

# A Study of Antimicrobial Activity of Few Medicinally Important Herbal Single Drugs Extracted in Ethanol, Methanol and Aqueous Solvents

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## ABSTRACT:

There is a currently worldwide upsurge in the use of herbal preparations and active ingredients of medicinal plant in health care. This is particularly true in the rural areas of Asian countries where herbal medicines are the only choice for treating human ailments. Present study reveals the difference in the antimicrobial activity pattern of *Hemidesmus indicus*, *Smilax china*, *Ocimum basilicum*, *Ocimum sanctum*, *Ocimum cannum*, *Azadirachta indica* A.Juss, *Trigonella Foenum graecum* and *piper cubeba* extracted in Ethanol, Methanol and Aqueous solvents against the pathogenic organisms *E.coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Bacillus subtilis*(L10969), *Bacillus subtilis* (SL5740) and clinically isolated strains *Shigella*, *Klebsiella*, *Proteus*, *Salmonella paratyphi*, *Salmonella typhi* and *Stapylococcus aureus*. Among all the extracts Ethanol shows more activity with a zone of inhibition ranges from 10mm to 22mm. And Aqueous extract shows less inhibition which ranges from 6mm to 11mm.

**Keywords:** Anti-bacterial, Herbal drugs, Extracts

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## INTRODUCTION

Infectious diseases caused by bacteria, fungi, parasites and viruses are still a major threat to public health, despite the huge progress in human medicine. Their impact is particularly large in developing countries due to unavailability of medicines and the emergence of widespread drug resistance<sup>[1]</sup>. Natural products especially, those used in ethno medicine provide a major source of innovative therapeutic agents for various conditions including infectious disease. The antimicrobial activity of different plant extracts have been reported by many authors<sup>[3,4,11]</sup>. Different classes of antibiotics have been used to control bacterial infections. However, the

usefulness of existing antimicrobial agents is rapidly fading, tipping the balance in favour of multi-drug resistant pathogens, including MRSA and there appears to be few, if any, new classes of drugs currently in use to fight against the multi-drug resistant pathogens. Multidrug-resistance (MDR) exhibited by many bacterial species is a major problem in treating both hospital and community acquired infections.

*Azadirachta indica* A. Juss (Stem bark )has for long been used in the traditional Unani system of medicine for its beneficial properties. The aqueous extracts of stem bark used as tonic, stimulant and as a remedy against various skin ailments<sup>[6]</sup>. The chemical constituents are Nimbin, Nimbinin and Nimbidin.

*Trigonella Foenum-Graecum* (Fabaceae) found in nature and is cultivated in India and Pakistan is a well known medicinal plant having properties of reducing blood sugar level <sup>[10]</sup>, antihelmentic, antibacterial <sup>[7]</sup>, anti-inflammatory, antipyretic <sup>[5]</sup> and antimicrobial <sup>[2]</sup>. The important chemical constituents are saponins, coumarin, fenugreekine, nicotinic acid, phytic acid, Scopoletin and Trigonelline. *Hemidesmus indicus* (Asclepiadaceae) posses potent anti-inflammatory, anti-pyretic, anti-oxidant property<sup>[8]</sup>, chemical constituents are Beta-Sitosterol, Beta-Amyrins, Lupeol, tannins and saponins.

### MATERIALS AND METHODS

#### PLANTS MATERIALS

Bark of *Azadirachta indica* A. juss (Fabaceae) were collected from local area (Hyderabad, Andhra Pradesh, India). Roots of *Hemidesmus indicus*, *Smilax china*, fruit of *Piper cubeba* and seeds of *Trigonella foenum graecum* were procured from local Herbal drugs dealers Hyderabad. Three species of *Ocimum*- *O.basilicum*, *O.sanctum*, *O.cannum* leaves were collected from Herbal garden CRIUM, Hyderabad. And were authenticated by Botanist at CRIUM Hyderabad, A.P, India).

#### BACTERIAL CULTURES

Microorganisms tested in this study were *Escherichia coli* (ATCC 259220), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (SL5740), *Bacillus subtilis* (L1O969), Clinical isolates - *Proteus*, *Salmonella paratyphi*, *Shigella*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella*.

#### EXTRACTION

The collected Herbal drugs were washed and air-dried for 48 h at the room temperature, chopped into small pieces and then soaked with three types of solvent at room temperature. The extract was filtered and then entire extract was concentrated to dryness using rotary evaporator under reduced pressure.

