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Pharmacological screening of some Egyptian marine algae and encapsulation of their bioactive extracts in calcium alginate beads

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ABSTRACT

The pharmacological screening of some crude extracts obtained from different marine algae collected from the Mediterranean in Alexandria region in Egypt has been conducted. The methanol, butanol, and ethyl acetate extracts of *Pterocladia capillacea*, *Ulva lactuca* and *Corallina mediterranea* showed inhibitory activity against the growth of *Lemna minor* plant. The methanol and petroleum ether extracts of *Ulva lactuca* (VII) exhibited significant antioxidant activity 92.2 and 84.6%, respectively, in the DPPH free radical scavenging assay. The methanol and ethyl acetate extracts of *Pterocladia capillacea* and *Corallina mediterranea* showed remarkable inhibitory activity against butyrylcholinesterase. The ether extract of *Pterocladia capillacea* (III) and the petroleum ether extract of *Ulva lactuca* (VII) exhibited high antiglycation activity (69.4% and 65% with IC_{50} of 0.0596 ± 0.0447 mM and 0.138 ± 0.0441 mM, respectively). The ether extract of *Ulva fasciata* (I) potently inhibited the oxidative burst activity of phagocytes from whole blood analysis (97.99%) at a concentration of 400 µg/mL. Scanning by electron microscope (SEM) showed successful encapsulation of the bioactive algal extracts into calcium alginate beads. Tested marine macroalgae have the potential to produce bioactive metabolites with significant antifungal, phytotoxic, antioxidant, anticholinesterase, antiglycation, and immunomodulating activities.

INTRODUCTION

Marine algae, also called macroalgae or seaweed, are photosynthetic eukaryotic species that thrive in coastal areas. Marine algae's chemical composition can be influenced by environmental factors such as temperature, salinity, sunlight, pH, physiological state, and CO_2 availability (**He *et al*., 2013a, b; Trivedi *et al*., 2015**). Given their ability to survive in harsh environments, macroalgae contain a number of natural bioactive compounds and metabolites, including polysaccharides, polyunsaturated fatty

acids, and phlorotannins (**Cheng *et al*., 2010; Hultberg *et al*., 2013; Wang *et al*., 2014**). Marine algae have been widely used in different applications (**Khaled *et al*., 2005; El Nemr *et al*., 2005, 2015, 2021; Shoaib *et al*., 2020a, b; Hassaan *et al*., 2021**).

Macroalgae and their extracts are excellent sources of naturally occurring antimicrobial compounds. They have been evaluated for their possible utility as therapeutic options for a variety of infectious diseases (**Wefky *et al*., 2009; Shobier *et al*., 2010, 2016; El Ashry *et al*., 2011; Abu-Ghannam *et al*., 2013**). Polyphenols, for example, are bioactive compounds with anti-cancer, anti-diabetic, and anti-inflammatory properties **(Fernando *et al*., 2016)**. The natural sources of antioxidant extracts from seaweeds have been analysed in detail, and it has been discovered that there is a strong association between total phenolic content and antioxidant activity (**Wang *et al*., 2009; Cox *et al*., 2010; Roohinejad *et al*., 2017**). Frequently, polysaccharides possess important antioxidant and immunomodulatory properties (**Hifney *et al*., 2016; Liu *et al*., 2017**).

Numerous marine algae species have been found to be involved in the treatment of a variety of human diseases; however, only a few have been studied in the treatment of neurological disorders such as Alzheimer's disease (AD) (**Suganthy *et al*., 2009**). Additionally, macroalgae such as *U. pinnatifida* and *F. vesiculosus* were discovered to have significant anti-glycation activity (**Fitton *et al*., 2016**).

Numerous bioactive extracts from macroalgae, especially the *Cystoseira genus*, have been investigated as potential leishmaniasis treatments (**Freitas-Junior *et al*., 2012; de Sousa *et al*., 2017**). Additionally, marine algae are a natural resource for the production of environmentally friendly and novel botanical insecticidal products (**Isman, 1995; Sahayaraji *et al*., 2012; Bantoto *et al*., 2013**). Their crude extracts facilitate the development of a novel approach to integrated pest management (**Rajesh *et al*., 2011; Asha *et al*., 2012; Hamed *et al*., 2018**). It has been shown that the chemical constituents of algal extracts have a beneficial effect on the growth and development of plants (**Khan *et al*., 2009; Chojnacka, 2012**).

Alginate molecules, an anionic polysaccharide derived from brown algae cell walls, can be cross-linked with di- and/or trivalent cations, especially Ca^{2+} cations, to form gels, which is advantageous for a variety of encapsulation applications (**Mikkelsen and Elgsaeter, 1995; Paredes Juárez *et al*., 2014; Puguan *et al*., 2015; Lee *et al*., 2016; Mahdi *et al*., 2016; Ching *et al*., 2017**); Due to its biocompatibility and non-toxicity, calcium alginate gel is well suited for bioencapsulation applications in fruit, pharmaceutical, and nutraceutical products (**Lee *et al*., 2016; Ching *et al*., 2017; Lee *et al*., 2017**).

To the best of our knowledge, the pharmacological screening especially (cytotoxicity, phytotoxicity, anti-leishmanicidal, insecticidal, anti-oxidant, acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), anti-glycation and the immunomodulating activities) of the Egyptian marine algae such as *U. lactuca*, *U.*

fasciata, C. mediterranea, and *P. capillacea* has been rarely or never studied. In this study, all of these pharmacological screenings in addition to the antibacterial and antifungal effect of the crude extracts of these algal species, collected from Alexandria coast, Egypt, were evaluated. Moreover, *U. lactuca* and *P. capillacea* extracts were encapsulated in calcium alginate beads and their surface morphology was studied.

MATERIALS AND METHODS

Materials

Extractions were carried out using distilled industrial grade solvents. Sisco Research Laboratories Pvt. Ltd., India provided the sodium alginate. Marine algae*; Ulva lactuca* (Linnaeus), *Ulva fasciata* (Delile), *Pterocladia capillacea* (Gmelin) Bornet and Thuret and *Corallina mediterranea* (Areschoug), were collected from the sub littoral rocks of Abu Qir Bay in Alexandria region of the Egyptian Mediterranean.

Preparation of Algal Extracts

The algae were washed with water and dried in the shade at room temperature (252 °C). The dried algae (100 g) were ground (200 mesh) and soaked for two weeks in methanol (1.5 L). Since evaporating the methanol extracts, they were extracted with petroleum ether, ether, and ethyl acetate. Each extract was evaporated and stored in the freezer before biological screening was performed. The methanol extract, on the other hand, was fractionated between *n*-butanol and water. The organic and aqueous layers were isolated and evaporated under reduced pressure, followed by biological screening of the final extracts (**El Ashry *et al*., 2011**).

