



PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIFUNGAL STUDIES OF *Pittosporum floribundum* Wight & Arn. LEAF, BARK, FRUIT AND SEED EXTRACTS.

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ABSTRACT

The herbal medicinal plant *Pittosporum floribundum* is a rare mountain tree species of Seshachalam Hill Ranges of Eastern Ghats. Local herbalists use this species in healing skin diseases, arthritis, inflammation, bronchitis, leprosy, narcotic and antidote to snake venom. Phytochemical screening reveals the presence of tannins, alkaloids, saponins, glycosides, fixed oils, steroids, flavonoids and phenols mainly in water, alcohol and methanol extracts of leaf, bark, seed and fruit. Methanol extracts of bark showed more effective inhibition zones against all selected bacterial strains and two fungal strains at 20-30mg/ml with 16-25mm Zone of Inhibition than leaf, seed and fruit. Minimum Inhibitory Concentration ranges between 0.312- 1.25 mg against all microbial strains.

KEYWORDS: *Pittosporum floribundum*, Fixed oils, Flavonoids, Methanol extracts, Skin diseases, Minimum Inhibitory Concentration.



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INTRODUCTION

Pittosporum floribundum (Pittosporaceae) is called 'Rakamuki' (Telugu), 'Kattu sampangi', 'Najundai', 'Tammata' (Tamil), 'Tumari', 'Vikhari', 'Vekhali' (Marathi). *P. floribundum* plant parts are used against skin diseases, piles and itches. Bark is aromatic, bitter and greenish black with resinous oil glands. In Ayurveda bark in high doses acts as narcotic used as antidote to snake poison, general weakness and also as a stimulant. The narcotic action of the bark is due to the presence of yellow oleoresins, and also contains saponins and Pittosporins^[1-5]. Bark powder 0.5-1.0 gm is taken orally daily twice in case of asthma^[6]. Bark is bitter and aromatic; possess narcotic properties used as febrifuge, chronic bronchitis which acts as good expectorant. Oil used for rheumatism, skin diseases, sprains, leprosy, bruises, sciatica, chest infections, ophthalmia, cutaneous diseases, secondary syphilis and chronic rheumatism, supports the presence of glycosides^[7]. In New Zealand Mori people used the gum, leaves, flowers and oils of *P. eugenoids* to anoint their bodies. Flowers, roots, bark and leaves are used as anti-inflammatory, antiseptic and in rheumatic disorders. Bark consists of oleoresins, triterpenoids, saponins, stigmasterols^[8-11]. *P. senecia* leaves are used for heart troubles, quinsy and diabetes, *P. neelgherrense* is used as an antidote against snake bite, *P. erocarpum* bark is aromatic and possesses narcotic effect used for chronic bronchitis. Root bark of *P. tetraspermum* is used as a remedy for rheumatic swellings^[3-4].

MATERIALS AND METHODS

Material Collection

Pittosporum floribundum was collected from Tirumala forest, during the months of July and December. The plant was authenticated by Prof. N.Yasodamma and voucher specimens KM11, KM21 were prepared as per the standard method^[12] and deposited in the herbarium, Department of Botany. Plant parts like leaf, bark, fruit and seed materials were collected and thoroughly washed further dried under shade at $28 \pm 2^\circ\text{C}$ for about 10 days. The dried parts were ground well into a fine

powder in a mixer grinder and sieved to give particle size of 50-150mm. The powders were stored in air sealed polythene bags at room temperature.

Phytochemical analysis

Solubility

The solubility was carried out in eight solvents with all parts powders parts (Hot water, Cold water, Benzene, Hexane, Ethyl acetate, Chloroform, Methanol and Ethanol) based on polarity gradient.

Extract preparation

Shade dried leaf, bark, fruit and seed powders were subjected to soxhlet extractions with Benzene, Hexane, Ethyl acetate, Chloroform, Methanol and Ethanol based on polarity gradient. Simultaneously cold water and hot water extracts were prepared. The above obtained semisolid extracts were preserved in air tight bottles at 4°C in a refrigerator until further use.

