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Phytochemical Investigation and Thin Layer Chromatographic Studies on the Fruits of *Solanum incanum* In Areza sub-zone, Zoba Debub, Eritrea

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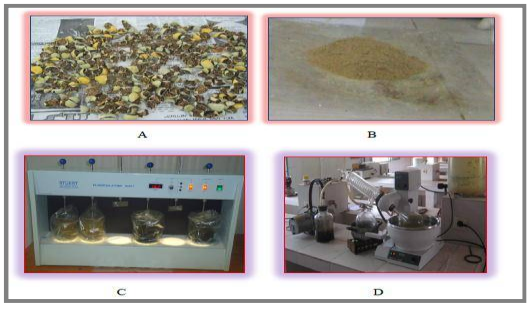
ABSTRACT

Solanum incanum (Solanaceae) is bushy herb up to 1.8 m tall, native to Northern and North-Eastern Africa including Eritrea. It is a well-known medicinal plant. Throughout tropical Africa a sore throat, angina, stomach-ache, colic, headache, painful menstruation, liver pain and pain caused by onchocerciasis, pleurisy, pneumonia and rheumatism are treated with Solanum incanum. This research project is aimed at Phytochemical screening of Solanum incanum fruit, in Areza sub-zone, Zoba Debub. The fruit of Solanum incanum (200 g) was macerated and extracted with 800mL 70% ethanol at room temperature for 48 h with occasional shaking. This process was repeated twice at room temperature, filtered and concentrated using Rota vapor to give yellowish extracts. Then the resulting extract was filtered using filter paper (S & S filter paper circles Ø 125mm). The filtrate was then evaporated to dryness in vacuum using Rota-vapor at 60°C to yield 41.23 g of crude extract. Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, phenols, flavonoids, glycosides, saponins, triterpenes, tannins and steroids as a major class of compounds. A qualitative analysis by Thin Layer Chromatography (TLC) also shows the different components like alkaloids, saponins, flavonoids, sugars and glucosides, phenol and tannin. The R_f values of the developed spots in the different solvent systems are calculated.

Graphical Abstract



A. Shade, B. dried powdered fruit, C. Extraction using 70% ethanol solution in incubation shaker, D. Evaporating solvent from extract in rota-vapor.

Keywords: *Solanum incanum*, fruit extract, Phytochemical screening, TLC, R_f values.

INTRODUCTION

Medicinal plants play a crucial role in the search for alternative antimicrobial components. According to the World Health Organization, it is estimated that around 80% of the earth's population use some form of herbal medicine in their health care, whereas natural products are a preferable option than synthetic ones [**1**]. The literature indicates that medicinal plants have secondary compounds that are of great importance in human life in terms of acting as antioxidants, anti-inflammatory, and being involved in the modulation of detoxification enzymes, the stimulation of the immune system, the modulation of steroid metabolism and antimicrobial effects [**2**]. Research findings also support the idea that many plants are used in the treatment of various diseases whose symptoms might involve microbial infection leading to the discovery of novel bioactive compounds [**3-5**].

Solanum species are the most potent plants against pathogenic microorganisms. *Solanum incanum* is one of the important traditional medicinal plants belonging to Solanaceae family. Antibacterial activity of *Solanum incanum* was studied [**6, 7**] and presence of phytochemicals was also studied [**8**]. Other solanum species, *Solanum torvum* (leaf, stem and roots) showed antibacterial and antifungal activity [**9**] and antibacterial activity of *Solanum surattense* whole plant extract [**10**] and leaf extract [**11**] were studied. Analysis, presence of phytochemicals and potent antibacterial activity of leaf, root and seed extracts were studied in *Solanum nigrum* [**12**]. The Solanaceae family members are well adapted to different agro ecological environments and hence show a good dispersal across the globe [**13**]. The fruit of *Solanum incanum* is used for the treatment of dandruff, skin diseases, sores and wounds in Tanzania [**14**]. In Ethiopia, the fruit juice is used by peasant farmers to control ticks [**15, 16**].

