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RESEARCH ARTICLE

PHYTOCHEMICAL AND THROMBOLYTIC ACTIVITY OF GREWIA ORBICULATA ROTTLER. LEAF

[1,*]Srinivasa Reddy, C. H., [2]Aravind, G., [2]Akhila, R., [2]Devi Priya, P. and [2]Aradhya Sarma, B. V. L.

Department of Botany, PB Siddhartha College of Arts and Sciences, Vijayawada, India

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ABSTRACT

Grewia orbiculata of family TILICEAE is widely used in the traditional medicine for the various diseases. In present study, the plant parts (leaves) were shade dried and phytochemicals were extracted with polarity based solvents like hexane, ethylacetate and methanol. The three different extracts showed great variation in terms of phytochemical composition. The various phytochemicals in leaf includes Alkaloids, tannins, terpenoids, flavonoids saponins. These wide range of chemical constituents and these can be useful for drug discovery and development of various new formulations. Among the three different solvent extracts, ethyl acetate extract showed strong thrombolytic activity.

INTRODUCTION

Medicinal plants are well known to have a very good immune modulation property and can act on both specific and non specific immunity by its stimulation on the site. These plants promote resistance against infections in the host and maintain the body in an equilibrium state and conditioning the tissues (Thomson, 1978; Madhuri, 2008; Madhuri and Panday, 2009; Agarwala *et al*., 2001and Panday, 2006). Humans were using the plants for the various treatments of various ailments since thousands years (Unial *et al*., 2006). Plants were being used medicinally in different countries and they were still using the rely on traditional medicine for their primary health care and even found in our day to day life. These medicines are relatively safer and even cheaper than the synthetic [or] modern medicine (Palambo and Semple, 2001; Ragavendra *et al*., 2006). Herbal molecules are more safer to overcome the resistance produced by pathogens as they exist in the form of cluster which is more than a one molecule in the protoplasm of the plant cell (Abdul moniem and Saadab, 2006; Dupui's *et al*., 1972). The secondary metabolites are synthesized in a specialized cell types at a distinct development stages making their extractions and their purification so difficult. Secondary metabolites that are used commercially as a biological activity compounds are generally high value with low volume products which are used in the drug manufacture by pharmaceutical industries.

Corresponding author: Srinivasa Reddy, C. H.
Department of Botany, PB Siddhartha College of Arts and Sciences, Vijayawada, India

Many medicines were discovered through these observations of Indigenous medicinal practices (Gilani and Atta-ur-Rahman, 2005) all over the world. Present study is carried out to know the important secondary metabolites with polarity based solvents like hexane, ethyl acetate and methanol and thrombolytic activity of these corresponding solvents.

Botanical Description

Botanically *Grewia orbiculata* is a small deciduous trees; branch lets velvety. Leaves oblong, ovate or orbicular, 3-8.5 x 2-5 cm, 5- nerved, pubescent above, glaucous and woolly below, base oblique, sub cordate or rotund, margin irregularly serrate, dentate, apex obtuse; stipules linear, subulate.

Flowers bright yellow, axillary and terminal leaf opposed umbels; bracts spathulate. Sepals 5, oblanceolate. Petals 5, oblong, apex obtuse to retuse. Stamens numerous. Ovary globose, furrowed, densely stiff-hirsute, 2-locular; ovules 2 per locule, axile, stigma 4-lobed or laciniate. Drupe subglobose, obscurely 2-or 4- lobed, woolly; seed stony.

Systematic Position

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Family : Tiliaceae
Genus : Grewia
Species : orbiculata

MATERIALS AND METHODS

Collection of Plant Materials

The leaf of *Grewia orbiculata* Rottler leaf of family Tiliaceae, commonly called Peda Janakayalu in telugu collected from hill slopes of Gunadala, Andhra Pradesh, India and authenticated by Ravi Kiran, BSI, Coimbatore. A voucher specimen is deposited in Department of Botany, PB Siddhartha College of arts and the specimen number is PBS121553. Collected leaf was shade dried till the moisture content is evaporated and finally pulverized in to small pieces.

Solvent Extraction

Crude plant extract was prepared by Soxhlet extraction method.100g of leaf was uniformly packed into the thimble and extracted with 300ml of different solvents separately. Polarity based solvents used were Hexane, Ethyl acetate and Methanol. The process of extraction continues till the solvent in siphon tube of an extractor become colorless. The extract was taken in a beaker and kept it for air dry till the solvent got evaporated. Dried extract was used for phytochemical analysis.