Preliminary Phytochemical screening

The extracts of the different parts were subjected to phytochemical screening for the presence of phytoconstituents like Alkaloids, Flavonoids, Phenols, Lignins, Anthroquinones, Steroids, Tannins, Saponins, Fixed Oils and Glycosides by using standard methods from the book Harborne and Kokate; Fransworth and Gibbs methods^[13-16].

Antimicrobial activity

Bacterial and Fungal cultures

The bacterial cultures *Bacillus subtilis* (MTCC-441) causes Pneumonia, diarrhea; *Staphylococcus aureus* (MTCC-737) causes bone and joint pains, skin infections, boils; *Escherichia coli* (MTCC-443) causes urinary tract infections, Pneumonia; *Pseudomonas aeruginosa* (MTCC-741) causes urinary infections, bones and joint pains, gastrointestinal tract infections and Pneumonia. The Fungal pathogens like *Aspergillus niger* (ATCC-16404) causes kidney and liver damage, convulsions, hemorrhages of lungs and brain; *Candida albicans* (ATCC-10231) causes infections of mucous membrane of the mouth (Thrush), vagina and alimentary tract,

physiological disorders and obesity. The above bacterial and fungal strains were procured from the Department of Microbiology S.V.University Tirupati.

Preparation of inoculums

Stock cultures were maintained at 4°C of nutrient agar slants. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient agar medium and were incubated without agitation for 24hrs at 37°C.

Well diffusion method

Antibacterial and Antifungal activities of the leaf, Bark fruit and seed alcohol, methanol and ethyl acetate extracts were used for Agar well diffusion method by Perez with slight modifications [17]. Nutrient agar and potato dextrose agar media were inoculated with the selected microorganisms by spreading the bacterial and fungal inoculums. Wells (9 mm diameter) were punched in the agar and filled with plant extracts of 10, 20, 30, 40 mg/well. Control wells containing pure solvents (negative control) and standard antibiotic (positive control) for bacterial *Ampicillin* (10 mg), for fungal *Nystatin* (10mg). The plates were incubated at 37°C±2°C for 24 hours for bacterial and 25±2°C for 48 hours for fungal activity. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. The data of crude drugs activity is given the mean of triplicates along with the standard error.

Minimum inhibitory concentration

Minimum inhibitory concentration was defined as the lowest concentration where no visible turbidity was observed in the test tube (Bacterial / Fungal).The Vollekova method of MIC was modified by Usman was employed

[18-19]. In this method broth dilution technique was used where the leaf, bark, fruit and seed alcohol, methanol and ethyl acetate extracts were prepared to the highest concentration of 10mg/ml (stock). By adding sterile distilled water and serially diluted (two fold) using the nutritive broth and later inoculated with 0.2ml standard suspension of the test organisms. After 18hrs of incubation at 37°C., the test tubes were observed for turbidity .The lowest concentration of the tube that did not show any visible growth can be considered as the Minimum inhibitory concentration.

RESULTS

Solubility

Highest solubility with *P. floribundum* leaf was observed in order of Methanol> Alcohol >Ethyl acetate> Cold water> Hot water extracts, where as Bark, Fruit and Seed in the order of Alcohol>Methanol> Cold Water>Hot water > Ethyl acetate was observed.

Preliminary Phytochemical Screening: (Table: 1)

The Secondary metabolites like alkaloids, flavonoids, phenols, lignins, steroids, tannins, saponins, fixed oils and glycosides are present in all parts. In leaf alkaloids, flavonoids and phenols are present in water extracts; in bark alcohol and methanol extracts; in fruit alcohol extracts; Flavonoids and phenols in water, alcohol and methanol extracts. Tannins, saponins, and fixed oils in all parts in water, alcohol, methanol extracts, and glycosides are absent in water extracts, of leaf and bark; but present in fruit water extracts.

