in flavonoids has been extensively investigated due to their occurrence in oolong green teas. It was reported time ago that they contain a mixture of catechin compounds which in-vitro inhibited Vibrio cholerae.
Streptococcus mutans (13.68,182.222). Shigella, and other microorganisms (227). Flavonoid compounds such as Swertifrancheide, Glycyrrhizin (from licorice) and Chrysin exhibited inhibitory effect against multiple viruses including RSV and HIV (16.74.100.163).

An isoavone found in a West African legume, alpinumisoavone, prevents schistosomal infection when applied topically (163). Phloretin found in certain serovars of apple may have activity against a variety of microorganisms (92). Galangin (2.5.6) (trihydroxyflavone) derived from perennial herbs - Helichrysum aureonitens seems to be particularly useful compound, since it has activity against a wide range of Gram positive bacteria as well as fungi and viruses in particular HSV-1 and Coxsakie B virus type 1 (4). Flavonoids lacking hydroxyl groups on their B ring are more active against microorganism than those with the OH groups (44).

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (81). They are found in almost every plant part - bark, wood, leaves, fruits, root (190). They are divided into two groups, hydrolysable and condensed tannins. Many human physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and wide range of anti-infective actions has been assigned to tannin (165). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions.

Tannins in plants inhibit insect growth (187) and disrupt digestive events in ruminal animals (35,96). Scalbert (183) who reviewed the antimicrobial properties of tannins
listed 33 studies that documented the inhibitory activities of tannins. According to these studies, tannins can be toxic to filamentous fungi, yeast and bacteria. Although this is still speculative, tannins are considered at least partially responsible for the antibiotic activity of methanol extracts of the bark of *Terminalia obtusa* found in Nepal (214). Coumarins are phenolic substances made of fused benzene and alpha pyrone rings. They are responsible for characteristic odor of hay. At least 1,300 had been identified. They show antithrombotic (216) anti inflammatory (165) and vasodilatory (146) activities. Warfarin is a well-known coumarin, used both as an oral anticoagulant and interestingly as a rodenticide (103). It may also have antiviral effects (19). Coumarin was found in-vitro to inhibit *Candida species*. During in-vivo tests on rabbits, the coumarin-spiked water supply was inadverstently given to all animals in research facility; it was discovered to be a contraceptive agent (218). Its estrogen effects were later described (202). As a group, coumarins have been found to stimulate macrophages (33). More specifically, seems to prevent re-occurrence of cold sores caused by HSV-1 in humans (19) but was found to be inefficient in leprosy (216,218).

The fragrance of plant is carried in its quinta essentia, or essential oil fraction. These oil are secondary metabolites that are highly enriched in compounds based on an isoprene structure (Fig. 1). They are called terpenes when these compounds contain additional element, usually oxygen. They are termed as terpenoid. Examples of common terpenoid are methanol, camphor (monoterpenes), farnesol and artemisin (sesquiterpenoids). Artemisin and its derivative alpha artemether are known by the name qinghaosu currently used as antimalarials (213,232).
Terpenes or terpenoids are active against bacteria, fungi (82.112,170.172.207.224), viruses (65.82,162.206.240) and protozoa (66.89). It was reported that 60% of the essential oil derivatives examined to date were inhibitory to fungi, while 30% inhibited bacteria. Betulinic acid is just one of the terpenoids which has been shown to inhibit HIV. A terpenoid constituent capsaicin exhibits a wide range of biological activities in humans, affecting the nervous, cardiovascular and digestive system (229) as well as it is used as an analgesic (46). The evidence for its antimicrobial activity is mixed. Cichewiez and Thorpe (52) found that capsaicin might enhance the growth of Candida albicans but it clearly inhibits various bacterial strains.

Another hot testing diterpene, aframodial from a Cameroonian spice is known to be a broad-spectrum antifungal agent. The ethanol soluble fraction of purple prairie clover yields a terpenoid called petalostemunol, which showed excellent activity against Bacillus subtilis and Staphylococcus aureus and lesser activity against Gram-negative bacteria as well as Candida albicans (9).

Residents of Mali used the bark of a tree called Pteleopsis suberosa for the treatment of gastric ulcers. Investigators tested a fraction-containing terpenoid in 10 rats before and after the rats were chemically induced to have ulcers. They found that the terpenoids prevented the formation of ulcers and diminished the severity of existed ulcers. Whether this activity was due to antimicrobial action or protection of the gastric mucosa is not clear (52) Kadota et al (98) found that trichorabdal A - a diterpene from a Japanese herb could directly inhibit H. pylori.
Heterocyclic nitrogen compounds are called alkaloids like morphine isolated in 1805 from the opium puppy *Papaver somniferum* (69 codeine and heroin are both derivative of morphine). Diterpenoid, an alkaloid commonly isolated from the plants of *Ranunculaceae* or buttercup (97) family (14) is found to have antimicrobial properties (153). Solamargine, a glycoalkaloids from the berries *Solanum khasianum* and other alkaloid may be useful against HIV infection (193) as well as intestinal infections associated with AIDS (132). Alkaloids have been found to have antibacterial and anti-protozoal properties too. Berberine is an important representative of the alkaloid it is potentially effective against trypanosomes and plasmodia (117,133,136,241).

Peptides, inhibitory to microorganism were first reported in 1942. They are positively charged and contain disulphide bond (247). Their mechanism of action may be the formation of ion channels in the microbial membrane (83) or competitive inhibition of adhesion of microbial protein to host polysaccharide receptor (215). Thionins are peptide commonly found in barley and wheat. They contain 47 amino acid residues and exhibit broad spectrum antimicrobial activity (47). Vabatin a newly identified 47-residue peptide from fava beans appears to be structurally related to gamma thionine from grain and inhibit *E.coli*, *P. aeruginosa* and *Escherichia coli* but not Candida or Saccharomyces. Larger molecules including mannose specific lectins from several plants (15). MAP30 from bitter melon (240), GAP31 from *Gelonium multiflorum* (32) and Jacalin, are inhibitory to HIV and CMV proliferation.

The chewing stick is widely used in African countries as an oral hygiene aid (in place of a tooth brush). Chewing sticks come from different species of plant and within one
stick the chemically active components may be heterogeneous. 5% crude extract of some spices used for this purpose *Sorunshia wernackeri* inhibited the periodontal pathogens *Porphyromonas gingivalis* and *Bacteroides melaninogenicus* in vitro. Active component of Nigerian chewing stick (*Ficus zanthoxyloides*) was found to consist of various alkaloids (152). Papaya (*Carica papaya*) yields a milky sap often chief among them is papain, a well known proteolytic enzyme (34). An alkaloid, carapine is also present and may contribute to its antimicrobial properties. Its latex was found to be bacteriostatic to *B. subtilis*, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus vulgaris* (228).

Ayurveda is a type of healing craft practiced in India. Ayurvedic practitioners rely on plant extract both pure single plant preparation and mixed formulations. The preparations have lyrical names such as Ashwagandha (*Withania somnifera* root). Curaevey 100 mixture and Livo-vet (129). These preparations are used to treat animals as well as humans. In addition to the antimicrobial activities, they have been found to have antidiarrheal, immunomodulatory, anticancer and Pychotropic properties (54, 116, 195).

C. COMBINATION THERAPY FOR INFECTIOUS DISEASES

The gift of bacteria to human in the new millennium is bacterial resistance to the conventional drugs used in the therapy. So in case of infections caused by methicillin resistant *Staph aureus*, multidrug resistant *Salmonella typhi*, *Pseudomonas Spp.*, and *Enterococci* more than one drug is used to treat the disease e.g. penicillin, aminoglycosides and vanomycin. aminoglycoside combination is used to treat *Enterococci* infection. The latter combination is alternative in patients who are
allergic to penicillins. The combination of penicillin G and Streptomycin is synergistic against viridans Streptococci. Combination of penicillin (Carbenicillin, ticarcillin, mezlocillin, azlocillin or piperacillin with an aminoglycoside (gentamicin, tobramycin, or amikacin) is synergistic against many strains of *Pseudomonas aeruginosa*. In-vitro synergism with cephalosporin and aminoglycoside combination has been demonstrated to be active against *Klebsiella pneumoniae* (166,236).

Synergism is defined as a four fold or greater decrease in the minimal inhibitory (MIC) or greater decrease in the minimal inhibitory or bacterial cidal concentrations of individual antibiotics when they are present together (11,12,175). It has been found that the synergism, which is due to the combination of an aminoglycoside and an inhibitor of cell wall synthesis, increases the entry of the aminoglycoside into the bacterium where it can interact with the ribosome and inhibits protein synthesis. Also in case of synergism between Amphotericin B and other drug like Fluconosin. Amphotericin B acts on fungal cell membrane in vitro and increases the entry of Fluconosin to its site of action inside the cell. This combination is especially found to be useful in Cryptococcal and Candidal infection in mice(168). Other factor that helps in the synergism is at times one drug inhibits the inactivation of the other drug. Clavulanic acid for example has little intrinsic antimicrobial activity, but it is an irreversible Beta lactamase inhibitor and is used in combination with penicillins and some cephalosporin against a variety of Beta lactamase producing enterobacteriaecae and *Staphylococcus aureus*. Fixed ratio of clavulanic acid and ampicillin is marketed under the trade name of Augmentin (237,149). In some synergistic preparations, drugs used inhibit different steps in a critical metabolic pathway.
Trimethoprim and Sulfamethoxazole when combined in a single preparation, sulfonamides inhibits synthesis of folic acid and trimethoprim inhibits the reduction of folate to tetrahydrofolate which is utilized as cofactor in one carbon metabolism in the cell (10). The combination of these two drugs has been shown to be synergistic against a wide variety of bacteria. Mecillinam is one of the unique Beta lactamase resistant compound that is very effective against enterobacteriaceae in particular Escherichia coli. Mecillinam interacts with one of the E. coli Pencillin binding proteins, a membrane bound enzyme that is required for maintaining the ‘rod’ shape of the organism. Synergy has been demonstrated in vitro when mecillinam is combined with penicillin or cephalosporin that interact preferentially with other penicillin binding proteins like the transpeptidases that are required for wall synthesis during elongation. This combination has also shown synergy in vivo against gram-negative bacillary infections in mice.

Other advantage of using combination of drug showing synergism is to decreased emergence of resistant organisms during therapy. This strategy has been effectively applied in the treatment of tuberculosis where initial intensive therapy of the disease is always carried with two or three drugs. Combined drug treatment of tuberculosis results in markedly reduced selection of resistant mutants. When combination of antibiotics is used it results in reduced toxicity. In case of amphotericin B and Flucytosine synergistic combination, as small amount of amphotericin B is used, for shorter period of time, patient treated with combination experienced less amphotericin B induced nephrotoxicity (28).
When Gram positive and Gram negative aerobic and an aerobic bacterial species are involved, therapy is initiated with a combination of antimicrobial agents. In these situations, aminoglycoside such as gentamicin to cover facultative anaerobes of the coliform group and another agent, such as Clindamycin, Cefoxitin, Metronidazole, Chloramphenicol or Ticarcillin to provide coverage for some Staphylococci but not enterococci as latter organism are mostly ignored in poly microbial or mixed infection. When it is necessary to provide coverage for enterococci in polymicrobial infection high dose intravenous penicillin or ampicillin is usually added to the treatment regime.

The most common clinical indication for combined therapy is the initiation of treatment of severe infection when etiological agent is not known. Combination therapy is especially common in neutropenic patient, patient with compromised host defense mechanism may be infected with wide variety of pathogens and early in the course of infection they may not present clear cut signs and symptoms of infection because infection progress rapidly in such a patient (58).

**D. MECHANISMS OF PATHOGENESIS OF BACTERIA**

Bacteria in natural environment have a prediction for colonizing solid surfaces. The initial association of marine bacteria with inert substances is thought to be reversible and it represents a balance between the attraction of wander walls forces and repulsion due to the net negative electrostatic charges present on most natural surfaces and on most of the bacteria. Attachment of the bacteria may become firmer over time. If polymeric material is synthesized which can form electrostatic
hydrophobic or hydrogen bond with substrate surface such interaction result in adhesion, which have been classified as non-specific. In contrast, specific adhesion is considered to occur when stereo chemical interaction take place which involve complementary components of the bacterium and those on the substratum i.e. lock and key mechanisms. However, in addition, parasitic bacteria often possesses lectins like ligand called adhesin on their surfaces which interact in a stereo chemical manner with saccharide moieties on the eukaryotic cells and results in specific adhesion which is often first step in an infectious process Both non-specific physical binding and specific stereo chemical interaction have been suggested to be involved in the attachment of bacteria to the acquired salivary pellicle covering the teeth (31,69).

i. CARIGENIC INFECTIONS

The oral cavity has long been considered as a potential reservoir for respiratory pathogens. The mechanism of infection could be aspiration into the lungs of oral pathogen capable of causing Pneumonia. Several anaerobic bacteria from periodontal pockets have been isolated from infected lungs.

Viridians Streptococci are not highly invasive but their entrance into tissue usually occurs through dental or surgical instrumentation, as they reside in oral cavity. Chewing hard candy or brushing of the teeth also provide port of entry to the blood stream which can lead to the condition called bacterial meningitis, abdominal infections and tooth abscesses but the most important condition is subacute endocarditis.
Another common disease caused by viridian Streptococci is dental carries, which finally leads to the destruction of the enamel. In elderly patients living in chronic care facilities the colonization of dental plaque by pulmonary pathogen is frequent. Inflammation caused by them leads to both periodontal disease and emphysema. This over reaction may explain the association between disease and chronic obstructive pulmonary disease. The fourth leading cause of death in developed countries (139).

These findings underline the necessity for improving dental hygiene and to find agents, which inhibit bacterial adhesion on the teeth surface, which can help in controlling carries and periodontal disease. Cell free enzyme glucosyltransferase (GTF) present in whole saliva incorporates itself into pellicle. It plays an essential role in the formation of biofilm known as dental plaque and in pathogenesis of dental carries. Mutant Streptococci produce three distinct GTF. (GTF, B, C and D), each of which catalyzes the formation of glucan polymer from sucrose (141,184). Glucan is a major constituent of plaque biofilm. It contributes to the virulence factor for the colonization of S.mutans and S. sobrinus as both of these organisms adhere to glucan and form the aggregates on the pellicle. S.mutans and S. sobrinus also develop multiple glucan binding protein (GBP). These include GTF, which are able to bind to product glucan and non-enzymatic 'lectin' like protein, which recognize linear sequences of x-1-6 linked glucose. Therefore glucan formed in sites in salivary pellicle and developing plaque could act as binding sites bearing surface associated GBP's. Thus interaction between cariogenic Streptococci and in situ formed glucan provides potential targets for the action of antiplaque agents (121,142).
The previously recognised species of the genus Streptococcus named ‘Sanguis’ has recently changed to ‘sanguinis’ is one of the primary colonizers of tooth surface. It colonizes several human oral surfaces including both hard and soft tissue. Large salivary mucin like glycoproteins bearing sialic acid residues is known to bind various S. sanguinis strains resulting in its adherence to saliva coated enamel surface and the formation of aggregates in saliva with the help of hydrophobic bonds (42,102,140,148,156). It is reported that S. sanguinis OMZ 9 binds to exfoliated human buccal epithelial cell HBEC in a sialic acid sensitive manner. The desialylation of such cells invariably abolishes adhesion of S. sanguinis OMZ 9 to the cell surface. Sialylated glycoprotein with ‘O’-linked carbohydrate chain behaves as a potent inhibitor of attachment of S. sanguinis OMZ to exfoliated HBEC(23). Also antibodies raised against fimbriated, adhesive strains of S. sanguinis were found to block the adhesion of this organism to saliva coated hydroxyapatite.

Fimbriae or fibrilla have been associated with adherence properties of Streptococci to epithelial cells and erythrocytes and other bacteria or to other S.H.A surface (93). A number of cell molecules which may be carried on fibrils have suggested to act as adhesin or to impart hydrophobic character or both (140,157). The distribution and prevalence of Streptococcus mutans and S. sobrinus were determined in plaque samples from cervical areas of all buccal, lingual and proximal tooth surfaces and from the fissures of all occlusal sites in 50 subjects harboring both species of S. mutans was detected more often and in higher numbers than S. sobrinus(86). The highest number of CFU for both species was detected on the molars with lowest prevalence onto the anterior teeth (118). The possible role that hydrophobic interaction may play in bacterial adhesion to teeth and to other body surfaces has
recently attracted much attention primarily because of the development of simple technique to evaluate the hydrophobic surface properties of bacteria. It has been found that most of the oral bacteria possess hydrophobic surface (140,234). Researchers have found correlation between their relative hydrophobicites with which they attach to experimental salivary pellicles. In addition, researches have isolated hydrophilic strains of S. sanguinis and S. mutans with impaired adhesion.

S. mutans with hydrophilic property do not adhere to saliva coated hydroxylapatite and have shown to lose their fbers by electron microscopy. It has been reported that cell wall of hydrophobic strains of S. mutans possess a number of high molecular weight protein which are absent from the cell walls of non adhering hydrophilic strains. It is not known whether any of these protein function as specic adhesin (126,131,176). As bacterial adherence to intraoral surfaces is, to a large extent, determined by hydrophobic interactions, it is possible to change the hydrophobicity of the tooth surface by coating it with a mixture of active molecules and if this coating is long lasting, bacterial adherence in vivo could be reduced. Selection of these active molecules could make it possible to formulate oral health care products without using antimicrobial agents (108, 151, 242).

Intrageneric co-aggregation is responsible for the complexity of the micro biota in human and dental plaque and is believed to be important in the initial bacterial colonization of human dental plaque. Actinomyces naeslundii are early colonizers of teeth surface. They may enhance subsequent colonization of Porphyromonas gingivalis, which is associated with adult periodontitis (243). Yamaguchi, T. et al., identified an AF factor that helps in A. naeslundii aggregation with P. gingivalis.
They found that an AF carbohydrate compound mediated coaggregation with P. gingivalis cells. An AF also inhibited coaggregation with other periodontal disease associated bacteria such as Peptostreptococcus micros, Neisseria cycloheximide, Streptococcus mutans, Actinomyces viscosus forms considerable portions of the micro flora on oral surfaces in humans and in animal species like the rat and hamster respectively. Adhesion of A. naeslundii involves two distinct fimbriae type 1 and 2. While type 2 fimbriae binds to β-linked galactose and glucosamine structures on epithelial and bacterial cell surfaces. Type 1 fimbriae are thought to participate in late plaque development and colonization of oral mucosa. Thus expression of type 1 and type 2 fimbriae and their variant binding types enable A. naeslundii to establish distinct intraoral colonization niches (60,78,220).