Preparation of Calcium Alginate Beads Containing Algal Extracts

In this study, the bioactive ether and methanol extracts of *P. capillacea*, as well as the aqueous extract of *U. lactuca* were encapsulated. Calcium alginate beads (CABs) were used as a supporting material for the entrapment of the bioactive algal extracts. They were prepared by cross-linking with the divalent calcium ions. Thus, a 2% solution of sodium alginate (Na Alg) in distilled water was prepared using a hot plate and gentle heating and stirring. The extract was then applied in the necessary quantity and stirred until fully dispersed in the Na Alg solution. Under stirring, this solution was applied dropwise to 100 mL of 0.2 M $CaCl_2$ solution. The calcium alginate beads resulting from this procedure were left in the calcium chloride solution for 1.5 hours. Filtration was used to separate the beads, which were then rinsed with distilled water and dried under vacuum (**Tanaka *et al*., 1984; Kenawy and Sakran, 1996**). Finally, they were connected to stubs with two-sided adhesive tape and coated with a sheet of gold (15-20 nm) before being tested at a 20 kV acceleration voltage. The scanning electron microscope SEM was used to examine the morphology of calcium alginate beads (JEOL JSM-6360LV-Japan).

Pharmacological Screening

Antibacterial test

By using the well diffusion method, the extracts were tested for antibacterial activity against Escherichia coli, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi isolates* (**Carron *et al.*, 1987**). As a positive control, imipenem (Primaxin), an intravenous *β*-lactam antibiotic, was used, while DMSO was used as a negative control.

Antifungal test

The antifungal bioassay was performed on human (*Aspergillus flavus, Candida albicans, Candida glaberata, Trichophyton longifusus*,), animal (*Microsporum canis*) and plant (*Fusarium solani*) pathogens using both organic and water extract fractions of the algae, following agar tube dilution assay (**Shaukat *et al.*, 1980; Choudhary *et al.*, 1995**). Growth in the extract-containing media was determined by measuring linear growth (mm), and inhibition of growth was calculated using the negative control as described in equation 1.

$$Inhibition\,\% = 100 - \left(\frac{\text{linear growth in test (mm)}}{\text{linear growth in control (mm)}}\right) \times 100 \qquad (1)$$

Cytotoxic activity test

Cytotoxicity of the crude extracts was checked through brine shrimp (*Artemia salina*) assay (**Meyer *et al.*, 1982; Dey, 1990; Kivçak *et al.*, 2002; Carball *et al.*, 2002**).

Phytotoxicity test

The *Lemna* bioassay was used to investigate the phytotoxicity of various algal extracts (**Itokawa *et al.*, 1917; Finney, 1971; Atta-ur-Rahman, 1991**). The results were expressed as a percentage of growth regulation, measured in comparison to the negative control.

Antileishmanicidal activity test

This bioassay was performed as previously described by **Ash and Orihel (1987)** and **Habtemariam (2003)**.

Insecticidal test

The impregnated filter paper technique was used for the assay (**Tbassum *et al.*, 1997; Atta-ur-Rahman and Choudhary, 2001**). The survival of insects was assessed and the percentage mortality was calculated using equation 2.

$$Mortalit\,\% = 100 - \left(\frac{\text{Number of insects alive in test}}{\text{Number of insects alive in control}}\right) \times 100 \qquad (2)$$

Antioxidant activity test

Both DPPH and nitric oxide free radical scavenging assays were used according to **Lee *et al*. (1998)** and **Badami *et al*. (2003)**.

Cholinesterase inhibition test

Acetylcholinesterase AChE (EC 3.1.1.7) and butyrylcholinesterase BChE (EC 3.1.1.8) inhibitory activities were measured by the spectrophotometric method (**Ellman *et al*., 1961**). The (%) inhibition = ((*E*-S)/*E* x 100); *E* is the activity of the enzyme without test extract and *S* is the activity of enzyme with test extract (**Ahmad *et al*., 2005**).

Antiglycation test

The *in vitro* antiglycation assay was performed according to **Matsuura *et al*. (2002)**, after modifications. The fluorescence activity was determined and the percent inhibition was estimated using the equation 3 below (**Kim and Kim, 2003**).

$$Inhibition\% = 100 - \left(\frac{\text{Fluorescence of sample}}{\text{Fluorescence of glycated}}\right) \times 100 \qquad (3)$$

Immunomodulating activity test (Chemiluminescence assay)

As defined by **Helfand *et al*. (1982)**, a luminol-enhanced chemiluminescence assay was performed.

RESULTS AND DISCUSSION

1. Pharmacological Studies

1.1. Antibacterial activity

Different types of pharmacological screening for the selected algal extracts were evaluated. The algal extracts of the marine algae *U. lactuca, U. fasciata, P. capillacea* and *C. mediterranea* were screened for their antibacterial activity. The results showed that these extracts possess no or insignificant activity against test bacteria (**Table 1**). The antibacterial activity of the ethyl acetate and methanol extracts of the same algae species against six fish and human pathogenic bacteria has been previously studied and showed that the highest activity was exerted by the methanol extract of *P. capillacea* and *U. lactuca* against the fish pathogens; *A. hydrophila, V. anguillaum, P. fluorescens* and *P. aeruginosa* (**Wefky *et al*., 2009**). The antibacterial activity of different crude extracts (hexane, chloroform, ethyl acetate, acetone and methanolic) of the brown alga; *Stoechospermum marginatum* (Ag) Kutz against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. flexneri* and *V. cholerae* has been evaluated (**Raj *et al*., 2015**). When compared to other extracts, the ethyl acetate extract demonstrated the highest activity against all test bacterial strains, with mean zones of inhibition ranging from 7.1 to 18.1 mm and MIC values between 125

and 500 g/mL (**Raj *et al*., 2015**). **Moubayed *et al*. (2017)** screened methanol and acetone extracts of three seaweed species obtained from the Red Sea and Arabian Gulf, Saudi Arabia: *Sargassum latifolium B, Sargassum platycarpum A,* and *Cladophora socialis*. The brown seaweed *S. latifolium* acetone extract exhibited the highest inhibitory activity against Salmonella sp., followed by *S. platycarpum* A and C. socialis acetone extracts. **El Shafay *et al*. (2016)** investigated the antibacterial activity of diethyl ether, methanol, ethanol, and chloroform extracts of several Egyptian marine macroalgae (*Ceramium rubrum*, *Sargassum vulgare*, *Sargassum fusiforme*, and *Padina pavonia*) against ten multidrug resistant (MDR) bacteria. The brown alga *S. fusiforme* diethyl ether extract demonstrated promising activity against *S. aureus* 2, while the ethanol extract of *S. vulgare* was the most effective against *K. pneumoniae*.

