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Antimicrobial Activity and phytochemical analysis of *Phyllanthus acidus*

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Abstract

Various medicinal plants have been used for years in daily life to treat disease all over the world. In this present study focus the antimicrobial and phytochemical activity of phyllanthus acidus leaf and fruit extracts obtained from different extracts (methanol, ethyl acetate and Diethyl ether) methanol extracts of the phyllanthus acidus showed highest toxicity. A qualitative phytochemical analysis was performed for the detection of alkaloids, flavonoids, steroids, terpenoids, anthroquinones, phenols, saponins, tannins, carbohydrates, oils and resins.

Keywords: *Medicinal plant, Antimicrobial, Phyllanthus acidus, Phytochemicals.*

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 source. Interest towards traditional natural products has increased on a larger scale (Taylor *et al*., 1996). In the traditional system of ayurvedic treatment, medicines consisting of plant products either single or in combination with others are considered to be less toxic and free from side effects when

compared to synthetic drugs (Latha *et al*., 1999). The disorders associated with the liver are also numerous and varied (Wolf, 1999).
Medicinal plants are plants that have at least one of their parts (leaves, stem fruit have barks or roots) used for therapeutic purpose (Bruneton, 1993). The availability and relatively cheaper, Cost of medicinal plants make them more attractive as therapeutic agents when compared to modern medicine (Agbor *et al*., 2005).

Phyllanthus acidus is commonly known as star gooseberry. It is quite a common tree found in the tropics and belongs to the plant family euphorbiaceae. *P. acidus* is consumed as herbs by the Indian tribal for remedy of gastro intestinal tract disorders (Supratic Kunder *et al*.,). *Phyllanthus sps* has long been used in folk medicine in many countries as antimicrobial and / or antioxidants (I.M.S Elden *et al.,* 2010). *Phyllanthus acidus* leaf extract have antioxidant, analgesic and anti-inflammatory activities (Raja chakraborthy *et al.*).

The phytotherapeutic can provides many modern drug development can provides many invaluable drugs from traditional medicinal plants. Search for pure phytochemicals as drug is time consuming and expensive. Numerous plants and polyherbal formulations are used for the treatment of liver diseases. World plant biodiversity is the largest source of herbal medicine and still about 60-80% world population rely on plant based medicines which are health care system. India is endorsed with a rich wealth of medicinal plants, which ranked our country in the list of top producers of herbal medicine. Based on this background the present study was intended to screen the plant *Phyllanthus acidus* (leaf and fruit) phytochemical analysis and antimicrobial activity.

Materials and Methods

Collection plant material

Leaves and fruits of the *Phyllanthus acidus* were collected in Salem, Tamilnadu. The plant parts were washed separately, air dried and powered. The collected leaves and fruits identified and confirmed by ABS botanical garden, Salem. The powder was extracted with different solvents (Methonal, Ethyl acetate and Diethyl ether).

Phytochemical Procedure

Preliminary phytochemicals analysis was carried out for all the extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

b) **Wagner's test**: Filtrates were treated with Wagner's reagent. Formation of brown / reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

a) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) **H_2SO_4 test**: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The color changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthraquinones

Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

Detection of Phenols

a) **Ferric chloride test**: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) **Lead acetate test**: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of Saponins

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Detection of Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of Carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Antimicrobial Activity

Screening of antibacterial activity

Bacteria tested

Totally five bacterial strains were used throughout investigation namely *Proteus vulgaris, Shigella boydii, Shigella flexneri, Klebsiella aerogenes* (Gram negative), *Corney bacterium* (Gram positive). All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of inoculums

Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37^{o}C and 25^{o}C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0 \cdot 10^{6}$ colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity. *Invitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Results and Discussion

The percentage of yield of each extract determined. The methanol extract of leaf and fruit showed the maximum yield. Leaf and fruit of the *P. acidus* methanol extract was found the most potent extract against the bacteria.

Table 1. Percentage of yield obtained in leaf extracts *Phyllanthus acidus*.

