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STUDIES ON PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF LEAVES OF *SOLANUM NIGRUM* L.

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ABSTRACT

Solanum nigrum is a well-known medicinal herb found all over India. For cloning, explants were harvested from rejuvenated shoot sprouts of selected plants of *Solanum nigrum.* Medicinal plants are of great importance to the health of individuals and communities. A large number of plants are claimed to possess the anti-diabetic, anti-fertility, antihyperlipidaemic, anti-inflammatory, anti-cancer, hepatoprotective and immunomodulatory activities in the traditional therapeutic systems. It is now believed that nature has given the cure of every disease in one way or another. *Solanum nigrum* a valuable medicinal plant possess many bioactive principles which includes diabetes mellitus, chronic bronchitis, goitre, mucous disorders and leprosy. The ethanolic extract of leaves of *Solanum nigrum was* investigated for its phytochemical properties and analysis for its active chemical ingredients. For qualitative and quantitative phytochemical analysis the ethanol extract of *Solanum nigrum* acts as a source of therapeutic agent.

KEYWORDS: *Solanum nigrum.*

INTRODUCTION

Plants which have one or more of its organ containing substances that can be used for the therapeutic purpose, are called medicinal plants. Sofowara (1993). Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts world. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins) however, other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils etc. also have been reported Farnsworth (1966). Phytochemical are very important in medicine and constitute most of the valuable drugs. Alkaloids are rich in medicine and constitute most of the valuable drugs. They have physiological effect on animals. Edeoga *et,al.* (2001).

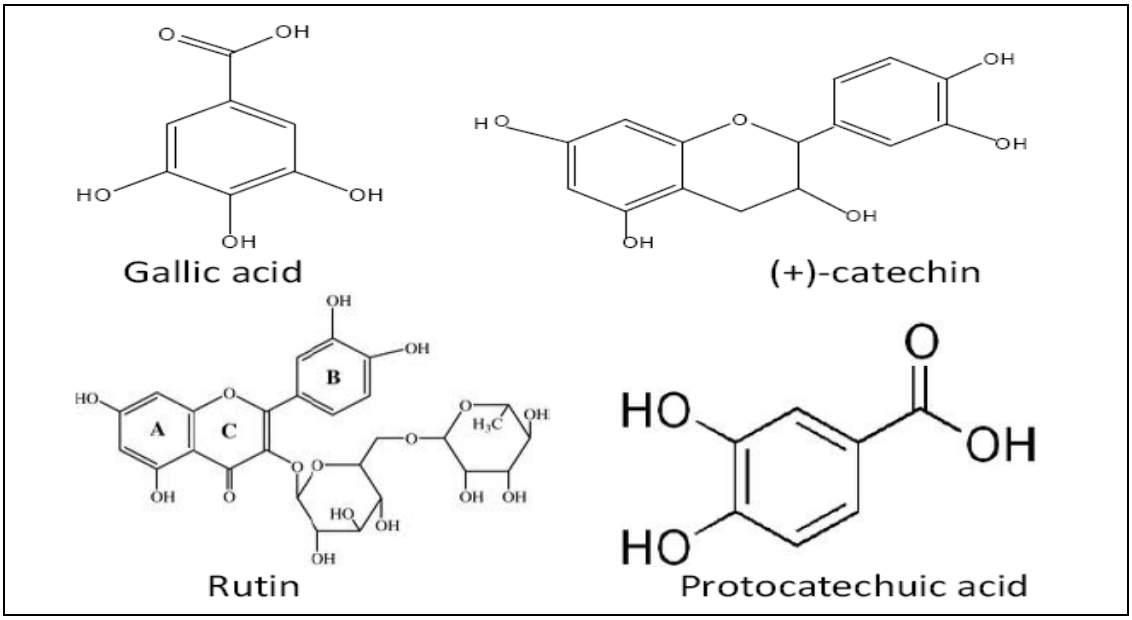
The *Solanaceae*, to which the genus *Solanum* L. belongs, is a cosmopolitan family containing many essential vegetables and fruits such as potatoes, tomatoes, aubergines, paprika, chillies, green and red peppers and black nightshade. Composed of approximately 90 genera and between 2000 and 3000 species, the family is widely distributed throughout tropical and temperate regions of the world, with centres of diversity occurring in Central and South America and Australia (Edmonds 1978a; D'Arcy 1991).Within this family, *Solanum* constitutes the largest and most complex genus; it is composed of more than 1500 species, many of which are also economically important throughout their cosmopolitan distribution. Examples of food plants are the potato *(S. tuberosum* L.), the aubergine or egg plant *(S. melongena* L.); horticulturally useful plants include the winter cherry *(S. pseudocapsicum* L.) and jasmine nightshade *(S. jasminoides* Paxt.); species cultivated for their drug use include bittersweet *(S. dulcamara* L.) and *S. viarum* Dun., both used as sources of corticosteroids.

The generic name *Solanum* is generally considered to be derived from the latin *Solamen,* and to refer to the quieting or sedative effects associated with many of the species particularly in South America. *Solanum nigrum* itself is a predominantly Eurasian species, which does not occur naturally in South America (Edmonds 1979a). India has a rich heritage of Knowledge on plant based drugs for use in preventive as well as curative therapies (Rai and Nath 2005). About 6000 plants in India have been in use in traditional, folk and herbal medicines (Dubery *et.al.* 2004). *Solanum nigrum* L. is a medicinally important plant of the family Solanaceae. The plant has been traditionally used as hepatoprotective agent in India. Fruits make a delightful Jam (Chopra *et.al.*, 1986) Watt *et.al.* 1962). Fruit of plant is also used as a nervous tonic in the Mexican medicine. Chemically, solasodine, solasonine and solanidine have been identified from plant (Wayne *et.al.*, 2011.) Fruits of plant have also been used as an antioxidant and cancer chemopreventive material (Son *et.al.*,, 2003).