1.2 Antifungal activity

The data of the antifungal assay showed that some of the extracts exhibited an inhibitory activity against different tested fungal pathogens (**Table 2**). The ether extract of *P. capillacea* (III) showed significant activity (80%) against *M. canis* and good activity (60%) against *T. longifusus*. Moreover, the methanolic extract of the same alga showed 60% activity against *M. canis* and moderate activity (50%) against *T. longifusus*. The methanol extract of *P. capillacea* (IV) possessed moderate activity against both *A. flavus* and *T. longifusus*, while the methanol extract of the same alga *P. capillacea* (V) did not inhibit the tested fungi. Further, among the tested extracts only, the methanol extract of *P. capillacea* (IV) showed antifungal activity (50%) against *A. flavus,* while the standard antifungal agent amphotericin B exhibited non-significant activity (20%). In the present study, the methanol extract of *P. capillacea* could not inhibit the growth of *C. albicans* and *F. solani* conversely; **Shobier *et al*. (2016)** reported that the extract was inactive against both pathogens. Lobophorolide isolated from the marine brown alga *Lobophora variegata*, collected from the islands of the Bahamas and from the Red Sea, Hurghada, Egypt, exhibited antifungal activity against wild-strain and amphotericin-resistant-strain *C. albicans*, with IC_{50} values of 1.3 and 0.5 µg/mL, respectively (**Kubanek *et al*., 2003; El-Hossary *et al*., 2017**). The butanol extract of *U. lactuca* (VII) showed good activity (70%) towards *M. canis*, and moderately inhibited the growth of *T. longifusus*. Furthermore, the petroleum ether extract of the same alga showed a moderate inhibition of *T. longifusus* (50%). The other extracts were inactive against the tested pathogens (**Table 3**). Ethyl acetate and methanol extracts of *U. lactuca* have been reported to possess antifungal activity against *F. solani,* and *C. albicans* (**Shobier *et al*., 2016**). In our study, butanol and petroleum ether extracts of *U. lactuca* had no activity against the two pathogens. Caulerprenylol B, isolated from the green alga *Caulerpa racemosa*, possessed potent antifungal activity against *T. rubrum*, with an MIC of 4 µg/mL. Capisterones A and B, isolated from the green alga *Penicillus capitatus,* exhibited potent

antifungal activity against Lindra thallasiae with LD_{50} of 0.03 and 0.94 µg/mL, respectively (**Puglisi *et al*., 2004; Liu *et al*., 2013; El-Hossary *et al*., 2017**).

Table 1: Antibacterial activity of different algal extracts.

Algal species	Tested extract	*Escherichia coli*	*Bacillus subtilus*	*Shigella flexenari*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhi*
U. fasciata I	Ether	-	12	-	-	-	-
C. mediterranea II	Methanolic	-	10	-	-	-	-
	Ethyl acetate	-	10	-	-	-	-
P. capillacea III	Methanolic	-	10	-	-	-	-
	Ether	-	12	-	-	12	12
P. capillacea IV	Methanolic	-	10	-	-	-	-
P. capillacea V	Methanolic	-	-	-	-	12	10
P. capillacea VI	Methanolic	-	-	-	-	-	-
	Aqueous	-	10	-	-	-	-
U. lactuca VII	Butanolic	10	12	-	-	11	-
	Petroleum ether	-	-	9	-	12	-
	Imipenem	30	33	27	45	24	25

Zone of inhibitions in mm; Conc. of samples: 3 mg/ml DMSO; *U. fasciata* I: alga collected in spring 2002; *C. mediterranea* II: alga collected in winter 2003; *P. capillacea* III: alga collected in spring 2003; *P. capillacea* IV: alga collected in summer, June 2003; *P. capillacea* V: alga collected in summer, July 2003; *P. capillacea* VI: alga collected in summer, June 2005; *U. lactuca* VII: alga collected in summer, July 2005.

Table 2: Antifungal activity of different algal extracts.

Algal species	Tested extract	*Aspergillus flavus*	*Candida albicans*	*Candida glaberata*	*Trichophyton longifusus*	*Microsporum canis*	*Fusarium solani*
U. fasciata I	Ether	0	0	0	20	0	0
C. mediterranea II	Methanolic	20	0	0	0	0	0
	Ethyl acetate	0	0	0	20	0	0
P. capillacea III	Methanolic	0	0	0	50	60	0
	Ether	0	0	0	60	80	0
P. capillacea IV	Methanolic	50	0	0	50	20	20
P. capillacea V	Methanolic	0	0	0	20	40	0
P. capillacea VI	Methanolic	20	0	0	0	0	0
U. lactuca VII	Aqueous	0	0	0	20	0	0
	Butanolic	0	0	0	50	70	0
	Petroleum ether	20	0	0	50	40	0
	Miconazole		110.8	110.8	70	98.4	73.3
	Amphotericin B	20					

% Inhibition of the fungs; Conc. of samples: 400 µg/ml DMSO; Incubation temperature 27 ^{o}C; Incubation period 7 days

1.3. Cytotoxicity activity

Cytotoxicity studies revealed that none of the extracts investigated were cytotoxic to brine shrimp. We investigated the cytotoxicity of 22 ethanol extracts of seaweed plants. Only five brown seaweeds, *S. marginatum*, *S. swartzii*, *S. binderi*, *S. asperum*, and *S. indica*, demonstrated an LC_{50} value less than 1000 µg/mL, while only one green seaweed, C. racemose, demonstrated significant activity. *n*-Hexane soluble fractions of *S. marginatum* and *S. swartzii* ethanol extracts were found to be active, while methanol soluble fractions of *S. asperum* and *S. binderi* displayed the greatest cytotoxicity. As compared to ethanol extracts and their fractions, water extracts of *S. indica* and *C. racemosa* exhibited the greatest potential activity (LC_{50} < 70 µg/mL), which could be due to the presence of compounds of different polarity (**Ara *et al.*, 1999**). The capacity of methanol and dichloromethane extracts from twelve algae to cause cytotoxicity in HepG-2 cells (1000 µg/mL; 24 h) was investigated. The most successful methanol extracts were those of seaweeds belonging to the Rhodophyta and Heterokontophyta divisions. Both *A. armata* extracts were extremely cytotoxic, killing 11.22 ± 2.98 and 1.51 ± 0.38 % of HepG-2 live cells, respectively. *S. coronopifolius* methanol and dichloromethane extracts both significantly reduced HepG-2 viability (14.04 ± 2.62 and 12.84 ± 3.82 % of HepG-2 live cells, respectively). However, algae belonging to the division Chlorophyta did not exhibit significant behaviour (**Alves *et al*., 2016**).

