PHYTOCHEMICAL SCREENING AND ANTI-OXIDANT ACTIVITY OF BORRERIA HISPIDA L. - AN ANTICANCER PLANT

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Abstract-Cancer is a neoplastic deadly disease that involves unregulated cell division and tissue invasiveness. Existing lines of cancer treatment include surgery, radiation, and chemotherapy. These modern lines of treatment produce serious side effects. Recent studies established that herbs and herbal medicine are free from serious side effects. This study was designed to determine the phytochemical components in the whole plant of Borreria hispida L. Methods: In the present investigation, the plant Borreria collected from places such as Thiruvannamalai, Thiruvallur, Vizhupuram, Pondicherry and Arani. The phytochemical screening of Borreria hispida with Ethanol, Aqueous, Petroleum ether, Acetone and Chloroform and Phytochemical screening was carried out to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. The Antioxidant property with various extracts were assessed by standard method by DPPH radical scavenging activity. Results: The Thiruvannamalai accession and the ethanolic extract of plant Borreria showed presence of strong positive phytochemicals for antioxidant property. Conclusion: The ethanolic extract of the plant is further subjected to TLC and GCMS analysis to investigate further phyto constituents for anticancer activity. Key words- Borreria hispida, Phytochemical screening, Antioxidant activity and Cancer.

I. INTRODUCTION

Plants constitute major source of drugs for prevention and spread of wide range of pathogenic carriers and also treating various diseases of human beings. Modern people increasingly prefer drugs of natural origin mostly from plant origin due to abundant accessibility and fewer side effects.

Whereas synthetic drugs and antibiotics often cause wide spread toxicity and harmful side effects to the end user other than targeted health condition pathogen carrier. Compounds isolated from plants are safer and have a lot of potential than the chemical drugs (1). Traditional herbal medicine is a rich source for modern, molecular target specific drug discovery (2). Plants have proven to be the most useful in curing diseases and provide an important source of pharmacy and medicine.

The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human (3). The major importance of these bioactive constitute of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Plants in all aspect of life have served as important material for drug development (4). Medicinal plants are the foundation of many important drugs of the modern world. Many of these local medicinal plants are used as spices and food items. Plants based drug is the major area of research. According to WHO calculations 80% of the world’s population presently uses medicinal herbs drug for their primary health care (5). Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used worldwide (6).

Synthetic drugs and antibiotics often cause wide spread toxicity and harmful side effects to the end user other than targeted health condition pathogen carrier. Compounds isolated from plants are safer and have a lot of potential than the chemical drugs (7). Traditional herbal medicine is a rich
source for modern, molecular target specific drug discovery (8). The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human (9). The major importance of these bioactive constitute of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Plants in all aspect of life have served as important material for drug development (10).

Medicinal Plants based drug is the major area of research. According to WHO calculations 80% of the world’s population presently uses medicinal herbs drug for their primary health care (11). Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used worldwide (12).

Natural products as chemotherapeutic agent is the recent trends adopted. As natural products causes less side effects it is preferred. It is used as chemoprevention to inhibit or reverses carcinogenesis and to suppress the tumour. Current chemotherapeutic drugs are not useful in all cases and have severe side effects on human health. So there is increased demand in identification of new plant based drugs. With the view to identify an herbal drug source Borreria hispida L. is selected and studied for the development of anti-cancer drug.

B. hispida L. has been extensively used in Siddha system of medicine for various conditions including decreasing the blood sugar levels. In traditional medicine, Spermacoce hispida is used to heal stomach ailments and also used as tonic and antidandruff. The flowers have been applied to boils, eruptions, swellings and also regarded as an emetic and as a remedy for coughs and malaria. The plant under study has been used since ages by folk because of its rich medicinal values. *Spermacoce hispida* Linn. Popularly known as “Nattaiccuri” in Tamil or “Shaggy button weed” in English, in Sanskrit it is known as “Madanghanti” and in Telugu it is called as “Madana Granthi” and belongs to family of Rubiaceae, Subfamily is Rubioideae, and the genus-Spermacoce. The whole plant is used for medicinal properties; it is widely distributed in the Western Ghats of Kerala and in Maruthamalai forest, which is an extension of Western Ghats in Tamil Nadu (7).

