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Research Article

IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF FIVE INDIGENOUS PLANTS EXTRACT AGAINST FIVE BACTERIAL PATHOGENS OF HUMAN

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ABSTRACT

Methanol and ethanol leaf extracts of five indigenous plants of *Piper betle* L., Punica *granatum* L., *Psidium guajava* L., *Gloriosa superb, L.* and *Mangifera indica, L.* were investigated for their in vitro antimicrobial proprieties against five bacteria species of *Escherichia coli, Klebsiella pneumonia, Salmonell typhi murium, Staphylococcus aureus* and *Bacillus cereus*. The antimicrobial activity of the extracts was evaluated and based respectively on the inhibition zone using the agar well diffusion assay and minimal inhibition concentration (MIC). The antibacterial activity of two solvent of five leaves of indigenous plants crude extract against five MTCC bacteria species of *Escherichia coli, Klebsiella pneumonia, Salmonell typhi murium, Staphylococcus aureus* and *Bacillus cereus* by the well diffusion showed zone of inhibition ranges from 11.0 ± 0.5 to 19.5 ± 1.0 for methanol extract and 9.0 ± 1.5 to 19.0 ± 0.5 for ethanol extract. MIC values for methanol extract against five bacterial species ranges from 0.5 to 0.00977 µg/ml and for ethanol extract are 2.5 to 0. 0.07813 µg/ml respectively. The strong *in vitro* antibacterial activity of the separated compound against gram positive and gram negative suggests the wide pharmaceutical application.

Keywords: Antibacterial activity, Agar well diffusion, Minimal Inhibition Concentration (MIC), Indigenous plants, p-iodonitrotetrazolium violet (INT).

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 50 000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world[1,2,3]·[4,5]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients [6,7]. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [8,9,1][0]. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [11,12,13]·[14,15,16]. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants [17,18].

Piper betle L. leaves is widely used as a mouth freshener after meal. This plant is extensively grown in Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Its common name is betel in English, paan in India and Bangladesh[19]. Indian system of medicine and health has adopted the use of betel leaves in various ways. In Indian folkloric medicine, betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for its healing effects. Essential oil extracted from betel leaf may be used as an industrial raw material for manufacturing medicines, perfumes, mouth fresheners, tonics, food additives etc[20].

Punica granatum L., commonly known as pomegranate, is a fruit-bearing deciduous shrub or small tree, native to Asia and belongs to the family Lythraceae[21]. The leaves are shiny and about 7.6 cm long[22]. Different parts of the plant such as bark, leaves, immature fruits and fruit rind have medicinal significance[23]. *Punica granatum* L.has been extensively used as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies[24]. Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties[25,26,27]. Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious[28].

Psidium guajava Linn., belonging to the Family Myrtaceae, is originated in the tropical South America[29] and grows wild in Bangladesh, India, Thailand, Brazil, Florida, West Indies, California and also in several other countries[30]. *Guajava* leaf extract contains guajava polyphenol that has an anti-oxidation action[31,32] and flower and leaf of the plant have been reported to have antibiotic activity[33]. The leaves contain various constituents such as fixed oil 6%, volatile oil 0.365% 3.15% resin, 8.5% tannin, fat, cellulose, chlorophyll and mineral salts and a number of other fixed substances[34,35].

Gloriosa superba Linn. is an important medicinal plant belonging to the family Liliaceae. Which is one of the endangered species among the medicinal plants[36,37]. Being native form Indian especially Southern India it is known as glory lily and climbing lily-in English. In the world market glory lily considered as rich source of colchicines and gloriosine[38]. The flower has analgesic, anti-inflammatory potential, antimicrobial, larvicidal potential, antipoxviral potential, antithrombotic potential, antitumor potential, enzyme inhibition potential, and also used in treatment of snake bite, Skin disease, respiratory disorders[39,40,41].

Leaves of *Mangifera indica, L.* contain alkaloids, anthracenosides, coumarins, flavonones, sugars, tannins, steroids and saponins. These, together with chlorophyll, are responsible for the extract colours observed. The fruity or sweet smell of the extracts is due to the presence of esters and essential oils in the plant extracts[42,43]. Some of these compounds have been reported to possess antimicrobial activity[44].

In this study, methanol and ethanol extracts of five plants, which had been described in herbal books and folklore medicine of India, were screened for their antimicrobial activity. The species tested were: *Piper betle* L. (Betel leaf or Betelvine) - leaf *Punica granatum* L. (Pomegranate) – leaf , *Psidium guajava* L. (Guava) - leaf, *Gloriosa superb, L.* (glorylilly) – leaf and *Mangifera indica, L.* (mango), - leaf against five bacteria pathogens of human: three gram negative (*Escherichia coli, Klebsiella pneumoniae Salmonell typhu murium*) and two gram positive (*Staphylococcus aureus,* and *Bacillus cereus*).

