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Phytochemical Screening of whole plant extract of *Phyllanthus amarus* Schum

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Abstract
Phyllanthus amarus is an important herb upto 30-50cm in height belongs to the family Euphorbiaceae. *Phyllanthus amarus* has been used traditionally in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies etc. Phytochemical screening of petroleum ether and methanol extracts shown the presence of alkaloids, flavonoids, terpenoids, tannins and phenolic compounds in the methanol extract. It suggests that plant can be used in treatment of many diseases.

Keywords: *phyllanthus amarus*, euphorbiaceae, phytochemical screening

Introduction
Phyllanthus amarus belonging to family Euphorbiaceae [1]. *Phyllanthus amarus*, is a small, erect, annual glabrous herb with 30-50 cm in length [2]. It comprises slender, leaf-bearing branchlets, distichous leaves. The leaves are subsessile elliptic-oblong, obtuse with rounded base. Flowers of the plant are found with 5 white sepals and apical acute anther and are yellowish, whitish or greenish in color, auxillary. Male flowers are found in groups of 1-3 whereas females are solitary. Fruits are depressed-globose like smooth green capsules and fruiting pedicels present underneath the branches. Seeds are trigonous, pale brown with longitudinal parallel ribs on the back.
Phyllanthus amarus has been used traditionally in several health problem such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds over 2000 years. The plant also has been found to have anti inflammatory, antihepatotoxic, antilithic, analgesic, hypotensive, antispasmodic, antiviral, antibacterial, diuretic, antimutagenic, hypoglycaemic, etc activities.

Material and Method
Collection of Plant Material
The Indigenous plants were collected from different locations of Bhopal (M.P.) region in the month of Sept.-Oct. 2012 and Jan.-Feb. 2013. The Plant *Phyllanthus amarus* Schum was selected on the basis of ethnomedicinal value for further study. The plants were acknowledged by a senior Botanist Dr. Tayaaf Safi Principal Gandhi P.R. college Bhopal and herbarium deposited at Safia Science college Bhopal.

Preparation of Extract
Plant material was washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were grinded by using the electronic grinder. The powder of the whole plants of *Phyllanthus amarus* S. was extracted according to (Harborne and Baxter., 1995) [5]. The dried plants sample was powered and filed into the soxhlet using petroleum ether and methanol respectively. Almost all the chlorophyll and lipid was deposited on the side of the flask and removed carefully. The extracts were stored in refrigerator till any further use.

Phytochemical Screening of crude extracts of Petroleum ether, and methanol from *Phyllanthus amarus* Schum.
This property of selective reactivity of photochemical present in extracts forms the basis of chemical tests for identification of compounds. Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailments. Phytochemical screenings of the extracts were investigated according to the standard procedures (Trease and Evans., 1989 and Kokate C.K. *et al*., 2006) [6, 7].

Test for Carbohydrates:
a) Molish Test
2 ml of aqueous extract was treated with two drops of alcoholic α-naphthol solution in a test tube and then 1 ml of conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

b) Benedict's test
Equal volume of benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Test for Proteins
a) Biuret's Test
The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper

sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.

b) Million's test
3ml of extract was mixed with 5ml of million's reagent. White precipitate formed which on heating turned to brick red, indicating the presence of proteins.

Test for amino acids
a) Ninhydrin test
3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Tests for Glycosides
b) Legal,s test
1ml of test solution was dissolved in pyridine. 1ml of nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

b) Keller-Killiani test
To 2ml of test solution, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of cardiac glycosides.

Test for Saponins
a) Froth test
The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Test for Alkaloids
To the extract, dilute HCl was added, shake it well and filtered. With the filtrate, the following tests were performed.
a) Mayer's test
To 2-3 ml of filtrate, few drops of Mayer's reagent were added along the sides of tube. Formation of white or creamy precipate indicates the presence of alkaloids.

b) Wagner's test
To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

c) Dragendroff,s test
To 1-2ml of filtrate, few drops of dragendroff,s reagent were added in a test tube. Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test
To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Test for Terpenoids and Steroids
a) Salkowski's test
The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated H_2SO_4, shaken and allowed to stand. If the lower layer turns red, steroids are present. Presence of golden yellow layer at bottom indicates the presence of Terpenoids.

b) Libermann-Burchard's Test
The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Test for Flavonoids
a) Lead acetate test
The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

b) Shinoda test
To the extract, 5 ml (95%) 0f ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drops wise addition of concentrated hydrochloric acid. Formation of pink colour indicates presence of flavonoids.

Tests for Tannins and Phenolic compounds
a) Ferric chloride test
Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5 % ferric chloride solution was added. Formation of blue, green or violet colour indicates the presence of phenolic compounds.

b) Lead acetate test
Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compound.

Results and Discussion
The phytochemical screening of petroleum ether and methanol extracts have shown that the main components i.e., alkaloids, flavonoids, terpenoids, tannins and phenolic compounds were present in the methanol extract.

Table 1: Showing Phytochemical Screening of crude extracts of Petroleum ether and methanol from *Phyllanthus amarus* Schum. (+) =Presence, (-) =Absent

Phytochemicals	Tests	*Phylanthus amarus* Schum	
		Pet ether extract	Methanolic extract
Alkaloids	Mayer's Test	-	+
	Wagner's Test	-	+
	Hager's Test	-	+
Flavonoids	Lead Acetate Test	-	+
	Alkaline Reagent Test	-	+
	Shinoda Test	-	+
Terpenoids and Steroids	Salkowski Test	-	+
	Libermann Burchards Test	+	+
Glycosides	Killer Killians Test	-	+
	Legal's Test	-	+
	Bortrager's Test	-	+
Saponins	Froth Test	+	+
Tannins and Phenolic compounds	$FeCl_3$ Test	-	+
	Lead Acetate Test	-	+
Carbohydrates	Molish Test	-	+
	Bendict's Test	-	+
Amino acid and proteins	Biuret's Test	-	+
Fat and Oils	Solubility Test	-	+

Conclusion

Phytochemical screening of both extract confirmed the presence of several bioactive compounds like alkaloid, flavonoids, Terpenoids, glycosides, saponin, and carbohydrate. It suggests that plant having various medicinal properties which can be used in the treatment of various diseases. Thus there is need of more study in order to evaluate the effects of these compounds on biological system.

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