

## Phytochemical analysis of some medicinal plants

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### Abstract

North-eastern India has been known for its rich biological diversity. For this study, seven medicinal plants such as *Bryophyllum pinnatum*, *Ipomea aquatica*, *Oldenlandia corymbosa*, *Ricinus communis*, *Terminalia bellerica*, *Tinospora cordifolia*, and *Xanthium strumarium*, were selected. The aim of the present study was to investigate the presence of phytochemicals and to determine the total phenolic and flavonoid contents of the selected medicinal plants. Soxhlet apparatus was used for the organic solvent extraction. Solvents used were water, methanol, ethanol, and acetone. Total phenolic contents of the aqueous extracts of the plants were determined by the Folin-Ciocalteus reagent method whereas total flavonoid contents of the aqueous extracts were determined by the Aluminium Chloride method. Proteins, carbohydrates, phenols, tannins, flavonoids, saponins, were detected in all of the plants tested. Total phenolic contents obtained were 18.4mg/gm, 18.8mg/gm, 11.6mg/gm, 29.2mg/gm, 29.6mg/gm, 40.8mg/gm, 12.8mg/gm, 71.6mg/gm of the extract and total flavonoid contents obtained were 8.4mg/gm, 37.6mg/gm, 4.4mg/gm, 6mg/gm, 42.8mg/gm, 18mg/gm, 6mg/gm, 28.8mg/gm of the extract for the plants *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves) respectively. Our findings provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases.

**Keywords:** *Bryophyllum pinnatum*, *Ipomea aquatica*, phytochemicals, phenols, tannins, flavonoids, etc.

### INTRODUCTION

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs [1], antimicrobial drugs [2], antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [3].

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4,5]. These compounds are synthesized by primary or rather secondary metabolism of living

organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [6]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [7].

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [8]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [9,10,11].

In the present work, qualitative and quantitative phytochemical analysis were carried out in seven plants, *Bryophyllum pinnatum*, *Ipomea aquatica*, *Oldenlandia corymbosa*, *Ricinus communis*, *Terminalia bellerica*, *Tinospora cordifolia*, and *Xanthium strumarium*, of Northeastern region of India.

### MATERIALS AND METHODS

#### Collection of plant materials

Fresh parts of seven medicinal plants, *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves/Stem), and *Xanthium strumarium* (Leaves) were collected from different regions of Sonitpur and Dibrugarh districts of Assam. The plant materials were taxonomically identified and authenticated by The Department Of Life Science, Dibrugarh University, Dibrugarh, Assam. The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were

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ground well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

### Preparation of plant extracts

#### Hot water extraction

5gm of dried finely powdered plant material was taken in a beaker and 200ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 30°-40°C for 20 minutes. Then the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis. The water extract was kept in refrigerator when not in use.

#### Solvent extraction

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were methanol, ethanol, and acetone. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis.

### Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods [12,13,14].

#### Test for proteins

##### Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

#### Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

#### Test for carbohydrates

##### Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

##### Benedict's test

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

##### Molisch's test

Crude extract was mixed with 2ml of Molisch's reagent and

the mixture was shaken properly. After that, 2ml of concentrated H<sub>2</sub>SO<sub>4</sub> was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

#### Iodine test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

#### Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of FeCl<sub>3</sub>. A blue-green or black coloration indicated the presence of phenols and tannins.

#### Test for flavonoids

##### Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

#### Alkaline reagent test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

#### Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

#### Test for glycosides

##### Liebermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H<sub>2</sub>SO<sub>4</sub> was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

##### Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

##### Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl<sub>3</sub>. The mixture was then poured into another test tube containing 2ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interphase indicated the presence of cardiac glycosides.

### Test for steroid

Crude extract was mixed with 2ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H<sub>2</sub>SO<sub>4</sub> and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

### Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

### Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

### Quantitative phytochemical analysis

#### Total phenolic content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na<sub>2</sub>CO<sub>3</sub> were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample

was measured at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) [15].

#### Total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) [15].

## RESULTS

The phytochemical characteristics of seven medicinal plants tested were summarized in the *table-1*. The results revealed the presence of medically active compounds in the seven plants studied. From the table, it could be seen that, proteins, carbohydrates, phenols and tannins, flavonoids and saponins were present in all the plants. Glycosides were absent only from the leaves of *Tinospora cordifolia*. Steroids were absent only in the leaves of *Xanthium strumarium* while terpenoids were absent in the leaves of *Ipomea aquatica*, roots of *Ricinus communis* and also in the leaves of *Xanthium strumarium*. Alkaloids were absent in the roots of *Ricinus communis*, leaves of *Terminalia bellerica* and also in the leaves of *Tinospora cordifolia*.

