ANTIMICROBIAL ACTIVITY OF HERBAL PLANTS AGAINST TEST BACTERIA

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ABSTRACT*:* Herbal plants are traditionally used for the treatment of human gall bladder infections. The present study was undertaken to investigate activity of herbal extracts against human gall bladder bacterial pathogens. Extracts were prepared in methanol. The activity of plant extract was evaluated against five bacterial pathogens including *E. coli, Salmonella sp, Klebsiella, Streptococcus, Staphylococcus* (test bacteria) using agar diffusion method. Most of the herbal plant extracts are less effective against gram positive bacteria specially *Streptococcus* sp. More resistance power is shown by Salmonella sp. against almost of the plant extracts. Most of the plant extract shows highest activity against *E. coli* and *Klebsiella s*p. The results from the study suggest that the herbal extract show antibacterial activity against different bacterial sp. They could be used alternatives to common antimicrobial agents for treatment of gall bladder infections.

Keywords: Herbal plants, Antimicrobial activity, plant extracts, Inhibition zone.

INTRODUCTION:

According to world health organization more than 80% of the world's population relies on herbal medicine for their healthcare need. The use of herbal plants as traditional medicine is well known in rural and urban areas of many developing countries.(1)

'Traditional healers claim that their medicine is more effective than advanced medicine. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use traditional medicine for the treatment of diseases. (2) pharmacological researchers are increasingly studying medicinal herbs, and many such herbs have a long history of medicinal use in Asia (3). These herbs have many potential and therapeutic applications in the modern medical field, as numerous studies have revealed that they contain active components, and have resulted in a better understanding of their physiological, therapeutic and clinical actions **(4)**. Medicinal plants are an important therapeutic aid for many microbial infections. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century **(5)**

MATERIALS AND METHODS:

Preparation of Plant extracts:

Soxhlet extraction apparatus (Borosil Co, Mumbai)

A glass Soxlet extraction apparatus with 500 ml flat bottom flask was used for the conventional Soxhlet extraction procedure.

Cellulose extraction thimble:

Prepare from Whatman's filter paper no.1 sized to fit the soxhlet extractor and to accommodate the desired amount of sample.

Heating mantle (Scientific Corporation, Delhi)

To provide uniform heating of the solvent for efficient extraction of plant extracts.

Methanol (Qualigens fine chemicals, GSK, Mumbai)

Used as a solvent for plant extract preparation.

Procedure

Plant material collection

The fresh leaves of plants were collected from the local area. The leaves were thoroughly washed with tap water followed by distill water and were shade dried. Dried leaves were crushed with the help of mortar and pastel and a fine powder was prepared.

Plant extract preparation

The powdered leaves were weighed and twenty-five gram of powdered leaves was extracted with the help of soxhlet apparatus. For Soxhlet extraction, thimble was prepared and powdered leaves were kept in it. The extraction was performed with the help of methanol as solvent. 250 m1 of methanol was used for the plant extract preparation from 25 grams leaves. The methanol was mixed with powdered leaves kept in thimble and were allowed to stand overnight. Next morning extraction was performed for 8 hours. The extracts obtained were dried and used for antimicrobial activity.

Table: List of plants used for antimicrobial analysis against bacteria associated with gallbladder patients:

Code	**Common name**	**Botanical name**	**Family**
HE-1	Coleus	*Coleus aromaticus*	Lamiaceae
HE-2	Gudmar	*Gymnema sylvestris*	Apocynanceae
HE-3	Mulethi	*Glycyrrhiza glabra*	Leguminoceae
HE-4	Custard apple	*Annona squamosa*	Annonaceae
HE-5	Karanj	*Pongamia glabra*	Leguminoceae
HE-6	Ginger	*Zinziber officinal*	Zinziberaceae
HE-7	Ritha	*Sapindus mukorossi*	Sapindaceae
HE-8	Bougainvillea	*Bougainvillea glabra*	Nyctaginaceae
HE-9	Champa	*Michelia champaca*	Magnoliaceae
HE-10	Neem	*Azadirachta indica*	Meliaceae
HE-11	Marigold	*Calendula officinalis*	Asteraceae
HE-12	Ashok	*Saraca indica*	Fabaceae
HE-13	Guava	*Psidium guajava*	Myrtaceae
HE-14	Amla	*Emblica officinalis*	Euphorbiaceae
HE-15	Mango	*Mangifera indica*	Anacardiaceae
HE-16	Boswellia	*Boswellia thurifera*	Burseraceae
HE-17	Jamun	*Eugenia Jambolana*	Myrtaceae
HE-18	Pomegranate	*Punica granatum*	Punicaceae
HE-19	Dhatura	*Dhatura stramonium*	Solanceae
HE-20	Gulmohar	*Delonix regia*	Fabaceae
HE-21	Methi	*Trigonella foenumgraecum*	Leguminoceae

