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QUALITATIVE PHYTOCHEMICAL ANALYSIS OF LEAVES AND STEM BARK OF SOLANUM PUBESCENS WILLD.

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Abstract

Solanum pubescens an important medicinal plant used in the treatment of malaria, Epilepsy, whooping cough and febrile convulsions. The identification of qualitative phytochemicals has become the important tool for knowing the active principles of different medicinal plants. Solanum pubescens is a wild shrub found in the forest and hilly areas of Chitradurga District of Karnataka State. It is commonly used by the local traditional practitioners for treating different diseases. The present work was aimed to know the preliminary phytochemical constituents of Ethyl acetate extracts of leaf and stem bark of Solanum pubescens. The present study shows the presence of carbohydrates, proteins, flavanoids, tannins, phenols, saponins, Betacyanin, quinones, resins, fixed oils and fats. This study helps us to know the antimicrobial and anti carcinogenic activity presumably play active role in treating diseases.

Key words: Solanum pubescens**,** phytochemical constituents, Chitradurga District.

1. Introduction

Plants are potent source of phytochemicals. Phytochemistry is mainly concerned with enormous varieties of secondary plant metabolites which are biosynthesized by plants. The beneficial physiological and therapeutic effects of plant materials typically result from the combinations at these secondary products present in the plants but only a small percentage have been investigated for its phytochemicals, only a fraction has undergone biological or pharmacological screening. Solanum pubescens is an annual erect wild shrub found in the forest and hilly areas of Chitradurga District of Karnataka State, commonly known as " Usti gida" in kannada, "Ushti chettu, Kasivuste" in telugu and "Kattusundal" in Tamil. Flowering and fruiting in the month from July-February. Plant is bitter in taste due to the presence of alkaloids. It is commonly used by the local traditional practitioners for treating different diseases like used in the treatment of headache, menstrual pain, rheumatoid arthritis, tuberculosis, ulcers etc (Sumalatha et al., 2013), it can also be used to treat whooping cough (Reddy et al.,2006). Pharmacological studies viz., Antidiabetic (Hemamalini K et al., 2012) Antidiarrheal activity (Hemamalini K et al., 2013), antinociceptive screening (Sumalatha et al., 2013), Anti inflammatory activity (Niyogi. P et al., 2012) antibacterial activity (Haseebur Rahman et al., 2014) Anticonvulsant and sedative effects (Suvarchala Kiranmai. A et al., 2013) in the treatment of Epilepsy and febrile convulsions.

Phytochemical screening of Solanum pubescens dried fruit material reveals the presence of carbohydrates, saponins, oils& fats, alkaloids and flavonoids (Haseebur Rahman et al., 2012). Methanolic

leaves extract indicates the potency of alkaloid, glycoside, saponins, phenolic compounds, tanins, flavonoids (Ayyadurai et al., 2017). n-hexane extract of Solanum pubescens leaves shows the presence of Myricetin methyl ethers (Krishna kumari G.N et al., 1985). Phytochemical profiling of successive extracts of Fruit and stem bark revealed oils& fats, alkaloids, flavonoids, carbohydrates, saponins, coumarins and phenolics are present in different extracts. (Haseebur Rahman et al., 2014). The present study was aimed for qualitative phytochemical analysis of ethyl acetate extract of leaves and stem bark of Solanum pubescens. This study helps us to know and evaluate the antimicrobial and anti carcinogenic activity presumably play active role in treating diseases.

2. Materials and Methods

2.1 Morphological Characters:

Solanum pubescens is an annual erect shrub, grows up to 1.5 m tall, younger parts pubescent, older parts glabrous. Leaves simple with entire margin and pubescent with yellow hairs; petiole up to 5 cm long, flowers axillary, in loose racemose cymes, sepals 5, lanceolate, corolla purple, up to 2.5 cm across, lobes 5, stamens 5, filaments 2 mm long, stigma capitate, berry globose, red, seeds scaly.

2.2 Plant material collection and identification:

Solanum pubescens fresh plant parts were collected from the hilly areas of Chitradurga District, Karnataka State, India located at 13.95°N 76.62°E. The plant was confirmed by referring Phytographia(Gamble 1883) authenticated by Prof. K.C. Chandini, Dept. of Botany, I. D. S.G. Government College, Chikkamagaluru, Karnataka- State. The plant herbarium is deposited at Department of Botany, I. D. S.G. Government College, Chikkamagaluru, Karnataka.