EXTRACTS	YIELD OBTAINED (Leaf)	PERCENTAGE OF YIELD (Leaf)	YIELD OBTAINED (Fruit)	PERCENTAGE OF YIELD (Fruit)
Methanol	9.86g	49.3%	8.73g	43.65%
Ethyl acetate	3.94g	19.7%	3.58g	17.9%
Di-ethyl ether	0.95g	4.75%	0.86g	4.3%

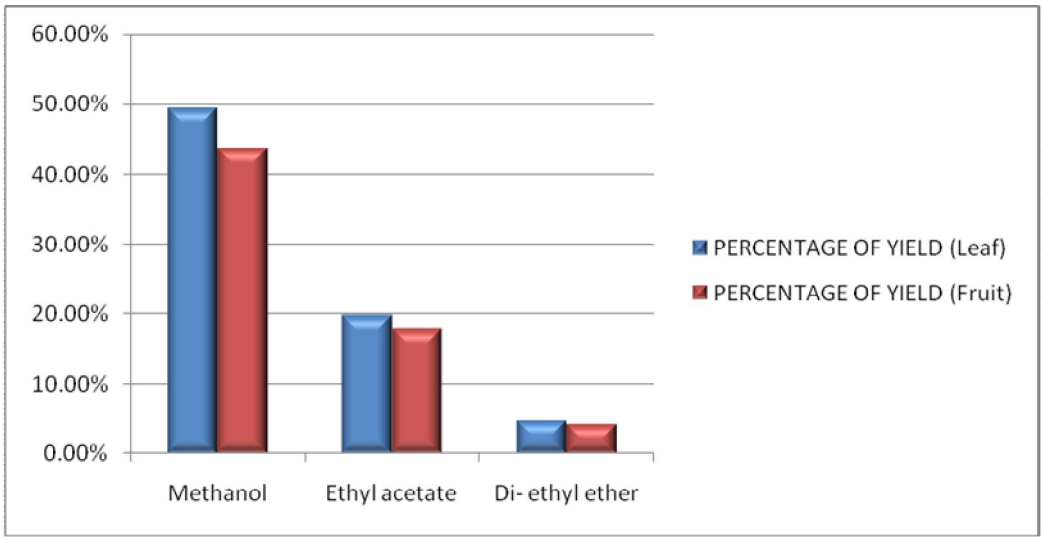

Fig 1. Percentage of yield obtained in leaf extract of *Phyllanthus acidus*

Table 2. Qualitative phytochemical analysis in leaf extract of *phyllanthus acidus.*

Phytochemicals	Extracts (*Phyllanthus acidus*)					
	Leaf			*Fruit*		
	Methanol	Ethyl Acetate	Diethyl ether	Methanol	Ethyl Acetate	Diethyl ether
Alkaloids						
Mayer's test	+	-	+	-	-	-
Wagner's test	-	-	+	-	-	+
Flavonoids						
Lead acetate test	+	-	-	+	-	+
H_2SO_4 test	+	-	-	-	+	+
Steroids						
Liebermann-Burchard test	+	-	+	+	-	-
Terpenoids						
Salkowski test	-	+	-	-	-	+
Arthroquinone						
Borntrager's test	-	-	-	-	-	-
Phenols						
Ferric chloride test	-	+	-	-	-	-
Lead acetate test	+	-	-		-	+
Saponin	-	+	+	+	+	-
Tanin	-	+	+	+	-	+
Carbohydrates	+	+	-	-	+	-
Oils & Resins	+	+	+	+	+	+

Table 3. Antimicrobial activity of *phyllanthus acidus*

MO	C	Leaf	Fruit
		P.acidus	***P.acidus***
P. vulgaris	21	9	17
C. bacterium	10	6	12
K. aerogenes	29	12	14
S. boydii	19	16	8
S. flexneri	19	9	14

Phytochemical screening correlated Alkaloids, flavonoids, steroids and phenols are present in leaf extract. Terpenoids, Anhtroquinone, saponin and tannin are absent in leaf. Leaf extract show better activity when compared to fruit extract. Phytochemical constituents such as alkaloids, flavonoids, phenols and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against many microorganisms. In this study P. acidus leaf extract was found more antioxidant than the fruit extract.

Conclusion

In this study it is concluded that *P. acidus* has high antimicrobial activity. Leaf extract shows effective result than the fruit extract. Of the three solvents, methanol extract reveals the presence of maximum phytochemical constituents. The antimicrobial assay also proves that the leaf extract obtained high concentration of yield. The presence of alkaloids in the solvent fractions could be well correlated with the antimicrobial activities. Phytochemicals possesses specific physical, chemical and biological activities that make them useful as drugs (Nathiya and Dorcus, 2012).

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