In general, *Solanum incanum* is one of the most important medicinal plants in Eritrea. Nevertheless, up to now there is no research report on extraction and characterization of active constituents of Eritrean species. Thus, this work is believed to fill the gap.

In Eritrea, the indigenous knowledge of medicinal plants has been documented. The traditional use for treatment of different diseases and a comprehensive datum of medicinal plants exists both on the number and types. Research in search of bioactive metabolites from these medicinal plants is in its preliminary stage and thus a systematic and concerted approach to this activity has not been maintained, for want of experts, sophisticated equipments and high cost chemicals. Despite of the documented number, the concept of applied research in the medicinal use of plants has not received much attention. This research paper will try to address/investigate the bioactive metabolites present in the fruit of *Solanum incanum*. The obtained results will be expected to fill the available traditional medicinal use as well as the traditional tannery process (vegetation tanning).

MATERIALS AND METHODS

Dry grinder (electronic blender), mechanical shaker (flocculator SW1), Rota vapor, TLC plates and filter paper (S and S filter paper circles Ø 125 mm). All chemical and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Acetic anhydride, glacial acetic acid, 10% ammonia, chloroform, conc. Sulphuric acid(H_2SO_4), dilute sodium hydroxide(NaOH), 10% sodium hydroxide(NaOH), sodium bicarbonate($NaHCO_3$), dilute hydrochloric acid(HCl), distilled water, 1% lead acetate, 10% lead acetate, 10% ferric chloride ($FeCl_3$) solution, Fehling solution A and B, Wagner's reagent(Iodine-potassium iodide solution), methanol, H_2SO_4 solution, NH_4OH(conc.) and acetone.

Survey, Plant collection: In addition to the existing data, information on the ethno botanical uses of the fruits of *Solanum incanum* was collected by formal and informal interview of traditional users and local people in Zoba Debub around Areza sub-zone. The plant was identified by Prof. Ghebrehiwet Medhanie biology department, botany EIT, Mai Nafhi. The fruits were collected from the plant and washed with water thoroughly to free from debris. The fruits were then sliced and shade dried for 20 days. The dried fruits were grounded finely by using dry grinder and passed through a sieve and stored for further use.

Preparation of Extracts: A sample of 100 g from the powder prepared above was dissolved in 400 mL of 70% ethanol solution in a 600 mL beaker. The solution was kept in an incubation shaker for two days and then filtered through the filter paper (S and S filter paper circles Ø 125 mm). The extraction was repeated twice for better quantity with the same amount of solvent and filtered again. The 70% ethanolic extract was finally evaporated to dryness in vacuum using Rota-vapor at 60^{o}C to yield 41.3 g of crude extract. The obtained crude extract was used for further studies, i.e. for evaluating the presence of alkaloids, saponins, steroids, phenols, flavonoids, glycosides and others.

