ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Screening of phytochemical compounds in brown seaweed (*Turbinaria conoides)* using TLC, UV-VIS and FTIR analysis

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ABSTRACT

The present work was carried out to investigate the medicinally active compounds present in the methanolic extract of *Turbinaria conoides* by using the analysis of TLC, UV-VIS and FTIR. In the present investigation, chromatographic techniques such as thin layer chromatography (TLC) analysis was used to separate and isolate flavonoid compound from the crude extract of *Turbinaria conoides*. The solvent system of TLC was n-Butanol, Acetic acid and water in the ratio of 4:1:5 was used and its R_f value was detected. For UV-VIS Spectrophotometric analysis, the extract of *Turbinaria conoides was* scanned in the wavelength ranging from 190-800 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks and their absorption values were detected. For FTIR Analysis, the extract of *Turbinaria conoides* was focused in the transmittance ranging between 400-4000cm^{-1} on a Perkin Elmer Spectrophotometer system and the characteristic peak values and their functional groups were detected. From TLC analysis result, a spot was identified with R_f value was 0.66. This R_f value was compared with literature data showed that the presence of flavonoid compound as Quercetin-3-galactoside. The UV-VIS profile showed the peaks at 200,224,232, and 669 nm with the absorption values 3.15, 4.25, 3.65 and 0.25 respectively. The result of UV-VIS spectroscopic analysis confirms the presence of phenols and Flavonoids in the *Turbinaria conoides* extract. The results of the present FTIR study confirms the presence of Phenol, Alkane, Alkene, Carboxylic acid, Aromatic compound, Nitro compound, Alcohol, Silicon, Ketones, Benzene and Bromo alkanes compounds. The results of the present study were revealed that the presence of phenols, flavonoids and functional groups of the *Turbinaria conoides* which indicates the medicinal importance of this Seaweed.

KEY WORDS: *Turbinaria conoides*, TLC, UV-VIS, FTIR, Phytochemicals, medicinally importance.

1. INTRODUCTION

The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites. It also represents great challenges that requires inputs from various scientific areas to bring the marine chemical diversity up to its therapeutic potential. Seaweeds are considered to be a rich source of antioxidants (Cahyana, 1992). Recently, the potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid) and polyphenols (phenolic acid, flavonoids, tannins). These compounds are widely distributed in plants and seaweeds and are known to exhibit higher antioxidant activities. Seaweeds are noted to contain not only labile antioxidants (ascorbate, glutathione) when fresh (Kakinuma, 2001), but also, more stable molecules such as carotenoids (Yan, 1999). Seaweeds are relatively simple photosynthetic plants with unicellular reproductive structures; they range from unicellular organisms to non-vascular filamentous or thyloid plants (Druehl, 2000).

Three major groups of seaweeds are recognized according to their pigments that absorb light of particular wavelengths and give them their characteristic colors of green, brown or red .The green algae (chlorophyll) are truly green with no pigments to mark the chlorophyll. The green seaweeds are very diverse and range from microscopic free swimming single cells to large membranous, tubular and bushy plants. Brown seaweeds (phaeophyta) are multicellular and are found in a variety of different physical forms including crusts and filaments. Like all photosynthetic organisms, brown algae contain the green pigments chlorophyll. They also contain other gold and brown pigments, which mask the green color of chlorophyll. The dominant pigment found in brown algae is called Fucoxanthin. The red algae (Rhodophyta) in addition to chlorophyll contain the pigments phycocyanin and phycocerythrin, which give the red coloration. Red algae are found in a variety of physical forms, including simple and branched filaments (Ramanigade, 2013).

Seaweed is one of the most extensively used functional foods and medicinal herbs with a long history in Asian countries. It refers to a wide variety of different species with different medicinal activities which is divided into two groups, namely, microalgae and macro algae or seaweed. Seaweeds are known as functional food because of their richness in lipids, minerals and certain vitamins, and also several bioactive substances like polysaccharides, proteins, and polyphenols, with potential medicinal uses against cancer, oxidative stress, inflammation, allergy, diabetes, thrombosis, obesity, lipidemia, hypertension, and other degenerative diseases. Since certain seaweeds have long been used in the treatment of cancer, many kinds of crude or partially purified polysaccharides from various brown and red algae were tested for their property and showed antitumor activity against experimental tumor (Ramberg, 2010; Harvey, 2004).

Antioxidants in biological systems have multiple functions, including defending against oxidative damage in the major signaling pathways of cells. Brown algae are rich sources of various bioactive compounds, including polyphenols, carotenoids and polysaccharides with different physiological effects (toxic or curative) on human health. The components of various brown algae (Phaeophyceae) species have been widely investigated and it has rich inorganic composition. More than 1140 secondary metabolites have been reported from Phaeophyceae. The brown algae are a rich source of various natural antioxidant such as polyphenols, which play an important role in preventing lipid peroxidation (Amsler and Fairhead, 2006).