2.3 Preparation of powder from plant parts:

The healthy plant leaves and stems were collected and thoroughly washed in distilled water and blotted. The leaves were shade dried for one week and stems for fifteen days. The dried leaves and stem bark were pulverized in a mixer, sieved with a fine mesh and used for study.

2.4 Soxhlet extraction:

The leaves and stem bark powder were subjected for consecutive extraction in a Soxhlet extractor by using Ethyl acetate solvent. The extracts were concentrated to dryness under reduced pressure in desiccators to yield dried extracts.

2.5 Qualitative Phytochemical analysis:

Ethyl acetate extracts of leaves and stem bark were used to analyse the different phytochemical constituents. The following methods employed to analyse the presence of phytochemicals.

Test for Carbohydrates - Molisch's Test (Sofowara. 1993): The extracts were treated with two drops of alcoholic α-naphthol solution in a test tube and two ml Conc. H_2SO_4 was added carefully along the sides of the test tube. Formation of dull violet / red ring at the interphase indicates the presence of carbohydrates.

Test for Acids: To one ml of extract one ml of sodium bicarbonate solution was added. Formation of effervescence indicates the presence of acids.

Test for Betacyanins (Harborne, 1973): For two ml of plant extract, one ml of 2N NaOH was added and heated for 5 minutes at 100° C. Formation of yellow colour indicates the presence of betacyanin.

Test for Quinones (Evans, 1996): To one ml of extract, one ml of Conc. H_2SO_4 was added. Formation of red colour indicates the presence of quinones.

Test for Coumarins: A few drops of ammonia were added on a filter paper, for this, a drop of the extract was added and the paper was observed for fluorescence.

Test for Alkaloids - Mayer's Test (Fvans, 1997): The extracts were treated with Mayer's reagent (1.36 g mercuric chloride and 5 gms of potassium iodide were dissolved in 100 ml distilled H_2O). The formation of a yellow cream precipitate indicates the presence of alkaloids.

Test for Aminoacids - Ninhydrin Test (Yasuma and Ichikawa, 1953): To the extract 0.25% Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acids.

Test for Proteins (Brain and Turner, 2006) Biuret Test: Extracts were treated with one ml of 10% NaOH solution and heated. To this a drop of 0.7% $CuSO_4$ solution was added Formation of purplish violet colour indicates the presence of proteins.

Test for reducing sugars - Benedict's test (Tiwari, 2011; Sofowara, 1993): The extracts were treated with Benedict's reagent and heated on a water bath Formation of an orange red precipitate indicates the presence of reducing sugars.

Test for Fixed oils and Fats - Stain Test: Small quantities of the extracts were pressed between two filter papers. Formation of an oily stain on the filter paper indicates the presence of fixed oils and fits.

Test for Flavanoids- Ferric Chloride Test (Raman, 2006): The extract was treated with a few drops of $FeCl_3$ solution; Formation of a blackish red colour indicates the presence of flavanoids.

Test for Gums and Mucilages (Whistler and Bemiller, 1993): About 5 ml of the extract was slowly added to 5 ml of absolute alcohol under constant stirring. The appearance of precipitation indicates the presence of gums and mucilages.

Test for Steroids (Kokate, 1994): Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with two ml H_2SO_4. Change in colour from violet to blue or green indicates the presence of steroids.

Test for Tannins (Trease and Evans, 1989): To one ml of the solvent extract, few drops of 1% $FeCl_3$ solution were added. The appearance of a blue, black, green or blue green precipitate indicated the presence of tannins.

Test for Resins - Acetone- H_2O Test: The extracts were treated with acetone. A small amount of water was then added and shaken; appearance of turbidity indicates the presence of resins.

Test for Phlobatannins (Harborne, 1973): About two ml of aqueous extract was added to two ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins.

Test for Terpenoids - Salkowski Test (Evans, 1997): To one ml of the solvent extract, two ml of chloroform was added. Then 3 ml of conc.H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

Test for Phenols - Ferric Chloride Test (Mace, 1963): To one ml of solvent extracts, 3 ml of distilled H_2O was added. To this, a few drops of neutral 5% $FeCl_3$ solution was added. Formation of a dark green colour indicated the presence of phenolics.

Test for Saponins - Foam Test (Kumar, 2009): About two ml of distilled H_2O and one ml of solvent extract were mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins.

