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EVALUATION OF ANTI-BACTERIAL ACTIVITY OF PLANT *SESBANIA SESBAN*

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ABSTRACT

The genus Sesbania sesban contains about 50 spieces, the majority of which are annuals. The greatest spieces diversity occurs in Africa with 33 spieces. The spieces *Sesbania sesban* belongs to sub-family Papilionoidea of the family Leguminosae. It is a small perennial tree with woody stems, yellow flowers and linear pods . *Sesbania sesban* is very common throughout Africa and in asian countries like India, Malaysia, Indonesia and Phillipines. Campesterol, β-sitosterol, Cyanidine, Delphinidin glycosides, α-Keto glutaric, Oxaloacetic and pyruvic acids, Oleanolic acid, saponins, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid and Linolenic acid are reported in Whole plant. Cyanidin and Delphinidin glycosides, Flavonols are reported in flowers. The plant has been reported to possess various activities such as anti-inflammatory activity, antinociceptive activity, antidiabetic activity, antifertility activity and antioxidant activity. The present study was designed to study the anti-bacterial activity of *Sesbania sesban*. The extracts of *Sesbania sesban* stem were prepared. Soxhlet extraction was carried out by petroleum ether (60-80°C), chloroform, hexane, methanol, ethanol and then distilled water. Plant is subjected to antimicrobial study. Chloroform and methanol extracts of stem of *Sesbania sesban* at a concentration of 25 mg/ml were equally inhibit *P. aeruginosa* after 48 hrs. Both chloroform and methanol extracts at a concentration of 100 mg/ml were more effective against *B. subtilis* than other two bacterial strains after 48 hrs. No antibacterial activity was observed for pet ether ethanol and water extracts in a range from 25 mg/ml to 100 mg/ml.

Keywords: Alkaloids, Flavnoids, Antimicrobial and Anti-bacterial activity

INTRODUCTION

The herbal medicines are used for treatment of various diseases since several years. A plant contains a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway. [1] Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. [2] The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. [3] But still the potential of many herbal plants is largely unexplored. From the large flora only a small percentage has been investigated phytochemically and fractions for pharmacological screening are even smaller. In current trend the extraction of bioactive compounds done mainly for pharmaceuticals, agrochemicals, flavor, fragrance ingredients, pesticides and food additives. Secondary metabolites are important chemical compounds of plant because they cause adaptation of plant to the environment and also due to their potent therapeutic appraise they represents important source for pharmaceuticals. [4] Now a days in pharmaceuticals herbal medicines gained more interest. These herbal medicines comprises of active ingredients from different parts of plants such as roots, stems, rhizomes, leaves, seeds and fruits. The active constituents so obtained are crude in nature. [5] Medicinal properties of plants are due many active compounds like alkaloids, glycosides, saponins, terpenoids, lactones, phenols and flavnoids. The

practice of Ayurveds therapeutics consisted of 8 sections divided into 180 chapters and listed 314 plants, which are used as medicines in India. About 15000 medicinal plants have been reported to have medicinal value in India but only 7000-7500 medicinal plants were used by traditional communities of India. [6] Ayurvedic medicines are now a days not only used by Indian peoples but also in developed countries like USA, Canada, Japan, China etc. [7] There are many plants which have been reported to have antimicrobial activity. The different parts used include stem, root, leaves, flowers, twigs exudates and modified plant organs. Although many plant species have been tested for antimicrobial activity, the vast majority of have not been adequately evaluated. [8] There are main three types of microbial infections i.e. bacterial infections, fungal infections and viral infections.

1. **Bacterial Infections**: The vast majority of bacteria are harmless or beneficial quite a few bacteria are pathogenic. The different types of bacterial pathogens and the diseases caused by them are enlisted in Table.1.
2. **Fungal Infections**: A fungus is a member of large group of eukaryotic organisms that includes yeast, molds and mushrooms. Many fungi are parasites on plants, animals, humans and other fungi. Some fungi may cause serious diseases in humans like aspergilloses, candidoses, coccodioidomycosis, cryptococcosis, hystoplamosis and mycetomas. Some fungi can attack eyes, nails, hairs and skin which are called dermatophytic and keratinophyllic fungi. They cause local infections such as ringworm and athlete's foot.

Fungal infections can be classified according to the site of infection as:-

1. Superficial mycosis
2. Subcutaneous mycoses
3. Systemic mycoses [9]

3. **Viral Infections**: A Virus is a small infectious agent that replicates only inside the living cells of other organism. They infect all types of cellular life including plants, animals and humans. Most virus infections eventually result in the death of the host cell. The causes of death include cell lysis, alterations to the cell's surface membrane and apoptosis.