Chelating agent such as acetyl acetone, citrate and EDTA inhibit coaggregation between the pairs of microorganisms. Carbonyl methylcellulose assays were conducted on eight pairs of periodontal pathogens and one pair consisted of E. coli and Saccharomyces cerevisiae. The inhibitory effect of chelating agents was reversible except for Actinomyces naeslundii 12104, the adhesin of which was irreversibly inactivated. Even though bacteria possessed different kinds of adhesin, the sensitivity to the chelating agent appears to be a common property. Non-toxic chelating agent appears to be a common property. Non-toxic chelating agents such carbonyl methylcellulose and citrate may prove to be useful anti adhesins. There are a variety of other preventive interventions against dental caries such as antibiotics used to treat cariogenic bacterial infections, the fluoridization of tooth surface, sugars that cannot be converted to water insoluble glucan (WIG) by glucosyl transferase (Gtase), antibodies against WIG-Gtose and inhibitory activities on WIG-Gtase. It has
been reported that *S. mutans* and *S. sobrinus* produce three kinds of GTF-I, SI and SH and four kinds (GTF-I, -S, -S2 and S3) of Gluase respectively.

A widely adopted approach for reducing plaque development is the topical application of bactericides. These agents e.g. triclosan, chlorhexidine and cetylpyridinium chloride act by inhibiting the plaque development and lowering the number of microorganisms in saliva (120). In general it is non-selective in its efficacy and its frequent use can lead potentially to a change in the oral micro biota. Antibodies raised against fimbriated adhesive strains of *S. sanguis* were found to block the adhesion of this organism to saliva coated hydroxyapatite. An alternative approach to inhibit plaque formation is to select molecules that can block or reduce bacterial adhesion. Vaccines against major cariogenic oral bacteria and chemical agents for coating the tooth surface or by interfering with bacterial binding have been investigated (208, 141, 242).

By the middle of the 19th century it has been known that cell agglutinating proteins notably haemagglutinins are widely distributed in nature. Such proteins have been found in plants and were therefore known as phyto haemagglutinin or phyto agglutinins. In year 1953 Boyd named them as 'Lectins' from Latin legere to pick up or to choose. Many simple reliable methods were developed to detect the anti adherence properties of these lectins. One of these methods is haemagglutination, which is based on aggregation of erythrocytes. Specific bacterial induced hemagglutination assay is performed to study the inhibition activity of the lectins. Many bacteria use their surface lectins for attachment to saccharide moieties on the surface of eukaryotic cells. Such adhesion is often the first step in an infectious
process. Adhesin present as pili or fimbriae on the bacterial surface binds to the saccharide molecules on the erythrocyte surface causing hemagglutination.

The inhibition of aggregation formation causes the erythrocytes to sediment, thereby enabling the identification of efficient inhibitors. During the last two decades many new lectins have been found in plants and other organisms from mammal to bacteria, over hundred of these lectins have been purified and well characterized (1).

It has been reported that poly phenol extracted from hop (Humulus lupulus L.) bract strongly inhibited the cellular adherence of Actinomyces naeslundii and S. mutans in presence of sucrose (142). In case of S. salivarius, Weekamp and Jacobs have identified galactose binding lectins which acts as vellonella-binding protein and other tissue protein which mediates adherence to host tissue. Bacterial polysaccharides have been shown to serve as receptor molecules for carbohydrate binding adhesin of genetically distinct bacteria in the human oral cavity. It is believed that the bacteria involved in this congregation interaction possess a selective advantage utilizing by sequential accretion. The binding of carbohydrate is specific as demonstrated by saccharide inhibition studies of two interacting bacterial partner cells, inhibition with purified polysaccharide or oligosaccharide and lack of polysaccharide on saccharide binding can be inhibited by using whole bacteria, purified lectins or by the use of Phyto Lectins present in the crude extracts of different plants and their parts (160).

It is reported that neuraminidase treatment has a selective effect upon the attachment of bacteria to salivary pellicles. This treatment markedly reduces the number of S. sanguis is attaching to the salivary pellicle. But it has little or no effect upon the
attachment of strains of _S. mutans_, *Actinomyces viscosus* or *Acinetobacter calcoaceticus*. In fact neuraminidase treatment appears to increase the attachment of certain _A. naeslundii_ presumably by exposing penicillin galactosyl binding fimbriae of the organisms.

Fluoride and other weak acids, such as benzoate, indomethacin, salicylate and sorbate were found to be sensitzers for acid killing of cells of *Actinomyces naeslundii ATCC 19246* and *Streptococcus sanguinis* NCTC 10904 in 343 pension or in monoo-organisms biofilm. These organisms from dental plaque and were killed when acidified to pH values between 3.5 and 4.0 (223). During screening for antiplaque agents of plant origin ethanol extracts from *Melaphia chinensis* (Bell), the Chinese Nutgall, exhibited strong inhibition e.g. glucosyl transferase (GTF) in vitro adherence and glucan induced agglutination of _S. sobrinus_ and _S. mutans_ (239). The glucan binding lectins of *Streptococcus cricetus AHT* and *Streptococcus sobrinus* 67115 were reversibly inhibited by sodium fluoride. Fluoride was superior to chloride, bromide, iodide and thiocyanate in preventing glucan-mediated aggregation of the bacteria. Fluoride was also an effective inhibitor of the sucrose dependent adhesion of _S. sobrinus_ to glass surface. The inhibition of sucrose binding lectins activities may be one of the mechanisms of action of fluoride in preventing dental disease (43.51).

ii. URINOGENITAL INFECTIONS

Urinary tract infections are caused mainly by _Escherichia coli_ are the most common infectious disease. Most isolate of the uropathogenic _E. coli_ can express type 1 and P fimbriae which contains adhesin. In vivo expression of pili by _E. coli_ in the urine of 41 adults with lower urinary tract infection was analyzed by immuno staining with polyclonal antiserum to type 1 and P pili. Type 1 pili were detected in 31 of 41 urine
specimens while P pili were detected in 6 of 18 specimens. P fimbriae recognize kidney glycolipid receptors and are involved in pyelonephritis. Type 1 fimbriated E. coli recognize uropakin 1a and 1b. two major glyco- proteins of urothelial apical plaques. Anchorage of E. coli to urothelial surface via type 1 fimbriae uropakin 'Y' interaction play a role in its bladder colonization and eventual ascent through the ureters against urine flow to invade kidneys(99).

Flow cytometry is a widely used method for analyzing the expression of cell surface and intracellular molecules (on a per cell basis), characterizing and defining different types in heterogeneous population, assessing the purity of isolated subpopulations and analyzing cell size and volume. This technique is predominantly used to measure fluorescence intensity produced by fluorescent - labeled antibodies or ligand that binds to specific cell associated molecules. Thus flow cytometer - analyses bacterial adhesion to substrata such as epithelial cells and RBC and offers the following advantages:

1. Low - affinity binding can be captured since attached versus non attached
2. Bacteria can be detected without a final wash step.

Up to 10,000 epithelial cell can be easily analyzed and depicted on a histogram for each data point, increasing the statically validity of attachment assay and providing for the detection of non- heterogeneous attachment assay. Phenomena suggested by flow cytometry results can be simultaneously monitored via fluorescent microscopy.
Further more this assay technique is enabled to be used to identify whether individual molecules and the sequence of events leading to strong attachment. Because this assay technique is able to quickly analyze thousands of epithelial cells and represent the data as histograms, subtle trends in adhesive potentials are revealed that would otherwise be overlooked by the methods that would disrupt loose adhesion or require microscopy as the source for data collection. This aspect of assay technique has been demonstrated by revealing a sub population of epithelial cells with noticeably more bacteria associated with them and will be used to further investigate this phenomenon.

Principal of haemocytometry: Cells can be analyzed or sorted. The cells enclosed in a sheath of fluid pass through a laser beam. The amount of light scattered and fluorescent light emitted are recorded. Either of these Signals can be used to place on the droplet so the cell can be deflected. The number of cells at each fluorescent unit is plotted against the number of cells at each fluorescent intensity. The dashed line separates two populations that quantitatively differ in staining. FACS analysis: Two-dimensional dot plot. Cells are stained with two different fluorescent dyes (e.g. bound to antibodies) and the relative intensity of one dye is plotted against that of other for each cell (represented by a dot) the dashed line separate four population differing in staining(235).

E. EFFECT OF PLANT PRODUCTS ON HUMORAL AND CELL MEDIATED IMMUNE RESPONSES

Body of the animals and humans are well equipped to fight against the invasion of pathogenic bacteria. The skin is the largest tissue that is very efficient in preventing
the entry of the bacterium by the virtue of physical properties and its ability to produce unsaturated fatty acids that have antibacterial action. The mucus membrane and their secretions help in prevention of bacterial invasion in the body. But in most cases pathogenic bacteria do overcome these barriers and successfully enter the body. Once bacterium has entered the body then it is confronted with a well orchestrated set of responses including antibody production, the complement system, inflammatory and intra cellular killing mechanism an overall effect is assumed to be the bacterial death. But quite a number of bacteria are well equipped to overcome the resistance of the immune system. In case of the people taking immuno suppressive drugs, AIDS patient immuno compromised patient and people suffering from diabetes mellitus the immune system is impaired and their body is unable to fight against the invading bacteria. In this case antibiotics therapy is given to the patient but due to the indiscriminate use of antibiotic. Most of the bacteria have developed resistance to available antibiotics too. But recently it has been known that the main factor that helps bacterium to fight against phagocytes is the firm attachment of bacterium to the host tissue. This attachment is due the hydrophobic bond formed between the tissue and the bacterium or sometimes certain molecules present on the bacterium help them to adhere to the host tissue. Now it has been realized that besides screening natural plant products for antimicrobial activity and immunomodulating properties, plant products should be explored for the anti adhesive properties also.

In the past, living and attenuated microorganisms autologous and heterologous proteins and injections of animal organ preparation have been used with the aim of restoring an impaired defense mechanism or immuno therapy. At present some biological response modifiers (e.g. interferon, tumor necrosis factors, interleukins)
chemically low molecular weight compounds (e.g. lentitan, Schizophyllan) and some plants with immunomodulatory actions are used for similar purpose. Immunopharmacology, as a new and developing branch of pharmacology aims at searching for immunomodulators. These are drugs that directly modify a specific immune function or have a net negative effect on the activity of immune system. The potential uses of immunomodulators in clinical medicine include the reconstitution of immune deficiency (e.g. the treatment of AIDS) and suppression of normal or enhancement of immune functions (e.g. the treatment of graft rejection or autoimmune diseases). Medicinal plants and their active components have been shown to be an important source of immunomodulators (21.119).

Basaran & et al investigated in-vitro immunomodulatory activities of some Turkish medicinal plants. The extracts of Arectium minus, Momordica charantia, Uricu and Viscum album were found active in chemotaxis and random migration test. Extracts of Arctium minus and Echallium elaterium and Viscum album were found to be useful in some diseases associated with free radical damage (17). Kroes and et al studied the modulatory effect of Artemisua annua petroleum ether, diethyl ether, ethyl acetate, ethanol and water extract. The extracts were screened for their in vitro effects on human complement, proliferation of T lymphocytes and chemiluminescence’s by Zymosan stimulated polymorph nuclear leukocytes. All extracts exhibited dose dependent inhibitory activities in our immune assays. The highest activity towards the classical pathway of complement was found in ethyl acetate and ethanol extract, where as in chemiluminescence’s assay, diethyl ether and ethyl acetate extracts showed the most pronounced inhibitory activity in T cell Proliferation assay the diethyl ether extract exhibited the most potent inhibitory effect (111).
Benencia & et al investigated in vitro activities of *Cedrela to biflora* aqueous leaf extracts on murine macrophages, polymorph nuclear leukocytes and complement. They observed significant reduction in the phagocytic capability and respiratory burst response (61.5% and 57.6% respectively) of murine peritoneal macrophages when these cells are incubated for 24 hours with medium containing 1 mg/ml extract. At concentration 4 mg/ml the extract reduced significantly the phagocytic activity of mouse polymorph nuclear leukocytes (81.5%) without altering the oxidative metabolism of these cells. Finally, a concentration of 2 mg/ml was required to inhibit the haemolytic activities of both pathways of mouse complement (22,192).

Extracts of the rhizomes of *Picrorhiza scrophularioides* (serophulariaceae) was investigated for in vitro and in vivo immunomodulatory properties. Diethyl ether extract showed potent inhibitory activity towards the classical pathway of the complement system, the respiratory burst of activated polymorphonuclear leukocytes and mitogen induced proliferation of T lymphocytes. The extracts showed anti-inflammatory activity towards carrageen induced paw edema (201). Ottendorfer et al investigated Dichloror methane, Methanol and 25% ethanol extracts of 20 African plants which were found to have immune enhancing activity of phagocytic cells, tumoricidal potential of macrophages and stimulation of oxidative burst of macrophages and granulocytes (111,159).

Anjupuri studied in vivo the immunostimulant activity of dry fruit and plant materials used in Indian traditional medical system for mothers after childbirth and invalids.
Feeding of *Prunus oxygelobus* (almond) and *Buchanania hungrum* (chirronjii) stimulated both cell mediated and humoral immunity in BALB/C mice as evident by enhancement of macrophage migration index, Haemagglutination titer and plaque forming cell count. *Euryale ferox* (Tel makhana), *Phoenix dactylifera* (cheohara) and *Zingiber officinale* (sonth) stimulated humoral activity to a greater extent than cell mediated immunity (167). *Picrorhiza scrophulariiflora pummell* and *Kurrooa* are extensively used in traditional medicine system of China Nepal, India and Sri Lanka for the treatment of various immune related diseases (217). Preparations from leaves, seeds, stem bark and roots of many plants belonging to the Meliaceae family have been widely used for antiviral, antihelmintic, antitumor, anti inflammatory and ant rheumatic activities specifically. Several reports have made it clear that some members of this family such as *Azadirachta indica*, *Mimusops paniculata*, *Melia azedarach* and *Cedrela tubiflora* posses potent anti-inflammatory and anti-rheumatic properties as a consequence of their action on immune system. It has been reported that aqueous leaf extract of *Muzacca rach 1*, *Cedrela tubiflora*, *Cedrela illii* and *Trichilia elegans* exerted inhibitory activities on both murine complement activation and phagocytes mediated by mouse peritoneal exudate cells and PMN leukocytes (22).

A number of polysaccharides have been demonstrated to have potent immuno stimulatory activities based on Chinese medicine. A number of herbs which contain some kind of polysaccharide have been found to have a capacity to strengthen immune function against a number of disease disorders e.g. panasginseng, *Astragalus mongholicus*, most of the herbs which have not been used for immunomodulatory properties showed immuo modulation properties. *Piptophila unaria*
(meadowsweet) is used in traditional medicine for treatment of several inflammatory diseases including gout and rheumatoid.

Hatkes et al investigated light petroleum, diethyl ether, ethyl acetate and methanol extracts of roots, barks and flower of *Filipendula ulmaria* for in vitro immunomodulatory properties. Strong inhibitory activity was found towards the classical pathway of complement in the ethyl acetate extracts of root and flowers, in all methanol extracts and in the aqueous root extract, except for the light petroleum extracts, all fraction tested inhibited the production of reactive oxygen species by human polymorph nuclear leukocytes. The diethyl root extract was found to be most potent in inhibiting lymphocyte proliferation (107).

Sharma and et al studied the immunomodulatory activity of Boswellia Acid (BA) (Pentacyclic triterpene acids) from *Boswellia serrata*. It was found to be non-toxic and immunomodulatory at dose 5.200 mg/kg inhibited the expression of delayed type of hypersensitivity and enhanced humoral antibody synthesis. Preincubation of macrophages with different concentration of BA increased the phagoeytic function of adherent macrophages (77,196). The fruit juice of *Morinda citrifolia* (noni) contains a polysaccharide rich substance (noni-ppt) with anti-tumour activity in the Lewis lung (L1.6) Peritoneal carcinomatosis model. It suppressed the tumour growth through activation of the host immune system. Noni-ppt stimulated the release of several mediators from marine effector cells including tumour necrosis factor-α (TNF-α), interleukin 1β (IL-1β), IL-10, IL-12, interferon-γ (IFN-γ), and nitric oxide (NO) (68).
Immunomodulatory polysaccharides are rapidly emerging as promising immuno therapeutic agent in the treatment of cancer. Preclinical studies of several polysaccharides isolated from higher plants, mushrooms and seaweeds have demonstrated anti-tumour activity against transplantable tumour in mice. Lentinan, a β-glucan from the edible mushroom *Lentinus edodes* (shiitake) is the best characteristic of the immuno modulatory polysaccharide. Activated macrophages, NK cells and cytotoxic T lymphocytes (CTLs) are generally involved with its antitumour activity (198).

Benencia and et al studied the efficacy of *Trichiula glabra* (Melaceae) aqueous leaf extract on mouse lymphocytes. The in vitro proliferation of T and B-lymphocytes were completely impaired. Besides extract significantly diminished both antibody and delayed hypersensitivity in treated mice. Thus it exerts marked immunomodulatory effect on murine immune system (244).

Mitra & et al studied the immunomodulatory effect of a polyherbal formulation which consist of aqueous extract of following herbs *Tinospora cordifolia* (stem, 40% w/w), *Withania somnifera* (roots 20% w/w), *Embilica officinalis* (fruit 20% w/w) and *Belimmon sancum* (Leaves 20% w/w). The in vivo treatment enhanced the delayed type of hypersensitivity response in mice, stimulated lymphocyte proliferation; while humoral immune system was unaffected but cell mediated immunity was enhanced (90,138).

A crude polysaccharide fraction F-5 from Juzen-Taiho-To, a Kampo (Japanese herbal) medicine prepared by decocting a prescription of 10 herbs enhanced the
production of interleukin-2 (16.2) by mouse spleen cell stimulated with concanavalin A (con-A). When F-5 was fractionated by certain precipitation, the neutral polysaccharide fraction (F-5-5). 14-2 production also showed anti-complementary activity.

Polysaccharides isolated from C. mexicana enhanced nonspecific immunity by increasing phagocytic function. The juices and infusions of Kalanchoe Pinnata (Crassulaceae) synonym Bryophyllum Pinnatum leaves are widely used in Brazil in the treatment of wounds, arthritis and ulcers showing no apparent toxicity. Bergmann et al studied the effect of leaves extract on immune system. It was found to cause significant inhibition of cell mediated and humoral immune response in mice. The spleen cells of animal pretreated with Kipinnata showed decrease ability to proliferate in response to both mitogen and antigen in vitro. It impaired the ability of mice to mount a delayed type of hypersensitivity reaction by almost completely abolishing the DTH reaction. The specific antibody response to ovalbumin were also significantly reduced by treatment. Together, these observations indicate that aqueous extract of K. pinnata possesses an immuno suppressive activity (155,179,238).

Global emergence of resistance in bacteria to the antimicrobial agents has made it difficult for the physicians to cure infectious diseases. It is now the responsibility of a microbiologist to find ways and means, which can enable physicians to control the infectious diseases caused by MRSA, MDR Salmonella, Ps. aeruginosa, Actinobacter, Candida etc. When microorganisms invade the host, they are attacked by the elements of non-specific immune system i.e. phagocytes. Many potent pathogens overcome this allele and get firmly attached to the host tissue with the help
of adhesin present in their pili. On the host tissue, most of the pathogens envelop themselves with the capsule, which makes them disguise and the elements of both non-specific and humoral system cannot recognize them. Thus they multiply on the host tissue producing toxic products.

Since long infectious disease are being cured by traditional practitioner with the help of herbs and higher plants. At present antibiotics, obtained from molds, fungus or synthetically prepared have lost their efficacy. The new trend to combat the pathogens is to use aqueous sand organic extract from different parts of medicinal plants. In this study aqueous and organic extracts from medicinal plants were investigated with the following specific objectives.

