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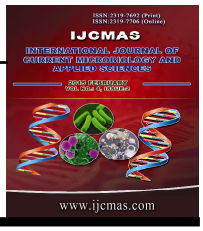

Original Research Article

Phytochemical Analysis and Antimicrobial Properties of Extracts from Aerial Parts of *Phyla nodiflora* (L) Greene

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Antimicrobial activity, Medicinal plants, Phytochemicals, Plant extracts, *Phyla nodiflora*.

A B S T R A C T

Bacteria develop resistant to many antibiotics leading to multi-drug resistant (MDR) strains and hence the discovery of efficient and novel antibacterial agents is essential. Crude extracts from aerial parts of *Phyla nodiflora* were screened for phytochemical and antimicrobial properties. Powdered plant material was sequentially extracted with solvents namely hexane, chloroform, ethyl acetate and methanol. The antimicrobial activity of the plant extracts was evaluated using human pathogenic bacteria and fungi by using disc diffusion assay. All the extracts from *Phyla nodiflora* had inhibitory effects in both bacteria and fungi. The results of this study clearly proved that plant is a potential source of natural antimicrobial agents.

Introduction

Medicinal plants represent a rich source of antimicrobial agents and are used as a source of many potential and powerful drugs in several countries (Srivastava *et al.*, 1996). Plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (Chopra *et al.* 1992) and have a great potential for producing new drugs for human benefit. Traditional medicine prepared using plants contain a vast array of substances that can be used to treat chronic and infectious diseases. With an increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds is very important.

Traditional medicine based on plants has played a key role in the health care system of many countries like India, China etc., (Sudhersan, 1998). About 60% of the total global population remains dependent on traditional medicines for their health care system (Kumar *et al.*, 2004).

Phyla nodiflora Linn. (Verbenaceae) known as *Lippia nodiflora* is an evergreen, creeping, branched herb is distributed in India, Sri lanka, Ceylon, South and Central America and Tropical Africa. In literature review it was found that the aerial parts are used as anodyne, antibacterial, diuretic, parasiticide, refrigerant, febrifuge and cooling (Agarwal, 1997). *Phyla nodiflora*

contains flavonoids, sugars, sterol, an essential oil, resin, non glucosidal bitter substance, tannin, large amount of potassium nitrate and other constituents (Anonymous, 1962) Several workers have reported many pharmacological properties including antispasmodic hair afflictions (Panniachamy *et al.*, 1989), anti-inflammatory, analgesic and antipyretic (Forestieri *et al.*, 1996), anti *Helicobacter pylori* activity (Yuan Chuen and Tung Liany, 2005), hypotensive activity (Akhtar, 1993).

Materials and methods

Collection of the plant material

Phyla nodiflora was collected from the medicinal plant garden at Irula Tribal Women's Welfare Society, Chengalpattu, Chennai, India.

Preparation of the extract

Aerial parts of *Phyla nodiflora* were collected, shade dried and were coarsely powdered using a mechanical grinder. It was then passed through sieve and the fine powder size was stored in air tight container. The sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using hexane, chloroform, ethyl acetate and methanol successively. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extract obtained was filtered, concentrated in rotary flash evaporator and dried in a vacuum oven. The percentage yield of each extract was calculated and the dried extract was stored in air tight containers for further studies.

Phytochemical analysis

The crude extracts were used for the qualitative analysis of the primary metabolites (Proteins and carbohydrates) and secondary metabolites (alkaloids, flavonoids, saponins, steroids, terpenoids, and glycosides). The screening for all the primary and secondary metabolites was done according to standard protocols (Trease and Evans, 1989; Wall *et al.*, 1952; Sofowara, 1993).

Test for proteins (Biuret test)

A qualitative test was performed by adding equal volumes of 1% sodium hydroxide and a 1% copper sulphate solution to the extract. The appearance of purple colour indicates the presence of proteins.

Test for amino acids (Ninhydrin test)

To 1 mL of the extract, few drops of 5% ninhydrin solution were added. It was then boiled in water bath for 5 minutes. The appearance of bluish purple colour indicates the presence of amino acids.

Test for starch (Potassium hydroxide test)

To 1 mL of the extract, 2 mL of 5% potassium hydroxide solution was added. Formation of canary yellow colour indicates the presence of starch.

