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Qualitative and Quantitative Analysis of Phytochemical Studies on *Chara* sp.

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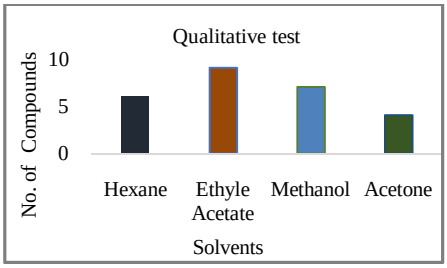
ABSTRACT

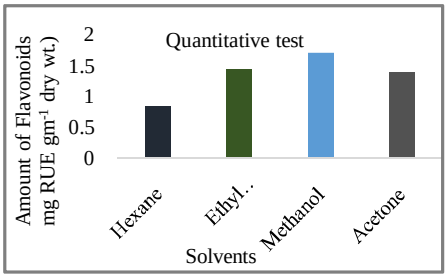
In the secondary metabolites i.e., phytochemicals have been extensively investigated as a source of the medicinal agents. The sample for the study of constitutes Chara braunii from the family Characeae. It was collected from the river Mahananda at Malda. Four completely different solvent extracts of genus Chara braunii were subjected to qualitative and quantitative phytochemical analysis of secondary metabolites each by preliminary phytochemical screening tests of 10 different chemical compounds alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides. The result of phytochemical screening of the Chara braunii showed in the presence of alkaloids, terpenoids, steroids, tannins, flavonoids, phenols, coumarins, quinones and glycosides besides the absence of saponins. Among the tested four different extracts showed that the presence of maximum number of compounds i.e. nine compounds in ethyl acetate, methanol extracts showed seven compounds, hexane extracts showed six compounds and acetone extracts only four compounds. However, the estimation of total phenolics, tannins and flavonoids were observed in different extracts of Chara braunii. The quantitative analyses of the various phytochemicals showed that the secondary metabolites such as phenolics (2.14 ± 0.15 mg GAE g^{-1} dry wt.) and flavonoids (1.72 ± 0.05 mg RUE g^{-1} dry wt.) in high amounts in methanol extract, where as tannins showed high amounts(2.12 ± 0.45 mg CAE g^{-1} dry wt.) in ethyl acetate extract.

Graphical Abstract

Collected Specimen
(Chara braunii)

Extract





Keywords: *Chara braunii*, Secondary metabolites, Phytochemical, Solvents.

INTRODUCTION

In the history of humanity, plants are contained various phytochemicals [1]. So, plants have always been present as a source of health. It is to refer herbs, herbal cosmetics dietary supplements or alternative medicine [2]. Approximately, 7350 species of algae present in fresh water ecosystem. Among these most of them are blue green algae and Green algae. The algae are autotrophs, a group of chlorophylls containing in thalloid plant, synthesize their food material. The mainly algae, fungi and lichens are grouped together under the division of thallophyta. The *Chara* is a genus of green algae belongs to Characeae. *Chara braunii* are most important because, it contains various phytochemicals. It is multicellular and superficially resembles of land plants because it shows stem like and leaf like structures. Which are found in the fresh water particularly in lime stone areas throughout the northern temperature zones and they grow submerged or attached to the muddy bottom. Therefore, it is preferredless oxygenated, hard water and which aren't found in and where mosquito larvae are present. They are covered with calcium carbonate crystals.

MATERIALS AND METHODS

Collection of Algae Sample: The algae *Chara braunii* were collected from Mahananda river near Mangal Bari Mahananda bridge, Malda Town, Malda district, West Bengal, India. There after species were identified in our laboratory and samples were shade-dried and coarsely powdered (Figure 1). The coarse powder was used for the extraction with various solvents, such as water, ethyl acetate, methanol, hexane and acetone.

Figure 1: Collected Algal Sample (*Chara braunii*) from The River Mahananda at Malda.

Preparation of Algae extract: Ten grams of dry powders of algae sample were taken in individual aspirator bottle; 100 mL of solvents (ethyl acetate, methanol, hexane and acetone) were used and the mixtures were shaken occasionally for 3 days. After, the extracts were filtered using Whatman's filter paper No. 1 on a Buchner funnel and the solvents were removed by vacuum distillation in a rotator evaporator at 40ºC. Finally, the water, ethyl acetate, methanol, hexane and acetone extracts of algae *Chara braunii* were used for the preliminary phytochemicals screening.