**AIMS AND OBJECTIVES**

1. Screening of aqueous and organic extracts of indigenous plant products for their antimicrobial activity against potential human pathogens.

2. Determination of Minimal Inhibitory and Bactericidal concentrations of potential antimicrobial plant products and their effect on bacterial growth kinetics.

3. To develop new synergistic combinations of plant products in combination with Beta Lactam antibiotics.

4. Modulation of bacterial pathogenic mechanisms by plant products specifically - *In Vivo* and *In Vitro* inhibition of Colonization, Adhesion / Attachment to host cells. Aggregation of Uropathogenic *E. coli* Type-I, Cariogenic *Strept. sanguins*, *Strept. agalactiae* Rat Vaginal Infection.
5. Determination of animal toxicity of plant products and new synergistic formulations in BALB/c mice.

MATERIALS
AND
METHODS
MATERIALS AND METHODS

The biggest challenge for today’s microbiologists and medical professionals is to control the spread of potential human pathogens, which have developed resistance to a large number of antibiotics. In order to overcome this problem ethno-biologist together with the microbiologist are looking forward to the higher plants as the source of new, more effective and less toxic antimicrobial substance.

A. PREPARATION OF PLANT EXTRACTS:

Twenty-eight different medicinal plants and their parts were studied as water, methanol, ethanol and acetone soluble extracts utilizing the techniques mentioned below.

1. Dried plant materials were ground into powder form. A 3% solution was made in sterile distilled water. It was kept soaked for 3 days and each day it was stirred with a magnetic stirrer for one hour. The mixture was filtered on sterile cheesecloth. The filtrate was divided into two portion, one portion was filter sterilized while the other portion was autoclaved.

2. Fresh plant were washed twice once with tap water then with distilled water. At 4°C Peels from Beta vulgaris and Persea americana mill were removed with the help of a peeler. Both the peel and the remaining portion of pulp were cut separately into smaller pieces and were soaked in different solvents namely water, ethanol, methanol and chloroform. Porphyra tenera consisted
of thin sheets, which were cut into small pieces and soaked in organic solvents and water. From 
*Solatunum tuberosum* three tissues were taken out i.e. peel, sub peel (1/4 cm thick layer from the pulp) and pulp. These tissues were separated and cut into small pieces and soaked in water. The mixtures of organic solvent/water with plant parts were stirred on magnetic stirrer for 1 hr. All mixtures were kept at 4°C for 48 hrs. After two days mixtures ware again stirred on magnetic stirrer for 1 hour and were filtered through sterile cheesecloth. Filtrate of each organic solvent was collected in separate evaporating dish. These dishes were kept in fume cupboard for the solvent to evaporate. After two days dried residues were collected. In case of water solution filtrate was centrifuged at 10,000 rpm for 15 minutes, supernatant was collected and was termed as 100% water solution /extract.

B. **ISOLATION AND IDENTIFICATION OF BACTERIAL CULTURES**

Bacterial cultures used in this study are listed in Table 2. All organisms were grown aerobically at 37°C for 24 hrs on Mueller Hinton agar. except *S. sobrinus* and *S. sanguinis*. *S. sanguinis* was grown anaerobically on tryptic soya broth and *S. sobrinus* was also grown anaerobically but in treated tryptic soya agar / broth the tryptic broth and agar were pre treated with 5 mg of dextranase / gm of the dry mass of the media at 55°C for 2 hrs and 1 mg of invertase/gm dry weight of the media at 37°C for 2 hrs. The bacterial culture was prepared by inoculating 3– 4 isolated colonies from agar plate in the broth tube and incubated in shaking water bath at 37°C for 1.5 hours.
C. DETERMINATION OF SUSCEPTIBILITY OF BACTERIAL ISOLATES TO STANDARD ANTIBIOTICS AND PLANT PRODUCTS

For the treatment of infectious diseases clinicians select the antibiotic on the basis of their minimal inhibitory concentration (MIC). A traditional method for screening the presence of antimicrobial potential of the plant product/drug is the agar well diffusion method. After confirming the antimicrobial of activity of this compound, in this study minimal inhibitory concentration of that product/drug is evaluated by the micro/macro broth dilution method. We have used micro broth dilution method to determine the MIC of the plant-derived substances. Small quantity of the broth culture and the substance is required for the micro method (59).

I. Agar well diffusion method:

Twenty-four hours old culture was inoculated in Mueller Hinton broth and was incubated for 2-12 hr. To determine the actually growing culture Turbidity was then adjusted to 0.5 McFarland. With the help of sterile cotton swab bacterial culture was spread on the Mueller Hinton agar plate. Wells were dug with the help of sterile metallic borer. Different dilutions of the extract prepared in water were poured in respective wells. The plates were incubated at 37° C for 24 hours and zone of inhibition were recorded (18).

II. Disc diffusion method:

The bacterial cultures (Table 2) were collected from different hospitals and laboratories were first identified according to the standard methods and then
screened against commercially available standard antibiotics (Table 3) for their susceptibility profiles by disk diffusion method of Baur and Kirby.

III. Minimum Inhibitory Concentration (MIC) by Micro broth Dilution Method:

In sterile flat-bottomed 96 well plates two fold dilution of aqueous and organic extracts of different plant parts were prepared in Mueller Hinton broth. 20 µl of bacterial culture containing $10^5$ CFU/ml was added in each well including positive control containing only broth. Negative control consisted of serial dilution of aqueous and organic extracts only. Plates were incubated at 37°C for 24 hrs and observed for the development of turbidity. The highest dilution of the extract showing no turbidity was recorded as MIC (64,144).

D. SCREENING OF PLANT PRODUCTS IN COMBINATION WITH ANTIBIOTICS:

Large number of antibiotics has lost their efficacy against many bacteria because of the development of drug resistant mutants. To overcome this problem microbiologist are combining two or more antibiotics with different mode of action to prepare the synergistic combination. In this study two synergistic combinations have been formulated, one containing amoxicillin and water solution of C. fistula and other consists of Shaminin and amoxicillin. The synergistic preparations were evaluated by the following methods.
I. Disc Diffusion Method

Filter paper discs were divided into two groups. Disc in-group 1 were impregnated with 10 μl of the combination (leaf stem or fruit), group 2 had aqueous fruit extract of *C. fistula* (150 μg/10 μl) or amoxicillin (15 μg/10 μl) alone. Leaf, stem and fruit extracts of *C. fistula* were tested for the synergistic activity by the disc diffusion assay (131). Bacterial inoculum of MDR *S. typhi* was prepared by suspending them in Mueller Hinton Broth from MacConkeys agar plate. Broth culture was incubated for 2-1/2 hrs. turbidity was adjusted to 0.5 McFarland standard. 15 minutes of adjusting the turbidity of the inoculum, with the help of the sterile cotton swabs the dried surface of the Mueller Hinton agar plate was streaked with the organisms. Filter paper discs were evenly placed and firmly pressed on the inoculated plate with the help of forceps. After 24 hours of incubation, the antimicrobial effects of the samples that produced zone of inhibition were recorded (18).

II. Checkerboard Titration

Combination of fruit extract with Amoxicillin gave synergistic effect against the multidrug resistant strains of *S. typhi*. Checkerboard titration was carried out to determine the synergy and MIC of Amoxicillin and fruit extract of *C. fistula* in micro titer trays with cation supplemented Mueller Hinton broth (Difeo USA). Fruit extract of *C. fistula* and Amoxicillin were tested at five different concentration from 195 – 3120 μg/ml and 0.39 – 12.5 μg/ml respectively. Fruit extract of *C. fistula* was dispensed alone in the first row. *C. fistula* and Amoxicillin were combined in the remaining row, and amoxicillin was also dispensed alone in the last column. Incubula were prepared by
suspending growth from MacConkeys agar plate in to Mueller Hinton broth. Density of broth culture at logarithmic phase was adjusted to that of 0.5 Mc Farland Standard. This was diluted such that final inoculum contained $5 \times 10^5$ CFU/ml. Trays were incubated aerobically over night. The lowest dilution containing no turbidity was considered as MIC. Fractional Inhibitory Concentration (FIC) were calculated as MIC of the amoxicillin and fruit extract of *C. fistula* in combination / MIC of the amoxicillin or fruit extract of *C. fistula* alone (Table --). In order to evaluate the outcome of combination of Amoxicillin and fruit extract of *C. fistula* fractional inhibitory (FIC) index was calculated as the summation of FIC = $FIC_{amoxicillin} + FIC_{fistula}$. Individual checkerboard was repeated three times for each isolate and combination. A mean FIC index was calculated practical to a commonly utilized definition of synergy and classified as either synergistic ($\leq 0.5$) Additive or indifferent ($\geq 0.6-2$) antagonistic (0.2-4) (39,233).

E. **EFFECT OF PLANT PRODUCTS ON GROWTH KINETICS OF SUSCEPTIBLE BACTERIA**

In order to determine the bactericidal effect of plant derived substances on the growth of bacteria. 1/10 of MIC of Amoxy-cassia, *C.fistula* and Amoxicillin were incorporated in the broth tubes separately. Each tube was inoculated with bacterial culture containing $10^5$ CFU/ml. Number of Cell forming unit (CFU) were counted at '0' minute, 30 minutes and then after each hour till 8 hours. Graph was plotted between log of number of bacteria versus time (241).
F. EFFECT OF PLANT PRODUCTS ON THE PATHOGENESIS OF CARIOGENIC BACTERIA

For the initiation of infectious diseases, the most important factor is the adhesion of bacteria to the host tissues. Oral bacteria cause infections due to their ability to adhere on the teeth and other surface of the oral cavity, aggregation of the bacteria to the product dextran produced by S. mutans species and coaggregation among different bacterial pathogens of the oral cavity.

In this study the bacterial species used were S. sobrinus, S. sanguinis, and A. naeslundii T14V. The organisms were pretreated with organic extracts at the concentrations of 25 mg/ml and 2.5 mg/ml and 50% and 5% of aqueous solution of Persea americana mill, Beta vulgaris (beet) root, Porphyra tenera (sea vegetable) and Solanum tuberosum (potato). Three models were set up to study the effect of plant products on infection of oral bacteria, which are as follows:

1. The first model consists of adherence of cariogenic S. sanguinis to human 'O' RBC. In this assay eleven two-fold dilutions of pretreated S. sanguinis at 1.0 A660 were prepared in sterile 96 well plates. Well number 12 was treated as negative control. In all wells 20μl of 20% human 'O' RBC were added. In positive control plate mannose sugar was also included along with bacteria and RBC. The plates were incubated at 37°C for 2 hours. Plates were observed for the formation of matrix/bacterial. To confirm the results plates were incubated at 4°C for 24 hours and observation was recorded again.
II. In the second model aggregation of *S. sobrinus* with high molecular weight dextran (produced by *Strept. mutans*) was studied using 96 well microtiter plates. First well was kept as control containing 0.065 ml of untreated culture. The remaining wells contained 0.065 ml of pretreated cultures of *S. sobrinus*. Then 0.020 ml of distilled water and finally 0.005 ml of high molecular weight dextran (0.5 μg/ml) was added in each well. OD of the wells was recorded at '0' minute and then after every 30 minutes for 2 hours. Graph was plotted between the OD of the culture and time in minutes. In negative control experiment low molecular weight dextran was included to inhibit the aggregation. Decrease in OD was observed in case of positive control (56).

III. In the third model *A. naeslundii* T14V co- aggregates with *S. sanguis* 35 when present in oral cavity. *In vitro* similar assay was set up in 96 well microtiter plates. *A. naeslundii* T14V was treated with the plant derived substances. The first well of microtiter plate was kept as positive control and 50μl of *S. sanguis* and untreated *A. naeslundii* T14V at OD 1.0 *A595* were added. In remaining wells *S. sanguis* 35 and pretreated *A. naeslundii* T14V were added and OD was observed at '0' minute and after every 30 minutes for 2 hrs. In negative control plate β-lactose (β-N-Gal (1-4)) was added to inhibit the reaction.

G. **EFFECT OF PLANT PRODUCTS ON THE ATTACHMENT OF UROPATHOGENIC E. COLI TYPE I.**
Mannose resistant *E. coli* type 1 attaches itself to the cells of the urinary tract lining with the help of pili. The pili have the ability to produce the capsule-like substance, which covers the bacteria and make them indistinguishable by the immune system. During this study, we developed an in-vitro model to determine the adherence of mannose resistant *E. coli* type 1. Mannose resistant *E. coli* type 1 was allowed to adhere to the guinea pig RBCs. In this assay, eleven two-fold dilutions of pretreated mannose resistant *E. coli* type 1 at an OD 1.0 *A* 530 were prepared in sterile 96 well plates. Well number 12 was treated as control, which contained saline. In all other wells 20 μl of 20% guinea pig RBC was added. In positive control plate mannose sugar was also included along with bacteria and RBC. The plates were incubated at 37°C for 2 hours. Plates were observed for the formation of matrix/button. To confirm the results plates were incubated at 4°C for 24 hours and results were recorded again.

II. FLOW CYTOMETRIC EVALUATION OF THE EFFECT OF PLANT PRODUCTS ON CARIOGENIC AND UROPATHOGENIC BACTERIA

Most important step in the pathogenesis of bacteria is its ability to adhere to the host surface. Because after firmly attaching themselves to the host tissue, bacteria secrete toxic substances and multiply in the body of the host. Thus plant derived substances that inhibit the adherence of bacteria to the host play an important role in treatment of infectious disease. Earlier in this study, in-vitro adhesion of cariogenic *S. sanguinis* and mannose resistant *E. coli* type 1 were studied in micro titer 96 well plate. The plant extracts, which were found to be effective in inhibiting bacterial adherence to human RBC thus reducing hemagglutination titer as compared to the control, were
further evaluated by the flow cytometry technique. Bacterial cultures of *S. sanguis* and *E. coli type I* were grown for 18 hours in tryptic soya broth then centrifuged at 4°C and 10,000 rpm for 10 minutes. The bacterial culture was washed twice with PBS 7.3; the optimal density of the culture was adjusted to density 1.0 at A600. The bacterial cultures were pretreated with aqueous solution and organic extracts of the plant. Bacterial culture used as control was left untreated. Fluorescent staining of bacteria was performed according to Logen et al. Fluorescent labeled *S. sanguis* were mixed with human 'O' RBC and *E. coli type I* with guinea pig RBC.

The reaction was analyzed on Fluorescin Activated Cell Sorter (FACS - Becton - Dickinson, Sparks, MD) Flow cytometric analyses were performed using cell quest software provided by the manufacturers (Becton Dickinson- USA ). Scattered plots consist of points representing each RBC, where the 'X' axis is a measure of the size of detected particles and the Y-axis measures internal cellular complexity i.e. (granularity). Histogram indicates the distribution of RBC based on their relative fluorescence intensity and Y is the number of particles counted at specific fluorescence and set as a basal of fluorescence resulting in histogram peak at the far left of the X axis is the number of particles counted at the specific fluorescence intensity. For histogram analysis MI marker was set to incorporate approximately 95% of the events for the auto fluorescence of RBC (Fig. 1-A). The median fluorescence intensity of subsequent histogram peaks falling outside. The median fluorescence intensity of subsequent histogram peaks falling outside the MI marker was recorded for adhesion analysis as an increase in overall fluorescence.
When analyzing the adhesion between bacteria and RBC bacterial clumps occasionally appeared with the size range of the RBC. The clump did not interfere with the analysis of the RBC due to their ability to exclude this data from analysis of RBC fluorescence intensity. The clump bacteria can be disregarded by running a sample of bacteria alone and gating out the resulting dot plot population therefore selecting a population that contained a majority of RBC. Furthermore histogram data representing clumped bacteria can be excluded from analysis by setting an additional marker around the clumped bacteria peak and disregarding any data that appears within this peak (which always appear outside the range of RBC intensity).

1. **EFFECT OF PLANT PRODUCTS ON S. AGALACTIAE VAGINAL INFECTIONS IN RATS.**

*S. agalactiae* are known to colonise in the female vagina, though they don’t cause any harm to the normal adult. But when they are present in pregnant women they are transferred to the baby during delivery and can cause a cause of fatal neonatal diseases. The plant products used to develop this infection model were polyphenoloxidase (PPO) and asparaginase. Both of these plant-derived enzymes are known to have the property of inhibiting the adhesion of bacteria to the eukaryotic cells.

For this infection model, virgin female Sprague Dawley rats (source) were infected with the inoculum containing $10^7$ *S. agalactiae*. Infection was allowed to develop for 2 days. They were then divided into three groups, first group was treated with 80 units of asparaginase, second group was treated with 200 units of PPO and the control group
was treated with saline. Again after 12 hours same treatment was given to each group of rats. Each day from day '0' to day '20', 10 μl of vaginal secretion was collected and was cultured on Columbia blood agar with 5% sheep blood supplemented with 10 μg/ml colistin sulfate and 15 μg/ml of nalidixic acid for the growth of S. agalactiae. Number of colony forming units (CFU) of S agalactiae 10μl of vaginal secretion in each rat were counted and recorded (122).

J. TOXICITY OF PLANT PRODUCTS FOR BALB/c MICE

In order to evaluate the toxicity level of various drugs, Lethal dose (LD₅₀) is usually determined in mice and other laboratory animals by exposing them to several different concentrations. In this study the plant extracts were administered in different doses to check their toxicity in BALB/c mice. Different group of mice A, B, C, D were injected by the intra peritoneal route with different dilutions of plant extracts. The group I that received only saline instead of plant products was included as control group. Animals in group A were injected intra-peritoneal with 1000, 500, 250 and 125 mg of aqueous fruit extract / Kg body weight of mice. Group B was given 100, 50, 25 and 12.5 mg of amoxicillin / body weight of the mice. Group C was given combination of fruit extract and amoxicillin in the concentration of 1000:100, 500:50, 250:25 and 125:12.5 mg/body weight of the mice. Group D was given 150,300,600, 1200mg of methanol extract of P.hormala. Animals were kept under observation and their mortality and behavior was observed up to two weeks. The LD₅₀ was calculated by Reed and Muench method. (23,115).

K. EFFECT OF PLANT PRODUCTS ON HOST IMMUNE RESPONSE
There are many plant derived substance and antimicrobial compounds, which affects the immune system. Some of them have positive effect on immune system as they enhance the activity of the specific and non-specific immune response to infection. They can play a very effective role in the treatment of different infectious diseases specially in immunocompromized patients. Immunomodulating properties of our synergistic formulation “Amoxy-cassia”, and each component i.e. *Cassia fistula* and amoxicillin was studied by evaluating the antibody titer and the number of antibody secreting splen cells in BALB/c mice. Assays employed were Haemagglutination and hemolytic plaque assay.