1.4. Antileishmanicidal activity

Leishmaniases are a group of infectious diseases caused by the Leishmania genus's obligate intracellular protozoa. Leishmaniases are confronted with parasite resistance to commercially available medications (**Freitas-Junior *et al.*, 2012**). The antileishmanicidal and insecticidal activities have been tested and the results showed that none of the test's algal extracts possess antileishmanicidal or insecticidal activities. In an early study, the antileishmanial activity of various seaweeds has been investigated. Crude seaweed extracts of *C. faridii* (IC_{50}<34 µg/mL), *C. flabellatum* (IC_{50}<34 µg/mL), *C. racemosa* (IC_{50}<37.5 µg/mL), *U. fasciata* (IC_{50}<50 µg/mL), *L. pinnatifida* (IC_{50}<6.25 µg/mL), *S. hatei* (IC_{50}<14.10 µg/mL), *M. afaqhusaini* (IC_{50}<32.6 µg/mL), *G. corticata* (IC_{50}<37.5 µg/mL) showed significant activity. While other species such as *S. indica* (IC_{50}<59.6 µg/mL), *C. clavulatum* (IC_{50} <57.89 µg/mL), *B. leptopoda* (IC_{50}<60.81 µg/mL), *C. iyengarii* (IC_{50}<60.40 µg/mL), *U. reticulata* (IC_{50}<64.75 µg/mL) and *U. rigida* (IC_{50}<65.69 µg/mL) exhibited good activity against Leishmania *in vitro* (**Sabina *et al*., 2005**). Two antileishmanial meroditerpenoids, (3*R*)- and (3*S*)-tetraprenyltoluquinol and (3*R*)- and (3*S*)- tetraprenyltoluquinone, from the hexane extract of macroalgae *C. baccata* have been isolated (**de Sousa *et al*., 2017**). These compounds inhibited the growth of the *L. infantum* promastigotes (IC_{50} = 44.9±4.3 and 94.4±10.1 µM), respectively. Tetraprenyltoluquinol was found to display potent antileishmanial activity and decrease the intracellular infection index (IC_{50} = 25.0±4.1 mM), while

tetraprenyltoluquinone eliminated 50% of the intracellular amastigotes at a concentration > 88.0 µM. Methanol extracts of different seaweed species belonging to Chlorophyta (*C. racemosa* and *C. bursa*), Phaeophyta (*C. barbata* and *C. crinata*) and Rhodophyta (*C. granifera*, *J. rubens*, *C. rubrum*, *G. verrucosa*, *D. pedicellata* and *G. crinale*) showed antileishmanial activity with IC_{50} values ranging from 16.76 to 69.98 µg/mL (**Süzgeç-Selçuk *et al*., 2011; Torres *et al*., 2014**). The sesquiterpenes obtusol (IC_{50} = 9.4 µM) and elatol (IC_{50} = 13.5 mM and 0.45 mM) isolated from the red alga *L. dendroidea* showed significant activity against *L. amazonensis* intracellular amastigotes (**Santos *et al*., 2010; de Silva *et al*., 2011**).

1.5. Insecticidal activity

Concerning insecticidal activity, various crude seaweed extracts from various species such as *C. scalpelliformis*, *P. pavonica*, *S. tenerrimum*, *U. fasciata*, and *U. lactuca* were found to be insecticidal against the cotton insect pest *Dysdercus* spp., which causes severe crop loss (**Rajesh *et al*., 2011; Sahayaraj and Kalidas, 2011; Asha *et al*., 2012; Kombiah and Sahayaraj, 2012; Hamed *et al*., 2017; Sahayaraj and Jeeva, 2012**). After 96 hours, chloroform extracts of *S. swartzii* and *P. pavonica* killed the nymphs of *Dysdercus cingulatus*. Both extracts were also found to be lethal to *D. cingulatus* nymphs in their third instar due to the presence of stigmastan-6, 22-dien 3,5-dedihydro and hexadecanoic acid methyl ester in *S. swartzii* and *P. pavonica*, respectively. Male and female durability of *D. Cingulatus* is reduced by chloroform and aqueous extracts of *S. swartzii*. Additionally, the hexane extract reduces the fecundity and hatchability of *D. cingulatus* (**Asharaja and Sahayaraj, 2013**). A paly-toxin-like insecticide has been isolated from the Japanese red alga *Chondria armata*. Palytoxin was found to be highly insecticidal and to be lethal against cockroaches at a concentration of 0.005 µg (**Mori *et al*., 2016**).

1.6. Phytotoxicity activity

The results of the phytotoxicity bioassay indicated that the crude extracts of tested algal species inhibited the growth of *Lemna minor* plant while those of others helped to promote its growth. The methanol extract of *P. capillacea* (VI) showed a potent inhibition (100%) of the growth of *Lemna* plant at a high concentration 1000 µg/mL, however, it stimulated its growth (15.3%) at the lowest concentration (10 µg/mL). Similarly, the methanol extract of the same alga (IV) exhibited a moderate inhibitory activity (55.1%) at the highest concentrationand a negative activity (-2.5%) which promoted the growth at the concentration 10 µg/mL. The ether extract of *P. capillacea* (III) exhibited a similar inhibitory activity (55.1%) at 1000 µg/mL while the methanol extract demonstrated a negative activity at all tested concentrations. Furthermore, the ethyl acetate extract of *C. mediterranea* (II) manifested a moderate inhibitory activity (61.5%) at the same concentration. The butanol extract of *U. lactuca* (VII) followed a

moderate inhibitory activity (60%) at 100 µg/mL, however; the aqueous extract of this alga had a negative activity at all concentrations. The other extracts showed weak activity against the growth of *Lemna minor* at different concentrations (**Table 3**). The phytotoxic activity obtained here is probably due to the accumulation of different natural products possessing this activity (**Rizvi and Shameel, 2003**). The methanol extract of *E. intestinalis* displayed 95% inhibition of the fronds of *Lemna aequinoctialis* plant at a concentration of 100 µg/mL, while *A. taxiformis* exhibited significant activity 100% at the highest concentration (1000 µg/mL) and a poor activity 12.5% at the lowest concentration (10 µg/mL) (**Rizvi and Shameel, 2003**). The methanol extract of the brown alga *S. marginatum* exhibited 77% of growth depletion against *Lemna* sp. at 400 mg/L. The *Laurencia* sp. species inhibited the growth by 59% on *Lemna* sp. at the same concentration whereas the *S. wightii* exhibited 40% of growth inhibition (**Manilal *et al.*, 2010**).

