

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY ETHANOLIC EXTRACTS OF DRIED FRUITS OF *SOLANUM TORVUM* (FAMILY-SOLANACEAE)

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ABSTRACT

Objective: To study the antimicrobial activity ethanolic extracts of dried fruits of *Solanumtorvum* (family-solanaceae) along with preliminary phytochemical analysis.

Methods: The ethanolic extract of dried fruits of the herb *Solanumtorvum* (family-solanaceae) were prepared and analyzed for phytochemical constituents using standard methods. The antimicrobial activity of the plant extracts were examined against 2 bacterial strains among one is gram positive and other is gram negative and 2 fungus using agar well diffusion method.

Results: The present experiment shows the phytochemical analysis, antimicrobial activity of the ethanolic extract of dried fruits of the plant *Solanumtorvum* (family-solanaceae). Various phytochemical analysis revealed the presence of alkaloids, saponin, flavonoids, carbohydrates, glycosides, Steroids, proteins and amino acids and tannins. All the extracts of the plant showed antimicrobial activity against most of the test organisms.

Conclusion: It can be concluded ethanolic extract of whole aerial parts of the *Solanumtorvum* (family-solanaceae) contain the high presence of phytochemicals. This extract was found to possess promising antimicrobial activity when compared with the standards.

Keywords: Antimicrobial, Zone of Inhibition, Solanumtorvum, Agar disc diffusion method

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INTRODUCTION

Search for naturally occurring materials which are having biologically active substance and show biological activity and in plant chemotherapy, the use of naturally occurring antifungal substances is gaining more importance and have more significant values [1].

Each and every plant contain near about hundreds or thousands of metabolites. Medicinal plants are a wonderful gift of nature and are being used against numerous infectious and harmful diseases in the world since from ancient period of time [2].

It is estimated that 2, 50, 000 to 5, 00, 000 plants species are there but only a small percentage has been checked phytochemical properties and the fraction submitted to biological screening is even smaller. The plant kingdom has a wide range of population that relies on plant and plant extract for health care [3].

Medicinal plants plays a great role for the health of individuals and communities. It has a huge importance in today's world. These plants have a great medicinal value that lies in some chemical substances which produce physiological action on the human body. Medicinal plants can also be used as food and spices. Medicinal plants contain a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, locations and oils (essential and fixed) [4].

Higher plants have the capacity of producing both primary and secondary chemical metabolites, primary chemical metabolites is important in development and reproduction of plants. On the other hand, secondary metabolites acts as defence mechanisms against adverse biotic and abiotic conditions for survival of the plant [5].

The World Health Organization (WHO) told that the best source of obtaining various qualities of the drug is none other than medicinal plants. According to them, about 80% population of this modern world use traditional medicine, which has compounds derived from medicinal plants [6]. Both plants and plant-based medicaments are the basic needs of the various pharmaceuticals products. The plant kingdom has numerous source of active ingredients which are

valuable in the treatment as well as management of different types of disease [7].

Medicinal plants have been tested for various kinds of activities such as antimicrobial, hypoglycemic, antihelmintic, hepatoprotective, antioxidant, analgesic, antipyretic activities, antipyretic, antileishmanial and insecticidal activities [8]. Medicinal plants such as *Ocinumgratissimum* and *Eugenia uniflora* are rich in volatile oils. It is also reported that they contain 75% thymol which has an antimicrobial effect against *Staphylococcus* sp., *Escherichia coli* and *Shigella* sp. They are also used in the treatments like diarrhea human ear infection [9].

The plant kingdom has varieties of plants which are capable of producing secondary metabolites and are used for medicinal purposes for several years [10]. Countries like India rely on such medicinal plants to content against the plant diseases. During seed borne phases the management of fungal pathogens is considered to be a cheap dress disease control strategy [11].

There are many locally available plants and they are rich in source of novel bio-compounds which are cheapest in the source. Isolation, Screening and studying the antimicrobial effect of such biochemical substances is needed to control and treat the diseases [12].

So the target of this present study is to identify the phytochemical components of *solanumtorvum* and to determine the antimicrobial effects of the dried fruit extract on *E. coli*, *S. aureus*, *Candida albicans* and *Trichophytonrubrum*.

MATERIALS AND METHODS

Collection of plant materials

Solanumtorvum was collected from Deepor Beel Bird Sanctuary, Guwahati India. The plant specimen was authenticated by Dr. G. C. Sarma, Curator, Department of Botany, Gauhati University, Guwahati, Assam. The voucher specimen has been deposited in the Herbarium of the Department of Botany, Gauhati University with voucher no Acc. No.-18209 dated 28-10-2016 for future reference.

Chemicals and reagents

Ethanol (ET), Petroleum Ether(PE), hydrochloric acid, Dragondorff reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, sulphuric acid, lead acetate, Molisch's reagent, Fehling solution A and B, sodium citrate, copper sulphate, ferric chloride, sodium hydroxide, glacial acetic acid, benzene, chloroform, ammonia, nitric acid, dimethyl sulfoxide (DMSO), potassium nitrite, gelatine, Beef extract, Peptone and agar. All the chemicals and solvents used were of standard analytical grades.

