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Research Article

PHYTOCHEMICAL ANALYSIS OF DIFFERENT SOLVENT EXTRACTS OF *SHUTERIA INVOLUCRATA* FROM KOLLI HILLS IN INDIA

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ABSTRACT

Phytochemicals are secondary metabolites produced by *Shuteriainvolucrata* has medicinal uses. This plant belongs to *fabiaceae* family respectively. The phytochemical analysis of whole plantparts used in different solvents such as aqueous, chloroform, ethanol, hexane, methanol, and petroleum ether extracts were investigated. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, carotenoids, coumerin, essential oil, flavonoids, glycosides, gum & mucilage, lipids & fat, phenols, proteins, quinines, resin, saponin, starch, steroids, tannin, terpenoids, vitamin C & ascorbic acid, and phytosterols in varying concentrations. The present study provides evidence that solvents extract of *Shuteriainvolucrata* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases including dental diseases.

INTRODUCTION

Natural products especially from plant sources, including herbal species have been investigated for their characteristics and health effects. Earliest records of the usage of plants are drugs are found in the Artharvaveda, mainly used for Ayurvedic medicine in India, the clay tablets in Mesopotamis (1700 BCE), and the Eber Papyrus in Egypt (1550 BCE).[1]

Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% of world populations still rely on plant based medicines. Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge. The medicinal value of plant lies in the bioactive phytochemical constituents of the plant, through phytochemical screening one could detect the various important compounds which could be as the base of modern drugs for curing various diseases.[2,3,4] Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological applications.[5] Medicinal plants are expensive gift from nature to human. For thousands of years, natural products have played a vital role in health care and prevention of life killing diseases. The phytochemicals are grouped into two main categories namely primary constituents and secondary constituents.[6,7] Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc.[8]

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease, Because of this property; many researchers have been performed to reveal the beneficial health effects of phytochemicals.[9,10] The present study revealed the qualitative phytochemistry of *Shuteriainvolucrata* medicinal plant *Fabiaceae* family used by the peoples of Kolli hills, Namakkal district, Tamilnadu, India.This research supports the local use of the *Shuteriainvolucrata* plant extracts to cure dental diseases.

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MATERIALS AND METHODS

Collection of plant Materials

The plant material was collected from Kolli hills, Namakkal district Tamilnadu. The identity was confirmed/authenticated by Dr.S. Susairaj, Department of Botany, St Joseph's college, Tiruchirappalli, Tamilnadu, India. The plant parts (stem, roots and leaves) were washed thoroughly in running water to remove soil and other foreign particles. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use. Chemicals used were of LR and AR grade.[10]

Preparation of plant extracts

The extraction was done with different solvents like chloroform, ethanol, hexane, methanol and petroleum ether. 20 g of powdered plant material and 150 mL of solvents were taken in soxhlet apparatus. The plant material and different solvents were heated to their boiling points for 8 ± 14 hours. The green colour extracts was obtained. Above procedure were repeated for other solvents. All the extracts were concentrated by distilling the solvents in a rotary vacuum evaporator. Water extraction was done by using soaking method. 25 g of plant material in 100ml of distilled water and kept aside for three days (soaked for 72 h). The filtrates were preserved in airtight containers and kept at 4-5 ^{0}C until further use.[11] The different extracts were filtered and the filtrates were used for qualitative phytochemical analysis.

Soxhletextraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds.[12, 13]

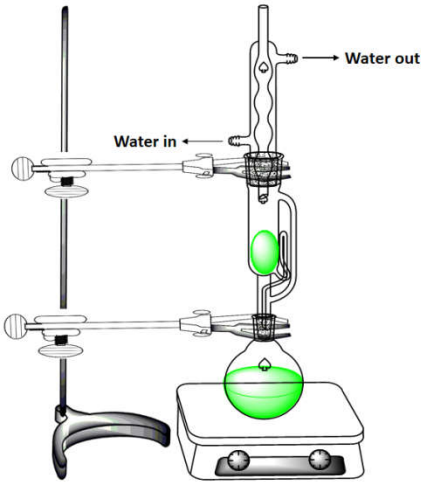

Fig 1 Schematic diagram of Soxhlet apparatus

Preliminary phytochemical analysis

The preliminary phytochemical analysis of the crude extracts of leaves, stem and roots of *Shuteriainvolucrata* were carried out according to the method described by Harborne, Trease and Evans.[14, 15, 16]A brief account of the different tests conducted was as follows:

Test for Alkaloids

Wagner's test: to the filtrates Wagner's reagent (iodine in potassium iodide) was added. A reddish brown precipitate indicated the presence of alkaloid.

