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Phytochemical screening and antimicrobial activity in leaves of *Gnetum ula,* Brongn

V Priya and PV Anjana

Abstract
The aim of the present study is to investigate the presence of phytochemical compounds and antimicrobial activities in the leaves of *Gnetum ula* using methanolic and hexane extract. Various concentrations of extracts have been used against the clinical microbes (fungal strains: *Aspergillus niger, penicillium* sp. and bacterial strains: *Escherichia coli, Serratia* sps. and *Bacillus subtilis)* using standard methods. Phytochemical screening in both extracts reveals the presence of alkaloids, flavonoid, glycoside, phenol, phytosterol, tannins, quinine, carbohydrate, protein and absence of saponin and triterpenoids. Antimicrobial studies indicate that the methanolic and hexane extracts inhibit the growth of microbes, but it may get altered due to different concentration levels. The study exhibits higher inhibition against the fungal strains than the bacterial strains.

Keywords: *Gnetum ula,* phytochemical screening, anti-microbial

Introduction
Gnetum ula is considered as sacred plant by different regions of India. It is a large evergreen woody climber with scaly bark. Leaves are ovate, oblong or elliptic in shape and are arranged in opposite manner. Flowers are monoecious, whorled in the axils of copular bracts in panicle spikes. Fruit is a drupe and reddish orange when ripened. Seed is solitary, ellipsoid and hard. Flowering and Fruiting time is December to June. Mainly it is found in Western Ghats near Khandala, Kerala, Nilgiris, Godavari district of Andhra Pradesh and Orissa. Seeds are used for edible purpose either roasted or boiled and the seed oil is used in rheumatism by folklore practitioners. Stem and leaf extracts are useful in jaundice and liver enlargement, while leaf paste is applied externally cures arthritis.
Natural compounds or phytochemicals play an important role in medicinal field. Plants-derived substance have recently become of great interest owing to their versatile application. Plants are the richest bio-resource of traditional system of medicine, food supplements, and chemical entities for synthetic drug. Today gymnosperms plants were commonly grown in garden only for ornamentation apart from this gymnosperm plants were also used as medicine. Microbial resistance is a world concerned problem. Efforts are being made to discover new antimicrobial agents from various sources. Constant research investigations may result in the discovery of novel effective agents. This study revealed that the plant extracts possessed bioactive compounds that have antibacterial and antifungal infectious diseases.

Materials and Methods
The plant materials are collected from Sree Andalur Kavu located nearly 20km away from Kannur town of Kannur district, Kerala. And leaves are shade dried and powdered. Both the extracts were prepared by using soxhlet method.

Gnetum ula -Habitat

Preliminary Phytochemical Analysis

Test for alkaloids

1ml of extract in two separate test tubes, 2-3 drops of Dragendorff's, Wagner's reagent and Mayer's reagents were separately added.

Test for flavonoids

1 ml of extract 1ml of ferric chloride is added.

Test for glycosides

Keller Kilimi Test: 2ml of extract 1ml of glacial acetic acid with ferric chloride and concentrated H_2SO_4 was added.

Test for saponins

1 ml of extract was taken in a test tube and 5 ml of distilled water was added and vigorously shaken.

Test for tannins

2ml of the extracts were diluted with distilled water in separate test tubes; 2-3 drop of 5% ferric chloride ($FeCl_3$) solution was added.

Test for steroids

2ml of the extract were taken in separate test tubes and evaporated to dryness. The residues were dissolved in acetic anhydride and then chloroform was added. Conc. H_2SO_4 was added by the side of the test tube.

Test for terpenoids

5ml of extract were mixed with 2ml of chloroform and con. H_2SO_4 to form a layer.

Test for tri-terpenoids

Chloroform Test: To 1ml of extract, few drops of chloroform were added and it was treated with concentrated sulphuric acid solution.

Test for phenols

5 ml of concentrated extracts were taken and 2ml of neutral ferric chloride solution was added.

Test for quinone

1 ml of extract added with 5 ml of conc. HCl

Test for Cardiac glycosides

Keller killani Test: To 1 ml of extract add 2 ml of glacial acetic acid and few drops of 5% ferric chloride solution. Then it was treated with few drops of concentrated sulphuric acid.

Test for Proteins

Biuret test: To 1 ml of the test solution, few drops of 0.7% copper sulphate solution and 1 ml of 10% NaOH were added and mixed thoroughly

Test for Carbohydrates

Benedict's test: To 1ml of extract, add 2mlof Benedict's reagent and heated in boiling water bath for 5minutes.

Antimicrobial Activity

To evaluate the antimicrobial activity of selected bacterial, that is, *Escherichia coli*, *Bacillus subtilis* and *Serratia spp.* and fungal strains, that is, *Aspergillus niger*, *Penicillium* by using plant extract and fractions analyzed by disc diffusion method (NCCLS, 1997), minimum inhibitory concentration (MIC) with some modification using method described by Sarker *et al*., (2007) [8].

Results and Discussion

1. Preliminary phytochemical analysis

Preliminary phytochemical analysis of hexane and methanol extract of *G. ula* leaves showed the presence of alkaloid, flavonoid, glycoside, phenol, phytosterol, tannins, quinine, carbohydrate and protein. And both the extracts show negative results for saponin and triterpenoids. The results are shown in Table. 1.

