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HPLC Profiling of Brown Seaweeds (*Turbinaria Conoides*)

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Abstract: Seaweeds having potential phytochemical constituents which are capable of curing various human ailments and prevent disorders. Among the phytochemical constituents, antioxidant and antimicrobial bioactive compounds are playing a key role in society especially in health care Industry. The bioactive compounds present in the samples were extracted with solvents like methanol, ethanol, chloroform and ether and analysed by standard procedures. High performance liquid chromatography (HPLC) has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in sea weed extracts because of its simplicity, sensitivity, accuracy, suitability for high throughput screening.HPLC analysis of the methanolic extract of *Turbinariaconoides* showed the presence of various constituents as evidenced by the chromatogram obtained at various retention times (3.643, 3.819 and 6.463)at λmax 254 nm.
Key words: Seaweeds,Antioxidant, Turbinariaconoides, HPLC

1. Introduction

Seaweed is a macroscopic, multicellular, marine algae that lives near the seabed. The term includes some members of the red, brown, and green algae. The study of seaweed is known as Phycology. They belong to three different groups, empirically distinguished since the mid-nineteenth century on the basis of thallus color.Seaweeds are far more complex organisms than generally realised. Many have specialised tissues and growth forms. They may have very complicated sex, with many of them producing sex pheremones (chemicals that attract males or male gametes), and with many different types of sex organs.Seaweeds are multi-cellular macro-algae used as potential renewable resource in the field of medical and commercial environment due to the presence of polyphenols such as phenolic acids, flavonoids, anthocyanidins, lignin, tannins, catechin, epicatechin, epigallocatechin and gallic acid. The polyphenolic compounds have much health benefits such as antioxidant, anticancer, antiviral, anti-inflammatory and an ability to inhibit human platelet aggregation. Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which serve as a component for nutraceutical and numerous pharmacologically important bioactive constituents such as flavanoids, carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Marine seaweeds characteristically contain sulphated polysaccharides that are not found in land plants. Until now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations. Recent findings evidenced that seaweeds possess antiviral, antibacterial, antifungal and antitumor potentials, among numerous others. Seaweeds have recently received significant attention for their potential as natural antioxidants.[8]

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, arotenes, phenolic acids, and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals[1].

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers [2]

2. Matreials & Methods

2.1. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in sea weed extracts because of its simplicity, sensitivity, accuracy, suitability for high throughput screening. Over the past decade, HPLC has been successfully used in the analysis of pharmaceuticals, plant constituents and bio-macromolecules [3]. A major advantage of HPLC is that it has the ability to easily separate a wide variety of chemical mixtures. From HPLC spectrum, this study confirmed the functional constituents like flavonoid and phenol presence in the methanolic extract of *Turbinariaconoides*..

Flavonoids fractions were analyzed by using a HPLC method [9] .The HPLC analysis of Extract were carried out with Chromatographic system consist of autosampler with 20µl fixed loop and an UV-Visible detector. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. The samples were run for 25min. and detection was done at 280 nm by UV detector (Lamp-D2). All chromatographic data were recorded and processed using autochro-software.

3. Result & Discussion

3.1 HPLC Analysis

HPLC profile of methanolic extract of *Turbinariyaconodies*.was given in Fig.2 and standard was give in Fig.1 and the peak values of *Turbinariyaconodies*was given in Table.1 and Table.2. HPLC analysis of the methanolic extract of *Turbinariaconoides*showed the presence of various constituents as evidenced by the chromatogram obtained at various retention times (3.643, 3.819 and 6.463).The separation of the peaks such as 3.643, 3.819 and 6.463 were compared with the retention times of isolated compounds with literature data. The phenolic acid like gallic acid was identified at Rt = 3.643[5] the flavonoids were identified like quercetin at Rt = 3.819 [12] and myricetin at Rt = 6.463[13] at λmax 254 nm.