HE-22	Dalchini	*Cinnamomum zeylanicum*	Lauraceae
HE-23	Belpatra	*Aegle marmelos*	Rutaceae
HE-24	Aak	*Calotropis procera*	Asclepiadaceae
HE-25	Sadabahar	*Vinca rosea*	Apocynanceae
HE-26	Ashwagandha	*Withania somnifera*	Solanacea
HE-27	Grapes	*Vitis vinifera*	Vitaceae
HE-28	Jackfruit	*Artocarpus heterophyllus*	Moraceae
HE-29	Mahogany	*Swietenia macrophylla*	Maliaceae

Table: Extract obtained from 25-gram leaves after soxhlet extraction:

Sr. no.	**Plant extracts**	**Total yield after extraction**
1.	*Coleus aromaticus*	3.99 gm
2.	*Gymnema sylvestris*	4.208 gm
3.	*Glycyrrhiza glabra*	4.563 gm
4.	*Annona squamosa*	4.11 gm
5.	*Pongamia glabra*	7.486 gm
6.	*Zingiber officinal*	3.319 gm
7.	*Sapindus mukorossi*	4.288 gm
8.	*Bougainvillea glabra*	4.97 gm
9.	*Michelia champaca*	2.4 gm
10.	*Azadirachta indica*	4.59 gm
11.	*Calendula indica*	3.319 gm
12.	*Saraca indica*	6.825 gm
13.	*Psidium guajava*	2.01 gm

14.	*Emblica officinalis*	6.31 gm
15.	*Mangifera indica*	6.47 gm
16.	*Boswellia thurifera*	4.818 gm
17.	*Eugenia jambolana*	3.865 gm
18.	*Punica granatum*	11.52 gm
19.	*Dhatura stramonium*	7.84 gm
20.	*Delonix stramonium*	3.02 gm
21.	*Trigonella foenumgracum*	3.882 gm
22.	*Cinnamomum zeylanicum*	6.269 gm
23.	*Aegle marmelos*	2.607 gm
24.	*Calotropis procera*	3.102 gm
25.	*Vinca rosea*	4.365 gm
26.	*Withania somnifera*	2.11 gm
27.	*Vitis vinifera*	3.1321 gm
28.	*Artocarpus heterophyllus*	3.431 gm
29.	*Swietenia macrophylla*	3.311 gm

Soxhlet apparatus

Preparation of media and plate for antimicrobial- analysis of plants extract

Antimicrobial susceptibility analysis was performed over Mueller Hinton Agar (Hi media) which is an only susceptibility medium validated by NCCLS (National committee for clinical laboratory standards).

Mueller- Hinton agar:

Composition:

Ingredients	Grams/Litre
Beefinfusion solids	4.0 gm
Starch	1.5 gm
Casein hydrolysate	17.5 gm
Agar	15.0 gm
Final pH	7.4 ± 0.2 at 37°C

The media was poured in petridishes and were allowed to solidify; the cork borer was used for the creation of well in the solidified media. Cork borer was sterilized with the help of alcohol and incineration after single use. Total four wells were punched in the Petri dish having 90 mm diameter.

Processing of plant extract before its activity

The 300 mg of plant extracts were dissolved in 500 µl of methanol. This solution was vortexes over vortex mixture for its homogenous mixing. 40 µl (Equivalent to 24 mg) of above described plant extracts were added to each well with the help of micropipette. These plates were kept overnight for evaporation of methanol and diffusion of extracts. This procedure was also helpful for checking contamination among the plates during pouring and well punching in media.