Qualitative Phytochemical Screening (Evans *et al*., 1989; Gokhale *et al*., 1993; Trease and Evans, 1996; Harborne, 1973 and Shanmugam *et al*., 2010)

Detection of Alkaloids

Mayer's Test: One or two drops of Mayer's reagent is added to plant extract by the sides of the test tube. White precipitate indicates the presence of alkaloids.

Wagner's test: One or two drops of Wagner's reagent is added to plant extract by the sides of the test tube. Reddish brown precipitate indicates the presence of alkaloids.

Hager's Test: One or two drops of Hager's reagent is added to plant extract. Prominent yellow precipitate indicates the presence of alkaloids.

Detection of Phlobatannins

To 0.5ml plant extract few drops of 10% ammonia solution was added. Pink color precipitate indicates the presence of Phlobatannins.

Detection of Coumarins

1ml of plant extract is added with 10% sodium hydroxide. Yellow colour indicates the presence of coumarins.

Detection of Anthraquinones

0.5ml of plant extract is treated with few drops of 2% HCL. Red color precipitate indicates the presence of anthraquinones.

Detection of Tannins

Ferric chloride test: 5mg of plant extract was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicates the presence of tannins.

Gelatin test: Few ml of plant extract was treated with gelatin solution. Formation of white precipitate indicates the presence of tannins.

Detection of Glycosides

Legalstest: 2ml of plant extract is treated with 3ml of chloroform and10% ammonia solution. Formation of pink colour indicates the presence of Glycosides.

Liebermann's test: Few ml of plant crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Conc.$H_2SO_4$was added carefully. A colour change from violet to blue to green indicates the presence of glycosides.

Keller-kilani test

Few ml of plant crude extract was treated with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into a test tube containing 2ml of concentrated H_2SO_4. Formation of brown ring at the interphase indicated the presence of cardiac glycosides.

Detection of Phytosterols

Salkowski reaction test

0.5 ml chloroform extract is treated with 1 ml of concentrated H_2SO_4 from the sides of the test tube. Formation of reddish brown colour in chloroform layer indicates the presence of phytosterols.

Detection of flavonoids

Watery plant extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Alkaline reagent Test: When plant extract was treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test: Plant extract treated with few drops of 10% lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Detection of Phenols

Lead acetate test

5 mg of plant extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. Formation of a bulky white precipitate indicates the presence of phenols.

Ferric chloride test: Plant extract was treated with 5% ferric chloride. Formation of intense colour indicates the presence of phenols.

Table 1. Phytochemical screening of *Grewia orbiculata* Rottler. leaf
"+" = Present "-" = Absent

S.No	Name of the Test	Hexane Extract	Ethyl Acetate Extract	Methanol Extract
1	Alkaloid test			
	Mayer's test	+	-	-
	Wagner's test	+	+	-
	Hagers' test	+	-	-
2	Phlobatanins	+	-	-
3	Coumarins	-	-	-
4	Anthraquinones	-	-	-
5	Tannins			
	$Fecl_3$ test	-	-	-
	Gelatin test	-	-	+
6	Glycosides			
	Legals's test	-	-	-
	Liebermann's test	+	-	-
	Keller-Kilani test	+	+	+
7	Phytosterols			
	Salkowski test	-	-	-
8	Flavonoids			
	Ammonium hydroxide test	+	+	+
	Alkaline reagent test	+	+	+
	Lead acetate test	+	+	+
9	Phenols			
	Lead acetate test	+	+	+
	$Fecl_3$ test	+	+	+
10	Saponins	+	-	+
11	Terpenoids	+	+	-

Detection of Saponins

The plant extract was mixed with 5ml of distilled water and was shaken vigorously. Formation of stable foam indicates the presence of saponins.

Detection of Terpenoids

0.5ml of plant extract was mixed with 2ml of chloroform and concentrated H_2SO_4 is added carefully. Formation of red brown color at the interface indicates the presence of terpenoids.

In vitro thrombolytic activity

The in vitro thrombolytic potential of hexane, ethylacetate and methanol solvent extracts were evaluated with the method developed by Daginawala using Urokinase as the standard substance (Prasad *et al*., 2006). To 9 mL venous blood was drawn from six healthy volunteers without a history of oral contraceptive or anticoagulant therapy was distributed in eight different pre-weighed sterile eppendrof tubes and incubated at 37 °C for 45 min. After clot formation, without disturbing the clot formed, serum was completely aspirated out using syringe and the weight of clot in each tube was measured. To each tube containing pre-weighed clot, 100 µL aqueous solution of three different solvent extracts with the concentration of 10 mg/mL was added separately. Then, 100 µL of Urokinase (UK) and 100 µL of distilled water were separately added to the control tube as positive and negative controls, respectively. All the tubes were then incubated at 37 °C for 90 min and observed for clot lysis. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (weight of released clot /clot weight) X100

Statistical Analysis

All values of thrombolytic activity were calculated as mean±SEM of three parallel determinations and were evaluated using t-test. $P< 0.001$ was regarded as statistically significant.