MATERIALS AND METHODS

The leafs of selected indigenous plants were collected from Prakasam district of Andhra Pradesh, South India. And they were identified and authenticated by K.Babu Rao, Incharge Scientist, Sai Lara Biotechnology, Hyderabad, Andhra Pradesh, India. The leaves of selected indigenous plants were separated and washed with sterile distilled water and dried using laminar air flow, ground into fine powder using a blender and stored in air tight container till further analysis.

Plant Materials

Piper betle L. (Betel leaf) - leaf *Punica granatum* L. (Pomegranate) – leaf**,** *Psidium guajava* L. (Guava) - leaf, *Gloriosa superb, L.* (glorylilly) – leaf and *Mangifera indica, L.* (mango), - leaf.

Extraction procedure

Ten grams of each plant fine powder of indigenous plants weighed into a 250 ml conical flask and 100 ml of methanol and ethanol was added separately for each plant powder then on a rotary shaker at 190 – 220 rpm for 24 hours[45]. This was filtered with whatman No1. Filter paper, the residue discarded, and the filter were evaporated to dryness in a water bath temperature at 80^{o}C.

Preparation of stock solution for each extract of leaves selected indigenous plants powder

Stock solution was prepared by weighing 10 mg of each dried solvent extract dissolved in 1 ml of dimethyl sulphoxide (DMSO) giving a final concentration of 10,000 µg/ml. The stock solution was kept in screw capped bottles for subsequent use.

Test Microorganisms

The test organisms were supplied by Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh – 160036, India. Three gram-neagtive bacteria: (*Escherichia coli* (MTCC 443), *Klebsiella pneumonia* (MTCC3384)*, Salmonell typhu murium* (MTCC 3241) and two gram positive (*Staphylococcus aureus* (MTCC3160), and *Bacillus cereus* (MTCC1305).

Agar well diffusion Method

The antibacterial activity of Methanol and Ethanol extract of five Indigenous medicinal plants against eight standard organisms by using agar well diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS). Briefly, 3-5 morphologically identical colonies from each agar plate were lifted with a sterile wire loop and transferred into a tube containing 5 ml of tryptic soya broth (TSB). The turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of 0.5 McFarland standards, resulting in suspension containing approximately 1 to 2 X 10^{8} CFU/ML. Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plates approximately 60° each time to ensure even distribution of the inoculum. As a final step the rim of the agar plate was also swabbed. After allowing the inoculum to dry at room temperature, 6mm diameter wells were bored with sterile metal borer. Each extract was checked for antibacterial activity by introducing 10µl of 100 mg/ml concentration into duplicate wells by using sterile micro pipette. The plates were allowed to stand at room temperature for 1 h to allow diffusion of extract into the agar. Then, plates were incubated at 37°C for 18 h in upright position. The presence of zone of inhibition was regarded as the indicator of antimicrobial action and the antimicrobial activity of extract was expressed in terms of average diameter of zone inhibition measured in millimeters. Each test was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) By Micro broth Dilution Technique

Minimal inhibitory concentration (MIC) was determined for the extracts by broth dilution method as described by Ayafor *et al.,* (1994)[46]. The concentration at which there was no visually detectable bacterial growth was taken as the MIC. MIC is defined as the lowest concentration that is able to inhibit any visible microbial growth and below that concentration which, there is no further inhibition.

MIC Method

Quantitative antimicrobial activity assay was carried for both methanol and ethanol extracts of test compound by determining minimum inhibitory concentrations (MIC). Bacterial species were cultured from 12-hour broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. All tests were performed in Muller Hinton broth (MHB). A two-fold serial dilution of each test compounds (100µl) in sterile normal saline was prepared in 96 wells microtiter plate and 50µl overnight fresh bacterial culture and suspensions were adjusted to 0.5 McFarland standard turbidity unit were added to each well. The antibiotic ciprofloxacin and normal saline were included as standard references in each assay. The plates were incubated overnight at 37°C. As an indicator of bacterial growth, 50µl of p-iodonitrotetrazolium violet (INT) 0.2 mg/ml was added to each well and incubated at 37°C for 30min. MIC values are recorded at the lowest concentration of test compound (both methanol and ethanol extracts) that completely inhibited bacterial growth that showed clear color less well. The colorless tetrazolidium salt acts as an electron accepter and is reduced to red colored formazan product by biological active organisms indicating bacterial growth., where bacterial growth was inhibited, the solution in the well remained clear color less after incubation with INT 0.2 mg/ml. The tested extract in this study was screened in three replicates against each organism.

