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Characterization of Three Brown Algae *Bifurcaria Bifurcata, Cystoseira Gibraltarica* and *Fucus Spiralis*

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ABSTRACT: In recent years, scientists have become interested in marine macroalgae, which represent a rich source of bioactive substances. These molecules are characterized by a multitude of forms and structures and have several biological activities. The present study aims to characterize the three brown algae *Bifurcaria bifurcata*, *Cystoseira gibraltarica* and *Fucus spiralis.* These were collected in the region of Cap Ghir north of Agadir. The results obtained show that the mineral analyses of the extracts of *F. spiralis*, *B. bifurcata* and *C. gibraltarica* highlighted the richness of these brown algae in macroelements (Ca, K, P, Na, N). Concerning the total sugar content of *C. gibraltarica* shows a significant difference with the other two algae *B. bifurcata* and *F. spiralis* and has the highest content. For the protein content, no significant difference was recorded between the three algal extracts. Indeed, the aqueous extract of *C. gibraltarica* has a protein content of 48.44 mg/g DM, the aqueous extract of *F. spiralis* showed an average protein content of 45.55 mg/g DM, and that of the aqueous extract of *B. bifurcata* is 44.03 mg/g DM. The three brown algae *C. gibraltarica*, *B. bifurcata* and *F. spiralis* have consistent levels of organic matter. *F. spiralis* and *C. gibraltarica* have significantly equivalent organic matter contents (73.74 and 73.44 g/100g DM, respectively). In this study, the analysis of total phenols shows that *F. spiralis* presents a significant difference with the two other algae and has the highest content (7.2 µg/mg dry matter). In general and whatever the method of flavonoid determination, the flavonoid content of the three algae is significantly different. The methanolic extract of *F. spiralis* shows the highest flavonoid content, followed by *B. bifurcata*. While the lowest content is presented with the methanolic extract of *C. gibraltarica.* The results of the antioxidant potential using DPPH reveal a significant difference between the three algae. Indeed, the extracts of the analyzed algae present an antioxidant activity varying between 33.61 and 88.3%, with a high antioxidant potential (88.3%) for *F. spiralis* and thus an important capacity to trap the DPPH radical. These three studied brown algae represent a promising source of biologically active molecules that can be used in several fields such as organic agriculture.

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KEYWORDS: characterization, brown algae, total phenols, antioxidant activity, total sugars and proteins.

I-INTRODUCTION

The aquatic and marine environment is home to many organisms producing a variety of bioactive molecules from several metabolic pathways (Smit, 2004). Among these marine organisms, algae are a huge reservoir of specific natural substances that provide them with a chemical defense capability in response to ecological pressures, such as competition for space, predation, and tidal variations (Pérez et al., 2016). These organisms have developed during evolution some specific physiological adaptations, including the elaboration of a number of secondary metabolites that they use as defense against several species including microorganisms (Águila-Ramírez et al., 2017).

In Morocco, marine currents and hydroclimatic conditions, rapidly favor the development and expansion of marine algae such as brown algae (Riadi, 1998) and many species of algae grow rapidly and efficiently, especially compared to land plants (Kindleysides et al., 2012). Brown algae are very abundant at the Atlantic coast especially in the Cap Ghir area. This requires a better management

of this biomass, while promoting lines of research aimed at the exploitation of these brown algae including *Cystoseira gibraltarica*, *Bifurcaria bifurcata* and *Fucus spiralis* in several areas especially the agricultural field. However, few studies have been conducted to highlight the different molecules contained in the brown algae harvested on the double maritime facade that Morocco has.
Using polar solvents, phenolic compounds, organic acids, tannins and salts can be extracted from seaweeds (Sabeena and Jacobsen, 2013). Phenolic compounds found in brown algae possess strong antioxidant activity and their potential to scavenge free radicals is stronger than other polyphenols derived from terrestrial plants (Ahn, 2007). Some studies indicate that algal extracts can partially substitute fertilizers (Hong et al., 2007; Zodape et al., 2010) because they contain both minor and major mineral elements. Saccharides existing in algal extracts can act as elicitors of plant defensive mechanism (Khan et al., 2009). Thus algae are considered as fertilizers and growth promoters (Sharma et al., 2014).

