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EVALUATION OF POTENTIAL ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS AGAINST COMMON FOOD-BORNE PATHOGENIC MICROORGANISM

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ABSTRACT

Indian Medicinal plants contain many antimicrobial agents and properties. Hence, an attempt has been made to find out newer components from the Indian Medicinal plants Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacy-therapeutic agents in medicinal plants. To determine the potential and promote the use of herbal medicine, in the present study of aqueous and ethanoloic extract of 5 medicinal plant tested leaves of *Azadirachta indica* (**Meliaceae**), *Portulaca oleracea* (**Portulacaceae**) , *Euphorbia hirta* ***(*Euphorbiaceae*)*** , *Gmelina asiatica* (**Verbenaceae**), *Santalum album*(**(Santalaceae),** were screened for their anti-microbial activity against seven species of micro organism *Escherichia coli*, ,*Bacillus subtilis*, ,, *Pseudomonas aeruginosa* , Salmonella typhimurium ,*Staphylococcus aureus*, *Candida albicans* and *Aspergillus Niger*. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics. The antimicrobial activities of these plants were discussed according to their phytochemical components.

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KEY WORDS

ANTIMICROBIAL ACTIVITY *Azadirachta indica*, *Portulaca oleracea*, *Euphorbia hirta*, *Gmelina asiatica*

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko *et al*. 2002). The increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Seeradski *et al*. 1999). For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents.

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [1,2]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [3].This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance [4],there is a constant need for new and effective therapeutic agents[5]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [17].

Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world[8, 11]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species seed in herbal medicine[12]. Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [13]. All medicinal, plant contains certain active constituent, it responsible to some pharmacological activity. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct.[15]

Table.1
List of medicinal plant

S.no	Plant name	*Family*	Part used	Chemical compound	Medicinal use
1.	**Azadirachta indica**	*Meliaceae*	Leaf	alkaloids,flavonoids, triterpenoids , phenolic compounds , Carotenoids , steroids and ketones ,azadirachtin	In treating eczema, ringworm, acne, anti-inflammatory,antiheperglycemic, wounds, soothes, swelling
2.	**Euphorbia hirta**	*Euphorbiacea e*	Leaf	sterols, alkaloids, tannins, glycosides, triterpenoids, alkenes, phenolic acids, choline, and shikimic acid	It is used against asthma, bronchitis, worm infestation, conjunctivitis and dysentery. The latex of the plant is used for warts and cuts.
3.	**Gmelina asiatica L**	*Verbenacea e*	Leaf	sterols, alkaloids, tannins, glycosides, triterpenoids, alkenes, phenolic acids, choline, and shikimic acid	Diabetes, antimicrobial activity
4.	**Santalum Album**	*Santalaceae*	Leaf	Santalol, Santyl acetate and Santalene	Can treat respiratory tract infections, astringent and it is used to helps and resolve mucous congestion. Headaches, insomnia, nervous tension, and anti -viral effect
5.	**Portulaca olerac**	*Portulacaceae*	Leaf	Oxalic acids, alkaloids, Omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides and anthraquinone glycosides. It has high contents of Omega-3 fatty acids and proteins	used as antidiarrhoeal, antihelminthic, antiphlogistic and bactericide in bacillary dysentery, hemorrhoids, enterorrhagia, antidiabetic, externally used as cataplasm of fresh leaves formaturing of abscesses

MATERIALS AND METHODS

Plant collection and extraction:

The Fresh plants/plant parts were collected in September 2010from nilagir dist, Tamil nadu, India. Department of botany Annamalai University. Identified these plants. Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles. Ethno-botanical information of all the plants screened is given in **table 1.** 10g of dried plant material was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 hr the supernatant was concentrated to make the final volume One-fifth of the original volume [17].The extract was then autoclaved at 121^{o} C and 15 lbs pressure, and stored at 4oC.in solvent extraction 10g of dried plant material was extracted 100ml of methanol kept on a

rotary shaker for 24 h thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume[17]. It was stored at 4°C in airtight bottles for further studies.

