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Phytochemical investigation of whole fruit of *Olax scandens Roxb*

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ABSTRACT

Whole fruit of Olax scandens Roxb. was analyzed for its phytochemical constitution following percolation method of extraction using different solvents. The screening tests revealed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, proteins, terpenoids, triterpenoids, steroids, coumarins, cardiac glycosides, quinones, anthraquinones and phytosterols. The method of extraction employed has been found to be very simple to extract a number of phytochemicals and the preliminary screening tests using standard techniques showed the presence of secondary metabolites which was specified by the solvent used. Ethanol was found to be more efficient in extracting maximum number of fruit constituents by percolation method. The chemical constitution of the fruit shows that it can be a potential source of future drugs and vital for good health.

Keywords : *Olax scandens* Roxb., phytochemicals, percolation, solvents, extraction, screening.

INTRODUCTION

In addition to food, clothing and shelter plants have been part of the human culture and source of medicine since time immemorial. The plant community forms a source of biologically active compounds (phytochemicals) with variety of chemical structures. These phytochemicals called secondary metabolites represent smaller quantities in higher plants, including alkaloids, steroids, flavonoids, terpenoids, tannins, and many others [1]. Their chemical and taxonomical diversity endows with ambiguous function within the plant body. However, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases [2]. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [3]. Evaluation of higher plants for phytochemicals turned out to be the basis of modern medicine, with a knowledge that the phytochemical composition varies from organ to organ, with the developmental stages and also the developmental conditions. The phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently will lead to the drug discovery and development.

Olax scandens Roxb. (Olacaceae), an important medicinal plant, distributed in tropical India [4] was evaluated for its chemical composition in the fruit. *O. scandens*, is a scandent shrub, grows up to 5 m height, bears white flowers in axillary racemes and globose or ovoid, yellow and fleshy fruit (drupe), with an accrescent calyx [4,5].
Swamynathan and Ramamoorthy [6] identified *Olax scandens* as a sacred groove plant, having socio-religious attachment to the local people of Cuddalore, Tamilnadu. Tender shoots of this plant with leaves are cooked as pot-herbs [7] fruit is edible and reported to be used in making sherbet [4].

Ethnomedicinal properties have been reported by various researchers. Tribes of certain districts of Orissa use leaves as food [8] and medicine [9]. Decoction of stem bark is taken internally to cure fever and cough, [10, 11] Fresh leaves and decoction is given to treat psoriasis [12]. Boiled leaves are tied in the forehead for two times to get relief from headache [13]. Microscopic characters and molecular characterization of *Olax scandens* Roxb. was done recently by random amplified polymorphic DNA markers [4]. Bark is used in anemia and as a supporting drug in diabetes; also in the treatment of fever [5]. Other areas of medicinal importance includes treatment for cancer [16, 17] and bacterial diseases [10].
Phytochemicals like, octacosanol, β-sitosterol, oleanolic acid, and glucosides of β –sitosterol, and glucosides of β –sitosterol were isolated from aerial parts of the plant [8].
Review of literature reveals that its fruits have not been studied for phytochemical constitution except a preliminary study by [9] for few of the phytochemicals. Hence, the present study was undertaken to explore qualitatively, the chemical array of whole fruit of *Olax scandens* Roxb. in a new dimension.

MATERIALS AND METHOD

Collection of Plant Material

Fresh plant material was collected for identification and processing from Sriramagiri, a village of Warangal district, Andhra Pradesh and the plant was authenticated by the Prof. P. Ramachandra Reddy, Professor, (Plant anatomy and taxonomy laboratory) Department of Botany, Osmania university Voucher number was given as 087, Deposited in herbarium of Hyderabadanse.

Processing

Fresh fruits were washed thoroughly under running tap water, sterilized by distilled water and dried in hot air oven at 45^{o} C until concurrent dry weights were obtained by electronic balance machine (Type BL-22OH, NO.D427600501). Fruits were ground into fine powder by using mechanical pulverizer. The powdered material was meshed through 0.3mm mesh and stored in airtight sterile container.