II - MATERIAL AND METHODS

II-1-Study area

Three species of brown algae, *Cystoseira gibraltarica*, *Bifurcaria bifurcata* and *Fucus spiralis* were collected at low tide, in the coastal area of Cape Ghir, (30°38'37 "N, 09°53'20 "W), located about 43 kilometers northwest of Agadir (Morocco). This area is characterized by a natural state preserved with an important abundance of macroalgae in particular brown algae.

II-2-Plant material

The species of brown algae, *Cystoseira gibraltarica*, *Bifurcaria bifurcata* and *Fucus spiralis* collected are washed on site and then put in sea water. In the laboratory, the samples are sorted, identified and then rinsed abundantly with fresh water in order to eliminate all impurities: sand, salt, shell debris and some epiphytes. For each wash, electrical conductivity measurements were performed. The algae were then dried at room temperature and protected from sunlight by spreading the samples on sieves for 10 days. After drying, the samples are reduced to a powder which is kept in a dry place. Subsequently, biochemical, phytochemical and mineral element analyses were performed on the powder of these three brown algae. Each analysis is performed in three repetitions.

II - 3 - Determination of mineral elements

The mineral analyses of the algae were carried out according to the classical method of Page et al, (1982), the following elements are quantified: Organic matter (OM), Nitrogen (N), Phosphorus (P), Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), Zinc (Zn), Iron (Fe), Manganese (Mn) and Copper (Cu).

II - 4 - Determination of biochemical parameters

II - 4 - 1 - Determination of total sugars (Dubois, 1956)

We homogenize 20 mg of algal powder with 2 ml of ethanol 70% (v / v), the mixture is centrifuged at 2000 g. After recovery of the supernatant, the pellet is rinsed twice with ethanol 70% (v/v). To the supernatants thus combined, 16 ml of distilled water are added. 200 µl of the solution to be determined is added to 200 µl of a 5% aqueous phenol solution, then 1 ml of concentrated sulfuric acid is quickly introduced into the reaction medium. The vortexed mixture is allowed to stand for 10 min and then placed in a water bath for 10 to 20 min at a temperature of 30°C. The optical density is read at 490 nm using the visible IC 6400 spectrophotometer. The blank is the reaction mixture without sample. The values obtained are converted into sugar content in mg/g of dry matter (DM) from a calibration curve (Appendix I, Fig. 1).

II - 4 - 2 - Protein assay (Lowry et al., 1951)

The method of Lowry (Lowry et al., 1951) consists in forming a complex between the peptide bonds and copper sulfate in alkaline medium. This complex then reduces the phosphomolybdic and phosphotungstic acids of the Folin-Ciocalteu reagent to give a second complex of blue color, measured by spectrophotometer (Frolund et al., 1995).
The assay reagent (solution R) is prepared extemporaneously from three solutions, respecting the order of addition of the solutions and stirring after each addition:

- Solution C: copper sulfate at 10 g/l,
- Solution B: sodium/potassium tartrate (Na/K) at 20 g/l,
- Solution A: Sodium carbonate (Na_2CO_3) at 20 g/l and soda (NaOH) 0.1 mol/l.

Protein extraction

0.1 g of algal powder is ground in 1 ml of lysis buffer to extract the proteins. The extract is centrifuged at 13000g for 10 min.
Lysis buffer is prepared by mixing 8 ml of 1M Tris-HCl pH=6.8, 2 ml of β-mercaptoethanol, 10 ml of SDS and 80 ml of water.