Micro-organisms tested:

The following strains of bacteria and fungi were used:, *Escherichia coli* (ATCC 25922), ,*Bacillus subtilis*, ATCC 11778,, *Pseudomonas aeruginosa* , (ATCC 27853), Salmonella typhimurium (ATCC 0650),*Staphylococcus aureus*, (ATCC 12228), , *Candida albicans (* ATCC 7596) and *Aspergillus Niger*. (ATCC 9763*)* (Ncl, pune). The organisms were further identified and maintained in stock culture.

A) Preparation of standard bacterial suspensions:

The average number of viable *Escherichia coli*, Bacillus *subtilis*, Pseudomonas *aeruginosa,* Salmonella typhimurium, Staphylococcus *aureus*, and organisms per ml of the stock suspensions was determined by means of the surface viable counting Technique (Miles and Misra, 1938). About (108-109) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

B) Preparation of standard fungal suspensions:

The fungal cultures (*Aspergillus Niger, Candida albicans* were maintained on Saboraud dextrose agar, incubated at 25ºC for 4days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100ml) of sterile normal saline and the suspension was stored in refrigerator till used.

In vitro testing of extracts for antimicrobial activity:

Testing for antibacterial activity:

The cup-plate agar diffusion method was adapted according to Kavanagh, (1972) to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial Stock suspensions (108-109) colony- forming units per ml was thoroughly mixed with 60ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into Sterile Petri dishes. The agars was left to set and in each of these plates 4 cups, 10 mm in Diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using micro titer-pipette and Allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

Testing for anti-fungal activity:

The same method as for bacteria was adopted. Instead of nutrient agar, yeast and mould extract agar was used. The inoculated medium was incubated at 25ºC for two days for the *Candida albicans* and three days for *Aspergillus Niger*. Their sensitivity to the reference antibiotics was checked (Table 2). Erythromycin and gentamycin (Sigma, USA) were used for the bacteria; Nystatin (Sigma, USA) was used for the Candida albicans.

Organisms	Mean Diameter Inhibition Zone(mm)									
	Azadirachta indica		*Euphorbia hirta*		*Gmelina asiatica L.*		*Santalum Album*		*Portulaca oleracea*	
	Aqueous	*Methanol*	Aqueous	*Methanol*	Aqueous	*Methanol*	Aqueous	*Methanol*	Aqueous	*Methanol*
E. coli,	(-)	*(-)*	(-)	4	(-)	(-)	(-)	4	(-)	(-)
B. subtilis	(-)	*27*	(-)	11	(-)	11	(-)	11	(-)	7
P. aeruginosa	(-)	*15*	12	23	11	(-)	(-)	5	(-)	11
S. typhi	(-)	*21*	11	25	(-)	(-)	(-)	8	(-)	10
S. aureus	(-)	*17*	(-)	14	(-)	(-)	(-)	6	(-)	11
A. Niger ,	(-)	*(-)*	(-)	(-)	(-)	(-)	(-)	2	(-)	(-)
C. albicans	(-)	*15*	(-)	8	(-)	(-)	5	17	0	9

Table 2
Antibacterial activity of aqueous and methanol extracts of screened medicinal

Plants:

RESULTS AND CONCLUSIONS

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure The methanol and aqueous extracts of the *Portulaca oleracea* , of *Azadirachta, indica* Euphorbia hirta, Gmelina asiatica L**,** and Santalum Album were subjected to a preliminary screening for antimicrobial activity against five standard bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Salmonella typhi*) and two fungi (*Aspergillus Niger* and *Candida albicans*). It was clear from table (1), that both Methanol and aqueous extracts of the Leaf.

