ABSTRACT

The Phytochemical activity of the methanol extract of whole plants of \textit{Borreria articularis} (family: Rubiaceae) was studied to fix the parameters for pharmacognostical standards. These created an interest to test the possible phytochemical activity of the plant. In the screening process of \textit{Borreria articularis} indicate the presence of carbohydrates, gums, steroids and tannins. This phytochemical study was performed by using standard procedure. The methanolic extract of whole plant of \textit{Borreria articularis} on carbon tetrachloride showed Hepatotoxicity in Albino rats. Further, detailed studies are needed to know whether in-vivo administration of the extracts is beneficial for patients.

Keywords: \textit{Borreria articularis}, Methanolic extract, Pharmacognostic Studies.

INTRODUCTION

Plants are vital component of the world’s biodiversity and essential natural resources for human well-being (Gadgil, 1996). Besides sustenance, the plants have been used as therapeutic aid for alleviating human ailments from very ancient times (Sullivan and Shealy, 1997). Such plants commonly referred as medicinal plants, have been one of the valuable tools in the traditional system of medicine and are also known to provide ingredients for formulation of new medicines in pharmaceutical industry. In fact, WHO has listed over 21,000 plant species to be of medicinal use around the world. More than 60% of the world’s human population relies on plant medicine for primary health care needs (Singh, 2002). Plants are critical to other life on this planet because they form the basis of all food webs. Most plants are autotrophic, creating their own food using water, carbon dioxide and light through a process called photosynthesis. Some of the earliest fossils found have been aged at 3.8 billion years. This fossils deposits show evidence of photosynthesis, so plants or the plant like ancestors of plants, have lived on this planet longer that most other groups of organisms. At one time, anything that was green and that wasn’t animal was considered to be a plant. Now, what were once considered “Plants” are divided into several kingdoms: Protista, Fungi and Plantae. Most aquatic plants occur in the kingdoms Plantae and Protista. Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way. “A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor of synthesis of useful drugs”.(Sofowora, 1982). Unfortunately, this definition of the WHO group includes only the medicinal plants whose therapeutic properties and chemical constituents have been established scientifically. But it does not take into consideration the vast majority of the medicinal plants, which have not yet been subjected to through scientific studies. These medicinal plants have been used in traditional medicine for hundreds of years with reputation as efficacious remedies although there may not be sufficient scientific data to substantiate their efficacy. Selection of the medicinal plants by early man, without any prior knowledge about them, was largely based on intuition, guesswork or trial and error. Curiosity and search for food had contributed considerably to his knowledge about the plants and their virtues. Superficial resemblance between a specific plant part and the affected organ or some symptoms of the ailment had also guided ancient man in his selection of medicinal plants.
The world health organization (WHO) has defined traditional medicine as the some total of all knowledge and practice, whether applicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance, relying exclusively on practical experience and observations handed down from generation, verbally in writing (WHO, 1976). However, the forms and practice of traditional medicine vary from the highly organized and long established Chinese and Ayurvedic systems to the largely herbalist and spiritualist type common in rural areas of Asia and Africa. As evident from historical records. Traditional medicine has been practiced in its various forms all over the world since time immemorial and even today about 80 percent of the rural population of most developing countries on traditional medicine for maintaining health and well-being. Approximately 80010 of the world’s population exclusively use plants for various healing purposes. In the industrially developed countries almost 35% of drugs contain active principles of natural origin and the consumption of medicinal plants is increasing (Irvine, 1995). The practice of traditional medicine in China is finely established. Approximately more than 5000 kinds of Chinese medicinal herbs are used medicinal plants. About 500 species are being used here in the purpose of traditional medication. From a survey from illness approximately 14% of them go to qualified allopathic doctors, 29% approach quack and 19% contact homeopaths. The survey represents an extensive use of medicinal plants most of which are served in a crude and substandard form by the different types of traditional practitioners. But the use of the medicinal plants in crude or substandard form in sometimes hazardous for the health. For which, we should standardize our traditional use of the medicinal plants. Thus to maintain a safer traditional practice, we should make more research with our medicinal plants to determine their chemical entities and biological activities properly. 

