# Qualitative and Quantitative Analysis of Phytochemical Studies on Brown Seaweed, Dictyota dichotoma

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***Abstract* - In recent years, the secondary metabolites (Phytochemicals) have been extensively investigated as a source of medicinal agents. The sample for the study constitutes *Dictyota dichotoma* from brown seaweed. It was collected from Hurghada, Red sea coast of Egypt. Four different extracts of *Dictyota dichotoma* were subjected to phytochemical analysis of secondary metabolites both qualitatively and quantitatively by preliminary phytochemical screening tests of ten different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides). The result of phytochemical screening of *Dictyota dichotoma* showed the presence of alkaloids, terpenoids, steroids, tannins, flavonoids, phenols, coumarins, quinones and glycosides and the absence of saponins. Among the four different extracts, ethyl acetate extract showed the presence of maximum number (9) of compounds. Next to that, Methanol extracts showed seven compounds. Hexane extracts showed six compounds and acetone extracts showed only four compounds. The estimation of total phenolics, tannins and flavonoids were observed in different extracts of *Dictyota dichotoma*. The quantitative phytochemical analyses revealed that secondary metabolites such as phenolics (2.14 ± 0.15 mg GAE/g dry wt) and flavonoids (1.72 ± 0.05 mg RUE/g dry wt) showed high amounts in methanol extract, while tannins (2.12 ± 0.45 mg CAE /g dry wt) showed high amounts in ethyl acetate extract.**

***Index Terms* - Seaweed, Secondary metabolites, *Dictyota dichotoma,* Phytochemical, solvents.**

## I. INTRODUCTION

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient and chemical composition. Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae [1]. Over the past decades, seaweeds have been used by humans as medicine and food and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. Seaweeds are the reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B1, B12, C, D and E [2].

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that have protected human from various diseases [3]. So, phytochemical analysis of the seaweeds will be a good preliminary approach to reveal its secondary metabolite constituents and the resultant medicinal values. Seaweeds are a known source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [4]. Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential [5].

Macroalgae produce a wide variety of chemically active metabolites including alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols that have a broad range of biological activities [6]. *Dictyota* is a marine seaweed belonging to Kingdom: Chromista, Subkingdom: Harosa, Infrakingdom: Heterokonta, Phylum: Ochrophyta, Subphylum: Phaeista, Infraphylum: Limnista, Superclass: Fucistia, Class: Phaeophyceae, Order: Dictyotales, Family: Dictyotaceae [7]. Species of Dictyotales (brown algae) produce a large array of bioactive secondary metabolites possessing a broad defensive action against herbivores in the marine environment [8]. Almost a third of the reported brown algal chemistry comes from a single genus, *Dictyota*, which has elaborated a wealth of terpenes [9].

Based on the above facts, the present study aims to screen different solvents of *Dictyota dichotoma* both quantitatively and qualitatively for the phytochemicals.

## II. MATERIAL AND METHODS

### 1. Study area

Collection site was along Hurghada shores, Red sea coast of Egypt (Fig. 1); it is one of the most important places of interest for algal growth in Egypt.


**Figure 1:** Collection site of *Dictyota dichotoma*

## 2. Collection and preparation of *Dictyota dichotoma*

The selected sample of *Dictyota dichotoma* is a known species of brown seaweed (Fig. 2). It was collected by hand, washed with seawater at the sampling site to remove the adhered sediments and impurities and then packed in polyethylene bags and brought to the laboratory for further analyses.

Then it was washed successively with tap water, distilled water to remove all the salt on the surface. The water was drained off and the seaweed was spread on blotting paper to remove excess water. The clean seaweed was shade dried and then kept in an oven 60 °C for 4hrs. The dried algal material was ground to 2 mm or smaller particle size.

