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ANTICOAGULANT AND ANTI-INFLAMMATORY POTENTIALS OF SOME EGYPTIAN MARINE ALGAE

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ABSTRACT

Marine algae are significant ingredients to formulate drugs for treating disease as they possess various bioactive potentials. Different marine algae (*Laurencia papillosa* and *cylindrica* (red algae) were collected from Red Sea (Faied and Ein Al-Sokhna- Suez). *Ulva fasciata* (green algae) was collected from the Mediterranean Sea (Abu-Qir near Alexandria) and *Dilophus fasciola* (brown algae) was collected from Marsa Matrouh. Phenolics, flavonoids and tannins content of the different algae species were estimated and the ethanol extract of these algae species were evaluated for their role as anticoagulant and anti-inflammatory. The results declared that the highest concentration of phenolics, flavonoids and tannins were detected in *D. fasciola* followed by *G. cylindrica* and *L. popillosa*. *U. fasciata* and *D. fasciola* exhibited high anticoagulant activities. Results also markedly demonstrated a significant increase in anti-inflammatory activity of *D. fasciola* and *G. cylindrica* as they recorded inhibition percent 40.62% and 43.64%, respectively at the dose of inhibitor 200 mg/ml. Thus, these bioactive compounds can be characterized and tested the efficacy of the future use for the public. Research institutions should collaborate with industry in order to design and develop suitably appealing products with these bioactive compounds.

KEYWORDS: **Marine algae, Phenolics, Flavonoids, Tannins, Anticoagulant, Anti-Inflammatory**

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INTRODUCTION

Algae represent valuable sources of a wide spectrum of secondary metabolite such as carotenoids, terpenoids, xanthophylls, chlorophyll, vitamins, polyphenols, flavnoids alkaloids and halogenated compound [1]. Macroalgae or seaweeds are one of the important marine living resources and are an excellent source of Vitamins (A, B, B12, C, D & E), riboflavin, niacin, panthothanic acid and folic acid as well as minerals such as Ca, P, Na and K [2]. The importance of macroalgae derives from their bioactive metabolites used in the pharmaceutical industry especially in drug development. Many of bioactive compounds are used to treat diseases mainly cancer, acquired immune-deficiency syndrome (AIDS), inflammation, pain, arthritis, in addition viral, bacterial, and fungal infections [3]. Marine algae have shown their potential as important sources of bioactive compounds [4]. Natural products from marine algae may use in developing anti-inflammatory drugs. Some marine natural products show useful pharmacological activities and are being developed either as analgesics or to treat inflammation [5]. Many sessile and soft bodied benthic organisms possess defensive mechanisms based on the use of chemical compounds, which often display high biological activity [4]. Recent study on marine organisms is in fact providing many bioactive natural products, in larger percentages than terrestrial organisms [6]. Red algae are known as the largest species among algae, present the greatest chemical diversity recorded to date in the literature, and supply many useful substances for the treatment diseases that affect living beings. These algae are a source of variable bioactive components, such as polyphenols, alkaloids, and terpenes, and particularly polyphenols, which considered as one of the most numerous and widely distributed chemical groups. Red algae are classified into different families with different contents of phenolic acids, phenylpropanoids, flavonoids, and due to their various pharmacological activities such as antioxidative, α-glucoside inhibitory, antimicrobial, aldose reductase inhibitory, antitumor, and anti-inflammatory activities [7]. Also, Lakmal et al. [8] concluded that the phenol compound from two species of green algae (*Chaetomorpha crassa* and *Caulerpa racemosa*), and brown algae species (*Sargassum cassifolium*) and three species of red algae (*Chondrophycus ceylanicus, Gelidiella acerosa,* and *Gracilaria corticata*), showed inhibitory effect on a human promyelocytic leukemia (HL-60), a human lung carcinoma (A549) and a mouse melanoma (B16F10) were assessed *in vitro*. This study aims to evaluate the biological activity of ethanol extract of some marine algae as anticoagulant and anti-inflammatory activities as well as, characterization of their chemical constituents.

MATERIALS AND METHODS

(i) Materials

1. *Collection of marine algal samples*

Four algal samples of *Laurencia papillosa* and *cylindrica* (red algae) were collected from Red Sea (Faied and Ein Al-Sokhna-Suez). *Ulva fasciata* (green algae) was collected from the Mediterranean Sea from (Abu-Qir near Alexandria) and *fasciola* (brown algae) was collected from Marsa Matrouh, and were used throughout this study.