Table 1
Preliminary Phytochemical Screening of *Pittosporum floribundum*

TEST	LEAF								BARK							
	CW	HW	AL	ME	EA	CH	H	B	CW	HW	AL	ME	EA	CH	H	B
Alkaloids	Mayers test	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-
	Wagner's test	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-
Flavonoids	Shinodons test	+	+	-	+	+	+	-	-	-	+	+	-	-	-	-
	Fecl3 test	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-
Phenols	Fecl3 test	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-
	Ellagicacid test	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Lignins		-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Anthroquinones		-	-	+	+	+	-	-	-	-	+	+	-	-	-	-
Steroids	Salkowski test	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+
	Liebermann's Burchard test	-	-	-	+	-	-	-	-	-	+	-	+	+	-	-
Tannins	Gelatin test	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-
	Fecl3 test	-	+	+	+	+	-	-	+	+	+	+	-	-	-	-
Saponins		+	+	+	+	-	-	-	+	+	+	+	-	-	+	-
Fixed Oils		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Keller-kilani test	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+

TEST	FRUIT								SEED							
	CW	HW	AL	ME	EA	CH	H	B	CW	HW	AL	ME	EA	CH	H	B
Alkaloids	Mayers test	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-
	Wagner's test	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-
Flavonoids	Shinodons test	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-
	Fecl3 test	+	+	+	+	+	-	-	+	-	-	+	+	-	-	-
Phenols	Fecl3 test	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-
	Ellagicacid test	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Lignins		-	-	+	+	-	-	-	-	+	+	+	-	-	-	-
Anthroquinones		+	+	+	+	-	-	-	-	+	+	+	-	-	-	-
Steroids	Salkowski test	-	+	+	+	+	-	-	-	-	+	+	-	+	+	-
	Liebermann's Burchard test	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-
Tannins	Gelatin test	+	+	+	+	-	-	-	+	+	+	+	-	+	+	-
	Fecl3 test	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-
Saponins		+	+	+	+	-	-	-	-	+	+	+	-	+	+	-
Fixed Oils		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Keller-kilani test	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+

+++ = Indicates the presence of Secondary Metabolites. - = Indicates the absence of Secondary Metabolites.
 CW: Cold water, HW: Hot water, AL: Alcohol, ME: Methanol,
 EA: Ethyl acetate, CH: Chloroform, H: Hexane, B: Benzene

Antimicrobial Activity: (Graph-1, Table 2-3, Plates 1-4)

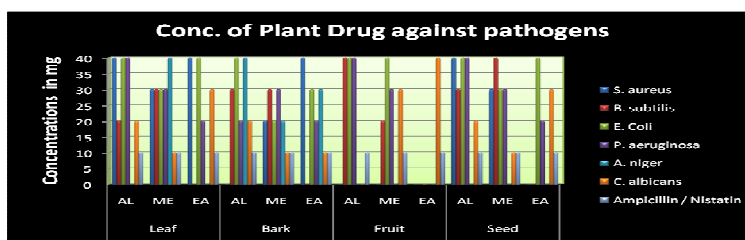
Antibacterial Activity

Antibacterial activity of methanol extracts with 20 to 30mg/well was more effective on all selected bacterial strains but with alcohol and ethyl acetate at 20-40mg /well the activity was observed. Over all bark methanol extracts exhibits effective inhibition with 16 – 25mm of Zone of Inhibition compared to that of the control drug Ampicillin with 14 – 18.4mm at 10mg/well. Fruit ethyl acetate extracts are not showing much better inhibition zones. *B. subtilis* and *E. coli* are more susceptible with all parts of all extracts of *P.floribundum*.

Antifungal Activity

Antifungal activity against the two selected fungal strains methanol extract of leaf and bark at 10-20mg/well showed effective inhibition with 16-25mm Zone of Inhibition to that of the control drug Nystatin 14 to 20 mm at 10mg/well. With Alcohol and Ethyl acetate extracts of the leaf and bark; activity showed from 20-40mg/ well. Fruit alcohol and ethyl acetate extracts showed least inhibition even at 40mg/well. Over all *C.albicans* is more susceptible than *A. niger* with *P. floribundum* plant extracts.

Graph 1
Inhibitory Concentrations of plant drug against bacterial and fungal strains



AL: Alcohol ME: Methanol EA: Ethyl Acetate

Table 2
Antibacterial Activity: mg/ well.

