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Screening of phytochemical and antimicrobial activity from the leaves of *Pleiospermium alatum* (Wall. & Arn.) Swingle

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ABSTRACT

The antimicrobial activity of Pleiospermium alatum leaves were extracted successively with different solvents viz., Petroleum ether, chloroform, ethyl acetate and methanol. Screened for their antimicrobial activity against Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae. Candida albicans, Candida parapsilosis and Candida tropicalis by using disc diffusion method, the extent of the inhibitory zones, Minimum Inhibitory Concentration (MIC) Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were also determined. The methanol extract of P. alatum showed the highest antimicrobial activity against all the bacterial and fungal strains tested than the other extracts. The mean zones of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.0 to 19.8 mm. The MIC values were between 125 and 250µg/ml, MBC values250 and 1000 µg/ml and MFC 250 and 1000 µg/ml values were recorded. Phytochemical analyses of different extracts of leaves P. alatum was analysed. The methanol extract of P. alatum leaves are showed the presence of strong phytochemicals viz., steroids, flavonoids, tannins, phenolic compounds and terpenoids than the other extracts. The highest mean of zone inhibition (19.8 mm) was observed in the methanol the extract of P. alatum against Staphylococcus aureus. These finding suggest that the methanol extract of Pleiospermium alatum can be used as a antimicrobial substance for the treatment of microbial infections.

Key words: Antimicrobial activity, *Pleiospermium alatum*, MIC, MBC, MFC

INTRODUCTION

Pleiospermum alatum belongs to the family Rutaceae and distribute in all parts of tropical and sub-tropical region of India. All parts of the plant have been recognized to have medicinal properties. The plants are commonly called as 'kurunthumulthazhai'. The leaves and bark are used for the fomentation of rheumatic pain; the dried fruit is useful in malignant and pestilent fevers and is used as an antidote for poisons [1]. Various extracts of the plants have been reported for its anti-inflammatory and analgesic activity [2]. Oils are obtained from fresh leaf juice used to cure rheumatic complaints by the Kanikkar tribals of Kalakad - Mundanthurai Tiger Reserve, Western Ghats, Tamil Nadu [3].

One of the more alarming recent trends in infectious diseases have been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Numerous classes of antimicrobial agents have now become less effective as a result of the effective pressure of antimicrobial usage [4].

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [5].

Staphylococcus aureus is a facultative anaerobic gram-positive coccal bacterium and its one of the most common causes of nosocomial post-surgical wound infections [6, 7], *S. aureus* and *Pseudomonas aeruginosa* are responsible for a significant number of biofilm related infections. *Staphylococci* are was currently the most common cause of nosocomial infections. Opportunistic *S. aureus* is involved in native valve endocarditis, otitismedia and all kinds of infections of implanted devices [8, 9, 10]. In recent years, nosocomial infections caused by *P. aeruginosa* has been recognized as an acute problem in hospitals due to its intrinsic resistance to many antibiotic classes and its capacity to acquire practical resistance to all effective antibiotics [11], *P. aeruginosa* is a gram negative bacteria, occasionally associated with opportunistic diseases of humans [12].

The major causative agents of diarrhea in humans include *Shigella flexneri*, *Staphlococcus aureus*, *Escherichia coli* and *Salmonella typhi* [13]. *Escherichia coli* is a gram-negative human enteric species that is generally a virulent but sometimes causes weakly virulent gastroenteritis and urinary tract infections. *Salmonella typhimurium* is a gram-negative pathogen that most commonly causes enterocolitis and *S. aureus* is a gram-positive pathogen that most commonly causes abscess, food poisoning and toxic shock syndrome; both are considered to be more virulent than *S. lactis* and *E.coli* [14]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [15, 16]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [17]. Skin diseases occur worldwide and amount to approximately 34 % of all occupational diseases encountered [18]. They affect people of all ages from neonates to the elderly and constitute one of the five reasons for medical consultation. Skin diseases have been of major concern recently due to their association with the Human Immuno-deficiency Virus and Acquired Immunity Deficiency Syndrome (HIV/AIDS) [19].