Crude Extraction

Crude extraction was carried out using the solvents, ethanol, methanol, chloroform, petroleum ether, and acetone separately by percolation method. 20gms of the powder was moistened with an appropriate amount of the specified solvent and allowed to stand for approximately 4hrs in a container, after which the mass is packed and the top of the percolator was closed. Additional solvent is added to immerse the marc and the mixture is allowed to macerate in the closed percolator for 48 h. The outlet of the percolator was then opened and the liquid contained therein is allowed to drip slowly. Additional solvent was added as to account for about 200ml. Whole percolate was collected, the solid mass was pressed and the expressed liquid is added to the percolate.

Aqueous extraction

30 g of the fruit powder was taken in a flask and heated with 240ml of distilled water for five hours by agitating gently at regular intervals. The contents were then filtered through Whatman's

No.1filter paper (W and R Balson Ltd, England) and the filtrate was used for preliminary phytochemical screening.

Qualitative tests

Phytochemical analysis was done to assess the chemical composition of different samples of crude extracts using commonly employed precipitation and coloration reactions to identify both primary and secondary metabolites in the fruit including alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, Proteins, terpenoids, Resins, phenols, steroids, coumarins, cardiac glycosides, quinones, leucoanthocyanins, anthraquinones, phytosterols, and amino acids. Each extract was analyzed using standard procedures described by Trease and Evans, Kokate and Wagner, etc. More than one tests were conducted for alkaloids, saponins, carbohydrates and proteins to confirm their occurrence with reference to the method of extraction.

Detection of alkaloids

Extracts were dissolved in dilute Hydrochloric acid and filtered

a) To a 2ml ml filtrate one to two drops of Mayers reagent were added by the side of the test tube a white or creamy precipitate indicated the test as positive [20]
b) To a 2ml ml of filtrate few drops of Wagner's reagent (Iodine in Potassium Iodide) was Added by the side of the test tube, formation of brown/reddish precipitate indicated the Presence of alkaloids [21]
c) 2ml ml of Filtrate is taken and 1 to 2 ml of Dragendroff's reagent was added. Appearance of a prominent yellow precipitate denoted the presence of alkaloids [22].
d) To 2ml ml of Filtrates 1 to 2 ml of Hager's reagent (saturated aqueous picric acid solution) was added. A prominent yellow precipitate indicated the presence of alkaloids [23].

Detection of carbohydrates

The extract was dissolved in 5ml of water and filtered; the filtrate was subjected to the following tests [24].

a) To 2ml of filtrate, two drops of alcoholic solution of α- napthol was added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand, appearance of a violet ring indicated the presence of carbohydrates.
b) One ml of filtrate was boiled on water bath with 1 ml each of Fehling solution 'A' and 'B'. A red precipitate indicated the presence of sugar.
c) To 1ml of filtrate, 1ml of Barfoeds reagent was added and heated on a boiling water bath for 2min. Appearance of red precipitate indicated the presence of sugars

Detection of glycoside):

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides [25].

a) 5ml of extract was taken , 5ml of 5% $feCl_3$ and 5ml dil. HCl were added. Contents were heated for 5min in boiling water bath followed by cooling. To this mixture, 10 ml of benzene was added and shaken well. Organic layer was separated and equal volume of dilute ammonia solution was added. Appearance of pinkish red color in the ammonical layer indicated the presence of glycosides

Detection of saponins

a) Extracts is diluted with distilled water and made up to 20ml and suspension was shaken in a graduated cylinder for 15 min. Formation of foam layer of about two centimeters indicated the presence of saponins [26].

b) In a test tube, about 5ml of the extract was taken and a drop of sodium bi carbonate was added .the mixture was shaken vigorously and kept for 3 min. Formation of honey comb like froth showed the presence of saponins.