Plants as antimicrobial agents

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. They may be used as the base for the development of a medicine and also a phytomedicine for the treatment of diseases. There are two major benefits of using plant derived medicines:

Therapeutic Benefit- The discovery of penicillin led to later discoveries of antibiotics such as streptomycin and chloromycetin etc. Though the most of clinically used antibiotics are produced by soil micro-organisms and higher plants e.g Lichens have bacteriostatic and fungicidal properties, antibiotic action of allinine in garlic etc . Economic Benefit- The interest in natural products is a result of many factors like consumer's belief that natural products are superior, consumer's dissatisfaction with conventional medicines and national concern for health care cost. The immediate source of financial benefit from plants based antimicrobials is from the herbal products. Some of the plants having antimicrobial activity are enlisted in Table 2.

Sesbania sesban

Sesbania sesban is very common throughout Africa and in asian countries like India, Malaysia, Indonesia and Phillipines. In India these crops have had a long history of agricultural use and as a source of forage. One of the major advantages of perennial *Sesbania sesban* spieces over other forage trees and shrubs is their rapid growth rates. *Sesbania sesban* grows well in the sub-tropics and is most suitable for altitudes between 200-500m. *Sesbania sesban* is outstanding in its ability to tolerate water-loging and is ideally suited to seasonally water-logged environments. The flowers of *Sesbania sesban* have been reported to have Cyanidin and Delphinidin glycosides, Flavonols. [10] The seeds of Sesbania sesban have been reported to have Saponins, Palmitic acid, Stearic acid, Lignoceric acid, Oleic acid, Linoleic acid and Linolenic acid. [11] The leaves of Sesbania sesban have been reported to have Kaempferol. [12]

MATERIALS AND METHODS

Plant Material: Dried stem portion of Sesbania sesban was collected from S V University, Tirupati, Andhra Pradesh.and botanical Authentication of Sesbania sesban has been obtained from Dr. K. Madhava Chetty, S V University, Tirupati, Andhra Pradesh.

Standard Drugs: Ciprofloxacin was used as standard drug for antibacterial activity. The drug obtained was purchased from Bansal Medical Hall, Gurusar Sudhar, Ludhiana, India. Ciprofloxacin is active against wide variety of micro-organisms such

as S. typhi, B. Lintus, K. pneumonia, S. griseus, B. subtilis, S. aureus, S. albus, E. coli, P. aeruginosa etc. [13]

Solvents Used: Petroleum ether (60-80°C), Chloroform and Methanol were employed for extraction of plant material using soxhlet apparatus and finally the drug was boiled with distilled water. Dimethyl sulphoxide ,was used as solvent for dissolving different extracts. It is colourless liquid with boiling point 189°C. It is miscible with water, chloroform, acetone, alcohol and petroleum ether.

Chemicals Used: Sodium hydroxide, Chloral hydrate, Copper sulphate, Ferric chloride, Sulphuric acid, Iodine, Lead acetate, Magnesium, Potassium iodide, Potassium mercuric iodide, Picric acid, Mercuric chloride, Nitric acid, Gelatin, Sodium chloride, α-napthol,, sodium nitropruside, pyridine, were used for phytochemical screening of the plant extracts.

Prepration of extracts: The stems of plant was dried under shade and coarsely powdered. Five hundred gram powder material was subjected to successive. The solvents used were petroleum ether (60-80°C), chloroform, hexane, methanol, ethanol and then distilled water. Soxhlet extraction was carried out by these solvents in an increasing order of their polarity for not less than 48 hours. After each extraction the powdered material was dried in air at room temperature. Finally, marc was digested with distilled water for 24 hours or more to obtain aqueous extract. Each extract was concentrated in vaccum using Rotatory evaporator.Extracts were weighed subsequently and the percentage yields were calculated of each extract obtained individually in terms of the air dried weigh of plant material.

Antibacterial Activity studies: Examination of antibacterial activity is studied from crude extracts of dried stem of Sesbania sesban against three selected bacterial strains. The antibacterial activity of crude extracts of Sesbania sesban has been evaluated on the basis of agar diffusion cup plate method. [14]

Microorganisms (bacterial strains): Clinically important microbial strains were obtained from MTCC and Gene Bank Institute of Microbial Technology, Sector-39A, Chandigarh. The list of microbial strains obtained are mentioned in Table 3.

Maintainence of culture

Nutrient agar slants were prepared for maintainence of culture which are further incubated in an incubator. All cultures were stored at 37°C so that sufficient growth of bacteria was maintained. The stock cultures were subcultured at regular intervals.

Sterilization: All the equipments and materials that were used during the experiment such as Glassware, forceps, scissors, inoculating loop, petriplates, borer, measuring scale and media were autoclaved (Calton, vertical autoclave, Narang Scientific works, India) at 15 lb pressure for 20 minutes.

Preparation of inoculums: Cell suspension of isolated bacterial strain, loopfull is taken and dipped into a sterilized nutrient broth. Stir loop for 2-5 sec. Nutrient broth is further incubated in incubator shaker (New Brulsiick Scientific, Edison NJ, USA) for 6 hrs at 30°C at 300 rpm. This cell suspension was used as inoculum.