RESULTS AND DISCUSSION

The methanol and ethanol extract of five indigenous plants were tested against the pathogenic microbes viz., *E.coli* , a most common bacteria of which virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis; *Klebsiella pneumonia* which is the causative organism of pneumonia; *Salmonell typhu murium* causes symptoms resembling typhoid fever in humans; *Staphylococcus aureus*, a wound infecting pathogen which can cause septicemia, endocarditis and toxic shock syndrome; *B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting and diarrhea.

Out of five plants tested for antimicrobial activity, five plant species showed antibacterial activity by inhibiting five microorganisms. The results of the antimicrobial activity of plant extracts tested against microorganisms by agar well diffusion method are shown in Table -1 and 2. Among the plants screened, the both methanol and ethanol extract of *Piper betle, Gloriosa superb* and *Mangifera indica,* showed significant inhibition of all tested bacteria followed by *Gloriosa superb < Mangifera indica < Piper betle < Punica granatum < Psidium guajava* against five pathogens.

Table 1: Antibacterial activity of selected indigenous medicinal plants Ethanol extracts (10mg/ml of DMSO crude extract each part of plant, (100µg concentration in well) and ciprofloxacin antibiotic (10 µg /ml) against, gram negative and gram positive microbial species tested by using agar well diffusion Diameter of Zone of inhibition (mm)

Microorgnisms	PB	PG	PGU	GS	MI	DMSO	Cf.
E.Coli	13.1 ± 0.25	11.0 ± 1.25	10.5 ± 1.25	15.5 ± 0.25	13.0 ± 1.25	--	21.0 ± 0.5
KP	12.0 ± 0.5	12.0 ± 1.0	11.0 ± 1.5	14.0 ± 1.0	11.5 ± 1.25	--	22.0 ± 0.58
SM	16.0 ± 1.5	13.5 ± 1.2	11.5 ± 0.25	16.5 ± 1.25	13.5 ± 0.5	--	22.0 ± 0.55
SA	17.0 ± 1.0	15.0 ± 1.0	14.0 ± 0.5	17.5 ± 0.5	17.0 ± 0.5	--	23.0 ± 0.5
BC	19.5 ± 1.5	11.0 ± 1.5	15.0 ± 1.25	18.0 ± 1.25	19.0 ± 1.5	--	25.0 ± 1.52

Table 2: Antibacterial activity of selected indigenous medicinal plants Methanol extracts (10mg/ml of DMSO crude extract each part of plant, (100µg concentration in well) and ciprofloxacin antibiotic (10 µg /ml) against, gram negative and gram positive microbial species tested by using agar well diffusion Diameter of Zone of inhibition (mm)

Microorgnisms	PB	PG	PGU	GS	MI	DMSO	Cf.
E.Coli	12.2 ± 0.25	9.2 ± 0.5	9.5 ± 0.25	10.2 ± 1.0	12.0 ± 1.0	--	17.0 ± 0.5
KP	9.0 ± 1.5	10.0 ± 1.25	7.5 ± 1.25	11.0 ± 1.5	9.0 ± 1.5	--	18.0 ± 0.58
SM	14.2 ± 1.5	11.5 ± 0.55	7.0 ± 0.25	14.0 ± 1.0	13.0 ± 0.25	--	17.0 ± 0.55
SA	17.0 ± 1.55	10.2 ± 0.25	10.7 ± 0.45	16.0 ± 1.5	14.2 ± 1.5	--	21.0 ± 0.5
BC	19.0 ± 1.0	8.6 ± 0.5	12.2 ± 0.55	17.0 ± 1.5	15.0 ± 0.25	--	22.0 ± 1.52

Plants name

Plants name*:* **PB**: *Piper betle* L. (Betel leaf or Betelvine) - leaf **PG**: *Punica granatum* L. (Pomegranate) – leaf **, PGU**: *Psidium guajava* L. (Guava) - leaf, **GS**: *Gloriosa superb, L.* (glorylilly) – leaf and **MI**: *Mangifera indica, L.* (mango), - leaf

Microorganisms: E.Coli: *Escherichia coli,* KP: *Klebsiella pneumoniae* SM: *Salmonell typhu murium,* SA: *Staphylococcus aureus,* and BC: *Bacillus cereus* Cf. Ciprofloxacin.

Note: Values of the observed zone of inhibition (in mm diameter) including the diameter of well (6 mm) after 24 hours incubation against different bacterial species when subjected to different extracts in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values ± Standard Deviation. In each well, the sample size was 10µl. Inhibition observed in extracts due to solvent were assessed through negative controls. "– "No Inhibition Zone was observed. "**Cf." -** Ciprofloacin (10 µg ml-1) was used as standard antibiotic.

