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COMPARATIVE PHYTOCHEMICAL INVESTIGATION OF LEAF, STEM, FLOWER AND SEED EXTRACTS OF *MACROTYLOMA UNIFLORUM* L.

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ABSTRACT

Phytochemicals are secondary metabolites produced by medicinal plants and reported to have therapeutic values. The major aim of the present study was to investigate the phytochemical screening of various extracts of *Macrotyloma uniflorum* L. leaves, stem, flower and seed. Petroleum ether, chloroform, ethyl acetate, ethanol and water extracts were prepared and subjected to phytochemical screening in which the secondary metabolites were confirmed based on tests of colouration and precipitation. The leaves and stem have shown the presence of all the bioactive constituents like alkaloids, flavonoids, saponins, terpenoids, tannins etc. Flowers and seed show limited amount of phytoconstituents when compared to that of leaves and stem. Among the various extracts used, the ethanolic extract of *Macrotyloma uniflorum* leaves and stem were found to have accountsable number of phytoconstituents.

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INTRODUCTION

Plants consist of a number of biologically dynamic ingredients hence they are used for the treatment of large number of infectious diseases. These biologically active ingredients are alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds [1]. Secondary metabolites derived from plants are found to be richest bio-resource of drugs used in traditional systems of medicine [2, 3]. A large number of African and Asian populations use medicinal plants as established medicines for their primary healthcare [4]. Today the massive traditional knowledge of medicinal plants is playing an important role in the development of new drugs. Various important drugs which were developed from plant resources are aspirin from *Filipendula ulmar*, morphine from *Papaver sominiferum* and ephedrine from Ephedra species [5].

Horse gram *Macrotyloma uniflorum* (Lam.) Verdc. is a minor legume used as a pulse crop in India and has been found to have good nutritional quality [6]. Horse gram seeds have recently been shown to prevent atherosclerosis in rats and may be a potential functional food for the prevention of hyperlipidaemic atherosclerosis [7]. An alpha amylase inhibitor from horse gram seeds has recently been shown to have antihyperglycemic potential [8]. Extracts from horse gram plants have shown potential therapeutic activity in treating several human infections [9].

Macrotyloma uniflorum has been used in traditional system of medicine for treating haemorrhoids, tumours, bronchitis, cardiopathy, nephrolithiasis, urolithiasis, splenomegaly, strangury, hiccough, ophthalmopathy, verminosis, inflammation and liver problem. An attempt was made to explore the hepatoprotective activity of *Macrotyloma uniflorum* in Wistar albino rats [10]. Leaves and stems were reported to have more number of amino acids and lectin like glycoprotein. Presence of coumesterol and psoralidin were also reported. Seeds of *M. uniflorum* contain lectins, glycoprotein, agglutinin, anti-A phytoagglutinin, glycosidase enzymes, allantoinase, diuretic dipeptide and pyroglutamyl glutamine [9]. Literature survey showed that Dolichin A & B, pyroglutaminylglutamine along with some flavonoids were isolated from this plant [11].

The present study was carried out to find the solvent system among the various solvents used and also to find the *Macrotyloma uniflorum* part that possess maximum number of phytoconstituents.

MATERIALS AND METHODS

Plant material

The plant specimens (Leaf, stem, flower and seed) for the proposed study were collected from Kothavadi village, Coimbatore district, Tamil Nadu, India. The plant was taxonomically authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/2013-14/Tech/1309.

Sequential solvent extraction

The extraction was carried out for 16 hours with the solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate, ethanol and water. 50 g of dried plant powder was extracted in 250ml of each solvents and kept in an orbitory shaker for 72 h. Repeated extraction was done with the same solvent till clear colorless solvent is obtained. Each time before extracting with the next solvent the residue was dried thoroughly to remove the solvent used. Obtained extract was evaporated and stored at 0-4˚C in an air tight container.

Extraction yield

The selected parts were extracted with different solvents. The percentage of yield is calculated by using the formula:

$$\text{Percentage of Yield (\%)} = \frac{\text{Amount of extract yield (g)}}{\text{Amount of dried plants used (g)}} \times 100$$

Qualitative analysis of secondary metabolites

The qualitative analysis of secondary metabolites was carried out by the standard methods [12, 13].

Test for alkaloids

2 ml aliquot of the extract was treated with the Dragenderoff's reagent. An orange red precipitate is produced immediately indicating the presence of alkaloids.

1 ml aliquot of the extract was treated with few drops of Mayer's reagent. Formation of white or pale yellow precipitate showed the presence of alkaloids.

Test for flavonoids

1 ml of the extract was treated with magnesium turnings and 1-2 drops of concentrated HCl. Formation of pink or red color shows the presence of flavonoids.

1 ml of the extract was treated with one ml of ferric chloride. The formation of brown color confirms the presence of flavonoids.