Preparation of extracts samples from dried residues: A suitable chemical which is able to dissolve plant extracts without its own activity is chosen. Good solvent for experimental purposes is DMSO (Merck, 2001), so it was used for dissolving various extracts obtained as a result of soxhlet extraction for antibacterial testing. Variable concentrations of extracts were prepared by dissolving dried residues in DMSO for testing inhibitory efficacy against selected bacterial strains. Stock solutions of extracts were diluted in DMSO to produce concentrations ranging from 100 mg/ml to 25 mg/ml.

Agar diffusion cup plate (**antibacterial activity screening) method:** Sensitivity of various extracts, (as prepared above) to a particular bacterial strain was measured in terms of zone of inhibition by agar diffusion method. From the inoculum, prepared as above 0.1 ml of E. coli suspension was spread uniformly on LB medium in petriplates. Similarly 0.1ml each of P. aeruginosa and B. subtilis were taken and spread uniformly on LB medium in petriplates. These inoculated Petri plates were incubated at 37°C-38°C for two days. Wells of 8 mm were cut out in agar plates using a sterilized steel cork borer and filled with aliquot volume of extracts samples using sterilised micropipette. [15]

Plates were then incubated in BOD incubator at 37°C up to 48 hours for two days. Diameter of any resultant zone of inhibition including well size was measured. For each combination of extract preparation and organism, the plates were kept in triplicate (n=3). DMSO was used as control and Ciprofloxacin was used as standard drug.

RESULTS AND DISCUSSION

The antimicrobial activity of pet.ether, chloroform, methanol and aqueous extracts of Sesbania sesban stem portion was studied against Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis by zone of inhibition and Minimum Inhibitory concentration method. The Zone of Inhibition study results are depicted in Table 4-7. The pictures of Zone of inhibition against Bacillus subtilis are (Figure 1A-1E), Escherichia coli (Figure 2A-2E) and Pseudomonas aeruginosa (Figure 3A-3D) respectively. The result indicates that after 48 hrs methanol extract (100mg/ml) showed zone of inhibition 16.2mm against Bacillus subtilis, 15.2 against Escherichia coli and 14.8 against Pseudomonas aeruginosa. The zone of inhibition showed by choloroform extract (100mg/ml) after 48 hrs is 12.3mm against Bacillus subtilis, 12.1mm against Escherichia coli and 12.6mm against Pseudomonas aeruginosa. No antibacterial activity was showed by pet ether and aqueous extracts. The graphical representation of Zone of inhibition Vs concentration of standard drug and extracts after different time intervals (12hrs, 24hrs, 36hrs and 48hrs) are shown in Figure (4-7) respectively.

Minimum Inhibitory Concentration:

A - Serial dilutions of five percent methanol extract from 500 µg/ml to 1200 µg/ml in nutrient broth containing B. subtilis from left to right.

B - Serial dilutions of five percent methanol extract from 1300 µg/ml to 1500 µg/ml in nutrient broth containing B. subtilis from left to right.

The MIC for 5% methanol extract is depicted in Table.8

DISCUSSION

Antimicrobial chemotherapy brought dramatic changes in microbial infections dealing with mankind. [16] Various synthetic antimicrobial drugs have prominent therapeutic effects in evading microbial pathogens. Methicillin and Vancomycin are used to evade gram positive S. aureus. Carbapenem and quinolone drugs help in treating diarrhoea due to E. coli. Sulfonamide drugs such as sulfadiazine and sulfamexthoxazole were used to cure pathogenicity caused by P. carinii. [17] Clotrimazole, Nystatin and Itraconazole antifungal drugs are clinically used to treat cutaneous mycoses . [18] Antimicrobial chemotherapy needs more attention or advancement due to re-emerging of microbial infections. S. aureus became resistant to Methicillin by producing an enzyme which inhibits binding of drug to target site. E. coli became resistant to quinolone by mutation in gyr A and par C target sites. [16] So there is need arrived for antimicrobial drugs to be devoid of having adverse effects. Various natural products are potent antimicrobial agents without side-effects. Methanol extract of Abutilon indicum (Malvaceae) inhibit Salmonella paratyphi, Vibrio mimicus, Shigella dysenteriae and boydii. Both flowers of Alangium savifolium (Alangiaceae) and rhizomes of Bergenia ligulata (Saxifragaceae) were showing antibacterial property. Annona squamosa is active against Aspergillus fumigatus and Candida neoformans due to presence of 11-hydroxy-16-hentriacontanone in plant. [19]Authentication of plant material is prerequisite before using it as research material. Therefore it was authenticated from Dr. K. Madhava Chetty, S V University, Tirupati, Andhra Pradesh. Plant is subjected to antimicrobial study. Chloroform and methanol extracts of stem of Sesbania sesban at a concentration of 25 mg/ml were equally inhibit P. aeruginosa after 48 hrs. Both chloroform and methanol extracts at a concentration of 100 mg/ml were more effective against B. subtilis than other two bacterial strains after 48 hrs. It was observed that with increasing concentration from 25 mg/ml to 100 mg/ml of methanol and chloroform extracts increases the zone of inhibition respectively. No antibacterial activity was observed for pet ether ethanol and water extracts in a range from 25 mg/ml to 100 mg/ml.

