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Phenolic composition and biological activities of brown alga *Dictyopteris polypodioides*

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Abstract
This paper provides an overview of work that suggests that *Dictyopteris polypodioides* were used to extract their bioactive compounds by alcoholic method and total phenolic, flavonoid and condensed tannins contents of the extracts were investigated.
The results showed that the methanol and ethyl acetate extracts are endowed the best antibacterial effect against *Staphylococus aureus* and *Aspergilus niger*. Moreover, the methanol extract presented the strongest inhibitory potential against alpha-amylase (86.36%) with low levels of inhibition lipase activity. Meanwhile, this extract was found to have the highest phenolic content. Our study also showed that there were positive relationship among total phenolic contents and biological activities.
It was noticed that the extents of antibacterial, antidiabetic and antiobesity activities of *Dictyopteris polypodioides* extracts are in accordance with their phenolic compounds.

Keywords: Algea, antioxidant, antimicrobial, diabetes, obesity

1. Introduction
The oceans cover more than 70% of the Earth's surface with marine species comprising approximately half of the total global biodiversity [1].
Hence, the big diversity of marine organisms is being recognized as rich sources of functional compunds. Among marine organisms, marine algae are usualy identified as an under-exploited plant resources although they have long been used in various pharmaceutical preparations [2].
The ancient tradition of consumption of marine algae has made a large number of epidemiological studies showing their health benefits. In recent years, many marine resources have attracted attention in the search for bioactive natural compounds, in order to develop new medicines and dietetic foods. In fact, many metabolites isolated from marine algae have been shown to possess biological. Therefore, a new trend to isolate and identify bioactive compounds and constituents from marine algae has emerged.
They act as trappers of free radicals against the protection of living organisms from damage oxidative agents [3], Antibiotics [4] and as antiproliferative agents [5].
Over the last 20 years, a theory has developed that the intake of natural antioxidants, such as polyphenols are a major phytochemical component, could protect against the damage caused by free radicals, membranes and cellular components, which are involved in disease progression [3].
Given the developments in both the food and medical sectors as well as in the search for new natural renewable phytotherapeutic resources, we thought it advisable to highlight the biological properties of the *Dictyopteris polypodioides* extracts (DPE) by *in vitro* tests for the validation of antimicrobial, antidiabetic, antiobesity properties.

2. Materials and methods
2.1 Sample Collection
Dictyopteris polypodioides was collected from Chebba, Tunisia; identified at the National Institute of Science and Technology of the Sea (INSTM), Sfax-Tunisia and the Stazione Zoologica 'A. Dohrn', Functional and Evolutionary Ecology Laboratory, Punta S. Pietro, Ischia, Italy.

2.2 Extraction Procedure
In brief, 20 g of powder sample was extracted at room temperature by the percolation method using three different solvents: Hexane, Ethyl acetate and Methanol. The resulting extract was concentrated over a rotary vacuum until a crude extract was obtained. The extraction was

executed in triplicate. Condensed tannins were measured according to the vanillin assay described by Sun *et al*. [6] using catechin as standard.

2.3 Phenolic compounds composition

Total content of phenolic compounds of algae sample extracts was determined by the Folin-Ciocalteau method using gallic acid as standard [7]. Total flavonoid content was determined according to the method of Dewanto *et al*. [8] and condensed tannins were measured according to the vanillin assay described by Sun *et al*. [6] using catechin as standard.

2.3 Antibacterial and antifungal activities of DPE

2.4.1 Microorganisms

The strains used in the present work was : *Escherichia coli* (ATCC 8739), *Escherichia coli* DH5 (alpha), *listeria monocytogene* (BUG 496), *Salmonella enteria* (ATCC 43972), *Agrobacterium tumefaciens, Pseudomonas aerigunosa* (ATCC 49189), *Staphylococus aureus* (ATCC6538), *Micrococcus luteus* (LB 14110), *Bacillus subtilis* (ATCC 6633), *Bacillus amyloliquefaciens* (ATCC 6633) and four species of fungi *Aspergilus niger*, *saccharomyces cerevisiae*, *Fusarium oxysporum* and *Candida albicans* (ATCC 90028), were obtained from the Microbiology Departement, Faculty of science, Sfax-Tunisia.

2.4.2 Antimicrobial assay disc-diffusion method

Evaluation of the antibacterial and antifungal activities was carried out by the agar diffusion method called the disk diffusion method [9].