Values are the average triplicate; includes the agar well diameter (6mm)

The agar well diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. Both methanol and ethanol extract of plants (10mg/10µl in each well of agar plate) exhibited the prominent antibacterial activity all the five bacteria but was more susceptible against *Staphylococcus aureus* and *Bacillus cereus* (Fig. 1 and 2) than *Escherichia coli, Klebsiella pneumonia* and *Salmonell typhu murium* (Fig. 3 and 4) whereas ethanol extract of five plant showed moderate zone of inhibition against gram negative bacterial pathogens of *Klebsiella pneumonia* and *Salmonell typhu murium.*

Both methanol and ethanol extracts of five plants extracts that showed maximum antimibacterial activity was taken for MIC assay. The result of MIC assay is shown in Table-3and4. *Piper betle* and *Mangifera indica* exhibited the highest antibacterial efficacy against gram positive and gram negative organisms at maximum lower concentrations (Table-3 and 4; Fig. 5 and 6) followed by : *Bacillus cereus< Staphylococcus aureus< Escherichia coli< Salmonell typhu murium < Klebsiella pneumonia*. Least efficacy was shown against gram positive organism of *Bacillus cereus and Staphylococcus aureus* which were inhibited at 0.00245 to 0.078125 mg/ml concentration.

Table 3: Minimum inhibitory concentration (MIC) of indigenous plants Methanol extracts (10mg/ml of DMSO crude extract each part of plant, (1000 µg concentration) and ciprofloxacin antibiotic (5 µg /ml) against gram negative, gram positive microbial species tested by using ELISA micro titer plate

Microorgnisms	PB	PG	PGU	GC	MI	CF
E.Coli	0.3125	2.5	0.15625	0.625	0.3125	0.15625
KP	0.3125	1.25	2.5	2.5	0.0625	0.3125
SM	0.78125	2.5	2.5	1.25	0.0625	0.15625
SA	0.00245	1.25	1.25	0.00977	0.00977	0.07813
BC	0.078125	0.3125	5.0	2.5	0.3125	1.3125

Minimum inhibitory concentration (MIC) mg/ml

Plants name*:* **PB**: *Piper betle* L. (Betel leaf) - leaf **PG**: *Punica granatum* L. (Pomegranate) – leaf **, PGU**: *Psidium guajava* L. (Guava) - leaf, **GS**: *Gloriosa superb, L.* (glorylilly) – leaf and **MI**: *Mangifera indica, L.* (mango), - leaf

Microorganisms

E.Coli: *Escherichia coli,* KP: *Klebsiella pneumoniae* SM: *Salmonell typhu murium,* SA: *Staphylococcus aureus,* and BC: *Bacillus cereus* Cf.: Ciprofloxacin

Table 4: Minimum inhibitory concentration (MIC) of indigenous plants Ethanol extracts (10mg/ml of DMSO crude extract each part of plant, (1000 µg concentration) and ciprofloxacin antibiotic (5 µg /ml) against gram negative, gram positive microbial species tested by using ELISA micro titer plate

Microorgnisms	PB	PG	PGU	GC	MI	CF
E.Coli	0.625	1.25	1.25	0.0625	0.3125	0.15625
KP	0.3125	1.25	2.5	2.5	0.0625	0.3125
SM	0.3125	2.5	2.5	1.25	0.0625	0.15625
SA	1.25	2.5	0.625	1.25	1.25	0.07813
BC	0.15625	0.07813	0.07813	0.15625	0.3125	1.3125

Minimum inhibitory concentration (MIC) mg/ml

Plants name*:* **PB**: *Piper betle* L. (Betel leaf) - leaf **PG**: *Punica granatum* L. (Pomegranate) – leaf **, PGU**: *Psidium guajava* L. (Guava) - leaf, **GS**: *Gloriosa superb, L.* (glorylilly) – leaf and **MI**: *Mangifera indica, L.* (mango), - leaf

Microorganisms:

E.Coli: *Escherichia coli,* KP: *Klebsiella pneumoniae* SM: *Salmonell typhu murium,* SA: *Staphylococcus aureus,* and BC: *Bacillus cereus* Cf.: Ciprofloxacin

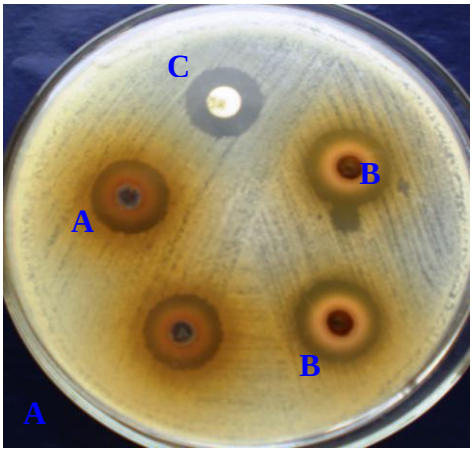

Fig. 1: Antibacterial activity of the Ethanol extracts of A. *Gloriosa superb, L.*– (leaf) *B. Mangifera indica, L.* (leaf) and C. Ciprofloxacin antibiotic against *Staphylococcus aureus*

