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EVALUATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT IN GONAD OF SEA URCHIN *Stomopneustes variolaris* AND SEAWEEDS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Authors KM and AS designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Both the authors read and approved the final manuscript.

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ABSTRACT

The Phenolic and Flavonoids are bioactive compounds which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions. In the present study total phenolic and flavonoid content was estimated in the gonads of sea urchin *Stomopneustes variolaris* collected from Arokiyapuram, Kanyakumari dt. (Tamilnadu, India). Seaweeds collected from the feeding grounds of *S. variolaris* were also analyzed for total phenolic and flavonoid content. The total phenolic and flavonoid content of the gonad was 59.3±3.4 and 18±1.3 mg/100 g tissue respectively. In seaweeds, phenolic content was ranged from 12.5±2.1 (*Chaetomorpha media*) to 16.4±2.1 mg GAE/100 g (*Stoechospermum marginatum*). Total flavonoid content in *Sargassum wightii*, *Stoechospermum marginatum* (Brown algae), *Gracilaria corticata, G. verrucosa* (Red algae), *Chaetomorpha media*, *Enteromorpha compressa*, *Ulva lactuca* (Green algae) were 4.1±1.2, 4.3±1.0, 3.7±1.4, 4.1±0.5, 3.2± 1.1, 4.0± 0.4 and 4.0± 0.5 mg RE/100 g respectively.

Keywords: Flavonoids; phenolic compounds; sea urchin gonad; seaweeds.

1. INTRODUCTION

Sea urchins belong to phylum echinoderm which also includes sea star, brittle star, sea cucumber and sand dollars. Sea urchins have rounded shell (test), covered by sharp spines with varied colour. In sea urchins, the gonad is the only edible part in both males and females and is called "Roe" regardless of sex [1]. They are herbivores grazing on attached marine plant (Kelp) and drifted algal fragments. *S. variolaris* is herbivore, feeding on a variety of sea weeds and occupies the habitats in close proximity to the rich algal growth.

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom [2]. Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development. Seaweeds are major sources for bioactive compounds since they produce wide range of biological activities such as antibacterial, antioxidant, anticancer, anticoagulant,

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and antiviral properties [3,4]. Seaweeds are low calorie food rich in vitamins, minerals, proteins, polyphenols, polysaccharides and dietary fibers [5,4].

The flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions [6,7]. They have potent antioxidants and have free radical scavenging abilities. Many have anti-allergic, anti-viral actions and some of them provide protection against cardiovascular mortality [8,9]. The flavonoids were shown to inhibit the growth of various cancer cell lines *in vitro* and reduce tumor development in experimental animals [10]. They also exhibited anti-coagulant, anti-hyperlipidic, anti-nepritic, vasodilative effects [11] and Human Immunodeficiency Virus (HIV) type 1 integrase inhibition [12]. The most active area of flavonoids research at the present time is in the possible therapeutic contribution that flavonoids make to human health [13]. As therapeutic potentials of phenolics and flavonoids are higher, present study was undertaken to estimate the total phenolic and flavonoid content in sea urchin gonad and seaweeds collected from its feeding grounds.

2. MATERIALS AND METHODS

2.1 Collection and Sample Preparation

The sea urchin *S. variolaris* was collected from Arokiyapuram (Lat. 08°04'N; Long. 77°36'E; Kanyakumari Dt., Tamilnadu, India). Animals with the dominant size group of 6 - 7 cm (shell diameter) was used for the present study. The gonad was separated out from the shell by cracked it into two halves along the vertical axis by cutting tool for further analysis.

The extraction and estimation of flavonoids was done in sea urchin gonad and sea weed (collected from the sea urchin feeding ground such as *Sargassum wightii*, *Stoechospermum marginatum* (Brown algae), *Gracilaria corticata, G. verrucosa* (red algae), *Chaetomorpha media*, *Enteromorpha compressa*, *Ulva lactuca* (Green algae).

2.2 Extraction

Total samples were extracted according to the method followed by Hertog et al. [14,15] with slight modification. 5 g of fresh gonad sample was homogenized with 40 ml of 75% methanol with 2 g/l t-butyl hydroquinone using a pestle and mortar. 10 ml of 6 M hydrochloric acid was added and carefully mixed and refluxed at 90°C for 2 hrs. After cooling the supernatant was filtered through whatman No.1 filter paper and transferred to a volumetric flask with methanol, further air was replaced with nitrogen gas to inhibit decomposition. The extracts were kept at -80°C until further analysis.

2.3 Estimation of Total Phenolic Content

The total phenolic content was determination by using Folin and Ciacalteau reagents [16,17]. The sample (1 ml, diluted to 50 to 25% of original concentration with methanol), 0.5 ml of Folin and Ciacalteau's phenol reagent (2.0N) and 3 ml of Na_2CO_3 (200 mg/ml) were mixed in the given order. The mixture was vortexed and the reaction was allowed to proceed for 15 mins. at room temperature and absorbance was measured at 725 nm in spectrophotometer (HITACHI 220S). Gallic acid was used as standard and the equivalents (mg/100 g) were determined from a standard concentration curve. Samples were analysed in triplicate.

2.4 Estimation of Total Flavonoid Content

Total flavonoid content was determined by Aluminium Chloride Calorimetric Assay [18]. 20 µL of sample extract was mixed with 20 µL of 10% aluminium chloride, 20 µL of 1 M potassium acetate and 180 µL of distilled water and kept at room temperature for 30 min. The absorbance was measured at 415 nm. A standard curve was plotted using different concentration of mehthanolic rutin solution and the amount of total flavonoid content was measured as rutin equivalents (RE) in mg/100 g of sample.