We used the diffusion method in solid medium (Muller-Hinton medium) for antibacterial activity and (Sabouraud medium) for antifungal activity. Consequently, each of the sterile Wattman paper disks N° 3 and of diameter 6 mm is impregnated with 20 µl of the polysaccharide extract at a concentration of 50 mg / ml and placed on the surface of the middle of the petri dish in presence of disks impregnated with aqueous solution (negative controls). Discs of ampicillin marketed (at 10 µg / disc) as positive controls, and discs of cycloheximide (10 µg / disc) which were taken as antifungal for the positive controls. The dishes are then incubated for 2 h at 4 °C. and then at 37 °C. for 24 h for the bacteria and at 30 °C. for 48 h for the fungi. The diameters of the zones of inhibition surrounding the discs containing the samples to be tested are measured.

2.4.3 Minimum inhibitory concentration

The liquid microdilution method using Elisa plates with 96 wells was used [10]. Pre-cultures are prepared in the same manner as described above for the diffusion test. The inoculum is prepared in order to obtain a final cell density of about 10^6CFU/ml.

In the 96 well plates, serial dilutions of the extract, suitably solubilized, are prepared, and an appropriate controls are used as positive and negative controls. The plates thus prepared are incubated with moderate stirring at the optimum growth temperature of the microorganism: at (37 °C) for 24 h for the bacteria and at (30 °C) for 48 h for the fungi.

Following incubation, the MIC is determined by following the turn of the brick red color of the phenol to a yellow coloration in the presence of growing microorganisms. Once the growth is blocked, it is observed that the red color of the phenol persists. The MIC corresponds to the first sample concentration of the first red well with no bacterial disorder or bacterial pellet. The tests were carried out twice at the rate of 3 wells / sample during each test.

2.5 Antidiabetic activity

The capacity of *D. polypodioides* extracts to inhibit the α-amylase activity is determined according to the method of (Kit Biomaghreb, Tunis).

80 µl of the sample at various concentrations (25, 50, 100 and 200 µg / ml in DMSO) are mixed with 120 µl of the enzyme α-amylase (7 U / ml) prepared in the 40 mM phosphate buffer, PH 6.8 and 480 µl of 2-chloro-4-nitrophenol-α-D-maltotrioside (CNPG3). The mixture is incubated for 5 min at 37°C. After incubation, the absorbance is measured at 405 nm. Acarbose is used as a reference. The sample less mixture is used as a control. The ability to inhibit α-amylase activity is calculated using the following equation:

$$\text{Inhibition}(\%) = \frac{\text{Abs540(Control)} - \text{Abs540(Extract)}}{\text{Abs540(Control)}} * 100$$

2.6 Antiobesity activity

The lipase activity is determined by the enzymatic method according to the reaction scheme according to Kit Biolabo, France.

The formation rate of the quinone compound is directly proportional to the lipase activity in the sample which is measured at 400 nm.

In a tube, 1 ml of the working reagent (composed of two reagents R1 and R2 (V / V)) is mixed with 20 µl of the sample. In the calibrating tube, the sample is replaced by the reagent R4 (calibrator = lipase), mixed and incubate for 4 min at 37°C, Add 350 µl of reagent R3. The complexe was mixed and incubated for 3 min at 37 °C. The absorbance was determinate every minute for 3 min at 400 nm. The results are expressed as percentage of control activity [11].

2.7 Statistical analysis

Data were expressed by mean ± SD. Statistical analysis was carried out by analysis of variance (ANOVA) and by Student's t-test. A $p<0.05$ was considered to be statistically significant.

3. Results and discussion

3.1 Phenolic analysis

Phenols are secondary plant metabolites that are present in all plant products. The Total phenolic content of *Dictyopteris polypodioides* extracts with the standard gallic acid is shown in (Figure 1).

The total phenolic content of studied seaweed ranged from 43.05 to 89.12 mg GAE/g of extract. Methanolic extract of this brown seaweed exhibited highest phenolic content; as compared to other extracts. Phenolic compounds are generally more soluble in polar solvents, and a methanol was recommended and commonly used for the extraction of phenolic compounds [12].