Test for alkaloids (Dragendorff's test)

0.5 gm extract was taken and to that 5 mL of 1% hydrochloric acid was added carefully along the sides of the test tube and then boiled and filtered. To 1 mL of filtrate, 2 drops of Dragendorff's reagent was added. The formation of reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids

i) Ferric chloride test

Extracts were treated with few drops of 10%

ferric chloride. Formation of green precipitate indicates the presence of flavonoids.

ii) NH_3-HCl test

2 mL of dilute ammonia solution was added to 1 mL of the extract. It was mixed well and 1 mL of concentrated HCl was added. Development of yellow colour which disappears on standing indicates the presence of flavonoids.

iii) 1% $AlCl_3$ test

Extracts were treated with 1% aluminium chloride. Formation of yellow colour indicates the presence of flavonoids.

Test for tannins

i) 5% $FeCl_3$ test

To the extract, 1% ferric chloride solution was added. Formation of brownish green or blue black colour indicates the presence of tannins.

ii) Iodine test

A few drops of iodine solution were added to 2 mL of the extract. Formation of faint bluish colour indicates the presence of tannins.

iii) H_2SO_4 - HCl test

To about 2 mL of the extract, 2 drops of sulphuric acid and 5 drops of 5% hydrochloric acid were added. Formation of green colour indicates the presence of tannins.

iv) $K_2Cr_2O_7$ test

1 mL of 10% $K_2Cr_20_7$ was added to 2 mL of the extract,. Formation of yellowish brown precipitate indicates the presence of tannins.

v) 10% lead acetate test

To 2 mL of the extract, a few drops of 10% lead acetate were added. The development of a yellow colour precipitate indicates the presence of tannins.

Test for Phlobatannins (1% HCl test)

To 1 mL of the extract, 2 mL of 1% hydrochloric acid was added and shaken well. Formation of red precipitate indicates the presence of phlobatannins.

Test for steroids
(Liebermann Buchard test)

1 mL of the extract was dissolved in 2 mL of chloroform and 1 mL of acetic anhydride. Concentrated sulphuric acid was added carefully along the sides of the test tube.

Formation of a brownish-green ring at the interface indicates the presence of steroidal terpenes.

Test for terpenoids (Salkowski test)

1 mL of the extract was dissolved in 2 mL of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicates the presence of terpenoids.

Test for Saponins (Foam test)

About 1 gm of the powdered sample was boiled in 5 mL of distilled water in water bath, cooled and filtered. To 2 mL of the filtrate, 1 mL of distilled water was added and shaken vigorously for the formation of stable persistent froth.

Development of the froth indicates the presence of saponins.

Test for cardiac glycosides
(Keller Killiani test)

To 1 mL of the extract, 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added. The solution was underlayed with 1 mL of concentrated sulphuric acid. A brown ring obtained at the interface indicates the presence of a deoxy-sugar typical of cardenolides. A violet ring appears below the brown ring. In the acetic acid layer, a greenish ring is formed just above the brown layer and gradually spread throughout the layer.

Test for Anthraquinones
(Borntrager's test)

1 mL of the extract was shaken well in 2 mL of chloroform. Equal volume of 100% ammonia solution was added. It was mixed well. The development of pink, violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Quantitative analysis of phytochemicals

a) Estimation of alkaloids
(Harborne, 1973)

5 gms of powdered sample was added to 200 mL of 10% acetic acid in absolute ethanol. The sample was covered and allowed to stand for 4 hrs. It was then filtered and the filtrate was concentrated to one fourth of its original volume on a water bath. Concentrated ammonium hydroxide was added drop-wise, to the extract until the precipitation was complete. The solution was allowed to settle in a beaker and the precipitates were washed with dilute ammonium hydroxide. The residue was transferred to Petri plates, dried and weighed. This was expressed as gram % of alkaloids on dry weight basis.

b) Estimation of total phenolic content (McDonald *et al.*, 2001)