Table 3: Phytotoxic activity of different algal extracts.

Algal species	**Tested extract**	**% Inhibition of the growth of *Lemna minor***		
		1000 µg/ml	**100 µg/ml**	**10 µg/ml**
U. fasciata I	Ether	35.8	34.6	-15.3
C. mediterranea II	Methanolic	10.2	-2.5	35.8
	Ethyl acetate	61.5	3.8	-8.9
P. capillacea III	Methanolic	-8.9	-21.7	-2.5
P. capillacea III	Ether	55.1	10.2	3.8
P. capillacea IV	Methanolic	55.1	29.4	-2.5
P. capillacea V	Methanolic	35.8	-2.5	10.2
P. capillacea VI	Methanolic	100	29.4	-15.3
U. lactuca VII	Aqueous	-28.2	-2.5	-15.3
	Butanolic	33.3	60	46.6
	Petroleum ether	2.3	24.8	17.3

Standard drug: Paraquat; Conc. of standard drug: 0.015 µg/ml

1.7 Antioxidant activity

In the present study, two techniques (DPPH and nitric oxide free radical scavenging assays) were used for screening of the *in vitro* antioxidant properties of methanol, ethyl acetate, ether, petroleum ether and aqueous extracts of the green algae (*U. lactuca* and *U. fasciata*) and the red algae (*C. mediterranea* and *P. capillacea*). In the nitric oxide free radical scavenging assay, all test extracts did not show any activity. Conversely, in the DPPH free radical scavenging assay, the methanol and petroleum ether extracts of *U. lactuca* (VII) exhibited strong antioxidant activity 92.2 and 84.6%,

respectively, while the aqueous extract of the same algae showed good activity (72.3%). Other extracts were found to be inactive (**Table 4**). Our results are not in consistence with the findings of **de Lima *et al*. (2016)** who reported that the methanol extracts of the brown (*D. dichotoma* and *S. vulgare*) and the red algae (*A. multifida* and *C. crenulata*) possess higher antioxidant activity than those of the green algae (*U. fasciata* and *C. crenulata*). However, at all concentrations the algal extracts of all species exhibited lower antioxidant activity compared with positive controls, with values of DPPH scavenging activity ranged between 53.96 and 64.96%. The reported values of DPPH scavenging activity in the present study are higher than those cited for various seaweeds. For example, extracts of the green alga *U. lactuca* showed higher antioxidant activities than the ethanol extract of *E. prolifera* (**Cho *et al*., 2011**). Similarly, the extracts of *U. lactuca* also presented higher DPPH scavenging activities than the extracts of *A. nodosum, L. hyperborea, P. canaliculata, F. vesiculosus* and *F. serratus* (**Òsullivan *et al*., 2011**) and those of *G. edulis, C. hornemanni, H. pannosa* and *J. rubens*, which varied from 5 to 25% (**Devi *et al*., 2008**). The methanol extract of the Tunisian green alga *C. fragile* shows antioxidant activity (IC_{50} 18 µg/mL) compared to vitamin E ($IC5_0$ 12 µg/mL) (**Kolsi *et al*., 2017**).

Table 4: DPPH free radical scavenging activity of different algal extracts.

Algal species	Tested extract	% Radical scavenging activity (RSA)
U. fasciata I	Ether	-
C. mediterranea II	Methanolic	-
	Ethyl acetate	-
P. capillacea III	Ether	-
P. capillacea IV	Methanolic	-
P. capillacea V	Methanolic	-
P. capillacea VI	Methanolic	-
U. lactuca VII	Aqueous	72.3
	Methanolic	92.2
	Petroleum ether	84.6
	Propyl gallate (+ve control)	90.0

Incubation period 30 min; Incubation temperature 37 °C

1.8. Cholinesterase inhibition activity

Marine algae contain compounds with special neuroprotective features (**Chacón-Lee *et al*., 2010**), however; published information about their neuroprotective effect is slightly rare (**Pangestuti and Kim, 2011, 2013**). Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder resulting in loss of memory and cognitive

functions (**Querfurth and La Ferla, 2010**). Inhibitors of acetycholinesterase (AChE) and butyrylcholinesterase (BChE) have been used for the development of therapeutic strategies for AD (**Rhee *et al*., 2004**). Methanol, ethyl acetate, ether, petroleum ether and aqueous extracts of *U. lactuca*, *P. capillacea* and *C. mediterranea* were screened for their enzyme inhibitory activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The ethyl acetate extract of *C. mediterranea* (II) and the methanolic extract of *P. capillacea* (VI) showed good inhibitory activity of 59.6% and 51.9%, respectively, against butyrylcholinesterase at a concentration of 200 µg/mL. In comparison, the positive control galanthamine showed maximum activity of 100%. The aqueous extract of *U. lactuca* (VII) wasn't found to possess any inhibitory activity against butyrylcholinesterase enzyme. Other extracts were found to possess only low activity against this enzyme (**Table 5**). Neither of the tested extracts exhibited noticeable AChE inhibitory activity. The DCM/MeOH crude extract of the Brazilian red alga *P. capillacea* showed weak acetylcholinesterase inhibition of 5.38% at 400 µg/mL (**Machado *et al*., 2015**). The non-polar extracts of the Indian brown alga *S. wightii* was found to possess significant BuChE inhibitory activity. Petroleum ether, hexane, benzene and dichloromethane extracts inhibited the BuChE activity with IC_{50} values of 17.91 ± 0.65, 32.75 ± 1.00, 12.98 ± 0.31, 36.16 ± 0.64 µg/mL, respectively at 100 µg/mL (**Syad *et al*., 2013**). The acetylcholinesterase inhibitory activity for methanolic extracts of the brown algae *P. tetrastromatica* and *P. australis* has been evaluated (**Hajimehdipoor *et al*., 2017**). Results indicated that the total extracts of *P. australis* and *P. tetrastromatica* demonstrated 15.6% and 23.7% of enzyme inhibition, respectively at a concentration of 300 µg/mL. **Ghannadi *et al*. (2013)** mentioned that the methanol extract of *Sargassum boveanum* exhibited high AChE inhibitory activity (IC_{50} = 1 mg/mL) while *Cystoseira indica* exhibited the lowest activity (IC_{50} = 11 mg/mL). The species *Gracilaria corticata* and *Gracilaria salicornia* from Rhodophyta had moderate activities (IC_{50} = 9.5, 8.7 mg/mL, respectively).

Table 5: Butyryl cholinesterase inhibition by different algal extracts.

