Article

Phytochemical investigation and antimicrobial activity of the fruit extract of *Solanum incanum* grown in Eritrea

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Received 4 July 2017; *Accepted 12 July 2017*; *Published 1 September 2017*

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 study is aimed at phytochemical screening and antimicrobial activities of *Solanum incanum* fruit, which is collected from Areza sub-zone, ZobaDebub, Eritrea. Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, phenols, flavonoids, glycosides, saponins, triterpens, tannins and steroids as a major class of compounds. Antimicrobial activities were estimated by measuring zones of inhibition through hole-plate diffusion method. The results of antimicrobial activities clearly showed that plant extracts were specific in action against the growth of bacterial and fungal species. Ethyl acetate, ethanol and chloroform fruit powder extracts were more effective followed by petroleum ether fruit powder extracts while aqueous extracts showed low inhibition zones against all the tested microorganisms. *S. typhimerium* was more sensitive to ethyl acetate and chloroform extracts with inhibition zones of 27±1.0 and 20±0.29mm diameter respectively. Similarly, *E. coli* was more sensitive toethyl acetate and chloroform extracts with inhibition zones of 27.8±0.29 and 29.3±0.7 mm in diameter respectively. Meanwhile, the *S. aureus* was resistant to all extracts except to ethanol extract which showed medium sensitivity of 10±0.91 mm in diameter (zone of inhibition).

Keywords *Solanium incanum*; fruit extract; phytochemical screening; antimicrobial activity; inhibition zone.

Ornamental and Medicinal Plants
ISSN 2522-3682
URL: http://www.iaees.org/publications/journals/omp/online-version.asp
RSS: http://www.iaees.org/publications/journals/omp/rss.xml
E-mail: omp@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 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 intheir health care, where natural products are a preferable option than synthetic ones. 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 (Alamri and Moustafa, 2012). 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 (Thankman, 2003; Narendra et al., 2009; Sequeira et al., 2009).

The Solanaceae family is an important family in the plant kingdom of the advanced order Solanales in the division Magnoliophyta, the angiosperms or flowering plant division (Bremer et al., 2003). It includes 91 genera and an estimated 2450 species with great variation in habit, morphology and ecology (Mabberley, 2008). The family is ranked as third in economic importance and is regarded as a source of many morphologically different domesticated crop species beneficial to human health, diet, beauty and ornamental use (Sekara et al., 2007). Tomato, potato, pepper, petunia, datura, tobacco and eggplant are some of the valuable family members. The Solanaceae family members are well adapted to different agro ecological environments and hence show a good dispersal across the globe (Knapp et al., 2004).

Solanum species are the most potent plants against pathogenic microorganisms. *Solanum* species, *Solanum torvum* (leaf, stem and fruits) extracts showed antibacterial and antifungal activities (Bari et al., 2010). Antibacterial activity of *Solanum surattense* whole plant extracts (Patil et al., 2009) and leaf extract (Sheeba et al., 2010) were studied. Analysis and presence of phytochemicals and potent antibacterial activity of leaffruit and seed extracts of *Solanum nigrum* were studied (Khizar et al., 2014; Dalal, 2016).

Solanum incanum is one of the important traditional medicinal plants. Many of the medicinal uses of *Solanum incanum* are based on its analgesic properties. Throughout tropical Africa a sore throat, angina, stomach-pain, colic, headache, painful menstruation and liver pain are treated with *Solanum incanum*. For these purposes, leaf, fruit and fruit decoctions are drank, fruits are chewed and sap swallowed, leaf sap is used for washing painful areas, and ash of burnt plants is mixed with fat and applied externally. Leaves are added to soup to improve the flavor. The large variation in toxicity makes it dangerous to transfer specific uses from one region to another. The fruit and seed are used in Africa and Asia to curdle milk and to make cheese. Also, the plant is employed in East and Southern Africa for the treatment of skin diseases, general infections, abdominal pains, fever, stomachache and indigestion. In addition, the fruit of *Solanum incanum* is used for the treatment of dandruff, skin diseases, sores and wounds in Tanzania (Habtamu et al., 2014).