1 gm of the powder was extracted three times each with 20 mL of acetone:methanol:water (7:7:6 v/v/v) at room temperature. It was kept in shaker for 1-2 hrs, filtered through Whatman No. 1 filter paper (125 mm) and centrifuged. To 1 mL of filtrate, 0.2 mL of Folin Ciocalteau reagent (0.5 N) was added and incubated in room temperature for 15 minutes. 5 mL of 2% sodium carbonate was added and it was incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm. Pyrocatechol (10 mg/100 mL) was used as a standard (Slinkard and Singleton, 1977) and the total phenolic content was expressed in terms of standard equivalent (mg/gm of the sample on dry weight basis).

c) Estimation of total carbohydrates by Anthrone Method
(Trevelyan and Harrison, 1952)

1 gm of powder was homogenized using 10 mL of distilled water and centrifuged at 5,000 rpm for ten minutes. 0.5 mL of the supernatant was transferred into a fresh tube and the volume was made up to 1 mL with distilled water. 4 mL of 0.2% Anthrone reagent was added to that and heated in water bath for 10 minutes and allowed to cool. The formation of green colour was observed and the absorbance was measured at 630 nm. Glucose was used as the standard and the amount of total carbohydrates was expressed in terms of the standard equivalent (mg/gm of the sample on dry weight basis).

d) Estimation of flavonoids
(Boham and Kocipal-Abyazan, 1974).

5 gm of the powder was taken and extracted with 50 mL of 80% (v/v) aqueous methanol

and covered and kept in shaker for 1-2 hrs. Then filtered through Whatman No.1 filter paper (125 mm) and transferred to Petri plates, was evaporated to dryness. After the extract dried, it was weighed and the presence of flavonoids was expressed as mg/g on dry weight basis.

e) Test for total free amino acids by Ninhydrin method (Chinard, 1952)

Crude drug powder was homogenized in 80% (v/v) ethanol and centrifuged at 5000 rpm for 10 minutes. 0.5 mL of the supernatant was taken and it was made up to 1 mL with distilled water and then 2 mL of 4% ninhydrin was added and heated in boiling water bath for 20 minutes. It was then cooled and observed for the change of colour to purple-violet and the absorbance was read at 570 nm in UV spectrophotometer (Systronics 117, India). Glycine was used as the standard and the total amino acid content was expressed in terms of the standard equivalent (mg/gm of the sample on dry weight basis).

f) Estimation of total soluble proteins by method of Lowry *et al.*, (1951)

Appearance of bluish-black colour was observed and absorbance at 620 nm was read. Bovine serum albumin was used as the standard and the amount of total soluble proteins was expressed in terms of the standard equivalent (mg/gm of the sample on dry weight basis).

g) Estimation of photosynthetic pigments by Lichtenthaler and Wellburn's method (1983)

Fresh sample of leaf (50 mg) was weighed and homogenized with pre-chilled 80% acetone solution. Then, it was centrifuged at 6000 rpm for 3 minutes. Resulting pellet was resuspended in acetone till the pellet became colourless successively, after each wash. The absorbance read at 470 nm, 647 nm and 663 nm.

h) Test for non-photosynthetic pigments (Mancinelli *et al.*, 1975)

Fresh leaf sample (50 mg) was weighed and homogenized and left in 80% acidified methanol (80:20:1) overnight and kept in dark to extract the flavonoids in the given sample. This extract was centrifuged and then the supernatant was removed and the absorbance read at 530 nm, 670 nm and 315 nm.

Anthocyanins = $(A_{530}) - (0.38 \times A_{670})$

Where, A_{530} = Absorbance at 530 nm; A_{670} = Absorbance at 670 nm.

Antibacterial activity

The bacterial strains used for the study were *Staphylococcus aureus* (+ MTCC 96), *Staphylococcus epidermidis* (+ MTCC 2639), *Klebsiella pneumoniae* (- MTCC 432), *Enterococcus faecalis* (+ MTCC 126), *Shigella flexneri* (- MTCC 1457), Methicillin resistant *Staphylococcus aureus* (+) clinical isolate, *Micrococcus luteus* (+ MTCC 106), *Salmonella paratyphi* B (-) clinical isolate, *Salmonella typhi* (- MTCC 733), *Pseudomonas aeruginosa* (- MTCC 424), *Escherichia coli* (- MTCC 2939), *Vibrio cholerae* (- MTCC 3906). All the bacterial cultures were maintained on Muller Hinton Agar (MHA) medium.