Part	Organisms	<i>S. aureus</i>				<i>B. subtilis</i>			
		10 mg/ well	20 mg/ well	30 mg/ well	40 mg/ well	10 mg/ well	20 mg/ well	30 mg/ well	40 mg/ well
Leaf	Alcohol	7.3±0.94	11.0±0.0	13.0±1.41	17.0±0.81	14.6±0.47	20.6±0.47	22.6±0.47	24.3±0.94
	Methanol	12.6±0.47	14.6±0.47	15.6±0.47	17.6±1.88	9.0±0.0	12.6±0.47	18.0±0.81	18.6±0.47
	Ethyl acetate	9.0±0.0	13.6±0.47	14.6±0.47	16.0±0.0	12.6±0.47	14.6±0.47	16.0±0.81	17.0±0.81
Bark	Alcohol	7.6±0.47	8.0±0.81	9.6±0.47	11.3±1.24	12.6±0.47	16.3±0.47	19.3±1.24	22.0±0.81
	Methanol	14.0±0.81	17.3±0.47	20.3±0.94	22.3±0.47	13.3±0.47	16.6±0.47	20.0±0.81	23.3±0.47
	Ethyl acetate	9.0±0.0	13.6±0.47	14.6±0.47	16.0±0.0	12.6±0.47	14.6±0.47	16.0±0.81	17.0±0.81
Fruit	Alcohol	7.6±0.47	9.0±0.0	11.6±1.24	12.6±0.47	7.6±0.47	9.3±0.94	14.0±0.0	18.0±0.81
	Methanol	8.3±0.47	8.6±0.47	11.0±0.0	14.3±0.47	17.6±0.47	18.0±1.41	18.3±0.94	21.1±0.12
	Ethyl acetate	7.0±0.0	8.6±0.47	9.0±0.0	10.0±0.47	9.0±0.0	9.0±0.0	11.0±0.0	11.6±0.47
Seed	Alcohol	7.2±0.64	10.6±0.24	12.3±0.84	16.8±0.61	13.3±0.3	19.6±0.84	21.3±0.68	24.1±0.84
	Methanol	12.3±0.2	13.±0.67	15.0±0.83	17.3±0.64	8.5±0.64	12.3±0.4	17.6±0.94	18.0±0.42
	Ethyl acetate	7.0±0.0	7.0±0.0	7.0±0.0	9.0±0.0	12.2±0.2	13.5±0.8	15.6±0.6	16.6±0.6
Ampicillin (10 mg/ Well)		15.2±0.25				18.4±0.45			

Part	Organisms	<i>E. Coli</i>				<i>P. aeruginosa</i>			
		10 mg/ well	20 mg/ well	30 mg/ well	40 mg/ well	10 mg/ well	20 mg/ well	30 mg/ well	40 mg/ well
Leaf	Alcohol	8.6±0.47	12.0±0.0	15.0±1.41	17.6±0.47	9.0±0.0	11.6±0.47	13.0±0.0	18.0±0.81
	Methanol	11.6±0.47	15.6±0.47	18.6±0.47	18.6±0.94	12.3±0.94	13.0±1.41	15.6±0.47	16.0±0.0
	Ethyl acetate	10.3±0.47	12.6±0.47	15.6±0.47	17.3±0.94	10.3±1.24	18.6±0.47	19.6±0.47	22.6±0.47
Bark	Alcohol	10.6±0.47	11.0±0.47	14.0±0.0	18.0±0.81	12±0.47	20.0±0.47	26.0±0.0	27.0±0.81
	Methanol	15.0±0.81	20.6±0.94	22.0±0.81	25.3±0.47	12.0±0.81	13.0±0.0	16.0±0.81	18.0±0.81
	Ethyl acetate	10.3±0.47	12.6±0.47	15.6±0.47	17.3±0.94	10.3±1.24	18.6±0.47	19.6±0.47	22.6±0.47
Fruit	Alcohol	7.0±0.0	10.6±0.94	14.3±0.47	21.0±0.0	7.3±0.94	8.0±0.0	12.0±0.0	17.6±0.47
	Methanol	10.3±1.24	13.0±0.0	15.3±0.94	20.3±0.47	8.6±0.47	12.3±0.94	18.0±0.0	18.0±1.41
	Ethyl acetate	7.3±0.94	11.6±0.47	14.0±0.0	15.3±0.94	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Seed	Alcohol	8.3±0.67	11.5±0.62	14.6±0.83	17.2±0.47	8.7±0.38	10.5±0.07	12.7±0.43	17.8±0.61
	Methanol	11.3±0.8	14.6±0.2	17.8±0.8	18.2±0.78	12.6±0.8	12.8±0.2	14.8±0.63	15.9±0.21
	Ethyl acetate	9.8±0.47	11.8±0.8	15.1±0.9	17.6±0.9	9.9±0.86	17.9±0.7	19.1±0.8	22.1±0.4
Ampicillin (10 mg/ Well)		16.2±0.20				14.4±0.40			

Values are mean Inhibition Zone (mm) ±S.D. of Triplicates.

