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PHYTOCHEMICAL EVALUATION OF DIFFERENT SOLVENT EXTRACTS OF A TRADITIONAL MEDICINAL PLANT

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary metabolites. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants are proved to have antifungal, antibacterial, antioxidant, antidiabetic, hepatoprotective and anti-inflammatory activities. *Solanum torvum* is a plant growing in many parts of the world. Their fruits are edible, can withstand heavy drought and with good nutritional content and is investigated in this study for its phytoconstituents in different solvent extracts. In the present work different extracts of *Solanum torvum* fruits were analyzed for their phytochemical constituents which may probable be responsible for various pharmacological activity of the plant and also to quantitate the essential constituents which is believed to be more contributory to these activities. The analysis revealed the presence of phytoconstituents such as flavonoids, alkaloids, phenolic compounds, saponins, terpenoids, tannins, carbohydrates etc. Further, some of these chemical constituents were also quantitatively estimated.

KEYWORDS: *Solanum torvum*, aqueous extract, phytoconstituents.

INTRODUCTION

Medicinal plants play a highly significant role in drug discovery and development process. They are of great help to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on

the human body. Natural products are directly or indirectly responsible for almost 40% of the drug use in modern therapeutics. Nature has provided many things for humankind over the years, including the tools for the first attempts at therapeutic intervention. Ancient civilization depended on plant extracts for the treatment of various ailments. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents, food additives, agrochemicals and industrial chemicals.[1] Plant based natural constituents can be derived from different parts of the plant like bark, leaves, flowers, roots, fruits, seeds, *etc*. The phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibres to act as defence system against disease or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids and tannins *etc.*[2] The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. But among the 250,000-500,000 plant species only a small percentage has been investigated phytochemically.

Medicinal plants are a group of species that accumulate different active principles, useful in treating various human or animal diseases. The long term use of herbs in medicine is a sure indication of their value and usefulness in the future. Phytotherapy is a source of treating and improving certain diseases by using the beneficial effects of medicinal plants. An important amount of therapeutic products are derived from medicinal plants. There are over 1700 medicinal plant species, of which more than 500 are cultivated. In modern medicine, the importance of medicinal plants is increasing.[3] Plant extracts were traditionally used as medicine without scientific investigations and the outcome was mostly unpredictable. Nutritional substances and secondary metabolites form the basis for their pharmacological actions. The family Solanaceae represent one of the most economically and medicinally important families of angiosperms. The genus Solanum is a hyper-diverse taxon of this family. There are about 2000 species of Solanum in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the temperate areas. About 21species and one variety in this genus are used as herbal medicines.[4] *Solanum torvum* L. is

a small solanaceous shrub, distributed widely in Pakistan, India, Malaya, China, Philippines, and tropical America.[5] For many decades, different ethnic groups have used the dried stem and root of this plant for treatment of various ailments. *Solanum torvum* is a pharmacologically important species of the family Solanaceae. Traditional medicinal uses of *S. torvum*, have been highlighted in the Ayurveda and Chinese pharmacopeia. *Solanum torvum* has been extensively explored for its chemical constituents. Various parts (fruit, leaves and roots) are being in use for the isolation of a wide range of compounds. This plant species is a very good source of alkaloids, flavonoids, saponins, tannins, glycosides and polyphenolic compounds.[6,7] The aim of this paper is to present the phytochemical screening of bioactive compounds found in the most widespread herb in the area which is edible and also used as a folkloric medicine. The obtained results were used as important key aspects in making recommendations concerning the cultivation of certain species, whose active principles can be valued as phytotherapeutical products, food supplements and cosmetics.

MATERIALS AND METHODS

Collection and identification of plant material

The *Solanum torvum* fruits were collected from the local area in the month of November and authenticated and a voucher specimen is deposited in the Rapinet herbarium, SJC, Trichy. The collected plant materials were thoroughly washed and then dried at 35 ºC in a thermostatically controlled oven until they attain a constant weight. The dried plant samples were ground well into a coarse powder in a mixer grinder, so as to enhance effective contact of solvents with sites of plant materials. The powdered samples were then stored in air tight containers for future use.

Preparation of plant extract

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of hexane, ethyl acetate and water separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. After that, the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Percentage yield was measured. Dried extract was kept in refrigerator at 4ºC till future use.

Preliminary Phytochemical Screening

Preliminary phytochemical analysis was carried out for all the extracts as per standard procedures.[8,9,10]

Test for Alkaloids

Crude extracts were dissolved individually in dilute hydrochloric acid and filtered.

The filtrates were used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.
b) **Wagner's test**: Filtrates were treated with wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colour disappears on standing.