The methanol extract of *Azadirachta indica* exhibited pronounced activity against *Bacillus subtilis* (27 mm), high activity against the Gram-positive organism s*taphylococcus aureus* (17mm), the Gram-negative bacteria *Salmonella typhi* (21 mm), low activity against *Pseudomonas aeruginosa* (15 mm) and inactive against *Escherichia coli* . These might be due to presence of triterpenoids , phenolic compounds , Carotenoids, steroids , ketones and tetratriterpenoids azadirachtin These results were similar to those reported by [8,9] All extracts were inactive against *Aspergillus niger*. The methanolic extracts of *Azadirachta indica* exhibited high activity against *Candida albicans* (15-18mm); while its aqueous extract was inactive against *Candida albicans.*

Methanol extract of *Portulaca oleracea* showed high activity against both Gram-positive organisms *Bacillus subtilis* (20mm) and *Staphylococcus aureus* (15mm) and only active against one Gram-negative bacteria namely *Pseudomonas aeruginosa* (18mm) These might refer to the presence of coumarins, flavonoids and saponins as chemical components of these plant[13], determined similar results and found that the methanol extract of *Portulaca oleracea* seeds (20mg/ml) was active against *Staphylococcus aureus*, *Bordetella bronchiseptica* and *Bacillus cereus*. All extracts of the seeds of *Portulaca oleracea* were inactive against *Aspergillus* antifungal properties against *Aspergillus Niger* and *Candida albicans*

The methanol extract of Euphorbita hirta exhibited pronounced activity against *Salmonella typhi(25mm), Staphylococcus aureus* (14mm) *Bacillus subtilis* (11 mm), and

Pseudomonas aeruginosa (23mm)high activity against the Gram-positive organism the Gram-negative bacteria , low activity against *Candida albicans* (8mm) and inactive against *Escherichia coli, Aspergillus Niger.*. the aquose extract Euphorbita hirta active against *Pseudomonas aeruginosa* (12mm)and *Salmonella typhi(11mm).*These sterols, alkaloids, tannins, glycosides, triterpenoids, alkenes, phenolic acids, choline, and shikimic acid. These results were similar to those reported by [4]

The methanol extract of *Santalum Album* activity against the Gram-positive organism the Gram-negative bacteria Such as *Bacillus subtilis* (11 mm), *Salmonella typhi(8mm), Staphylococcus aureus* (6mm) and *Pseudomonas aeruginosa* (5mm)high activity against *Candida albicans* (17mm) and inactive against *Escherichia coli, Aspergillus Niger.*. The .

extract *Santalum Album* Contains High level of Santalol, Seaquiterpenol, Fragrance oil. Reported [13]

The methonolic extract of gemelina asitica active against only in Bacillus *subtilis* (11 mm), the aqueous extract of gemelina asitica effective against *Pseudomonas aeruginosa* (11mm).and inactivity against *Escherichia coli, Aspergillus Niger, and Candida albicans.* All extracts were inactive against *Aspergillus Niger*. The and methanolic extracts of *Azadirachta indica* exhibited high activity against *Candida albicans* (15-18mm); while its aqueous extract was inactive against *Candida albicans*. It is not surprisingly that there are difference in the antibacterial activities of the extracts of the different extracts of tested plants this could be due to the phytochemical differences between them.

Fig 1

Antimicrobial activity of some medicinal plant

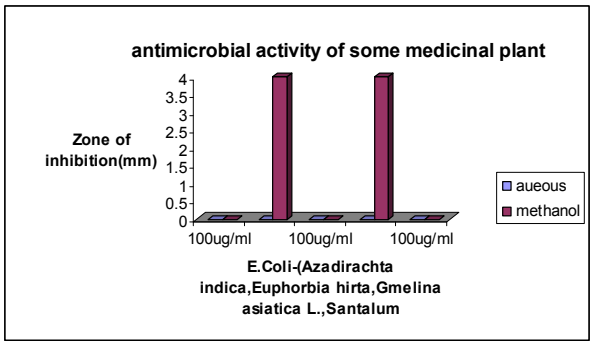
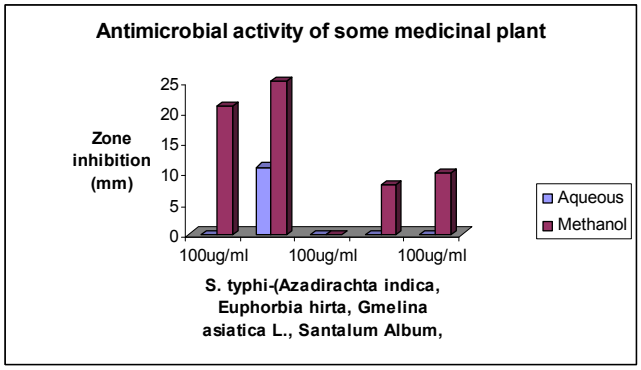