The antibacterial activity of *P. nodiflora* was evaluated by agar disc diffusion method. Microbial cultures were grown in Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi. Petri plates were prepared with 20 mL of sterile MHA. 200 µL of culture suspension

containing 10^8 CFU/mL bacteria was swabbed on solidified MHA medium and allowed to dry for 10 minutes. In the study, 2.5 mg per disc concentration was used. The discs were impregnated on the seeded agar plate and incubated at 37 °C for 24 hrs. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used. The experiment was repeated thrice. The antimicrobial activity was evaluated by measuring the inhibition zone diameter in millimeters (mm) around the wells. 10 µg was used as positive controls.

Antifungal activity

The fungal cultures used for the study were *Candida albicans (*MTCC 183), *Candida krussei (*clinical isolate), *Candida tropicalis* (MTCC 184), *Trichophyton mentagrophytes* (MTCC 7687), *Microsporum gypseum* (MTCC 8469) *Malassezia pachydermatis* (ATCC 14522). The cultures were obtained from the standard stock cultures of Microbiology Laboratory, MTCC.

The fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28^{o}C. The antifungal activity was performed according to the standard reference method (NCCLS, 2002). The antifungal agent Amphotericin (150 µg) was used as positive control and the plates were incubated for 24 hrs at 28 °C.

Determination of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) determined for the highly active plant parts that showed significant antimicrobial activity against the test microorganisms according to the methods of Nakamura *et al.,* 1999 and Dulger, 2009 with some modifications. Selected plant extracts were subjected to a serial dilution (5 to 0.039 mg/ mL) using sterile nutrient broth medium as diluents. In a 96- well titre plate 175 µl of an individual microorganism and 25 µl of selected plant extract were loaded and incubated at 37°C for 24h.

The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract. All samples were examined in triplicate. A control experiment was run in parallel to study the impact of the solvent alone (without plant extracts) on growth of the test organisms.

d) Minimum Bactericidal Concentration (MBC)

Equal volume of the extract at different concentrations and Mueller Hinton Broth (Oxoid, UK) were mixed in micro-tubes to make up 0.5 mL of solution. 0.5 mL of McFarland standard of the organism suspension was added to each tube (Bonjar, 2004). The tubes were incubated aerobically at 37°C for 24 hrs. Two control tubes were maintained for each test batch. These included tubes containing extract without inoculum and the tubes containing the growth medium and inoculum.

The MBC was determined by subculturing the test dilution on MHA and further incubated for 24 hrs.

The minimal dilution that yielded no single bacterial colony was taken as the Minimum Bactericidal Concentration (Akinyemi *et al.,* 2005). This was carried out on some of the extracts with high antimicrobial activity and some of the highly sensitive organisms (Aibinu *et al.,* 2007).

Result and Discussion

Phytochemical Screening

Phytochemical screening of the four different solvent extracts from the aerial parts *P. nodiflora* revealed the presence of various phytochemicals which are summarized in Table 1. More compounds were detected in methanol extract. Proteins, amino acids, starch, alkaloids, flavanoids, saponins, tannins, steroids and terpenoids were present. The presence of terpenoids in plants exhibited cytotoxicity activity against microorganisms. Plants possess natural anti-infecting agents such as triterpenoids, steroids and saponins which react against infection-causing agents. Flavonoids are the important phytochemicals which are responsible for the free radical scavenging activity.

Quantitative analysis of phytochemicals

Amino acids, proteins, carbohydrates, flavonoids, phenolics and alkaloids were quantitatively determined and are presented in Table 2. Total proteins in aerial parts were 1.356 mg/gm dry weight, while the total amino acids were 2.214 mg/gm dry weight, total carbohydrates were estimated to be 1.421 mg/gm, the amount of alkaloids was found to be 0.589 mg/gm and total phenolic content was 0.411 mg/gm dry weight. Flavonoids were quantified to be 0.312 mg/gm dry weight.