Inoculums preparation

Each culture to be tested was streaked onto a no inhibitory agar medium to obtain isolated colonies. After incubation at 37°C overnight, few *well-isolated* colonies were selected with an inoculating loop and the bacteria were inoculated to a tube of nutrient broth at 37°C. Then the turbidity of bacterial suspension was compared to 0.5 McFarland standards. Viewing the tubes against a sheet of white paper on which sharp black lines are drawn made this comparison. The turbidity standard was agitated on a vortex mixer immediately prior to use. If the bacterial suspension does not appear to be the same density as the McFarland 0.5, the turbidity was reproduced by adding sterile broth or increased by adding more bacterial growth. 0.5 and 1.0 McFarland standard were prepared prior to beginning of susceptibility testing.

Preparation of McFarland Standard

0.5 McFarland standard was prepared by adding 0.05 ml of a 1.175% (wt/vol) barium chloride dehydrate ($BaCl_2 \cdot 2H20$) solution to 9.95 ml of 1 % (vol/ vol) sulfuric acid. The turbidity standard was then aliquot into

test tubes identical to those used to prepare the inoculums suspension. McFarland standard tubes were sealed to prevent evaporation. McFarland standards were stored in the dark at room temperature (22° to 25°C). Before each use, the tubes were shaked and mixed; so that the fine white precipitate of barium sulfate in the tube develop turbidity. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with an I c.m. light path; for the 0.5 McFarland standard, the absorbance at a wavelength of 625 nm should be 0.08 to 0.1.

McFarland standards preparation table:

Mc Fenland Standard No.	0.5	1	2	3	4
1.0% $Bacl_2$ (in ml.)	0.05	0.1	0.2	0.3	0.4
1.0% H_2SO_4 (in ml.)	9.95	9.9	9.8	9.7	9.6
Approx. Cell density	1.5	3.0	6.0	9.0	12.0

RESULTS:

Most of the herbal plant extracts are less effective against gram-positive bacteria specially *Streptococcus sp.* More resistance power is shown by *Salmonella sp.* against almost of the plant extracts. Most of the plant extract shows highest activity against *E. coli* and *Klebsiella sp.* It is found Mulethi (*Glycyrrhiza glabra*), Dalchini (*Cinnamomum zeylanicum*), Vitis (*Vitis vinifera*), Dhatura (*Dhatura stramonium*) and pomegranate (*Punica granatum*) shows good activity (zone diameter is large) against most of the organisms. Among these Dhatura (10m.m.-20m.m.), Mulethi (12m.m.-17m.m.) shows highest activity. Other plant extracts shows very moderate/least activity against test bacteria and some was resistant to it and some shows no activity.

This concludes Mulethi and Dhatura extract are very beneficial to stop the growth of bacteria. Dalchini, Vitis, Pomegranate, Sitaphal (*Annona sp.),* Mango (*Mangifera indica*) also shows good activity against gram positive and gram negative except some isolates. It means these extracts are useful in infective GB complication. But Marigold *(Tagetes patula*), Neem (*Azadirachta indica*), Gulmohar (*Delonix regia*) shows least activity. Neem (*Azadirachta indica*) and Gulmohar (*Delonix regia*) shows least activity (small inhibition zone) against all of the isolates. In recent time these plant extracts are used at large level for herbal medicines.