Table 3
Antifungal Activity: (mg/well)

Part	Organisms	<i>A. niger</i>				<i>C. albicans</i>			
		10 mg/ well	20 mg/ well	30 mg/ well	40 mg/ well	10 mg/ well	20 mg/ well	30 mg/ well	40 mg/ well
Leaf	Alcohol	12.0±0.45	13.0±0.0	14.0±0.49	15.0±0.83	13.6±0.47	14.6±0.47	15.3±0.94	17.0±0.81
	Methanol	14.3±0.47	15.3±0.47	16.6±0.94	17.0±1.0	22.3±0.47	24.0±0.0	24.3±0.0	25.0±0.47
	Ethyl acetate	8.6±0.47	9.0±0.0	9.0±0.0	10.0±0.0	11.6±0.47	13.3±0.47	15.3±0.94	16.0±0.0
Bark	Alcohol	10.0±0.0	11.6±0.04	15.0±0.81	19.0±0.0	12.0±0.0	18.0±1.41	20.0±0.0	24.6±0.94
	Methanol	15.6±0.47	20.0±0.81	21.0±0.0	24.6±0.47	15.0±0.0	16.3±0.94	17.0±0.0	19.0±0.0
	Ethyl acetate	13.0±0.04	13.0±0.08	16.0±0.04	18.3±0.09	20.3±0.47	20.6±0.47	21.6±0.47	22.3±0.94
Fruit	Alcohol	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	11.3±0.47	12.0±0.0	12.0±0.94	12.6±0.94
	Methanol	11.3±0.47	14.0±0.0	14.0±1.24	15.0±0.0	12.0±0.0	12.6±0.47	14.3±0.47	15.0±1.41
	Ethyl acetate	11.6±0.47	12.3±1.24	13.3±0.94	15.3±0.94	11.0±0.0	12.0±0.0	13.3±0.94	15.6±0.47
Seed	Alcohol	11.5±0.67	12.6±0.73	13.7±0.73	14.9±0.24	13.1±0.63	14.9±0.84	14.4±0.86	16.6±0.96
	Methanol	14.0±0.23	14.9±0.93	15.9±0.84	16.6±0.24	21.6±0.63	23.6±0.89	24.0±0.46	24.6±0.84
	Ethyl acetate	8.2±0.64	8.6±0.94	8.7±0.84	9.7±0.0	11.4±0.68	12.8±0.98	14.6±0.68	15.6±0.93
Nystatin (10 mg)		16.0±0.0				14.0±0.0			