In this experiment, 4 groups’ 20 female BALB/c mice were used. Each group consisted of 5 mice each. On day zero, first group of mice were intra-peritoneally injected with 10 µg/ml of Amoxy-cassia. Group 2 were treated with 10 µg/ml of *Cassia fistula*. Group 3 mice were treated with 1 µg/ml of amoxicillin. Group 4 animals were treated with saline. On day ‘1’ same treatment as day ‘0’ was given to each animal and then 0.2 ml of 10% washed SRBCs were injected in the peritoneum of group 1, 2, 3 and 4. On day 2, in group ‘1’ animals, 5 µg of Amoxy-cassia was injected intra peritoneally. In group 2 mice, 5 µg/ml of *C. fistula* fruit solution was introduced. In group 3 animals 0.5 µg/ml of amoxicillin was introduced. The control group of animals, Group 4 was treated with saline instead of Amoxy-cassia. Effect of plant-derived substance on the immune system of BALB/c mice was determined by anti-SRBC hemagglutination titer in test and control animals.

I. Anti-SRBC antibody titer by haemagglutination (H.A)
Blood from the treated and control mice were collected on day 4, 5, 6 and 7. Serum was separated. Twenty-three, two fold serial dilutions of serum from each group of mice were prepared in microtiter plate. The contents of the wells were mixed thoroughly. Then 20 μl of 1% sheep RBC were added in each well. Plates were incubated at 37°C for 2 hrs. And then wells were observed for matrix button formation. Plates were incubated again at 4°C over night to confirm the results. Reciprocal of the highest dilution showing hemagglutination was considered as hemagglutination titer (II.A).

II. Hemolytic plaque assay for detecting anti-SRBC antibody secreting plasma cells

In this experiment each the animal was killed on day 4 by cervical dislocation and the spleen was removed aseptically. The spleen was macerated and washed in tissue culture medium. Then the cells were filtered and centrifuged. The pellets were diluted in the tissue culture and cell density was adjusted to 10⁵ cells/ml. Spleen cells were stored on ice.

The spleen cells were mixed with equal volume of 100μl ml of washed 10% SRBC and 300μl of plating medium (1% agarose in Hanks balanced salt). The suspension was kept in water bath at 50°C and was mixed thoroughly. Spleen cell mixture was poured on to the pre-coated glass slides (0.1% agarose coated). Slides were incubated at 37°C for one hour. Then 8 ml of 1:2 diluted guinea pig or rabbit complement in veronal saline was poured on the slide and incubated for 30 minutes at 37°C. Number of plaques/10⁵ cells were counted.
To confirm the results, slides were incubated at 4 °C over night and plaque were counted again (211).
RESULTS
RESULTS

A. ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS AND THEIR SUSCEPTIBILITIES AGAINST STANDARD ANTIBIOTICS

In this study, thirty medicinal plants listed in Table - 1 and their parts were processed to prepare twenty-eight aqueous, twenty methanolic, five ethanol and five acetone extracts were prepared. Plant extracts were evaluated for their bioactivity against potential human pathogens ( Table -2 ) which were isolated from pus, blood, urine, stool, and throat swabs and dental plaque. They were collected from different pathological laboratories and they were identified by standard methods ( Table 4-6 ).

B. ANTIMICROBIAL ACTIVITY OF PLANT PRODUCTS

Extracts of medicinal plants were screened for their anti- microbial susceptibility against twenty-three bacterial isolates (Table 2). Bacterial isolates, which were inhibited by the plant products, were further tested for their susceptibility against the standard antibiotics listed in Table -3. As shown in table in Table 7-8, most of the bacterial isolates against which plant products exhibited activity, they were found to be resistant to standard antibiotics. Of all the eleven spices studied, aqueous extract of Syzygium aromatum (Clove) was found to be the only spice which showed broad spectrum antimicrobial effects against S. aureus, S. epidermidis, C. aeruginosa, B. subtilis, P. aeruginosa, Proteus, S. typhi, S. para typhi, S. agalactiae and Klebsiella (Table 9). Of the remaining 17 extracts, only Rosa centifolia (rose) gave weak antimicrobial
activity against *M. lysoditicus* and potential pathogens like *S. pyogenes* *Staph. aureus*, *C. pseudodiphtheroid* and *Sh. dysenteric*. Water extract of fruit of *C. fistula* was found to possess weak antibacterial activity against *S. typhi*, *E. coli* and *S. pyogenes*. Four methanolic fractions including P1-P2, 80MR, EA2 and ALCIR from the fruit of *C. fistula* were also screened for their antibacterial activity. These fractions were found to inhibit MDR *Salmonella typhi* at a concentration range of 125 – 500 µg/ml (Table 11).

Methanolic extract of another plant - *P. harmala* exhibited strong antimicrobial activity against *Staph. aureus*, *Achromobacter lwofii* and *Candida albicans* (Table 10). Minimal inhibitory concentrations for *Staph. aureus* was in the range of 40 µg/ml – 80 µg/ml. *A. lwofii* 125-250µg/ml and *Candida albicans* 25 – 200 µg/ml. *B. Subtilis* 62.5-250 µg/ml and *S. typhi* 250-500µg/ml. *Ficus carica* methanol extract exhibited antimicrobial activity for *S. pyogenes*, *S. epidermidis* and *Pseudodiphtheroid*.

Minimal inhibitory concentration for *S. epidermidis* was in the range of 40µg/ml to 80µg/ml (Table 8). *Olea euflorosa* methanolic extract showed weak antibacterial activity against *M. lysoditicus*, *S. pyogenes* and *Pseudodiphtheroid* and strong activity for *S. epidermidis*. MIC for *S. epidermidis* was found to be in the range of 40-320µg/ml. *Bombax ceiba* methanolic extract exhibited activity against *Pseudodiphtheroid*, *S. epidermidis* and *S. pyogenes* and *S. typhi*. MIC value for *S. epidermidis* is in the range of 40 – 160 µg/ml and *S. typhi* 80-240 (Table 10). Shamamin - a pure compound isolated from the leaves of *Bombax ceiba* exhibited anti-microbial activity against *P. aeruginosae*, *L. monocytogenes* and *B. Subtilis* (Table 12) with Minimal inhibitory
concentration for *Pseudomonas aeruginosa* (ATCC) < 62.5 µg/ml *Pseudomonas aeruginosa* 125-250µg/ml, *L. monocytogenes* 125-250µg/ml and *B. Subtilis* 62.5-250µg/ml.

C. **SYNERGISTIC ACTIVITY OF AMOXY-CASSIA**

Disc diffusion assay (DDA) was carried out to screen synergistic activity of aqueous extract of leaf, stem and fruit of *C. fistula* with amoxicillin against clinical isolates. Mixture of fruit extract and amoxicillin showed synergistic effect against the clinical isolates of *Salmonella typhi* and *E. coli*. Size of zone of inhibition for *S. typhi* and *E. coli* ranged from around the disc containing mixture of 150 µg of the extract and 15 µg of amoxicillin / disc which was greater then the zone around the amoxicillin alone (13). *Salmonella typhi* isolates were resistant to the leaf and stem extract of *C. fistula* alone or in combination with amoxicillin. However inhibitory activity was recorded against *E. coli* with a big zone of inhibition (15 mm) around the disc containing the bark extract of *C. fistula*. Growth of *Proteus vulgaris, Klebsiella pneumoniae* were not affected by any of the substances tested.

**Checkerboard Titration of Synergistic Activity of Amoxycassia**

Antimicrobial activity of combination of Amoxicillin and aqueous extract of *C. fistula* (Amoxycassia) was studied against multidrug resistant *S. typhi*, non-MDR *S. typhi* and Methicillin resistant *Staphylococcus aureus (MRSA)*.

**Multi drug Resistant S. typhi:**

MIC of amoxicillin alone was >750 µg/ml for all the isolate tested. The MIC of *C. fistula* alone ranged from 3750 -7500 µg/ml. MIC of amoxicillin in combination
ranged 23.4-93.75 μg/ml and C. fistula 468-1875 μg/ml. FIC values for amoxicillin ranged in 0.03-0.5 where as FIC value for C. fistula is found to be 0.12 to 0.5. FIC index for ≤ 0.5 indicating synergism for 100% of the organisms tested (14).

**Drug sensitive S. typhi:**

MIC of amoxicillin and C. fistula alone ranged from 1.56 - 6.25 μg/ml and 390 - 3120 μg/ml respectively whereas MIC value of amoxicillin and C. fistula in combination ranged from 0.39 - 1.56 and 390 - 1560 μg/ml respectively. Fractional inhibitory concentration (FIC) of amoxicillin ranged from 0.06 - 0.5 for 87.5% of the organism tested and FIC value of 1 for 12.5% of the strain studied. In total 62% of the organism tested synergy FIC <0.5 was observed and additive property in 87.5% of the organism tested (Table 15).

**Methicillin Resistant Staph aureus (MRSA).**

MIC of C. fistula alone was in the range of 375-750 μg/ml, amoxicillin in the range of 9.37-37.5 μg/ml whereas in combination MIC of Amoxicillin was in the range of 1.6-9.5 μg/ml. FIC indices ranged in the value 0.36-0.5 Which indicates synergism (16).

**E. coli:**

MIC of amoxicillin alone was found to be in the range of 0.156 - 1.25 μg/ml and C. fistula alone is 31.2 - 62.5 μg/ml whereas MIC in combination of amoxicillin is in the range of 0.039 - 0.078 μg/ml and C. fistula is 78 - 156 μg/ml. FIC index against all the organisms tested was found to be > 0.5 which indicates the presence of synergism (Table 17).
D. **EFFECT OF PLANT PRODUCTS ON GROWTH KINETICS OF BACTERIA**

The time kill kinetic graph shown in Fig 1 indicates that from '0' hour to 4 hours Amoxy-cassia treated MDR *S. typhi* remain at lag phase till one hour. During 1-4 hours they start multiplying at much slower rate as compared to MDR Strains growing in the presence of amoxicillin or and *C. fistula* alone. After the 5th hour they start dying out rapidly. in the case of control without Amoxycassia or Amoxicillin or *C. fistula*. MDR *S. typhi* goes into log phase much earlier as compared to treated bacteria.

MDR *S. typhi* treated with amoxicillin are multiply at log phase till 4 hours after which they start dying and at 5th hour they again start multiplying and their CFU increases but there is an abrupt fall of CFU from log 10 to log 6 in the case of Amoxy-cassia treated *S. typhi*. There is a sharp rise in CFU of both the amoxicillin and *C. fistula* treated MDR *S. typhi*. The difference in CFU in the case of bacteria growing in presence of Amoxycassia and with just sublethal concentration of Amoxicillin or *C. fistula* remains to ≈ log 2. *Cassia fistula* treated MDR *S. typhi* increases at 1 hour then there is decline. At 3rd hours then there is increase in the growth at 6 hours it remains at stationary phase from 6 to 7 hours.(Fig 1-2). Nafisa – pl. check this.

**Combination of PE and amoxicillin**

*S. typhi* treated with PE in combination with Amoxicillin grows at the same rate as Amoxicillin till 2 hours. Then from 2 to 4 hours there is a decrease in CFU. Then there is rise in the CFU till 6 hours. Amoxicillin treated grows at faster rate but the log
difference of $>\log 2$ was present at 6 hours. The control \textit{S. typhi} and those growing in presence of PE alone grows at a faster rate (Fig -3)²

**Effect of plant product on time kill kinetics of MRSA**

Methicillin resistant \textit{Staph aureus} was found to grow with the same rate for one hour in presence of all compounds. Approximately equal logarithmic increase takes place in all the preparations then from 8 -10 hours rapid decline in the Amoxy-cassia treated CFU took place. There is $>2\log$ CFU difference between the CFU in Amoxy-cassia and those growing with amoxicillin alone. By the 10th hour the CFU in positive control and C fistula treated MRSA becomes equal (Fig 4 ).²

**E. EFFECT OF PLANT PRODUCTS ON THE PATHOGENESIS OF CARIOGENIC BACTERIA.**

Effect of plant extracts on the adherence of \textit{S.sanguis} to ‘O’ blood group RBC was evaluated by the Hemagglutination assay ( HA). A total of 16 extracts were screened . The observations are summarized as follows:

All extracts (100% ) of \textit{Beta vulgaris} (beet root) decreased the adherence of \textit{S. sanguinis} to ‘O’ group RBC which was recorded as a significant decrease in HA titer as compared to the control. Active aqueous and organic extracts includes water peel pulp 5% and 50% acetone peel 25 and 2.5 mg/ml acetone pulp 25 and 2.5 mg/ml; ethanol peel 25 and 2.5 mg/ml and Ethanol pulp 25 and 2.5 mg/ml ( Fig -7).
63% of *Persea americana* mill aqueous and organic extracts decreased the adherence titer of *S. sanguinis* to 'O' blood group RBC. Active extracts are water peel and pulp 5% and 50% acetone peel 25 and 2.5 mg/ml acetone. Pulp 25 mg/ml. Total 16 extracts/solutions were tested. (Fig 5-6)

75% of the *Porphyra tenera* solutions/extracts reduced the adherence of *S.sanguinis* to 'O' group blood RBC extracts responsible for this reduction are water 5 & 50% acetone 2.5 and 25 mg/ml. Ethanol 25 mg/ml and methanol 25 mg/ml. Total extracts or solutions tested were eight. (Fig-7-8).

50% water solution of *Solanum tuberosum* decreased the adherence titer of *S. sanguinis* to 'O' blood group RBC. The solutions causing this effect are water peel 50% water sub peel 5% and 50%. Total solutions checked are six. (Fig -8).

**Effect of plant products on aggregation of S. sobrinus with high molecular weight dextran.**

Aggregation of cariogenic bacteria plays an important role in the initiation of infectious process. The effect of various plant extracts on the aggregation of bacteria alongwith the bacterial products including high molecular dextran. The observations are summarized below:

69% of the *Beta vulgaris* extracts decreased the aggregation of *S. sobrinus* to high molecular weight dextran a product released by *Strept mutans*. Aqueous and organic extracts which decreased the aggregation potential were water pulp, 5% and 50%
acetone peel 2.5 and 25 mg/ml, ethanolic peel 2.5 and 25 mg/ml, ethanolic pulp 2.5 and 25 mg/ml and methanolic pulp 2.5 and 25 mg/ml (Fig. 11-12).

25% of the *P. americana mill* solutions/extract decreased adhesion of *S. sobrinus* with high molecular weight dextran. The solutions or extracts are pulp ethanol 2.5 and 25 mg/ml, pulp methanol 2.5 mg/ml and pulp acetone 2.5 mg/ml (Fig. 9-10). 50% of *Porphyra tenera* extracts/solutions reduced the aggregation of *S. sobrinus* to high molecular weight dextran. The solutions/extracts responsible for the aggregation were water solution, 50% and methanol extract 25 mg/ml. Total 8 extracts or solutions were checked (Fig 13).

33% of the *Solanum tuberosum* water solutions decreased the aggregation of *S. sobrinus* to high molecular weight dextran. These solutions are water peel 50% and water sub peel 50%. Total 6 water solutions were checked (Fig--)?

**Effect of plant products on the coaggregation of *A. naasblundii* to *S. sanguis***.

40% of the *Beta vulgaris* extracts reduced the potential of *A. naasblundii* to aggregate with *S. sanguinis*. These extracts were acetone pulp 2.5 mg/ml, Ethanol pulp 25 mg/ml, Methanol pulp 2.5-25 mg/ml. A total 10 aqueous and organic extracts were checked (Fig 16-17).

25% of the *P. americana mill* solutions/extracts decrease the coaggregation of *A. naasblundii* to *S. sanguis*. Active extracts, which reduced the coaggregation, were pulp ethanol 2.5 mg/ml and 25 mg/ml, pulp methanol 2.5 mg/ml and pulp acetone 2.5 mg/ml. A total of 16 extracts were checked (Fig 14-15).
100% extracts/solutions of *Porphyra tenera* reduced the coaggregation of *A. naevilundii* to *S. sanguis*. These solutions/extracts are water 50% and methanol 25 mg/ml. (Fig 18).

66% of the *Solanum tuberosum* water solutions decrease the coaggregation of *A. naevilundii* to *S. sanguis*. The extract/solutions responsible for the reaction are water peel 5 and 50%, sub peel 50% and water pulp 50%. Total six water solutions were checked (Fig19).

F. EFFECT OF PLANT PRODUCTS ON THE PATHOGENESIS OF UROPATHOGENIC BACTERIA.

Effect of various plant products on the attachment of uropathogenic *E.coli* type 1 to guinea pig RBC was assessed by the Haemagglutination titer in the presence and absence of mannose. The results are summarized below:

Of the 16 extracts/solutions of *Beta vulgaris* tested only acetone pulp at 25 mg/ml reduced the haemagglutination activity (Fig20 -21).

Out of 16 extracts/solutions tested, 33% of the aqueous and organic extracts including water peel and pulp 50% and 5 % ethanol peel 2.5 mg/ml and methanol pulp 2.5 mg/ml decreased the hemagglutination titer of mannose resistant *E. coli* type 1 to guinea pig RBC.
Out of 4 extract of *Porphyra tenera*, 25% of the extracts reduced the haemagglutination titer of guinea pig RBC. This only solution showing reduced activity was water solutions 5% (Fig 22).

G. FLOW CYTOMETRIC ANALYSES OF THE EFFECT OF PLANT PRODUCTS ON THE ADHERENCE OF BACTERIA TO GUINEA PIG RBC.

Combination of plant products and bacterial isolates that exhibited anti-adhesion property were rechecked by flow cytometry assay. Median fluorescence intensity of the plant treated with the extracts was lower as compared to the control (Fig 23-24).

H. EFFECT OF PLANT PRODUCTS ON *S. AGALACTIAE* VAGINAL INFECTION IN RATS.

In order to determine the inhibition of attachment of *Strept agalactiae* to the vaginal membrane lining and modulation of infection in the presence and absence of plant products, a rat infection model was developed and the sequence of in-vivo growth of bacteria was monitored. For this purpose, rats were infected with actively growing culture. Twenty-four hours after the infection animals were treated with different plant enzymes. In case of animals who were treated with polyphenoloxidase as well as the control untreated but infected group, *S. agalactiae* could not be isolated from vaginal secretions on day one suggesting phagocytic killing or engulfment or attachment.
In case of saline treated rats, the CFU count was positive for 2-3 consecutive days 2 days after the infection. In case of rats treated with Polyphenol Oxidase (PPO) and Asparaginase, a similar duration was required by the bacteria to reappear in the vaginal secretions after the host-parasite adjustment period. The difference was observed after day 4 and 5. The average number of CFU obtained from the PPO treated was higher as compared to the Asparaginase treated rat group on day 4 post treatment. In case of Asparaginase treated rats, CFU of *S. agalactiae* appeared after 24 hours. The early appearance of a large number of bacteria in the vaginal secretions in case of enzyme treated rats may be due to the anti-attachment effect of plant substances. The bacteria may attach to the host cells and also multiply during 24 hours prior to enzyme treatment. Later, when they interact with the enzymes, the attachment to new cells is inhibited, thus the infection eventually ends up in the treated animals whereas vaginal secretions of saline treated control rats persists as evident from the late reappearance of high numbers of bacteria on day 12 onwards.