CONCLUSION

The present study was designed to study the anti-bacterial activity of Sesbania sesban. The antimicrobial activity of pet ether, chloroform, methanol, ethanol and aqueous extracts was evaluated by cup-plate method. All the extracts were evaluated in concentration range from 25 mg/ml to 100 mg/ml. The chloroform and methanol extracts showed antibacterial activity while no antibacterial activity was showed by pet ether and aqueous extracts. The methanol extract of stem of Sesbania sesban has more potent antibacterial activity than chloroform extract. It was observed that, as the concentration of methanol extract was increasing from 25 mg/ml to 100 mg/ml the antibacterial activity was also increased respectively. The order of bacterial strains inhibition of methanol extract after 48 hrs was as B. subtilis>E. coli> P. aeruginosa. So, it is concluded that Sesbania sesban possesses good antibacterial activity. The antibacterial activity is may be due to the presence of flavonoids and phenolic compounds.

ACKNOWLEDGEMENTS

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Table 1: List of Bacterial Pathogens and Human bacterial infections

S.no	Name of Bacterial Pathogen	Transmission	Diseases Caused
1	Bacillus anthracis	Contact with goat, sheep and horse	Cutenous & Pulmonary Anthrax
2	Bordetella Pertussis	Contact with respiratory droplets	Whooping Cough
3	Borrelia burgdorferi	Ticks, deer and mice	Lyme disease
4	Brucella abortus	Direct contact with infected animal	Brucellosis
5	Chlamydia pneumonia	Respiratory droplets	Pneumonia
6	Chlamydia trachomatis	Sexual and pessage through birth canal	Nongonococcal Urethritis.
7	Clostridum botulinum	Spores from soil and aquatic sediments	Botulism
8	Clostridum perfringens	Spores in soil and Human flora	Gas gangrene & food poisoning
9	Clostridum tetani	Spores in soil infecting puncure wounds	Tetanus
10	Corynebacterium diptheriae	Respiratory droplets	Diptheria
11	Escherichia coli	Parts of gut flora	Urinary tract infections & diarrhea.
12	Haemophilus influenza	Droplet contact	Bacretial meningitis, Pneumonia & bronchitis.
13	Helicobacter pylori	Colonising stomach	Peptic Ulcer, Risk factor for gastric carcinoma
14	Leptospira interrogans	Contaminated food and water	Leptospirosis
15	Lysteria monocytogenes	Dairy products	Listeriosis
16	Mycobacterium leprae	Prolonged human-human contact through exudates from skin lesions	Leprosy
17	Mycobacterium tuberculosis	Droplet contact	Tuberculosis
18	Salmonella Typhi	Human – human through food and water	Typhoid fever
19	Vibrio Cholerae	Contaminated food and water	Cholera
20	Yersinia pestis	Fleas from animals Ingestion of animal tissues	Plague

Table 2: Plants having antimicrobial activity

S.No	Common Name	Scientific Name	Compound	Activity Against
1	Aloe	*Aloe barbadensis*	Latex	*Cornybacterium, Salmonella.*
2	Ashwagandha	*Withania somniferum*	Withafarin A	Bacteria and fungi
3	Bael Tree	*Aegle marmelos*	Essential oil	Fungi
4	Black pepper	*Piper nigrum*	Piperine	Fungi, *Lactobaccilus Micrococcus*
5	Cashew	*Anacardium pulsatilla*	Salicylic acids	Bacteria and fungi
6	Chamomile	*Maticaria chamomilla*	Anthemic acid	*M.tuberculosis and S.aureus*
7	Clove	*Syzygium aromaticum*	Eugenol	General
8	Dill	*Anethum graveolens*	Essential oil	Bacteria
9	Eucalyptus	*Eucalyptus globules*	Tannin	Bacteria and virus
10	Garlic	*Allium sativum*	Allicin and ajoene	General
11	Ginseng	*Panax notoginseng*	Saponins	*E.coli, Staphylococcus*
12	Henna	*Lawsonia inermis*	Gallic acid	*S.aureus*
13	Licorice	*Glycyrrhiza glabra*	Glabrol	*S.aureus, M.tuberculosis*
14	Onioin	*Allium cepa*	Allicin	Bacteria and *Candida*
15	Quinine	*Cinchona* Spp	Quinine	*Plasmodium*
16	Senna	*Cassia angustifolia*	Rhein	*S.aureus*
17	Thyme	*Thymus vulgaris*	Caffeic acid, Thymol	Bacteria and Fungi Virus

Table 3: List of microbial strains

Escherichia coli	MTCC 1302
Pseudomonas aeruginosa	MTCC 2295
Bacillus subtilis	MTCC 1133