Fig.2

Antimicrobial activity of some medicinal plant:



The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used, as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic Antimicrobials and undergo further pharmacological evaluation

Fig.3

Antimicrobial activity of some medicinal plant:

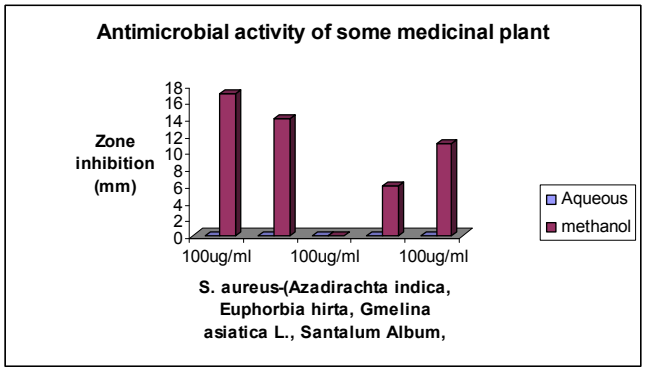


Fig.4

Antimicrobial activity of some medicinal plant:

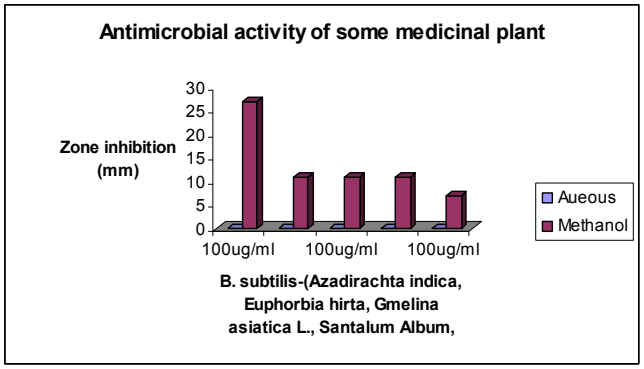


Fig.5

Antimicrobial activity of some medicinal plant:

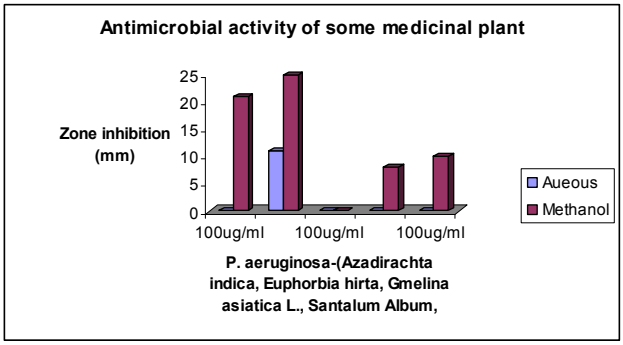
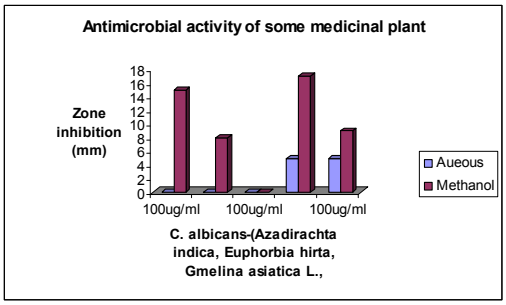


Fig.6

Antifungal activity of some medicinal plant:



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