Qualitative Phytochemicals Analysis: Qualitative phytochemical analysis of ethyl acetate, methanol, hexane and acetone extracts of *Chara braunii* was carried out on the extract using standard procedure to identify the constituents as described by Sofowora (1993), Trease and Evans (1989a) and Harborne (1973) [3].

Test for Alkaloids (Mayer's reagent test): One millilitre of algae extract, 5 mL of 0.1N HCl was added, shaken well and filtered. 3 mL of filtrate, few drops of Mayer's reagent (Potassium Bismuth Iodide solution) were added. Formation of red creamy precipitate indicates the presence of alkaloids.

Test for Terpenoids (Salkowski's test): Five millilitre of algae extracts, 2 mL of chloroforms and 3 mL of conc. H_2SO_4 was added to form of a layer. Formation of a reddish-brown colour of the interface indicates the presence of terpenoids.

Test for Steroids: Two millilitres of algae extracts, 2 mL of acetic acid and 2 mL of conc. H_2SO_4 was added. The colour changed from violet to blue or green indicates the presence of steroids.

Test for Tannins (Ferric Chloride test): One of algae millilitre extract was boiled 20 mL of distilled water in a test and then filtered. A few drops of 1% ferric chloride solution were added and formation of green or blue-black indicates the presence of tannins.

Test for Saponins (Foam test): Two and half millilitre of algae extracts was boiled in 10 mL of distilled water in a water bath and filtered. The 10 mL of filtrate solution and 5 mL of distilled water was mixed together and thereafter shaken vigorously for a stable persistent forth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, formation of emulsion indicates the presence of saponins.

Test for Flavonoids (Alkaline reagent test): Three of algae millilitre extracts were added with few drops of 1% NaOH solution separately in a test tube. Formation of yellow colour and added with 0.1N H_2SO_4 solution. The yellow colour disappeared indicates the presence of flavonoids.

Test for Phenols (Ferric Chloride test): One of algae millilitre extract was dissolved in 10 mL of distilled water and added was few drops of ferric chloride solution, formation of dark green in colour, which indicates the presence of phenolic compounds.

Test for Coumarins: One millilitre of algae extracts and 1ml of 1N NaOH solution was added. The test tubes were kept in boiling water bath for 10 min and shaken well. Finally, the formation of yellow colour indicates the presence of coumarins.

Test for Quinones: One millilitre of extract and 1mL of conc. H_2SO_4 were added. The formation of red colour indicates the presence of quinones.

Test for Glycosides (Bontrager's test): Three millilitres of algae extracts and 0.1N H_2SO_4 was added, boiled for 5 min and filtered. After cooled down of filtrate, equal volume of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated ammonia solution was added to it. Formation of pink to red in colour in ammonical layer indicates the presence of glycoside compounds.

Quantitative Phytochemicals Analysis: The phytochemicals which are present in the ethyl acetate, methanol, hexane and acetone extracts of *Chara braunii* was determined and quantified by standard procedures [**4, 5**].

Determination of total phenols: Hundred millilitres of the extract of the sample was weighed accurately and dissolved in 100 mL of triple distilled water (TDW). The 1 mL of extract solution, 0.5 mL 2N of the Folin-Ciocalteu reagent and 1.5 mL 20% of Na_2CO_3 solution was added, ultimately the volume was made up to the 8 mL of with TDW followed by the vigorous shaking and finally allowed to stand for 2 h after which the absorbance was taken at 765 nm wave length. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid [**6**].

Determination of total tannins: Five hundred milligrams of the sample and 50 mL of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up with the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [7].

Determination of total flavonoids: The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at wave length 415 nm. The 100 µL of the sample extracts in methanol (10 mg mL^{-1}) was mixed with 100 µL of 20 % aluminium trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 mL. The absorption at the 415 nm was read after 40 min. Blank samples were prepared from 100 mL of sample extracts and a drop of acetic acid, and then diluted to 5mL with methanol. The absorption of standard ruin solution (0.5 mg mL^{-1}) in methanol was measured under the same conditions. All determinations were carried out in triplicates [8].