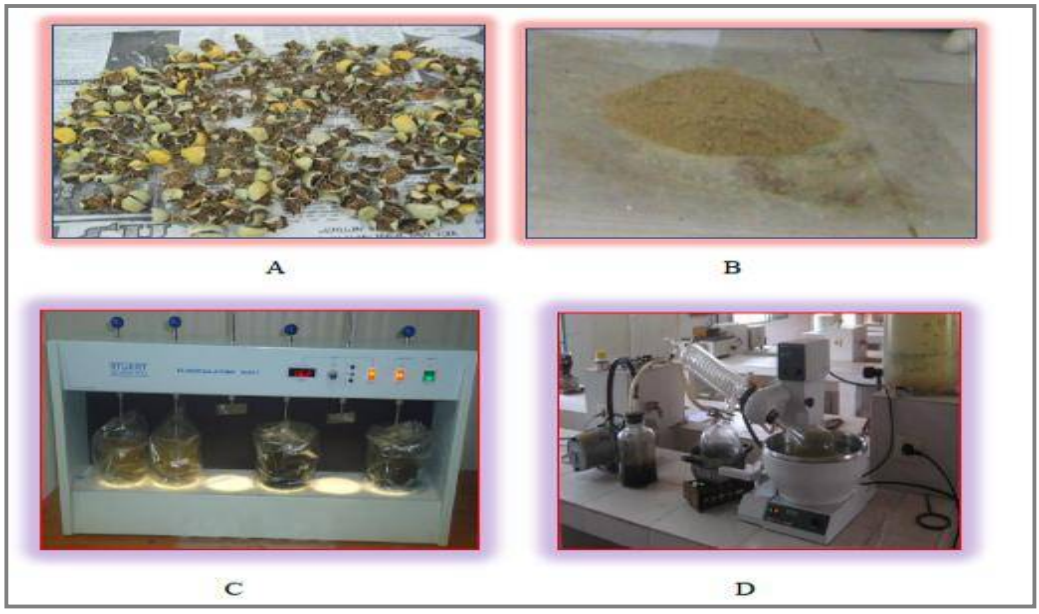


Figure1. A. Shade dried, B. Powdered fruit, C. Extraction using 70% ethanol solution in incubation shaker, D. Evaporating solvent from extract in rota-vapor.

Qualitative phytochemical analysis: The extracts obtained were subjected to preliminary phytochemical screening, to identify the chemical constituents. Chemical tests were carried out on the extracts of *Solanum incanum* using standard qualitative procedures. The methods of analysis employed were those described by Kokate C.K [**17**] and Harbone J.B [**18**]. The result of the phytochemical screening was described qualitatively. The presence of phytochemicals in *Solanum incanum* extract was confirmed by the following tests.

Alkaloids: 2 mL of the *Solanum incanum* extract was acidified by adding 1.5% v/v HCl and a few drops of Wagner's reagent (Iodine-potassium iodide solution 1.2 g of iodide was dissolved in 2.0 g of sulphuric acid and the solution was diluted to 100 mL with distilled water) was added. Formation of yellow or brown precipitate confirmed the presence of alkaloid.

Flavonoids: To 1 mL of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow color was observed, which become colourless on the addition of few drops of dilute HCl acid, which indicates the presence of flavonoids. The presence of flavonoids was also confirmed by another

test i.e. few drops of 10% ferric chloride solution were added to 1ml ethanolic extract. A green or blue color indicated the presence of phenolic nucleus.

Saponins: In a test tube containing about 5 mL extract of *Solanum incanum*, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 min. A honey comb like froth formation confirms the presence of saponins.

Fehling's test (Reducing Sugar test): 1mL of water and 20 drops of boiling Fehling's solution (A and B) were added to 1 mL ethanol extract. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars.

Ninhydrin Test (Proteins Test): 3 mL of the extract and 3 drops of Ninhydrin solution were heated in boiling water bath for 10 min. Appearance of purple color shows the presence of amino acids/proteins.

Phenols Tests

(a)Ferric chloride Test: 2 mL of distilled water followed by drops of 10% aqueous $FeC1_3$ solution were added to 1ml of the extract. Formation of blue or green indicates the presence of phenols.

(b) Lead acetate Test: One mL of *Solanum incanum* extract was diluted to 5 mL with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate was formed which indicates the presence of phenols.

Glycosides: A small amount the extract was dissolved in 1mL of water and aqueous sodium hydroxide solution was added. Formation of yellow color indicates the presence of glycosides.

Terpenoids: 5 mL of *solanum incanum* extract was mixed with 2 mL of chloroform and concentrated sulphuric acid (3 mL) was carefully added to form a layer. A reddish-brown coloration was formed in the interface, which indicates the presence of terpenoids.

Anthraquinone (Born Tragers test): About 0.5 g of the extract was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone.

