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Phytochemical Screening and Bioactivity of Some Indian Medicinal Plants

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Medicinal plants are having various phytochemicals which show diverse bioactivity. In this study, various medicinal plants were collected and analyzed for their bioactivity and also screened for their phytochemical constituents. TLC-bioautography for antioxidant activity confirmed the presence of antioxidant molecule in *Aegle marmelos*, *P. murex* and *H.enneasperms*. *Aegle marmelos* and other plants too showed antibacterial activity. Antibacterial activity was confirmed by SEM analysis

Key words: Phytochemicals; Bioactivity; TLC-bioautography, SEM - scanning electron microscopy.

There are 250,000 to 500,000 species of plants inhabiting the land throughout the globe, of which only a few have been explored [1]. It is evident that those with biological activity especially in the protective and disease preventive properties are considered as medicinal plants and are fervently used in therapeutic purposes. These medicinal plants have attracted the modern medicine towards them, as they possess some diverse chemical substances like alkaloids, tannins, flavonoids and phenolic compounds with novel bioactivity that are safe with fewer or no side effects. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines[2]. Drug resistance is becoming a serious threat for the management of infectious diseases[3]. Moreover most antibiotics have been withdrawn from the market because of serious adverse effects. Plant derived antimicrobial agents have got two reasons to be worked more one for its fewer side effects and the other to overcome multi drug resistance in microorganisms. The plants chosen for the study are based on their prophylactic importance in the Indian natural system of medicine. *Aerva lanata* belonging to the family *Amaranthaceae* are commonly used for treating diabetes[4] and it also known to possess antimicrobial activity[5]. *Hybanthus enneaspermus* of the family *Violaceae*are used to treat diarrhea, dysuria, urinary tract infections and diabetes[6,7]. *Phyllanthus emblica* with a rich source of vitamin C belonging to *Euphorbiaceae* has got good antioxidant and other bioactive properties[8], *Tribulus terrestris* belonging to the family *Zygophyllaceae* are valuable herb which has been proven to possess various pharmacological properties[9]. *Pedalium murex* and *Aegle marmelos* have been validated and proven to have antioxidant and antimicrobial activity[10,11].

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MATERIALS AND METHODS

Collection of medicinal plants

Leaves of *Pedalium murex, Hybanthus enneaspermus, Phyllantus emblica, Tribulus terrestris,* and *Aerva lanata,* were collected from Dindigul, Tamil Nadu, India and the leaves of *Aegle marmelos* from Chennai, Tamil Nadu, India. The collected leaves were washed with water and shade dried for 14 days, after which it was pulverized and stored in air tight containers for further use**.**

Preparation of the extract

The powdered plant sample (Leaves) were subjected for both aqueous and ethanol extraction. Aqueous extraction was done by grinding 100g of samples in distilled water with the help of mortar and pestle, the crushed samples were centrifuged and supernatant was collected and dried.

Ethanolic extract was taken by adding 99% dehydrated ethanol with 100g of the powdered sample and kept in the conical flask for 72 hours in a shaker. Then the extract was collected and filtered using standard filter paper. The filtrate was dried using rotary vacuum evaporator. Extract thus obtained was stored for further uses.

Phytochemical screening - tests for secondary metabolites

Tests for phytochemicals like Tannins, Saponin, Flavonoids, Steroids, Terpenoids, Glycosides and Carbohydrates were done following the method of Edeoga *et al* [12].

Determination of antimicrobial activity

The antimicrobial assay was performed using agar well diffusion method[13]. The crude plant extract of 100mg was dissolved in 500µl of the solvent. The extract was added at a concentration of 5, 10, 15, 20 µg/ml concentrations into wells. All the plant extracts were subjected to antibacterial activity against *Bacillus subtilis, Klebsiella pneumoniae* and *E.coli* and antifungal activity against *Hypocrea lixii, Aspergillus tereus* and *Phoma* sp. Standard antibiotic disc were used as positive control. Plates were closed and left for some time till the extract diffuse into the medium which were then swabbed with overnight grown microbial cultures using sterile cotton swab and incubated at 37°C for 24 hour. After incubation the plates were observed for zone of inhibition.