Traditional Uses

S. nigrum has been used traditionally to treat various ailments such as pain, inflammation fever and enteric diseases. It possess many activities like antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic agent, antibacterial, mycotic infection, cytotoxicity, anti-convulsant, antiulcerogenic. It is also used against sexually transmitted diseases.

Chemical constituents



S. nigrum possesses numerous compounds that are responsible for pharmacological activities. Its active components are glycoalkaloids, glycoproteins, and polysaccharides, polyphenolic compounds such as gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin.

MATERIALS AND METHODS

S.nigrum plants were collected from Ramagiri fort Mahadevapur reserve forest south region Karimnagar District, The study plant were identified with the help of available Indian literatures and the identities were verified with the help of Herbarium, SRR Govt. Arts & Science College Karimnagar.

Preparation of Powder

The collected fresh leaves root and fruits were shade dried at room temperature for 3 days and sun dried for 3days and then milled into coarse powder bya mechanical grinder (Harborne, 1988).

Preparation of Aqueous Extract

The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No. 42 (125 mm) (Rao *et al.* 1995).

Phytochemical Screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for Tannis

About 0.5 g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Phlotannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973). 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated

with 10ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for Steroids

2ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski Test)

5ml of each extracts was mixed in 2 ml of chloroform and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani Test)

5ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Quantitative Determination of the Chemical Constituency Preparation of Fat free Sample

2g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

Determination of Total Phenols by Spectrophotometric Method

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of ammounium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. It was measured at 505 nm.

Alkaloid Determination using Harborne (1973) Method

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to onequarter 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 precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin Determination by Van-Burden and Robinson (1981) method

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. Then 5ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Saponin Determination

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 ^{0}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{0}C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n- butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoid Determination by the Method of Bohm and Kocipai-Abyazan (1994)

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

RESULTS AND DISCUSSION

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of the *S.nigrum* investigated are summarized in.

Table 5.1 Photochemical screening test of Leaf extracts of *S.nigrum* .

Sl No	Chemical components	Methanol	Ethanol	Chloroform	Pet Ether	Water
1	Alkaloids	+	+	+	+	+
2	Terpenoids	+	+	+	+	+
3	Flavonoids	+	+	+	+	+
4	Anthraquinones	-	-	-	-	-
5	Tannins	+	+	-	-	-
6	Saponins	+	+	+	+	+
7	Glycosides	-	+	+	-	+
8	Pholotannins	+	+	+	+	+
9	Steroids	+	+	+	+	+
10	Cardiac glycosides	+	+	+	+	+
11	Phenol	-	-	-	-	-

The qualitative screening of phytochemical constituents on leaf extracts of *S.nigrum* reveals the presence of alkaloid, saponin, tannins, flavonoids, proteins etc. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bacterial effects (Stray 1998).They exhibit marked physiological activity when administered to animals.

In the present study, the observed alkaloid content in *S.nigrum* could be responsible for their much acclaimed medicinal values though the exact mode of action is poorly understood. Saponins are a special class of

glycosides which have soapy characteristics. It has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponin include formation of forms in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness (Sodipo *et.al.* 2000).These properties bestow high medicinal activities on the leaf extract from *S. surattense*. Tannins are also known antimicrobial agent. Tannins (commonly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods. Tannins are water soluble plant polyphenols that precipitate proteins. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Sodipo *et.al.* 1991).The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et.al.* 1998). Phytotherapatically tannin containing plants are used to tract nonspecific diarahoea, inflammations of mouth and throat and slightly injured skins.

Biological activities

In this study, the presence of tannins might have accounted for the sharp taste of *S.nigrum* and have been reported to hasten the healing of wounds and inflamed mucous membrane. Flavonoids are potent water soluble antioxidants and free radical scanvengers, which prevent oxidant cell damage, have strong anticancer activity (Salah *et.al.*, 1995). Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity. This may be reason *S.nigrum* have been used for the treat ment of wounds, burn and ulcers in herbal medicine. Apart from these secondary metabolites, due to the abundantly presence of protein in leaf of *S.nigrum* which can serve many of the medicinal properties exhibited by the plants. For example, a variety of proteins have been isolated in medicinal plants and found to be bioactive against certain ailments (Tsao *et.al.*, 1990).

The presence of the above said phytochemical constituents could account for the much medicinal properties of *S.nigrum* for the treatment of various diseases/ailments such as cough, liver problem, stomach-ache, skin diseases, inflammation, jaundice, tooth ache etc which are reported by various workers (Pronob Gogoi *et.al.* 2012).

Alkaloids are significance for defense and survival of plants. The significance of medicinal plants is directly associated with the wide range of chemical compounds produced by different biochemical pathway. High alkaloid value of *Solanum* xanthocarpum and *Nicotiana plumbaginifolia* justify the wide use in traditional system of medicine.

OBJECTIVES

Solanum nigrum is paramount in medicinal perspective and belongs to family Solanaceae. From different parts of the plant, significant pharmacological and biological activities have been reported previously. This study was aimed to analyze the presence of various phyto constituents and to determine the antioxidant potential, *in vitro*.

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