Detection of proteins

The extract was dissolved in 10ml of distilled water and filtered through Whatman no. 1 filter paper and the filtrate is subjected to tests for proteins [27, 28].

a) To 2ml of filtrate few drops of millon's reagent was added. Formation of a white precipitate indicated the presence of proteins [29].

b) To 2 ml of filtrate few drops of biuret reagent was added. Appearance of pink color indicated the presence of proteins [30].

Detection of amino acids

Two drops of ninhydrin solution (10mg of ninhydrin in 200 ml of acetone) were added to two ml of aqueous filtrate. Formation of a characteristic purple color indicated the presence of amino acids in the extracts [31].

Detection of cardiac glycosides

2ml filtrate was taken, to this 1ml of glacial acetic acid, 1ml ferric chloride and 1ml concentrated sulphuric acid were added. Green-blue coloration of solution appeared indicating the presence of cardiac glycosides [32].

Detection of flavonoids)

In a test tube containing 0.5 ml of the fruit extract, 5-10 drops of dilute HCL and small piece of Zn were added and the solution was boiled for few minutes. Presence of flavonoids resulted in reddish pink or dirty brown [33].

Detection of resins

To 2 ml of extract, 5-10 drops of acetic anhydrate was added, dissolved by gently heating and then 0.5 of sulphuric acid was added. Bright purple color was produced indicating the presence of resins [34].

Detection of terpenoids

1ml of the extract was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added. Formation of a layer of reddish brown coloration at the interface indicated positive test for the presence of terpenoids [34].

Detection of triterpenoids

The extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added followed by the addition of 2ml of conc. H_2SO_4. Formation of reddish violet color indicated the presence of triterpenoids [34].

Detection of steroids *Liebermann-Burchardt test*: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride, and 1 to 2 drops of concentrated sulfuric acid were added. Appearance of dark green color showed the presence of steroids [32].

Detection of phenolic compounds

To 1ml of extract, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added formation of blue or green indicates the presence of phenols [35].

Detection of tannins 1 ml of extract was taken and few drops of 1% lead acetate were added. Formation of yellowish precipitate indicated the presence of tannins [36].

Detection of phytosterols *Libermann Burchard's test*: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled followed by the addition of concentrated sulfuric acid. Formation of a brown ring at the junction indicated the presence of phytosterols [35].

Detection of quinones

Dilute NaOH was added to 1ml of crude extract blue green or red coloration indicated the presence of quinines [37].

Detection of coumarins

3ml of 10% NaOH was added to 2ml of aqueous extract. Yellow coloration of the contents indicated the presence of Coumarins [38].

Detection of leucoanthocyanins)

1ml of aqueous extract was added to 1ml of isoamyl alcohol. Upper layer turned red in color indicating the presence of leucoanthocyanins [38].

Detection of anthraquinone

To 1gr of the powdered plant material, chloroform was added and shaken for 5mints. Contents were filtered and to the filtrate, 5ml of was ammonia solution was added and agitated gently. A bright pink color in the upper aqueous layer indicated the presence of anthraquinone [39].

Detection of fixed oils

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils [26].

RESULTS AND DISCUSSION

Qualitative assay of the whole fruit of *Olax scandens* Roxb. revealed the presence of various phytochemicals. The results are presented in table.1 which helps to enable their comparative study with respect to the solvent used for extraction.

Table1. Qualitative Phytochemical Analysis of Whole fruit of *Olax scandens* Roxb.