1. **IN-VIVO TOXICITY OF PLANT PRODUCTS IN BALB/c MICE:**

A number of antibiotics as well as plant origin substances cannot be used for treatment purposes beyond a certain concentration due to their toxicity for the host cells. In order to make sure that the newly developed synergistic product - Amoxycassia which was found to be very effective in inhibiting the growth of MDR Salmonella typhi, MRSA and E.coli, we determined its toxicity level in BALB/c mice. Different groups of animals were treated with 5 different concentrations of Amoxycassia, as well as Amoxicillin and *C. fistula* alone. Introduction of 1000mg/kg of the body weight of *C. fistula* alone and in combination with 100mg/kg of the
body weight of the mice did not affect bring any noticeable changes in the physical conditions of treated mice. All the mice remained alive for 3 – 4 weeks / until observed after treatment with Amoxycassia and C. fistula. Lethal dose - LD<sub>50</sub> of P horrnala was found to be 300mg/Kg of the body weight of mice.

J. EFFECT OF PLANT PRODUCTS ON HOST IMMUNE RESPONSE.

A number of plant products and antibiotics are known to enhance or suppress the protective immune response to infections diseases. In an attempt to determine the effect of Amoxycassia on humoral immune response, BALB/c mice treated with Amoxycassia were immunized with sheep red blood cells (SRBC) as antigens. Antibody response was monitored in treated and control mice by determining the Anti-SRBC by hemagglutination assay and the number of Anti-SRBC antibody producing plasma cells in the spleens of immunized mice. Table 19, the control animals and animals treated with amoxicillin exhibited H.A. titer of 1: 32, whereas the animals treated with Amoxycassia exhibited a much higher H.A titer (1: 128). The animals treated with C. fistula alone also had a slightly higher H.A. titer (1:64) suggesting an immunoenhancing effect of Amoxycassia and C. fistula.

Similar immune-enhancement effect was also observed in case of treated immunized animals by the hemolytic plaque assay. As shown in Table 20, the number of cells producing anti-SRBC antibody per spleen of control mice as compared to Amoxycassia treated mice was significantly higher, suggesting an impressive immunomodulating property of the newly formulated synergistic compound. Animal treated with amoxycassia produced un-countable plaques/ 10<sup>5</sup> spleen cells. Aqueous
fruit extract of *C.fistula* alone is also found to have potent immunomodulatory property with 220 plaques/10^3
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<tr>
<th>S.#</th>
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<th>Parts used</th>
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<td>Dr. Kelly Cowan</td>
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<td>Dr. Shaheen Faizi</td>
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# TABLE 2

## LIST OF BACTERIAL ISOLATES USED

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<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella typhi</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>4</td>
<td>MDR <em>Salmonella typhi</em></td>
<td>Ms. Nuzhat(^1)</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>6</td>
<td><em>Proteus vulgaris</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>7</td>
<td><em>Streptococcus faecalis</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>8</td>
<td><em>Shigella dysenteriae</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>9</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>10</td>
<td><em>Bacillus anthracis</em></td>
<td>Mr. Shahid(^3)</td>
</tr>
<tr>
<td>11</td>
<td><em>Bacillus subtilis</em></td>
<td>Mr. Shahid(^3)</td>
</tr>
<tr>
<td>12</td>
<td><em>Escherichia coli</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>13</td>
<td>EPFC</td>
<td>Prof. Dr. Shahana U. Kazmi(^1)</td>
</tr>
<tr>
<td>14</td>
<td><em>Vibrio cholerae</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>15</td>
<td><em>Corynebacterium xerosis</em></td>
<td>Mr. Jamil(^2)</td>
</tr>
<tr>
<td>16</td>
<td><em>Streptococcus agalactiae</em></td>
<td>Prof. Dr. Kelly Cowan(^4)</td>
</tr>
<tr>
<td>17</td>
<td><em>Streptococcus sanguinis</em></td>
<td>&quot;</td>
</tr>
<tr>
<td>18</td>
<td><em>Streptococcus sobrinus</em></td>
<td>&quot;</td>
</tr>
<tr>
<td>19</td>
<td><em>Actinomyces naeslundii T1V4</em></td>
<td>&quot;</td>
</tr>
<tr>
<td>20</td>
<td><em>Streptococcus sanguinis 35</em></td>
<td>&quot;</td>
</tr>
<tr>
<td>21</td>
<td><em>Streptococcus pyogenes</em></td>
<td>Prof. Dr. Shahana U. Kazmi(^1)</td>
</tr>
<tr>
<td>22</td>
<td>EPFC</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

---

1. Prof. Dr. Shahana Urooj Kazmi, HDRL, Department of Microbiology, University of Karachi
2. Mr. Jamil, Karachi Laboratory.
3. Ms. Nuzhat, Liaquat National Hospital Laboratory.
4. Prof. Dr. Kelly Cowan, Dept. of Microbiology, Miami University, Oxford, OHIO.
5. Mr. Shahid, Department of Microbiology, University of Karachi.
# TABLE 3

**STANDARD ANTIBIOTICS USED IN THE STUDY**

<table>
<thead>
<tr>
<th>S.#</th>
<th>Antibiotics</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sulfmethoxazole</td>
<td>25 µg</td>
</tr>
<tr>
<td>2.</td>
<td>Tetracycline</td>
<td>30 µg</td>
</tr>
<tr>
<td>3.</td>
<td>Ampicillin</td>
<td>10 µg</td>
</tr>
<tr>
<td>4.</td>
<td>Chloramphenicol</td>
<td>30 µg</td>
</tr>
<tr>
<td>5.</td>
<td>Fosfomycin</td>
<td>30 µg</td>
</tr>
<tr>
<td>6.</td>
<td>Amoxicillin</td>
<td>10 µg</td>
</tr>
<tr>
<td>7.</td>
<td>Velosef</td>
<td>30 µg</td>
</tr>
<tr>
<td>8.</td>
<td>Nalidixic</td>
<td>30 µg</td>
</tr>
<tr>
<td>9.</td>
<td>Ceftazidime</td>
<td>30 µg</td>
</tr>
<tr>
<td>10.</td>
<td>Tobramycin</td>
<td>5 µg</td>
</tr>
</tbody>
</table>
### TABLE 4

**IDENTIFICATION SCHEME FOR GRAM NEGATIVE RODS**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Organism</th>
<th>Oxidase</th>
<th>Indole</th>
<th>TSI</th>
<th>H2S</th>
<th>Citrate</th>
<th>Urea</th>
<th>Motility</th>
<th>Pyucinin Production</th>
<th>Lactose</th>
<th>Mannitole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td>-</td>
<td>+</td>
<td>A/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td><em>Ps. aeruginosa</em></td>
<td>+</td>
<td>-</td>
<td>K/K</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td><em>S. typhi</em></td>
<td>-</td>
<td>-</td>
<td>A/K</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td><em>A. hoffii</em></td>
<td>-</td>
<td>+</td>
<td>K/K</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td><em>P. vulgaris</em></td>
<td>-</td>
<td>-</td>
<td>K/K</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td><em>S. dysenteriae</em></td>
<td>+</td>
<td>+</td>
<td>K/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td><em>K. pneumoniae</em></td>
<td>-</td>
<td>-</td>
<td>A/A</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

### TABLE 5

**IDENTIFICATION SCHEME FOR GRAM POSITIVE RODS**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Organism</th>
<th>Catalase</th>
<th>Spore</th>
<th>Glucose</th>
<th>VP</th>
<th>7% NaCl</th>
<th>Motility</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>Nitrate</th>
<th>Lecithinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>B. subtilis</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>B. anthracis</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

### TABLE 6

**IDENTIFICATION SCHEME FOR GRAM POSITIVE COCCI**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Organism</th>
<th>Catalase</th>
<th>Coagulase</th>
<th>Hippurate</th>
<th>Hemolysis</th>
<th>Bile Esculin</th>
<th>Mannitol</th>
<th>8.5% NaCl</th>
<th>Lactose</th>
<th>Inulin</th>
<th>Bacitracin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staph. aureus</em></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>β</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td><em>Staph. epidermidis</em></td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>β</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td><em>S. pyogenes</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>β</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>Sensitive</td>
</tr>
<tr>
<td>4</td>
<td><em>S. pyogenes</em></td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td><em>S. faecalis</em></td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>α</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td><em>S. faecalis</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>β</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

**KEY**
- + Positive
- - Negative
ND Not done
A Acidic
K Alkaline
α Alpha
β Beta
### TABLE 7

**ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES AGAINST STANDARD ANTIBIOTICS**

<table>
<thead>
<tr>
<th>S. no</th>
<th>Organisms</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>L. monocytogenes</em></td>
<td>ND</td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
<td>20</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td><em>B. subtilis</em></td>
<td>38</td>
<td>35</td>
<td>20</td>
<td>38</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aeruginosa</em></td>
<td>17</td>
<td>10</td>
<td>9</td>
<td>20</td>
<td>ND</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td><em>P. aeruginosa</em></td>
<td>9</td>
<td>10</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>10</td>
<td>8</td>
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<td></td>
<td>ATCC</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>E. cloacae</em></td>
<td>ND</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td><em>S. aureus</em></td>
<td>25</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>17</td>
<td>12</td>
<td>21</td>
<td>26</td>
<td>17</td>
</tr>
</tbody>
</table>

**KEY**

1. Sulfamethoxazole
2. Tetracycline
3. Ampicillin
4. Chloramphenicol
5. Fosfomycin
6. Amoxicillin
7. Piperacillin
8. Velocef
9. Cefizidime
### TABLE 8

**MINIMUM INHIBITORY CONCENTRATION (MIC) OF STANDARD ANTIBIOTICS AGAINST BACTERIAL ISOLATES**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organisms</th>
<th>Minimum Inhibitory Concentration (MIC) in µg / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td><em>A. lwofii</em></td>
<td>6.25 - 12.5</td>
</tr>
<tr>
<td>2</td>
<td><em>S. epidermidis</em></td>
<td>6.25 - 12.5</td>
</tr>
<tr>
<td>3</td>
<td><em>S. aureus</em></td>
<td>0.78 - 12.5</td>
</tr>
<tr>
<td>4</td>
<td><em>E. subilis</em></td>
<td>6.25</td>
</tr>
<tr>
<td>5</td>
<td><em>S. typhi</em></td>
<td>≤ 0.78</td>
</tr>
</tbody>
</table>

**KEY**

1. Tetracycline
2. Ampicillin
3. Chloramphenicol
4. Fosfomycin
5. Sulfamethoxazole
<table>
<thead>
<tr>
<th>No.</th>
<th>Organisms</th>
<th>Syzygium aromatum</th>
<th>C. fistula</th>
<th>P. harmala</th>
<th>Olea europaea</th>
<th>Ficus carica</th>
<th>Bombax ceiba</th>
<th>Shaminin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staph. aureus</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>S. epidermidis</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>B. subtilis</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>S. typhi</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>A. calcoccaceus</td>
<td>N.D</td>
<td>-</td>
<td>4+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>P. vulgaris</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>S. agalactiae</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>K. pneumoniae</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>S. pyogenes</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>M. lysodeikticus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>C. pseudopilum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>P. aeruginosa</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>13</td>
<td>C. albicans</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>C. xerosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Listeria monocytogenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

**KEY**
- **+** Weak Activity
- **++** Strong Activity
- **-** No Activity
- **ND** Not Done
# Table 10

**Minimum Inhibitory Concentration (MIC) of Medicinal Plant Extracts for Bacterial Isolates**

<table>
<thead>
<tr>
<th>No.</th>
<th>Organisms</th>
<th>Number</th>
<th><em>P. hamala</em></th>
<th><em>Olca euphorosa</em></th>
<th><em>Ficus carica</em></th>
<th><em>Bombax ceiba</em></th>
<th><em>C. fistula</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staph. aureus</em></td>
<td>n=6</td>
<td>40-80</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>750</td>
</tr>
<tr>
<td>2</td>
<td><em>S. epidermidis</em></td>
<td>n=5</td>
<td>N.A</td>
<td>40-320</td>
<td>40-80</td>
<td>40-160</td>
<td>N.A</td>
</tr>
<tr>
<td>3</td>
<td><em>A. ivesii</em></td>
<td>n=6</td>
<td>125-250</td>
<td>N.A</td>
<td>N.D</td>
<td>N.D</td>
<td>N.A</td>
</tr>
<tr>
<td>4</td>
<td><em>B. subtilis</em></td>
<td>n=6</td>
<td>62.5-250</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
</tr>
<tr>
<td>5</td>
<td><em>C. albican</em></td>
<td>n=10</td>
<td>25-200</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
</tr>
<tr>
<td>6</td>
<td><em>S. typhi</em></td>
<td>n=10</td>
<td>250-500</td>
<td>N.A</td>
<td>N.A</td>
<td>80-240</td>
<td>390-3120</td>
</tr>
<tr>
<td>7</td>
<td><em>E. coli</em></td>
<td>n=5</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>N.A</td>
<td>31.2-62.5</td>
</tr>
</tbody>
</table>
**TABLE 11**

**MINIMUM INHIBITORY CONCENTRATION (MIC) OF DIFFERENT FRACTIONS OF C. FISTULA AGAINST MULTIDRUG RESISTANT SALMONELLA TYPHI**

<table>
<thead>
<tr>
<th>No.</th>
<th>Fraction</th>
<th>Number n</th>
<th>MIC in µg / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1 = P3</td>
<td>n = 4</td>
<td>125 - 500</td>
</tr>
<tr>
<td>2</td>
<td>80 MR</td>
<td>n = 4</td>
<td>250 - 500</td>
</tr>
<tr>
<td>3</td>
<td>FA 2</td>
<td>n = 4</td>
<td>250 - &gt;1000 µg/ml</td>
</tr>
<tr>
<td>4</td>
<td>EP 2</td>
<td>n = 4</td>
<td>62.5 - 500</td>
</tr>
<tr>
<td>5</td>
<td>AI CR</td>
<td>n = 4</td>
<td>125 - 500</td>
</tr>
</tbody>
</table>

**TABLE 12**

**MINIMUM INHIBITORY CONCENTRATION (MIC) OF SHAMIMIN (PURE COMPOUND OBTAINED FROM BOMBAX CEIBA) FOR BACTERIAL ISOLATES**

<table>
<thead>
<tr>
<th>No.</th>
<th>Organism</th>
<th>Number n</th>
<th>MIC in µg / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Ps. aeruginosa</em> ATTC</td>
<td>n = 1</td>
<td>≤ 62.5</td>
</tr>
<tr>
<td>2</td>
<td><em>Ps. aeruginosa</em> clinical</td>
<td>n = 5</td>
<td>125 - 250</td>
</tr>
<tr>
<td>3</td>
<td><em>Listeria monocytogenes</em></td>
<td>n = 5</td>
<td>125 - 250</td>
</tr>
<tr>
<td>4</td>
<td><em>B. subtilis</em></td>
<td>n = 5</td>
<td>62.5 - 250</td>
</tr>
</tbody>
</table>
# TABLE 13

SYNERGISTIC ACTIVITY OF AQUEOUS EXTRACTS OF DIFFERENT PARTS OF *C. FISTULA* WITH AMOXICILLIN BY AGAR WELL DIFFUSION METHOD

<table>
<thead>
<tr>
<th>S. #</th>
<th>Organism</th>
<th>Amoxicillin 15 μg/ml disc</th>
<th>Leaf 150 μg/ml disc</th>
<th>Leaf + Amoxicillin disc</th>
<th>Fruit 150 μg/ml disc</th>
<th>Fruit + Amoxicillin disc</th>
<th>Bark 150 μg/ml disc</th>
<th>Bark + Amoxicillin disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>St. Sonnet</td>
<td>10 mm</td>
<td>0.0 mm</td>
<td>15 mm</td>
<td>0.0 mm</td>
<td>15 mm</td>
<td>15 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>2.</td>
<td><em>E. coli</em></td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>15 mm</td>
<td>15 mm</td>
<td>30 mm</td>
<td>15 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>3.</td>
<td><em>S. typhi</em></td>
<td>10 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>14 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
</tr>
<tr>
<td>4.</td>
<td><em>S. typhi Para B</em></td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>14 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
</tr>
<tr>
<td>5.</td>
<td><em>S. Para typhi B</em></td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>14 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
</tr>
<tr>
<td>6.</td>
<td><em>S. para typhi A</em></td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>9 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
</tr>
<tr>
<td>7.</td>
<td><em>S. typhi</em></td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>9 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
<td>0.0 mm</td>
</tr>
</tbody>
</table>
## TABLE 14
RESULT OF CHECKER BOARD SYNERGY TESTING OF AQUEOUS EXTRACT OF FRUIT OF *C.FISTULA* AND AMOXICILLIN AGAINST MDR *SALMONELLA TYPHI*

<table>
<thead>
<tr>
<th>No.</th>
<th>C.F alone ug/ml</th>
<th>C.F combination ug/ml</th>
<th>Amoxicillin alone ug/ml</th>
<th>Amoxicillin combination ug/ml</th>
<th>FIC C.F ug/ml</th>
<th>FIC amoxicillin ug/ml</th>
<th>FIC index ug/ml</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3750</td>
<td>1875</td>
<td>&gt;750</td>
<td>46.87</td>
<td>0.5</td>
<td>0.06</td>
<td>&lt;0.56</td>
<td>synergy</td>
</tr>
<tr>
<td>2</td>
<td>3750</td>
<td>468</td>
<td>&gt;750</td>
<td>93.75</td>
<td>0.12</td>
<td>0.125</td>
<td>&lt;0.245</td>
<td>synergy</td>
</tr>
<tr>
<td>3</td>
<td>7500</td>
<td>1875</td>
<td>&gt;750</td>
<td>23.43</td>
<td>0.25</td>
<td>0.03</td>
<td>&lt;0.28</td>
<td>synergy</td>
</tr>
<tr>
<td>4</td>
<td>3750</td>
<td>1875</td>
<td>&gt;750</td>
<td>23.48</td>
<td>0.5</td>
<td>0.03</td>
<td>&lt;0.53</td>
<td>synergy</td>
</tr>
<tr>
<td>5</td>
<td>3750</td>
<td>468</td>
<td>&gt;750</td>
<td>46.4</td>
<td>0.12</td>
<td>0.5</td>
<td>&lt;0.62</td>
<td>synergy</td>
</tr>
<tr>
<td>6</td>
<td>7500</td>
<td>937.5</td>
<td>&gt;750</td>
<td>46.4</td>
<td>0.12</td>
<td>0.06</td>
<td>&lt;0.18</td>
<td>synergy</td>
</tr>
</tbody>
</table>

C.F: *Cassia fistula*

FIC: Fractional Inhibitory Concentration
### TABLE 15

RESULT OF CHECKER BOARD SYNERGY TESTING OF CASSIA FISTULA, AMOXYCASSIA AND AMOXYCILLIN FOR SALMONELLA TYPHI.

