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SCREENING OF PHYTOCHEMICALS FROM DIFFERENT SOLVENT EXTRACTS IN *Borreria hispida* (Linn.)

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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. The present study to check the presence or absence of the phytochemical constituents. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, steroids, alkaloids, carbohydrate, cardiac glycoside, flavonoids, saponins, tannins, terpenoids and, antioxidants and microbial activity were found to be present in afore mentioned medicinal plants. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.

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INTRODUCTION

Medicinal plants play a key role in human health care. In recent years, there has been an increasing awareness about the importance of medicinal plants due to the presence of bioactive compounds which plays a dynamic role to discover the new therapeutic agents for drug development and to prevent various human diseases. The plant kingdom in all aspect of life has served as a precious starting material for drug development. Drugs from the plants are easily available, less expensive, and safe and rarely have side effects. Mankind experiences the trial and error method to know more about the medicinal properties of different plants.

Secondary metabolites from the plant possess many medicinal applications for drug delivery [1].

Herbal based drugs remain an important source because of the availability, relatively cheaper cost and no side effects when compared to modern medicine [2]. Nature has been a potential source of many therapeutic agents for thousands of years and an impressive number of modern drugs have been derived from plants. It is estimated that roughly 1500 plant species in Ayurveda, 1200 plant species in Siddha have been used for drug preparation. Though the Indian traditional systems of medicine are time-tested and practiced successfully from time immemorial, there is lack of standardization with regard to identification of crude drugs, methods of

preparation and quality of finished products. Pharmacognosy in a broad sense, embraces the knowledge of the history, distribution, cultivation, collection, selection, preparation, commerce, identification, evaluation, preservation and use of drugs and economic substances that affect the health of men and other animals. *Borreria hispida* K. Sch. (syn. *Spermacoce hispida* L.) popularly known as „Nattaichuri“ in Tamil and „Shaggy button weed“ in English, belongs to the family Rubiaceae. *Borreria hispida* is a procumbent herb; stem quadrangular, hirsute, hispid, with usually long internodes. Leaves sub sessile, 1- 3.5 cm long, oblong or elliptic, often rounded at the tip, scabrid, pubescent. Flower very small, 4-6 in a whorl within the stipular cup. The calyx-teeth are linear-lanceolate. The corolla is pale blue or white and it is 5 to 10mm in length. The fruit is hairy capsule about 5mm in length. The seeds are oblong; granulate, opaque, usually variable and 3mm or less in length. [3]



Figure; 1 *Borreria hispida*(Linn.)

Borreria hispida(Linn.) its belongs to the family Rubiaceae. It is known as *Nattaichuri* in Tamil. It is used in the indigenous systems of medicine. The other vernacular names are given below.

Bengali :	Madana - banta-Kadu
Gujarathi :	Madhurijadi
Hindi :	Guthri, Madanaghanti
Kannada :	Madanabudumaegiddah
Marathi :	Ghanti-chi-bhaji, gondi
Oriya :	Solagathi
Sanskirit :	Madanaghanta
Telugu :	Madanagrath

MATERIALS AND METHODS

Preparation of sample Extraction:

Sample collection

Ten medicinal plants Leaves of *Borreria hispida* (*rupisiya*L.) were collected locally from thanjavur in tamilnadu that is *Borreria hispida* was thoroughly washed with tap water. The plants were used for the purpose of their phytochemical

analysis. The plants collected were identified botanically in department of Botany A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu. Fresh and tender leaves of selected plants were used for phytochemical analysis.

Preparation of plant extract:

The leaves of the *Borreria hispida* plants were removed from the plant and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder and stored in polythene bags for use. About 1.5 kg of above dry powdered material was successively extracted with Ethyl acetate (40-60°C) by continuous hot percolation method in Soxhlet apparatus [4]. The extraction was continued for 72 hours. The Ethyl acetate extract was filtered and concentrated to dry mass by using vacuum distillation. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The dark green residue was obtained. (21.5gms) The marc left after Ethyl acetate extract was taken and subsequently extracted with Acetone for 72 hours. The Acetone extract was then filtered and concentrated to a dry mass. A dark green residue was obtained. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained (19gms) The extraction was continued for 72 hours and subsequently extracted with chloroform (15gms), methanol (10 gms) and hydro alcohol (7gms) extract was then filtered and concentrated to a dry mass. A dark green residue was obtained. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained above gms. The residue were taken and used for further phytochemical analysis. supernatant is decanted off and make up to 100 ml with distilled water.

IDENTIFICATION OF PHYTOCHEMICAL ACTIVE CONSTITUENTS

Preliminary phytochemical studies [11-12]

The extracts obtained (benzene, hexane and aqueous) was subjected to the following preliminary phytochemical studies.

Test for Alkaloids

Dragendorff's test: To 1 ml of the extract, 2 ml of distilled water was added; 2 M hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of

orange or red precipitates indicates the presence of alkaloids.

Hager's Test: To 1 ml of the extract was taken in test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirms the presence of alkaloids.

Wagners Test: 1 ml of extracts was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagners reagent were added. A yellow or brown precipitate indicates the presence of alkaloids.