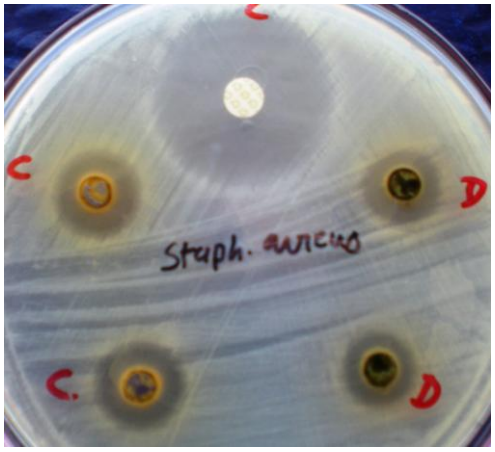

Fig. 2: Antibacterial activity of the Ethanol extracts of C. *Piper betle* L. – (leaf) *D. Punica granatum* L. – (leaf) and C. Ciprofloxacin antibiotic against *Staphylococcus aureus*

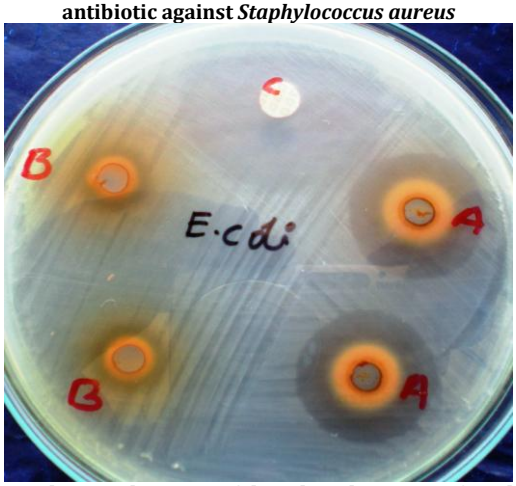

Fig. 3: Antibacterial activity of the Ethanol extracts of A. *Gloriosa superb, L.*– (leaf) *B. Psidium guajava* L. - (leaf) and C. Ciprofloxacin antibiotic against *Escherichia coli*

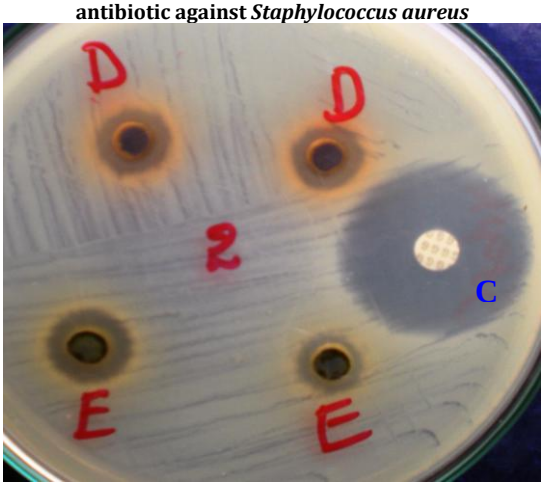

Fig. 4: Antibacterial activity of the Ethanol extracts of D. *Punica granatum* L. – (leaf) *E. Psidium guajava* L. (leaf)and C. Ciprofloxacin antibiotic against *Escherichia coli*

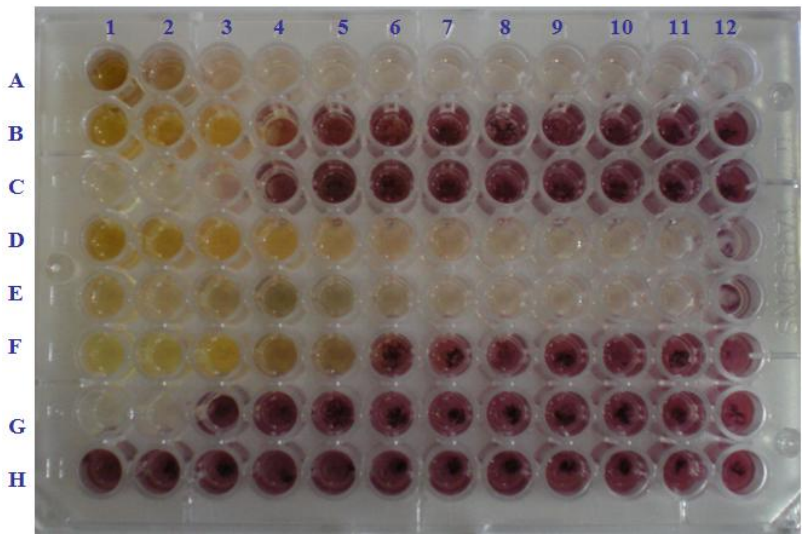

Fig. 5: Minimum Inhibitory Concentration of the Methanol extract of A. *Piper betle* L. (Betel leaf) *B. Punica granatum* L. (Pomegranate) – leaf *C Psidium guajava* L. (Guava), *D. Gloriosa superb, L.* (glorylilly) – leaf *)* and *E. Mangifera indica, L.* (mango), - leaf ciprofloxacin oxacin against *Staphyloccus aureus and E. Coli*