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>MIC µg/ml</th>
<th>MIC µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amx alone</td>
<td>Amx comb</td>
</tr>
<tr>
<td>20</td>
<td>6.25</td>
<td>0.78</td>
</tr>
<tr>
<td>40</td>
<td>3.12</td>
<td>0.39</td>
</tr>
<tr>
<td>50</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>50</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>140</td>
<td>6.25</td>
<td>0.78</td>
</tr>
<tr>
<td>160</td>
<td>3.12</td>
<td>0.39</td>
</tr>
<tr>
<td>130</td>
<td>6.25</td>
<td>0.78</td>
</tr>
<tr>
<td>120</td>
<td>3.12</td>
<td>0.78</td>
</tr>
<tr>
<td>270</td>
<td>3.12</td>
<td>0.39</td>
</tr>
<tr>
<td>200</td>
<td>12.3</td>
<td>0.39</td>
</tr>
<tr>
<td>370</td>
<td>3.125</td>
<td>0.78</td>
</tr>
<tr>
<td>310</td>
<td>3.12</td>
<td>0.39</td>
</tr>
<tr>
<td>70</td>
<td>3.125</td>
<td>0.39</td>
</tr>
<tr>
<td>260</td>
<td>3.125</td>
<td>1.56</td>
</tr>
<tr>
<td>170</td>
<td>6.25</td>
<td>1.56</td>
</tr>
<tr>
<td>SR11</td>
<td>1.56</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Amx: Amoxicillin, C.F: Cassia fistula, Comb: Combination, ADD: Additive, Syn: Synergy
### TABLE 17

RESULT OF CHECKER BOARD SYNERGY TESTING OF *CASSIA FISTULA*, AMOXYCASSIA AND AMOXICILLIN FOR *E. COLI*

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>MIC µg/ml</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amx alone</td>
<td>Amx comb</td>
<td>FIC amox</td>
<td>CF alone</td>
<td>CF comb</td>
<td>FIC CF index</td>
</tr>
<tr>
<td><em>E. coli</em> NTCC'</td>
<td>0.624</td>
<td>0.07</td>
<td>0.02</td>
<td>31.2</td>
<td>7.8</td>
<td>0.25 0.32 Syn</td>
</tr>
<tr>
<td><em>E. coli</em> 3</td>
<td>0.156</td>
<td>0.039</td>
<td>0.24</td>
<td>62.5</td>
<td>15.6</td>
<td>0.5 0.74 Syn</td>
</tr>
<tr>
<td><em>E. coli</em> 4</td>
<td>≥ 2.5</td>
<td>0.039</td>
<td>0.15</td>
<td>62.5</td>
<td>15.6</td>
<td>0.249 0.39 Syn</td>
</tr>
<tr>
<td><em>E. coli</em> 7</td>
<td>1.25</td>
<td>0.078</td>
<td>0.06</td>
<td>62.5</td>
<td>15.6</td>
<td>0.25 0.31 Syn</td>
</tr>
</tbody>
</table>

**KEY**

Amx : Amoxicillin  
CF : *Cassia fistula*  
Comb : Combination  
Syn : Synergy
# Table 18

**Results of Checker Board Synergy Testing of Shemamin and Amoxicillin Against *Salmonella Typhi***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>Shemamin alone</th>
<th>Shemamin Comb</th>
<th>Amoxicillin alone</th>
<th>Amoxicillin Comb</th>
<th>FIC Shemamin</th>
<th>FIC Amx</th>
<th>FIC Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. Paratyphi</em></td>
<td>500</td>
<td>7.78</td>
<td>12.5</td>
<td>0.78</td>
<td>0.0154</td>
<td>0.56</td>
<td>0.57ADD</td>
</tr>
<tr>
<td>2</td>
<td><em>S. typhi</em></td>
<td>&gt;500</td>
<td>7.78</td>
<td>6.25</td>
<td>0.78</td>
<td>0.0154</td>
<td>0.112</td>
<td>0.127SYN</td>
</tr>
<tr>
<td>3</td>
<td><em>S. typhi B</em></td>
<td>&gt;250</td>
<td>7.78</td>
<td>6.25</td>
<td>0.78</td>
<td>0.03</td>
<td>0.112</td>
<td>0.142SYN</td>
</tr>
<tr>
<td>4</td>
<td><em>S. typhi B</em></td>
<td>&gt;250</td>
<td>7.78</td>
<td>12.5</td>
<td>0.156</td>
<td>0.03</td>
<td>0.012</td>
<td>0.042SYN</td>
</tr>
<tr>
<td>5</td>
<td><em>S. typhi</em></td>
<td>&gt;250</td>
<td>62.5</td>
<td>6.25</td>
<td>0.156</td>
<td>0.25</td>
<td>0.024</td>
<td>0.27SYN</td>
</tr>
</tbody>
</table>

**FIC**  Fractional Inhibitory Concentration
### Table 19

**Hemagglutination Titer in Mice Treated with Different Antimicrobial Substances**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hemagglutination titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>32</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>32</td>
</tr>
<tr>
<td>Amoxy-cassia</td>
<td>128</td>
</tr>
<tr>
<td><em>Cassia fistula</em></td>
<td>64</td>
</tr>
</tbody>
</table>

### Table 20

**Anti-SRBC Secreting Spleen Cells in Balb/c Mice Treated with Different Antimicrobial Substances**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total no. of cells/spleen</th>
<th>Percentage of viable cells</th>
<th>No. of plaques/slide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>$1.35 \times 10^7$</td>
<td>90%</td>
<td>09</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>$1.20 \times 10^7$</td>
<td>90%</td>
<td>15</td>
</tr>
<tr>
<td>Amoxy-cassia</td>
<td>$1.1 \times 10^7$</td>
<td>95%</td>
<td>Uncountable</td>
</tr>
<tr>
<td><em>Cassia fistula</em></td>
<td>$1.0 \times 10^5$</td>
<td>92%</td>
<td>122</td>
</tr>
</tbody>
</table>
FIGURE 2

TIME KILL KINETICS OF AMOXY-CASSIA IN COMPARISON WITH
AMOXICILLIN AND C. FISTULA

![Graph showing time kill kinetics comparison with Amoxicillin, C. fistula, Amoxy-Cassia, and Control.](image-url)
FIGURE 3

TIME KILL KINETICS OF PE2 (FRACTION OF AMOXYCASSIA) WITH AMOXICILLIN AND WITHOUT AMOXICILLIN
FIGURE 5

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA* MILL (AVOCADO) ON THE INCREASE OF HAEMAGGLUTINATION (H.A) TITER OF *S. SANGUIS* TO HUMAN 'O' RBC
FIGURE 6

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL.* (AVOCADO) ON THE DECREASE OF HAEMAGGLUTINATION (H.A.) TITER OF *S. SANGUIS* TO HUMAN 'O' RBC
FIGURE 7

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF BETA VULGARIS (BEET ROOT) ON THE HAEMAGGLUTINATION (H.A) TITER OF S. SANGUIS TO HUMAN 'O' RBC
FIGURE 8

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF SOLANUM TUBEROSEUM (POTATO) ON THE HAEMAGGLUTINATION (H.A) TITER OF S. SANGUIS TO HUMAN 'O' RBC
AGGREGATION OF *S. SOBRINUS* IN PRESENCE OF HIGH MOLECULAR WEIGHT DEXTRAN IN PRESENCE OF *PERSEA AMERICANA* MILL. WATER SOLUTIONS AND ORGANIC EXTRACTS THAT INCREASED ADHESION
AGGREGATION OF S. SOBRINUS IN PRESENCE OF HIGH MOLECULAR WEIGHT DEXTRANE IN PRESENCE OF PERSEA AMERICANA MILL WATER SOLUTIONS AND ORGANIC EXTRACTS THAT DECREASED ADHESION

![Graph showing the effect of different solutions on the optical density at 540 absorbance over time in minutes. The graph includes data points for Control (Untreated S. sobrinus), Pulp acetone 2.5mg/ml, and Pulp ethanol 2.5mg/ml.](image-url)
FIGURE II

AGGREGATION OF *S. SOBRINUS* IN PRESENCE OF HIGH MOLECULAR WEIGHT DEXTRAN IN PRESENCE OF *BETA VULGARIS* (BEET ROOT) WATER SOLUTIONS AND ORGANIC EXTRACTS THAT INCREASED ADHESION

![Graph showing aggregation of S.sobrinus with different treatments over time.](image-url)
AGGREGATION OF S. SOBRINE WITH HIGH MOLECULAR WEIGHT DEXTRAN IN PRESENCE OF BETA VULGARIS (BEET ROOT) WATER SOLUTIONS AND EXTRACTS THAT DECREASED THE ADHESION
FIGURE 13

AGGREGATION OF S. SOBRinus WITH HIGH MOLECULAR WEIGHT DEXTRAN IN PRESENCE OF PORPHYRA TENERA WATER SOLUTIONS AND EXTRACTS

![Graph showing optical density at absorbance 540 over time in minutes for different conditions: water 5%, water 50%, methanol pulp 25mg/ml, methanol pulp 2.5mg/ml, and control.](image-url)
FIGURE 14

COAGGREGATION OF *A. NAESEA* WITH *S. SANGUIS* IN PRESENCE OF *PERSEA AMERICANA MILL* (AVACADO) EXTRACTS WHICH INCREASES THE ADHESION

![Graph showing optical density at absorbance 540 over time in minutes.]

- Untreated *A. naesea* with *S. sanguinis*
- Water peel 60%
- Paste acetone 2.5mg/ml
- Acetone pulp 25mg/ml
FIGURE 15

COAGGREGATION OF A. NAESHULNDII WITH S. SANGUIS IN PRESENCE OF PERSEA AMERICANA MILL. (AVOCADO) EXTRACTS WHICH DECREASES THE ADHESION
FIGURE 16

COAGGREGATION OF A. NAESII/LUNDII WITH S. SANGUIS IN PRESENCE OF BETA VULGARIS (BEET ROOT) EXTRACTS WHICH INCREASES THE ADHESION
FIGURE 17

COAGGREGATION OF A. NAESHLUNDII WITH S. SANGUIS IN PRESENCE OF BETA VULGARIS (BEET ROOT) EXTRACTS WHICH DECREASES THE ADHESION

[Graph showing optical density at absorbance (540) over time in minutes with legend indicating different treatments: control (untreated A. naeslundii), Ethanol pulp 25mg/ml, Methanol pulp 25mg/ml, Methanol pulp 2.5mg/ml]
FIGURE 18

COAGGREGATION OF A. ANAESHLUNDII WITH S. SANGUIS IN PRESENCE OF PORPHYRA TENERA (SEA WEED, NORI) EXTRACTS
FIGURE 19

COAGGREGATION OF A. NAESIILUNDII WITH S. SANGUIS IN PRESENCE OF SOLANUM TUBEROSUM (POTATO) EXTRACTS

![Graph showing coaggregation with different potato extracts at different percentages and time points.]

- Peel 50%
- Peel 5%
- Core 50%
- Core 5%
- Subpeel 50%
- Subpeel 5%
- Control
FIGURE 20

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA* MILL. (AVOCADO) ON THE HAEMAGGLUTINATION TITER (H.A) OF UROPATHOGENIC *E. COLI* TYPE 1.
FIGURE 21

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT) ON THE HAEMAGGLUTINATION TITER (H.A) OF UROPATHOGENIC *E. COLI* TYPE 1

![Graph showing the effect of different extracts on haemagglutination titer.](image-url)
FIGURE 22

EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF PORPHYRA TENERA (SEA VEGETABLE, NORI) ON THE HAEMAGGLUTINATION TITER (H.A.) OF UROPATHOGENIC E. COLI TYPE 1

- Water 50%
- Water 5%
- Acetone 25mg/ml
- Acetone 2.5mg/ml
- Control
FIGURE 23

FLOW CYTOMETRIC EVALUATION OF HAEMAGGLUTINATION OF S. SANGUIS TO HUMAN 'O' GROUP RBC IN PRESENCE AQUEOUS AND ORGANIC EXTRACTS OF Beta vulgaris (BEET ROOT)
FIGURE 24

FLOW CYTOMETRIC EVALUATION OF HAEMAGGLUTINATION OF S. SANGUIS TO HUMAN 'O' GROUP RBC IN PRESENCE AQUEOUS AND ORGANIC EXTRACTS OF PERSEA AMERICANA MILL
FLOW CYTOMETRIC EVALUATION OF AQUEOUS AND ORGANIC EXTRACTS OF PERSEA AMERICANA MILL (AVOCADO) ON ADHERENCE OF E. COLI TYPE 1 TO GUINEA PIG RBCS
FIGURE 26

FLOW CYTOMETRIC EVALUATION OF AQUEOUS AND ORGANIC EXTRACTS OF SOLANUM TUBEROSUM (POTATO) ON ADHERENCE OF E. COLI TYPE 1 TO GUINEA PIG RBCs
FIGURE 27

LOG OF NUMBER OF *S. AGALACTIAE* IN VAGINAL SECRETIONS OF RATS TREATED WITH SALINE.
FIGURE 28

LOG OF NUMBER OF *S. AGALACTIAE* IN THE VAGINAL SECRETIONS OF RATS TREATED WITH ASPARAGINASE

![Graph showing the log of number of CFU against the number of days for different days: day 0, day 1, day 1+36, day 1+48, day 4, day 6, day 10, day 12, day 14, day 15, day 18, day 20.](image)
FIGURE 29

LOG OF NUMBER OF S. AGALACTIAE IN THE VAGINAL SECRETIONS OF THE RATS TREATED WITH PPO

![Graph showing the log of number of CFU against the number of days for various days: day 0, day 1, day 1+36, day 1+48, day 4, day 6, day 8, day 10, day 12, day 14, day 16, day 18, day 20.](image-url)
DISCUSSION
DISCUSSION

In spite of very impressive advancements in the field of science and technology, infectious diseases are still the leading cause of death. Millions of children die everyday due to typhoid, malaria, tuberculosis, septicemia, cholera, dysentery, diarrhea in mostly in the developing countries including Pakistan. The spread of infectious diseases results from changes in human behavior, unhygienic, overcrowded living conditions increased trade and travel, and inappropriate use of antibiotics.

Twenty well-known diseases, including tuberculosis (TB), malaria, and cholera have reemerged or spread geographically since 1973. often in more virulent and drug-resistant forms. At least 30 previously unknown disease agents have been identified since 1973, including HIV, Ebola, Hepatitis B and C, Crimean Congo Fever- for some of them no cures are available. Of the seven biggest killers worldwide, TB, malaria, hepatitis, and in particular, HIV/AIDS continue to surge, and are likely to account for the overwhelming majority of deaths from infectious diseases in developing countries by 2020.

In the mid of twentieth century, 'wonder drugs' were introduced and were prescribed by the physicians without recognizing the pathogen involved in the disease. And this led to the emergence of resistance in potential human pathogens against the conventional antibiotics used for the treatment. Few examples of pathogens, which have acquired resistance, are multi-drug resistant (MDR) Salmonella typhi, Methicillin resistant Staphylococcus aureus, Vancomycin resistant Streptococcus epidermidis, MDR, Mycobacterium tuberculosis and Pseudomonas aeruginosa.
In order to treat patients, we have to explore look for alternative sources of drugs to
cure the infectious diseases caused by the drug resistant bacteria. Higher plant
products have been used by traditional and Ayurvedic practitioners and in folklore
medicine since long, but without any scientific basis for use. In recent years World
Health organization is also encouraging their member countries to explore their natural
plant wealth before it is lost due to the urbanization. Herbal remedies used in
traditional folk medicine provides an interesting and still largely unexplored source
for the creation and development of potentially new drugs for chemotherapy which
might help to overcome the graving problem of resistance and also toxicity of
currently available commercial antibiotics. The traditional medicinal plants still play a
vital role to cover the basic health needs in developing countries. Therefore it is of
great interest to carryout a screening of medicinal plant, in order to validate their use
in folk medicine, to reveal the active principal by isolation and characterization of
their constituents and to develop better drugs against cancer as well as viral and
microbial infections. It is necessary that the documentation of medicinal plants be
treated as a matter of extreme urgency as change in land use due to urbanization to
destroy much of the habitat of useful plants would result in irreversible loss of plant
species.

In this study different parts from 30 medicinal plants were evaluated for their ability
to inhibit the growth of bacteria, their colonization on host surfaces and preventing
them to initiate disease in the host. To this end we screened the aqueous and organic
extracts of various plant products for antimicrobial activity, in-vitro and in-vivo anti-
adhesion activity, immunomodulatory property and their toxicity in BALB/c mice.
ANTIMICROBIAL PROPERTIES OF PLANT PRODUCTS

A total of twenty-eight aqueous extracts, 24 organic extracts including 19-methanol extracts, 5 ethanol and 5 acetone extractors were prepared. The logic behind using the water extract is that most of the plant studied are edible and are cooked with water; dry plant parts are consumed as tea dissolved in hot water and in some cases vapors of heated water solutions are inhaled. Very rarely they are consumed as tincture or solution in alcohol (49,61).

In this study medicinal plants were screened against 20 potential human pathogens including MDR Salmonella typhi, MRSA, Staph aureus, Streptococcus pyogenes (STSS), S. epidermidis, S. agalactiae, Klebsiella pneumonia, Vibrio cholera, E. coli type I, S. para typhi A, S. para typhi B, Shigella dysenteriae, Corynebacterium xerosis, Proteus vulgaris, Sh. Sonnei, C. diphtheriae, diphtheroid, E cloacae, Acinetobacter calcoaceticus, Serratia, HHEC and EPEC.

Out of 28 water solutions studied eleven solutions were prepared from the plants used as spices. These were Foeniculum vulgare (Saute), Arecham Sowa (Sowa), Cuminum Cuminum (zeera), Nigella sativa (Kalonchhi), Sesamum sudan (Jawauntari), Myrica fragrans (Jaiphal), Trachyspermum ammi (ajiwain), Cinnamom Tamala (Tezpat), Piper nigrum (Black pepper), Syzygium aromaticum (Clove) Annona subulatum (Bari ilaiichi). They were screened for the presence of anti-microbial properties by agar well diffusion method. Of the eleven spices studied, water solution of Syzgium aromaticum (Clove) was found to be the only one which showed broad spectrum antimicrobial effect against S. aureus, S. epidermidis, C. xerosis, B. subtilis, Ps.
aeruginosa, Proteus, S. typhi, S. para typhi, S. agalactiae and Klebsiella. The antibacterial component found in the clove was found to be heat resistant since autoclaving of the solution did not affect the antibacterial activity. Broad-spectrum antibacterial activity in the ethanol extract of clove has been reported earlier by other groups. In our study, we recorded broad-spectrum antibacterial activity in a more natural and commonly used water solution of clove against difficult pathogens like Ps. aeruginosa and S. agalactiae and others. Ps. aeruginosa is mostly nosocomially-acquired pathogen. It is the cause of severe epidemics, diarrhea in infants, ocular infection, burn infection, cystic fibrosis, folliculitis, osteomyelitis and malignant external otitis. Mostly third generation antibiotics are employed to cure the infection caused by Ps.aeruginosa. Most of these antibiotics are very expensive and poor patients cannot afford to buy them. S. agalactiae is a leading cause of neonatal meningitis. The newborn baby usually gets infected while passing through the birth canal. Since most of the allopathic drugs are becoming expensive, cheaper antibiotics derived from clove will prove to be a blessing for people living in poverty stricken areas. Clove is commonly used as a spice and its oil is a good remedy for toothache. It has been incorporated in some toothpaste as the anti-microbial agent. Our observations provide a further confirmation of its antimicrobial efficacy.