Resins: 2 g of the ethanolic extract was dissolved in 10mL of acetic anhydride then a drop of concentrated sulphuric acid was added. Appearance of purple color, which rapidly changes to violet, is an indication for the presence of resins.

Steroid: 2 mL of the extract was dissolved in 10 mL of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and the sulphuric acid layer showed yellow color with green fluorescence. This indicates the presence of steroids.

Tannins: 5 mL of the extract and a few drops of 1% lead acetate were mixed. A yellow precipitate was formed, which indicates the presence of tannins.

Qualitative Analysis by thin Layer Chromatography: Extract was checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel. TLC was carried out to isolate the principal components that were present in the fruit extract of the plant. Different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

RESULTS AND DISCUSSION

Phytochemical Analysis: The plant possesses numerous biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine. It was reported that most of the plants of Solanaceae contain alkaloids, tannins, steroids, saponins, as well as reducing sugars [19]. Results of the qualitative phytochemical tests also confirmed that point. The fruit extract was identified to have alkaloids, flavonoids, phenols, carbohydrates, tannins, triterpenoids, glycosides, steroids, resins and saponins, as illustrated in table 1.

Table 1. Results of the Phytochemical Screening of *Solanum incanum*

S.No.	Phytochemical	Reagent	Color Change	Result	
				Ethanol Extract	H_2O Extract
1	Alkaloids	Wagner Test	Yellow or brown ppt	+	+
2	Phenols	Ferric Chloride Test	Blue or green color	+	+
3	Flavonoids	Alkaline reagent test	Intense yellow color	+	+
4	Tannin	Drops of 1% lead acetate	Yellow precipitate	+	+
5	Glycosides	2 mL glacial acetic acid and a drop of $FeCl_3$	Yellow color	+	+
6	Anthraquinone	Bomtrager's test	Pink violet or red color	-	-
7	Saponins	Sodium bicarbonate and shake for 3 min	Honey comb froth formation	-	+
8	Terpenoids	Anisaldehyde	Reddish brown color	+	+
9	Steroid	10 mL $CHCl_3$ and 10 mL conc. H_2SO_4	Upper layer tums red	+	+
10	Resins	10 mL CH3COOH + cons. H_2SO_4	Purple color	+	+
11	Proteins	Million's test		+	+
12	Rrducing sugar	Fehling's test	Red-brick ppt	+	+

Figure 2. Colour change and precipitate formation in Phytochemical Analysis.

Figure 3. Observation of colors in TLC.

TLC profiling of the extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals give different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by Column Chromatography. Compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract (Figure 2 and 3). The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system. In the present state of affairs, TLC profile of all the plant extract of the fruits in different solvent system indicates the presence of diverse type of phytochemicals in this plant (table 2). Different Rf values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from this plant extracts.

Table 2. TLC Results of the Phytochemicals

Chemical Name	Solvent System	Ratio	Chemical spray	No. of components	R_f Values
Alkaloids	$CHCl_3$/MeOH/H_2O	20:15:10	Wagner's reagent	06	0.64,0.70,0.74,0.79,0.85,0.99
	MeOH/ NH_4OH	34:6		11	0.39,0.49,0.55,0.59,0.63,0.69, 0.76,0.85,0.90,0.94,0.99
Phenols	Acetone / H_2O	40:10	$FeCl_3$(aq)	08	0.10,0.18,0.59,0.68,0.74,0.78, 0.91,0.96
	H_2O /MeOH /$CHCl_3$	5:17.5:325	H_2SO_4 (aq and	06	0.11,0.20,0.25,0.49,0.54,0.86
Sugars, Glucosides	H_2O/MeOH/$CHCl_3$	5:17.5:32.5	heat for 10 min at 120 ^{0}C	06	0.65.0.74,0.78,0.83,0.91,0.99
Flavonoids	$CHCl_3$/MeOH	36:4	UV at 254nm	04	0.18,0.25,0.62,0.99
Tanins	Acetone/H_2O	20:20		04	0.68,0.75,0.87,98
Saponins	$CHCl_3$/ACOH/MeOH/H_2O	24:8:4:4	I_2 vapor	05	0.66,0.67,0.79,0.81,0.93