The presence of terpenoids in plants exhibited cytotoxicity activity against microorganisms. Plants possess natural anti-infecting agents such as triterpenoids, steroids and saponins which react against infection-causing agents. Flavonoids are important phytochemicals which are responsible for the free radical scavenging activity. Earlier studies using leaf extracts from *Lippia nodiflora* and *Wattakaka volubilis* showed the presence of various phytochemicals (Jeyachandran *et al.*, 2010). Balamurugan and Ignacimuthu (2011) revealed the presence of sterols, saponins, coumarins, quinines, tannins and flavonoids in methanol extract from aerial parts of *Lippia nodiflora*. The methanol extract from *Lantana camara* showed the presence of glycosides, carbohydrates, phenolic compounds, saponins, alkaloids, flavonoids, steroids and triterpenoids (Mamta and Jyoti, 2012). The phenolic components are important in pharmacy due to their relationship with cancer activity (Singh *et al.*, 2012).

Anti-Microbial Activity

Antibacterial activity

The present study revealed that test organisms exhibited varying degrees of multidrug resistance to the standard antibiotic disc used in this present study. The test organisms used in this study were sensitive to standard antibiotic disc Ciprofloxacin 10 µg (Table 3). The antibacterial activity of specific concentrations of hexane, chloroform, ethyl acetate and methanol extract from different plant parts is shown in Table 3. Antibacterial activity was determined against five gram positive bacteria and seven gram negative bacteria. The results revealed that the plant extracts showed significant antibacterial activity with varying magnitudes. Among the crude extracts, the ethyl acetate extract from aerial parts and methanol extract from roots exhibited broad spectrum activity against all test bacteria in comparison to other extracts. The ethyl acetate and chloroform extract from aerial parts exhibited high antibacterial activity with zone of inhibition ranging from 9 to 18 mm and 9 to 14 mm respectively. Hexane extract

from aerial parts showed moderate activity of 10 to 13 mm, and methanol extract from aerial parts exhibited mild activity ranging from 10 to 12 mm. The ethyl acetate extract from aerial parts of *P. nodiflora* showed highest inhibition zone against *Staphylococcus aureus* with an inhibition zone of 18 mm, and least activity was observed against *Vibrio cholerae* (9 mm). The inhibition zone in chloroform extract was the highest (14 mm) against *Shigella flexneri* and the lowest was observed in *Vibrio cholerae* with a zone measuring 9 mm. Hexane extract exhibited a zone of inhibition of 13 mm against *P. aeruginosa* and the least activity was observed against *Salmonella paratyphi* B with an inhibition zone measuring 10 mm and in *Salmonella typhi* it was 10 mm. In the case of methanol extract from aerial parts, the highest activity was observed against methicillin resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* with a zone of inhibition measuring 12 mm in each.

b) Antifungal activity

The fungal strains used in this study exhibited sensitivity to standard antibiotic disc amphotericin (Table 3) Results of antifungal activity of extracts from *P. nodiflora* are summarized in Table 4. The ethyl acetate extract from aerial parts of *P. nodiflora* exhibited the highest zone of inhibition measuring 14 mm against *Candida albicans.* Methanol and hexane extracts showed antifungal activity against only two fungal strains and chloroform extract did not show any inhibitory activity against fungi.

In *P. nodiflora*, the highest antimicrobial activity was observed in methanol extract from roots against *Salmonella typhi* (24 mm) and ethyl acetate extract from aerial parts against *Staphylococcus aureus* (18 mm). Earlier studies show that methanol extract from the seeds of *P. nodiflora* and methanol and ethyl acetate extract from leaves significantly inhibited the growth of bacteria (Jeyachandran *et al.,* 2010). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999). *L. citriodora* showed the presence of essential oils such as geranial, neral and limonene (Argyropoulou *et al.,* 2007). The possible reasons for the higher antibacterial activity of methanol extracts may be due to the stronger extraction capacity of biologically active components such as alkaloids, flavonoids, essential oils, terpenoids, etc., by methanol.

c) Minimum Inhibitory Concentration (MIC)

Results of MIC studies of ethyl acetate extract from aerial parts and methanol extract from roots are presented in Table 5. The MIC of the ethyl acetate extract from aerial parts ranged between 0.078 and 0.312 mg/mL. Similarly for methanol extract from roots the MIC ranged between 0.625 and 2.5 mg/mL. The ethyl acetate extract inhibited growth of *Staphylococcus aureus*, *Salmonella typhi* and *Malassezia pachydermatis* at 0.312 mg/mL and *Klebsiella pneumoniae* at 0.078 mg/mL. Methanol extract from roots inhibited the growth of *S. aureus* at 0.625 mg/mL and also inhibited the growth of *K. pneumoniae*, *Salmonella typhi* and *M. pachydermatis* at 2.5 mg/mL. The standard Ciprofloxacin had MIC values varying between 0.001 to 0.125 mg/mL.