2. *Identification of algae species*

After preparation of herbarium specimens of the algae, they were identified by Dr**.** Rauhaiya Abdul-Latif, Professor of Botany Department, Faculty of Science, Al-Azhar University, Cairo, Egypt with the authentication number 0011325 for *Ulva fasciata,* 3705399:11 for *fasciola*, 2231271:19 for *Laurencia papillosa* and 1871:62 for *cylindrica*.

(ii) Methods

1. *Preparation of marine algae samples*

Marine algae collected from different sites were washed several times with tap water, air dried in shaded area. The dried samples were grinded into fine particle by electric mill and stored in glass containers at room temperature for further experiments.

2. *Preparation of algal extract*

Ten grams of the dried powder from marine algae were soaked with 100 ml 80% ethanol and shaking at room temperature for 48 hours. The extracts were filtered and re-extracted twice. The final extract was used for the determination of total phenolics, flavonoids, and tannins and their anticoagulant and anti-inflammatory activity [2].

3. *Phytochemicals*

a. Phenolics content

Phenolics of algal ethanol extract were determined by using Folin-Ciocalteu's reagent [9]. The reaction mixture was prepared from mixing 0.5 ml of algal ethanol extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The samples were incubated in a thermostat at 45^{o}C for 45 min. The absorbance was spectrophotometrically recorded at 760 nm. A standard series concentrations of Gallic acid was prepared in ranges 2-12 µg/ml and were treated as the samples.

b. Flavonoids content

Flavonoids content of marine algae extract was spectrophotometrically determined by the aluminum chloride method using quercetin as a standard [10]. 0.2 ml of extract or standard solution (quercetin, 20-120 mg/l) was mixed with 0.3 ml 5 % $NaNO_2$. After 5 min, 0.3 ml of 10% $AlCl_3$ was added then incubated for 6 min and 2 ml of 1 mol/l NaOH was subsequently added. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using a spectrophotometer.

c. Tannins content

Tannins content of marine algae extract was measured using the Folin-Ciocalteu reagent assay according to Tambe and Bhambar [11]. About 0.1 ml of algal ethanol extract or standard solution of (tannic 20-120 mg/l) was added to 7.5 ml distilled water then add 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution. The volume was made up for 10 ml with distilled water and absorbance was measured against prepared reagent blank at 725 nm by using spectrophotometer.

4. *Biological evaluation of algal ethanol extract*

a. *Anticoagulant activity of algal ethanol extracts*

Anticoagulant activity of the algal ethanol extract was determined according to the method of Hassan et al.[12]. Blood was collected from NRC rates into 8% sodium citrate solution in the proportion of 1:19 volumes of blood. The mixture was immediately agitated by gentle inversion, centrifuged at 5000 rpm at 4˚C and the separated canary yellow plasma was pooled. Algal ethanol extract (0.8 ml), and standard heparin sodium solution (0.5 U.S.P. units / 0.8 ml) were used as positive control, or 0.8 ml saline solution as negative control was placed in glass tubes. Then, 1 ml plasma and 0.2 ml of 1% calcium chloride solution were added to each tube. Tubes were stopper immediately and the time was recorded, and inverting three times in such a way mixed the contents that the entire inner surface of the tube was wet. The time required for clotting was determined.

b. *In-vitro anti-inflammatory activity of algal ethanol extract*

Anti-inflammatory of algal ethanol extract was tested using the method of Rahman et al.[13] .The different concentration of algal extract or standard drug diclofenac sodium (50, 100, 150,200 ug/ml) was mixed with 0.45ml bovine albumin serum. The sample extracts were incubated at 37°C for 20 min and then heated to 57°C for 3 min after cooling the samples was added 2.5 ml phosphate buffer pH 6.4. The absorbance was measured using UV visible spectrophotometer at 255 nm.

5. *Statistical analysis*

Data were analyzed by analysis of variance (ANOVA), SPSS coupled with Co-stat computer program, where unshared letter is significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

