ANTIMICROBIAL AND ANTHELMINTIC POTENTIAL OF *GLINUS OPPOSITIFOLIUS (LINN) FAMILY: MOLLUGINACEAE.*

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Summary

In the present study, the petroleum ether ,chloroform and alcoholic extract of Glinus oppositifolius (L) aerial parts were subjected to preliminary phytochemical screening , antimicrobial and anthelmintic activity. The preliminary phytochemical screening Results of preliminary phytochemical tests suggest that petroleum ether extract shows the presence of cardiac glycosides and flavonoids, chloroform extract shows the presence of cardiac glycosides and phenolic compound and methanol extract shows the presence of steroid, saponin glycosides ,alkaloids and phenolic compounds. The alcoholic extract exhibited significant anti bacterial, antifungal activity, comparable to the standard drug norfloxacin. The all three extracts were evaluated for Anthelmintic activity on adult Indian earthworms, '*Pheretima posithuma'*. The alcoholic extract produced more significant Anthelmintic activity than other extracts and the activities are comparable with the standard drug albendazole. The activity of methanolic extract may be due to the presence of phytoconstituent present on it.

Keywords: Glinus oppositifolius (L), Antimicrobial, Anthelmintic, Norfloxacin, Albendazole.

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Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine(1). Plants remain the most common source of antimicrobial agents and anthelmintic agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa (2). The most of the medicinal preparation now a day available in the market are either not effective upto the mark or has developed resistance resulting in reoccurrence again. Plant derived drug serve as prototype to develop more effective and less toxic medicines Glinus oppositifolius (L) is commonly known as Jima in West Bengal .It belongs to family Molluginaceae.

It has a lot of therapeutic importance in the ancient system of medicine. The plant is highly esteemed in India as bitter vegetable, eaten occasionally on account of its stomachic, aperient and antiseptic properties. An infusion of the plant is given to women to promote the menstrual discharge. It is used as blood purifier, improves digestion, stimulate the action of liver and cures burning sensation and skin diseases. Traditionally, the plant is also used as diuretic, anthelmintic, digestive, antioxidant, antimalarial and antiviral property (3-4). On account of its use as antimicrobial and anthelmintic, this study was undertaken to evaluate the antimicrobial and anthelmintic potential of different solvent extract of Glinus oppositifolius (L).

Materials and Methods

Plant Material

The plant Glinus oppositifolius were collected from the rural belt of Midnapore district, West Bengal in July 2010. P. G.Diwakar, scientist E& head of office, Botanical survey of India, Koregaon road, Pune did authentication of the plant. The herbarium of the plant specimen has been deposited at B.S.I. Pune, the voucher specimen Number Pattanayak-1. (Reference number-BSI/WC/Tech/2010/429).

Drugs and chemicals

The following drugs and chemicals were used. Drugs:

Albendazole (BANDY, Mankind Pharma Ltd., New Delhi), Norfloxacin (Mankind Pharma Ltd., New Delhi), Chemicals:Petroleum ether A.R. (SD FINE, Mumbai), Chlorform A.R. (SD FINE, Mumbai), Methanol A.R (SD FINE, Mumbai), Dimethyl formamide (DMF) (SD FINE, Mumbai),Mumbai).

Extraction of Plant Material

The aerial parts of the plant Glinus oppositifolius (L) were air-dried, coarsely powdered and were then extracted by using different polarity solvents.The extraction was done by soxhelation untill the solvent became colourless.Each extract was filtered and then it was concentrated vacuum distillation and then dried in open air. The percentage yield of petroleum ether, chloroform and methanol extract of Glinus oppositifolius was found to be 1.18%, 2.03% and 6.29 % respectively.

Anti microbial Study

Micro Organisms:

Four strains of *Bacillus subtilis, Staphylococcus* aureus , *Pseudomonas aeruginosa* and *Escherichia coli*, were used for assessing the anti bacterial activity .One fungal strains Asperigillus niger was used for anti-fungal activity. The microorganisms were obtained from the

Department of Pharmaceutical Microbiology, Shardchandra Pawar College of Pharmacy, Pune, Maharashtra, India.

Study Protocol:
Antimicrobial activity was determined by cup-plate agar diffusion method. Muller Hinton and Saboured Dextrose Broth were used as medium for bacterial and fungal strains respectively. The petridishes with the bacteria and fungal cultures were incubated at 37±20^{0}C for 24 hrs and 27±20 ^{0}C for 48 hrs respectively. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. Both positive and negative controls were determined, for negative control the three solvents (pet ether, chloroform and methanol) were also used to determine their effect on test organisms. Norfloxacin in 10µg/ml and 20µg/ml were used to compare the effectiveness of the extracts against bacteria and fungi. The assessment of anti microbial activity was based on the measurement of diameter of inhibition zone formed. The experiment was repeated thrice, averaged and the mean values were tabulated (5, 6, 7,8).