Table 4: Showing Activity after 12 hrs

	Activity after 12 hrs		
Extracts	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Control (DMSO 5%)	0	0	0
Standard (50 µg/ml)	38	26	24.7
Standard (250 µg/ml)	40.1	29.2	26.7
Standard (500 µg/ml)	44	32.7	30.8
Methanol (25 mg/ml)	10	8.7	9.3
Methanol (50 mg/ml)	14	11.5	10.5
Methanol (75 mg/ml)	14.2	12.8	13.2
Methanol (100 mg/ml)	14.7	13.3	13.7
Chloroform (25 mg/ml)	8	7.7	8.2
Chloroform (50 mg/ml)	9.3	8.5	8.4
Chloroform (75 mg/ml)	10.1	9.4	10.3
Chloroform (100 mg/ml)	11	10.4	11.5
Water (25 mg/ml)	0	0	0
Water (50 mg/ml)	0	0	0
Water (75 mg/ml)	0	0	0
Water (100 mg/ml)	0	0	0
Pet Ether (50 mg/ml)	0	0	0
Pet Ether (75 mg/ml)	0	0	0
Pet Ether (100 mg/ml)	0	0	0

Table 5: Showing Activity after 24 hrs

	Activity after 24 hrs		
Extracts	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Control (DMSO 5%)	0	0	0
Standard (50 µg/ml)	38.4	26.3	25.1
Standard (250 µg/ml)	40.5	29.5	26.9
Standard (500 µg/ml)	44.2	32.9	31.2
Methanol (25 mg/ml)	11.1	9.1	9.7
Methanol (50 mg/ml)	14.3	11.9	10.9
Methanol (75 mg/ml)	14.7	13.1	13.8
Methanol (100 mg/ml)	15.2	13.8	14.1
Chloroform (25 mg/ml)	8.2	7.9	8.8
Chloroform (50 mg/ml)	9.8	8.9	8.6
Chloroform (75 mg/ml)	10.6	9.8	10.7
Chloroform (100 mg/ml)	11.4	11	11.7
Water (25 mg/ml)	0	0	0
Water (50 mg/ml)	0	0	0
Water (75 mg/ml)	0	0	0
Water (100 mg/ml)	0	0	0
Pet Ether (50 mg/ml)	0	0	0
Pet Ether (75 mg/ml)	0	0	0
Pet Ether (100 mg/ml)	0	0	0

Table 6: Showing Activity after 36 hrs

	Activity after 36hrs		
Extracts	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Control (DMSO 5%)	0	0	0
Standard (50 µg/ml)	38.9	26.8	25.5
Standard (250 µg/ml)	41.5	30.4	27.6
Standard (500 µg/ml)	44.6	33.4	31.7
Methanol (25 mg/ml)	11.6	9.8	10.3
Methanol (50 mg/ml)	14.8	11.9	10.9
Methanol (75 mg/ml)	15.3	13.7	14.3
Methanol (100 mg/ml)	15.7	14.3	14.6
Chloroform (25 mg/ml)	8.7	8.5	9.5
Chloroform (50 mg/ml)	10.4	9.4	9.8
Chloroform (75 mg/ml)	11.2	10.4	11.4
Chloroform (100 mg/ml)	11.8	11.7	12.2
Water (25 mg/ml)	0	0	0
Water (50 mg/ml)	0	0	0
Water (75 mg/ml)	0	0	0
Water (100 mg/ml)	0	0	0
Pet Ether (50 mg/ml)	0	0	0
Pet Ether (75 mg/ml)	0	0	0
Pet Ether (100 mg/ml)	0	0	0

Table 7: Activity after 48 hrs

	Activity after 48hrs		
Extracts	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Control (DMSO 5%)	0	0	0
Standard (50 µg/ml)	39.1	30.4	25.7
Standard (250 µg/ml)	41.8	30.8	27.9
Standard (500 µg/ml)	44.9	33.5	32.1
Methanol (25 mg/ml)	11.8	10.2	10.7
Methanol (50 mg/ml)	15.2	12.7	11.9
Methanol (75 mg/ml)	15.7	13.9	14.7
Methanol (100 mg/ml)	16.2	15.2	14.8
Chloroform (25 mg/ml)	9.1	8.7	9.7
Chloroform (50 mg/ml)	10.7	9.7	10.1
Chloroform (75 mg/ml)	11.7	10.8	11.6
Chloroform (100 mg/ml)	12.3	12.1	12.6
Water (25 mg/ml)	0	0	0
Water (50 mg/ml)	0	0	0
Water (75 mg/ml)	0	0	0
Water (100 mg/ml)	0	0	0
Pet Ether (50 mg/ml)	0	0	0
Pet Ether (75 mg/ml)	0	0	0
Pet Ether (100 mg/ml)	0	0	0