Fungal associated diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosuppressed persons [20]. *Candida albicans* is the most common species linked with candidiasis and is the most commonly improved species from hospitalized patients. Candidiasis encompasses infections that range from superficial, such as oral thrush [21] and vaginitis, to systemic and potentially life intimidating diseases. The increase of *Candida albicans* infections equivalents medical developments such as invasive procedures, immunosuppressive treatments for organ transplants and extensive use of broad-spectrum antibiotics [22]. Candida and *Aspergillus* species have been found to be the most common etiological agents in nosocomial blood stream fungal infections (BSI). The most common species accounting for more than 90% of all *Candida*-associated BSIs are *C. albicans, C. glabrata, C.parapsilosis, C. tropicalis* and *C. krusei*, while *Aspergillus fumigatus, A. flavus, A. nigers* and *A. terreus* are the most common isolated species in *Aspergillus*-associated BSIS [23] .

The aim of the present study was to evaluate the antimicrobial activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Pleiospermum alatum* leaves against bacterial and fungal strains.

EXPERIMENTAL SECTION

Collection of Plant material and preparation of extracts

The fresh leaves of *Pleiospermium alatum* (Rutaceae) were collected from Silambur (Lat, 11.35 ºN; Long, 79.31ºE), Ariyalur District, Tamil Nadu, India. During the months of March to April 2014. Specimens were was deposited in the Herbarium of Department of Botany, Annamalai University, Annamalai nagar. Collected leaves were washed with water, then surface sterilized with 10% sodium hypochlorite solution rinsed with sterile distilled water and shade dried under room temperature. The samples were ground in to a coarse powder. One hundred grams of coarse powder was extracted with different organic solvents like non-polar to polar *viz*., Petroleum ether, chloroform, ethyl acetate and methanol for 8 hours using Soxhlet apparatus. The solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4˚C until further use.

Phytochemical analysis Screening of Extracts

The Petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves of *Pleiospermium alatum* were used for qualitative phytochemical analyses. Phytochemicals such as, flavonoids, tannins, steroids, glycosides, saponins, phenolic compounds, terpenoids and alkaloids were analyzed according to described by [24, 25].

Microorganisms

Seven clinical bacterial strains isolates Gram - positive bacteria: *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis* and Gram - negative bacteria: *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and three fungal species: *Candida albicans, Candida parapsilosis* and *Candida tropicalis* were used in the present study. The stock cultures were maintained on Muller Hinton Agar medium and Sabouraud Dextrose Agar at 4°C for bacterial and fungal respectively. The isolates obtained from Raja Muthaih Medical College Hospital, Annamalai University, Tamilnadu. *In vitro* antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB). *In vitro* antifungal activity was determined by using Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) (fungi) they were obtained from Himedia Mumbai.

Antibacterial and Antifungal assays

Disc diffusion method

The agar diffusion method [26] was employed for the initial assessment of antibacterial potential of the extracts. Petri plates were prepared by pouring 20 ml of MHA and SDA allowed solidifying for the use in susceptibility test against bacteria and fungi. The standard inoculum using bacterial suspension containing10^8 CFU per ml, yeast suspension containing 10^6 CFU per ml were swabbed on the top of the solidified sespenture media and allowed to types for 10 minutes. Plates were dried and uniformly spread. The excess inoculums were drained and the plates were allowed to dry for 5 min. After drying, the disc with extracts were placed on the surface of the plate with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacin (5µg/disc) for bacteria and Amphotercin B (100units/disc) for yeast was used as positive control. 10 per cent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37 °C for 24 h bacteria and yeast. The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated three times.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of plant different extracts were tested in MHB for bacteria and SDB for fungi described by [27]. The plant extracts were dissolved in 10% DMSO to obtain 2 mg/ml and 0.5 ml of stock solution was incorporated into 0.5 ml of MHA to get a concentration of 1000, 500, 250, 125, 62.5, 31.2 and 15.6 µg/ml, 100µl of standardized. Bacterial suspension of the test organism was transferred into each tube. The control tube contained only organism and devoid of plant extracts. The culture tubes were incubated at 37°C for 24 h for bacteria and yeast. The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation were determined as MIC.

Table. 1. Phytochemical analysis of the different extracts of leaves *Pleiospermium alatum*

S.NO	Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	+	+	+
2	Flavonoids	-	_	+	+ +
3	Phenolic compounds	+	+	+	++
4	Saponins	-	_	-	+
5	Tannins	+	_	+	++
6	Cardicac glycosides	-	+	_	+
7	Steroids	+	_	+	+ +
8	Terpenoids	+	+	+	+ +

(++) = Strong; (+) = Positive (present); (-) =Negative (absent)

Minimum Bactericidal Concentration (MBC)

The MBC of the different extracts were determined by plating 100µl of samples from each MIC assay tube with growth inhibition into freshly prepared MHB and the plates were incubated at 37°C for 24 h bacteria, The MBC values were recorded as the lowest concentration of the extracts that did not permit any visible bacterial colony growth on the agar plate during the period of incubation.