Another widespread use of *Solanum incanum* is in the treatment of venereal diseases. Different plant parts arealso used to treat snake bites.In Senegal, Kenya, Uganda and Zimbabwe a decoction of the fruits is drunk, fruits are chewed and sap is swallowed, and young chewed leaves or pulped fresh fruits are applied to the bite wound. In Niger, Sudan, Rwanda and Namibia the fruits are used as an ingredient of arrow poison and in Mozambique as fish poison (Alamri and Moustafa, 2012). In Ethiopia, Fruit sap is mixed with butter and applied to cattle to control ticks.The boiled fruits are used as soap and in tanning leather. In southern part of Ethiopia, Haddiya people use the fruitof the plant toget relief from stomach problem, the fruit is chewed and sap is swallowed (Habtamu et al., 2014).

Solanum incanum is one of the most important medicinal plants in Eritrea. 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 exist 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 lack of experts, sophisticated equipment 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 work attempted to address/investigate the bioactive metabolites present in the fruit of *Solanum incanum* and its antimicrobial activity; identify the constituents which are

important for tannery and milk curdling processes. Hence, the obtained results are expected to contribute scientific findings to the available traditional medicinal use as well as the traditional tannery process (vegetation tanning).

2 Materials and Methods

2.1 Survey, collection and preparation of plant material

In additionto 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 ZobaDebub around Arezasub-zone. The collection was made in February 2015 and was identified at the Department of Plant Biology herbarium, EIT, Mai-nefhi. The collected fruits were 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.

2.2 Preparation of extracts

A sample of 100gm from the powdered fruit was dissolved in 400mL of 70% ethanol solution in a 600mL beaker. The solution was kept in an incubation shaker for two days and then filtered through the filter paper (S & S filter paper circles Ø 125mm). 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 41.3g 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.

2.3 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 described by Kokate (2001) and Harbone (1998). The result of the phytochemical screening was described qualitatively. The presence of phytochemicals in *Solanum incanum* extract was confirmed by the following tests.

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

Flavonoids: To 1mL of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow color was observed, which become colorless 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 5mL extractof *Solanum incanum*, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3minutes. Ahoney comb like froth formation confirms the presence of saponins.

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

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

Phenols:(a) Two 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)** One mL of the friut extract

was diluted to 5mL 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: Five mL of fruit extract was mixed with 2mLof chloroform and concentrated sulphuric acid (3mL) was carefully added to form a layer. Areddish brown coloration was formed in the interface, which indicates the presence of terpenoids.

Anthraquinone: Borntragers test was performed. About 0.5 g of the extract was taken into a dry test tube and 5mL of chloroform was added and shaken for 5 minutes. 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: Two grams of the ethanolic extract was dissolved in 10mL of acetic anhydride then adrop of concentrated sulphuric acid was added. Appearance of purple color, which rapidly changes to violet, is an indication for the presence of resins.

Steroid: Two mL of the extract was dissolved in 10mL 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: Five 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.

2.4 Microbial assay

2.4.1 Preparation of sample extract

Two grams of dried powder of the plant material was added to 10mL of sterile distilled water; ethanol, ethyl acetate, petroleum ether and chloroform in order to obtain extracts (200 mg fruit powder/mL) in a closed 50mL test tubes. The extraction was done at room temperature for 24 hours. To separate the plant residue and the filtrate, it is centrifuged in a centrifuge machine. The extract obtained was stored in sterile capped reagent bottles and refrigerated at 4^{o}C until required for use. Three different concentrations were prepared from the extract.

2.4.2 Proofing the sterility of fruit powder extract

According to Ronald(1995) the extract was tested for sterility by introducing 2 mL of the extract into 10mL of sterile nutrient broth. This was incubated at 37^{o}C for 24 hours. A sterile extract was indicated by the absence of turbidity or clearness of the broth after the incubation period.

2.4.3 Selection of microorganisms

Escherichia coli NCTC 12241/ATCC 25922, Salmonella typhimerium and *Staphylococcus aureus NCTC12981/ATCC25923, Candida albicana* were the pathogenic microorganisms used in the study. These bacteria are selected for this study because they are the main bacteria for the cause of diarrhea and stomach pain. All the cultures were obtained in pure form from the culture collection of Institute of Microbial Technology and cultured in agar Media where they can best grow. For example, *E. coli* and *S. typhimerium* grows best in macConkey agar and *S. aureus* and *C. albicana* grows in Maniton Salt Agar (MSA) and Saboradi agar respectively**.**

2.4.4 Media preparation

Thirty eight grams of Muller Hilton agar (CM0337) (OXOID) was mixed with distilled water and then sterilized in autoclave at 121^{o}C and 151Barr pressure for 15 minutes. The sterilized media were allowed to cool to a temperature of about 50^{o}C and poured into petridishes with the thickness of the media about 4 mm

inside the safety cabinet. The solidified plates were kept in the refrigerator of about 2-8^{o}C and bored with 6 mm diameter cork borer. The plates with wells were used for the antibacterial studies.