*B. hispida* (Linn.) K. Schum. (Syn.: *S. hispida* L.) is being used as an alternative therapy for diabetes (8). In India, decoction of the plant is used for headache (9) and the seeds as stimulant (10) and for the treatment of internal injuries of nerves and kidney(11).

*Borreria* is a procumbent, branched, hairy herb, 10 to 14 centimetres long. Branches are greenish or purplish, ascending, stout and 4-angled. Leaves are ovate, spatulate, or elliptic, 1 to 3.5 centimetres long, 0.8 to 1.7 centimetres wide, pointed or rounded at the tip. Flowers are 4 to 6, occurring in whorls in the axils of leaves. Calyx-teeth are linear-lanceolate. Corolla is white in colour, 5 to 10 millimetres in length.

**II. PLANT COLLECTION**

Fresh plants of the *Borreria* were collected from Thiruvannamalai, Thiruvarur, Vizhupuram, Pondicherry and Arani. The plant was identified using floras and authenticated by Retired Professor, Anatomist Dr Jayaraman, Thambaram.

**Phytochemical Screening of whole plant Extracts of Borreria hispida :**

The phytochemical screening of Borreria hispida extracts were assessed by standard method as described by Harbourne (7). Phytochemical screening was carried out on the whole plant extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds.

Phytochemical analysis

1. **Test for flavonoids**

3 ml of the leaf extract was mixed with 4 ml of 1N NaOH in a test tube. Formation of dark yellow colour was observed which indicated the presence of flavonoids.
2. Test for saponins

Plant extract (0.5 g) was dissolved in 2 ml of boiling water in a test tube, allowed to cool and shaken well to mix thoroughly. The appearance of foam indicates the presence of saponins.

3. Test for alkaloids

Mayer’s reagent: Mercuric chloride (0.355 g) was dissolved in 60 ml of water and 5 g of potassium iodide was dissolved in 20 ml water. Two solutions were mixed and volume was made up to 1000 ml with distilled water.

One ml of the Plant extract (0.5 g) was mixed with about 1 ml of 1% HCl, warmed and filtered. 2 ml of filtrate were treated separately with Mayer’s reagent. Turbidity or precipitation, green colour was observed to indicate the presence of alkaloids.

4. Test for tannins

About 0.5 g of plant extract was boiled in 20 ml of distilled water in a test tube and then filtered. 1 ml of the leaf extract added with 5 % FeCl3 (1 ml) was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins.

5. Test for Coumarins

One ml of the plant extract (0.5 g) was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow florescence to indicate the presence of coumarins.

6. Test for Anthocyanin and Betacyanin

To 2 ml of the leaf extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100 C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of Betacyanin.

7. Test for Glycosides

Two ml of the leaf extract added with three ml of chloroform and 1 ml of the 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

8. Test for cardiac glycosides

One ml of the leaf extract added with 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

9. Test for Terpenoids

To 1 ml of the leaf extract added with 2 ml of chloroform was added and added 1.5 ml concentrated Sulphuric acid was added carefully. Formation of reddish brown colour at the interface indicates the presence of Terpenoids.

10. Test for Phenols

To 1 ml of the leaf extract, 2 ml of distilled water followed by 0.5 ml of sodium carbonate and Folin Ciocalteau’s reagent (0.5 ml) added to the extract. Formation of blue / green colour indicates the presence of phenols.

11. Test for Quinones

To 1 ml of the leaf extract added 1 ml of concentrated sulphuric acid. Formation of red colour indicates the presence of quinones.

12. Test for Steroids

To 1 ml of the leaf extract, 2 ml of chloroform and 1 ml of Sulphuric acid (H2SO4) were added. Formation of reddish brown ring at interface indicates the presence of steroids.

Extraction of plant material

Plant materials were shade dried and coarsely powdered. Measured amount of air-dried powdered plant materials was taken in an aspirator bottle and was soaked Ethanol, Aqueous, Petroleum ether, Acetone and Chloroform for 2 days at room temperature. On 3rd day the extract was distilled off and residue subjected to further analysis. Respective chemicals were added subsequently in the order of increasing polarity and extracts were obtained after distilling off the...
solvents. Then the extracts obtained were filtered and evaporated using a vacuum rotary evaporator at 40°C.