d) Minimum Bactericidal Concentration (MBC)

The results revealed that Minimum

Bactericidal Concentration **(**MBC) of ethyl acetate extract from aerial parts against *Staphylococcus aureus, Salmonella typhi* and *Malassezia pachydermatis* was at 0.625 mg/mL and *Klebsiella pneumoniae* was at 0.156 mg/mL. The MBC value of methanol extract from roots ranged between 1.25 mg/mL to 5 mg/mL (Table 5).

Preliminary screening of phytochemicals was carried out for different extracts of *P. nodiflora*. Ethyl acetate extract from aerial parts showed potent antimicrobial and antifungal activity.

Table.1 Qualitative phytochemical screening of *P. nodiflora* (Aerial parts)

Phytochemicals	Test/Reagents used	Solvents used			
		Hexane	Chloroform	Ethyl acetate	Methanol
		AP	AP	AP	AP
Proteins	Biuret test	-	-	+	+
Amino acids	Ninhydrin test	-	-	+	+
Starch	KOH	-	-	+	+
Alkaloids	Dragendorff's Reagent	-	-	-	+
Flavonoids	1%$AlCl_3$	+	-	+	+
	10% $FeCl_3$	+	-	+	+
	NH_3-HCl	-	-	+	+
Tannins	5%$FeCl_3$	-	-	+	-
	Iodine	+	-	-	+
	$H2SO_4$-HCl	+	-	+	+
	$K_2Cr_2O_7$	-	-	-	+
	10% Lead acetate	+	-	-	+
Phlobatannins	1% HCl	-	-	-	-
Steroids	Liebermann buchard test	+	+	+	+
Terpenoids	Salkowski test	+	+	+	+
Saponins	Foam test	-	+	+	+
Cardiac glycosides	Keller- Killiani test	-	-	-	-
Anthraquinones	Borntrager's test	-	-	-	-

Table.2 Quantitative analysis of *P. nodiflora* (mg/gm dry weight)

Parameters	Aerial parts (mg/gm)
Alkaloids	0.589
Phenolics	0.411
Carbohydrates	1.421
Flavonoids	0.312
Amino acids	2.214
Proteins	1.356
Chlorophyll a	10.975
Chlorophyll b	1.835
Total chlorophyll	12.810
Carotenoids	2.079
Anthocyanins	0.183

Table.3 Antibacterial activity of *P. nodiflora* by disc diffusion method

Bacterial strains	Zone of inhibition (mm)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Ciprofloxacin 10
Staphylococcus aureus (+)	_	_	18	_	36
Staphylococcus epidermatitis (-)	12	12	14	-	21
Klebsiella pneumoniae	_	12	14	12	28
Enterococcus faecalis	10	13	12	_	25
Shiegella flexneri	_	14	12	_	27
Staphylococcus aureus (Methicillin resistant)	_	_	14	12	20
Micrococcus luteus	_	_	12	_	29
Salmonella Paratyphi B	10	_	13	_	28
Salmonella typhi	10	_	13	10	25
Pseudomonas aeroginosa	13	10	_	10	25
Escherichia coli	_	_	13	_	24
Vibrio cholerae	_	9	9	_	21

Table.4 Antifungal activity of *P. nodiflora* by disc diffusion method

Fungal strains	Zone of inhibition (mm)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Amphotericin
Candida albicans	9	-	14	-	9
Candida krussie	-	-	-	-	9
Candida tropicalis	_	_	11	12	8.5
Trichophyton mentagrophytes	-	-	12	12	8
Microsporum gypseum	_	_	12	_	8
Malassezia pachydermatis	11	_	11	_	8

Table.5 MIC and MBC of crude extracts from *P. nodiflora*

Microorganisms	AP		R		Cip	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus (+)	0.312	0.625	0.625	1.25	0.125	0.125
Klebsiella pneumoniae	0.078	0.156	2.5	1.25	0.031	0.062
Salmonella typhi	0.312	0.625	2.5	2.5	0.001	0.003
					Amp	
Malassezia pachydermatis	0.312		2.5	5		

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