2.4.5 Inoculum preparation

For inoculum preparation three/four colonies for single species were selected. Test organism was inoculated in peptone water and incubated for 3 hours at 35^{o}C.Turbidity of the suspension was adjusted to match 0.5 McFarland and used for antibacterial sensitivity assay.

2.4.6 Antibacterial and anti-fungal activity

Antibacterial and anti-fungal activity of the plant extract was tested using well diffusion method (Mariajancyrani et al., 2012). The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 6mm cork borer. 0.1µL of the extracts were poured into the well using sterile syringe. The plates were incubated at 37 ± 2 ºC for 24 hours for bacterial and 25 ± 2 ºC for 48 hours for fungal activity. The plates were observed for the zone formation around the wells and measured in millimeter. For each treatment three replicates were maintained. The diameter of inhibition zones was measured in millimeter and the results were recorded.

2.5 Statistical analysis

The mean zones of inhibitions caused by the solvent extracts of the plant's fruit materials were calculated and reliability of thesamples was assessed by calculating standard deviation.

3 Results

3.1 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 (Amadiet al., 2010). Results of this qualitative phytochemical test also confirmed similar findings. The fruit extract was identified to have alkaloids, flavonoids, phenols, carbohydrates, tannins, triterpenoids, glycosides, steroids, resins and saponins, as given in Table 1.

3.2 Antibacterial and antifungal bioassays

In this investigation aqueous, ethanol, chloroform, ethyl acetate and petroleum ether extracts from the fruits of *Solanum incanum* exhibit antimicrobial activity against *E. coli, C. albicana, S. typhimerium* and *S. aureus.* The sensitivity assay of these pathogens is shown in the figure below. Antibacterial and antifungal potential of fruit extract were assessed in terms of zone of inhibition of microbial growth. The triplicate results of the effects of different extracts of test plant on bacterial and fungal species was shown in Table 2 (a, b and c). The results clearly showed that plant extracts were specific in action against the growth of bacterial and fungal species. Ethyl acetate, ethanol and chloroformfruit powder extracts were more effective followed by petroleum ether fruit powder extracts while aqueous extracts were low inhibition zones against all the tested microorganisms. *S. typhimerium* was more sensitive to ethyl acetate and chloroform extracts with inhibition zones of 27±1.0 and 20±0.29mm diameter respectively. Similarly, *E. coli* was more sensitive to those extracts with inhibition zones 27.8±0.29 and 29.3±0.7 mm in diameter respectively. Meanwhile, the *S. aureus* was resistant to all extracts except to ethanol extract which showed medium sensitivity of 10±0.91 mm in diameter (zone of inhibition).

Table 1 The phytochemical screening of the *Solanum incanum*.

S.N	Phytochemicals	Reagents	Color change	Results	
				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 $FeCl_3$	Yel Yellow color	+	+
6	Anthraquinone	Borntrager's test	Pink violet or red color	—	—
7	Saponins	ium bicarbonate and shaken for 3 min	Honey comb froth formation	—	+
8	Terpenoids	Anisaldehyde	Reddish brown color	+	+
9	Steroids	10 mL $CHCl_3$ and 10 mL conc.H_2SO_4	Upper layer turns red	+	+
10	Resins	10mL CH_3COOH + conc.H_2SO_4	Purple color	+	+
11	Proteins	M Million's test		+	+
12	Reducing sugar	F Fehling's test	Red-brick ppt	+	+