### Table 1. Phytochemical test results:

<table>
<thead>
<tr>
<th>Phytochemicals Tested</th>
<th>Ethanol Extracts of <em>Borreria hispida</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thiruvalur</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Cardiacglycosides</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>-</td>
</tr>
<tr>
<td>Beta cyanin</td>
<td>+</td>
</tr>
</tbody>
</table>

++ = strong positive  
+  = positive  
= negative

### Qualitative analysis of antioxidant activity of *Borreria hispida*:

The antioxidant activity of whole plant extracts of *Borreria hispida* was determined by following the method as described by George et al., (1996); 50µl of extract of *Borreria hispida* was taken in the microtiter plate. 100µl of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

### Quantitative analysis of free radical scavenging activity of *Borreria hispida*:

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of leaf extracts were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee et al., 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

\[
\text{% DPPH radical-scavenging} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test Sample}}{\text{Absorbance of control}} \right) \times 100
\]

### Table 2 Qualitative analysis of antioxidant activity of *Borreria hispida*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extracts</th>
<th><em>B. hispida</em> (Thiruvannamalai)</th>
<th><em>B. hispida</em> (Thiruvallur)</th>
<th><em>B. hispida</em> (Vizhupuram)</th>
<th><em>B. hispida</em> (Pondicheery)</th>
<th><em>B. hispida</em> (Arni)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Ethanol</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>S2</td>
<td>Aqueous</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S3</td>
<td>Petroleum ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S4</td>
<td>Acetone</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S5</td>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
+++ = very strong positive  
++ = strong positive  
+ = positive  
- = Negative

Fig.2 Qualitative analysis of antioxidant activity of Borreria hispida - Ethonolic extracts of the whole plant taken in micrititer plate.

Fig.2 Quantitative analysis of antioxidant activity of Borreria hispida

A1: Control; A2: Standard (BHT), A3: Ethanol; A4: Aqueous; A5: petroleum ether; A6: Acetone; A7: Chloroform – Accession I (Thiruvannamalai)

B1: Control; B2: Standard (BHT), B3: Ethanol, B4: Aqueous; B5: petroleum ether; B6: Acetone; B7: Chloroform – Accession II (Thiruvallur)

C1: Control; C2: Standard (BHT), C3: Ethanol, C4: Aqueous; C5: petroleum ether; C6: Acetone; C7: Chloroform – Accession III (Vizhupuram)

D1: Control; D2: Standard (BHT), D3: Ethanol, D4: Aqueous; D5: petroleum ether; D6: Acetone; D7: Chloroform – Accession IV (Pondicherry)

E1: Control; E2: Standard (BHT), E3: Ethanol; E4: Aqueous; E5: petroleum ether; E6: Acetone; E7: Chloroform – Accession V (Arni)


Table 3 Quantitative analysis of antioxidant activity of Borreria hispida collected from various places

<table>
<thead>
<tr>
<th>Place where plants collected</th>
<th>BHT</th>
<th>Ethanol</th>
<th>Aqueous</th>
<th>Petroleum ether</th>
<th>Acetone</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiruvanamalai</td>
<td>97.20%</td>
<td>84.40%</td>
<td>57.10%</td>
<td>22.85%</td>
<td>38.50%</td>
<td>21.40%</td>
</tr>
</tbody>
</table>

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Thiruvallur  97.20%  62.80%  47.40%  11.40%  22.80%  11.40%
Vizhupuram  97.20%  67.80%  32.90%  20%  27.80%  12.40%
Pondicherry  97.20%  48.60%  34.30%  12.90%  22.80%  14.30%
Arni  97.20%  46.80%  34.30%  18.10%  28.60%  20%

III. RESULTS AND DISCUSSION

Among the fresh plants collected from Thiruvannamalai, Thiruvallur, Vizhupuram, Pondicherry and Arani, Thiruvanamalai accession showed best result and possess more phytocompounds than the other accessions. Ethanolic extract of the whole plant of Borreria hispida showed more antioxidant activity than Aqueous, Petroleum ether, Acetone and chloroform extract. Further ethanolic extract of the plant Borreria hispida L has to be subjected to TLC and HPTLC for identification of potent anticancer active substance.

REFERENCES