Table 1. Phytochemical constituents of seven medicinal plants studied.

Plants	Proteins	Carbohydrates	Phenols/Tannins	Flavonoids	Saponins	Glycosides	Steroids	Terpenoids	Alkaloids
<i>Bryophyllum pinnatum</i> (leaves)	+	+	+	+	+	+	+	+	+
<i>Ipomea aquatica</i> (leaves)	+	+	+	+	+	+	+	-	+
<i>Oldenlandia corymbosa</i> (leaves)	+	+	+	+	+	+	+	+	+
<i>Ricinus communis</i> (leaves)	+	+	+	+	+	+	+	-	-
<i>Terminalia bellerica</i> (leaves)	+	+	+	+	+	+	+	+	-
<i>Tinospora cordifolia</i> (leaves)	+	+	+	+	+	-	+	+	-
<i>Tinospora cordifolia</i> (stems)	+	+	+	+	+	+	+	+	+
<i>Xanthium strumarium</i> (leaves)	+	+	+	+	+	+	-	-	+

Total phenolic contents obtained were 18.4mg/gm, 18.8mg/gm, 11.6mg/gm, 29.2mg/gm, 29.6mg/gm, 40.8mg/gm, 12.8mg/gm, 71.6mg/gm of the extract and total flavonoid contents obtained were 8.4mg/gm, 37.6mg/gm, 4.4mg/gm, 6mg/gm, 42.8mg/gm, 18mg/gm, 6mg/gm, 28.8mg/gm of the extract for the

plants *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves) respectively.

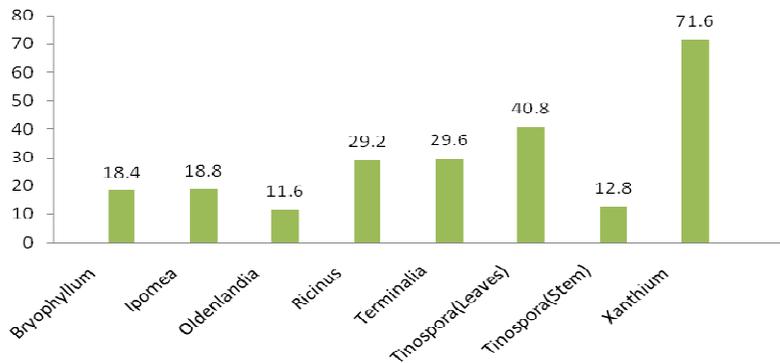


Fig 1. It shows the total phenolic contents of *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves) respectively.

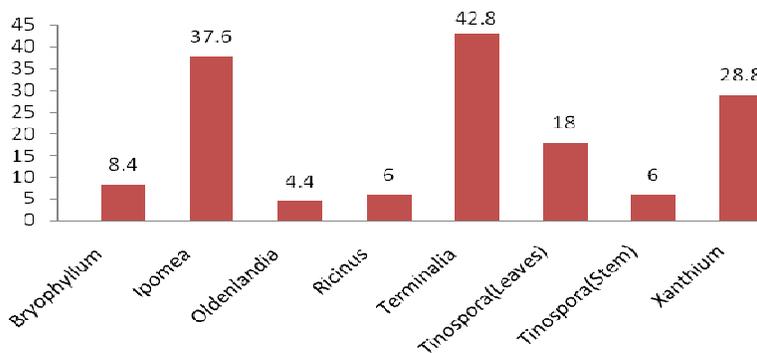


Fig.2. It shows the total flavonoid contents of *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves) respectively.

**DISCUSSION**

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [12]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids.

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [16]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [17]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [18,19]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. [20]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [21]. They also are effective antioxidant and show strong

anticancer activities [22,23,24].

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [25]. Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [26,24]. Steroids have been reported to have antibacterial properties [27] and they are very important compounds especially due to their relationship with compounds such as sex hormones [28]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [29]. Several workers have reported the analgesic [30,14], antispasmodic and antibacterial [31,32] properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports [33]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

**CONCLUSION**

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be

bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts.

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