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HPLC Analysis of Flavonoids in *Acanthophora Specifera (Red Seaweed)* Collected from Gulf of Mannar, Tamilnadu, India

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Abstract: ***Acanthophora Specifera is a species of marine red seaweed and in the family Rhodomelaceae. They were freshly collected from Mandapam Coastal Area, Rameswaram Tamilnadu, India and rinsed in seawater and packed in aseptic bags for further proceedings to laboratory. Seaweeds are potential renewable resources in the marine environment. It has been used as antioxidant and antimutagen. Methanol extract was prepared for further analysis. HPLC analysis of Acanthophora Specifera was carried out with Chromatographic system (Shimadzu Class-VPV6.14SP2, Japan) consist of autosampler with 20µl fixed loop and an UV-Visible detector.This investigation was carried out to determine the possible flavonoids components from Acanthophora specifera by HPLC analysis. In the present study total six flavonoidal compounds were detected. The percentages of major flavonoids contents were chlorogenic acid(69.64%), Caffeic acid (12.86%), Vitexin-rahmnose (5.20%), Querecetin (0.59%) and Catechol (1.41%). These compounds have main physiological role and pharmaceutical effect on the Acanthophora specifera and also same role on the animal and human physiology.***

Keywords: Red Seaweed, HPLC, *Acanthophora specifera*, Chromatogram, Flavonoids

1. Introduction

Seaweed or benthic marine algae are the group of plants that live either in marine or brackish water environment. Like the land plants seaweed contain photosynthetic pigments and with the help of sunlight and nutrient present in the seawater, they photosynthesize and produce food. Seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available. Plant pigments, light, exposure, depth, temperature, tides and the shore characteristics combine to create different environment that determine the distribution and variety among seaweeds. They are basically classified according to colour into three main groups i.e. green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta).

Seaweeds are potential renewable resources in the marine environment. It is generated enormous amount of bioactive compounds with immense medicinal potential. Nowadays, the uses of antibiotics have increased due to infections.[1]

The first investigation antibiotic activity carried out by Pratt *et al*., (1944)[2]. Since algae have been used in traditional medicine for a long time and also some algae have bacteriostatic, bactericidal, antifungal, anti viral and anti tumor activity, they have been extensively studied by several researchers. Seaweed is rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes. Seaweeds are the most excellent source of Vitamin A, B1, B12, C, D and E[3].

The flavonoids are a large family of polyphenolic compounds synthesized by plants and structurally derived from the parent substance flavone. Flavonoids present in fruits and leafy vegetables are thought to provide potential and versatile health benefits through radical scavenging and chelating activity. The in-vitro antioxidant activities of the flavonoids are due to their ability to reduce the free radical formation and hence exhibit several biological activities.

Many studies have suggested that flavonoids like rutin, kaempferol, quercetin, apigenin etc. are well-known for its antiinflammatory, anti-allergic, anti-thrombitic, hepatoprotective, anti-spasmodic and anticancer properties (Kumar, 2012)[4]. Each different fruits and leafy vegetables are capable to display different extent of antioxidant activities owing to the presence of varied amount of free phenolic and flavonol contents. Ascorbic acid, a water soluble vitamin is essential nutrient in human diets and found mainly in fruits and vegetables. Due to the remarkable antioxidant properties of this compound, it is widely employed in pharmaceutical and cosmetic industry and also exerted several biological activities (Prabhakar, 2010)[5]. Phenolic compounds are ubiquitous in plants and these are secondary metabolites which shield the plants against UV-radiation or resist the pathogenic aggression.

Phenolic acids play a potential protective role against different kinds of oxidative damaged diseases through consumption of fruits and vegetables. The amazing antioxidant cum nutraceutical properties of phenolics attracted global attention over the past decades. The biological activities like antimutagenicity, anti-bacterial action, anti-viral activity, anti-inflammatory traits, apoptotic actions etc. can only be rationalized by detecting and quantitating such compounds (Mattila, 2007)[6]. It is worthy to be noted that only long-term ingestion leads to mitigation. The mineral nutrient present in seaweeds are diverse and the main elements being magnesium, sodium, potassium and calcium. The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions [7].