Rows A, B, C, D & E: Methanol extract of test compound in serial dilution + broth culture (*Staphylococcus aureus*) + saline + ^{INT} indicator

Rows F& G: Methanol extract of test compound in serial dilution (A. *Piper betle* L. (Betel leaf) *B. Punica granatum* L. (Pomegranate)) + broth culture (*E.Coli*) + saline + ^{INT} indicator

Row H: Ciprofloxacin in serial dilution + broth culture (*Staphylococcus aureus*) + saline + ^{INT} indicator)

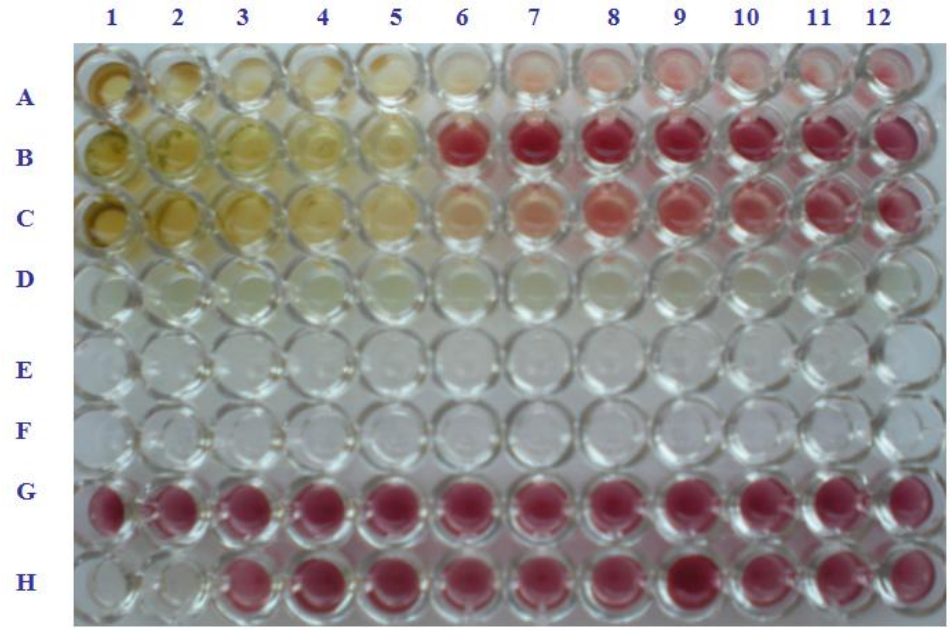

Fig. 6: Minimum Inhibitory Concentration of the Methanol extract of A. *Psidium guajava* L. (Guava),*B. Gloriosa superb, L.* (glorylilly) – leaf *)* and C*. Mangifera indica, L.* (mango), - leaf ciprofloxacin oxacin against *E. Coli*

Rows A, B and C: Methanol extract of test compound in serial dilution + broth culture (*E.Coli*) + saline + ^{INT} indicator

Row: F: only saline

Row G: Normal saline+ broth culture + ^{INT} Indicator

Row H: Ciprofloxacin in serial dilution + broth culture +saline + ^{INT} indicator)

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores[47]. The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical constituents of the selected plants investigated. Analysis of plant extracts revealed the presence of coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis[48]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls[49]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell[50]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes[51]. In this study, *Bacillus cereus and Staphylococcus aureus* were found to be sensitive to five plant extracts. The highest sensitivity of *Bacillus cereus* than *S.aureus* may be due to its cell wall structure and outer membrane[52]. Our results suggest that gram-positive bacteria are generally more sensitive to the herb extracts. This was consistent with the previous studies on other herbs.

CONCLUSION

Piper betle, Gloriosa superb and *Mangifera indica* exhibited the highest antimicrobial activity at a minimum concentration against both gram positive and gram negative pathogenic microorganisms. The results provide justification for the use of these plants in folk medicine to treat various infectious diseases.