S. No	Name of Phytochemicals	**Solvent used**	EtOH	MeOH	$CHCL_3$	Petroleum ether	Acetone	Aqueous
1	ALKALIODS							
	a. Mayers test		+	+	+	-	+	+
	b. Wagner test		+	+	+	-	+	+
	c. Dragendroffs test		+	+	+	-	+	+
	d. Hagers test		+	+	+	-	+	+
2	FLAVANOIDS		+	-	-	-	-	-
3	TANNINS		+	+	-	+	+	+
4	SAPONINS a. Froth Test b. Foam test		 - -	 + +	 - -	 - -	 - -	 + +
5	CARBOHYDRATES		-	-	-	-	-	-
	a. Molish test		-	-	-	-	-	-
	b. Fehling test		-	-	-	-	-	-
	c. Barfoed test		-	-	-	-	-	-
	d. Benedict test		-	-	-	-	-	-
6	GLYCOSIDES Borntrager's Test (modified)		 +	 +		 +	 +	 +

					+			
7	PROTIENS							
	a. Millons test		+	+	-	-	-	-
	b. Biuret test		+	+	-	-	-	-
8	TERPINOIDS		+	+	+	+	+	-
9	TRITERPINOIDS		+	+	+	+	+	-
10	RESINS		-	-	-	-	-	-
11	PHENOLS		-	-	-	-	-	-
12	STERIODS		-	-	+	-	-	-
13	COUMARINS		+	+	-	+	+	+
14	CARDIAC GLYCOSIDES		+	-	+	+	+	-
15	QUINONES		-	+	-	-	-	-
16	LEUCOANTHOCYANINS		-	-	-	-	-	-
17	ANTHRAQUINONES		-	-	-	+	+	-
18	PHYTOSTEROLS		+	-	+	+	+	+
19	AMINOACIDS		-	-	-	-	-	-
20	FIXED OILS		-	-	-	-	-	-

The phytochemical screening in the present study following percolation method of extraction, revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, proteins, terpenoids, triterpenoids, steroids, coumarins, cardiac glycosides, quinones, anthraquinones and phytosterols in the *O. scandens* fruit. None of the solvents could give positive test for carbohydrates, resins, phenols, leucoanthocyanins, amino acids and fixed oils by this method of extraction using different solvents.

Ethanol was found to be more efficient to extract the secondary metabolites by percolation method from the seeds of *O. scandens* compared to other solvents used and methanol being next. Glycosides could be extracted with all the solvents used whereas carbohydrates, resins, phenols, leucoanthocyanins, amino acids and fixed oils were not traced in any of the crude extracts. Screening test was positive for flavonoids, steroids and quinones only in ethanol, chloroform and methanol respectively. Flavonoids could be identified only in Ethanolic extract. Methanolic and aqueous extracts gave positive reaction for saponins. ethanolic and methanolic extracts gave positive reaction for proteins also. Alkaloids were detected in all the extracts except petroleum ether. Terpenoids, including triterpenoids were found in all the crude extracts except the aqueous extract and tannins and coumarins were present in all the extracts except chloroform extract. Similarly phytosterols were efficiently extracted by this method by using said solvents except

methanol. Anthraquinones were identified in petroleum ether and acetone extracts and cardiac glycosides were found in chloroform, petroleum ether and acetone extracts.
So far no work has been reported regarding phytochemical composition of fruits of *O. scandens* except preliminary phytochemical analysis of methanolic extract of the fruit for few of the phytochemicals [19]. The results differed depending upon the method of extraction. Quite a number of biologically active phytochemicals were identified in the extraction by the method followed and screening tests done.

CONCLUSIONS

The whole fruit of *O. scandens* was analyzed in the present study for its phytochemical constitution following percolation method of extraction using different solvents. The screening tests revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, proteins, terpenoids, triterpenoids, steroids, coumarins, cardiac glycosides, quinones, anthraquinones and phytosterols. The method of extraction employed has been found to be very simple to extract a number of phytochemicals and the preliminary screening tests using standard techniques showed the presence of secondary metabolites which was specified by the solvent used. The chemical constitution of the fruit shows that it can be a potential source of future drugs and vital for good health. The outcome of the analyses done will certainly facilitate their quantitative estimation and isolation of pharmacologically active chemical compounds.

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