Test for carbohydrates

Molisch test: To 2–3 ml of the aqueous extract, add two drops of alpha napthol solution in alcohol, shake and add conc. $H_2SO_4$from the sides of test tube. Violet ring is formed.[17]

Test for Carotenoids

Small amount of extract were treated with concentrated H_2SO_4 in ether or chloroform, gives an intense blue color indicates presence of carotenoids.

Test for Coumerin

Extract solution is concentrated to yield a residue. Dissolve residue in hot water. After cooling divide solution in two test tubes. To one test tube add 10% (w/v) Ammonium Hydroxide. Other test tube is used as control. Fluorescence color indicates the presence of coumerin.

Test for Essential oil

To 2 ml of the extract, 0.1 ml of 2 M sodium hydroxide was added, followed by a small quantity of 2 M hydrochloric acid and shaken well white precipitate indicated presence ofessential oil.

Test for Flavonoids

Zinc chloride test: extract is subjected to reaction with zinc metal and few drops of con. HCl brick red colour gives the presences of flavonoids.

Test for Glycosides

Keller-Killani test:1ml of glacial acetic acid containing traces of Ferric chloride and 1ml of concentrate sulphuric acid were added to the extract carefully. A reddish brown colour formed at the junction of two layers and upper layer turned Bluish green indicated the presences of glycosides.[18]

Test for Gum and mucilage

The extract was dissolved in 5 ml of distilled water and to this 25ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage's.

Test for Lipids & Fat

***Spot test*:** A small quantity of extract is pressed between two-filter papers. Oil stains on the filter paper indicates the presence of lipids and fat.

Test for Phenols

Ellagic Acid Test*:* The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$solution. The solution turned muddy or Niger brown precipitate occur

Test for proteins

Biuret test: Added 4% of NaOH and few drops of 1% $CuSO_4$solution to 3 ml of the extract. Formation of violet or pink colour indicates the presence of proteins.

Test for Quinines

To a small amount of extract, concentration of sulphuric acid is added. Appearance of red colour indicates presence of quinines.

Test for Resins

One ml of extract were treated with few drops of acetic anhydride solution followed by one ml of conc. H_2SO_4. Resins give colouration ranging from orange to yellow.

Test for Saponins

About 2 ml of 1% sodium bicarbonate was added to 1 ml of methanolic bark extract and shacked. Lather like formation persistent for some time is indicative of presence of Saponins.

Test for Starch

50% Iodine solution, Blue black spot was observed.

Test for Steroids

***Liebermann's test*:** some few mg. of residues in a test-tube, few ml of acetic anhydrides was added and heated. The contents of the test-tube were cooled. Few drops of concentrated sulphuric acid were added from the side of the test-tube's blue colour gave the evidence presence of steroids.[19]

Test for Tannin

Ferric chloride test:1gm of sample added with 100ml of distilledwater, boiled and cooled, and then filtered. 1% ferric chloride was added drop wise to the filtrate. Green black precipitate shows the presence of tannin.[20]

Test for Terpenoids

Salkowski test: To 0.5 g of each extract, 2 ml of chloroform was added, followed by a further addition of 3ml of concentrated H_2SO_4 to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.[21]

Test for vitamin C (Ascorbic acid)

Dilute 1ml of 2% w/v solution with 5 ml of water and added 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2ml dilute NaOH solution. Added 0.6ml of hydrochloric acid drop wise and stir, the yellow colour turns blue.

Test for phytosterols

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

RESULTS AND DISCUSSION

The phytochemical characteristics of *Shuteriainvolucrata* tested are summarized in the table1 solvents used in extracts. The results revealed the presence of medically active compounds in the medicinal plant studied. From the table, it could be seen that, alkaloids, carbohydrates, carotenoids, coumerin, essential oil, flavonoids, glycosides, gum & mucilage, lipids & fat, phenols, proteins, quinines, resin, saponin, starch, steroids, tannin, terpenoids, vitamin C & ascorbic acid, and phytosterols were present in all the extracts such as aqueous, chloroform, ethanol, hexane, methanol and petroleum ether from *Shuteriainvolucrata.* Among the six different polar and nonpolar solvents ethanol has maximum phytoconstituents present compare to other solvents, details are given in Fig. 2 and 3.The extraction of phytochemicals dependent on the dissolution of each compound in the plant material matrix and their diffusion into the external solvent, therefore the choice of the extraction solvent is one of the most important matters to consider solid-liquid extraction.[22]