Chandini *et al*. [13] reported that brown seaweed extracts had a phenolic content of 24.61 and 49.16 mg GAE/g of extract, while other studies reported that the crude methanolic extracts of the brown species *Turbinaria conoides* has low levels of phenolic compounds (1,23±0.173 mg GAE/g extract) [14].

Total flavonoids content varied from 22.06 ± 1.45 to 59.86 ± 2.96 mg/g extract (Figure 2). The best described property of flavonoids is their ability to act as an antioxidant, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as potential therapeutic agents against a wide variety of diseases [15, 16].

Besides, condensed tannins are present in low concentrations (5.56mg CE/g extract, 15.95mg CE/g extract and 10.47mg CE/g extract respecticly) compared to total phenols and

flavonoids compounds (Figure 3). Recent studies from cox *et al*. [17] have shown that the content of tannins from the brown seaweed, *Himanthalia elongata* was significantly higher than the other species. Brown species *H. elongata, Laminaria saccharina* and *Laminaria digitata* contained 38.34, 6.17 and 5.44 mg CE/g extract, respectively ; and many other studies have shown that phlorotannins are the only phenolic group detected in brown algae [18, 19].

The results of the present study are promising as algal polyphenolic compounds are effective antioxidants, therefore the seaweed extracts could have potential in food applications [20]. They are regarded for their important dietary roles as antioxidants and chemo preventive agents.

3.2 Antimicrobial analysis

All the extracts of algae was subjected to an antibacterial test against bacteria and fungi strains. In this study, all algal extracts tested showed an important antibacterial activity especially against gram positive bacteria. Our results showed that the methanol extract caused better halo-zones followed by the ethyl acetate than hexane (Table 1). The type of extraction solvent had a big influence on the antimicrobial properties of obtained extracts, suggesting that antimicrobial activity depends on both algal species and the efficiency of the extraction method. The best activity was detected against *Staphylococus aureus* and *Aspergilus niger* with a diameter inhibition of 27 mm and 15 mm respectively. The highest resistance in Gram (-) bacteria might be explained by the presence of their outer membrane covering the cell wall, which limits the diffusion of hydrophobic compounds through its phospholipids coating. For Gram (+) bacteria the absence of this barrier allows the direct contact of *Dictyopteris polypodioides* extracts with the phospholipids bilayer of the cell membrane, which causes an increase of ion permeability and passage of vital intracellular constituents or impairment of the bacterial enzyme systems.

This high activity may be related to the presence of phenolic compounds. In the natural environment, the light, temperature, mineral salts and motions of the water constitute the essential ecological parameters in the determination of the fertility of algae, light and temperature are at the origin of a seasonal and spatial variation of the algal flora, they act equally well on the growth of algae than on their morphological characters. Temperature whose fluctuations are mainly related to the seasons, is a determining factor in the seasonal marine flora. In this context, showed that the growth of algae is not identical throughout the year, it is maximal when the light and temperature conditions are favorable, this is the case in temperate regions where fertility of a large number of species is maximal at spring and autumn [21], however some species are known for their fertility throughout the year. This phase of active growth and sexual maturity of the seaweed corresponds to the period of synthesis secondary metabolites responsible for the biological activities [22]. Therefore the influence of ecological parameters on biology and the physiology of algae can also reach production of secondary metabolites.

Table 1: Antibacterial activity of *Dictyopteris polypodioides* extracts using agar disc.

Strains	DDHE	DDEAE	DDME	DD Controls	MIC (mg/ml)
Bacterial strains Gram (-)					
Escherichia coli	na	20	18	22	25
Salmonella enterica	na	14	19	26	6.25
Bacterial strains Gram (+)					
Staphylococus aureus	11	24	27	25	50
Micrococcus luteus	na	16	20	20	12.5
Fungal strains					
Aspergilus niger	13	15	15	21	25
saccharomyces cerevisiae	na	10	na	25	na

DDH: Disc Diameter of inhibition (halo size) in (mm) of Hexane extract 100 µg/disc, DDEAE Disc Diameter of inhibition (halo size) in (mm) of Ethyl acetate extract, Disc Diameter of inhibition (halo size) in (mm) of methanol extract. DD (controls): Disc Diameter of inhibition zone of ampicillin (10 µg/disc) and cycloheximide (10 µg/disc), were used as positive controls for bacteria and fungi, respectively, MIC: minimum inhibitory concentration (mg/ml), (na) no activity.