3. RESULTS

In the present study the mean total phenolic content in the gonad was recorded as 59.3±3.4 mg GAE/ 100 g and in seaweeds, phenolic content was ranged from 12.5 ± 2.1 (*Chaetomorpha media*) to 16.4± 2.1 mg GAE/100 g (*Stoechospermum marginatum*) (Table 1). Total flavonoid content of *S. variolaris* gonad was 18±1.3 mg (RE)/100 g tissue. In seaweed total flavonoid content was recorded highest in *S. marginatum* (4.3±1.0) and lowest in *C. media* (3.2±1.1), the values are presented in Table 1.

4. DISCUSSION

Phenolic compounds in plants were found extensively in the form of aromatic secondary metabolites and it also exhibits various biological activities including antioxidant properties. [19]. Polyphenolic compounds in sea weeds (algal polyphenolics) were called as phlorotannins [20]. This phlorotannins and fucoxanthin were found highest in brown sea weeds [21-23]. The results of the preset study matches with

Table 1. Total phenolic and flavonoid content of seaweeds[a]

Sea weeds	Total phenolic content mg GAE/100 g	Total flavonoid content mg (RE)/100 g
Sargassum wightii	16.2 ± 2.3	4.1 ± 1.2
Stoechospermum marginatum	16.4 ± 2.1	4.3 ± 1.0
Gracilaria corticata	12.5 ± 2.0	3.7 ± 1.4
Gracilaria verrucosa	16.1 ± 1.5	4.1 ± 0.5
Chaetomorpha media	12.3 ± 2.1	3.2 ± 1.1
Enteromorpha compressa	15.9 ± 1.5	4.0 ± 0.4
Ulva lactuca	16.0 ± 1.0	4.0 ± 0.5

[a] *Value are expressed as mean ±SD; (n = 3)*

previous works, total phenolic content was recorded higher in brown seaweed *S. marginatum* (16.4±2.1 mg GAE/100 g) followed by *Sargassum wightii* (16.2±2.3 mg GAE/100 g) and red seaweed *Gracilaria verrucosa* (16.1±1.5 mg GAE/100 g), which act as phytoalexins, antifeedants, contributors to the pigmentation, antioxidants and protective agents against UV light [24,25]. Similar result was also obtained by Angelina Mei Ling et al. [26] in red seaweed *Kappaphycus alvarezii*. In the present study, among the green seaweed maximum phenolic content was estimated (16.0±1.0 mg GAE/100 g) in *Ulva lactuca* followed by *Enteromorpha compressa* (15.9 ± 1.5 mg GAE/100 g). *Ulva clathrata* collected from lower intertidal and middle intertidal area showed higher concentration of phenolics and flavonoids due to the prolonged exposure to solar UV radiation may result in producing bioactive compounds such as phenolics and flavonoids [27].

In the present study flavonoid content (brown seaweed (*S. wightii* 4.1 ± 1.2 and *S. marginatum* 4.3 ± 1.0 mg RE/100 g), green seaweed (*Enteromorpha compressa* 4.0 ± 0.4 and *Ulva lactuca* 4.0 ± 0.5 mg RE/100 g) and red seaweed (*Gracilaria corticata* 3.7 ± 1.4 and *Gracilaria verrucosa* 4.1 ± 0.5 mg RE/100g) was well correlated with the previous workers on brown seaweed *Sargassum horneri* [28], green seaweed *Ulva clathrata* and *U. prolifera* [27] and Red seaweed *Gracilaria bursa-pastoris* [29] and *Kappaphycus alvarezii* [26].

Sea urchins are severe grazers of marine algae, mainly on brown seaweeds. The bioactive compounds such as phenolics and flavonoids present in seaweeds find their way into sea urchins through diet [30,31]. Archana and Babu [32] recorded 9.90 mgGAE/g of total phenolic content in the gonad of *S. variolaris* collected from Visakhapatnam. In *E. mathaei*, total phenolic content was estimated in methanol extract of gonad was 0.0044 ± 0.0003 mg GAE/g whereas, total flavonoid content in Ethyl acetate extract of gonad was 3.9 ± 0.41 mg BHT/g when compared with other body parts such as shell, spine and Aristotle lantern [31]. In present study total phenolic and flavonoid content in gonad of *S. variolaris* showed highest value (59.3 ± 3.4 mg GAE/100 g and 18 ± 1.3 mg RE/100 g) when compared with that of previous records. Since, *S. vriolaris* consumes more of brown and green seaweed especially in the middle and lower intertidal areas. Current results states that increase in total phenolic contents was also increase the total flavonoid content in seaweeds. But previous researchers (Angelina Lee Mei Ling et al. [26], Shipeng et al. [28], Farasat et al. [33], Farasat et al. [27], Ramdani et al. [29]) was recorded vice-versa to the present result may due to the physcio-chemical parameters of the study area.

Studies on Phenolics and flavonoids reveled that it remarkably contributes to the anti-lipidimic [34], anti-inflammatory [35] and antioxidants [36,37] properties extracted from plants and seaweeds. Many of the previous studies shown that a high dietary intake of natural phenolics and flavonoids was strongly associated with longer life expectancy, reduced risk of various types of cancer, diabetes, obesity, improved endothelial function and reduced blood pressure [38-40].

In seaurchin very few works was done on phenolics and flavonoids with great opportunity as a new source of bioactive substance for therapeutic uses [41,33]. Phenolics and Flavonoids are increasingly appreciated as being an important component of the human diet [32]. In recent years, food manufacturers and consumers moving towards functional food with specific health effects.

5. CONCLUSION

The finding of the present study on the presence of Phenolics and flavonoids in the gonad of the sea urchin *S. variolaris* indicates a new source of bioacitve compound from marine. From the author's point of view sea urchin gonad is a new seafood

commodity to the mankind can be used as supplementary food and to extract therapeutic compounds for the pharmaceutical industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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