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A PRELIMINARY PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *SOLANUM XANTHOCARPUM*

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ABSTRACT
Identification of primary and secondary constituents has become the utmost important tool for the presence of active moiety. The present study was aimed to investigate phytochemicals present in the leaf extract of *Solanum xanthocarpum*. The leaves of *Solanum xanthocarpum* contain phytoconstituents like Alkaloids, Glycosides, Tannins and Phenolic compounds, Flavonoids, Proteins and Amino Acids, Sterols, Triterpenoids, Carbohydrates and Fats. TLC profiling of extracts also gives an idea about the presence of various phytochemicals. The Petroleum ether, Chloroform, ethyl acetate and Methanol extracts were proceed to T.L.C. TLC resulted in identification of 2 spots for Petroleum ether extract, 4 spots for Chloroform extract, 2 spots for Ethyl acetate extract and 2 spots for Methanol extract.

KEY WORDS: *Solanum xanthocarpum*, Phytochemical screening, Petroleum ether extract, Thin Layer Chromatography.

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INTRODUCTION

The green plants are the store houses of many chemical components. In recent years the popularity of complementary medicine has increased. Over 50% of all modern drugs are natural product [1,2,3] origin and they play an important role in drug development programs of the pharmaceutical industry[4]. The present investigation is aim to focus the light on the chemical constituents of leaves of a valuable medicinal plant *Solanum xanthocarpum (Schrad and Wendl). Solanum xanthocarpum (Schrad and Wendl)* is known as Indian night shade or yellow berried night-shade plant[5]. The common name is Kantakari, synonym *Solanum surattense* and it belongs to the family Solanaceae. The plant is rich in many pharmaceutical ingredients like alkaloids[6,7], phenolics[8], flavonoids, sterols[9], saponins[10,11] and their glycosides and also carbohydrates, fatty acids, tannins[12] and amino acids. This plant is known for its medicinal benefits for times immemorial. Roots, stem, leaves, flowers and fruits are useful parts of this herb as Ayurvedic medicinal herb[13,14]. Studies indicate that Solanum xanthocarpum possesses antifertility, antipyretic[15], anticancer[16,17], anti-allergy, anti-inflammatory, anti-histaminic, hypoglycemic[18], antiasthmatic[19], antitussive[20],antioxidant[21], antibacterial, antifungal[22], anthelmintic and larvicidal activities.

MATERIALS AND METHODS

The plant specimen was collected from Jawalka, Osmanabad district, Maharashtra, India in the month of October 2010.The specimen was authenticated by perusing through the floristic literatures.

Preparation of material for analysis work

The leaves of the plant were dried under shade and made to a fine powder using electrical grinder. The powdered drug (approx.25 gm) were then packed in the thimble of soxhlet apparatus[23] and was extracted successively[24-27] with petroleum ether (40-60^0) (Merck, India), chloroform (Merck, India) , ethyl acetate (Merck, India), methanol (Merck, India), ethanol (Merck, India) and distilled water. The excess solvent in the extracts were removed by distillation and concentrated on water bath to get thick syrup. The extracts were then collected in petridish and stored in a desiccators at room temperature. The yield values and other physical properties were observed.

Preliminary phytochemical screening

It involves testing of different extracts of *Solanum xanthocarpum* for various phytochemicals by qualitative chemical tests to give general idea regarding the nature of constituents present in crude drug. The qualitative chemical tests for various phytoconstituents[28-32] were carried out for all the extracts of *Solanum xanthocarpum* as explained below

Test for Alkaloids

Mayer's test Alkaloids give cream colour precipitate with Mayer's reagent [Potassium mercuric iodide solution].

Dragendorff's test Alkaloids give reddish brown precipitate with Dragendorff's reagent [Potassium bismuth iodide solution].

Wagner's test Alkaloids give a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide].

Hager's test Alkaloids give yellow colour precipitate with Hager's reagent [saturated solution of Picric acid].

Tannic acid test Alkaloids give buff colour precipitate with 10% Tannic acid solution.

Test for Glycosides

The extracts were tested for free sugars The extract is hydrolyzed with mineral acid and then tested for the glycone and aglycone moieties.