Mayers Test: To a few drops of the mayers reagent, 1 ml of extract was added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Anthrone Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 2 ml of anthrone reagent solution was added. Formation of green or blue colour indicates the presence of reducing sugars.

Benedict's Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 5 ml of Benedict's solution was added and boiled for 5 min. Formation of brick red coloured precipitate indicates the presence of reducing sugars.

Fehling's Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 1ml mixture of equal parts of Fehlings solution A and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicates the presence of reducing sugar.

Molisch's Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To these 2 drops of freshly prepared 20% alcoholic solution of α -naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red-Violet ring appear, indicating the presence of carbohydrates which disappear on the addition of excess of alkali.

Test for flavonoids

Shinods test: 1 ml of extract was dissolved in 5 ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.

With Con. Sulphuric acid test: Yellow colour (anthocyanins), yellow to orange colour (flavones) and orange to crimson (flavonones).

Test for Glycosides

Molisch Test: 1ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 2-3 drops of Molisch reagent was added, mixed and 2ml of conc. sulphuric acid was added carefully through the side of the test tube. Reddish violet ring appears, indicating the presence of glycosides.

Test for proteins and free amino acids

Millions reagent-Appearance of red colour shows the presence of protein and free amino acid.

Ninhydrin reagent-Appearance of purple colour shows the presence of protein and free amino acids.

Biuret test: Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution was added. Appearance of pink or purple shows the presence of proteins and free amino acids.

Test for gums and mucilage

Precipitation with 95% alcohol: Small quantities of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test for anthraquinones

About five ml of the extract solution was hydrolysed with diluted Conc. H₂SO₄ extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Saponins

Foam test: In a test tube containing about 5 ml of extracts, a drop of sodium bicarbonates solution was added. The test tube was shaken vigorously and left for 3 min. Formation of honeycomb like froth indicates the presence of saponins.

Test for Sterols

Liebermann-Buchards test: 1 ml of extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green color indicates the presence of steroids.

Salkowski reaction: 1 ml of extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

Test for fixed oils

Spot test: Small quantities of various extracts were separately pressed between the two filter papers.

Appearance of oil stains on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for triterpenoids

About two ml of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H2SO4. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for phenolic compounds and tannins

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaf extracts of *Borreria hispida* collected from namely Thanjavur, Tamil Nadu has been shown in Tables 1 respectively. In the present study, phytochemical screening was performed with ethyl acetate, acetone, chloroform, methanol and hydro alcohol of the leaf extracts of *Borreria hispida*. Preliminary phytochemical analysis of leaf extracts of *Borreria hispida* collected from different places namely Thanjavur, Tirunelveli and Cuddalore has been shown in Tables 1-3, respectively. In the present study, phytochemical screening was performed with aqueous, ethanol, chloroform, acetone and petroleum ether of the leaf extracts of *Borreria hispida*. The phytochemical screening of five different extracts studied, showed that the ethanolic extract of leaf of *Borreria hispida* (Thanjavur) were rich in secondary metabolites such as tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, phenol, steroid, coumarins and alkaloids followed by other accessions.

Table 1: Qualitative analysis of phytochemicals in *Borreria hispida* plant roots

Phytochemicals	Ethanol	Aqueous	Chloroform	Petroleum ether	Acetone
Tannins	+	+	-	-	+
Saponins	+	+	-	-	-
Saponins	+	+	-	-	-
Quinones	+	+	-	-	+
Terpenoids	+	+	+	+	+
Steroids	+	+	-	-	+
Flavonoids	-	++	-	+	+
Phenol	-	+	++	+	++
Alkaloids	-	+	-	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	-	+	-	-	+
Coumarins	-	+	-	-	-
Antho cyanin	-	-	-	-	-

(+) = Presence; (-) Absence = Moderate concentration

In the present study, methanol extract of *S. hispida* contains alkaloids, tannins and steroids in maximum amount, while, the aqueous extract of *S. hispida* showed higher amount of saponins. The previous finding reported that saponins in plants are responsible for the tonic and stimulating activities [4]. Secondary metabolites like alkaloid contained in plants are used in medicine as anaesthetic agents [5]. Tannins are used as anti-insecticidal and tannic acid is used as astringent in burn case. Steroids are used as stimulant so due

to absence of steroids it has less possibility of stimulant effects [6]. The previous finding reported that methanolic leaf extract of *S. articularis* contains phytochemical compounds such as alkaloids, glycosides, steroids, flavonoids and tannins [7]. Ethanol has been found to be the most commonly used solvent for the extraction of tannins rather than other organic solvents [8]. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes [9]. Estimation of total phenol

content shows the sufficient amount of phenol present in the test samples of this study. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [10].

CONCLUSION

The many diseases in human being have been controlled mostly by medicinal plants.

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Borreria articularis (Family: Rubiaceae) is a medicinal plant which has many therapeutic effects. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, steroids, alkaloids, carbohydrate, cardiac glycoside, flavonoids, saponins, tannins, terpenoids and, antioxidants and microbial activity were found to be present in aforementioned medicinal plant.

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