2. MATERIALS & METHODS

2.1. Thin Layer Chromatography: Thin layer chromatography is based upon the principles of column and partition chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces (Harborne, 1973; 1984; Stahl, 1969).

2.1.1. Procedure: A thin-layered plate is prepared by spreading aqueous slurry of Silica gel G on the clean surface of a glass or rigid plastic. Calcium carbonate or starch is also added to the adsorbent to increase adhesion. The plate is then heated in an oven for about 30 minutes at 105°C to activate the plate. It is then cooled inside the oven itself. Test samples (1mg/ml of all extracts in respective solvents) were applied in the form of spots using capillary tube. The choice of solvents depends upon the nature of compound to be separated and also on the adsorbent used. The solvent is poured into the chamber and closed tightly and the chamber is saturated for a few hours before running the chromatogram.

The extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base. The plate was then dipped into a suitable solvent system (mobile phase) (n-Butanol : Acetic acid : Water (4:1:5)). The plate is then placed in a container with enough solvent in a well-covered tank. The solvent migrates up the plate. As the solvent rising through thin layer separates different components of the mixture at different rates which appear as spots in the thin layer. After the solvent has reached almost the top edge of the plate, nearly $3/4^{th}$ of the plate, the plate is removed from the tank and dried briefly at moderate temperatures 60-120°C. The presences of secondary metabolites in the extracts were detected by TLC using suitable spraying reagents.

2.1.2. Detection of spots by using spraying reagents: Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced colored areas in the regions, which they occupy (Harborne, 1973). The presence of flavonoid was detected by the formation of color in the plate a positive reaction was formation of yellow color spot by exposure of ammonia (Harborne, 1973; 1984).

2.1.3. R_f Value: It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$R_f = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

2.2. UV and FTIR Spectroscopic analysis: The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation (Neha and Jyoti, 2013) .

2.3. UV –VIS Spectrophotometric Analysis: The methanolic leaf extract was examined under UV Visible spectral analysis. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 190-800 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected (Iqbal, 2010; Nanzeen, 2012).

2.4. Quantitative Determination of total phenols by spectrophotometric method: Total phenols estimated by the method of Edeoga (Edeoga, 2005).

2.4.1. Procedure: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. This was measured at 505 nm.

2.4.2. Determination of Flavonoid: Flavonoids were determined by the method of Bohm and Kocipai (1974).

2.4.3. Procedure: 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper No.42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3. RESULTS & DISCUSSION

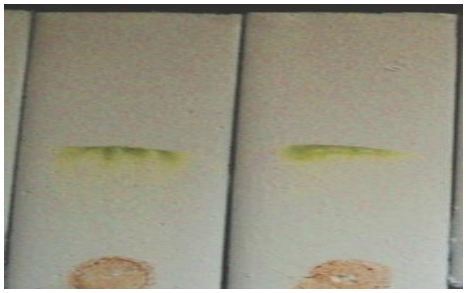
3.1. Thin Layer Chromatography: Thin layer chromatogram of methanolic extract of *turbinaria conoides* was given in fig 1 and its R_f value was given in Table.1.TLC of methanolic extract of *turbinaria conoides* revealed the presence of a spot having R_f value of 0.66 when a solvent phase of n-Butanol : Acetic acid : Water (4:1:5) solvent system was used. This R_f value 0.66 was compared with literature data and it was identified as flavonoid compound as quercetin in the methanolic extract of *Turbinaria conoides* (Kaya, 2012).

Table.1.Analysis of flavonoid by TLC

Phytoconstituents	R_f Value	Results	Compound
Flavonoid	3.2/4.8	0.66	Hyperoside (Quercetin-3-galactoside)

Values are expressed from triplicates

Fig.1.Analysis of flavonoid by TLC



3.2. UV and FTIR Spectroscopic analysis: The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR spectrum and its peak values with functional groups were represented in Fig 2 and Table 2. When the *Turbinaria conoides* extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, and benzene and bromo alkanes compounds, The maximum peak is obtained for phenols.