1. *Phenolics, flavonoids and tannins content of different marine algae*

Table (1) showed that the highest concentration of phenolics, flavonoids and tannins in *D. fasciola* (113.30, 51.97 and 82.24 mg/g, respectively) followed by *G. cylindrica* in phenolics (54.69) and *L. papillosa* in flavonoids and tannins (16.07 and 44.15mg/g, respectively). Polyphenols are well-known potent biologically active agents, but their wide diversity and chemical complexity makes it challenging to correlate their inflammation potency *in vitro* with specific biological activity *in vivo* [1]. Red algae provides a rich source of polyphenols as presented in this study that can be used to design and develop novel potentially useful therapeutic agents. Recent findings regarding the activities of flavonoids such as antiviral, antifungal, antioxidant, anti-inflammatory, antithrombic, anticarcenogenic, hepatoprotective and cytotoxic have generated interest in studies of flavonoids containing plants [14]. The presence of quinone was observed in *Padinatetra stromatica.* Similar result was earlier reported by Thinakaran et al. [15], in the same alga *Padina tetrastromatica,* coumarins were recorded in four solvent extracts except ethanol. In the present study, the presence of various secondary metabolites in the marine algae is a clear indication of their pharmaceutical potential. The secondary metabolites may be useful in containing the infection, act as hypoglycemic agents, reduce blood pressure and regulate cholesterol levels [14]. Coumarins are a phenolic compounds were found to inhibit telomerase activity in tumor cells [16]. In most tumors the maintenance of telomeres occurs with the telomerase expression[17]. Marine algae have shown to be a good source of unsaponifiable, nontoxic sterols that have medicinal value [13]. Flavonoids comprise a large group of naturally occurring compounds widely distributed in the plant kingdom and some of these compounds have been reported to contain various and potent biological activities including antioxidative tissue protective and tumoristatic effects as well as the inhibition of hepatic cholesterol biosynthesis [18]. Phytosterols, carboxylic acid and saponins were found in most of marine algae. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, antifeedent and hemolytic effects [14]. Earlier reports showed that different marine algae possess various activities in different solvents[19]. This could be attributed to the presence of various kinds of phytoconstituents such as phenols, carbohydrates, proteins, tannins, flavanoids, saponins, terpenoids etc [19]. Kayalvizhi et al. [20] reported that the acetone extract of brown microalgae showed significant antifungal activity against five fungal species. Phenolic toxicity to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound [21]. Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection and are often found effective *in vitro* as an antimicrobial substance against a wide array of microorganisms [21, 22].

2. *Anticoagulant activity of different marine algae*

Table (2) demonstrated blood anticoagulant activity *via* prothrombin time (PT) test. Results revealed that *U. fasciata and D. fasciola* exhibited remarkably high anticoagulant activities after 10 and 20 minutes. While, low anticoagulant activity was recorded for *L. papillosa* and *G. cylindrica*. The anticoagulation mechanism of algae may be attributed to direct inhibition of thrombin and potentiating of antithrombin III. Prolonged activated partial thromboplastin time (APTT), suggesting inhibition of intrinsic factors and increased intrinsic pathway-dependent clotting times. Generally, the anticoagulant activities of the extracts were less than heparin which was used as standard anticoagulant. The present results showe that the different algal extracts possess anticoagulant activity. This anticoagulation activity may be due to the presence of uronic acids. The present results are in concomitant with Toshihiko et al. [23] and El-Baroty et al. [24] who explained that, the polysaccharides containing uronic acids, carrying a negative charge, have the ability for binding calcium ions and therefore prevent the formation of clot. In addition, variation in anti-coagulating activity of the different extracts is probably due to the quantity of uronic acids in polysaccharides and some conformational differences in the molecules of these polysaccharides [23,24]. While, Mao et al. [25] declared that the anticoagulant activities of the sulfated polysaccharides from the green algae *Ulva conglobate*

were weaker than heparin on the same concentration. Anticoagulant activity is largely dependent on the sugar composition, sulfate content, sulfate position and molecular weight of the compound. Shanmugam and Mody [26] suggested that suitable length and/or conformation and moderate extent of negative charge density of the polysaccharide molecule would be required for expression of its effective anticoagulant activity.