Test for Cardiac glycosides - Keller-Killani Test (Sofowara, l984): The extract was dissolved in glacial acetic acid containing traces of $FeCl_3$. The tube was then held at an angle of 45^0 and one ml of Conc.H_2SO_4 was added along the sides of the tube. Formation of a purple ring at the interface indicates the presence of cardiac glycosides.

Test for anthroquinones - Borntrager's Test (Sofowara, 1993: Harborne, 1998): Small portion of the extract was shaken well with 10 ml benzene and filtered. 5 ml of 10% ammonia solution was added to the filtrate and stirred. The production of a pink red or violet colour indicates the presence of free anthroquinones.

Test for volatile oils (Trease and Evans, 1989): To one ml of the extract, one ml of 90 % ethanol was added, followed by the addition of a few drops of $FeCl_3$ solution. Formation of a green colour indicates the presence of volatile oils in the given sample.

Test for Emodols: The dry extract was added to 25% ammonia solution. The formation of a cherry-red solution indicated the presence of emodols.

Test for starch (Harborne, 1998): To one ml of the extract 10 ml of saturated NaCl solution was added. It was then heated. After heating, starch reagent was added. Formation of a blue-purplish pink colour is a positive test for the presence of starch.

Test for fatty Acids (Ayoola, 2008): 0.5 ml of extract was mixed with 5 ml of ether. This mixture was allowed to evaporate on the filter paper and then the filter paper was dried. The appearance of transparency areas on filter paper indicates the presence of fatty acids.

3. Results and Discussion

The present study on qualitative phytochemical analysis of leaves and stem bark of Solanum pubescens revealed the presence of medicinally important bioactive compounds. The phytochemical compounds in Solanum pubescens plant was evaluated in leaf and stem bark using Ethyl acetate solvent and results are shown in table - 1. Results indicated presents or absences in compounds of leaf extracts showed was present in high intensity followed by phenols, carbohydrates, betacyanins, proteins,fixed oils &fats, Flavanoids, tannins, resins and saponins. While the stem bark extract showed was present in intensity followed by resins, carbohydrates, betacyanins, quinones and proteins. Methanolic leaf extract showed the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, flavonoids (Ayyadurai et al., 2017) the ethyl acetate leaf extract showed the presence of carbohydrates, betacyanins, proteins and resins. Stem bark ethyl acetate extract showed the presence of alkaloids, flavonoids, tannins and phenols(Haseebur Rahman et al.,

2014) Present study showed the presence of carbohydrate, betacyanin, quinones, proteins and resins. Furthermore studies needed in the Solanum pubescens plant to isolate and characterize the active compounds for standardization of herbal drugs are a matter of great concern.

Table No.1 Phytochemical Analysis of leaves and stem bark of Solanum pubescens

Sl. No	Tests/Chemical constituent	Ethyl acetate extract	
		Leaves extract	Stem bark extracts
1.	Carbohydrates	**+ ve**	**+ ve**
2.	Acids	**- ve**	**- ve**
3.	Betacyanins	**- ve**	**+ ve**
4.	Quinones	**- ve**	**+ ve**
5.	Coumarins	**- ve**	**- ve**
6.	Alkaloids	**- ve**	**- ve**
7.	Aminoacids	**- ve**	**- ve**
8.	Proteins	**+ ve**	**+ ve**
9.	Reducing sugar	**- ve**	**- ve**
10.	Fixed oils &fats	**+ ve**	**- ve**
11.	Flavanoids	**+ ve**	**- ve**
12.	Gums and mucilages	**- ve**	**- ve**
13.	Tannins	**+ ve**	**- ve**
14.	Resins	**+ ve**	**+ ve**
15.	Phlobatannins	**- ve**	**- ve**
16.	Terpenoids	**- ve**	**- ve**
17.	Phenols	**+ ve**	**- ve**
18.	Saponins	**+ ve**	**- ve**
19.	Cardiac glycosides	**- ve**	**- ve**
20.	Anthroquinones	**- ve**	**- ve**
21.	Volatile oils	**- ve**	**- ve**
22.	Emodols	**- ve**	**- ve**

(+ ve Present, -ve Absent)

4. Conclusion:

Qualitative phytochemical screening in ethyl acetate extracts of leaves and stem bark of Solanum pubescens has a potent source of phenols, resins, saponins, fixed oils & fats, proteins, carbohydrates, betacyanin and quinones. The results obtained in the study suggested that the identified phytochemical compounds may be the bioactive constituents of the plant Solanum pubescens is a valuable medicinal deserve.

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