Algal species	Tested extract	% Inhibition of butyryl cholinesterase
C. mediterranea II	Ethyl acetate	59.6
P. capillacea III	Ether	43.7
P. capillacea IV	Methanolic	42.1
P. capillacea V	Methanolic	32.4
P. capillacea VI	Methanolic	51.9
U. lactuca VII	Aqueous	-
	Petroleum ether	17.6
	Galanthamine (+ve control)	100

Incubation period 15 min; Incubation temperature 25 ^{o}C

1.9. Antiglycation activity

In processed foods and *in vivo*, glycation is a non-enzymatic reaction of reducing sugars with amino acids and/or proteins (**Anguizola *et al.*, 2013**). External factors, such as UV damage, pollution and smoking, can speed this process (**Fitton *et al.*, 2016; Gasser *et al.*, 2011**). The protein glycation inhibitory activity of the methanol, ethyl acetate, ether, petroleum ether and aqueous extracts of the three algae and *U. fasciata* as well, was evaluated *in vitro* by using model system of bovine serum albumin and glucose. The most active extract was *P. capillacea* (III) ether extract which showed a high glycation inhibitory activity (69.4 %) with an IC_{50} = 0.0596 ± 0.0447 mM. The petroleum ether extract of *U. lactuca* (VII) showed a high inhibitory activity (65 %) with an IC_{50} value of 0.138 ± 0.0441 mM. Interestingly, *U. lactuca* (VII) aqueous extract was found to accelerate the glycation (–0.1%), while the methanol extract of the same alga showed a moderate inhibitory activity (55 %). The other algal extracts exhibited low glycation inhibitory activities as indicated in **Table 6**. The acetone extract of *F. vesiculosus* and its fractions strongly inhibited protein glycation mediated by glucose (**Liu, 2011**). The ethyl acetate fraction exhibited more potent antiglycation activity than the acetone extract and other fractions. Scavenging of reactive carbonyls was suggested to be a major mechanism for algae extracts to inhibit protein glycation. Several compounds including phenolic (**Tsuji-Naito *et al.*, 2009; Choudhary *et al.*, 2010**), oligo and polysaccharides (**Yang *et al.*, 2009; Meng *et al.*, 2011**), carotenoids and unsaturated fatty acids (**Sun *et al.*, 2010, 2011**), have been reported to possess anti-glycating activity.

Table 6: Antiglycation activity of different algal extracts.

Algal species	Tested extract	% Inhibition of protein glycation	IC_{50} (mM)
U. fasciata I	Ether	14	
C. mediterranea II	Ethyl acetate	9	
P. capillacea III	Ether	69.4	0.0596 ± 0.0447
P. capillacea IV	Methanolic	43	
P. capillacea V	Methanolic	34	
P. capillacea VI	Methanolic	39	
U. lactuca VII	Aqueous	-0.1	
	Methanolic	55	
	Petroleum ether	65	0.138 ± 0.0441
	Rutin (+ ve control)	85.9	

Incubation period 1 week; Incubation temperature 37 ^{o}C

1.10. Immunomodulating activity

The immunomodulating effect of the algal extracts has been evaluated. Preliminary screenings on whole blood oxidative burst activity indicated that all of the

extracts possess an inhibitory activity. A potent inhibitory activity (97.99 %) was shown by *U. fasciata* (I) ether extract at a concentration of 400 µg/mL. Moreover, a moderate inhibitory activity (53.52 %) was exerted by this extract at a concentration of 12.5 µg/mL. Other extracts exhibited a weak inhibitory activity at low concentrations as shown in **Table 7**. The immunomodulatory activity of two sulphated polysaccharides isolated from *Ulva intestinalis* has been investigated. The fractionated and unfractionated sulphated polysaccharides FSP30 and UPS were found to enhance the production of pro-inflammatory cytokines, including nitric oxide (NO), tumour necrosis factor-α (TNF-α), and interleukin-1β (IL-1β), in macrophage J774A.1 cells, revealing that these fractions were able to functionally activate the macrophages and could be used as potential immunomodulatory agents. FSP30 showed stronger immunomodulatory activity than UPS (**Peasura *et al.*, 2016**). Fucoidans extracted from the brown algae, *S. crassifolium* and *P. australis* had the ability to stimulate intestinal immunological activity via Peyer's patch cells (**Yuguchi *et al.*, 2016**). The immunostimulatory potential of algae-derived water-soluble *β*-1,3/1,6-*β*-glucan on mouse cells was investigated by (**Bobadilla *et al.*, 2013**). Seaweed-derived *β*-glucan exhibited no adverse effects on the survival of cells and had an increase in activated $CD19^+$ B lymphocytes (**Bashir and Choi, 2017**). **Vetvicka *et al*. (2007)** investigated the immunostimulatory effects of seaweed-derived *β*-glucans (phycarine). Significant stimulation of phagocytosis in peripheral blood cells by phycarine was observed. Phycarine possessed strong immunostimulatory effects on experimentally-induced leukopenia and helped in Lewis lung carcinoma chemotherapy (**Bashir and Choi, 2017**).

Table 7: Immunomodulating activity of different algal extracts.

Algal species	Tested extract	% Inhibition of immunomodulating activity (µg/mL)						IC_{50}
		400	200	100	50	25	12.5	
U. fasciata I	Ether	97.99	90.60	81.84	69.28	74.85	53.52	
C. mediterranea II	Methanolic	59.11	37.72	35.01	16.61	15.31	23.61	294.9±9.2
	Ethyl acetate	91.85	70.21	37.95	47.99	47.50	29.42	127.8±13.0
P. capillacea III	Ether	72.03	43.46	40.51	34.25	23.16	9.86	235.4±60.9
P. capillacea IV	Methanolic	91.83	45.46	41.60	39.64	27.36	16.11	207.9±2.2
P. capillacea V	Methanolic	86.03	42.43	48.43	30.51	37.70	17.64	225.2±17.2
P. capillacea VI	Methanolic	92.48	39.71	38.71	35.04	26.87	17.16	227.7±17.9
U. lactuca VII	Aqueous	45.94	12.89	13.05	14.46	14.32	26.60	312.3
	Methanolic	68.52	5.74	17.30	-31.11	-7.07	-4.74	230.0±22.8
	Petroleum ether	82.00	48.57	35.70	28.58	37.13	0.09	198.1±26.1

2. Scanning Electron Microscopic Study of Surface Morphology of Alginate Beads

Sodium alginate is a linear polysaccharide with varying amounts of 1,4-linked *β*-D-mannuronic acid and α-L-guluronic acid residues arranged block-wise along the backbone. Alginate can be used as a matrix for controlled release (CR) preparations due

to its biodegradability, where it is degraded and absorbed by the body during and/or after drug release without any toxic effects. Hence, it can be a suitable matrix for sustained release of various drugs (**Shilpa *et al.*, 2003**).