Thin layer chromatography (TLC) - bioautography for antioxidants

The Thin layer chromatography was performed for the leaf extracts of the Indian medicinal plants with silica gel as the stationary phase and chloroform as the mobile phase [14]. The chromatogram was allowed to dry and was then sprayed with DPPH to qualitatively identify the separated compounds for antioxidant property[15] and their corresponding retention factor (Rf) values were measured.

Analysis of biofilm inhibition using scanning electron microscope

Sterile cover slips were coated with plant extracts and kept inside the boiling tube containing sterile nutrient broth. *E.coli* was inoculated into the broth and allowed to grow for 24 to 48 h at 37^0C on the cover slip coated with the different extracts, after incubation the coverslips were rinsed with sterile saline and viewed under the scanning electron microscope.

RESULTS

The phytochemical analysis for the aqueous and ethanolic extracts of the Indian medicinal plants shows moderate presence of sterols, glycosides, saponins, tannins, primary amines while excess presence of flavonoids for *Tribulus terrestris. Aerva lanata* has also the same phytochemicals that of the *Tribulus terrestris* with carbohydrates occurring in addition. *Pedalium murex* extracts were found to contain carbohydrate, glycosides, tannins, aminoacids, and flavonoids in moderate level while sterols are found in high concentration. *Phyllanthus emblica* has phenols, tannins, primary amines in excess while saponins and glycosides are found at a moderate level and the other phytochemicals are absent in the extract. *Hybanthus enneaspermus* has carbohydrates, glycosides, sterols and primary amines whereas phenols and flavonoids are found to be moderately present (Table 1).

Antibacterial activity of the aqueous and ethanolic extracts of the Indian medicinal plants *Aegle marmelos, Aerva lanata, Phyllanthus emblica, Tribulus terrestris, Pedalium murex* and *Hybanthus ennaesperms,* was tested for their antimicrobial activity against three different bacterial strains namely *E.coli, Klebsiella*

pneumoniae and *Bacillus subtilis*. Among all the plant extracts the ethanolic extract of *Pedalium murex* and *Aegle marmelos* was found to be very effective against the bacterial pathogens (Table 2) (Figure 1). The study indicates that the aqueous extract of *H.enneaspermus, P.murex, A.lanata, and T.terrestis* did not show any activity where as the ethanolic extract of *H.enneaspermus* was showing good activity for all the concentrations used. The ethanolic extracts of *A.lanata, T.terrestris* were found to be active against *K.pneumoniae* and *B.subtilis*. Both the aqueous and ethanolic extracts of *P.emblica* were found to be active against *E.coli, B.subtilis* and no activity against *K.pneumoniae.* The ethanolic extracts of *P.murex* did not respond to *B.subtilis.*

Ethanolic extract of *Pedalium murex, Phyllanthus emblica* and *Aegle marmelos* were found be effective against the fungal pathogens too (Table 3). The ethanolic extracts of *Phyllanthus emblica, Hybanthus enneasperms, Aegle marmelos* and *Tribulus terrestris* were active against *A.tereus* and not against any other organisms taken for this study. The zone of

Table 1. Phytochemical analysis of some of the Indian medicinal plants

Phytochemical	*Tribulus terrestris*	*Aerva lanata*	*Pedalium murex*	*Phyllantus emblica*	*Hybanthus enneaspermus*	*Aegle marmelos*
Starch	-	-	-	-	-	-
Cellulose	-	-	-	-	-	-
Carbohydrate	-	+	+	++	++	-
Phenols	-	-	-	++	+	-
Sterols	+	+	++	-	++	-
Glycosides	+	+	+	+	++	-
Saponin	+	+	-	+	-	-
Tannin	+	-	+	++	++	+
Amino acid	-	-	+	-	-	-
Primary amine	+	+	-	++	++	-
Flavanoids	++	++	+	-	+	-