3. *Anti-inflammatory activity of marine algae*

Table (3) demonstrated significant increase in anti-inflammatory activity of *D. fasciola* and *G. cylindrica* as they recorded inhibition percent reached to 40.62 and 43.64% at the dose of inhibitor 200 ug/ml. The high concentration of inhibitors, the high percentages of inhibition i.e dose- dependent manner was notice for all algae species at the different concentrations (50-200 ug/ml). While, *U. fasciata* and *L. papillosa* recorded percentages of inhibition 37.48 and 24.66 % at the same concentration (200µg/ml) as compared to reference drug (88.47%). The species provided the best results for avoiding the release of inflammatory mediators at non-cytotoxic concentrations were arranged respectively *G. cylindrica*, *D. fasciola*, *U. fasciata* and finally *L. papillosa*. These strains showed also the highest content of phenolics, flavonoids and tannins (Table 1). Since inflammation is caused by the release of chemicals from tissues and migrating cells, thus, the active extracts could be useful in avoiding inflammation and pain 6. Inflammation is a defense response in a wide variety of physiological and pathological processes caused by stress, injury and infection [27]. This process contributes to impairment on the immune system when persisted for a long period of time as activated macrophage produces toxic factors. Macrophages activated by lipopolysaccharide (LPS) or interferon gamma secrete nitric oxide (NO), cyclooxygenase-2 (COX-2) and other intermediates to destroy the remaining microorganisms in the inflammation response. In particular, exposure to LPS activates macrophage to secrete pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 through the expression of inducible nitric oxide synthase (iNOS) and NO whereas prostaglandins production is mediated by COX-2. [1] Moreover, this process activates a set of extracellular stimuli-dependent signal transduction cascades such as the mitogen-activated protein kinases (MAPKs) pathway [1]. Chao et al. [28] declared that, flavonoid compounds has anti-inflammatory effect through modulation of NFκB. In addition, Engler and Engler [29] attributed the ability of immune function modulation to flavonoids as they also emerging as a potential cardioprotective effects, specifically, reduction of pro-inflammatory cytokines. However, Hatcher et al. [30] demonstrated the anti-inflammatory features of resveratrol active phytochemicals compounds such as, curcamin, resvatol, capsaicin, catechins, vitamins, beta carotine, tannins, flavonoids and polyphenols to their abilities to moderate cell Mitogin-Activated Protein Kinases (MAPK) signaling pathways, proliferation, apoptosis, redox balance besides, being a protective agents towards many diseases such as, cancer, neurodegenerative disorders and cardiovascular. Moreover, M Kowalski et al. [31] declared that the polyphenols anti-inflammatory properties may be mediated by suppressing effect of NF-kB and AP-1 transcription. Also the anti-inflammatory effects of polyphenolics compound can be attributed to their readily metabolization to phenolic acids and aldehydes by the microflore of the intestine [32].

Table 1
Phenolics, flavonoids and tannins content of different marine algae

Algae species	Phenolics (mg/g)	Flavonoids (mg/g)	Tannins (mg/g)
U. fasciata	2.01±0.001 [a]	14.66±1.43 [a]	6.57±0.56 [a]
D. fasciola	113.31±2.77 [c]	51.97±2.22 [b]	82.24±3.90 [c]
L. papillosa	5.61±0.22 [e]	16.07±1.90 [a]	44.15±1.89 [b]
G. cylindrica	54.69±2.89 [b]	12.32±1.10 [a]	5.30±0.21 [a]
LSD	4.30	18.17	6.7

Data were expressed as Mean±SD of three replicates and analyzed using analysis of variance (ANOVA), SPSS (version 8) coupled with Co- stat computer programs, where unshared letter is significant at P≤ 0.05.

Table 2
Anti-coagulant activity of ethanol extracted of different marine algae

Algal strain	Concentration (50 µg/ml) Time of clotting	
	10 minutes	20 minutes
U. fasciata	++++	++++
L. papillose	+++	+++
G. cylindrica	+++	+++
D. fasciola	++++	++++

Table 3
Anti-inflammatory activity of ethanol extracted of different marine algae

Algal lipid concentration (µg /mL)	Inhibition % Algal species				
	G. cylindrica	*D. fasciola*	*U. fasciata*	*L. papillosa*	Diclofenac Sodium
50	23.64± 5.12^{ijk}	22.26 ± 0.39^{jk}	27.73 ± 2.77^{hi}	12.82± 1.31^{i}	74.04 ± 0.37^{c}
100	27.42± 0.78^{hi}	32.07 ± 3.6^{gh}	32.57 ± 1.46^{g}	19.85± 6.16^{k}	81.74 ± 0.63^{b}
150	34.79± 2.58^{fg}	37.60 ± 3.77^{ef}	35.27 ±1.67^{fg}	24.27± 0.61^{ijk}	85.5 ± 0.21^{ab}
200	43.64± 6.16^{d}	40.62±2.35^{de}	37.48±1.21^{ef}	24.66± 1.22^{ij}	88.47 ± 6.30
LDS	4.98	4.45	4.76	3.56	2.87

Data were expressed as Mean±SD of three replicates and analyzed using analysis of variance (ANOVA), SPSS (version 8) coupled with Co- stat computer programs, where unshared letter is significant at P≤ 0.05.

CONCLUSION

Marine algae are rich sources of many active compounds such as dietary fiber, minerals, proteins, vitamins, and antioxidant activity of these seaweeds would elevate their value in the human diet as food and pharmaceutical supplements. Indeed, marine algae contains polyphenols, carotenoids and flavonoids referred to as antioxidants, protect the body's tissues against oxidative stress and associated pathologies such as cancer and inflammation. Moreover, flavonoids can be used clinically to treat patients with hypercholesterolemia and hypertension.

CONFLICT OF INTEREST

Conflict of Interest declared none.

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