*Borreria articularis* has been claimed to be useful in treating fever, bladder stones, sores, wounds, headache and constipation. A decoction of the plant is useful in relieving headache and toothache. Seeds are used to treat dental problems, fevers, diarrhoea and dysentery. Leaves are used against haemorrhoids and conjunctivitis. The leaf extract can also be used for treating haemorrhoids and gallstones. Roots and seeds have been used in the treatment of dysentery, fever and head cold. 

Plant pacifies vitiated vata, pitta, arthritis, pain, muscle ache, edema, trauma, indigestion, colic, skin disease, menorrhagia and leucorrhea.

**Figure 1:** The whole plant of *Borreria articularis*.

**MATERIALS AND METHODS**

**Collection**
The plant selected for present work was *Borreria articularis* (Family: Rubiaceae) which was collected from the Hatajari, Chittagong University Area, Bangladesh in September, 2011 at day time. The plant is available from September to November and the whole plants were collected from fresh plants.

**Synonyms:** *Spermacoce exilis, Borreria hispida.*

**Family:** Rubiaceae

**Preparation of crude extract**

**Drying and Grinding**
The plant was washed and cut into small pieces and kept in the open air under sunlight for 15 days. Then the plants were grinded into a coarse powder with the help of a suitable grinder. The powder was stored in a cool, dark and dry place until analysis commenced.

**Cold extraction (Methanol extraction)**

In this project work we used the cold extraction process with the help of methanol. About 125 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 650 ml of methanol. The container with its contents were sealed and kept for a period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of clean, white cotton wool.

**Evaporation of Solvent**
The filtrates (methanol extract) obtained were evaporated under ceiling fan into a stainless steel tray until they had dried. They rendered a gummy concentrate of greenish black.

**% yield value of methanol extract from whole plant of Borreria articularis:**

<table>
<thead>
<tr>
<th>Powder taken for extraction</th>
<th>Weight of empty beaker</th>
<th>Weight of beaker with extract</th>
<th>Weight of extract obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>= 125 gm</td>
<td>= 42.70 gm</td>
<td>= 53.48 gm</td>
<td>= (53.48-42.70) gm</td>
</tr>
<tr>
<td>Weight of beaker with extract</td>
<td></td>
<td></td>
<td>= 10.78 gm</td>
</tr>
</tbody>
</table>
% of yield of methanol extract = (Weight of extract/Powder taken for extraction) x 100
= (10.78/125) x 100
= 8.624 gm

Phytochemical screening of *Borreria articulare*:

Reagents used for the different chemical group test:
The following reagents were used for the different chemical group tests (Trease *et al.*, 1983; Ali, 1998; Dev, 2002).

a) Fehling’s solution A (Copper sulphate solution)
34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

b) Fehling’s solution B (Alkaline tartrate solution)
173 gm of sodium potassium tartrate and 50 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use.

c) Mayer’s reagent
1.358 gm of HgCl$_2$ was dissolved in 60 ml of water was mixed with a solution containing 5 gm of potassium iodide & the volume was adjusted by adding sufficient amount of distilled water to make it 100 ml.

d) Benedict’s Reagent
With the aid of heat, 173 gm sodium citrate and 100 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 800 ml with water. After filtration the solution was diluted to 850 ml. 17.3 gm of CuSO$_4$·5H$_2$O was dissolved in 100 ml distilled water. Then the two solutions was mixed with constant stirring & made upto1000 ml.

e) Molish Reagent (α–naphtha solution)
15 gm of pure α-naphtha was dissolved in 100 ml of ethanol or chloroform.

f) Salkowski reagent: Chloroform & a few drops of concentrated sulphuric acid.

f) Libermann-burchared reagent: Chloroform & few drops of concentrated sulphuric acid & 2-3 drops of acetic anhydride.