#### EVALUATION OF ANTI-BACTERIAL ACTIVITY

Anti-Bacterial activity of the extract was determined by Agar diffusion assay <sup>[9]</sup>. Bacterial strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 24 h at 37°C and after the incubation period 0.1ml of the test the inoculum was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. The seeded plates were allowed to dry in the incubator

at 37°C, wells were made using sterile 6mm cork borer in the inoculated MHA plate. The wells were filled with 200µl of the extracts (re-suspended in respective solvents). The concentration of stock extracts were 200 mg/ml. The inoculated plates were incubated at 37°C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics Ciprofloxacin (30mg). The photograph was taken in U.V-Visible documentation system.

### RESULTS AND DISCUSSION

The results of antibacterial activities in terms of zone of inhibition (mm) were presented in (Table 1). The antibacterial activities of single Herbal drugs extracts were tested against eleven bacterial strains. The results showed promising antibacterial activity against the bacteria tested. Among these Ethanol and methanol extracts were found to have a more potent inhibitory effect than Aqueous extracts. The observed activity may be due to the presence of potent phyto-chemical constituents in the drug extracts. Among all the drugs extracts of *Azadirachta indica* (Bark) and *Hemidesmus indicus* (Root) shows more activity on all the strains of bacteria. *Staphylococcus aureus* and *Salmonella paratyphi* was observed to be the most susceptible organism using Ethanol extracts of *Azadirachta indica* (Bark), at the same Ethanol extracts of *Hemidesmus indicus* shows no activity on *Salmonella typhi* and *Salmonella paratyphi*. Strains of *Bacillus subtilis* was found more susceptible to ethanol extracts of *Smilax china* (root). The Ethanol and Methanol extracts of *Trigonella foenum graecum* (seeds) were affective on *E.coli*, *Staphylococcus aureus* and *Salmonella typhi* and *Salmonella paratyphi* strains. Among the three *Ocimum* species extracts of Ethanol and Methanol of *Ocimum cannum* and *Ocimum sanctum* shows promising activity and least activity was found using Aqueous extracts. *E.coli*, *Staphylococcus aureus* and *Bacillus subtilis* strains showed good activity using *Piper cubeba* Ethanol and Methanol extracts compare with other bacterial strains. The susceptibility of tested Bacterial strains to Aqueous, Ethanol and methanol crude extracts of these Herbal drugs used is an indication of the potential of the extract as a drug that can be used against this organisms. *Ocimum basilicum* extracts and *Klebsiella* did not show any result in the entire test.

The phytochemical compounds are known to play an important roles in bioactivity of medicinal plants. Flavonoids exhibit as anti-inflammatory, anti-allergic,

**Table -1: Antimicrobial activity pattern of few Herbal single drugs presented as diameter of Zone of inhibition.**

ZONE OF INHIBITION (mm).																
S.No	Micro organisms	Hemidesmus indicus.			Piper cubeba			Smilax china			Azadirachta indica A. juss			Trigonella foenum graecum		
		Aq	Eth	Meth	Aq	Eth	Meth	Aq	Eth	Meth	Aq	Eth	Meth	Aq	Eth	Meth
1	E.coli (ATCC-25922)	0	14	16	N	13	12	0	11	0	13	16	14	0	14	17
2	Staphylococcus aureus (ATCC-25923)	0	12	12	N	13	14	0	14	12	14	20	16	0	18	16
3	Pseudomonas aeruginosa (ATCC-27853)	14	0	0	N	11	0	0	13	12	10	21	9	0	15	14
4	Bacillus subtilis (SLS740)	0	17	16	N	16	0	0	20	14	11	13	12	0	11	0
5	Bacillus subtilis (L10969)	11	0	16	N	13	0	0	22	16	12	14	11	0	18	16
6	Proteus sp	0	15	14	N	12	0	0	0	10	12	13	11	0	0	0
7	Salmonella paratyphi	0	0	0	N	11	0	0	0	0	10	20	12	0	16	18
8	Shigella	0	14	13	N	10	0	0	0	13	15	19	18	0	8	11
9	Salmonella typhi	0	0	0	N	10	0	0	0	0	14	18	17	0	14	12
10	Staphylococcus aureus	11	14	13	N	13	11	0	14	12	15	17	18	7	7	11
11	Klebsiella	0	0	0	0	0	0	0	0	0	12	17	16	0	0	0