From the remaining 17 water solutions, Rosa centifolia (rose) extract inhibited the growth of M. lysodeikticus and potential human pathogens like S. pyogenes Staph. Aureus C. pseudodiptheroid and Sh. dysenteriae at high concentration. S pyogenes is known to cause pharyngitis, tonsillitis, sinusitis, lymphadenitis, pyoderma, osteomyelitis, endocarditis and meningitis.
Water extract of fruit of *C. fistula* was found to inhibit the growth of *S. typhi*, *E. coli* and *S. pyogenes* at significantly higher concentrations. Methanolic fractions including, P1–P2, 80MR, EA2 and ALCR prepared from the fruit of *C. fistula* were found to inhibit MDR *Salmonella typhi* at Minimal Inhibitory Concentration (MIC) range of 125 – 500 µg/ml. Ethanol extract of *C. fistula* has been reported to have activity only against the Gram positive bacteria. In our study we found that the methanolic leaf extract of *C. fistula* effectively inhibits *S. epidermidis*, *S. pyogenes*, and *M. lysodeicticus*. *Cassia fistula* (Leguminaceae) commonly known as Amaltaas, is a tree of moderate size, indigenous to Pakistan, India, Tropical Africa, South America and West Indies. Its leaves and bark are used in roundworm infection, facial paralysis and rheumatism. Raw pulp from pod of *C.fistula* is a good laxative, its confection is used to treat diabetes and other extract is reported to have broad-spectrum antibacterial activity (25,26,57,145).

The significance of our study lies in the fact that unlike previous reports, we have found broad spectrum antibacterial activity in the water extracts of the fruit which includes pod too. Most people consume Amaltaas fruit as the syrup in water for various ailments whereas majority of the investigators reported antibacterial activity in organic extracts. The other most important observation of our study is the anti *Salmonella* activity in the fractions of *C. fistula* against MDR *S. typhi*, causative agent of Typhoid Fever – the most prevalent infection in Pakistan.

Of the 19-methanolic extracts tested, *Ficus carica*, *Olea europhosa*, Peganum harmala and *Bombax ceiba* were found to have potential antimicrobial activity. Methanol extract of *Ficus carica* and *Olea europhosa* was found to be effective...
against *S. pyogenes*, *C. pseudodiphtheroid* and *S. epidermidis* Ahmed et al. (2001) and B. Ali (2001) have reported similar results but with ethanol extract of *P. carica*. In traditional medicine fig or *P. carica* is used in prevention of colic pain, for headaches, toothache and for gout. The seeds are used to cure ulcers and to reduce swelling of the spleen.

In our study, methanolic crude extracts of another plant - *P. harmala* was found to inhibit *Staph aureus* with an MIC value < 40 - 80 µg/mL. Aqeel et al who also used methanolic extract fractions of *P. harmala*, namely harmine, harmaline, harmol, harmatal, tetrahydroharmane and tetrahydroharmal found antibacterial activity but at a much higher MIC value in the range of range of 250 - > 500 µg/mL (3). The possible reason for the lower MIC values recorded in our study could be that the crude extract used by us consisted of a combination various fractions instead of just one. There is a possibility that more than one antimicrobial constituent is present in the crude methanolic extract that may have exerted a synergistic action on the overall antimicrobial efficacy of *P. harmala*. Besides it also arrested the growth of Acinetobacter lwlsii and Candida albicans, both of these are serious but opportunistic microbes and major cause of nosocomial infections especially in the immuno compromised patients like AIDS and people suffering from malignancies or receiving chemotherapy (20). The LD50 of *P. harmala* was found to be 300mg /Kg of the body of the BALB/c mice, which is very high, suggesting that the crude extract as used in very small quantity for various ailments is almost non toxic. There are many reports of multi drug resistant Acinetobacter spp. Most of the infections due to Candida and Acinetobacter are treated with combination therapy. Since Candida species have developed resistance to the conventional drugs used. New array of anti-
fungal agents like triazoles fluconazole and itraconazole were introduced. But new fluconazole resistant strains of \textit{C. albicans} have already been isolated. Thus \textit{P. harmala} seems to be a promising alternative source of antibacterial agents for the treatment of nosocomial infections. \textit{P. harmala} was also found to be effective against multidrug resistant \textit{S. typhi} – causative agent of typhoid fever in developing and developed countries of the world. \textit{Peganum harmala} linn (harmala) is commonly found in N.W. India, Sind, Punjab and Kashmir. In native work, it is used as an alternative and purifying medicine supposed to arise from cold, such as palsy lembago (113,191).

In Punjab, the seeds are considered as narcotic and given in fevers and colic. The decoction of the leaves is given for rheumatism and the root mixed with mustard oil is applied to the hair to destroy vermin. In Gujarat it is burnt in the sick room as an antiseptic and deodorizer when any person suffers from wounds, ulcers or small pox. Seeds are narcotic, antispasmodic, hypnotic, anodyne, nauseant, emetic and emmenagogue. It gives relieve in simple cough, pain and procure sleep. It is also a good nauseant and depressant emetic in its largest medicinal doses. The action of harmaline, the constituent of methanol extract of harmal is practically same in action as quinine. It is possible that harmaline may be used as substitute for quinine. In this study a new aspect and use of \textit{P. harmala} has been explored, as it is the plant found in our country. Cheaper medicines can be developed from it and its manufacturing plants can provide job to our talented youth.

Crude leaf extract of \textit{Bombax ceiba} was found to be effective against \textit{S. epidermidis}, \textit{S. typhi}, \textit{B. Subtilis}. \textit{Bombax ceiba} Linn (syn. \textit{Bombax malabaricum} DC, \textit{Sideralia}
*Gossypium malabarica* DC Schott and Endl and *Gossypium malabarica* DC Merr known in vernacular as silk cotton tree belongs to family Bombacaceae. It is widely cultivated in Pakistan, India, China and Australia. The plant is well reputed for the treatment of diarrhea and fever. Shamimin, a novel pure compound isolated from the leaf extract of *Bombax ceiba* showed antimicrobial activity by well diffusion method against *Listeria, B. subtilis, Ps. aeruginosa, Sh dysenteri, C. pseudo diptheroid* and *Enterococci*. Minimum inhibitory concentration for *Ps. aeruginosa* ATCC strain was found to be 62μg/ml whereas for clinical extract it is ≥ 100. From this study it is revealed that *B ceiba* leaf extract can also be employed to cure disease like typhoid.

**AMOXY-CASSIA: A NEW SYNERGISTIC FORMULATION FOR MDR SALMONELLA AND MRSA.**

Indiscriminate use of antibiotics and development of resistance in large number of pathogens, for example multi drug resistant (MDR) Salmonella, Methicillin resistant Staphylococci and Pseudomonas species has left the physicians with very few antibiotics to treat such infections. Most of the commonly prescribed anti-microbial drugs like amoxicillin, sulphonamethoxazole, chloramphenicol have lost their therapeutic efficacy. This situation is quite alarming and has led to the use of combination therapy to combat drug resistant infections. The combined drug used must show synergism, which is two to four fold decreases in the minimal inhibitory concentration (MIC) or bactericidal concentration of individual antibiotics, when they are present together.
In an attempt to develop new synergistic compounds, we screened aqueous and organic extracts of various plants for the presence of the synergistic activity when combined with various Beta Lactam antibiotics. Amoxicillin – a Beta Lactam antibiotic that has been widely used due to its previously known broad-spectrum antimicrobial activity and non-toxic nature was chosen to develop synergistic drugs (71). A number of pathogens including MDR S. typhi, Methicillin resistant Staph aureus, E. coli and Pseudomonas species have developed resistance to Amoxicillin. These bacteria express resistance either by producing beta lactamase – an enzyme that inactivates Amoxicillin or modify the penicillin – binding protein (PBP). Commercially amoxicillin clavulanic acid combination is available in the market, by the name of augmentin or clavamox. Clavulanic acid is a chemical that inhibits beta lactamase enzyme. It has given extended life to penicillinase sensitive beta lactam antibiotics.

In this study, an attempt was made to formulate a new synergistic preparation using plant products and amoxicillin. Combinations containing Amoxicillin and Shamim in were found to be effective against S. typhi and E coli while Amoxicillin and aqueous fruit extract of C.fistula combination was found to be very effective against S.typhi - both MDR and Non MDR. We have named the new Synergistic Preparation as Amoxy-cassia. This new product of our lab has already received a Patent serial number 137124 by the Government of Pakistan. We preferred the use of water solution as both amoxicillin and C.fistula fruit have proven efficacy in oral dosage in aqueous form.
In case of multidrug sensitive *S. typhi*, MIC of amoxicillin and *C. fistula* ranged from 1.56 - 6.25 μg/ml and 390 - 3120 μg/ml respectively when tested alone. The value decreased whereas MIC value ranged from 0.39 - 1.56 and 390 - 1560 μg/ml when they were tested in combination. Synergy was observed against 87.5% of the organism tested with fractional inhibitory concentration (FIC) of amoxicillin ranging from 0.06 - 0.5. FIC value of 1 for 12.5% of the strain studied. In case of MDR *S. typhi*, MIC of amoxicillin alone was >750 μg/ml for all the isolate tested. The MIC of *C. fistula* alone for MDR *S. typhi* ranged from 3750-7500 μg/ml. MIC of amoxicillin in combination ranged 11.7 - 93.75 μg/ml and *C. fistula* 3750 - 7500 μg/ml. FIC values for amoxicillin ranged in <0.015 - 0.125 where as FIC value for *C. fistula* is found to be 0.12 to 0.5. FIC index for < 0.5 indicating synergism for the entire range of organisms tested.

None of the combinations investigated showed additive property. In case of *E. coli*, MIC of amoxicillin alone was found in the range of 0.156 - 72.5 μg/ml and *C. fistula* alone is 156 - 625 μg/ml whereas MIC in combination of amoxicillin is in the range of 0.039 - 0.078 μg/ml and *C. fistula* is 78 - 156 μg/ml. FIC index against all the organisms tested was found to be > 0.5 which indicates the presence of synergism. In case of MRSA *C. fistula* and amoxicillin was in the ranged of 1875 and >750 μg/ml respectively when tested alone. In combination value ranged from 420-468 and 9.7-11.7μg/ml respectively.

Time kill assays were performed against MDR *S. typhi* and MRSA. In the presence of *Anoxy cassia* we recorded a ≥ log 2 decrease in colony forming unit (CFU) as compared to the CFU present in the broth containing amoxicillin alone. The decrease
in log ≥ 2 further confirmed the synergistic effect of Amoxy-cassia. Time kill synergistic kinetics of the amoxicillin with the fraction of fruit of C.fistula against MDR S. typhi showed the synergistic effect at 8 hrs. The number of colony forming units (CFU) in the culture medium containing PE and amoxicillin reduced approximately by log 2. Further work on this combination is under progress.

In the animal toxicity studies, Amoxy-cassia (Amoxicillin plus C.fistula) as well as C.fistula alone was found to be non-toxic in BALB/c mice at a concentration of 1000 mg/kg body weight of the mice. These results proves that Amoxy-cassia has the potential to evolve as an important drug in near future as it was found to be effective against the difficult organism like MDR S. typhi, MRSA and E. coli and was free of any toxic effects.

Typhoid fever is an acute febrile disease caused by S. typhi. It is endemic in Pakistan and other under developed countries of Asia, Africa and Latin America. This is due to poor sanitation and presence of chronic carriers. Out breaks of S. typhi often spread by eating faecally contaminated, uncooked food, raw vegetable or fruit. In a previous study from our lab, 2,808 typhoid cases were reported in Karachi (Pakistan) in just 1½ years. In this study, 87% of the blood samples were found to be positive for S. typhi. Till 1987, all S. typhi were uniformly sensitive to all the conventional drugs used against typhoid fever. Then emerged the multi drug resistant strains of S. typhi (MDR – S. typhi), which were resistant to conventional anti typhoid drugs. The treatment of MDR S. typhi was possible with quinolones and third generation cephalosporins, but recently it has been reported that 3 – 14% of the MDR S. typhi
have developed resistance to quinolones as well as the newly introduced third generation cephalosporins. This situation is quite alarming (37.62,106,158).

MRSA is often associated with patients in hospitals. Patients with hip and knee implantation usually get nosocomial infection of MRSA. They are referred to as MRSA because they are resistant to Methicillin. But these organisms are also resistant to commonly prescribed antibiotics like erythromycin and cephalosporins. Some MRSA occur in epidemics, indicated by 'an' before MRSA e.g. EMRSA 16, EMRSA 3. MRSA when transmitted to same one who is already ill, then more serious infection may occur in that individual. For the treatment of infection caused by MRSA drugs of choice at present are Vancomycin and teicoplanin. But they are quite expensive, at times prove to be toxic and can be administered by the intravenous infusion. Since 1999 reports of clinical isolates with intermediate susceptibilities to Vancomycin have appeared in Japan and United States. Thus the scientists feel the need to develop effective alternative antimicrobial treatment such as combination therapy. So, most of the workers are busy in developing various synergistic combinations from commercially available antibiotics Vancomycin with rifampin or gentamicin.

Formulation of a novel synergistic compound Amoxy-cassia is our most important original achievement. Amoxy-cassia has the potential of being developed as a cost effective drug of choice for the treatment of difficult infections caused by MRSA and MDR S. typhi.

IMMUNOMODULATING PROPERTIES OF AMOXY-CASSIA.
The body of the human is covered with the skin, which is the biggest organ to prevent the entry of microorganism or foreign substance inside the host. Still most of the pathogens make their way into human body through cut in the skin, food eaten or are inhaled through nose. As soon as the pathogen/antigen enter the body, immune system is activated. There are two types of immunity: non-specific or cellular other is specific or humoral. Non-specific or cellular based immune system carries out the function of phagocytoses that engulfs the bacteria or foreign substance, which enter the host. In case of humoral immune system specific antibodies are produced that gets attached to the specific sites on the pathogen or antigen and destroys them. Antibodies are prepared in the lymphoid tissues of the spleen within three to four days of invasion of antigen. The antibody forming cells are activated in the spleen that helps in the production of antibodies. In our study we injected sheep red blood cells as antigen intra-peritoneal in the BALB/c mice. As the maximum number of cells are produced on fourth day after the entry of antigen in the body. Animals were dissected on fourth day. By Jerne Plaque assay it was shown that antibodies produced by lymphoid cell against red cell caused hemolysis of the sheep RBCs in presence of complement resulting in hemolytic plaques. Every plaque appeared as a result of an antibody-producing cell in its center. It was therefore possible to compute the number of specific antibody forming cells, in a given lymphoid cell suspension from spleen. These direct plaques were formed largely due to cells producing IgM class of antibody. This is assumed because of higher efficiency of IgM to bind to the complement and the fact that rise in the number of direct plaques after primary immunization occurs at 4 days that parallels the high titer of specific IgM in the serum. Results of our assay showed uncountable plaque produced in spleens from of by the spleen of the mice treated with Amoxy-cassia, control mice treated with saline
showed 90 plaques/spleen *Cassia fistula* fruit solution and amoxicillin 220 and 150 plaques/spleen respectively. Hemagglutination titer of the antibodies present in the serum for the animal treated with normal saline, Amoxy- cassia. *C fistula* and amoxicillin was found to be 32, 128.64 and 32 respectively.

Thus the *in-vivo* effect of Amoxy- cassia on immune system proves that besides being an ant microbial agent, it is an excellent immuno- enhancing compound. The water solution of *C. fistula* alone can be developed into an immuno-enhancing drug. They both will prove to be the excellent drug for the patient having impaired immune system like AIDS patients. person suffering from malignancies and immune-compromised host.

**ANTI-ININFECTIVE PLANT PRODUCTS FOR CARIOGENIC AND UROPATHOGENIC BACTERIA**

The relationship between human and their oral micro flora is a complex, dynamic micro ecosystem. The mouth contains a diversity of surface for colonization. Including the tongue, teeth, gingival palate and cheeks and it provides numerous aerobic and anaerobic and microaerophilic microhabitats for the estimated 1,000 different oral species. The habitat of the oral cavity is warm, moist and greatly enriched by the periodic infusion of food. In most humans, this association remains in balanced with little adverse effect, but in people with poor or nonexistent oral hygiene, it teeters constantly on the brink of disease. Adhesion of bacteria to the substrate is the first step in an infection process. As the organisms with the ability of firm attachment to the host tissue are able to overcome all the defense mechanisms of
the body which includes mucosal secretions, phagocytosis and antigen antibody reaction etc. Potential pathogens exhibit selectivity for the surfaces on which they colonize. Many studies have linked the ability of adherence of bacterial cells to specific animal tissues with their ability to initiate disease. Dental caries and periodontal disease are among the most prevalentable infections in human, which are the direct result of bacterial colonization A freshly cleaned tooth is a perfect landscape for colonization by microbes. Within a few moments, it develops a thin mucus coating called acquired pellicle, which is made up of adhesive salivary proteins. This structure presents a potential substrate upon which certain bacteria first gain foothold. The most prominent pioneering colonist are the cariogenic genera of Streptococcus and Actinomyces, these gram positive bacteria have adhesive receptors such as fimbriae and slime layers that allow them to cling to the tooth surfaces and to each other, forming a foundation for dense, whitish mass called plaque. Many researchers have emphasized the importance of controlling dental plaque to maintain good oral health to prevent dental caries and periodontal disease. Pathogen have proteins molecules possibly lectins that binds to the receptor molecules present on eukaryotic cell. The receptor consists of carbohydrate molecule that can be glycoprotein or glycolipids and it is complementary to the bacterial lectins. The binding between them is specific and is followed by a physiologically relevant response (41,125,172,219).

A wide variety of sources have been explored in the search for effective antiplaque agent. It has been known that cell agglutinating proteins notably haemagglutinins or phytohaemagglutinins are widely distributed in plants and they interfere with the
attachment of the bacteria to the host tissue. There are many reports on the isolation and identification of natural plaque inhibiting substances from plant (197).

In this study we have screened 16 water extracts and 30 organic extracts from three terrestrial plant including *Beta vulgaris* (*Beetroot*), *Persea americana mill* (*Avocado*), *Solanum tuberosum* (*Potato*) and one sea vegetable *Porphyra tenera* (*Nori*) for their *in-vitro* antiplaque forming ability / anti attachment / anti aggregation or anti co-aggregation by developing three model assays. In this study medicinal plants were evaluated for their anti-adherence properties are *Solanum tuberosum* (Potato), *Persea americana mill*, *Beta vulgaris* and *Porphyra tenera*. *Solanum tuberosum* it is staple food eaten worldwide. They are used for making fodder ethanol and butanol. Potato is a folk remedy for burns, corns, cough, cystitis fistula, prostatitis, scurvy, spasm tumor and warts. The tea made from the peels of the tuber is said to be remedy for corns. The oxidative product of phenolic compounds appear to be involved in the defense of plant against invading pathogens including bacteria, fungi and virus (130,135).