Minimum Fungicidal Concentration (MFC)

The MFC of the different extracts were determined by plating 100µl of samples from each MIC assay tube with growth inhibition into freshly prepared SDA and the plates were incubated at 37°C for 24h yeast. The MFC values were recorded as the lowest concentration of the extracts that did not permit any visible bacterial colony growth on the agar plate during the period of incubation.

Table 2. Antimicrobial activity of different extracts of leaves of *Pleiospermium alatum*

S. No.	Microbial strains/solvents	Mean zone of inhibition (mm) Concentration of the extracts (µg/disc)				MIC (mg/mL)	MBC/ MFC (mg/mL)
		1000	500	250	Ciprofloxacin (10 mg/disc)		
1	*Staphylococcus aureus*						
	Petroleum ether	14.0 ± 0.50	12.8 ± 0.76	9.5 ± 0.50	29.3 ± 0.57	250	500
	Chloroform	14.5 ± 0.50	13.1 ± 0.78	10.3 ± 0.57	28.6 ± 0.76	250	500
	Ethyl acetate	18.0 ± 0.50	14.6 ± 0.76	11.0 ± 0.50	28.0 ± 0.50	125	250
	Methanol	19.8 ± 0.76	15.1 ± 0.78	11.3 ± 0.57	27.5 ± 0.50	125	250
2	*Streptococcus pyogenes*						
	Petroleum ether	14.1 ± 0.78	11.8 ± 0.76	8.5 ± 0.50	30.1± 0.28	250	500
	Chloroform	14.6 ± 0.76	12.5 ± 0.50	9.8 ± 0.76	29.8 ± 0.50	250	500
	Ethyl acetate	15.3 ± 0.57	12.6 ± 0.76	10.0 ± 0.50	28.3 ± 0.50	125	250
	Methanol	16.1 ± 0.28	13.0 ±0.50	10.3 ± 0.57	27.3 ± 0.50	125	250
3	*Enterococus faecalis*						
	Petroleum ether	12.3 ± 0.57	10.8 ± 0.76	8.0 ± 0.50	28.3 ± 0.67	500	1000
	Chloroform	12.8 ± 0.16	11.0 ± 0.76	9.3 ± 0.57	28.0 ± 0.57	500	1000
	Ethyl acetate	13.6 ± 0.76	11.1 ± 0.78	9.6 ± 0.76	29.3 ± 0.58	250	500
	Methanol	14.1 ± 0.78	11.8 ± 0.76	10.0 ± 0.50	30.0 ± 0.50	250	500
4	*Escherichia coli*						
	Petroleum ether	10.3 ± 0.57	9.3 ± 0.76	7.1 ± 0.28	30.0 ± 0.50	500	1000
	Chloroform	10.8 ± 0.76	9.8 ± 0.76	8.5 ± 0.76	29.8 ± 0.76	500	1000
	Ethyl acetate	11.6 ± 0.76	10.1 ± 0.28	9.0 ± 0.57	27.8 ± 0.76	250	500
	Methanol	12.0 ± 0.50	10.6 ± 0.76	9.3 ± 0.57	27.0 ± 0.50	250	500
5	*Proteus vulgaris*						
	Petroleum ether	10.1 ± 0.78	9.0 ± 0.50	7.0 ± 0.50	26.5 ± 0.50	500	1000
	Chloroform	10.6 ± 0.76	9.1 ± 0.28	7.8 ± 0.70	27.0 ± 0.70	500	1000
	Ethyl acetate	11.3 ± 0.50	9.8 ± 0.76	8.5 ± 0.50	28.5 ± 0.50	250	500
	Methanol	11.8 ± 0.76	10.3 ± 0.57	9.1 ± 028	29.3 ± 0.50	250	500
6	*Pseudomonas aeruginosa*						
	Petroleum ether	9.8 ± 0.76	8.8± 0.80	7.0 ± 0.50	29.7 ± 0.73	500	1000
	Chloroform	11.0 ± 0.50	9.6 ± 0.76	7.3 ± 0.57	30.0 ± 0.50	500	1000
	Ethyl acetate	12..0 ± 0.50	10.8 ± 0.76	8.0 ± 0.50	28.1 ± 0.28	250	500
	Methanol	12.5 ± 0.50	10.3 ± 0.57	8.5 ± 0.50	26.9 ± 0.38	250	500
7	*Vibrio cholerae*						
	Petroleum ether	10.8 ± 0.76	9.6 ± 0.76	7.1 ± 0.28	29.0 ± 0.50	500	1000
	Chloroform	11.3 ± 0.50	9.0 ± 0.50	7.5 ± 0.50	28.5 ± 0.50	500	1000
	Ethyl acetate	13.3 ± 0.50	10.8 ± 0.76	8.3 ± 0.57	27.8 ± 0.36	250	500
	Methanol	14.0 ± 0.50	10.8± 0.76	9.0± 0.50	28.0 ± 0.50	250	500
8	*Candida albicans*						
	Petroleum ether	11.0 ± 0.50	9.8 ± 0.76	7.8 ± 0.76	16.1 ± 0.28	500	1000
	Chloroform	12..1± 0.78	10.0 ± 0.50	7.8 ± 0.76	15.0 ± 0.50	500	1000
	Ethyl acetate	13.0 ± 0.50	10.0 ± 0.50	8.8 ± 0.67	14.7 ± 0.38	250	500
	Methanol	13.8 ± 0.66	10.8 ± 076	9.6 ± 0.76	16.0 ± 0.57	250	500
9	*Candida parapsilosis*						
	Petroleum ether	11.0 ± 0.50	9.8 ± 0.76	7.8 ± 0.76	16.1 ± 0.28	500	1000
	Chloroform	12.0 ± 0.50	10.0 ± 0.50	8.1 ± 0.78	13.8 ± 0.76	500	1000
	Ethyl acetate	13.5 ± 0.50	103 ± 0.50	9.1 ± 0.78	15.3 ± 0.57	250	500
	Methanol	14.1± 0.28	11.0 ± 0.50	9.8 ± 0.76	14.0 ± 0.50	250	500
10	*Candida tropicalis*						
	Petroleum ether	9.3 ± 0.57	8.1 ± 0.28	7.0 ± 0.50	15.3 ± 0.57	500	1000
	Chloroform	9.8 ± 0.76	8.3 ± 0.57	7.3± 0.57	14.8 ± 0.76	500	1000
	Ethyl acetate	10.1 ± 0.28	9.0 ± 0.50	8.1 ± 0.28	13.0 ± 0.50	500	1000
	Methanol	10.8 ± 0.76	9.8 ± 0.76	8.3 ± 0.57	16.0± 0.50	500	1000

