

Research Article



In-vitro antimicrobial activities of *Pongamia glabra* and *Phyllanthus niruri*

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Abstract

The antibacterial and antifungal activities of chloroform, ethanol, acetone and aqueous extracts of *Pongamia glabra* and *Phyllanthus niruri* leaves were screened. In antibacterial activity assay followed by standard disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterobacter aerogens*. Among the all the treatments, chloroform extract of *P. glabra* showed the highest activity against bacterial and fungal strains. Chloroform extract of *P. glabra* showed the presence of phalobatannin, saponin, flavanoids, terpenoids, cardiac glycosides, alkaloids, steroids and aminoacids. *P. niruri* leaf extract showed the presence of tannin, phlobatannin, flavanoids, terpenoids, cardiac glycosides, steroids and aminoacids.

Keywords: *Pongamia glabra*, *Phyllanthus niruri*, Antibacterial, Antifungal, Phytochemicals

1. Introduction

Plants are invaluable sources of new drugs and there is an ever - growing interest in investigating different species of plants to identify their potential therapeutic applications (Rego 1995). Traditional medicine is still followed as an alternate for chemical drugs which cause residue problem and associated side effects (Cunha 2001). The antimicrobial compounds of medicinal plants often have fewer side effects, better patient tolerance, relatively less expensive and are being renewable in nature (Vermani and Garg 2002). Plants have a great potential for producing new drugs for human benefit, are used in traditional medicine to treat chronic and even infectious diseases (Chowdhury et al., 2009). According to a report of World Health Organization (WHO), more than 80% of world's population depends on traditional medicine for their primary health care needs (Duraipandian et al., 2006; Antonisamy et al., 2015; Balamurugan 2015; Barathi and Agastian 2015; Nandhini and Bai 2015; Rathi et al., 2015; Narendran et al., 2016; Puthur 2016; Noorudheen and Chandrasekharan 2016; Santhosh et al., 2016; Greeshma 2016; Sreeshma et al., 2016; Nair et al., 2016). The plant extracts and essential oils constitute a natural source of antimicrobial compounds and have a great usage in folk medicine (Demirie et al., 2008).

Pongamia glabra and *Phyllanthus niruri* plants are important medicinal values. *P. glabra*, leaves used as the patients of arthritis (Allen and Allen 1981). Internally, karanja is a valuable remedy for a vast range of diseases. In hepatosplenomegaly, the decoction of bark skin works well with rock salt (Burkill 1966). Karajin is a good blood purifier, hence is salutary in blood disorders as an adjunct and is one of the best herbs in various skin infections. The *P. niruri* have been shown to exhibit beneficial effect against various pathological stages including liver disease like hepatitis and fatty liver and no side effect have been reported in clinical studies (Bagalkotkar et al., 2006). The demand for more and more drugs from plant source is continuously increasing, it is therefore essential for the systematic evaluation of plants used in traditional medicine for promising biological activity (Chowdhury et al., 2009). The treatments in most cases administrated by traditional healers generally consist of crude plant material and extracts (Reid et al., 2005) and many of the plant materials used in traditional medicine are readily available in rural areas at a relatively cheaper cost (Mann et al., 2008).

The medicinal value of these plants lies in the chemical substance that produces a definite physiological action on the human body (Karunyadevi et al., 2009). A large number of plant species harbours in it bioactive compounds exhibiting health beneficial properties. The medicinal effects of the plant material typically result from the combination of secondary metabolites such as alkaloids, steroids, tannins and flavonoids which are synthesized and deposited in specific parts of plants. The alkaloids, tannins, flavonoids and phenolic compounds also play a major role in preventing number of chronic diseases (Graig 1999). In this context, the present study was envisaged with the aim of testing the in vitro antimicrobial activity of the leaf extracts of *P. glabra* and *P. niruri*.

2. Material and methods

2.1. Collection of plant material

Fresh leaves of the selected botanicals such as *P. glabra* and *P. niruri* were collected from the campus of Yadava College and Thiagarajar College, Madurai, Tamil Nadu. They were brought to the laboratory in plastic bags and washed twice with tap water and then with distilled water and shade dried (2 - 3 weeks). After complete drying, the plant materials were powdered separately using a domestic grinder and stored in plastic containers for further use.