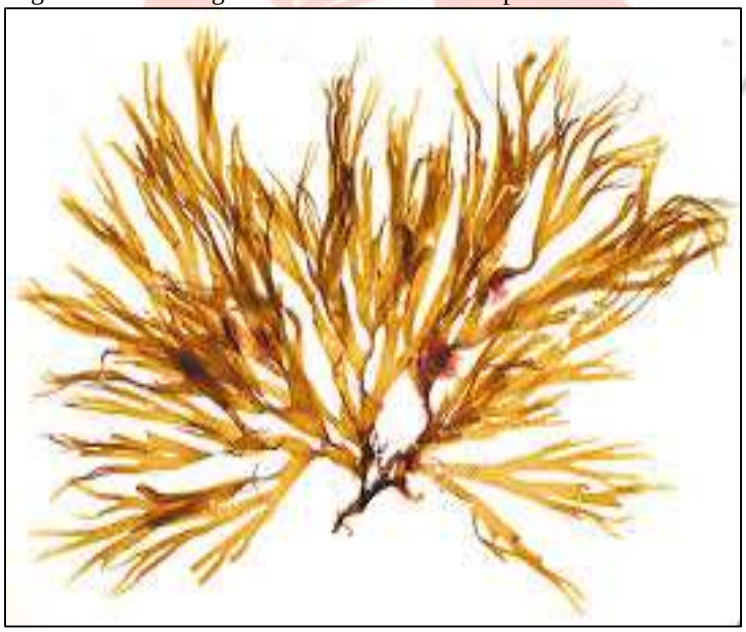

**Figure 2:** Selected sample of *Dictyota dichotoma*.

## 3. Preparation of algal extracts

The seaweed powder was successively extracted using solvents of increasing polarity according to Arokiyaraj *et al* (2009) [10] with some modifications. 15 g powder was initially soaked in 60 ml of hexane in air tight conical flask for two days. The flask was periodically subjected to shaking on an electronic shaker and then it was first filtered through double layered muslin cloth and then filtered through Whatman no 1 filter paper and filtrate was collected into sterile air tight bottle. Likewise, the above methods were repeated using ethyl acetate, acetone and methanol.

## 4. Qualitative analysis of phytochemical substances in algal extracts

The phytochemical screening of different algal extracts was assessed by standard method as described by Savithramma et. al. (2011) [11]. Phytochemical screening was carried out to identify the major natural chemical groups such as alkaloids, terpenoids,

steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the algal extracts tested.

***Phytochemical analysis***

**1. Test for Alkaloids**

For Alkaloid identification, 2 mL of concentrated Hydrochloric acid (HCl) was added to 2 mL algal extract. Then few drops Mayer's reagent was added**.** Presence of green color or white precipitate indicates the presence of alkaloids.

**2. Test for Terpenoids**

For Terpenoids identification, 2 mL of chloroform along with concentrated Sulphuric acid were added to 0.5 ml of the algal extract. Formation of reddish brown color at the interface indicates the presence of Terpenoids

**3. Test for Steroids**

For steroids identification, 2 mL of chloroform and 1 mL of sulphuric acid ($H_2SO_4$) were added to 0.5 mL of the algal extract. Formation of reddish brown ring at interface indicates the presence of steroids.

**4. Test for Tannins**

For tannins identification, one mL of ferric chloride (5% $FeCl_3$) was added to 1 mL of the algal extract. Formation of dark blue or greenish black color indicates the presence of tannins.

**5. Test for Saponins**

For saponins identification, 2 mL of distilled water was added to 2 mL algal extract and shaken in graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

**6. Test for Flavonoids**

For flavonoids identification, 1 mL of 2N sodium hydroxide (NaOH) was added to 2 mL of algal extract. Formation of yellow color indicates the presence of flavonoids.

**7. Test for Phenols**

For phenols identification, 2 mL of distilled water followed by few drops of 10 % ferric chloride was added to 1 mL of the algal extract. Formation of blue / green color indicates the presence of phenols

**8. Test for Coumarins**

For coumarins identification, 1 mL of 10 % NaOH was added to 1 mL of algal extract. Formation of yellow color indicates the presence of coumarins.

**9. Test for Quinones**

For Quinone identification, 1 mL of concentrated sulphuric acid ($H_2SO_4$) was added to 1 mL algal extract. Formation of red color indicates the presence of quinones.

**10. Test for Glycosides**

For glycosides identification, 3 mL of chloroform and 10% ammonium solution was added to 2 mL of the algal extract. Formation of pink color indicates the presence of glycosides.

**5. Quantitative analysis of phytochemical substances in algal extracts**

**1. Estimation of phenols**

Total phenols in different algal extracts were determined by a method described by Siddhuraju P et. al. (2007) [12] with slight modifications in the concentration of $Na_2CO_3$. The assay involved gallic acid as the standard. One mL of 10 % Folin-Ciocalteu reagent was added to 20 µL of algal extract or standard. The reagents were mixed well and incubated for 5 min before adding 700 µL of 10 % $Na_2CO_3$. The solutions were further incubated for 2 h before reading the absorbance at 765 nm. Gallic acid in the range of 20–200 mg/L was used to construct a calibration curve. Estimation of the total phenols was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg gallic acid equivalent (GAE)/g dried weight.

**2. Estimation of flavonoids**

Total flavonoids in different algal extracts were determined by aluminium chloride colorimetric method described by Brighente et. al. (2007) [13]. 0.5 ml of 2% aluminium chloride in methanol was mixed with the same volume of algal extract. After 1 hour-incubation at room temperature, the absorbance of the mixtures was measured at 415 nm using UV/Vis spectrophotometer. Rutin in the range of 20–200 mg/L was used to construct a calibration curve. Estimation of the total flavonoids was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg rutin equivalents (RU)/g dried weight.