Values are mean Inhibition zone (mm) ± S.D of Triplicates

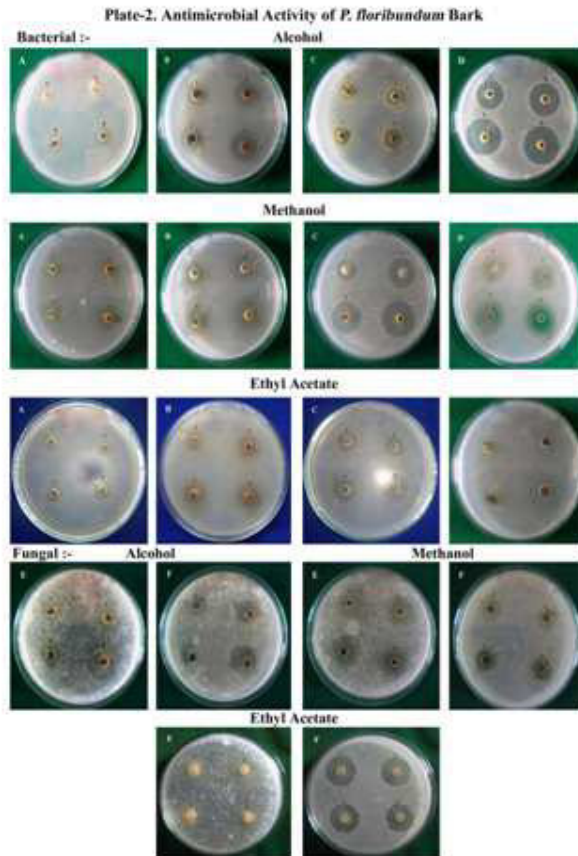
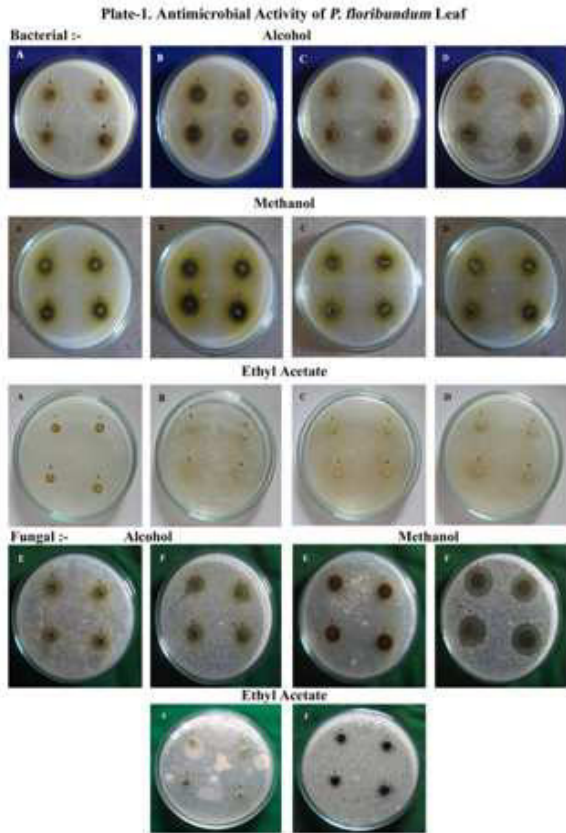