Table 8: MIC for five percent methanol Extract

Reading after 24 hrs	
Conc. of methanol extract	**Turbidity**
500 (µg/ml)	+++
600 (µg/ml)	+++
700 (µg/ml)	++
800 (µg/ml)	++
900 (µg/ml)	++
1000 (µg/ml)	+
1100 (µg/ml)	+
1200 (µg/ml)	+
1300 (µg/ml)	-
1400 (µg/ml)	-
1500 (µg/ml)	-

+++: High Turbidity, ++: Medium Turbidity, +: Less Turbidity, -: No Turbidity

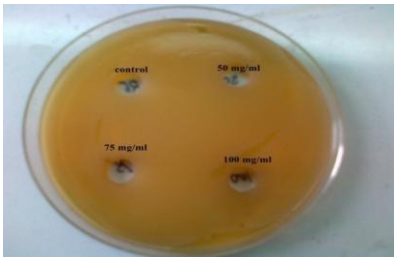

FIG: 1A

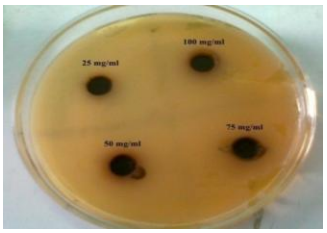

FIG: 1B

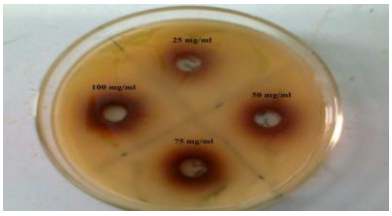

FIG: 1C

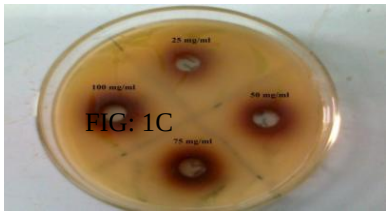

FIG: 1D

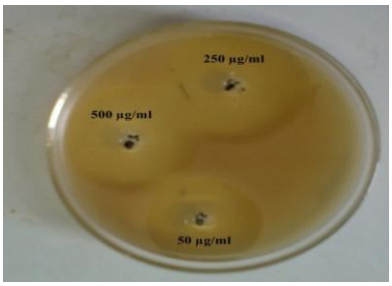

FIG 1E

A: Pet Ether extract and Control DMSO 5%
B: Chloroform Extract
C: Methanol Extract
D: Water Extract
E: Standard Drug Ciprofloxacin

Figure 1 (A-E): Represent the Zone of Inhibition of extracts on *B. subtilis*.

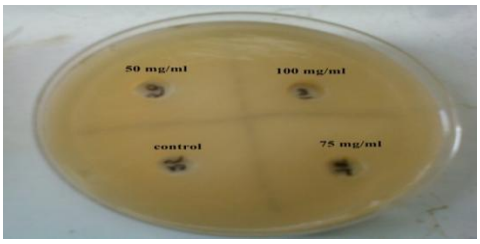

FIG: 2A

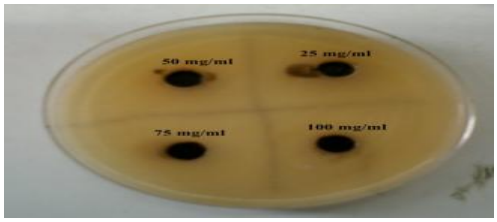

FIG: 2B

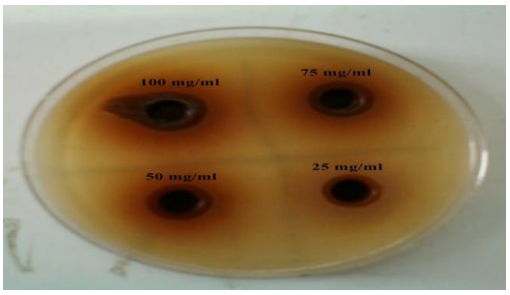

FIG: 2C

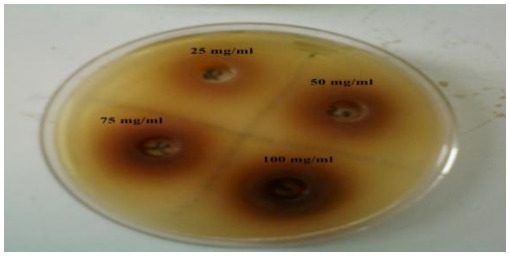

FIG: 2D

A: Pet Ether extract and Conrol DMSO 5%
B: Chloroform Extract
C: Methanol Extract
D: Water Extract
E: Standard Drug Ciprofoxacin