(+) indicates moderate, (++) indicates high, (-) indicates absence

Table 2. Antibacterial activity of different concentrations of the extracts of some of the Indian medicinal plants by cup diffusion assay

Organism	Conc (µg)	*Hybanthus enneasperms*		*Pedalium murex*		*Aerva lanata*		*Phyllanthus emblica*		*Tribulus terrestris*		*Aegle marmelos*	
		A	E	A	E	A	E	A	E	A	E	A	E
E.coli	5	-	1	-	-	-	-	-	-	-	-	-	0.6
	10	-	1	-	0.8	-	-	0.9	-	-	-	-	0.7
	15	-	1.2	-	1.2	-	-	1.1	-	-	-	-	0.9
	20	-	1.9	-	1.8	-	-	1.6	1.4	-	-	-	1.3
K. pneumoniae	5	-	0.6	-	-	-	0.5	-	-	-	0.4	-	Nil
	10	1	0.6	-	-	-	0.8	-	-	-	0.5	-	0.6
	15	1.4	0.8	-	1	-	1.4	-	-	-	1	-	0.8
	20	1.4	1.3	-	1.5	-	1.5	-	-	-	1.2	-	1.2
B.subtilis	5	0.5	0.7	-	-	-	0.4	-	-	-	0.5	-	0.5
	10	0.7	0.9	-	-	-	0.5	0.5		-	0.5	-	0.5
	15	0.8	1.1	-	-	-	0.7	0.9	1.2	-	0.6	-	0.7
	20	1	1.6	-	-	-	1	1.1	1.5	-	1	-	1.1

Zone of inhibition was measured in cmA-aqueous extract E- ethanol extract

Table 3. Antifungal activity of different concentrations of the extracts of some of the Indian medicinal plants by cup diffusion assay

Organism	Conc (µg)	*Hybanthus enneasperms*		*Pedalium murex*		*Aerva lanata*		*Phyllanthus emblica*		*Tribulus terrestris*		*Aegle marmelos*	
		A	E	A	E	A	E	A	E	A	E	A	E
Aspergillus terreus	5	-	0.5	-	-	-	-		0.5	-	0.7	-	0.6
	10	-	0.5	-	-	-	-	-	0.6	-	0.8	-	0.6
	15	-	0.7	-	-	-	-	-	0.8	-	0.9	-	0.7
	20	-	0.8	-	-	-	-	-	1	-	1	-	0.8
Phoma sp	5	-	-	-	-	-	-	-	-	-		-	
	10	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-	-	-
Hypocrea lixii	5	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-	-	-

A-aqueous E-ethanol

Table 4. Rf value of some of the fractions showed antioxidant activity

Fraction	*H.enneasperms*	*P.murex*	*A.marmelos*
1	0.18	0.52	0.14
2		0.45	0.22
3		0.39	
4		0.3	

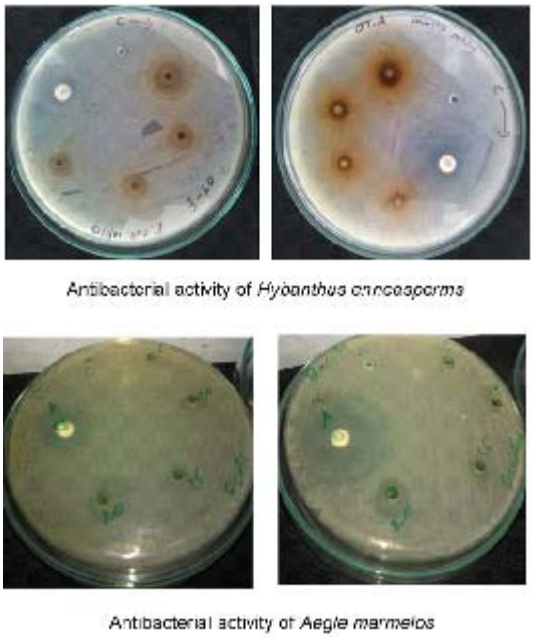

Fig. 1. Antibiogram of some medicinal plants

inhibition increased with the increase in concentration and is maximum for 20 µg concentration.