Among the different compounds with functional properties, antioxidants are the most widely studied. Antioxidants are the substances, which can defend serious human diseases including melanoma, cardiac disorders, diabetes, cancer, inflammatory that explain their potential use in increasing shelf life of food and as medicine [8].

Free radical induced oxidation is one of the major reasons in deterioration of nutritional quality and other physical attributes of food items under storage. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit progression of carcinoma formation [9].

Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of micro organisms. Therefore, the present study was carried out to demonstrate the preliminary phytochemical constituents with the aid HPLC analysis of *Acanthophora specifera* (Red Algae)

2. Materials and Methods

Collection of Seaweeds

Acanthophora specifera were collected from Gulf of Mannar, Rameswaram, Tamilnadu, India. The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. Then the samples were washed with tap water and distilled water and spread in the dark room for drying, after which the dried samples were powdered and stored in an air tight container.

Preparation of Extract

10g of sample were dissolved in 30mL of methanol, and then agitated in ultrasonic bath for 30minutes. The extract was filtered on whatman filter paper 0.5µm to remove the fibers and un dissolved textures. The extract was pre concentrated by steam of nitrogen to about 0.5mL and then completes the volume to 1mL by mobile phase solvent. Then 20µL of the aqueous filtrate was injected to HPLC column.

HPLC Instrument

(Shimadzu Class-VPV6.14SP2, Japan) consist of autosampler with 20µl fixed loop and an UV-Visible detector.

HPLC ANALYSIS

Flavonoids were analysed by using a HPLC method (Weerasak Samee *et al*., 2007)[10].The HPLC analysis of *Acanthophora specifera* was carried out with Chromatographic system (Shimadzu Class-VPV6.14SP2, Japan) 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 auto chro-software.