3.3 Antidiabetic evaluation

Diabetes was multifactorial in origin and one therapeutic approach to treat early stage of diabetes was to retard the absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as alpha amylase and alpha glucosidase.

The high prevalence of diabetes as well as its long-term complications has led to a continuous search for hypoglycaemic agents from natural sources [23] with high demand in the pharmaceutical sector.

Dictyopteris polypodioides extracts was also evaluated for its alpha amylase inhibitory potential and consequently type 2 diabetes.

A range of polyphenol-rich extracts inhibited α-amylase activity *in vitro* (Figure 4) with various levels of effectiveness dose-dependent. The inhibition activity was highest in methanol extract (86.36 %) and lowest in hexane extract (10.59%). Methanolic extract of *Dictyopteris polypodioides* was found to significantly ($P < 0.05$) inhibit α-amylase at different doses with 80.79 µg/ml of IC_{50} value.

Therefore, partial but significant inhibition of α-amylase by the *Dictyopteris polypodioides* extracts could help to modulate the release rate of glucose from starch, whereas the inhibition of α-amylase is key carbohydrate hydrolysing enzymes responsible for breaking α,1-4 bonds in disaccharides and polysaccharides resulting in decreasing the release of glucose [24, 25] so, the ability of the extracts to entrap glucose and amylolysis kinetics were also evaluated.

This appreciable α-amylase inhibitory activity could be associated with the presence of phenolic compounds. There is a positive relationship among total phenolic contents and alpha-amylase inhibition activity. It also confirms that brown seaweeds are not only consumed as functional foods but also used as pharmaceutical medicines.

3.4 Antiobesity evaluation

Obesity, a serious metabolic and energy disorder, became a major public health problem, the prevalence of this disease continues to grow rapidly and remarkably. Indeed, changing life styles and increased consumption of dietary fat actually contribute to the epidemic explosion of this high-risk pathology [26]. In this respect, the use of complementary and alternative medicine could be an effective, cheaper and less damaging solution. One of the therapeutic approaches to

obesity is to delay digestion and absorption of fat via the use of pancreatic lipase inhibitors extracted from natural sources to reduce postprandial [27].

In order to confirm the antihyperlipidemic effect of *Dictyopteris polypodioides*, we performed a preliminary *in vitro* test of the inhibition of pancreatic lipase activity by various organic extracts (Figure 5). Polyphenol-rich extracts of algae, were tested for their ability to inhibit pancreatic lipase *in vitro*, the different extracts caused slight but significant inhibition with a maximum of 49.85% in the mathanolic extract. The results obtained show that the hexane, ethyle acetate and metanolic extract have a comparable IC_{50} values indicating their medium lipase inhibitory activity. Previous data have shown that the presence of many flavonoids and polyphenols is required and necessary for the improvement of pancreatic lipase inhibition [28].

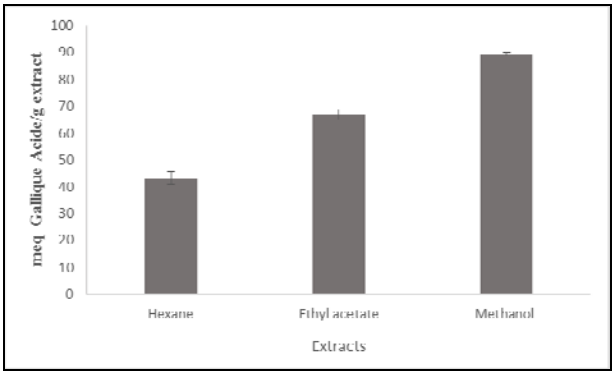

Fig 1 : Polyphenol contents in different extracts of *Dictyopteris polypodioides.*

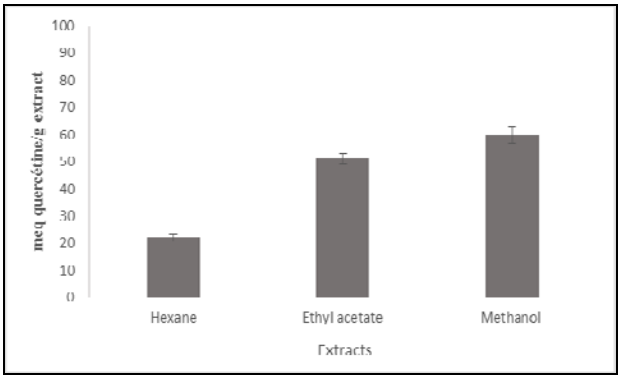

Fig 2 : Flavonoid contents in different extracts of *Dictyopteris polypodioides.*