**3. Estimation of tannins**

Total tannins content in different algal extracts were determined according to the method of Julkunen-Titto (1985) [14] .Briefly, 50 µl of algal extract was mixed with 1.5 ml of 40% vanillin (prepared with methanol), and then 750 µl of HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. The absorbance of the mixtures was measured at 500 nm using UV/Vis spectrophotometer. Catechin in the range of 20–200 mg/L was used to construct a calibration curve. Estimation of tannins content was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg catechin equivalents /g dried weight.

## III. RESULTS AND DISCUSSION

**Qualitative analysis of phytochemical substances in screening algal extracts**

Preliminary phytochemical screening of ten different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides) were tested in four different extracts. In the present study, the phytochemical screening was performed with hexane, ethyl acetate, acetone and methanol extracts of *Dictyota dichotoma*. Saponins did not show any positive result for their presence in any of the four extracts tested as shown in table 1.

Among the four different extracts, ethyl acetate extract showed the presence of maximum number (9) of compounds. Next to that, Methanol extracts showed seven compounds. Hexane extracts showed six compounds and acetone extracts showed only four compounds.Ethyl acetate extract showed the presence of alkaloids, terpenoids, steroids, tannins, flavonoids, phenols, coumarins, quinones and glycosides. Methanol extract showed the presence of alkaloids, tannins, flavonoids, phenols, coumarins, quinones and glycosides. Hexane extract showed the presence of terpenoids, steroids, tannins, flavonoids, phenols and quinones. Acetone extract showed the presence of alkaloids, terpenoids, flavonoids and glycosides. The presence or absence of the phytochemicals depends upon the solvent medium used for extraction.

Alkaloids were found in ethyl acetate, acetone and methanol extracts. Alkaloids have cytotoxic activity that is due to the presence of microtubule interfering agents that can bind to beta tubulin, thus inhibiting the formation of the mitotic spindle fibre required for cell division [15]. Terpenoids were found in hexane, ethyl acetate and acetone extracts. Terpenoids from seaweeds displayed wide spectrum of cytotoxic, nematicidal activity and antitumour activities [16], [17], [18]. Steroids were found only in hexane and ethyl acetate extracts. Steroids of seaweeds are known to be important for insecticidal, antimicrobial, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition, herbal medicine and cosmetics [19]. Tannins were found in hexane, ethyl acetate 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 [20]. Saponins did not show any positive result in any extract of *Dictyota dichotoma.*

Table 1: Qualitative analyses of phytochemical substances in different extracts of *Dictyota dichotoma*

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

++: intensely present, +: Present, - : Absent

Flavonoids showed its presence in all tested extracts. Flavonoids have antimicrobial, antiviral, antioxidant and spasmolytic activity. Flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. Phenols showed its presence in hexane, ethyl acetate and methanol extracts of *Dictyota dichotoma*. In general, phenolic compounds possessed specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-viral, anticancer actions [21]. Coumarins were found only in ethyl acetate and methanol extracts. Coumarins have been used as anti-coagulant to treat lymphedema [22]. Quinones showed its presence in hexane, ethyl acetate and methanol extracts of *Dictyota dichotoma.* 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, acetone and methanol extracts . Glycoside was present in all the organic extract of *acanthopora spicifera*. Glycoside was absent in ethanol extract of *sargassum wightii* [23].

**Quantitative analysis of phytochemical substances in algal extracts**

Phenolics, flavonoids and tannins contents of *Dictyota dichotoma* were varied according to solvents used in extraction processes. The highest total phenolics (2.14 ± 0.15 mg GAE/g dry wt) and flavonoids (1.72 ± 0.05 mg RUE/g dry wt) was recorded in methanol extract, while the highest total tannins (2.12 ± 0.45 mg CAE /g dry wt) was recorded in ethyl acetate extract of *Dictyota dichotoma* (Table-2). Simon et al. [24] demonstrated that extraction solvents have an effect on phenolic and flavonoid contents

Table 2: Quantitative analyses of phytochemical substances present in different extracts of *Dictyota dichotoma*

| Solvents | Total phenolics (mg GAE/g dry wt) | Total flavonoids (mg RUE/g dry wt) | Total tannins (mg CAE /g dry wt) |
|---|---|---|---|
| Hexane | 1.99 ± 0.08 | 0.91 ± 0.03 | 1.85 ± 0.24 |
| Ethyl acetate | 2.02 ± 0.11 | 1.42 ± 0.04 | 2.12 ± 0.45 |
| Acetone | 0 | 1.35 ± 0.06 | 0 |
| Methanol | 2.14 ± 0.15 | 1.72 ± 0.05 | 1.68 ± 0.48 |

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

## IV. CONCLUSION

This study concluded that different extracts of brown seaweed, *Dictyota dichotoma* possess 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 *Dictyota dichotoma*.

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