Encapsulation is a physico-mechanical method in which active ingredients are protected by a natural carrier polymer such as algal polysaccharides, alginate, carrageenan, or agarose (**Murano, 1998**). Bio-encapsulation involves the envelopment of tissues or biologically active substances in semipermeable membranes. It increases the efficiency with which various metabolites and therapeutic agents are generated (**de Voa *et al.*, 2009**). Nontoxic, biocompatible, and biodegradable are all advantages of algal polysaccharides. To monitor the gradual release of the extract from the beads, this process is used in the pharmaceutical, chemical, cosmetic, and food industries (**Gluza and Kennedy, 2007; Shobier *et a*l., 2016**).

The morphological examination of the algal extract encapsulated in calcium alginate beads was analysed by means of Scanning Electron Microscope (SEM). The SEM micrographs of the bioactive algal extracts (ether, methanol and aqueous) encapsulated in calcium alginate beads from *P. capillacea* (III), *P. capillacea* (IV) and *U. lactuca* (VII), respectively, are represented in **Fig. 1**.

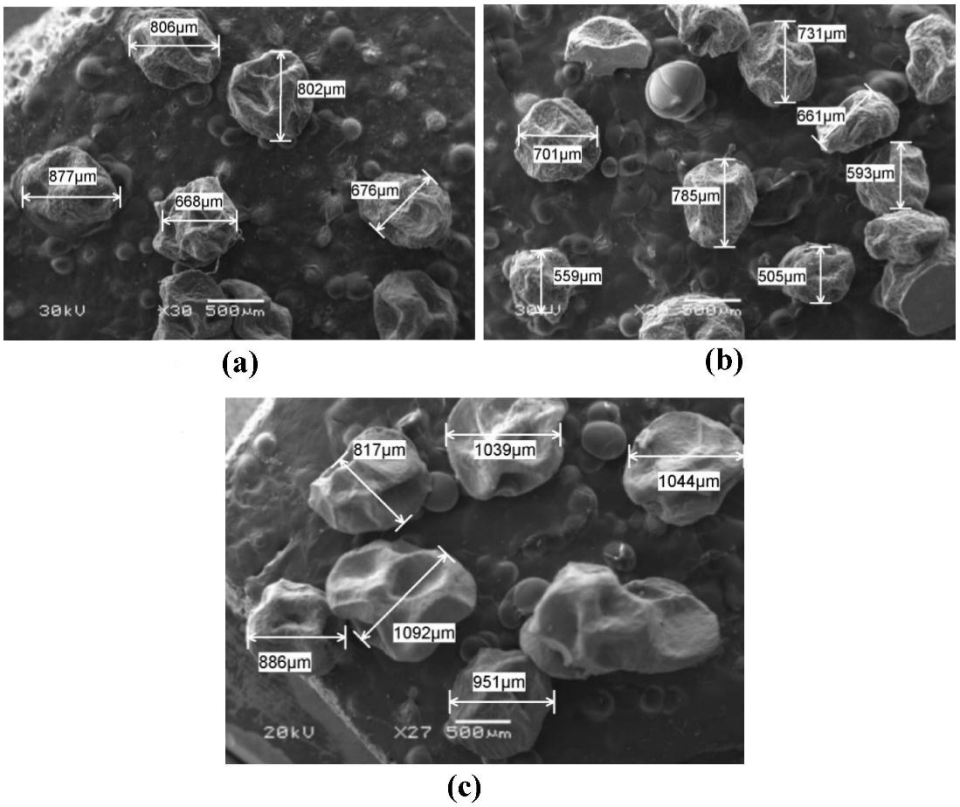

Fig. 1. SEM micrographs showing the size distribution of bioactive algal extract capsulated in calcium alginate beads from (**a)** *P. capillacea* IV, (**b)** *P. capillacea* III and (**c)** *U. lactuca* VII.

The obtained beads, encapsulated with *P. capillacea* (III), *P. capillacea* (IV) and *U. lactuca* (VII) extracts were spherically shaped and uniform in size with an average diameter of 647.9, 765.8 and 971.5 µm**;** respectively. Beads exhibited some irregularities such as blisters, and small cavities. Detailed examination of the outer surface structure (**Fig. 2**) showed the appearance of several wrinkles and pores with diameters of a few micrometres.