[a]*Diameter of zone of inhibition (mm) including the disc diameter of 6 mm*

[b]*Mean of three assays; ± - Standard deviation ; Significant at P<0.05*

RESULTS

The petroleum ether, chloroform, ethyl acetate and methanol extracts of *P. alatum* leaves were used to analyses the phytochemical contents. The ethyl acetate and methanol extracts of *P. alatum* leaves showed the presence of phytochemicals namely alkaloids flavonoids, phenolic compounds, saponins, tannin, glycosides steroids, and terpenoids strongly than the other extracts. In chloroform extracts showed all the phytochemicals absent. All the phytochemicals were present in ethyl acetate extract except cardiac glycosides and saponins. In petroleum ether extract all the phytochemical analysis are absent except, phenolic compounds, tannins, steroids and terpenoids (Table-1).

The present study different solvent *viz*., petroleum ether, chloroform, ethyl acetate and methanol extracts of *P. alatum* leaves was showed varied level of activities against the bacterial and fungal strains tested. All the extracts of *P. alatum* possessed significant antibacterial and antifungal activity against all the bacterial and fungal strains tested. The mean values are presented in Table- 2. When the different extracts were tested against the test bacterial and fungal strains by using disc diffusion method MIC, MBC, and MFC were also determined. The mean zone of inhibition obtained were between 7.0 to 19.8mm. The ciprofloxacin (10µg/disc) antibacterial positive control produced the mean zone o inhibition were from 26.5 to 30.0mm and Amphotercin-B (100µg/disc) antifungal positive control produced the mean zone of inhibition were between 13.0 and 16.1 The 10% DMSO did not produced the any zone of inhibition. The MIC values of different extracts of *P. alatum* ranged between 125 and 500 µg/ml, while the MBC values were between 250 and 1000 µg/ml and MFC values were between 250 and 1000 µg/ml. The highest mean of zone of inhibition (21.3) and the lowest MIC (125 µg/ml), MBC (250 µg/ml) values were obtained the methanol extract of *P. alatum* against *Staphylococcus aureus.* The highest mean zone of inhibition (14.1 mm) and the lowest MIC (250µg/ml) and MBC (500 µg/ml) values were reduced in methanol extract against *C. papapsilosis.*