Table-31. Inhibition zone diameter of herbal plants

S.no.	Plant extracts	E.coli	Klebsiella sp.	Salmonella sp.	Streptococcus sp.	Staphylococcus sp.
1.	*Coleus aromaticus*	10 m.m.	R	R	10 m.m.	R
2.	*Gymnema sylvestris*	12 m.m.	10 m.m.	11 m.m.	0	11 m.m.
3.	*Glycyrrhiza glabra*	12 m.m.	15 m.m.	R	17 m.m.	R
4.	*Annona squamosa*	27 m.m.	17 m.m.	10 m.m.	0	10 m.m..
5.	*Pongamia glabra*	12 m.m.	0	R	0	R
6.	*Zinziber officinal*	10 m.m.	11 m.m.	0	0	0
7.	*Sapindus mukorossi*	17 m.m.	0	10 m.m.	0	10 m.m.
8.	*Bougainvillea glabra*	13 m.m.	16 m.m.	R	0	R
9.	*Michelia champaca*	28 m.m.	0	R	0	R
10.	*Azadirachta indica*	15 m.m.	17 m.m.	0	0	0
11.	*Calendula officinalis*	0	0	R	0	10 m.m.
12.	*Saraca indica*	0	0	0	13 m.m.	0
13	*Psidium guajava*	0	19 m.m.	0	R	0
14.	*Emblica officinalis*	11 m.m.	10 m.m.	R	R	R

R=Resistent (<10 mm)

S.no	Plant extracts	E.coli	Klebsiella sp.	Salmonella sp.	Streptococcus sp.	Staphylococcus sp.
15.	*Mangifera indica*	16 m.m.	13 m.m.	10 m.m.	0	10 m.m.
16.	Boswellia thurifera	13 m.m.	0	R	0	R
17.	*Eugenia Jambolana*	10 m.m.	17 m.m.	R	0	R
18.	*Punica granatum*	18 m.m.	R	12 m.m.	10 m.m.	12 m.m.
19.	*Dhatura stramonium*	20 m.m.	11 m.m.	10 m.m.	20 m.m.	10 m.m.
20.	*Delonix regia*	R	17 m.m.	0	0	0
21.	*Trigonella foenumgraecum*	19 m.m.	0	0	0	0
22.	*Cinnamomum zeylanicum*	12 m.m.	18 m.m.	0	0	0
23.	*Aegle marmelos*	10 m.m.	10 m.m.	10 m.m.	R	10 m.m.
24.	*Calotropis procera*	13 m.m.	18 m.m.	10 m.m.	0	10 m.m.
25.	*Vinca rosea*	10 m.m.	0	0	0	0
26.	*Withania somnifera*	11 m.m.	16 m.m.	0	0	0
27.	*Vitis vinifera*	10 m.m.	15 m.m.	0	12 m.m.	0
28.	*Artocarpus heterophyllus*	17 m.m.	10 m.m.	0	0	0
29.	*Swietenia macrophylla*	16 m.m.	10 m.m.	0	10 m.m.	0

R=Resistent (<10 mm)