S.No	Micro organisms	Ocimum basilicum			Ocimum sanctum			Ocimum cannum			Standard antibiotic.
		Aq	Eth	Meth	Aq	Eth	Meth	Aq	Eth	Meth	Ciprofloxacin (30 mcg)
1	E.coli (ATCC-25922)	0	0	0	0	8	9	0	9	0	35
2	Staphylococcus aureus (ATCC-25923)	0	0	0	6	11	11	0	11	10.5	32
3	Pseudomonas aeruginosa (ATCC-27853)	0	0	0	0	0	12	0	10	12	35
4	Bacillus subtilis (SLS740)	8	0	0	0	12	11	0	11	12	30
5	Bacillus subtilis (L10969)	0	0	0	6	9	16	0	11	9	31
6	Proteus sp	9	0	0	0	10	9	0	9	10	25
7	Salmonella paratyphi	0	0	0	6	0	11	9	0	0	31
8	Shigella	0	0	0	10	9	0	0	8	0	35
9	Salmonella typhi	0	0	0	7	0	10	6	0	0	30
10	Staphylococcus aureus	0	0	0	0	10	11	0	12	11	15
11	Klebsiella	11	0	0	0	0	8	6	0	0	20

analgesic and antioxidant as well. Rather than that flavonoids also exhibit a wide range of biological activities such as scavenge for hydroxyl radicals. The presence of the saponin compound in these herbal drugs which is supported the usefulness of these drugs in the managing inflammation. . The crude Herbal extracts tested were compared with control Ciprofloxacin (30mcg) Standard

antibiotic. The Ethanol and methanol crude extracts showed the most significant zone of inhibition against almost all the bacterial strains.

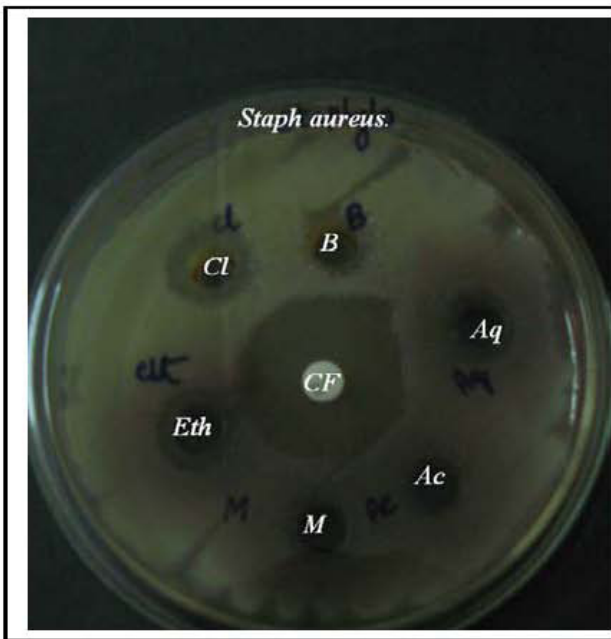
**CONCLUSION**

As part of on going study to screen local plants for anti-bacterial activity *Hemidesmus indicus*, *Smilax china*,

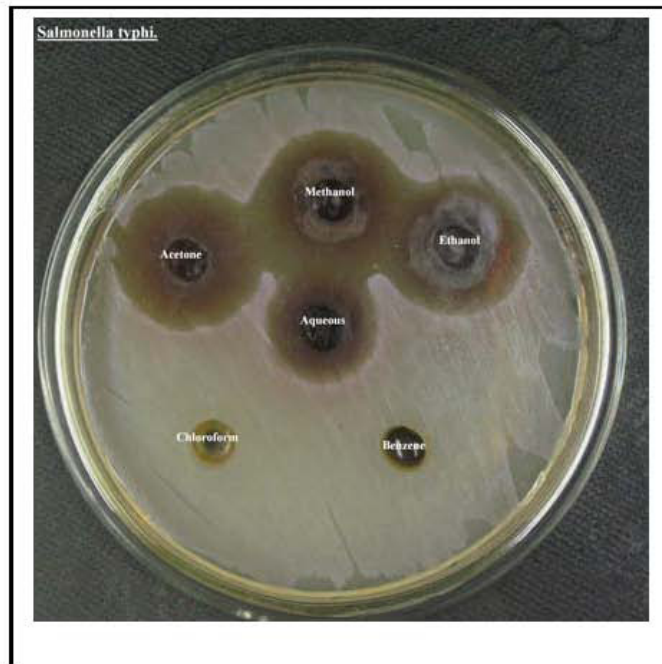
*Ocimum basilicum*, *Ocimum sanctum*, *Ocimum cannum*, *Azadirachta indica* A.Juss, *Trigonella Foenum graecum* and *piper cubeba* were assessed for its actions against different strains of microorganisms extracted in Ethanol, Methanol and Aqueous solvents. We here report the effect of these three extracts on standard ATCC strains and few clinically isolated strains and also compared with standard antibiotic Ciprofloxacin respectively.