RESULTS AND DISCUSSION

The three different extracts from leaf found to contain Alkaloids, Phlobatanins, Tannins, Glycosides, Flavonoids, Phenols, saponins and terpenoids. From the analysis hexane extract contains more phytoconstituents followed by Ethyl acetate and methanol in equal proportion. Results are tabulated in Table-1**.** Thrombolytic activity of three different extracts of hexane, ethyl acetate and methanol of *Grewia orbiculata* leaf is presented in Table 2. The maximum activity was recorded in Ethyl acetate leaf extract which amounted to (25.303±0.213) % of clot lysis while Urokinase exhibited a clot lysis of (50.005±0.277) %. The mean difference in clot lysis percentage between positive and negative control (sterile distilled water) was found statistically significant. Nevertheless, all extracts demonstrated statistically significant thrombolytic activity. Isolation of bioactive compound from ethyl acetate extract will helpful for solving cardiovascular problems.

Table 2. Thrombolytic activity of different extracts of *Grewia orbiculata* Rottler. Leaf

S.No	Sample	% of Clot lysis
1.	Hexane Extract	13.876±0.283
2.	Ethyl Acetate Extract	25.303±0.213
3	Methanolic Extract	7.841±0.067
4.	Urokinase (Positive control)	50.005±0.277
5.	Water (Blank)	2.610±0.360

REFERECES

Abdul moniem, M.A. and Saadab, A.M.A. 2006. Anti fungal activity of some saudi plants used in the traditional medicine. 5:907/909.

Agarwala, S.K., Chetarjee, S. and Mishra, S.K. 2001. Immune potentiation activity of a Polyhedral formulation immu-21" (Research Name). *Phytomedica*. 2(1and 2):1-22.

Dupui's, G., Johri, B., Bandoni, R. J. and Towers, G.H. 1972. Sinna Mylphenols as inhibitors of fungal growth. *Kanadian J. Microbial*, 18:129/932.

Evans, W.C., Trease and Evans, 1989. Pharmacognosy13th ed., Bailliere Tindall, London.

Gilani, A.H. and Atta-ur-Rahman. 2005. Trends in Ethno pharmacology. *Journal of Ethnopharmacol*, 100 (1-2): 43-49.

Gokhale, S.B., Kokate, C.K. and Purohit, A.P. 1993. A text book of Pharmacognosy, Nirali Prakshan, Pune, India,pp. 1–50.

Harborne, J.B. 1973. Phytochemical methods, Chapman and Hall Ltd., London. 49-188.

Madhuri, S. 2008. Studies on oestrogen induced uterine and ovarian carcinogenesis and effect of Proimmu in rats. Phd thesis. RDVV, Jabalur MP, India.

Madhuri, S. and Panday, G. 2009. *Some Anticancer medicinal plants of foreign Origin. Curr Sci.,* 96(6):779-783.

Palambo, E.A. and Semple, S.J., 2001. Antifungal activity of traditional medical plants. *J. Ethanopharmacol*, 77:151/157.

Panday, G. and Madhuri, S. 2006. Medicinal Plants: Better remedy for neoplasm. Indian Drugs 43(11):869-874.

Prasad, S., Daginawala, H.F., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J. and Taori, G.M. 2006. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. *Thromb J.*, 4: 14.

Ragavendra, M.P. Satish, S. and Ravisha, K.A. 2006. Phytochemical analysis and anti bacterial activity of oxalis corniculata- a known medicinal plant. my science 1:72/78.

Shanmugam, S., Kumar, T.S., Selvam, K.P. 2010. Laboratory hand book on biochemistry, PHI learning Private limited, New Delhi, India.pp.129-133.

Thomson, W.A.R. 1978. Medicines from the earth, maiden head, Mc GRA - Hill Book Co.

Trease, G.E., Evans, W. C. 1996. A textbook of Pharmacognosy.14th ed., Bailliere Tindall Ltd, London,

Unial, S.k., Singh, K.N., Jamwal, P. and LAL, B. 2006. Traditional use of medical plants among the tribal communities of Chhota Bhangal, West himalayan. Ethnobiol, Ethnomed.

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