Table 1 Phytochemical Screening of Different Solvent Extracts of *Shuteriainvolucrata*

S.No	Phytoconstituents	Aqueous	Chloroform	Ethanol	Hexane	Methanol	Pet.ether
1	Alkaloids	+	+	+	+	+	-
2	Carbohydrates	+	-	+	-	-	-
3	Carotenoids	+	-	-	-	-	-
4	Coumerin	-	+	+	+	+	+
5	Essential oil	+	-	-	-	-	-
6	Flavonoids	+	+	+	+	+	+
7	Glycosides	+	+	+	+	+	+
8	Gum & mucilage	+	-	-	-	-	-
9	Lipids & fat	+	-	+	-	-	-
10	Phenols	-	-	+	-	+	-
11	Proteins	+	+	+	+	+	-
12	Quinines	-	-	+	+	+	-
13	Resin	-	-	+	+	-	-
14	Saponin	+	+	+	+	+	+
15	Starch	-	+	+	+	-	-
16	Steroids	-	+	+	-	+	-
17	Tannin	+	-	+	+	+	-
18	Terpenoids	+	-	+	-	+	+
19	Vitamin C & Ascorbic acid	-	+	+	+	+	+
20	Phytosterols	+	+	-	+	+	+

Note: '+' indicates presence and '−' indicates absence

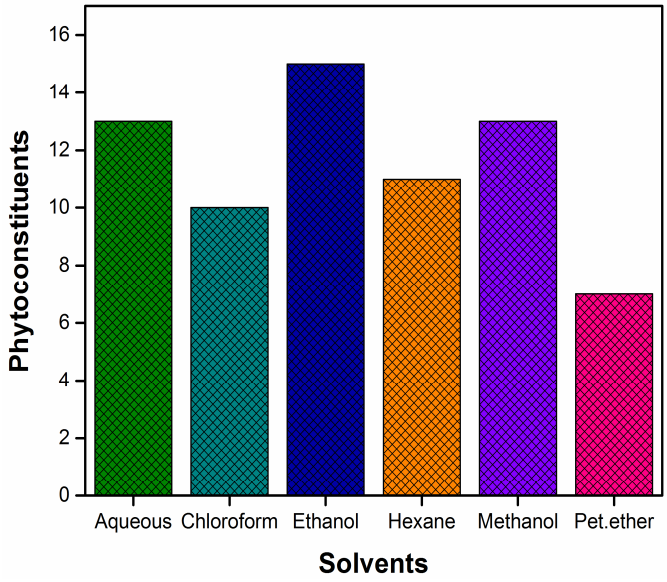

Fig 2 Graphical Representation of Secondary Metabolites Distribution in the Different Extracts of *Shuteriainvolucrata*

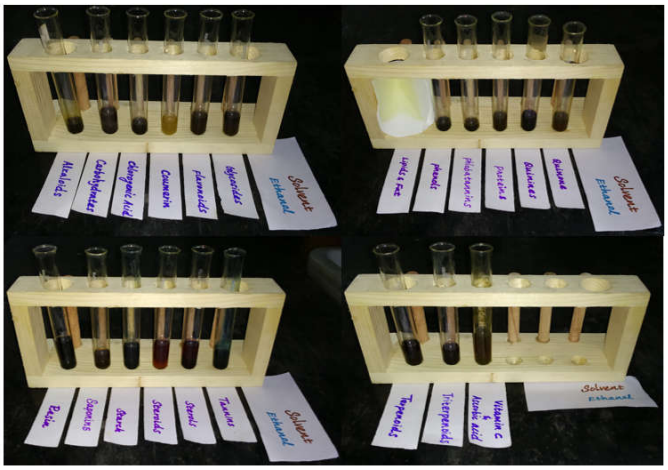

Fig 3 Confirmation testfor Phytoconstituents

CONCLUSION

The present work describes the phytochemical screening of different extracts of the plant *Shuteriainvolucrata.* The results revealed the presence of important secondary metabolites in all parts of the plant. It is noteworthy to mention that, the extract contains twenty major phytoconstituents which can be considered as active medicinal compounds. However, further study is necessary to isolate, characterize, quantify and to evaluate pharmacological action of the particular compound for drug development.

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