h) Ferric chloride (5%): 5 gm ferric chloride in 100 ml distilled water.

i) Potassium dichromate (10%): 10 gm Potassium dichromate in 100 ml distilled water.

j) Dragendorff’s reagent: 8 gm bismuth nitrate is dissolved in 20 ml of concentrate nitric acid & 27.2 gm of potassium iodide in 50 ml of distilled water. The two solutions are mixed & allowed to stand. When potassium nitrate crystallizes out, the supernatant is decanted off and make up to 100 ml with distilled water.

k) Wagner’s reagent: (Iodo-potasssium iodide solution) 2gm of iodine & 6 gm of potassium iodide in 100 ml of water.

l) Hager’s reagent: (picric acid solution) 1 gm of picric acid in 100 ml of water.

Tests procedure for identifying different chemical groups
The following tests were performed for identifying different chemical groups (Trease *et al.*, 1983; Ghani, 1998; Dev, 2002).

Tests for reducing sugar
(a) Benedict’s test
0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict’s solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

(b) Fehling’s Test (Standard Test)
2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling’s solutions A and B and was boiled for few minutes.

Tests for carbohydrates & gums
Molish test:
5 ml sample was taken and then a few drops of molish reagent were added. Then the tube was inclined and 1 ml of sulphuric acid was added gradually at the bottom of the test tube through one side.

Tests for alkaloids
(a) Mayer’s test
2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer’s reagent was added.

(b) Dragendorff’s test:
2 ml sample and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendorff’s reagent was added.

(c) Wagner’s test:
2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of iodine solution was added.

(d) Hager’s test: 2 ml sample and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of picric acid was added.

Tests for tannins
(a) Ferric Chloride Test
5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added.
b) Potassium dichromate test
5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added.

Test for steroids
(a) Salkowski reaction:
A few mg of sample was dissolved in chloroform and a few drops of concentrated sulphuric acid are added to the solution.
(b) Libermann-burchared reaction:
A few mg of sample was dissolved in chloroform and a few drops of concentrated sulphuric acid are added to the solution followed by the addition of 2-3 drops of acetic anhydride.

Test for flavonoids
A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material.

Test for Saponins
1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.

RESULTS AND DISCUSSION
Phytochemical study showed that carbohydrate, gums, steroids and tannins were present and reducing sugar, glycosides, saponins and flavonoid are absent and alkaloids may present or absent in the extract of Borreria articularis because it found present in only one test of alkaloids out of four. Different chemical group tests of Borreria articularis and their results are given at (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Phytochemical tests of Borreria articularis</th>
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<tbody>
<tr>
<td>Group test</td>
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<tr>
<td>Carbohydrate &amp; gums</td>
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<tr>
<td>Reducing sugar</td>
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<tr>
<td>Alkaloids</td>
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<td>Glycosides</td>
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<td>Tannins</td>
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<tr>
<td>Flavonoids</td>
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<td>Saponins</td>
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</tbody>
</table>

DISCUSSION
Alkaloids have different pharmacological activity such as they are used as analgesic, stimulant, some are used to rise blood pressure and some are used to fall blood pressure. Due to presence of alkaloid in only one test of alkaloid is possible to have any one of the pharmacological activity. So further experiment should be needed to examine pharmacological activity. Tannins are used as anti-insecticidal and tannin 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 effect. All these uses of different type of chemicals in the extract of Borreria articularis indicate that the necessity of further examination.

CONCLUSION
Borreria articularis (Family: Rubiaceae) is a medicinal plant which has many therapeutic effects. The whole plant was collected, washed, dried, crushed and then soaked with methanol. Later methanol extraction was found by fractional distillation and evaporation. The whole extract of the plant Borreria articularis has been tested for the identification of the chemical group present in that plant. There found that Carbohydrates, gums, steroids and tannins were present. All the tests had been done in laboratory base chemical and Borreria articularis has a great aspects of research. So, further research on it should need.

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