Legal's test Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red colour appears.

Bromine water test Test solution when treated with bromine water gives yellow precipitate.

Chemical tests for specific glycosides

Test for Saponin Glycosides

Froth test Place 1 ml solution of drug in water in a semi-micro tube and shaken well and noted for a stable froth.

Hemolysis test Add 0.2 ml soiution of saponin (prepared in 1% normal saline) to 0.2 ml of v/v blood in normal saline and mix well, centrifuge and note the red supernatant, compare with control tube containing 0.2 ml of 10% blood in normal saline diluted with 0.2 ml of normal saline.

Test for Tannins and Phenolic Compounds

Gelatin test Test solution with 1% gelatin solution containing 10% sodium chloride gives white precipitate.

Ferric chloride test Test solution gives blue green colour with ferric chloride.

Alkaline reagent test Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.

Test for Flavonoids

Shinoda test (Magnesium Hydrochloride reduction test): To the test solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid dropwise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

Zinc Hydrochloride reduction test To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red colour after few minutes.

Alkaline reagent test: To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow colour, which turns to colourless on addition of few drops of dil.acid, indicates presence of Flavonoids

Test for Proteins and Amino Acids

Millons test Test solution with 2 ml of Millons reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.

Ninhydrin test Amino acids and Proteins when boiled with 0.2% solution of Ninhydrin, Violet colour appears.

Test for sterols and Triterpenoids

Libermann - Burchard test Extract is treated with few drops of acetic anhydride, boil and cool, conc.sulphuric acid is added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of Triterpenoids.

Salkowski test Treat extract in Chloroform with few drops of conc. Sulphuric acid, shake well and allow standing for some time, red colour appears at the lower layer indicates the presence of Steroids and formation of yellow coloured lower layer indicates the presence of Triterpenoids.

Test for Carbohydrates

Molisch's test Treat the test solution with few drops of alcoholic alpha naphthol. Add 0.2 ml of conc.sulphuric acid slowly through the sides of the test tube, a purple to violet ring appears at the junction.

Benedict's test Treat the test solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

Camnelisation Carbohydrates when treated with strong sulphuric acid, they undergo charring with the dehydration along with burning sugar smell.

Fehling's test Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and

boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

Test for Fats and Fixed oils

Stain test Press the small quantity of extract between two filter papers, the stain on filter paper indicates the presence of fixed oils.

Saponification test Add a few drops of 0.5 N of alcoholic potassium hydroxide to small quantities of various extracts along with a drop of Phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of Fixed oils and Fats.

Thin Layer Chromatography Thin layer chromatography is a chromatography technique used to separate mixtures[33,34]. Thin layer chromatography is performed on a sheet of glass, plastic or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action because different analytes ascend the TLC plate at different rates, separation is achieved. The petroleum ether,chloroform, ethyl acetate and methanol extracts of the *Solanum xanthocarpum* were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical tests. Precoated TLC plates were used for the analysis. The Petroleum ether extract was dissolved in Petroleum ether, Chloroform extract in Chloroform, Ethyl acetate extract in Ethyl acetate and Methanol extract was dissolved in Methanol were applied as a thin band, about 2cm from the edge, by using capillary tubes. To make a choice of suitable solvent system, firstly elutropic series of different solvents was tried by running on the TLC plate. The TLC plate containing the sample was placed at 45^0 angles in the development chamber covering the bottom of the plate by the solvent up to nearly 1 cm. The solvent front was marked and the plate was finally allowed to dry.

Development of chromatogram

The eluted coloured substances were visual on the chromatogram. Colourless components were detected by using visualizing agent, iodine vapours. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of R_f Value.