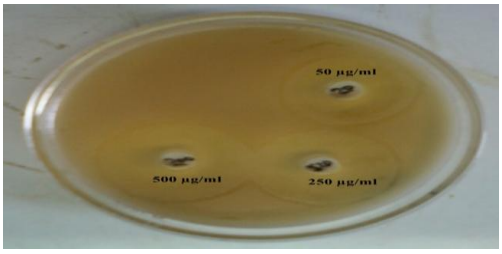

FIG: 2E

Figure 2 (A-E): Represent the Zone of Inhibition of extracts on *E. coli.*

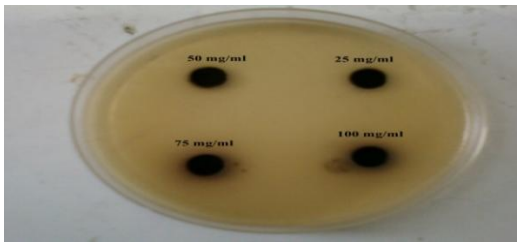

FIG: 3A

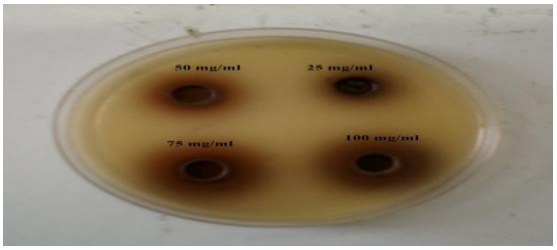

FIG: 3B

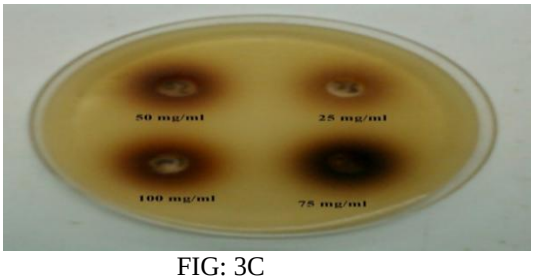

FIG: 3C

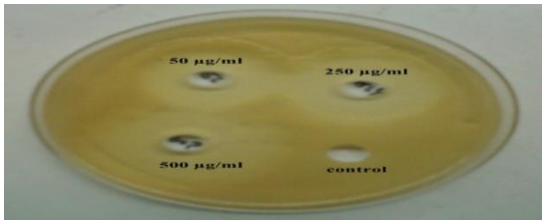

FIG: 3D

A - Chloroform extract
B - Methanol extract
C - Water extract
D - Standard drug ciprofloxacin and control DMSO (5%)

Figure 3 (A-D): Represent the Zone of Inhibition of extracts on *Pseudomonas aeruginosa*

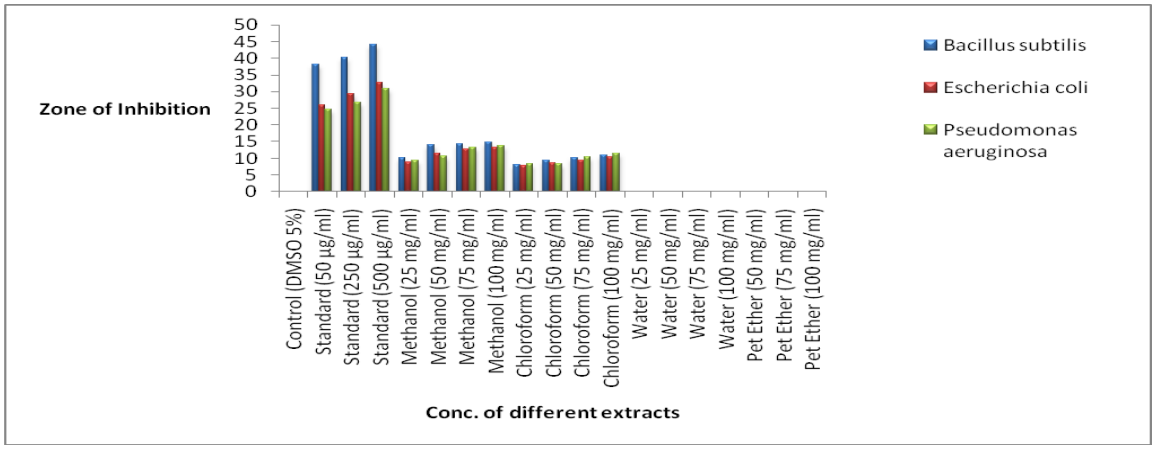

Figure 4: Graphical Representation of Antibacterial activity of extracts after 12 hrs