Anthelmintic Activity Study

The method, described by Pattanayak *et al. Pharmacologyonline* 1: 35-39 (2009) was followed for this study.

Animals
Indian adult earthworms (*Pheretima posthuma*) collected from moist soil were used for the Anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (9, 10).

Anthelmintic activity
All the extracts of Glinus oppositifolius were dissolved in minimum amount of DMF and then volume is adjusted to 10 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. Five groups of six earthworms each were released into 10 ml of desired formulations as follows; vehicles (5% DMF in normal saline), Albendazole (20 mg/ml), petroleum ether, chloroform, and methanol of G.*Oppositifolius* (20mg/ml, each) in normal saline containing 5% DMF. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors (11).

Results
Phytochemical constituents present in the plant extract included steroid, glycoside, alkaloid, phenolic compound and flavoineds.

Table1. Results of phytochemical screening of extracts of Glinus oppositifolius (L).

Sr.no	Extacts	Phyto chemical presents
1.	Pet ether extract	cardiac glycosides and flavonoids.
2.	Chloroform extract	cardiac glycosides and phenolic compound
3.	Methanol extract	Steroid, saponin glycosides, alkaloids, phenolic compounds.

Results of the antimicrobial activity of the plant extracts are shown in Table 2. The result shows both gram positive and gram negative organisms. The highest activity was demonstrated by the methanol extracts of Glinus oppositifolius while the lowest activity was demonstrated by the pet ether extract against microorganisms

Table 2. Results of antimicrobial activities of extracts of Glinus oppositifolius (L).

Sr.no	Microorganism	Zone of inhibition(mm) of doses in µg/ml concentration							
		Pet ether extract (µg/ml)		Chloroform extract (µg/ml)		Methanol extract (µg/ml)		Norfloxacin	
		2000	2500	2000	2500	2000	2500	10	20
1.	*Bacillus subtilis*	-	-	1	1	3	3	4	6
2.	*Staphylococcus aureus*	-	-	1	2	3	3	4	5
3.	*Pseudomonas aeruginosa*	-	-	1	1	2	3	1	2
4.	*Escherichia coli*	-	-	1	1	2	3	2	3
5.	*Aspergillus niger*	-	-	1	2	3	4	4	6

In this anthelmintic assay, extract of Glinus oppositifolius (L).not only produced paralysis but also cause death of both species of worms. As shown in Table 3, ethanolic extract exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 20 mg/ml concentration. Similar eff ects were observed for the tested standard drugs (i.e., Nitazoxamide and albendazole), at 20mg/ml dose concentration.

Table 3. Anthelmintic activity of Glinus oppositifolius (L).

Sr.no		Time for paralysis (min)	Time for Death (min)
1.	Control	No paralysis upto 30 min	No death upto 30 min
2.	Albendazole (20mg/ml)	4.14	10.52
3.	Petether extract	No paralysis upto 30 min	No death upto 30 min
4.	Chloroform extract	18.10	No death upto 30 min
5.	Methanol extract	4.50	12.40

Discussion

Phytochemical constituents such as alkaloids, flavonoids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores (12,13). This may therefore explain the demonstration of antimicrobial activity by the aerial parts extracts of Glinus oppositifolius. The demonstration of antibacterial activity against both gram positive and gram negative bacteria anti fungal activity may be indicative of the presence of broad spectrum antibiotic compounds (14). This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. Out of the three solvents used for extraction, the methanol extracts showed the highest activity against the test organisms, followed by the chloroform extracts and Petether

extracts. Different solvents have been reported to have the capacity to extract different phytochemical depending on their solubility or polarity in the solvent.
The highest anthelmintic activity shown by the methanol extract as described herein against earthworms suggests that it could be effective against parasitic infections of humans. Albendazole on the other hand causes death of the parasite. The lethal effect of Albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake (15).
It could be concluded that the ethanolic extract showed most potent antimicrobial and anthelmintic activity. The other two extracts e.g., petroleum ether and chloroform extracts, exhibited lesser activity than the ethanolic extract.
The present study revealed that the antimicrobial and anthelmintic activity increases with increasing polarity of solvents. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for antimicrobial and anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of Glinus oppositifolius as an antimicrobial and anthelmintic drug.

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