Key: + = Present − =absentppt= precipitate

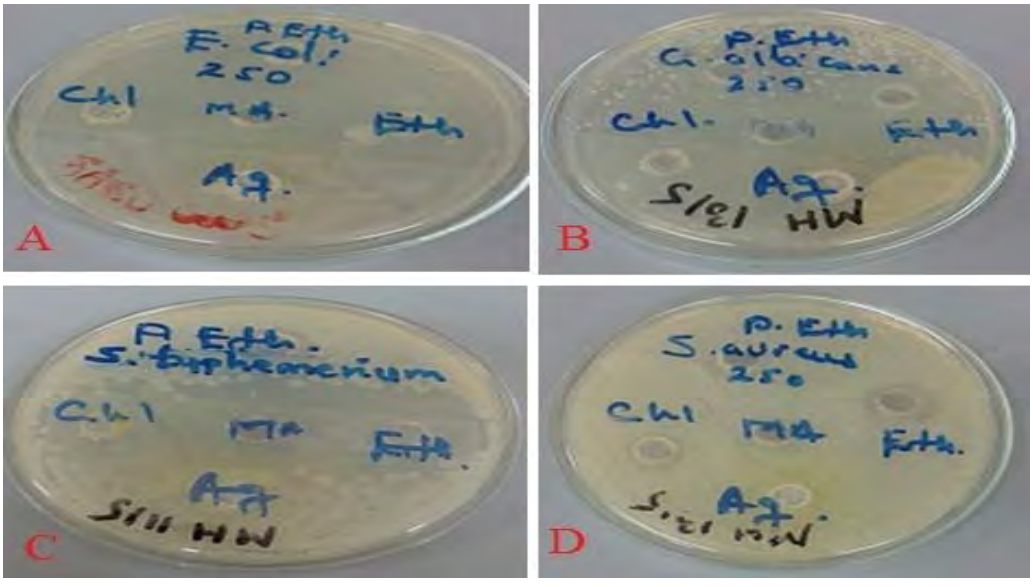

Fig. 1 Zone of inhibition for aqueous, ethanol, ethyl acetate, chloroform, petroleum ether against microorganisms: (A) *E. coli*, (B) *C. albicana* (C) *S. typhimerium* and (D) *S. aureus*.

Table 2 Assay of antibacterial activity.

a. Triplicate results of zone of inhibition in 100 mg fruit powder/mL solvent extracts.

Name of organisms	Zone of inhibition (mm in diameter) (M±SD) (n=3)						
	St.	PE	chloroform	EAc	Ethanol	Water	Con
E. coli	27±0.12	6.7±0.58	27.8±0.29	29.3±0.73	24±0.04	4±1.00	-
S. aureus	11.2±0.1	5.5±0.5	6.3±0.57	5.2±1.2	10±0.91	-	-
S. typhimerium	29.1±0.3	14.3±1.15	20±0.29	27±1.0	20.3±0.7	7±0.81	-
C. albicana	28±0.29	29.3±1.15	18.8±0.29	15±0.92	13.5±0.5	6.7±0.29	-

Key: St.= Chloronphenicole, con**.**= Control, PE = petroleum ether and EAc = ethyl acetate

b. Triplicate results of zone of inhibition in 50 mg fruit powder/mL solvent extracts.

Name of organisms	Zone of inhibition (mm in diameter) (M±SD) (n=3)						
	St.	PE	chloroform	EAc	Ethanol	water	Con
E. coli	14.2±0.29	3.2±0.2	11.7±0.57	14.7±0.76	9.9±0.15	1.9±0.15	-
S. aureus	5.1±0.12	3±0.45	1.75±0.25	1.5±0.41	5.3±0.29	-	-
S. typhimerium	15.0±0.0	7.3±0.41	9.7±0.38	12.7±0.58	10.2±0.6	2.5±0.25	-
C. albicana	13.2±0.29	14.3±0.6	9±0.51	5.3±1.25	6.9±0.52	3.2±0.21	-

Key: St.= Chloramphenicol, con.= Control, PE = petroleum ether and EAc = Ethyl acetate

c. Triplicate results of inhibition zones in 25 mg fruit powder/mL solvent extracts.

Name of organisms	Zone of inhibition (mm in diameter) (M±SD) (n=3)						
	St.	PE	chloroform	EAc	Ethanol	water	con
E. coli	6.9±0.15	0.9±0.14	5.4±0.38	6.7±0.29	4.2±0.43	1.1±0.28	-
S. aureus	3.2±0.31	0.78±0.25	-	0.7±0.29	2.2±0.31	-	-
S. typhimerium	7.1±0.00	3.5±0.00	4.75±0.25	6.8±0.15	5±0.17	1.2±0.29	-
C. albicana	6.8±0.35	7.3±0.35	4.6±0.19	2.3±0.15	3.7±0.15	1.0±0.00	-

Key: St.= Chloramphenicol, con.= Control, PE = petroleum ether and EAc = Ethyl acetate

4 Discussion

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites. Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Munoz et al., 2003; Dalal, 2016). The phytochemical active constituents of fruit powder extracts may be responsible for antibacterial activity against stomach pain and diarrhea pathogens. So *Solanum incanum* may prove it useful and might be used for the treatment of stomach pain and diarrhea. With advances in phytochemical techniques, several active principles of many medicinal plants have been isolated and introduced as valuable drugs in the modern systems of medicine. However, in our country, the isolation of active constituents and introducing them as valuable drugs is yet in its infancy.