RESULTS AND DISCUSSION

Qualitative analysis of the phytochemical substances in screening algal extracts: Preliminary phytochemicals screening of 10 different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides) were tested in four different extracts (hexane, ethyl acetate, acetone and methanol).The bio active phytochemicals such as alkaloids, flavonoids etc. are very important for human beings [9]. In the present study of the phytochemical screening was performed with extracts of *Chara braunii*. Saponins did not show in any positive result for their presence of the four extracts tested as shown in table 1 and figure 2.

Table 1. Qualitative analyses of phytochemical substances in different extracts of *Chara braunii*

S. No.	Phytochemical parameters	Ethyl acetate	Methanol	Hexane	Acetone
1	Alkaloids	++	++	-	+
2	Terpenoids	++	-	++	+
3	Steroids	++	-	++	-
4	Tannins	+++	++	++	-
5	Saponins	-	-	-	-
6	Flavonoids	++	+++	++	++
7	Phenols	++	+++	+	-
8	Coumarins	++	++	-	-
9	Quinones	+	++	+	-
10	Glycosides	+	++	-	+

Legend: +++ (Much abundant), ++ (less abundant), + (minute), - (absent)

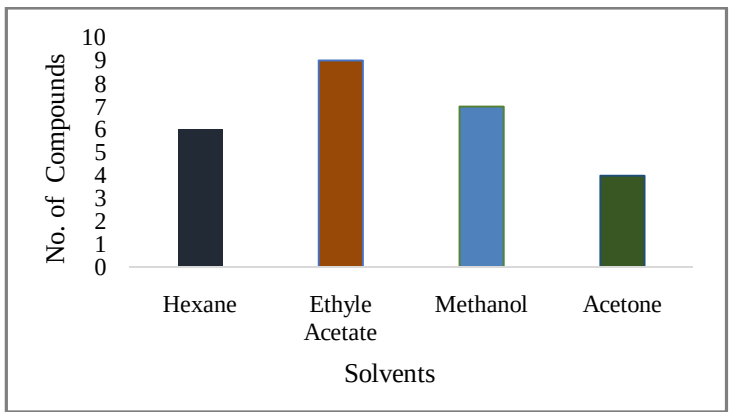


Figure 2. Qualitative tests of extracts.

Among the four different extracts, ethyl acetate extract showed the presence of nine (09) compounds which is maximum number of compounds. Next to that, Methanol extracts showed seven (07) compounds. Hexane extracts showed six (06) compounds and acetone extracts showed only four (04) compounds. Alkaloids were presence of ethyl acetate, acetone and methanol extracts. Alkaloids have cytotoxic activity which due to the presence of microtubule interfering agents that can bind to beta tubulin, thus inhibiting the formation of the mitotic spindle fiber required for cell division [10]. Terpenoids were found in ethyl acetate, hexane and acetone extracts. Terpenoids have wide spectrum of cytotoxic, nematocidal activity and antitumor activities [11]. The steroids were found only in the hexane and ethyl acetate extracts. Steroids have insecticidal, antimicrobial, antiparasitic and cardiotonic properties. Tannins were found in ethyl acetate, hexane and methanol extracts. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [12]. The saponins did not show in any positive result in any extract of *Chara braunii*. Flavonoids have antimicrobial, antiviral, antioxidant and spasmolytic properties. Phenols showed its presence in ethyl acetate, hexane and methanol extracts of *Chara braunii*. The phenolic compounds possessed in specific physical, chemical and biological activities that make them useful as drugs and phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-viral, anticancer actions [13]. Coumarins were found in only ethyl acetate and methanol extracts. Coumarins mainly used in anti-coagulant to treat lymphedema [14]. Quinones showed its presence in ethyl acetate, hexane and methanol extracts of *Chara braunii*. Quinones confer cytotoxic activity via interference of DNA and RNA replication and mitochondrial oxidative pathways, as well as through the formation of peroxide, superoxide and hydroxyl radicals in the cell [15]. Glycosides were found in ethyl acetate, methanol and acetone extracts.