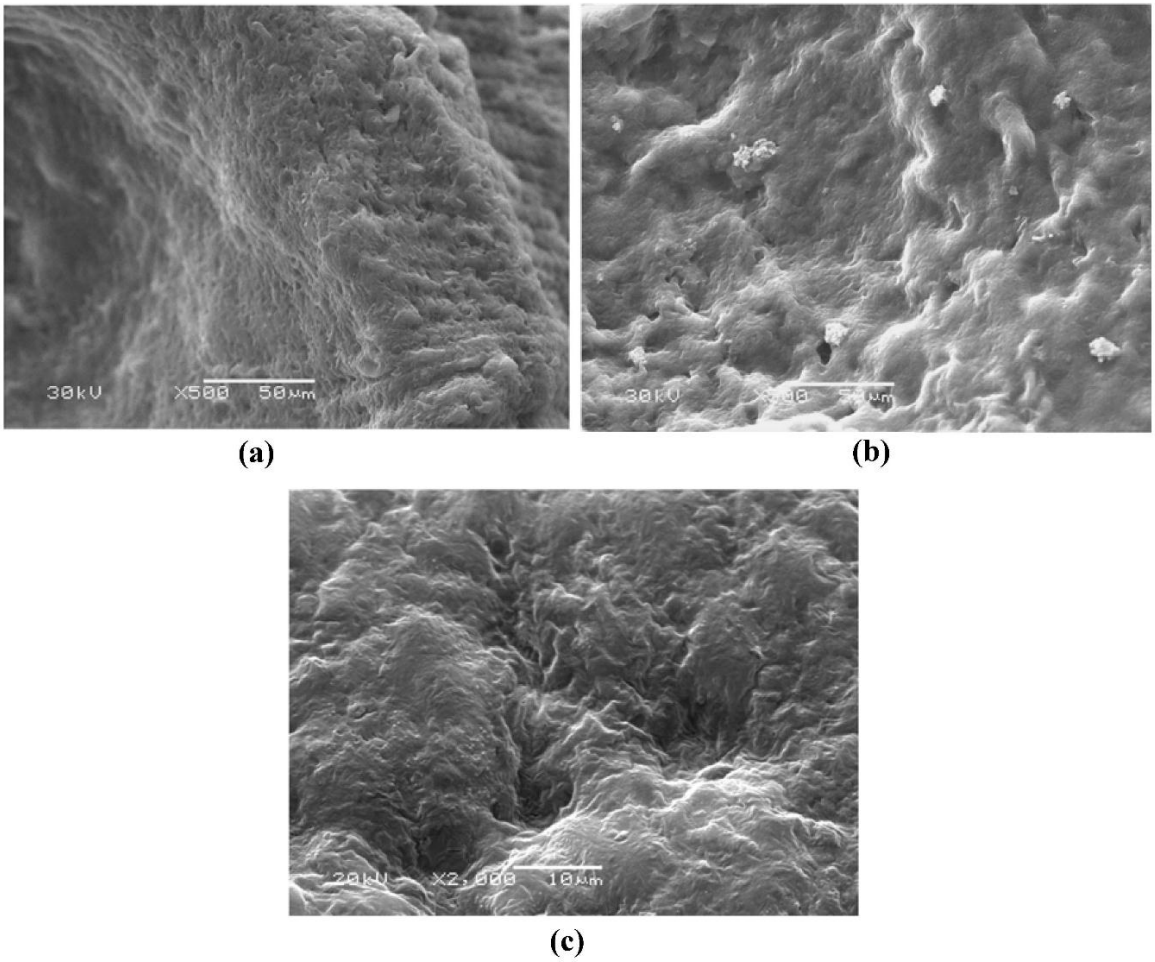

Fig. 2. SEM micrographs of the outer surface of bioactive algal extract capsulated in calcium alginate beads from (**a)** *P. capillacea* IV, (**b)** *P. capillacea* III and (**c)** *U. lactuca* VII.

The morphological characteristics of the internal surface of the encapsulated beads are shown in **Fig. 3**. It can be seen that the beads have a relatively rough surface with a more porous and hollow internal structure. Thus, the bioactive extract can be entrapped inside the porous structure that may act as a second barrier for the release of the trapped extract. These results are in agreement with those reported by **Shobier *et al*.**

(2016). The effect of the encapsulation process of algal extracts into calcium alginate beads on the antibacterial activity of methanol-L extracts of *U. lactuca* and *P. capillacea* against three fish pathogens; *A. hydrophila, V. anguillaum* and *P. fluorescens* have been studied. Results indicated that the encapsulated extract of *U. lactuca* showed good antibacterial activity against *V. anguillarum* and that of *P. capillacea* exhibited significant activity against the tested pathogens revealing that the encapsulation process improved the antibacterial activity and could be used successfully to control the growth of fish pathogenic bacteria.

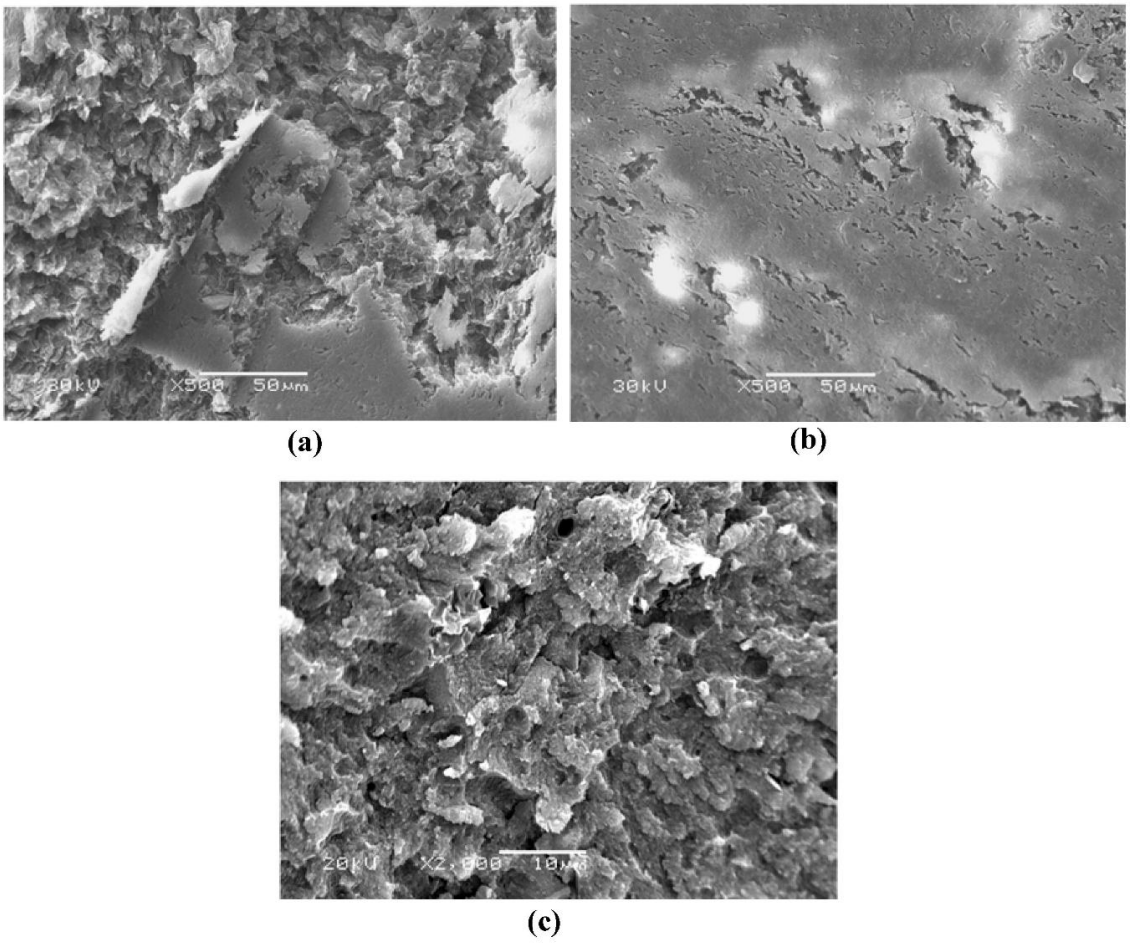

Fig. 3. SEM micrographs of the internal surface of bioactive algal extract capsulated in calcium alginate beads from (**a)** *P. capillacea* IV, (**b)** *P. capillacea* III and (**c)** *U. lactuca* VII.

CONCLUSION

The results of pharmacological screening revealed that the red alga *P. capillacea* and the green alga *U. lactuca* can be a good source for antifungal, phytotoxic, and antiglycating agents. Further, the green alga *U. lactuca* can be a good source for antioxidant agents. The red algae *P. capillacea* and *C. mediterranea* can offer a good source for butyrylcholinesterase inhibitors. The green alga *U. fasciata* can be used as a

good source for immunomodulating agents. SEM examination showed that calcium alginate gel beads might be an effective carrier for the encapsulation of bioactive algal extracts.

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