REFERENCES

1. Piddock, K.J.V., Wise, R., Mechanisms of resistance to quinolones and clinical perspective. Journal of Antimicrobial Chemotherapy, 1989: 23, 475–483.
2. Singh, M., Chaudhry, M.A., Yadava, J.N.S., Sanyal, S.C., The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* collected from 1984–1986 in Northern India. Journal of Antimicrobial Chemotherapy 1992 29, 159–168.
3. Mulligen, M.E., Murry-Leisure, K.A., Ribner, B.S., Standiford, H.C., John, J.F., Karvick, J.A., Kauffman, C.A., Yu, V.L., Methicillin resistant *Staphylococcus aureus*. American Journal of Medicine, 1993 : 94, 313–328.
4. Davis, J., Inactivation of antibiotic and the dissemination of resistance genes. Science, 1994: 264, 375–382.
5. Robin, E.H., Anril, W., Alexander, M., Loeto, M., Keith, K., Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Heamophilus influenzae* Type b in children under 5 years of age in Botswana. International Journal of Infectious Diseases Diseases, 1998: 3 (1), 18–25.
6. Rinaldi, M.G., Problems in the diagnosis of invasive fungal diseases. Review of Infectious Diseases, 1991: 13, 493–495.
7. Diamond, R.D., The growing problem of mycoses in patients infested with human immunodeficiency virus. Review of Infectious Diseases, 1993: 13, 480–486.
8. Iyengar, M.A., Study of Crude Drugs, 2nd edn. College of Pharmaceutical Sciences, Manipal, 1985: pp. 13–78.
9. Chopra, R.N., Nayer, S.L., Chopra, I.C., Glossary of Indian Medicinal Plants, 3rd edn. Council of Scientific and Industrial Research, New Delhi, 1992: pp. 7–246.
10. Harborne, S.B., Baxter, H., Phytochemical Dictionary. A Handbook of Bioactive Compounds from Plants. Taylor and Francis, London.