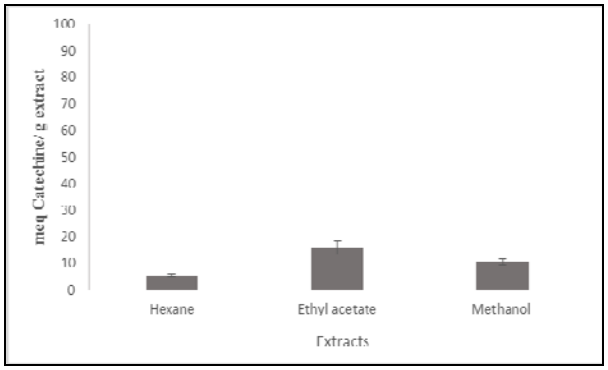

Fig 3: Condensed tannins contents in different extracts of *Dictyopteris polypodioides.*

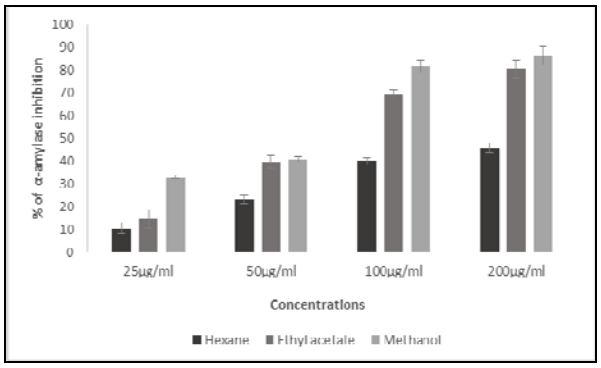

Fig 4: Evaluation of antidiabetic activity of different extracts of *Dictyopteris polypodioides.*

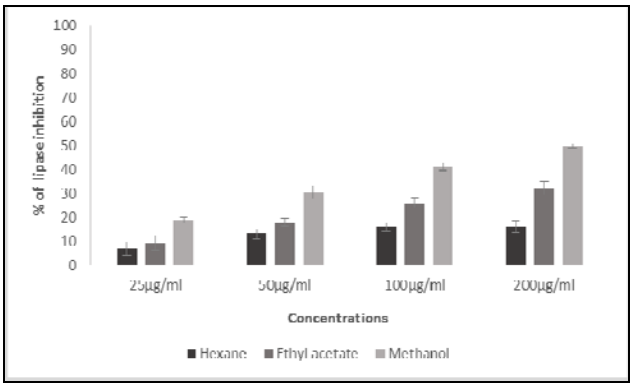

Fig 5: Evaluation of antiobesity activity of different extracts of *Dictyopteris polypodioides.*

4. Conclusion

In conclusion, our investigation clearly demonstrated the high content in total phenolic and flavonoids in *Dictyopteris polypodioides* marine algea wich exhibited a powerful antioxidant activity. Moreover, these extracts are endowed with a high antibacterial activity especially against gram-positive bacteria. Furthermore, the present study demonstrated the ability of this seaweed to inhibit key carbohydrate and lipids hydrolysing enzymes and unravelled their mode of inhibition, data gathered suggest that methanolic fraction was active enzyme inhibitors.

Our study provides the possible pharmacologic rationale to the medicinal use of *Dictyopteris polypodioides* in the development of pharmaceutical medicines.

Declaration of interest

No conflict in this work.

5. References

1. Kim SK, Wijesekara I. Development and biological activities of marine-derived bioactive peptides : A review. Journal of Functional Foods. 2010; 2(1):1-9.
2. Heo SJ, Hwang JY, Choi JI, Han JS, Kim HJ, Jeon YJ. Diphlorethohydroxycarmalol isolated from Ishige okamurae, a brown algae, a potent α-glucosidase and α-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. European journal of pharmacology 2009; *615*(1):252-256.
3. Qi H, Zhang Q, Zhao T, Chen R, Zhang H, Niu X *et al*. Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta) *in vitro*. International journal of biological macromolecules. 2005; 37(4):195-199.