The antioxidant property was analyzed by using thin layer chromatography spraying of DPPH, antioxidant activity was indicated by

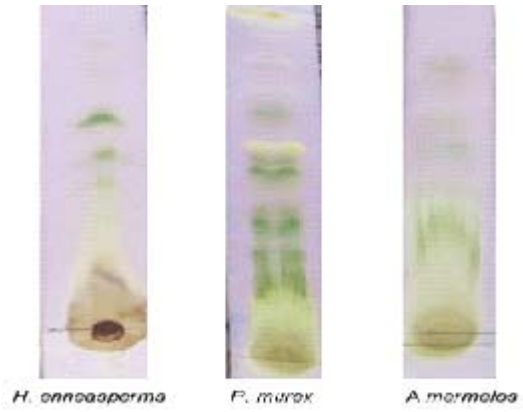

Fig. 2. TLC Bioautography analysis for antioxidants

fluorescence coloured band. The TLC chromatogram of *H.enneasperms* showed the presence of antioxidant activity in a single band with a corresponding rf value of 0.18 (Table 4) (Figure 3). *P.murex* extracts separated into 4 distinct fractions with the retention factor values 0.52, 0.45, 0.39, 0.3 as active fractions. The *A.marmelos* chromatogram showed two active fractions with Rf value as 0.14 and 0.22.

In experiments that we carried out to check the antibiofilm property of the plant extracts *Hybanthus enneaspermusv* and *Aegle marmelos* was found to alter the morphology of the bacterial species. These results were significant enough on comparison with that of the controls. The extracts of *Phyllantus emblica* reduced the bacterial biofilm inhibition and *Pedalium murex* did not have any considerable effect. The images of the biofilms were acquired by the Scanning electron microscope.