2.2. Preparation of extracts

Hundred and fifty grams of the powdered material of *P. glabra* and *P. niruri* were extracted with 450 ml of ethanol in a soxhlet apparatus for 24 - 48 h. The resultant material was distilled and the extract was further concentrated using a desiccators and the concentrated extract was stored in a plastic container. Similar methodology was followed for the ethanol, acetone, and chloroform extracts. For the aqueous extraction, 150 g powdered material of *P. glabra* and *P. niruri* were infused in 450 mL hot distilled water taken in a conical flask, stirred with a sterile glass rod and then kept in a shaker for 120 h. At the end of the period, the liquid part was passed through Whatman filter paper no: 1, the greenish filtrates so obtained were evaporated using a dessicator and it yielded a sticky black extract. The extract was stored in a plastic container and refrigerated at 4° C prior to use.

2.3. Antibacterial assay

The bacterial strains used for the screening such as *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterobacter aerogens* were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory. The antimicrobial

activity of ethanol, acetone, chloroform and aqueous extracts of *P. glabra* and *P. niruri* were tested against those organisms following the standard disc diffusion method. The stock cultures were maintained at 4^o C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture to test tubes of Muller Hinton Broth (MDB) that was incubated without agitation for 24 h at 37^o C. Muller Hinton agar (MHA) plates were prepared and the inoculum (18–24 h old broth culture) of the bacteria were spread on them using sterile swab. The extract loaded disc (20 µL) was placed on the plates using a sterile forceps. The discs loaded with the respective solvent and gentamycin served as controls and they were also placed on the same plate. The plates were then incubated at 37^o C for 24–48 h and observed for clear zone of inhibition and the inhibition zone was measured in mm.

2.4. Fungicidal assay

The chloroform, acetone, ethanol and aqueous leaf extracts of *P. galbra* and *P. niruri* were screened for antifungal activity by disc diffusion method. The stock cultures obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India were maintained in the laboratory on Potato dextrose agar slant. Subcultures for the experiments were prepared by transferring a loop full of culture to test tubes of Potato dextrose broth (PDB) that was incubated for 48 h at 27^oC. The molten Potato Dextrose Agar (PDA) was poured in to a petri dish by pour plate method. After solidification, the fungal spores were distributed uniformly on the surface of the agar plates with the help of sterile cotton swab. The extract loaded disc (20 µl) was placed on the plates using a sterile forceps. The discs loaded with the respective solvent and Nystatin served as controls and they were also placed on the same plate. Incubation period of 24-48 h at 28^o C was maintained for observation of antifungal activity of the plant extracts. The antifungal activity was determined by measuring the zone of inhibition of fungal growth surrounding the discs loaded with extracts and was measured with antibiotic zone scale in mm.

2.5. Preliminary phytochemical analysis

Preliminary phytochemical analysis of all there plant and respective solvent extracts followed by the method of Horborne (1984).

2.6. Statistical analysis

The data of average MIC values of antibacterial and antifungal values were compared with one way analysis of variance (One Way ANOVA) using SPSS 11. 5 version software. Results with P<0.05 were considered to be statistically significant.

3. Result

In the present study, the chloroform, acetone, ethanol and aqueous extracts of *P. glabra* and *P. niruri* were screened for their biological properties. For the antibacterial assay were tested against *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterobacter aerogens* in the in vitro conditions using the standard disc diffusion method. The formation of the inhibition zone was considered as the measure for the antibacterial activity. The maximum zone of inhibition produced by the *P. glabra* chloroform extract against *E.coli*, *S. aureus*, *M. luteus* and *E. aerogens* were 9.30, 5.60, 8.60 and 6 mm. Acetone extract ethanol extract against *E. coli*, *S. aureus*, *M. luteus* and *E. aerogens* were 5, 5.30, 6 and 5 mm respectively, (Table 1). No activity was observed with water extract of *P. glabra*. Less activity was observed with ethanol extract and no activity was observed against *M. luteus*. Similarly, the inhibition zone produced only by *P. niruri* ethanol

extract against *S. aureus* (3.40 mm). Maximum bacterial inhibitory activities were observed in *P. niruri* chloroform extract against *E. coli* (5.60 mm), *S. aureus* (5.60 mm), *M. luteus* (5.00 mm) and *E. aerogenes* (5.30 mm) respectively (Table 2).