4. Abdelmalek BE, Sila Ad, Krichen F, Karoud W, Martinez-Alvarez O, Ellouz-Chaabouni S *et al*. Sulfated polysaccharides from Loligo vulgaris skin: Potential biological activities and partial purification. International journal of biological macromolecules. 2015; 72:1143-1151.
5. Kanabar V, Hirst SJ, O'connor BJ, Page CP. Some structural determinants of the antiproliferative effect of heparin-like molecules on human airway smooth muscle. British journal of pharmacology. 2005; 146(3):370-377.
6. Sun B, Ricardo-da-Silva JM, Spranger I. Critical factors of vanillin assay for catechins and proanthocyanidins. Journal of Agricultural and Food Chemistry. 1998; *46*(10):4267-4274.
7. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965; 16(3):144-158.
8. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of agricultural and food chemistry. 2002; 50(10):3010-3014.
9. Rahal JJ. Novel antibiotic combinations against infections with almost completely resistant Pseudomonas aeruginosa and Acinetobacter species. Clinical infectious diseases. 2006; 43(Supplement 2):S95-S99.
10. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A *et al*. Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. Food chemistry. 2007; 103:1449-1456.
11. Ben Abdallah Kolsi R, Ben Gara A, Chaaben R, El Feki A, Paolo Patti F, El Feki L *et al*. Anti-obesity and lipid lowering effects of Cymodocea nodosa sulphated polysaccharide on high cholesterol-fed-rats. Archives of physiology and biochemistry. 2015; 121(5):210-217.
12. Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M *et al*. Phenolic composition of Cynara cardunculus L. organs, and their biological activities. C. R. Biologies. 2008; 331:372-379.
13. Chandini SK, Ganesan P, Bhaskar N. *In vitro* antioxidant activities of three selected brown seaweeds of India. Food chemistry. 2008; 107(2):707-713.
14. Vijayabaskar P, Shiyamala V. Antioxidant properties of seaweed polyphenol from Turbinaria ornata (Turner) J. Agardh, 1848. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(1):S90-S98.
15. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annual review of Nutrition. 2002; 22(1):19-34.
16. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules?. Free radical biology and medicine. 2004; 36(7):838-849.
17. Cox S, Abu-Ghannam N, Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal. 2010; 17:205-220.
18. Jormalainen V, Honkanen T. Variation in natural selection for growth and phlorotannins in the brown alga Fucus vesiculosus. Journal of Evolutionary Biology. 2004; 17(4):807-820.
19. Koivikko R, Loponen J, Pihlaja K, Jormalainen V. High-performance liquid chromatographic analysis of phlorotannins from the brown alga Fucus vesiculosus. Phytochemical Analysis. 2007; 18(4):326-332.
20. Yan XJ, Li XC, Zhou CX, Fan X. Prevention of fish oil rancidity by phlorotannins from Sargassum kjellmanianum. Journal of Applied Phycology. 1996; 8:201-203.
21. Farid Y, Etahiri S, Assobhei O. Activité antimicrobienne des algues marines de la lagune d'Oualidia (Maroc): Criblage et optimisation de la période de la récolte. J Appl. Biosci. 2009; 24:1543-1552.
22. Hornsey IS, Hide D. The production of antimicrobial compounds by British marine algae. IV. Variation of antimicrobial activity with algal generation. British Phycological Journal. 1985; 20(1):21-25.
23. Nicasio-Torres MP, Erazo-Gómez JC, Cruz-Sosa F. *In vitro* propagation of two antidiabetic species known as guarumbo: Cecropia obtusifolia an d Cecropia peltata. Acta Physiologiae Plantarum. 2009; 31(5):905-914.
24. Gropper SS, Smith JL. Advanced Nutrition and Human Metabolism, Cengage Learning, Hampshire, UK, 2012.
25. Rolfes SR, Pinna K, Whitney E. Understanding Normal and Clinical Nutrition, Cengage Learning Inc., Belmont, Calif, USA, 2008.
26. Haslam DW, James WP. Obesity. *The Lancet* 2005; 366:1197-1209.
27. Rahul B, Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources unexplored potential. Drug Discovery Today. 2007; 12:879-889.
28. McDougall GJ, Kulkarni NN, Stewart D. Berry polyphenols inhibit pancreatic lipase activity *in vitro*. Food Chem. 2009; 115:193-9.