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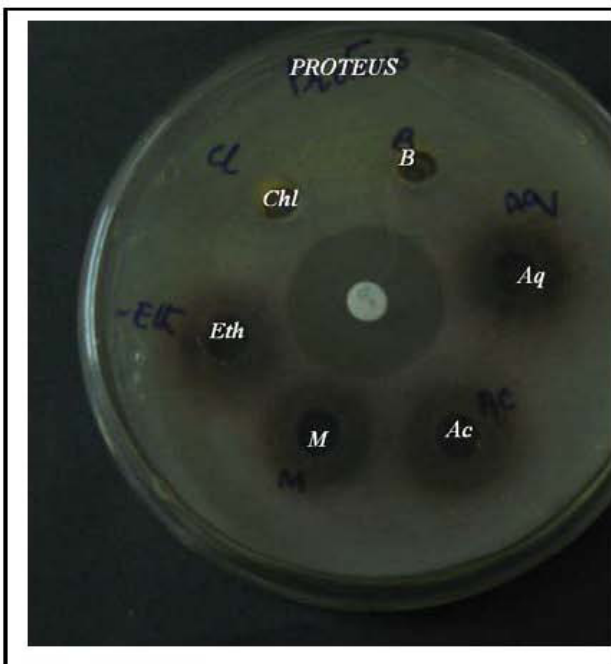
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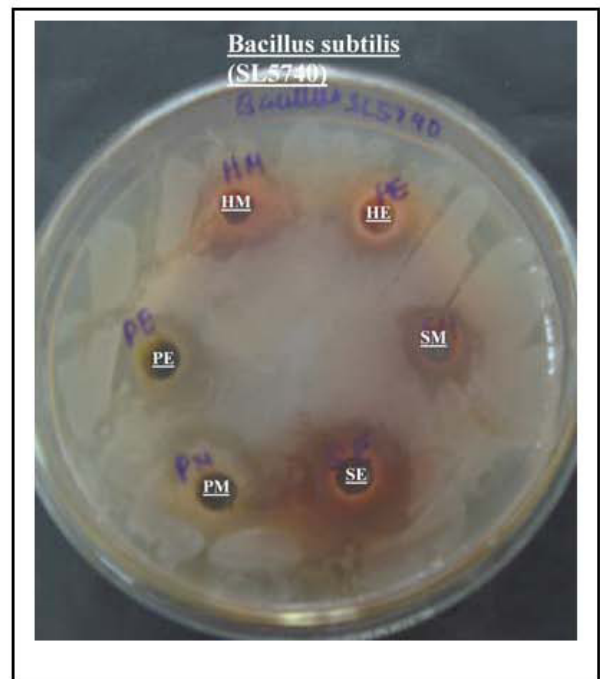
**Azadirachta indica A.Juss**