Solanum incanum as traditional medicine: A large number of plants produce secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, steroids and quinines that are used in pharmaceuticals, cosmetics and pesticide industries [**20, 21**]. Thus the present study confirms the traditional medical practice and previous pharmacological observations and supplement treatment for other health problems such as allergic reactions, arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production etc are due to these secondary metabolites. The phytochemical screening for the extract significantly showed the presence of metabolites. Alkaloids, Saponins, Tannins, Amino acids, Flavonoids and Terpenoids, were found to be present in the extract of *Solanum incanum* fruit. The result of this research shows the presence of phenols. Phenolic acids are regarded as one of the functional food components in fruits and have significant contribution to the health effects of plant-derived products by scavenging free radical species, inhibiting free radical formation, and preventing oxidative damage to DNA due to the presence of hydroxyl groups.

Hydroxyl groups can react with active oxygen radicals, such as hydroxyl radicals, superoxide radicals and lipid peroxyl radicals and inhibit the lipid peroxidation at an early stage [22]. The present study also supports the study conducted by Saad *et.al* [23] which indicates that the ethanol extract of fresh fruits of *Solanum incanum* have antimicrobial activity. Also, the extract of *Solanum incanum* showed the presence of saponins that have healing properties as a natural blood cleanser, expectorant and antibiotic.

APPLICATION

Application of Solanum incanum in traditional Tanning process: Tanning hides is a process of making leather from the skins of animals that otherwise would tend to decompose. The term comes from the word 'tannin' which is an acidic chemical compound that alters the nature of the protein fibers in the hide in such a way that they resist decay. The conversion of raw animal hides into leather has traditionally been carried out with plant derived tannins. Animal skins are made up of protein called collagen (among other things). This protein is readily degraded by bacteria and fungi. When tannins bond to the collagen, the crosslinked fibers are no longer susceptible to attack. Tannins bind to the collagen proteins in the hide and coat them causing them to become less water-soluble, and more resistant to bacterial attack. The process also causes the hide to become more flexible [24]. The study also reveals the presence of tannins which have advantage for tanning process that are used in traditional tanning i.e. vegetation tanning.

Fruit sap of Solanum incanum for curdling of milk: In plants, alkaloids generally exist as salts of organic acids like acetic, oxalic, citric, malic, lactic, tartaric, tannic and other acids. Some weak basic alkaloids (such as nicotine) occur freely in nature. A few alkaloids also occur as glycosides of sugar such as glucose, rhamnose and galactose, e.g. alkaloids of the solanum group (solanine), as amides (piperine), and as esters (atropine, cocaine) of organic acids [25, 26]. The pH of the sap was measured to have pH value on the range of the above mentioned organic acids. Fruit sap of *Solanum incanum* contains alkaloides which are responsible for maintaining the pH for milk curdling process. The tanninic acids also play a role on breaking down of the proteins in the milk thereby form curdles.

CONCLUSION

In the present study, fruit of *Solanum incanum* showed the presence of bioactive compound such as alkaloids, flavonoids, terpenoids, saponins, tannins, steroids, amino acids and reducing sugars etc . This study also leads to the further research in the way of isolation and identification of the active compound from the fruit using chromatographic and spectroscopic techniques. The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. These findings suggested that *Solanum incanum* fruit could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. Due to the presence of tannins the fruits of *Solanum incanum* are used for traditional tanning process as well as for their use of milk curdling. The alkaloids which are found as salts of organic acids maintain the pH and the tanninic acids breakdown proteins during traditional curdling process.

The plant under investigation can be a potential source of useful drugs. However, further studies are required to isolate the pure active principal from the crude plant extracts for proper drug development. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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