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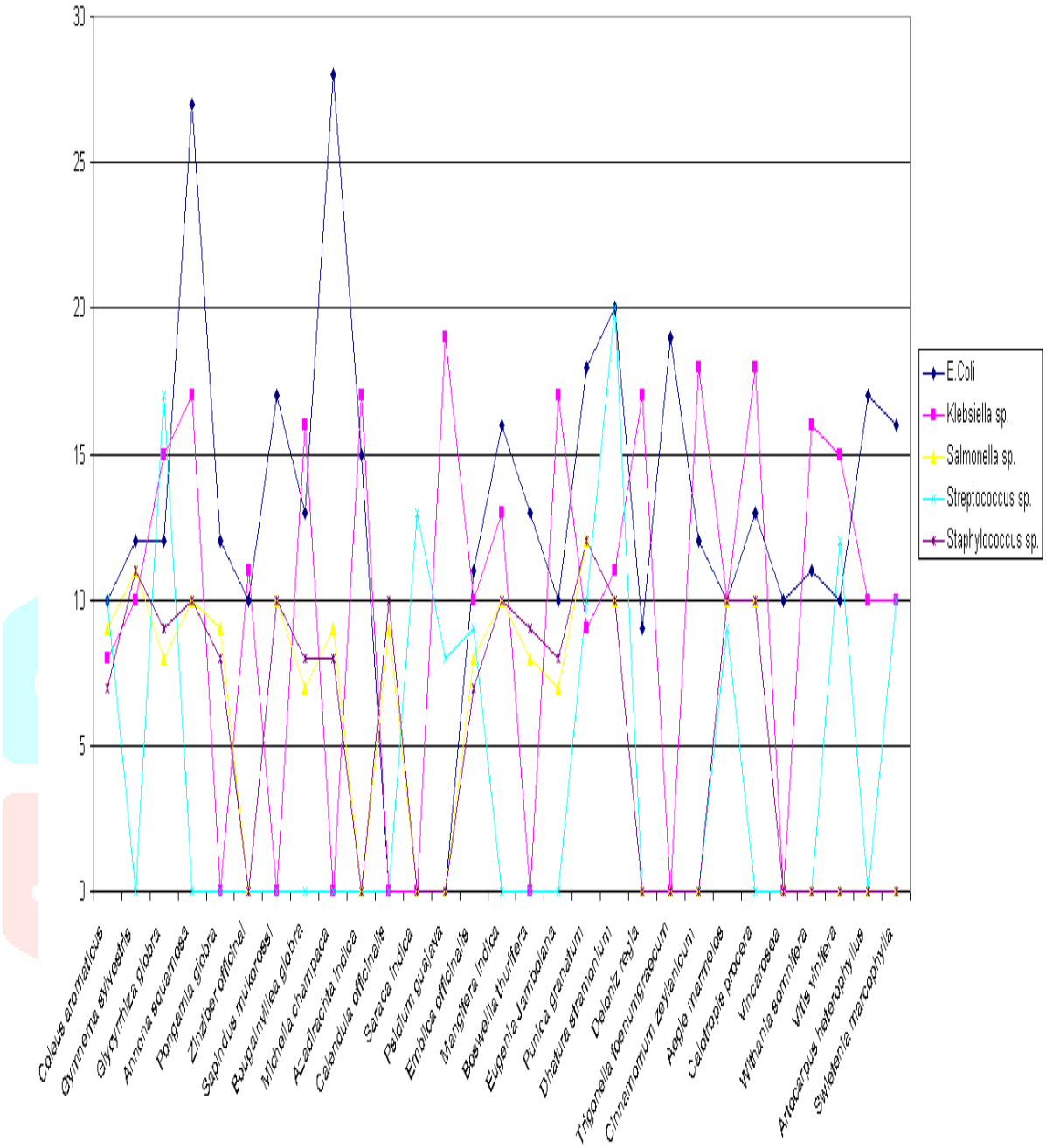
30
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E.Coli
Klebsiella sp.
Salmonella sp.
Streptococcus sp.
Staphylococcus sp.
Coleus aromaticus
Gymnema sylvestre
Glycyrrhiza glabra
Annona squamosa
Pongamia glabra
Zingiber officinal
Sapindus mukorossi
Bougainvillea glabra
Michelia champaca
Azadirachta indica
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Eugenia Jambolana
Punica granatum
Dhatura stramonium
Delonix regia
Trigonella foenumgraecum
Cinnamomum zeylanicum
Aegle marmelos
Calotropis procera
Vinca rosea
Withania somnifera
Vitis vinifera
Artocarpus heterophyllus
Swietenia marcophylla

DISCUSSION:

Components of *Glycyrrhiza glabra*, inhibit against the growth of *Helicobacter pylori in vitro*. (6). In present study, *Glycyrrhiza glabra* showed inhibition zone of 15 m.m. diameters against *Staphylococcus* sp., 17m.m. diameter against **Streptococcus s**p., 12 m.m. diameters against E.coli and 15 m.m. diameters against *Klebsiella* sp. *Glycyrrhiza glabra* (liquorice) root extracts showed various antibacterial activities (7-11 mm/20 µl inhibition zone) against the microorganisms tested. The alcohol extracts did not inhibit *B. subtilis* (7). In our study, methanolic extract of *Glycyrrhiza glabra* did not inhibit *Salmonella* sp.

Study suggests the potential of *Annonna squamosa* fruit pericarp for the development of modern medicine for the treatment of cancers. (8). A. squomosa seed extracts were evaluated for antimicrobial activity against human pathogenic bacteria strain of Gram-negative and Gram-positive Staphylococcus aureus and Streptococcus pyogenes. Benzene and methanol extracts showed high activity against *E. coli, P. aeruginosa* and *Staphylococcus aureus.* (9). Present study showed high activity against *E. coli, Klebsiella* sp., *Staphylococcus* sp., *Salmonella* sp., Extract was inactive against *Streptococcus* sp. The antimicrobial properties of mango seed kernel ethanol extract (MKE) had a broad antimicrobial spectrum, and was more active against gram-positive than gram-negative bacteria with a few exceptions (10).