Table 1: Phytochemical screening of methanol and hexane leaf extract of *G. ula*

S. No	Name of the compound	Hexane	Methanol
1	Alkaloid	+	+
	Mayer's test		
	Dragendorff's test	+	+
	Wagner's test	+	+
2	Flavonoides	+	+
3	Cardiac glycoside	-	+
4	Glycoside	+	+
5	Terpenoides	-	+
6	Saponin	-	-
7	Phenol	+	+
8	Phytosterol	+	+
9	Triterpenoids	-	-
10	Tannins	+	+
11	Quinone	+	+
12	Carbohydrate	+	+
13	Protein	+	+

Gnetum ula has a medicinal value due to the presence of phytoconstituents. These phytoconstituents make the plant useful for treating different ailment and have a potential of providing useful drugs of human use (Gourav, *et al*., 2013) [9]. Yogesh Kumar and Krishna Swamy, 2013, reported that the bark of *G. ula* show the presence of alkaloids, flavonoids, phenols and tannins. The phytochemical analysis of the leaves of *G. africanum* shows the presence of alkaloid, tannin, sterol, saponin, flavonoid and terpenoid (Chinyere Ilodibia, *et al*., 2015) [10]. Flavonoids are most commonly known for their antioxidant activity and they possess anti-cancerous, anti-inflammatory and antimicrobial activity (Balch and Balch, 2000), alkaloids are known to have antipyretic properties, saponins have antifungal properties (Ogu, *et al*., 2012), and similarly phenols are known to inhibit the mutagenesis of cell DNA (Heinonen, *et al*., 1998).

2. Antimicrobial study

Antimicrobial activities of methanol and hexane extracts of *G. ula* leaves were studied at different concentrations (25,50,75,100 mg/ml) against three human pathogenic bacterial strains (*E. coli*, *Serratia* spp., *Bacillus subtilis*) and two fungal strains (*Aspergillus niger* and *Penicillium* sp.). Antimicrobial potentials of extracts were assessed in terms of zone of inhibition of microorganism's growth. The results are shown in table 2-3. The higher antibacterial activity is exhibited by the methanol extract than the hexane, in which the zone of inhibition is higher in *E. coli* in 100mg/ml in methanolic solvent (12mm). In the case of antifungal study also methanolic extract show higher zone of inhibition in penicillium in 100 mg/ml (22mm).

In the study Chinyere Ilodibia, *et al*., 2015 [10] antimicrobial activity of *G. africanum* leaves also exhibit antifungal property than the antimicrobial activity like the present study shows. The antimicrobial activity of *G. gnemon* in Adolf Jan

Nexson Parhusip and Azis Boing Sitanggang, 2011 explained that in correlation with the present study the methanolic extract shows more zone of inhibition than the hexane extract.

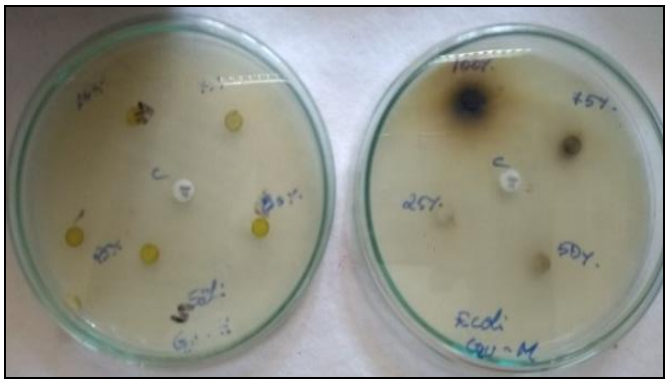
Table 2: Antibacterial activity of *Gnetum ula* leaves in different solvents

S. No	Name of Organism	Zone of inhibition (mm)								
		Standard (Ampicillin)	Sample Concentration (mg/ml)							
			Hexane				Methanol			
			25	50	75	100	25	50	75	100
1	*E. coli*	-	1	1	2	2	5	8	9	12
2	*Serratia*	-	0	0	1	1	3	4	5	6
3	*Bacillus subtilis*	-	0	1	1	2	4	5	8	11

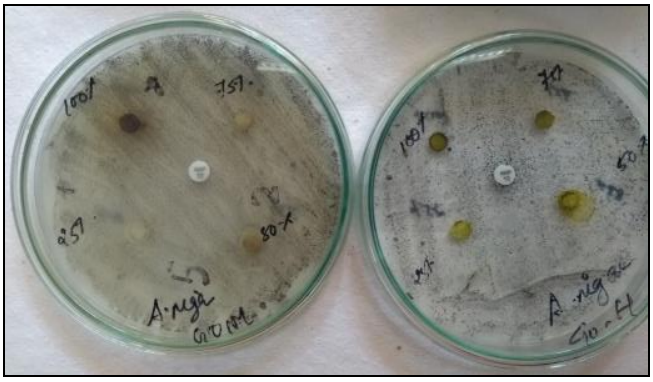
Table 3: Antifungal activity of *Gnetum ula* leaves in different solvents

S. No	Name of Organism	Zone of Inhibition (mm)							
		Standard (Ampicillin)	Sample Concentration (mg/ml)						
			Hexane				Methanol		
			25	50	75	100	25	50	75	100
1	*Aspergillus niger*	-	2	4	9	13	8	9	14	16
2	*Penicillium*	-	3	4	10	11	10	12	17	22

Antimicrobial activity in different solvent of *Gnetum ula* leaves



E. coli streaked plate



Penicillium sp. streaked plate

Bacillus subtilis streaked plate

Conclusion

In the present study the phytochemical screening and antimicrobial activity of G. ula leaf extract composed of various phytoconstituents and some of them are responsible for antimicrobial activity and others have several medicinal properties. Thus, this type of analyses are the primary step towards understanding the nature of active principles in this medicinal plant which will be helpful for further research.

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