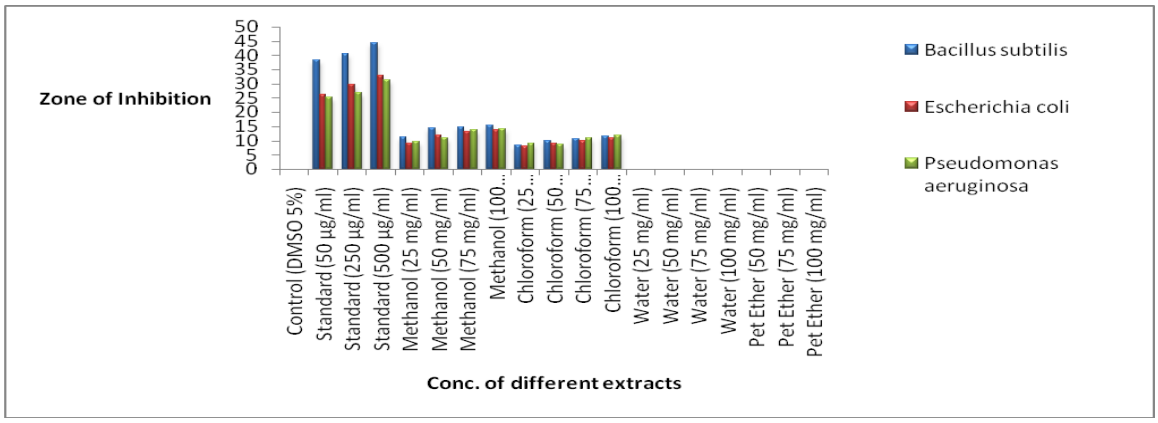

Figure 5: Graphical Representation of Antibacterial activity of extracts after 24 hrs.

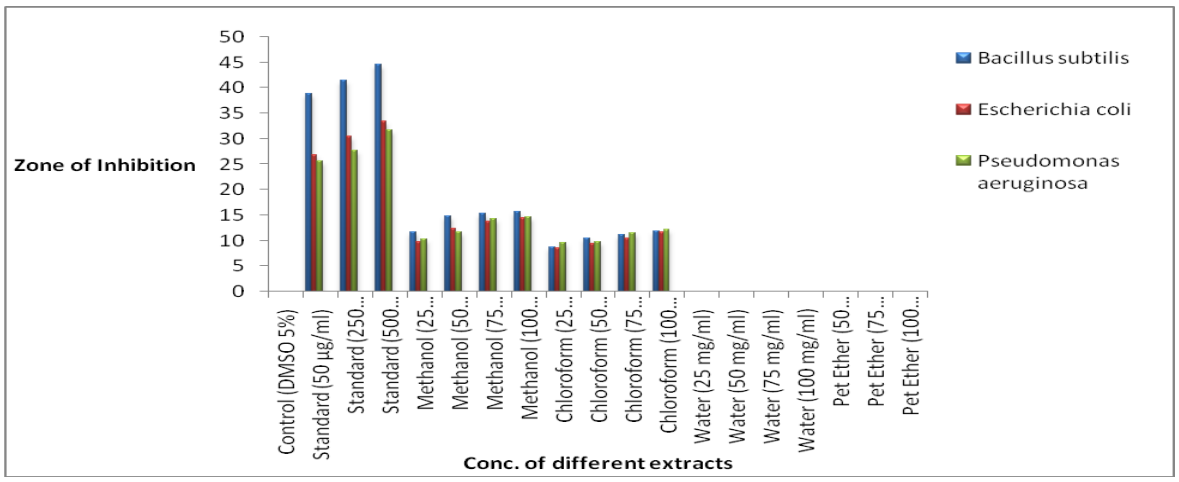

Figure 6: Graphical Representation of Antibacterial activity of extracts after 36hrs.

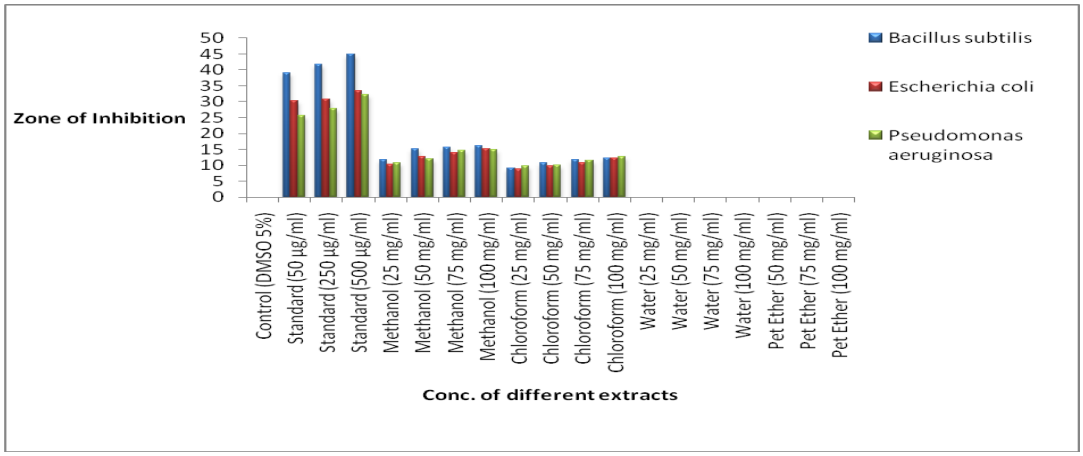

Figure 7: Graphical Representation of Antibacterial activity of extracts after 48hrs.

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