RESULT AND DISCUSSION

Table 1 shows the physical properties of the extracts of the leaves. All the extracts were almost viscous in nature with characteristic smell. Percentage yield of the petroleum ether, chloroform, ethyl acetate, methanol, ethanol and aqueous extracts of *Solanum xanthocarpum* were 0.81, 1.12, 1.02, 1.74, 0.51, 2.01 respectively. Table 2 represents the various phytochemicals present in different extracts. The petroleum ether extract contain sterols & terpenoids and fats &fixed oils. The chloroform extract contain glycosides,tannins & phenolic compounds, flavonoids, sterols and terpenoids. The ethyl acetate extract contain alkaloids and flavonoids. The methanol extract contain carbohydrates, proteins & amino acids, steroids & Terpenoids, flavonoidsand glycosides. The ethanol extract contain alkaloids, flavonoids and sterols & terpenoids. The aqueous extract contain carbohydrates, sterols & terpenoids and saponins.The solvent system selected for the TLC of petroleum ether extract (Fig.1) was Hexane: Ethyl acetate of the ratio 95:5. TLC resulted in the identification of 2 spots with the R_f value 0.785&0.642.The solvent system selected for the TLC of chloroform extract (Fig.2) was Chloroform: Ethyl acetate of the ratio 4:6. TLC resulted in the identification of 4 spots with the R_f value 0.144, 0.550, 0.782&0.942. The solvent system selected for ethyl acetate extract (Fig.3) was Toluene: Ethyl acetate of the ratio 8:2. TLC resulted in the identification of 2 spots with the R_f value 0.681&0.942. The solvent system selected for methanol extract (Fig.4) was Ethyl acetate: Formic acid: Acetic acid: Water of the ratio 100:11:11:27. TLC resulted in the identification of 6 spots with the R_f value 0.142, 0.171, 0.385, 0.642, 0.714& 0.785.

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TABLE 1: THE PHYSICAL PROPERTIES OF THE EXTRACTS OF THE LEAVES.

Physical characteristics	Percentage yield (gm)	Colour	Odour	Consistency
Petroleum ether extract	0.81	Greenish brown	Characteristic	Viscous pasty mass
Chloroform extract	1.12	Greenish	Characteristic	Pale green
Ethyl acetate extract	1.02	Greenish	Characteristic	Characteristic
Methanol extract	1.74	Pale green	Viscous	Highly Viscous
Ethanol extract	0.51	Greenish	Characteristic	Viscous
Aqueous extract	2.01	Reddish brown	Characteristic	Viscous

TABLE 2: PHYTOCHEMICAL TESTS FOR VARIOUS EXTRACTS.

Phytochemicals		**Petroleum ether extract**	**Chloroform extract**	**Ethyl acetate extract**	**Methanol extract**	**Ethanol extract**	**Aqueous extract**
Alkaloids	Mayer's Test	-	-	++	-	+	-
	Dragendorff's Test	-	-	++	-	-	-
	Wagner's Test	+	-	++	-	+	-
	Hager's Test	-	-	++	-	-	-
	Tannic Acid Test	-	-	+	-	-	-
Glycosides	Legal's Test	-	+	-	+	-	-
	Bromine Water Test	-	-	-	-	-	-
Tannins & Phenolic Compounds	Gelatin Test	-	++	-	-	-	-
	Ferric Chloride Test	-	++	-	-	-	-
	Alkaline Reagent Test	-	-	-	-	-	-
Flavonoids	Zinc Hydrochloride Reduction Test	-	++	+	++	++	-
	Alkaline Reagent Test	-	++	-	++	++	-
Proteins and Amino Acids	Millon's Test	-	-	-	++	-	-
	Ninhydrin Test	-	-	-	++	-	-
Sterols And Triterpenoids	Libermann-Burchard Test	++	++	-	++	++	++
	Salkowski Test	++	++	-	++	++	++
Carbohydrates	Molisch's Test	-	-	-	+	-	+
	Benedict's Test	-	-	-	-	-	-
	Fehling's Test	-	-	-	+	-	-
	Camnelisation	-	-	-	-	-	-
Saponin Glycosides	Froth test Hemolysis test	- -	- -	- -	+ +	- -	++ ++
Fats & Fixed oils	Stain Test	++	-	-	-	-	-
	Saponification Test	++	-	-	-	-	-

1) + sign indicates positive test
2) -sign indicates negative test

Figure No. 1 Image of TLC plate of Petroleum ether extract

Figure No. 2 Image of TLC plate of Chloroform extract

Figure No. 3 Image of TLC plate of Ethyl acetate extract

Figure No. 4 Image of TLC plate of Methanol extract

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