Table 1. Antibacterial activity of the different solvent extract of *Pongamia glabra* leaves on the tested pathogens.

Bacterial pathogens	Inhibition Zone in mm				
	Gentamycin*	Extracts			
		Chloroform	Ethanol	Acetone	Water
<i>Escherichia coli</i>	22.60 ± 0.04a	9.30 ± 0.04b	5.30 ± 0.04bc	5.00 ± 0.02c	-
<i>Staphylococcus aureus</i>	23.0 ± 0.08a	5.60 ± 0.04b	4.60 ± 0.04c	5.30 ± 0.04cd	-
<i>Micrococcus luteus</i>	22.0 ± 0.02a	8.60 ± 0.04b	-	6.0 ± 0.08c	-
<i>Enterobacter aerogenes</i>	22.0 ± 0.09a	6.00 ± 0.01b	3.82 ± 0.20d	5.0 ± 0.08c	-

-; No zone, *Positive control, Mean ± SD followed by a same letter do not differ significantly (Turkey's test, $P < 0.05$ level).

Table 2. Antibacterial activity of the different solvent extract of *Phyllanthus niruri* leaves on the tested pathogens

Bacterial pathogens	Inhibition Zone in mm				
	Gentamycin*	Extracts			
		Chloroform	Ethanol	Acetone	Water
<i>Escherichia coli</i>	20.60 ± 0.04a	5.60 ± 0.04b	-	4.85 ± 0.24c	2.80±0.08d
<i>Staphylococcus aureus</i>	23.0 ± 0.08a	5.60 ± 0.04b	3.40±0.24d	4.28 ± 0.15c	2.20±0.19e
<i>Micrococcus luteus</i>	22.0 ± 0.04a	5.00 ± 0.09b	-	5.0 ± 0.06b	3.60±0.12c
<i>Enterobacter aerogenes</i>	22.0 ± 0.09a	5.30 ± 0.03b	-	4.50 ± 0.22c	-

-; No zone, *Positive control, Mean ± SD followed by a same letter do not differ significantly (Turkey's test, $P < 0.05$ level).

In the case of antifungal activity chloroform extract of *P. glabra* showed maximum activity against tested fungal pathogens (*A. niger*, *A. flavus*, *A. fumigatus* and *Microsporium* sp) the inhibitory ranges between 5 to 8mm. Moderate antifungal activity were exhibited in ethanol extract of *P. glabra* i.e *A. niger*, *A. flavus*, *A. fumigatus* and *Microsporium* sp were 4.71, 5.28, 6.28 and 5 mm. Water extract had less activity against fungal pathogens (Table 3).

Table 3. Antifungal activity of the various different solvent of *Pongamia glabra* leaves on the tested pathogens.

Bacterial pathogens	Inhibition Zone in mm				
	Nystatin*	Extracts			
		Chloroform	Ethanol	Acetone	Water
<i>Aspergillus niger</i>	11.0 ± 0.02a	5.0 ± 0.07b	4.71 ± 0.25bc	5.30 ± 0.22b	5.20 ± 0.08 b
<i>Aspergillus flavus</i>	13.0± 0.05a	6.71 ± 0.09b	5.28 ± 0.21d	4.10 ± 0.30c	-
<i>Aspergillus fumigatus</i>	15.0 ± 0.03a	6.57 ± 0.12b	6.28 ± 0.29bc	6.20 ± 0.17bc	-
<i>Microsporium</i> sps	18.0 ± 0.06a	8.00± 0.23b	5.0 ± 0.13c	-	3.40 ± 0.10d

-; No zone, *Positive control, Mean ± SD followed by a same letter do not differ significantly (Turkey's test, $P < 0.05$ level).