The logic in using different solvents when screening for phytochemicals in plant materials was clearly validated in this study. For instance, the results shows that terpenoids were exceptionally present in hexane extracts but absent in water extract. Terpenoids were slightly present in methanol extract. The results indicate that majority of the secondary metabolites are contained in the extracts of fruit of *Solanum incanum.* So this medicinal plant holds promises as source of pharmaceutically important phytochemicals. Alkaloids, generally present in all extracts, play some metabolic role and control development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins are known to inhibit pathogenic fungi. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic, etc (Lalitha et al., 2012).

The antibacterial activity of the plant extract was assayed in vitro by agar well diffusion method against the three bacterial species (*E. coli, S. aureus and S. typhimerium*) and a fungus (*C. albicana*). Table 2 summarizes the microbial growth inhibition of the different extracts of the screened plant material. The maximum antimicrobial activity was shown by *E.coli, S. typhimerium* followed by *C. albicana* respectively, and a minimum activity was shown by *S. aureus.* Extracts of the investigated plant material showed maximum antibacterial activity against Gram-negative *S. typhimerium and E. coli* which are the main cause for the stomach pain and diarrhea. The plant material extracts showed minimum antibacterial activities against gram positive *S. aureus* which is the most active bacteria for the wounds and infections. The ethyl acetate, chloroform and ethanol fruit powder extracts of the plant showed maximum antibacterial activity followed by petroleum ether fruit powder extracts. The aqueous extracts showed minimum sensitivity against all the pathogens under this investigation. This is because the diffusivity of water is very low as compared to the other solvents used. It is quite possible that the plant material does not possess antibiotic properties in below 100 mg fruit powder/mL water concentrations, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in water. The drying process may have caused conformational changes to occur in some of the chemical constituents found in this plant. The above statements can be correlated to the results of the phytochemical screening shown in Table 1. The active secondary metabolites like alkaloids, terpenoids and tannins were found in high concentrations in the moderately polar solvents ethyl acetate and chloroform. For example, alkaloids and steroids were present in high concentration in ethyl acetate and chloroform extracts. Terpenoids, steroids and tannins were absent in water extract. As the concentration of the fruit powder extracts is reduced to half, the sensitivity is also reduced to half. For example, the inhibition zone of the 100mg fruit

powder/mL ethyl acetate extract against *S. typhimerium* was 27±.0.12 mm. However, it is reduced to 12.7±0.58 mm. in 50mg fruit powder/mL ethyl acetate concentration. The results of antimicrobial sensitivity assay were comparable with the standard chloramphenicol.

Several workers have reported that many plants possess antimicrobial properties including the parts which include flower, bark, stem, leaf, etc. It has been shown that when solvents like ethanol, hexane and methanol were used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (Bushra et al., 2003; Khizar, 2014).

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 (Wagner et al., 1996). 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 result of this research shows the presence of phenols which are regarded as one of the functional food components in fruits 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. Mitali et al. (2012) reported that 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.

The present study also support the study conducted by Alamri and Moustafa (2012) 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.

The study revealed the presence of tannins which have advantage for tanning process that are used in traditional tanning i.e. vegetation tanning. Tannin 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. Similar results reported by Covington (1997) indicate that 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.

According to the studies of Ramawat (2008) and Wink (2000), in plants alkaloids generally exist as salts of organic acids like acetic, oxalic, citric, malic, lactic, tartaric, tannic and other acids. In this study the pH of the fruit sap was measured to have pH value on the range ofthese 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.

5 Conclusions and Recommendation

In the present study, fruit of *Solanum incanum* showed the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, tannins, steroids, amino acids and reducing sugars and the extract showed significant antimicrobial activity. 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. This study leads to the further research in the way of isolation and identification of the active compounds from the fruit using chromatographic and spectroscopic techniques for proper drug development.

Acknowledgements

I am much indebted to the National central laboratory of microbiology for their support during this study.

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