Preparation of extracts of *Solanumtorvum* fruits

The *Solanumtorvum* fruits were dried under shade and then at tray drier for 370C and undergone crushing in an electric blender to form powdered. Then it was defatted by Petroleum ether and extracted with ethanol using Soxhlet's extractor. The percent yield of ethanolic extract was then calculated out.

Preparation of extract/drug stock solution

The stock solution of *Solanumtorvum* seed extract was prepared on each occasion by careful weighing and dissolving in a suitable volume of Dimethyl sulphoxide (DMSO) to get a concentration of 100 mg/ml. A tablet of ciprofloxacin was dissolved in an appropriate volume of water to get 5 mg/ml of stock solution.

Phytochemical screening

Phytochemicals screening were done using standard method. All the experiment has been repeated in triplicate for final confirmation of the result.

1. Test for saponins: To 1 ml of aqueous extract was added to few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

2. Test for alkaloids: Two methods were used to test for alkaloids. First, evaporate 20 ml of ethanol extract, the dry residue dissolved in 5 ml of HCl (2N) and filtered. A few drops of Mayer's reagent and Wagner was added, the presence of precipitate indicates the alkaloids.

Second, 3 to 15 ml of the aqueous extract was added 2 ml of NH₄OH to 10%. The alkaloid was extracted 3 times with 10 ml chloroform. The chloroform layer was washed 3 times with 2 ml of HCL (10%). This was divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other. The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids.

3. Test for sterols and steroids: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpene.

4. Test for the phenolic compounds: Flavonoids: The ethanol extract 5 ml was added to a concentrated sulphuric acid (H₂SO₄) (1 ml) and 0.5g of Mg. A pink or red coloration that disappears on standing 3 min. indicates the presence of flavonoids.

5. Tannins: Two methods were used to test for tannins. First, about 1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride (FeCl₃) solution was added and

observed for green to blue-green or blue-black coloration. Second, 2 ml of the aqueous extract was added to 2 ml of water, 1 to 2 drops of diluted ferric chloride (FeCl₃) solution were added. A dark green or blue-green coloration indicates the presence of tannins.

6. Test for Glycoside: 2 ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring i. an aglycone portion of the glycoside. [13,14]

Culture media

The media employed for the study was solid agar media.

Microbial strains

Three bacterial strains [*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and two fungi [*Candida albicans* (ATCC 10231), and *Trichophyton rubrum* (ATCC 28188)] were used. Stains were obtained from Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati

Sterilization of materials

The petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 170 °C for 1 h at each occasion. A solution of the extract and culture media were autoclaved at 121 °C for 15 min.

Antibacterial activity

The antimicrobial activity of the different extracts of the plant was assayed by agar-well diffusion method as described in NCCLS, 1993 [15]. Petri plates containing 20 ml nutrient agar medium was seeded with bacterial strains. Wells of approximately 10 mm was bored using a well cutter. Plant extracts were prepared in DMSO (stock: 1 mg/ml DMSO). The plant extracts of 25, 50, and 100 µl concentrations were added. Ciprofloxacin (20 µl) and DMSO (100 µl) were used as positive and negative controls respectively.

The plates were then incubated at 37 °C for 24 h. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact with the test organisms in the freshly seeded plate. The diameter of the zone of inhibitions was measured in millimetres after 24 h.

Antifungal activity

The potato dextrose agar plates were prepared and inoculated with a fungal culture. Wells of approximately 10 mm was bored using a well cutter and samples of different concentration were added. The zone of inhibition was measured in millimetres after overnight incubation and compared with that of standard antifungal (Fluconazole) (10 µl) which was used as positive control and DMSO (10%) as the negative control [16].

RESULTS

The present systematic examination shows the phytochemical analysis, antimicrobial activity of the ethanolic extract of the plant *solanumtorvum*. The yield % of the extraction of ethanol was 20.28%. It was a dark brown solid powder (ET) in appearance.

Table 1: Phytochemical screening of Ethanolic (ET) extract of dried fruits of *Solanumtorvum* (family-solanaceae)

S. No	Phytochemical test	Reagent used (test performed)	Observation	Result
1	Alkaloids test	Mayer's test Wagner's test Dragendroff's test	Frothing observed	+
2	Saponin test	Foam test	Turbidity obtained	+
3	Flavonoid test	Lead acetate test	Golden yellow colour observed	+
4	Carbohydrate test	Molisch's test Benedict's test Fehling's test	Yellow colour observed	+
5	Glycoside test	Modified Borntrager's	Brownish black ppt not observed	+
6	Steroid test	Salkowski's test	Ring not formed	+
7	Proteins and amino acids	Xanthoproteic test	Reddish black not seen	+
8	Tannins test	Gelatin test	A Reddish brown color formed	+

+sign indicates the presence and–sign indicates absence.