The inhibition zone produced by the chloroform extract of *P. niruri* against *A. niger*, *A. flavus*, *A. fumigatus* and *Microsporium* sp. and it was 8.25, 7.80, 5.87 and 7 mm and water extracts was 2.70, 4.71, 5.40 and 3.14 mm respectively. Inhibition zone produced by the acetone extract of *P. niruri* were 6.28, 6 and 7.42 mm against *A. Niger*, *A. flavus* and *Microsporium* sp respectively (Table 4).

Table 4. Antifungal activity of the different solvent extract of *Phyllanthus niruri* leaves on the tested pathogens.

Fungal pathogens	Inhibition Zone in mm				
	Nystatin*	Extracts			
		Chloroform	Ethanol	Acetone	Water
<i>Aspergillus niger</i>	11.0 ± 0.21a	8.25 ± 0.45bc	-	6.28 ± 0.45b	2.70 ± 0.05c
<i>Aspergillus flavus</i>	13.0 ± 0.08a	7.80 ± 0.49b	-	6.0 ± 0.53c	4.71 ± 0.45cd
<i>Aspergillus fumigatus</i>	15.0 ± 0.017a	5.87 ± 0.34b	-	-	5.40 ± 0.49bc
<i>Microsporium sp</i>	18.0 ± 0.23a	7.0 ± 0.53b	-	7.42 ± 0.49b	3.14 ± 1.34c

-, No zone, *Positive control, Mean ± SD followed by a same letter do not differ significantly (Turkey's test, $P < 0.05$ level).

Maximum antibacterial and antifungal activity showed in chloroform extract of *P. glabra* and *P. niruri*). Chloroform extract of *P. glabra* showed the presence of alkaloids, flavonoids, saponins, steroids, phlobatannin, cardiac glycosides, terpenoids and aminoacids. In the case of *P. niruri* chloroform extract showed the presence of flavonoids, phenols, steroids, tannin, phlobatannin, cardiac glycosides, terpenoids and aminoacids respectively (Table 5).

Table 5. Phytochemical test for different extracts of *Pongamia glabra* and *Phyllanthus niruri*.

Plant	Solvent extract	Phytochemicals*												
		1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Pongamia glabra</i>	Chloroform	+	-	-	+	-	-	+	+	-	+	+	+	+
	Ethanol	-	+	-	-	+	+	-	+	+	-	-	-	-
	Acetone	-	-	-	+	-	-	-	-	-	-	+	-	+
	Aqueous	-	+	+	-	-	-	+	+	-	-	-	-	-
<i>Phyllanthus niruri</i>	Chloroform	-	-	-	+	+	-	-	+	+	+	+	+	+
	Ethanol	+	+	-	-	-	+	-	-	-	-	-	-	-
	Acetone	-	-	+	-	-	-	-	+	+	-	-	-	-
	Aqueous	-	-	+	+	-	-	+	-	-	-	+	+	-

'+' Present, '-' Absent, *1-Alkaloids, 2-Anthraquinones, 3-Coumarins, 4-Flavonoids, 5-Phenols, 6-Quinones, 7-Saponins, 8-Steroids, 9-Tannin, 10-Phlobatannin, 11-Cardiac glycosides, 12-Terpenoids and 13-Aminoacids.

4. Discussion

The selected plant species have the property of curing diseases and disorders related to respiratory ailments, blood and liver, urinary system, diabetes, animal bites and parasitic related problems, rheumatism, mental aberrations, strengthening the gums. *P. glabra* and *P. niruri* were selected based on their relevant ethno-medical data and the antimicrobial activity was evaluated. Screening of medicinal plants for their potential pharmacological properties is highly essential (Vlietinck et al., 1995; Grosvenor et al., 1995). Plants contain many chemicals such as alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids that are responsible for various biological properties.