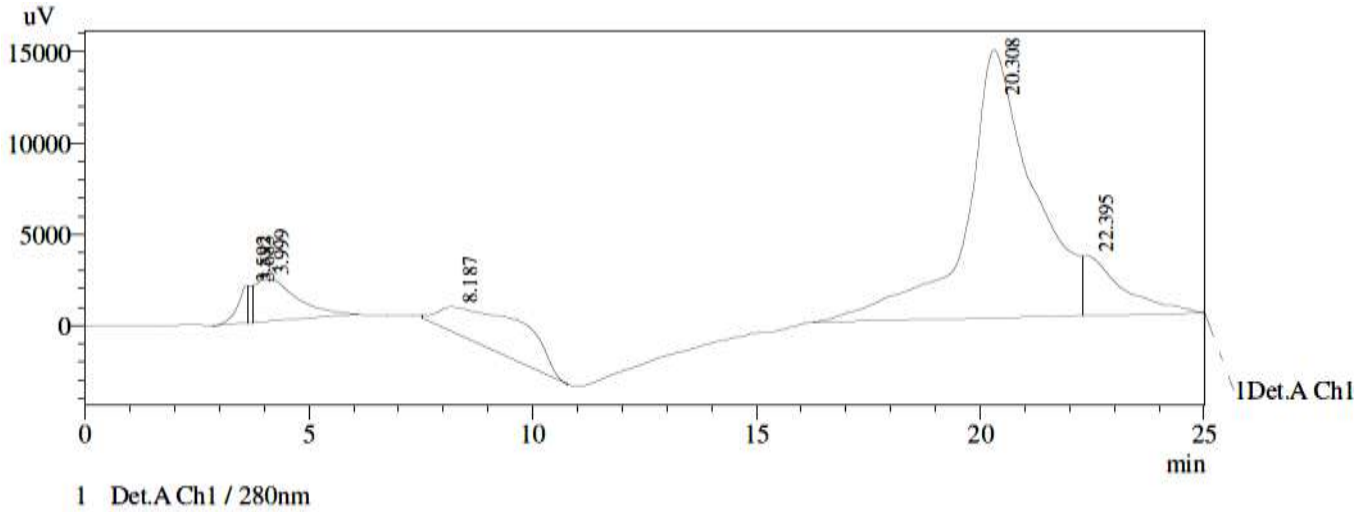

Figure 1: HPLC profile of Flavonoids

Table 1: HPLC profile of Flavonoids

Peak	Ret. Time	Area	Height	Area %	Height %	Compounds identified by literature **
1	3.592	31136	2017	1.411	7.846	Quercetin
2	3.683	13037	2010	0.591	7.817	Catechol
3	3.999	149984	2346	6.795	9.124	Unidentified
4	8.187	272662	1338	12.353	5.204	Vitexin-rahmnose
5	20.308	1537129	14691	69.642	57.143	Chlorogenic acid
6	22.395	203226	3308	9.208	12.867	Caffeic acid
Total		2207174	25709	100.000	100.000	

** (Hamahameen and Jamal, 2013)[11]

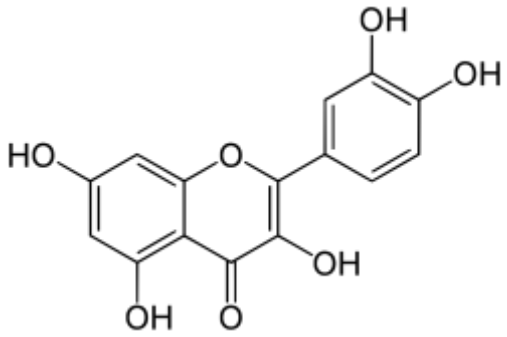

Figure 2: Structure of Quercetin

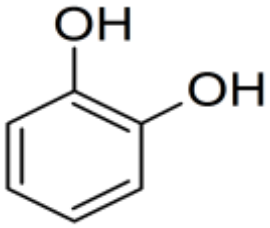

Figure 3: Structure of Catechol

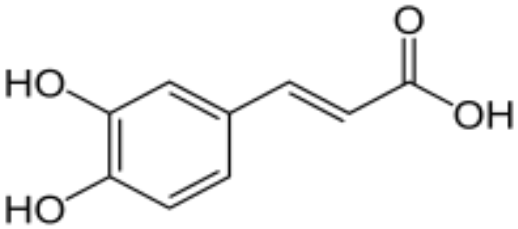

Figure 4: Caffeic acid

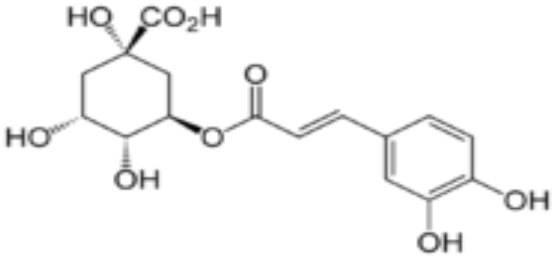

Figure 5: Chlorogenic acid

3. Results and Discussion

HPLC analysis was carried out in crude extract of seaweed *Acanthophora Specifera*. In the present study six chemical constituents have been identified from methanolic extract of the *Acanthophora Specifera* by HPLC analysis. The total chromatogram of methanolic extract showing the HPLC profile of the compounds identified is given in the figure 1.

Flavonoid contents of *Acanthophora specifera* was determined and it shown that the content of Quercetin (1.41%), Catechol (0.59%), Vitexin-rahmnose (12.35%), Chlorogenic acid (69.64%) and caffeic acid (12.86%) respectively and given in the table 1.

4. Conclusion

Acanthophora specifera is rich source of vitexin-rahmnose, chlorogenic acid and caffeic acid which are main effective flavonoids and mentioned in the introduction for medicinal purpose. Isolation of individual phytochemical constituents from *Acanthophora Specifera* and subjecting them to meticulous biological screening can give fruitful results. From the results it could be concluded that *Acanthophora Specifera* contains various bioactive compounds. Therefore it is recommended as seaweed of phyto pharmaceutical importance.

5. Conflict of Interest

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

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