In our study, methanolic extract of mango was more active against gram positive and gram negative both. The extract from different Indian mango varieties was tested against different bacteria to evaluate its anti-microbial activity. Extract was least effective against *Escherichia coli* while *Bacillus cereus* was most sensitive to it. The extract did not show antimicrobial activity even at higher concentrations (11). Current study showed extract of *Mangifera indica* least effective against Streptococcus sp. Leaf extracts of *Mangifera indica* (L.), a medicinal and horticultural plant were investigated for antibacterial activity against *Staphylococcus aureus, Esherichia coli* and *Pseudomonas aeruginosa.*

Punica granatum were tested (in vitro) for their antibacterial activity. The methanolic extract was found to be most effective against all tested microorganisms (12). Antibacterial activity of acetone, MeOH, and water extracts of *Punica granatum* was evaluated by pour plate method against a few Gram-positive and Gram-negative bacteria, In our study methanolic extracts are very effective against test bacteria.

The antibacterial activity of was investigated. The extracts showed activity against Gram (+) bacteria. Little or no antibacterial activity was found against *Escherichia coli* (13). Our study showed antibacterial activity of Datura sp. was high (20 m.m.) against *E. coli* and less against *Salmonella* sp.

Extracts of cinnamon were compared for their effect on Helicobacter pylori growth and urease activity. Extract was found to inhibit growth of *H. pylori.* Cinnamon extract (from methylene chloride) inhibited *H. pylori* at concentration range of common antibiotics. (14). Cinnamon extract (CE), at 130 mg/disc, exhibited antibacterial activity against *E. coli.* (15). In present study cinnamon extract was more active against *E. coli, Klebsiella* sp., *Staphylococcus* sp. and inactive against *Streptococcus* sp and *Salmonella* sp.

The grape seed extracts at 20% concentration inhibited all the bacteria except *B. amyloliquefaciens.* (16). Present study showed maximum inhibitory effect of grape extract against Streptococcus sp. followed by Klebsiella sp. and minimum or no effect against Salmonella sp. The grape extract was tested for antibacterial activities by pour plate method against Bacillus cereus, Bacillus coagulans, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. (17). Our study explained that grape extract was active against both gram positive and gram-negative bacteria (except Salmonella sp.). Current study described Vitis extract was more active against test bacteria.

The investigation was based on antibacterial properties of extracts of guava (Psidium guajava) and neem (*Azadirachta indica*) against a number of common bacteria. Guava and neem extracts exhibited higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. None of the extracts showed activity against Escherichia coli and Salmonella enteritidis. Use of guava and neem can guard against some of the pathogens (18). Our study shows neem and guava are less active against test bacteria. Several authors studied antimicrobial properties of *Azadirchata indica*. Rao *et al* reported the antimicrobial activity of the seed oil against a variety of pathogens. We planned the present study to find out the antibacterial activities of 'neem' leaves. (19). Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis* (20). Present work demonstrated neem extract showed good activity against *Klebsiella* sp., *E. coli* and no activity seen against *Streptococcus* sp., *Salmonella* sp., *Staphylococcus* sp. was resistant to this extract.

The antibacterial activity of Karanj (*Pongamia pinnata*) and Neem (*Azadirachta indica*) seed extracts in vitro against fourteen strains of pathogenic bacteria was assessed,using the tube dilution technique. The activity with both the extracts was bactericidal. (21). Present study explained Karanj are least active against test bacteria.

Extracts of *Calendula officinalis* plant was studied by diffusion method using *Staphylococcus aureus Escherichia coli* as test organisms. Zone of inhibition range from 10 to 20 mm for both *S. aureus* and *E. coli*. *C. officinalis* was recorded a maximum of 24 mm zone of inhibition against *S. aureus* (22). In our study Calendula extract are not effective against test organisms.

CONCLUSION:

Herbal plant extract have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Their single effect or synergistic effect against bacteria leads to new choice for the treatment of infectious diseases

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