Test for tannins and phenolic compounds

1 ml of the extract was treated with few ml of 5% neutral ferric chloride. A dark blue or bluish black color product shows the presence of tannins.

1 ml of the extract was treated with few ml of gelatin solution; a white precipitate is formed revealing the presence of tannins and phenolic compounds

1 ml of the extract was treated with few ml of lead tetra acetate solution. A precipitate production shows the presence of tannins and phenolic compounds.

Test for amino acids and proteins

To 1 ml of extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. Development of purple color indicates the presence of proteins.

The extract was treated with one ml of 40% sodium hydroxide solution and two drops of 1% copper sulphate reagent. Appearance of violet color indicates the presence of proteins.

Test for carbohydrates

Fehling's test

The extract was treated with 5 ml of fehling's solution (A and B) and kept at boiling water bath for 5 min. Formation of yellow or red color precipitate indicates the presence of reducing sugar.

Benedict's test

To 1 ml of the extract, added 5 ml of Benedict's solution and kept at boiling water bath for 5 min. Red, yellow or green precipitate indicates the presence of reducing sugars.

Test for glycosides

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer which indicates the presence of glycosides.

Test for saponins

About 1 ml of alcoholic extract was diluted separately with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. A one cm layer of foam indicates the presence of saponins.

To 1 ml of the extract, 1 ml of alcoholic vanillin solution was added which was followed by the addition of few drops of concentrated sulphuric acid. A deep violet color confirms the presence of saponins.

Test for fixed oils and fats

Spot test:

A small quantity of extract is pressed between two-filter papers. Oil stains on the filter paper indicates the presence of fixed oil.

Test for terpenoids

Horizon test

To 1 ml of extract, 2 ml of trichloroacetic acid was added. The formation of yellow to red precipitate shows the presence of terpenoids.

Libermann test

To 1ml of extract 3 ml of acetic acid and few drops of concentrated sulphuric acid were added. Color changed from red to blue indicating the presence of terpenoids.

Test for steroids

Libermann-Burchards test

To 1.0 ml plant extract, 1.0 ml of concentrated sulphuric acid was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and it turned blue to indicate the presence of steroids.

Salkowski reaction

To 2.0 ml sample extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the tube. A red colour was produced in the chloroform layers.

RESULT AND DISCUSSION

The secondary metabolites contribute much in the direction of the biological activities of medicinal plants such as hypoglycemic, anti-diabetic, antioxidant, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities, antimicrobial activity etc [14]. Several therapeutic effects of *Macrotyloma uniflorum* was established in Indian system of medicine. Various phytochemicals that are present in different parts of *Macrotyloma uniflorum* are responsible for this therapeutic effect.

Table 1: Percentage of yield Extract.

Extracts	Plant parts	Percentage of yield (%)
Petroleum ether	Leaf	2.12
	Stem	1.8
	Flower	0.8
	Seed	1.0
Chloroform	Leaf	3.94
	Stem	3.34
	Flower	1.22
	Seed	1.46
Ethyl acetate	Leaf	2.02
	Stem	1.74
	Flower	1.06
	Seed	1.3
Ethanol	Leaf	4.11
	Stem	2.92
	Flower	1.3
	Seed	1.96
Water	Leaf	1.84
	Stem	1.52
	Flower	1.2
	Seed	1.72

The yield of sequential extracts (g) is shown in [Table 1].
Presence of phytochemicals were analysed by the qualitative test which are shown in table (2-5).

Table 2: Phytochemical analysis of *M.uniflorum* Leaves.

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	-	+	-	+	+	-	+	+	+	-
Chloroform	-	+	-	+	+	-	+	+	+	-
Ethylacetate	-	+	-	+	+	+	+	-	+	+
Ethanol	+	+	+	+	+	+	+	+	+	+
Aqueous	-	+	+	+	+	+	+	-	-	+

AL - Alkaloids
SA - Saponins
TP - Tannin and phenolic compounds
FL - Flavonoids
ST - Steroids
CG - Cardioglycosides
OF - Oils and Fats
TN - Terpenoids
AP - Amino acids and Proteins
CH - Carbohydrates
"+" Present "-"Absent

In table 2, the petroleum ether extract of *M. uniflorum* recorded the presence of flavanoids, amino acids, proteins, carbohydrates, saponins, oils, fat and terpenoids. Chloroform extract shows the presence of phytoconstituents as same as in petroleum ether extract. Ethyl acetate extract was found to have maximum number of phytoconstituents except alkaloids, tannins, phenols, oils and fat. Aqueous extract shows the presence of all phytoconstituents except alkaloids, terpenoids, oils and fat, where as the ethanolic extract of leaves contain all the phytoconstituents.

Table 3: Phytochemical analysis of *M.uniflorum* flowers.