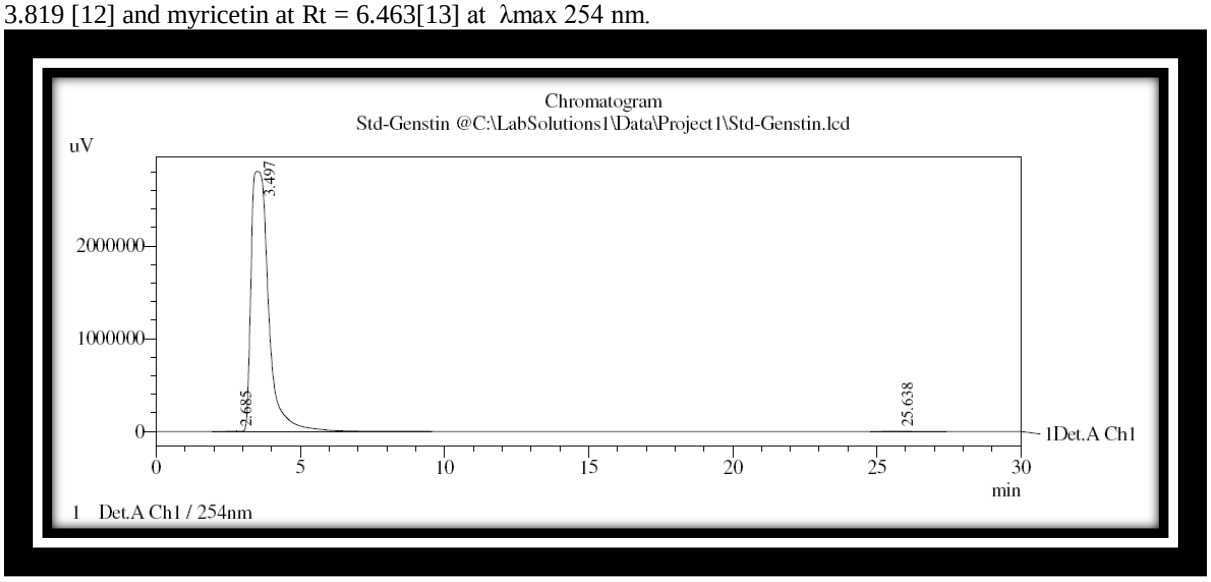

Fig 1: Standard Chromatogram of Genstin

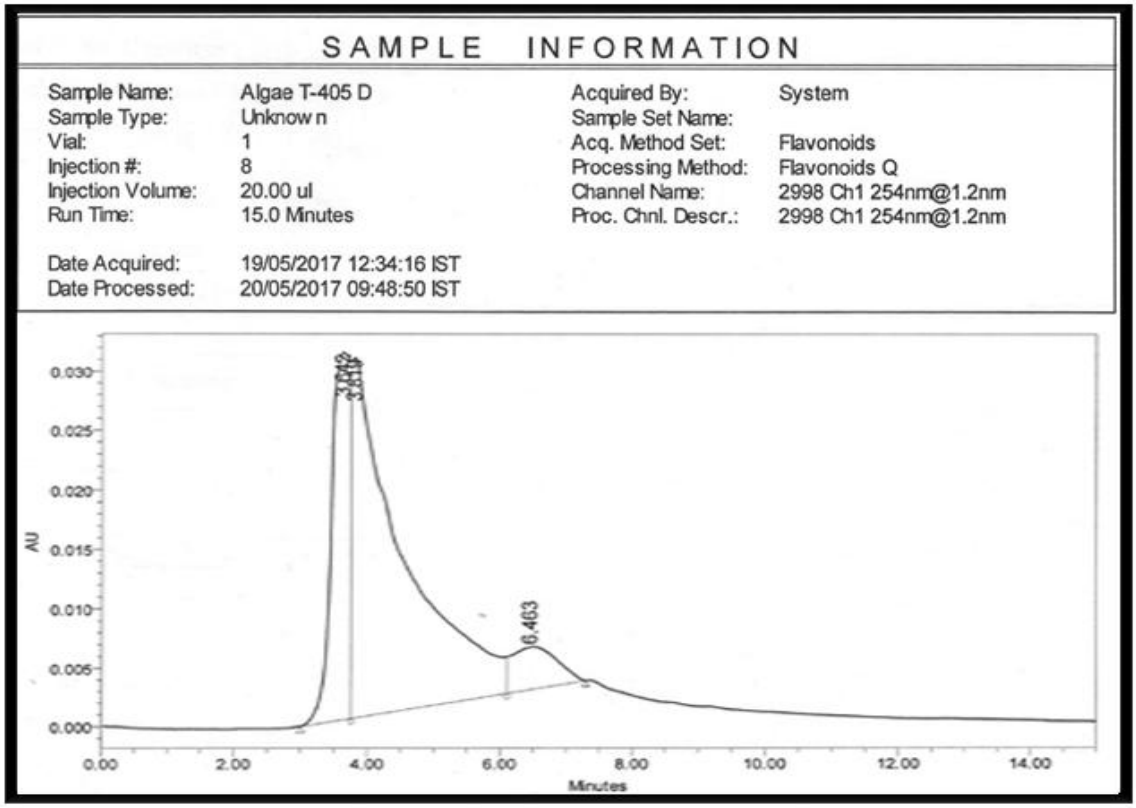

Fig .2: HPLC Chromatogram of Methanolic Extract of *Turbinariaconoides*

Table:1 Retention Times and Peak Areas of Methanolic Extract of *Turbinariaconoides.*

Peak#	Ret. Time	Area	Height	Area%	Height%	compounds
1	3.643	30150	1217	0.025	0.043	Chrysoeriol-7-o-glucuro-glucuronide
2	3.819	119003403	2805379	99.943	99.936	Quercetin-3-o arabinoside
3	6.463	38175	575	0.032	0.020	Saponarin
Total		119071727	2807171	100.000	100.000	

Table 2: Analysis of Phenolic and Flavonoid Compounds by HPLC

S.no	RT	Phytoconstituents	compounds
1	3.643	Galic acid	Phenol
2	3.819	Quercetin	Flavooid
3	6.463	Myriceti	Flavooid

High performance liquid chromatography (HPLC) has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in plant extracts and herbal drugs because of its simplicity, sensitivity, accuracy, suitability for high throughput screening. Over the past decade, HPLC has been successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules [5]. A major advantage of HPLC is that it has the ability to easily separate a wide variety of chemical mixtures. From HPLC spectrum, this study confirmed the functional constituents like flavonoid and phenol presence in the methanolic extract of *Turbinariaconoides*. The results of the present study was also supported by the findings of Rooban*et al*.,(2009) who have indicated the presence of flavonoid and phenolic compounds and suggested the antioxidative, anti-inflammatory, anti cancer and anti viral activity of *Turbinariaconoides* by using of HPLC study.

4. Discussion:

Bioactive principles in methanolic extract of *Turninariaconoides* were identified by TLC & HPLC, analysis. Thin layer chromatogram showed the presence of Hyperoside (Quercetin-3-galactoside) as flavonoid compound. HPLC profile showed the presence of phenolic and flavonoid compounds at RT 3.643, RT 3.819 and RT 6.463.

On the basis of results in this study, It is evident from the present study that the Methanolic extracts of *Turbinariaconoides* could be utilized as a good natural source of antioxidants and a possible food supplement or as an antimicrobial agent in pharmaceutical industry. The data may contribute to a rational basis for the use of antioxidant rich marine algal extracts in the therapy of diseases related to oxidative stress. In addition, the results indicate that phenolic compounds might be major contributors to the antioxidant activities of *Turbinariaconoides*. The finding of the current report appear useful for further research aiming to isolate, identify and characterize the specific phenolic compounds in *Turbinariaconoides* for its industrial and pharmaceutical applications.

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