Quantitative analysis of the phytochemical substances in algal extracts: The Phenolics, flavonoids and tannins contents of *Chara braunii* were varied according to solvents used in extraction processes. The highest total phenolics (2.14 ± 0.15 mg GAE g^{-1} dry wt.) and flavonoids (1.72 ± 0.05 mg RUE g^{-1} dry wt.) are shows in methanol extract, while the highest total tannins (2.12 ± 0.45 mg CAE g^{-1} dry wt.) are shows in ethyl acetate extract of *Chara braunii* (Table 2 and Figure 3). Simon *et al*., [16] demonstrated that extraction solvents have an effect on phenolic and flavonoid contents.

Table 2. Quantitative analyses of phytochemical substances present in different extracts of *C. braunii*

Solvents	Total phenolics (mg GAE g^{-1} dry wt.)	Total tannins (mg CAE g^{-1} dry wt.)	Total flavonoids (mg RUE g^{-1} dry wt.)
Hexane	1.83 ± 0.06	1.94 ± 0.18	0.84 ± 0.03
Ethyl acetate	2.08 ± 0.14	2.12 ± 0.45	1.44 ± 0.04
Methanol	2.14 ± 0.15	1.66 ± 0.50	1.72 ± 0.05
Acetone	--	--	1.38 ± 0.08

Values are means of three analyses of the extract ± standard deviation (n=3) GAE: Gallic acid equivalent, RUE: Rutin equivalent, CAE: Catechin equivalent, -- Blank

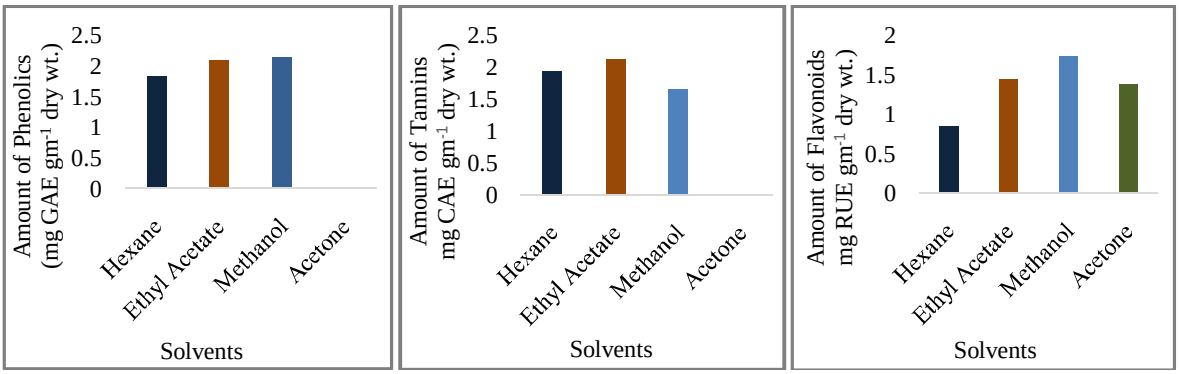


Figure 3. Quantitative test of extracts.

APPLICATION

The presence of these compounds can be used as pharmaceutical exploration in the field of carcinogenic disease prevention.

CONCLUSION

The qualitative analyses of phytochemical showed that, four different extracts, ethyl acetate extract showed the presence of maximum nine compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides) and the absence of saponins, methanol extract showed the presence of seven compounds, hexane extract showed the presence of six compounds and acetone extract showed the presence of four compounds. The quantitative analyses of the phytochemicals showed that the secondary metabolite such as phenolics (2.14 ± 0.15 mg GAE g^{-1} dry wt.) and flavonoids (1.72 ± 0.05 mg RUE g^{-1} dry wt.) showed high amounts in methanol extract, whereas tannins showed high amounts(2.12 ± 0.45 mg CAE g^{-1} dry wt.) in ethyl acetate extract. This study can be concluded that different extracts of *Chara braunii* contains several chemical compounds including alkaloids, terpenoids, steroids, tannins, flavonoids, phenols, coumarins, quinones and glycosides but lacks saponins. Extraction solvents have an effect on yield of total phenolics, total flavonoids and total tannins from *Chara braunii*. So, these compounds will enhance pharmaceutical exploration in the field of carcinogenic disease prevention.

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