DISCUSSION

In the present study, different solvent *viz*., petroleum ether, chloroform, ethyl acetate and methanol extracts of *P. alatum* leaves was showed varied level of activities against the bacterial and fungal strains tested. All the extracts of *P. alatum* possessed significant antibacterial and antifungal activity against all the bacterial and fungal strains. In the present study methanol extract was found to be most effective in antibacterial and antifungal activity and followed by petroleum ether, chloroform and ethyl acetate. The methanol extract of *P. alatum* leaves showed the highest antibacterial activity against S. aureus followed by other strains. The mean zone inhibition is 19.8 mm and the lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in methanol extract. In antifungal activity, the methanol extract of *P. alatum* showed highest antifungal activity and it was followed by petroleum ether, Chloroform and ethyl acetate. The methanol extract of *P. alatum* showed the highest antifungal activity against *C. parapsilosis.* The mean zone of inhibition of *C. parapsilosis* is 16.1 mm. The lowest MIC (250 µg/ml) and MFC (500µg/ml) was observed in the methanol extract of the leaves of *P. alatum*. Similarly, reported the leaf extract of *P. alatum* possess antibacterial and antifungal activity against the mutli-drug resistant strains of *E. coli, Candida albicans* and *S. aureus*. Our findings correspond with findings of [29]. Who observed that the methanol extracts of *Pterospermum suberfolium, Trachyspermum ammi, Peltaphorum pterocarpum, Ixora coccinea, Persicaria glabra, Terminalia elliptica* and *Cicca acida* showed potential antidermatophytic activities against *Trichophyton mentagrophytes* and *Microsporum gypseum*. Same significant results were obtained with *Piper aduncum* leaves against *Trichophyton rubrum* and *T. interdigitale* [30].

The methanol extracts of *Cassia siamea* showed a broad spectrum of antibacterial activity against *Bacillus subtilis, B. pumilus, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* with the mean zones of inhibition ranged from 7 to 24 mm [31]. The same trend in *Cassia fistula* extract showed strong antibacterial and antifungal activities than *Mesua ferrea* [32]. The methanol extract of *Canthium coromandelicum* showed the broad spectrum of antifungal activity. The MIC value at 128 µg/mL against *Aspergillus niger, Auricularia polytricha* and *Candida albicans* and 512 µg/mL against *Arthrobotrys oligospora* and *Chaetomella raphigera* [33].

Phytochemical constituents such as alkaloids, phenolic compounds, saponins, glucosides and terpenoids were detected in the methanol leaves extract of *P. alatum*.

Methanol extracts of *Pleiospermium alatum* showed potential inhibitory action on tested fungal strains. The solvent methanol is known for its ability to isolate more antimicrobials from plants including tannins, polyphenols, terpenoids, saponins, xanthoxyllines, totarol, quassinoids, lactones, flavones and phenones, while the water extracts could isolate only anthocyanins, starches, tannins, saponins, terpenoids, polypeptides and lectins. In general, phenolic compounds possess specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-feedant, anti-viral, anticancer and vasodilatory actions [35] Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [36]. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [37].

In this study, the methanol extract of leaves of *P. alatum* showed the highest antibacterial activity against the gram positive bacteria than the gram negative bacteria.

The MIC and MBC values of methanol extract were lower than that of other extracts of the *Pleiospermium alatum* showed potential antibacterial effect against bacterial strains tested. Similar observations were made while studying the antimicrobial activity of seeds of *Syzygium jambolanum* [38], FAME extracts of certain marine macro algae [39] and leaves of *Ipomoea pes-caprae* [40]. The majority of plant derived antimicrobial compounds generally have higher MICs than bacterial or fungal produced antibiotics, thus limiting their therapeutic potential [41] reported that seaweed extracts tested had strong inhibitory activity against gram positive bacteria (*S. aureus* and *B. subtilis*) but it showed weak activity against gram negative bacteria (*E. coli* and *proteus mirabilis*). The greater resistance of gram negative bacteria to plant extracts has been documented previously for seeds of *S. jambolanum* [42] and bark of *Cassia siamea* [43].

CONCLUSION

The antimicrobial activity of the *Pleiospermium alatum* methanol leaf extract, probably due to the recognized phytoconstituents, further confirms its use as a health cure in traditional medicine. Bioactive substances from this plant as a result be employed in the formulation of antifungal agents for the healing of a variety of microbial infections. Detection of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the scrutiny of formulating fresh chemotherapeutic agents should be the future direction for research.

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