Test for Steroids

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski's test)

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Tests for Anthraquinones (Borntrager's test)

About 0.2g of the extract was boiled with 10% hydrochloric acid for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% ammonia were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

Test for Phenols (Ferric chloride test)

Plant extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Test for Saponins: Crude extracts of different solvents were mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Tannins

1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N Hydrochloric acid was added and observed for blue-black coloration

Test for Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates by adding 5 ml of 2% anthrone reagent followed by concentrated sulphuric acid. A dark green colour indicates the presence of carbohydrates.

Test for Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Quantitative Determination of Phytoconstituents

Determination of Alkaloids

5 g of the dried powder of the plant extract was weighed into a 250 ml beaker and 200ml of 10 % acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated.[10]

Estimation of Flavonoids

10 g of each plant sample was extracted repeatedly with 100 ml of 80 % of aqueous methanol at room temperature. The whole solution was filtered through whattman filter paper No. 41(125 mm). The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight.[11]

Estimation of Total Phenolic Compounds

Total phenolic content of the aqueous extract of *Solanum torvum* fruits was determined by standard method[12] with little modifications, using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order

to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml, 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin-Ciocalteu reagent. The samples were mixed well and then allowed to stand for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm on using spectrophotometer after incubating the samples for 90 min. All the experiment was conducted in three replicates.

RESULTS AND DISCUSSIONS

The present work mainly aims at preparing different solvent extracts of edible fruits of *Solanum torvum* and analyzing the various phytoconstituents present in them. The data shown in Table 1 shows the screening of aqueous extract of fruits of Solanum torvum based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations and inference made in the phytochemical tests are presented below. The non-polar hexane extract reveals the presence of different chemical constituents such as alkaloids, flavonoids, terpenoids, saponins, carbohydrates and oil and resins whereas the mid polar ethyl acetate extract contains only alkaloids, saponis, carbohydrates and oil and resins. The aqueous extract also contains almost all the constituents except sterols, tannins and oil and resins. This shows hexane and aqueous extracts of fruits are almost equally effective and taking it to consideration the edible nature of fruits as it is commonly employed as vegetable in most of the countries of the world, this study mainly aims at studying the medicinal properties of the aqueous extract of the *Solanum torvum* fruits. Since alkaloids and flavonoids contribute to most of the pharmacological activities of plants, quantitative determination of these constituents is carried out to find out the percentage composition of these constituents in the fruit extracts. The results are given in Table 2.

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antileprosy activities etc.[13] The hexane extract of the fruits shows the presence of alkaloids, flavonoids, terpenoids, saponins, carbohydrates, oils and resins whereas the ethyl acetate extract reveals the presence of alkaloids, saponins,

carbohydrates, oils and resins and the aqueous extracts shows the presence of almost all the phytoconstituents except anthroquinones, sterols, oils and resins. The study shows that of all the three extracts aqueous extract of Solanum torvum fruits showed maximum phytoconstituents. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides.

Table 1: Phytochemical screening of various solvent extracts of *Solanum torvum* fruits

Phytochemicals	Extracts		
	Hexane	Ethyl acetate	Aqueous
Alkaloids	-	+	+
Flavonoids	+	-	+
Steroids	- -	- -	- -
Terpenoids	+	-	+
Arthroquinone	-	-	-
Phenols	- -	- -	-
Saponin	+	+	+
Tannin	-	-	-
Carbohydrates	+	+	+
Oils & Resins	+	+	-

Table 2: Percentage composition of phytoconstituents in the aqueous extract of *Solanum torvum*

S.No	Phytoconstutients	Percentage (%)
1	Alkaloids	0.18
2	Flavonoid	10.46

The quantitative estimation of the aqueous extract also reveals the presence of high amount of flavonoids followed by alkaloids. Flavonoids are potent water soluble antioxidants and free radical scavenger which prevents oxidative cell damage and also have strong anticancer activity. It also helps in managing diabetes induced oxidative stress.[14]

Moreover alkaloids represent a class which affects the central nervous system, reduces appetite and behaves as diuretic.[15] Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomoulatory properties.[16,17] In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to hae insecticidal properties as well.[18] Many studies have proved that saponins possess the unique property of precipitating and coagulating red blood cells.[19] Our

study reveals the presence of considerable amount of therapeutic phytochemicals like alkaloids, flavonoids, terpenoids and saponins. These result might provide scientific support to the traditional claims in using these plants in throat congestion, cough and cold.[20] The presence of various potentially important compounds justifies exploration of its medicinal qualities reinforced by its importance in indigenous herbal and conventional medicines. In addition there is a need to explore the local indigenous uses of this plant in different communities.

CONCLUSION

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

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