Plate-3. Antimicrobial Activity of *P. floribundum* Fruit

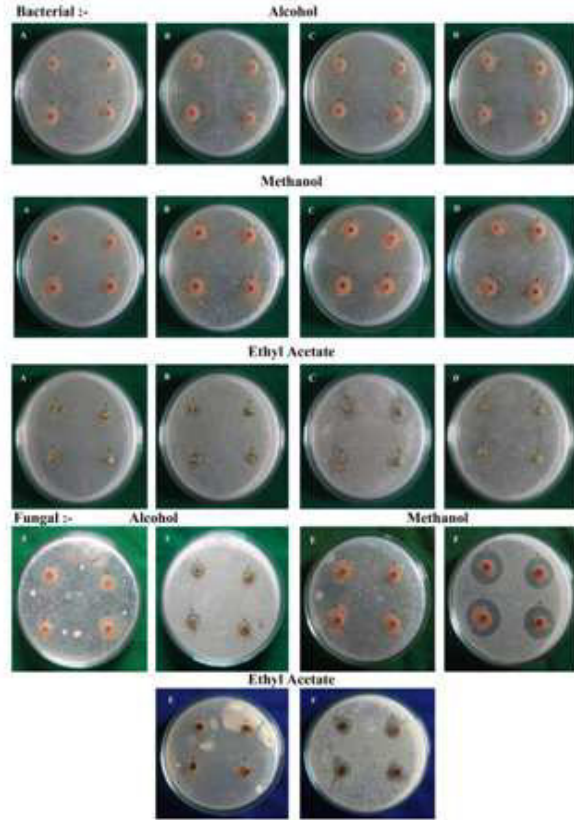
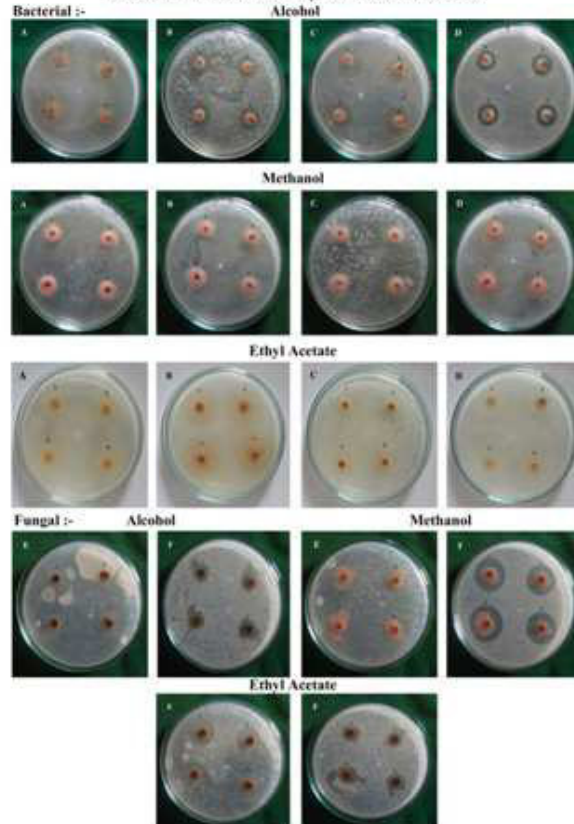
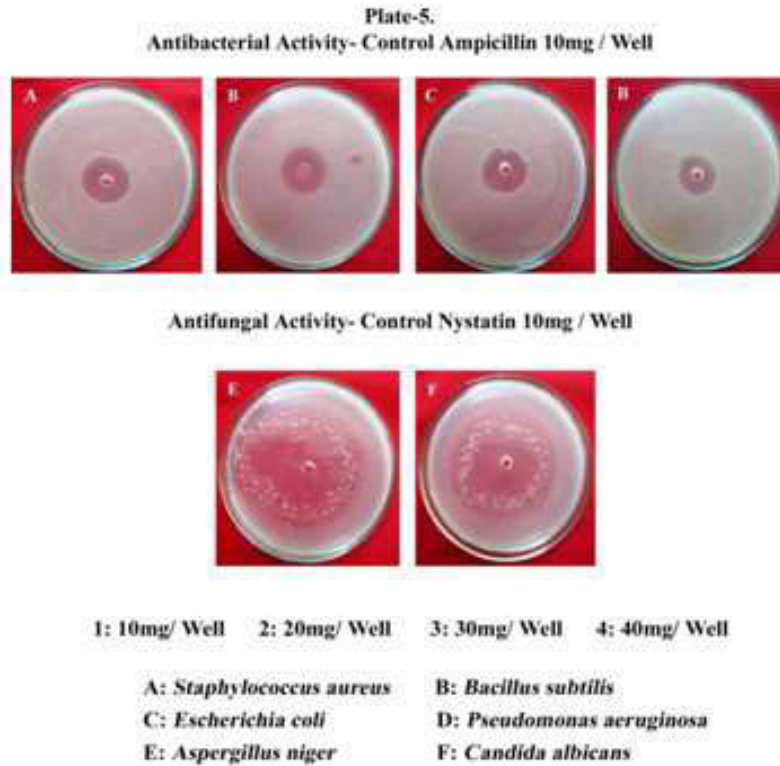


Plate-4. Antimicrobial Activity of *P. floribundum* Seed

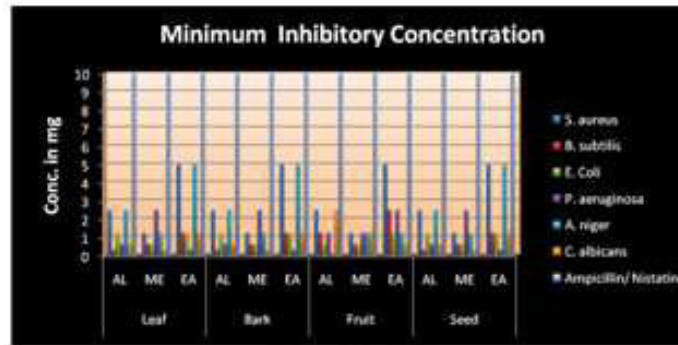




Minimum Inhibitory Concentrations: (Graph: 2, Plate: 5)

Minimum Inhibitory Concentrations of all parts with all extracts against all pathogens of bacterial and fungal strains ranges from 0.312 to 1.25mg at its lowest.