11. Grosvenor, P.W., Supriono, A., Gray, D.O., 1995. Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2, Antibacterial and antifungal activity. Journal of Ethnopharmacology, 1995: 45, 97–111.
12. Ratnakar, P., Murthy, P.S.,. Purification and mechanisms of action of antitubercular principle from garlic (*Allium sati6um*) active against isoniazid susceptible and resistant *Mycobacterium tuberculae* H37RV. Indian Journal of Clinical Biochemistry, 1995: 10, 14–18.
13. Silva, O., Duarte, A., Cabrita, J., Gomes, E., Antimicrobial activity of Guinea — Bissau traditional remedies. Journal of Ethnopharmacology, 1996: 50, 55–59.
14. David, M., Antimicrobial activity of garlic. Antimicrobial Agents and Chemotherapy, 1997: 41, 2286.
15. Saxena, K., Antimicrobial Screening of Selected Medicinal Plants from India. Journal of Ethnopharmacology, 1997: 58 (2), 75–83.
16. Saxena, V.K., Sharma, R.N., Antimicrobial activity of essential oil of *Lantana aculeata*. Fitoterapia, 1999: 70 (1), 59–60.
17. Hasegawa, H., Matsumya, S., Yamasak, K., Reversal of efflux mediated tetracycline resistance in *Staphylococcus aureus* clinical isolates by *Ginseng prosaponenins*. Phytotherapy Research, 1995: 9 (4), 260–263.
18. Lee, C.K., Kin, H., Moon, K.H., Shun, K.H., Screening and isolation of antibiotic resistance inhibitors from herb materials — resistance inhibition of volatile components of Korean aromatic herbs. Archives of Pharmaceutical Research, 1998: 21 (1), 62–66.
19. Datta A, Ghoshdastidar A, and Singh M. Antimicrobial Property of *Piper betel* Leaf against Clinical Isolates of Bacteria. *Int J Pharma Sci and Res.2:* 2011: 104-109.
20. Guha P. Betel Leaf: The Neglected Green Gold of India. *J Hum Ecol*. 2006: 19: 87- 93.
21. Altuner E.M., Investigation of antimicrobial activity of *Punica granatum* L. fruit peel ash used for protection against skin infections as folk remedies especially after male Circumcision, *Afr. J. Microbiol Res*., 2011: 5(20), 3339-3342.
22. Qnais E.Y., Elokda A.S., Abu Ghalyun Y.Y. and Abdulla F.A., Antidiarrheal activity of the aqueous extract of *Punica granatum* (Pomegranate) peels, *Pharm. Biol*., 2007: 45(9), 715–720.
23. Arun N. and Singh D.P., *Punica granatum*: a review on pharmacological and therapeutic properties, *IJPSR*, 2012: 3(5), 1240-1245.
24. Choi J.G., Kang O.H., Lee Y.S., Chae H.S., Oh Y.C., Brice O.O., Kim M.S., Sohn D.H., Kim H.S., Park H.,Shin D.W., Rho J.R. and Kwon D.Y., In vitro and in vivo antibacterial activity of *Punica granatum* peel ethanol extract against *Salmonella*, *Evid. Based Complement,Alternat. Med.,* 2011: 1-8
25. Dahham S.S., Ali M.N., Tabassum H. and Khan M., Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.), *American-Eurasian J. Agric. and Environ. Sci.,* 2010: 9(3), 273-281.
26. Inabo H.I. and Fathuddin M.M., In vivo antitrypanosomal potentials of ethyl acetate leaf extracts of *Punica granatum* against *Trypanosoma brucei brucei,*Adv. Agr. Bio., 2011: 1, 82-88.
27. Moussa A.M., Emam A.M., Diab Y.M., Mahmoud M.E.and Mahmoud A.S., Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats, *IFRJ*, 2011: 18, 535-542
28. Prakash C.V.S. and Prakash I., Bioactive chemical constituents from pomegranate (*Punica granatum*) juice, seed and peel-a review, *Int. J. Res. Chem. Environ.,* 2011: 1(1), 1-18
29. RK Pathak, CM Ojha: Genetic resources of guava, Vol. I, Fruit Crops, Part 1, In; Advance in Horticulture [C].Chad ha KL, Pareek OP, editorss, Malhotra Publishing House, New Delhi, 1993: 143–147.
30. LH Bailey: The standard encyclopedia of horticulture [C]. Vol. II. Macmillan Co, New York. 1960: 1415.
31. T Okuda, T Yoshida, T Hatano, K Yazaki, Y Ikegami, T Shingu,. *Chem. Pharm. Bull.,* 1987: 35, 443-446.
32. A Jimenez-Escrig, M Rincon, R Pulido, F Saura-Calixto*, J Agric Food Chem*, 2001: 49, 5489-5493.
33. JM Watt, MG Breyer-Brandwijk,. Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed., Edinburgh and London: E and S Livingstone Ltd, 1962: pp 1457-1458.
34. HM Burkill, The useful plants of West Tropical Africa. Families M-R. Royal Botanic Gardens Kew, 1997: Edition 2. Vol. 4.
35. KM Nadkarni, AK Nadkarni, Indian Materia Medica - with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies. Popular Prakashan Private Ltd., Bombay, India, 1999: Vol.1.
36. Badola HK. Endangered medicinal plant species in Himachal Pradesh. A report on the International Workshop on "*Endangered Medicinal Plant Species in Himachal Pradesh'*, organized by G.B. Pant Institute of Himalayan Environment and Development at Himachal Unit, Mohal-Kullu during 18-19 March 2002. *Curr. Sci* 2002: 83: 797-798.
37. Sivakumar, G. and Krishnamurthy, K.V. *Gloriosa superba* L. - a very useful medicinal plant. In: *Recent Progress In Medicinal Plants*, USA, 2002: 465-82.
38. Trease, S.E. and Evans, D. Colchicum seed and corn. In: Pharmacognosy, 12th edn. Balliere Tindall, London, 1983: 593-59.
39. Alagesaboopathi, C, Antimicrobial screening of selected medicinal plants in Tamilnadu, India. *Journal of Microbiology*, 2011: 5(6), 617-621.
40. Rehana banu, and Nagarajan N. Antibacterial Potential Of Glory Lily , *Gloriosa Superba* Linn, *International Research Journal Of Pharmacy*, 2011: 2(3), 139-142.
41. Abhishek Mathur, Satish K Verma, Santosh K Singh, Deepika Mathur, Prasad GBKS, and Dua VK, Investigation Of Anti-Inflammatory Properties of *Swertia Chirayta* And *Gloriosa Superba*, *Recent Research in Science and Technology*, 2011: 3(3), 40-43.
42. Marjorie, M.C. Plant products as antimicrobial agents. Clinical Microbiology. Reviews, American Society for Microbiology. Department of Microbiology, Miami University, Oxford, OH, USA, 1999: 12, 564–582.
43. Hirte, M. Benefit of Mango for the Human Health. http:// www.preda.org/archives/2002/r02111802.html (last accessed 2nd January, 2007).
44. Dweck, A.C. (2001) Article for cosmetics & toiletries magazine ethnobotanical plants from Africa. Black Medicare Ltd, Iltshire, UK. http://www.dweckdata.co.uk/ 2002: (last accessed 2nd January, 2007).
45. Harbone JB. Phytochemical Methods. London: Chapman and Hill; 1973:
46. Ayafor J.F., Tchuendem M.H.K. and Nyasse B., Novel bioactive diterpenoids from framomum aulacocarpos. J. Nat. Prod. 1994: 57:917-923.
47. Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biol Sci 2004: 4: 405-412.
48. Shimada T. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol. 2006: 32 (6): 1149-1163.
49. Marjorie C.Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999: 12: 564-582.
50. Zablotowicz RM, Hoagland RE, Wagner SC. Effect of saponins on the growth and activity of rhizosphere bacteria. Adv Exp Med Biol.1996: 405:83-95.
51. Raquel F. Epand, Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. Biochimica et Biophysica Acta. 2007: 2500–2509.
52. Zaika LL. Spices and herbs - their antimicrobial activity and its determination. J Food Safety 1988: 9: 97-118.