Table 2: Antimicrobial activity ethanolic (ET) extract of dried fruits of *Solanum torvum* (family-solanaceae) using disc diffusion assay

Name of the compounds and their concentration	Antibacterial activity diameter of inhibition zone (mm)		Antifungal activity diameter of inhibition zone (mm)	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>C. albicans</i>	<i>Trichophyton rubrum</i>
Standard Ciprofloxacin (20 µl)	42	40	-----	-----
Standard Fluconazole (20 µl)	-----	-----	32.21	30.01
Plant extract (25 µl)	14.5	11.2	12.5	10
Plant extract (50 µl)	18.33	16.51	17.3	11.2
Plant extract (100 µl)*	20.3	18.58	21.5	16.9

*significant activity

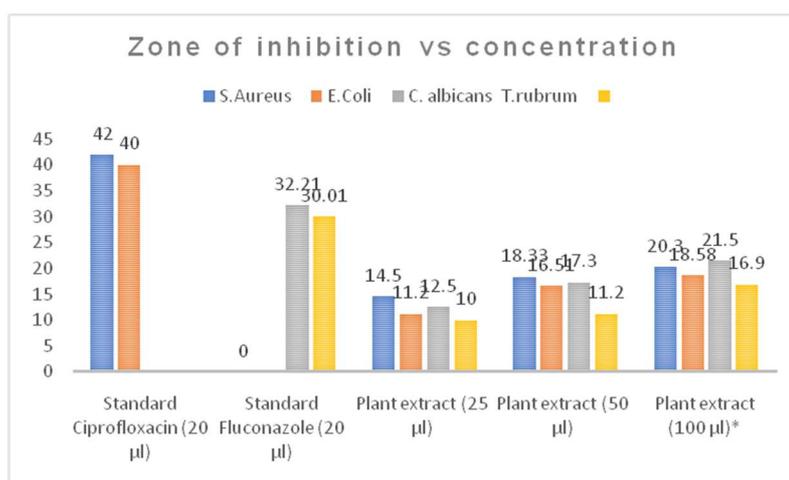


Fig. 1: Zone of inhibition vs concentration

Phytochemical analysis

The phytochemical analysis with the ethanolic extracts of the dried fruit of plant *solanumtorvum* showed the presence of alkaloids, saponin, sterols, Steroids, terpenoids, protein and amino acid, tannins, carbohydrate are given in (table 1).

Antimicrobial activity

Generally, Plant extracts are rich in antimicrobial compounds. The *in vitro* antimicrobial study of the ethanolic extracts of *solanumtorvum* under different concentration with the standard are mentioned in (table 2). The ethanolic extracts of dried fruits of the plant *solanumtorvum* showed antimicrobial activity against most of the test organisms.

DISCUSSION

Antimicrobial activity of dried fruits of plant *solanumtorvum* has been evaluated. Phyto-constituents present in plants namely alkaloids, saponin, sterols, terpenoids are having an exciting set of circumstances that makes it possible to do something for more extensive of modern therapies against a wide range of microorganisms. The present study was done against the variety of Gram-positive, Gram-negative bacteria, and fungal strains were selected for screening antimicrobial impact of the extracts to see the antimicrobial spectrum. Results of this study shows that the ethanolic extracts of the plant *solanumtorvum* were highly successful in producing the desired result against most of the Gram-positive bacteria, Gram-negative bacteria, and fungal strains in agar well diffusion method.

Phytochemical constituents of plants such as tannins, alkaloids, flavonoids, phenolic compounds and several other aromatic compounds are secondary metabolites which can be used in achieving a defence mechanism against plundering by many microorganisms.

The practical exhibition and explanation of antimicrobial activity against both Gram-positive and Gram-negative bacteria and on various fungal strains may be an indication of something presence of broad spectrum antibiotic compounds in the extracts which is shown in fig. 1. The 100µg/ml concentration of dried fruit extract of *solanumtorvum* have an influencing antimicrobial activity.

CONCLUSION

Medicinal Plant in plant kingdom are the important source of drug and plays a great role for the health of individual and communities. Many plants have been tested for the presence of compounds with therapeutic activity. Therefore, it is absolutely necessary to evaluate the antimicrobial activity of *Solanumtorvum*.

During the study, the antibacterial activity of the dried fruits of *Solanumtorvum* was judged by using disk diffusion method. The microorganisms that have been selected for study were Gram-positive, *S. aureus* and Gram-negative *E. coli* and fungus like *C. albicans*, and *T. rubrum*. Study of this micro-organism was done as they are important pathogens and also they have the capacity of rapidly developed antibiotic resistance as antibiotic use increases.

At the end, the study concluded that ethanolic extract of *Solanumtorvum* showed the presence of various phytochemicals of which include alkaloids, saponin, sterols, Steroids, and terpenoids.

The present study makes us know about the significant antimicrobial activity of the plant extracts when estimated with standards. So, after lots test, decision and thoughts it was found that the minimum inhibitory concentration of the plant extracts was less than 100µg/ml and therefore the plant was considered to have a very good antimicrobial activity.

However, further studies are definitely required to put light on the unknown biological activities of *Solanumtorvum* and its effect on living organisms, tissues or cells against various diseases.

CONFLICT OF INTERESTS

Declare none

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