Fig.2.Identification of functional groups using FTIR techniques

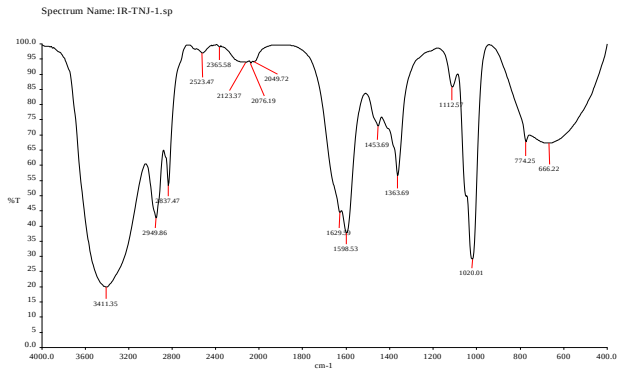


Table.2.Identification of functional groups using FTIR techniques

Peak Values	Functional Groups
3411.35	Alcohols, Phenols
2949.86	Alkanes
2837.47	Carboxylic acid
2365.58	Alkene
2049.72	Silicon and boron compounds
1629.59	Ketones
1598.53	Aromatics
1112.57	Aliphatic amines
1020.01	Carboxylic acid
774.25	Alkyl halides

3.3. UV visible spectral analysis of sample: The qualitative UV-Vis spectrum profile of methanolic extract of *turbinaria conoides* was selected from 190 nm to 800 nm due to sharpness of peaks and proper baseline. UV-Vis spectrum profile of methanolic extract of *turbinaria conoides* was given in Fig.3 and its absorption values were given in Table.3.The profile showed the peaks at 200, 224, 231and 669 nm with respectively. The spectra for Flavonoids typically lie in the range of 190-800 nm (Neha and Jyoti, 2013). The result of UV-VIS spectroscopic analysis confirms the presence of flavonoids in the Seaweed extract.

Fig.3.UV visible spectral analysis of sample

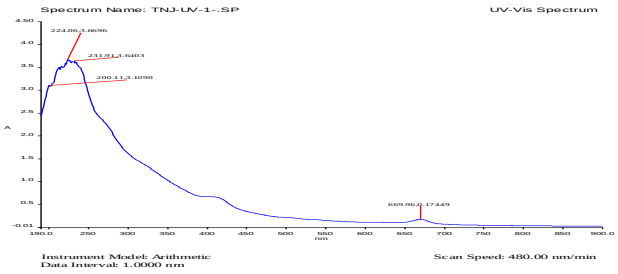


Table.3.UV-Vis Peak Values of Methanolic Extract of *Turbinaria conoides*

Nanometers	Absorption values	Compounds
200	3.15	Phenol and Flavonoid (Neha and Jyoti, 2013)
224	**4.25**	
232	**3.65**	
669	0.25	

3.4. Quantitative Determination of total phenols by spectrophotometric method: Total phenols were estimated by Edeoga Method [13] and Flavonoids was determined by the Bohm and Kocipai-Abyazan methods (Boham and Kocipai, 1974). The results were tabulated in Table.4.

Table.4.Quantitative Analysis of plant extract

Phyto constituents	Results (mg/gm)
Total Phenolics	203.45
Flavonoids	106.85

Values are expressed from triplicates

Discussion: At present, a number of analytical tools (chromatographic and spectroscopic) have been used to analyze flavonoids in plant samples or crude drugs. Thin Layer Chromatography (TLC) is one of the most popular and widely used separation techniques because of its ease of use, cost effectiveness, high sensitivity, speed of separation as well as its capacity to analysis multiple samples simultaneously. The technique can be utilized for separation, isolation, identification and quantification of components in a mixture. It can also be utilized on a preparative scale to isolate a particular component. The present study of TLC results revealed that the presence of flavonoid as quercetin in the extract of *turbinaria conoides*. The results also suggest that the extract of *Turbinaria conoides* has antioxidant and anti-inflammatory properties (Chandrappa, 2012).

Spectroscopic methods have become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of the pharmaceutical and biological materials. The present work, UV-VIS spectrophotometer revealed that the presence of phenolic compound like tannin and flavonoid compound which indicates the medicinal properties of this seaweeds. Phenolic compound tannin was used as antioxidant, anti inflammatory and anti cancer and flavonoid compound used as antioxidative activity, hepatoprotective, anti-inflammatory, anticancer and antiviral activity of this seaweed extract also observed form this study (Sarika, 2012).

By using FT-IR spectrum, we confirmed the functional constituents present in the given leaf extract. The results of the present study also revealed the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, benzene and bromo alkanes compounds in methanolic extract of *turbinaria conoides*. The results of the present study confirms the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, benzene and bromo alkanes compounds in methanolic exrtract of *turbinaria conoides*. The results of the present study also suggested that various medicinal properties of the *turbinaria conoides* [21]. This findings of the present study developed novel phytochemical marker to identify the medicinally important seaweeds.

4. CONCLUSION

The present study demonstrated that *Turbinaria conoides* has rich source of secondary metabolites. These findings recommended that *Turbinaria conoides* could be a potential source of natural antioxidant having great importance as a therapeutic agent and preventing oxidative stress related degenerative diseases. Further purification, identification and characterization of the active compounds would be our priority in future studies.

5. ACKNOWLEDGEMENT

Authors sincerely thank Dr.S.Velavan, Associate professor, Maruthupaniyar college of Arts & Science, Thanjavur. For his kind help & support.

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