In the present study, the leaf extracts of *P. glabra* and *P. niruri* were tested for its antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterobacter aerogens* in the in vitro conditions using the standard disc diffusion method. The

maximum zone of inhibition was observed in the ethanol extract of *V. negundo* against the tested bacterial pathogens such as *E. coli* (11.35 mm), *S. aureus* (12.25 mm), *M. luteus* (13.48 mm) and *E. aerogens* (11.54 mm) respectively. The other two plants extracts showed moderate activity against the tested bacterial pathogens. The extracts of *P. niruri* and *P. glabra* also had inhibitory action against the tested pathogens. The pharmacognosy researches showed the presence of alkaloids (Joshi et al., 1986), terpenoids (Singh et al., 1991), and lignans (Huang et al., 1992) in *P. niruri* and *P. glabra* contains the compounds such as alkaloids, flavonoids, tannin, polyphenols and oil (Irobi et al., 1994; Brantner et al., 1996) which had a wide range of biological activity.

The resistance of microorganisms to drugs, side effects of modern drugs and emerging diseases where no medicines are available have stimulated great interest in plants as significant source of new medicines (Patwardhan et al., 2004). Several plants have been screened for their possible antimicrobial properties (Sahin et al., 2003; Nair et al., 2005). In the present study, the chloroform, ethanol, acetone and aqueous extracts of *P. glabra* and *P. niruri* respectively were tested for its antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Microsporum* sp. in the in vitro conditions using the standard disc diffusion method. Ethanol, acetone and aqueous extracts of *P. niruri* showed the highest activity against *A. niger* (8.25 mm), *A. flavus* (7.0 mm) and *A. fumigatus* (9.14 mm) respectively. The highest activity against *Microsporum* sp. was observed in acetone extract of *P. glabra* (4.50 mm). Among the three leaf extracts, the maximum activity was observed in the acetone extract of *P. niruri* against *A. fumigatus* (9.14 mm).

The relatively low potency values in some of the extracts evaluated in these assays could be due to the impure form and/or low concentration of the active compound(s) in the extracts (Rabe and Van Staden 1997). It is also possible that the presence of certain other compounds in these extracts had an antagonistic effect on the active compound(s). However, some of these plant extracts, as with some drugs, may be more potent in vivo due to metabolic transformation of their components into highly active intermediates or their interaction with the immune system (Garcia et al., 2003; Ngemenya et al., 2006). In the present study, *P. niruri* showed the highest activity because of the presence of flavonoids, terpenes, benzenoids, ligans, lipids and vitamin C. The herb, *P. niruri* has been used widely for the treatment of jaundice (Manjrekar et al., 2008). Tona et al. (2004) reported the antimalarial activity of *P. niruri* in the in vivo condition. The knowledge of extent and mode of inhibition of specific compounds which are present in the plant extracts, may contribute to the successful application of such natural compounds for treatment of fungal and bacterial diseases.

The medicinal effects of the plant material typically result from the combination of secondary metabolites such as alkaloids, steroids, tannins and flavonoids which are synthesized and deposited in specific parts of plants. *P. niruri* extracts contains tannin, phlobatannin, flavonoids, terpenoids and cardiac glycosides. The pharmacognosy researches of *P. niruri* showed the presence of alkaloids (Joshi et al., 1986), terpenoids (Singh et al., 1991) and lignans (Huang et al., 1992). Some of these compounds, such as phyllanthin, and hypophyllanthin, have been reported to be hepatoprotective (Syamasundar et al., 1985) whereas Niruriside appears to be a specific inhibitor of HIV replication (Qian-Cutrone et al., 1996). Four lignans (phyllanthin, hypophyllanthin, phyltetralin and niranthin) were found in *P. niruri* with the highest amount of lignans found in the leaves and the least amount in the roots (Murugaiyah and Chan 2007).

P. glabra extract contains phlobatannin, saponin, flavonoids, terpenoids, cardiac glycosides and alkaloids. *P. glabra* showed significant bioactivity, this may be due the presence of alkaloids, flavonoids, tannin, polyphenols and oil (Gary and Kasera 1983; Irobi et al., 1994; Brantner et al., 1996). The thorough photochemistry of the extracts must be carried out with the

modern techniques and the bioactive compound should be separated, identified and incorporated in the drug discovery.

Conflict of interest statement

We declare that we have no conflict of interest.

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