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	-	+	-	+	-	-	+	+	-	-
Chloroform	-	+	-	+	+	-	+	+	+	-
Ethylacetate	-	+	-	+	+	+	+	+	-	-
Ethanol	+	+	-	+	+	+	+	-	-	+
Aqueous	-	+	+	+	+	+	+	-	-	+

In Table 3, the petroleum ether extract revealed the presence of flavanoids, amino acids, proteins, oils, fat and saponins. Chloroform extract shows the presence of all phytoconstituents except alkaloids, tannins, phenols, cardioglycosides and steroids. Flavanoids, amino acids, proteins, carbohydrates, cardioglycosides, oils and saponins were found in ethyl acetate extract. All the major phytoconstituents except terpenoids, tannins, phenols and oil were present in ethanolic extract. Aqueous extract shows the presence of flavanoids, tannins, phenols, amino acids, proteins, carbohydrates, cardioglycosides, saponins and steroids.

Table 4: Phytochemical analysis of *M.uniflorum* Seeds.

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	-	-	-	+	-	+	+	-	-	+
Chloroform	-	-	-	+	-	+	+	-	-	+
Ethylacetate	+	-	-	+	+	+	+	-	-	+
Ethanol	-	-	-	+	-	+	+	+	-	+
Aqueous	-	-	+	+	-	+	-	-	-	+

From table 4, it is evident that the petroleum ether extract recorded the presence of some phytoconstituents like amino acids, proteins, cardioglycosides, saponins and steroids. Chloroform extract shows the presence of phytoconstituents that are similar to petroleum ether extract. Ethyl acetate extract shows the presence of maximum phytoconstituents except flavanoids, tannins, phenols, oils, fat and terpenoids. Ethanolic extract shows the presence of amino acids, proteins, cardioglycosides, saponins, oils, fat and steroids, where as aqueous extract shows the presence of some phytochemicals like tannins, phenols, amino acid, proteins, cardioglycosides and steroids.

Table 5: Phytochemical analysis of *M.uniflorum* Stem.

Solvent extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petroleum ether	-	-	-	+	-	+	+	-	-	+
Chloroform	-	-	-	+	-	+	+	-	-	+
Ethylacetate	-	+	+	+	+	+	+	-	-	+
Ethanol	+	+	+	+	+	+	+	+	+	+
Aqueous	-	-	+	+	+	+	-	-	-	+

From Table 5, it is clear that the petroleum ether and chloroform extract contains similar phytoconstituents such as amino acids, proteins, cardioglycosides, saponins and steroids. Ethyl acetate extract shows the presence of all phytochemicals except alkaloids, oils, fat and terpenoids. Ethanolic extract recorded the presence of all phytochemicals tested, where as aqueous extract shows the presence of tannins, phenols, amino acids, proteins, carbohydrates, cardioglycosides and steroids.

The phytoconstituents are well known for its curative activity against several human problems such as diuretic, choleretic, spasmodic, chronic eczema, diarrhea, dysentery and menstrual disorders [15]. A variety of herbs and herbal extracts contain different phytochemicals with biological action that can be of valuable therapeutic index. Much of the protective effect of herbal plants has been attributed by phytochemicals, which are the non-nutrient compounds [16].

Existing literatures signify that medicinal plants are the backbone of traditional medicine [17]. Alkaloids, flavonoids, glycosides and phenols have been reported to exert multiple biological effects like anti-inflammatory, anti allergic, antioxidant, antidiabetic, anti-viral and anti cancer activities [18]. Alkaloids are formed as metabolic by products and have been reported to be responsible for the antibacterial activity. Phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be chief contributors to the antioxidant capacity of plants and miscellaneous biological activities may be related to their antioxidant activity. Anti- inflammatory properties of saponins, terpenoids, flavonoids, tannins, steroids and alkaloids were reported [19, 20]. Phytochemical compounds like steroids, saponins and triterpenoids were found to have sedative properties [21, 22]. Hypocholesterolemic and antidiabetic activities of saponins was well documented [23]. Flavonoid compounds such as quercetin and genistein excerted antitumor activity. These flavonoid compounds are highly cytotoxic to tumour cells but have no or insignificant activity in normal cells [24]. Saponins prevent the excessive intestinal absorption of cholesterol and thus diminish the risk of cardiovascular diseases such as hypertension [25].

CONCLUSION

This study provides evidences that different extracts of *M. uniflorum* found to have phytoconstituents and support that the ethanolic extract of *M. uniflorum* leaves and stem have more number of phytoconstituents when compared to other parts of this plant. *M. uniflorum* leaves and stem showed abundant amount of phytochemicals as secondary metabolites which participate in various pathophysiological conditions of different diseases. Intense study in this plant will help to identify the active principle and this can be used as clues for developing new drugs which may be used in the pharmaceutical industries for modern drug discoveries.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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