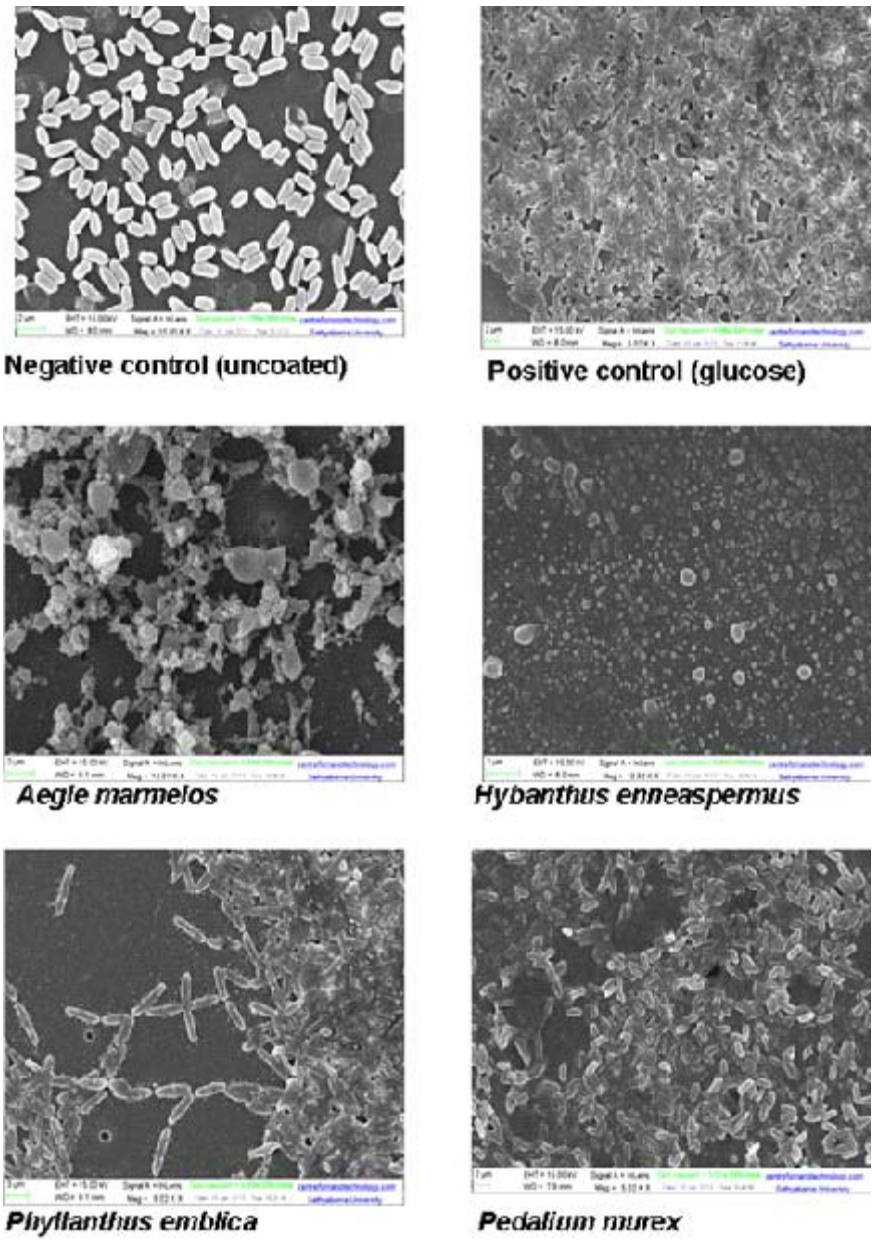

Fig. 3. SEM analysis of biofilm inhibition assay

DISCUSSION

The phytoconstituents responsible for the activity was analysed initially and it was found that carbohydrates, phenols, sterols, glycosides, saponin, tannin, amino acids and primary amines were found. Some plants had these phytochemicals in excess and the others in moderate levels. There are several reports that phytochemicals are responsible for the different bioactivities of the plants. Ethanolic extract of *Aegle marmelos* was proven to have antimicrobial property[16]. James and Friday[17] have observed the wound healing property of *Euphorbia heterophylla* due to the presence of different phytoconstituents. Raghavendra *et al*[18] found the antimicrobial property of *Oxalis corniculata* was due to the presence of phenolic compounds.

Ethanolic extract showed better antimicrobial activity than the aqueous. This may be because the phytochemicals responsible for the activity is poorly present in the aqueous extract. However all extracts did not respond alike which infers the concentration of active constituents is not uniform and needs to be quantified and standardised.

TLC bioautography assay is being used to detect antioxidant which has got simple procedure and highly sensitive assay[19,20]. In this study, three medicinal plants were observed to have antioxidant activity, which has been confirmed by TLC-followed with spraying of DPPH. Wang et al [21] isolated three antioxidant molecules from *B. textilis* by TLC bioautography-guided fractionation. There has been a similar result from Samrot *et al*[11] on the antioxidant activity in some common Indian medicinal plants like *H. suaveolens, E.hirta* etc. In this experiment, it was not only the antimicrobial effect of the extracts analyzed but also the ability of the plant to inhibit the biofilm formation. The success of plant extracts in inhibiting cell attachment as shown in this study is a promising tool for reducing microbial colonisation on surfaces of epithelial mucosa which subsequently leads to infections. The medicinal plants especially *Aegle marmelos* and *H.enneaspermus* had good biofilm inhibition property against which is evident from the SEM results. Namasivayam and Roy[22] have also found the antibiofilm activity of *Azadirachta indica, Vitex negundu, Tridax procumbens* and *Ocimum tenuiflorum*i against *E.coli* by SEM analysis. Khan and Ahmad[23] found eugenol and cinnamaldehyde to exhibit antibiofilm activity against *Candida albicans*, which have been proven by SEM analysis.

CONCLUSION

Still these plants should be studied intensively both in vitro and in vivo for safety so that they can be used to prevent the oxidative stress and also evaluated further to treat multidrug resistant microorganisms.

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