Graph 2



AL: Alcohol ME: Methanol EA: Ethyl Acetate

DISCUSSION

P. senecia leaf oil contains sesquiterpene δ -cadinene (11.3%), α murolol (15.9%) and α -cadinol (19.0%) [20]. *P. viridiflorum* leaf oil contains sesquiterpene δ -cadinene (10.6%) and α -cadinol (18.3%). The major fruit oils sabinene (13.2%), decanal (10.3%), β -elemene (9.5%), β -pinene (8.7%), α -pinene

(8.0%) and α -cadinol (8.1%). The leaf and fruit oils had similar inhibitory effects on all bacterial strains except fruit oil against *Pseudomonas aeruginosa*, with less activity [21]. The leaves of *P. viridiflorum* consists of 15 components and yield 85.4% of oils where as the mature fruits contains 26 components and

yield 94.5% of oils which showed effective antimicrobial activity against gram negative bacteria like *S. aureus* and *Salmonella typhi*. The leaves and fruits of *P. neelgherrense* contain 21 components comprising of 97.6% of oils; fruits consists 20 components comprising of 81.3% of oils. Undecane (62.2%) as the major component in the leaf followed by caryophyllene oxide (9.0%), β -caryophyllene (8.7%), β -Selinene (11.9%), fruit oil consists of Undecane (11.3%), nonane (8.8%) and α -pinene (8.4%). Oils show moderate activity against most of the tested gram-positive and gram-negative bacteria [22]. Essential oils from the bark of *P. dasycaulon* consists of dodencanal (53.43%), undecane (20.84%), hexadecanal (9.95%) dodecanoic acid (3.6) and 1-tridecanol (2.15%). These oils also shows effective antimicrobial activity against all gram positive and gram negative bacteria except on *Bacillus subtilis*. The Minimum Inhibitory Concentration ranges from 25-100 μ l/ml [23]. *P. undulatum* contain monoterpenoids, diterpenoids, sesquiterpenoids and alkanes, showed effective antimicrobial activity against *P. aureus*, *S. epidermis* and *S.aeruginosa* [24]. Essential oils antifungal activity against *A. flavus* found the inhibition of the aflatoxin B₁ production [25]. Crude saponin mixture of *P. tetrasporum* leaves showed effective antifungal activity 13.3mm Dz than that of the control *Nystatin* 12mm Dz [26]. Water, Ethyl acetate and chloroform extracts of *P. phylli-raeoides* major phytoconstituents such as alkaloids, flavonoids, phenols, saponins and proanthocyanidins, show effective anti-

microbial activity against 14 bacterial strains and 1 fungal strain. But no activity against *Candida albicans*. [27-28]

CONCLUSION

The present study supports the presence of fixed oils, alkaloids, glycosides, tannins, steroids, flavonoids and phenols in all the extracts with large quantities in all plant parts. Leaf, bark, fruit and seed crude extracts of alcohol, methanol, ethyl acetate exhibits an effective antibacterial activity against all tested bacteria to that of control drug. The most susceptible bacteria are *P. aeruginosa*, *B. subtilis*, and fungi *C. albicans* with *P. floribundum* bark methanol extracts. In earlier studies *P. neelgherrense* leaf, bark, fruit and seed resulted major percentage of essential oils like decanols, which exhibits moderate antimicrobial activity. Species like *P. viridifolium*, *P. undulatum*, *P. dasycaulon*, and *P. senacia* also possess essential oils of different groups and terpenoids expressed effective antibacterial activity. The above work supports the herbal and traditional uses against skin diseases, arthritis, inflammatory, spasmodic, sciatica, sprains, bronchitis, chest pains, antidote to snake bite, narcotic and also in curing leprosy may be due to the presence of the major secondary metabolites in the crude extracts and the effective activity with lowest concentrations on all bacterial and fungal strains which may cause the health disorders to that of the herbal uses of *Pittosporum floribundum*.

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