*Persea americana mill* (avocado) belongs to the family of Lauraceae. Twenty-four molecular species of triglycerides have been identified from Hass and Fuerte variety of Avocado. Their ground seeds extract prepared in different buffer solution (pH 2.0 - 12.0) showed hemagglutination activity towards A, B, AB and O human erythrocytes. It inhibited the interleukin 6 production when mixed with soya bean. A methanol extract of avocado fruits showed potent inhibitory activity against acetyl-CoA carboxylase a key enzyme in fatty acid biosynthesis. Avocado has been shown to have extra ordinarily potent liver injury suppressing activity, selective activity
against six human tumor cell line in culture and show selectivity for human prostate adenoma carcinoma (PC-3) cells with one of them as potent as Adriamycin, also when tested against yellow fever mosquito larva, it was more effective than rotenone, a natural botanical insecticide and positive control(8,27,29,45,53,87,88,150,245).

Beta vulgaris (beet root) is now is widely used as dietary vegetable and colourant. Oral ingestion of betalain from beet root inhibited TPA induced promotion of mice skin tumour. In case of lung cancer there was 60% reduction in the tumour of lung with crude extract. The combined findings indicate that beetroot is a useful cancer preventive vegetable. Nutrient Beta vulgaris (beet) root contains antioxidant betalain major betanin that is a beta glucoside betanin, catechin and alpha antioxidant. They inhibited the linoleate per oxidation by cytochrome. Porphyra tenera (nori). It is popular edible seaweed in Japan. It is rich in cobalamine. Porphyra tenera is found to have chemo-preventive effects against diethylnitramine (DEN) induced hepatocarcinogenesis (110).

In our first model, we studied the hemagglutination titer of the S.sanguinis to human “O” group RBC. The importance of this assay lies in the fact that erythrocyte membrane is the best-studied natural membrane that demonstrates distinct cell surface containing saccharide, to which specific bacterial adhesion that is present in their pili or fimbriae adhere. The specificity of the test can be confirmed by the hemagglutination inhibition assay. Result of our study show that aqueous extracts of Beta vulgaris, Persea americana mill, Porphyra tenera and sub peel of Solanum tuberosum inhibited the adhesion of S.sanguinis to ‘O’ RBC. In our experiment we treated the organism with 50% and 5% concentration of the water solution and 2.5
mg/ml and 25 mg/ml of organic extract for one hour, then the bacteria were washed to remove all the extracellular traces of water solution and extract present on the bacterial surface. They were then mixed with human ‘O’ RBC. This signifies that molecules of phytolecin present in the plant material gets attached to the specific molecules on the bacterial surface, which results in the inhibition / reduction in haemagglutination titer of S. sanguinis to ‘O’ RBC surface. The importance of the inhibition of the attachment of S. sanguinis to tooth surface, lies in the fact that it has high affinity for the pellicle, which is the salivary coat always present on the surface of the teeth. It is found in the nearest tooth in the multi-layer plaque that can reach thickness of 300 – 500 cells. It is present in large number in plaque as compared to the other streptococcal species. The adhesion of S. sanguinis on to the pellicle results in subsequent accumulation of S. mutans and other bacteria. Thus it plays an important role in the genesis of plaque formation. It is used as a model for oral streptococcal adhesion studies since its adhesion to teeth is known to be least partially specific. In addition an in-vitro study shows that once bound, the bacteria has a low capacity to dissociate. Thus it is considered to have a higher affinity to the pellicle-coated teeth than other bacteria found in plaque. This affinity, as well its foremost presence on the pellicle makes the study of its inhibition by the plant product to be an important attempt to inhibit plaque formation (85,200).

Second model was the inhibition of aggregation of the S. sobrinus with high molecular weight dextran – (produced by Strept. mutans another oral pathogen) in the presence of plant material. The specificity of this test was confirmed by inhibiting the reaction with the low molecular weight dextran T 10. The results of our study with pretreated bacteria revealed that the aqueous extracts of pulp of Beta vulgaris,
Porphyra tenera. Sub peel of Solanum tuberosum and among organic extract acetone, ethanol and methanol extract of Beta vulgaris (beet) root, Persea americana mill avocado and methanol extract of Porphyra tenera inhibited the aggregation S. sobrinus with high molecular weight dextran. In another study ( ) it was observed that pretreatment of S. mutans with spermine, indocacetic acid, galactosamine and mannose amine inhibited the attachment of S. mutans to SHA (saliva coated hydrophatite ). In another study, glucosamine had no effect on interaction whereas ethanolamine, lysine and ammonium chloride affected some strains only. The possible reason of anti adherence is thought to be due to the amine complexing to the salicylic acid molecules on the Streptococci surface. Glucan is a major constituent of plaque biofilm that helps in the colonization of S. mutans and S. sobrinus. Both these organisms adhere to glucan and aggregates. Though, S. mutans could persist without unfavorable consequences in the absence of sucrose in the mouth. Those people whose diet is rich in glucose, sucrose and certain complex CHO in them Streptococcus mutans and S. gordonii produce a gummy polymer of glucose called dextran that helps them to attach to smooth enamel surface that holds the plaque together, and add to its bulk. As these primary invaders continue to grow and plaque begins to build up, the scene is set for the invasion and aggregation of additional species of bacteria. Among secondary invaders are species of Lactobacillus, Bacteroids, Fusobacterium, Neisseria and Tryponema. The metabolism of sucrose also causes drop in pH so that demineralization of the calcium of tooth enamel occurs. This is the beginning of the carious lesion, while the presence of sucrose in the oral cavity ensures that S. mutans cells and large a large volaminous plaque will accumulate on the tooth surface, S. sobrinus and S. mutans develops multiple glucan binding protein (GBP). This includes GT1, which are able to bind to product glucan and non-enzymatic ‘lectin’
like protein, which recognizes linear sequence of x-1-6 linked glucose. Therefore, glucan formed in sites in salivary pellicle and developing plaque could act as binding sites bearing surface associated GEP's. Thus interaction between cariogenic Streptococci and in sites formed glucan provides potential targets for the action of antiplaque agent (51).

Our third model was the coaggregation of Actinomyces naeslundii T14V with S. sanguinis 35. The specificity of the reaction was checked by inhibition of the reaction with lactose. Results of our studies shows that water solution of Persea americana mill (avocado) and Porphyra tenera (sea vegetable) and sub peel of Solanum tuberosum and organic extracts of Beta vulgaris (beet) root is found to be effective in inhibition of coaggregation of A. naeslundii T14V with S. sanguinis 35. The importance of this model lies in the fact that intrageneric coaggregation is responsible for the complexity of micro biota in human and dental plaque and is believed to be an important element in the initial bacterial colonization of dental plaque. Actinomyces naeslundii are early colony of teeth surface. They may enhance subsequent colonization of Porphyra gingivalis that is associated with adult periodontitis (67). It is reported that T14V have fibrils that are laden with the virulent associated antigen and active molecule present in them may be the protein or glycoprotein. Active molecules on S. sanguinis 34 are primarily carbohydrate. The coaggregation between S34 and T14V and S. sanguinis 34 was found to be reversible. The coaggregation of the cells had been completely disaggregated upon the addition of lactose, calcium chelating agent or the amino group acetyllating agent N-acetyl succinimide. Gibbon and colleagues (3, 4) reported many examples of specific coaggregation between paired oral bacteria were certain strains of Streptococcus sanguinis coaggregating with
specific strains of *Actinomyces viscosus* and *A. naeslundii*. The study of this coaggregation is also of importance due to the possible role of actinomyces in root caries and periodontal disease. The result of our study reveals that all the four plants studied *Beta vulgaris* (beet) root, *Persea americana* mill (avocado), *Solanum tuberosum* (potato) and *Porphyra tenera* (sea vegetable) has the potential to interfere with the adhesion of all the models of oral bacteria. The most significant feature of this finding is that the water solutions of all the plants are playing an important role in the anti-adhesion property of these plants. From this we can say that when avocado, Beetroot, Potato and sea vegetable is eaten, the components of the plant material get in contact with the bacteria present in plaque. There is the need to carry out a research to study the relation ship between the formation of plaque and the number of people consuming above plants orally. Because there is the possibility that only eating of these vegetable and fruit can help in reducing the plaque formation. The basis of this hypothesis is the fact we treated the bacteria for one hour with the plants solutions/extracts then we did washed it with distill water still the anti-adherence property was maintained. So when we eat these plants their components cover the whole area of the oral cavity and they remain there for quite a long time which can be sufficient enough to loosen or inhibit the adhesion of *S.sanguinis*. aggregation of *S.sobrinus* to dextran and coaggregation of *A. naeslundii* to *S.sanguinis*. It is reported that when oral surfaces were treated with neuraminidase, it markedly reduced the attachment of *S. sanguinis* to the salivary pellicle. The incorporation of antiplaque agents into oral hygiene products is a growing area in dental caries prevention programs. These plants being non-toxic can easily be incorporated into the toothpaste. As these plants do not have appreciable antibacterial property there is no chance of organism being getting resistant to it and it can be used be in use for quite a
long time. They can simultaneously help in controlling dental caries, periodontal disease and plaque formation. Many different chemicals have been proposed as antiplaque agents. Amines and tin fluorides are antimicrobial agents that exhibit beneficial anti-caries activity when used alone or in combination. They also possess substantively and an activity sufficient to reduce the net adhesions of \textit{S. sanguinis} to conditioned surface (Ebleton) several others established or experimental mouthwash contains oxygenated agents like metal salts of Copper and Tin have demonstrated antibacterial activity. Ant adhesive agents Octopinoi and delonopinoi have shown some promising result but long-term effect on gingivitis and caries is lacking. Extended daily use of rinses with chlorohexidine reduced the number of bacteria in saliva 30 - 15 % and in plaque by 55 - 90%. At present many ant microbal compounds are used as anti-plaque agent too. Within them first generation antimicrobial agents possess activity usually bacteriostatic or bacterial cidal but no substantively. Second generation antimicrobial agents are mainly bacteria static or bactericidal agents that possess same degree of substantality third generation agents possess same degree of bacterial cidal or bacterial static and they possess anti-adhesion property which may disrupt intragenic or intergenic adhesion (109.203.221.226).

Urinary infections caused mainly by \textit{E.coli} type I are among the most common infectious diseases. Most of the uropathogenic isolaces of \textit{E.coli} can express type P. fimbriae and I that contain adhesin, which recognize cell receptors. P. fimbriae recognize kidney glycolipid receptors and are involved in pyelonephritis. \textit{E.coli} type I anchor to urethelial surface via uroplakin '1' interaction. This anchorage plays a role in its bladder colonization and eventual through ureters against urine flow to cause infection in kidney.
The type I pilus of *E. coli* is a prototype of this class of hair-like multimeric adhesive organelles. The pilus mediates adherence to mannose-containing receptors on mucosa epithelia and other cells. Pili have other roles in disease, like capsule they can be antibacterial. They are also highly channelable and permit some organism to put on a succession of distinguished that to flank the immune system. The type I pilus is one of several serological variants that are expressed by all *E. coli* strains and its promotion of colonization by pathogenic bacteria. The protective effect of purified pilus vaccines suggests that it is important as a virulent factor. It is reported that *E. coli* 0517:H7 (EHA) bacterial induced haemaggutination to group 'O' erythrocytes was inhibited by *Tamarind indica* (tamarind), *Spinacia oleracea* (plum), *Psidium guava* (guava), *Cydonia vulgaris* (quince) *Crataegus mexicanus* (arjocete), *Ricinus communis* (castor oil), *Glycine max* (soybean), *Phaseolus vulgaris* (bean), *Vicia faba* (the fava bean) and *Solanum tuberosum* J. Two new specific lectins were identified from tamarine and guava. Lectin in guava was found to be lactose specific; it prevented adhesion of *E. coli* 0157:H7 to human 'O' RBC (246).

Water extract of *P. tenes* (sea vegetable) and *B. vulgaris* (beet) root inhibited the adhesion of *E. coli* to guinea pig RBC. Most significant result is shown by the *P. americana* (avocado). Its water solution is also effective in inhibiting the adhesion of *E. coli* type 1. It is reported that cranberry juice is also effective in the cure of urinary tract infection. It is given to the women and the sugar present in it is does not let *E. coli* to adhere to the urinary lining. Clinical trials of the avocado juice need to be carried out. There will be an added advantage, of consuming juice of avocado as it will further help in eliminating the dental plaque too.
The adhesion of bacteria to the epithelial cells and the kinetics of its adherence have been studied by the flow cytometry since last two decades. (Hasty 1992). We have utilized flow cytometry technique to confirm the result of in-vivo bacterial haemagglutination of S. aureus to human 'O' group and mannose resistant E.coli type 1 to guinea pig RBC. Both the bacteria were treated with water solutions and extracts of peel and pulp of Beta vulgaris (root) beet and fruit of Persea americana mill and Solanum tuberosum (potato). We investigated those combination of the extracts which in which the number of bacteria attached to RBC were decreased. Thus the titer of haemagglutination obtained from the 96 well plates and results obtained from flow cytometer supported each other.

Even though there have been numerous advances in assay techniques in recent years particularly in the realm of molecular analysis, but most of the good experimental techniques require the component to be well separated and clean. Most of the assay involves rinsing procedure that destroys low affinity associations. The flow cytometry approach preserves loose binding. This study found flow cytometry to be particularly well situated to detect bacterial binding, anomalies and heterogeneity within population this is significant improvement over the vast majority of adhesion assays, which yield averaged data. In same combination for adhesion a secondary shoulder to main right of main peak is discernable on the histogram plot and reveals a sub population of RBC with noticeably more bacteria associated with them as compared to majority of RBC. Detection of this sub population would only be possible with a non averaging technique such as flow cytometry. It should be noted that while there have been several studies of bacterial adhesion using flow
analysis of bacterial adhesion to substrate such as collagen offers the following advantages.

Flow cytometer detects events based on the size of the particles and unbound bacteria do not interfere with the analysis. This study found flow cytometer to be particularly well suited to detect bacterial binding anomalies and heterogeneity within population. This is a significant improvement over the vast majority of adhesion assays which yield averaged data up to 10,000 red blood cells can be easily analyzed and depicted on a histogram for each data point increase the statistical validity of the attachment assay and providing for deletions of non-homogenous attachment. Phenomenon suggested by flow cytometer results can be simultaneously monitored via fluorescence microscopy.

In the present study we have developed a rat model to study in-vivo the anti-adherence properties of plant enzymes asparaginase and polyphenol oxidase. The vagina of the virgin rats was infected with *S. agalactiae*, they were than treated with either polyphenoloxidase (PPO) or asparaginase. Rats in control group received saline. From day '0' to day 20 regularly the weight of the rats were taken and CFU of *S. agalactiae* in vaginal secretions was monitored. Number of CFU of *S. agalactiae* obtained from test group was compared with that of the control group.

Group B Streptococci (GBS) strains are present in the female vagina, pharynx and large intestine. GBS is easily transferred to the infant during delivery. In some new
born, these bacteria become the part of the normal flora without infection. But in
infants, with weakened defense it causes two form of clinical disease. The early onset
type that develops few days after birth is accompanied by sepsis, pneumonia and high
mortality (50%). The late onset disease comes on in two to six weeks with symptoms
of meningitis, fever, vomiting and seizure. Since most cases occur in hospital,
personnel must be aware of the risk of passively transmitting this pathogen especially
in the neonatal and surgical unit. There are some well-defined material risk factors
that favour the development of infection in premature birth, maternal chorioamnionitis,
prolonged membrane rupture, intrapartum fever and GBS bacteriuria (24,73).

Pneumonia is the primary presentation of early onset GBS disease. Its initial site of
infection is the neonatal lung. The ability to adhere to epithelial surface has been
demonstrated to be an important virulence factor for many bacterial pathogens. GBS
adhere to surface receptors present on epithelial cells (126,209,216). These receptors
include fibronectin and laminin. Teilli et al observed that pretreatment of neonatal
buccal and vaginal epithelium cells with lipoteichoic acid induced a marked
inhibition in the adherence of all strains of GBS tested (72,205). Pretreatment of
bacteria with substance known to bind to lipoteichoic acid such as monoclonal,
polyclonal anti polyglycerophosphate antibodies and albumin also resulted in
adherence inhibition. Grischke et al studied 2,373 mothers and their newborn for 2
years with respect to GBS colonization and contamination. Vaginal and oral smears
were taken from mothers at the beginning of parturition as well as amniotic and
stomach aspiration and smears from the ear of the newborn was taken. In case of GBS
colonized mother amniotic showed the highest contamination (43%) followed by
stomach content (26%) and ear smears (taken from each side separately) 28% and
30% respectively. The rate of contamination of newborn dropped significantly from 50% to 20% after intraoral antibiotic prophylaxis. The antibiotic treatment of choice for group B Streptococcal infection is penicillin G. Alternatives are erythromycin and cephalosporins. Some physicians advocate routine penicillin prophylaxis in colonized mothers and infants but other fear that practice can increase the rate of allergies and infection by resistant strains. A typical passive immunization with human immunoglobulin is currently being considered for treatment and prevention of disease in mothers and infants at high risk. Treatment of enterocolitis infection usually requires combined therapy with penicillin and amino glycoside (gentamicin) to take advantage of synergism of these drugs and to overcome antimicrobial resistance. It is proposed that vaccine might be an excellent tool against GBS infections in mothers, infants and even in non-pregnant adult but they are still under investigation and are not yet commercially available. The pathogenesis of the infection includes the invasion of the pathogen in the host tissue. Once the bacteria has enter the host in normal healthy individual the non-specific immune system is activated. Most of the bacteria are engulf by the phagocytes. In most of the cases from among the group of invading bacteria escapes the immune mechanism and gets themselves to attach to the host tissue. For some time they remain there and then they multiply.

As in this study S. agalactiae were directly introduced in the vagina where they normally reside. Twenty four hours after the infection of S. agalactiae are not detected in any of the groups, indicating that those organism that were free moving in the secretion has been destroyed by the immune system. In case of saline treated rat the CFU count was positive for 2-3 consecutive days after the elapsed of the 50 hours of infection. In case of rats treated with PPO and aspartianase same duration was
required by the bacteria to reappear in the vaginal secretion. The number of CFU in the vagina of the control rats were more than in PPO treated rats. The difference was observed on day 4 and 5. The average number of CFU obtained from the PPO treated was higher as compared to the control and asparaginase treated rat. This finding can lead to the conclusion that large number of attached bacteria detached from the vaginal lining. In case of saline model the organisms reappear on 12th day whereas with PPO treated rats they reappear after 16 to 18 days. In case of Asparaginase treated rats on day 1 CFU of S. agalactiae appears after 50 hours. In most of the rats after the treatment they reappear on 6th day or 12 day like the control. But their CFU count is very high indicating that asparaginase helps in adhesion of bacteria to the host surface.

Thus in-vivo, the anti-adhesion activity of PPO to control the growth of S. agalactiae on the vagina of the virgin rat will go far and help in the control of neonatal disease.

Polyphenol oxidase is the plant constituent also known as tyrosinase. It is an oxidoreductase present in many vegetables and most European not citrus fruits. PPO catalyzes the conversion of monophenol and O-dihydroxy phenols to quinones. These darkly pigmented products are responsible for enzymatic browning of food such as mushroom, potatoes and apples PPO has a major role in defense against herbivores, insects and micro organism as its expression is often increased upon wounding or microbial colonization. The polymeric phenolic compounds it produces are more toxic to pathogens than are the monomeric phenols from which they are made. Further more it is speculated that polymerization process acts 93 type of wound healing mechanism.
CONCLUSIONS
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REFERENCES


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