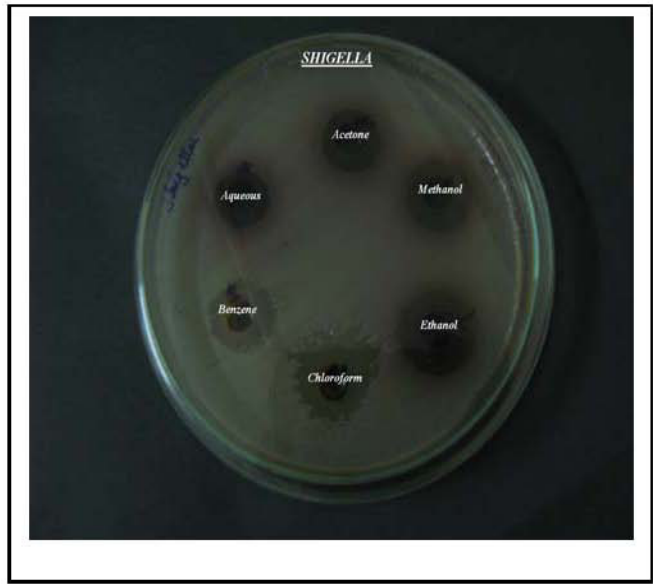
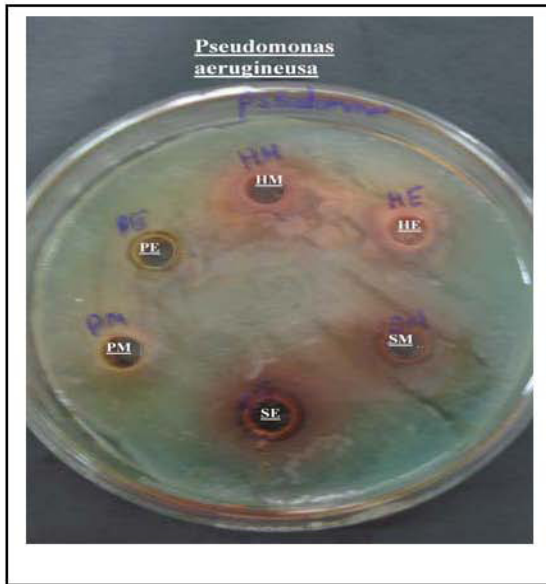
**Azadirachta indica A.Juss**



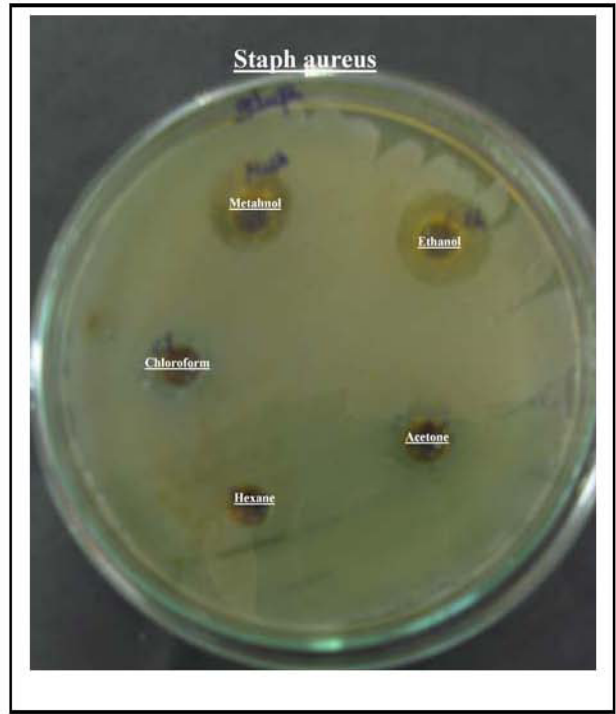
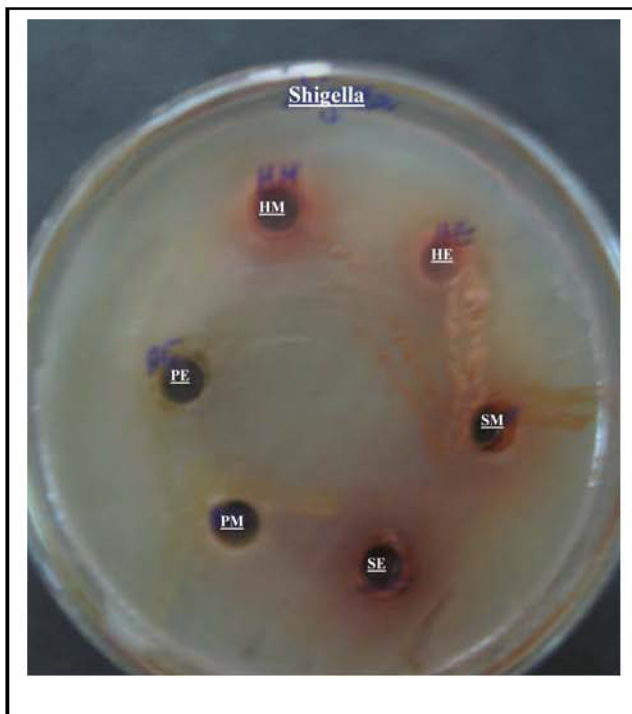
**Azadirachta indica A.Juss**







**Azadiracht indica A.Juss**



**Trigonella foenum graecum**

**Figure 1:** Photographs of Antimicrobial Activity of Herbal Drugs Used In The Study.

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