Assay method

To 10 µl of the supernatant are added 990 µl of water and 5 ml of solution R (3 ml of solution C, 3 ml of solution B and 300 ml of solution A). The tubes are incubated for 10 min in the dark, then 0.5 ml of a 50% (v/v) Folin-Ciocalteu reagent solution is added and the mixture is vortexed. The stabilization of the color takes a few minutes. The intensity of the color obtained is evaluated by measuring the absorbance at 750 nm using the visible IC 6400 spectrophotometer. At the same time, a calibration line of bovine serum albumin (BSA) (2mg/ml) is performed (Appendix I, Fig. 2). Protein concentrations are expressed in milligram per gram of dry matter (mg/g DM) of sample.

II - 5 - Determination of phytochemical parameters

II - 5 - 1 - Extraction

50 mg of algae powder, put in an Eppendorf tube, are homogenized in 1 ml of Methanol-water (8 : 2, v/v). The mixture is sonicated for 20 min and then centrifuged for 15 min at 10000 g. The extract obtained is used for the quantification of phenolic compounds (total phenols and total flavonoids) and for the determination of the antioxidant activity of the different algae.

II - 5 - 2 - Determination of total phenols

Phenolic compounds undergo a redox reaction with the complex of phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent. This reaction varies according to the number of hydroxyl groups (OH) of the phenolic compounds (Singleton et al., 1999). However, this method is non-specific because the reagent can react with some amino acids (tyrosine and tryptophan), reducing sugars and sulfur compounds (Boizot and Charpentier, 2006). Bruneton (1999) reported that phenolic compounds are generally soluble in polar organic solvents and aqueous solutions and are poorly soluble in apolar organic solvents, hence the choice to extract optimally with methanol-water (80-20; v/v). Similar results have been reported by several studies using the same extraction system and conditions from different plant parts (Ben Ahmed et al., 2016).

To 25 µl of algal extract, previously prepared, 110 µl of Folin-Ciocalteu reagent is added, shaking for 3 minutes and then 200 µl of sodium carbonate is added to the mixture. Then 1.9 µl of distilled water is added and vortexed. After a 30-minute incubation in the dark, the optical density (OD) of each sample is measured by spectrophotometer at 750 nm (Makkar, 2003). The calibration range is performed by gallic acid (Appendix II, Fig. 3). The OD values obtained are then transformed into the unit microgram of gallic acid equivalents per milligram of dry matter (µg GAE/mg DM).

II - 5 - 3 - Determination of total flavonoids

The dosage of flavonoids is carried out using two different methods:

II - 5 - 3 - 1 Method of Andary (Andary, 1990):

2 ml of algal extract are added with 100 µl of the reagent (2 amino-ethyl diphenyl borate) (Neu, 1956). The OD reading is done at a wavelength of 404 nm. The flavonoid content is calculated according to the following formula:

$$T\ flavonoïdes = Aext \times 0.05 \times 100/Aq \times Cext$$

Aext: Absorption of the extract.

Aq: Absorption of quercetin (0.05 mg/ml).

Cext: Concentration of the extract in mg/ml.

The results are given in micrograms of quercetin equivalents per mg of dry matter (µg quercetin/mg DM).

II - 5 - 3 - 2 Jay's method (1975)

The determination of flavonoids is performed according to the method of Jay, (1975) as described by Harnafi et al, (2007) with a difference in the extraction solvent. To 1ml of algal extract are added 0.5 ml of aluminum chloride ($AlCl_3$), left to stand for 30 minutes at room temperature, then the OD is measured at 430 nm by a visible IC 6400 spectrophotometer. The calibration range is performed by quercetin. The OD values obtained are then transformed into the unit µg quercetin equivalents/mg dry matter (DM) with reference to the calibration curve (Appendix II, Fig. 4).

II - 6 - Determination of antioxidant activity

The evaluation of the antioxidant activity of the extracts of three brown algae is performed using the Scavenger method. The chemical compound 2,2-diphenyl-1-picrylhydrazyl (α-diphenyl-β-picrylhydrazyle) (DPPH) is one of the first free radicals used to study the structure-antioxidant activity relationship of phenolic compounds (Blois, 1958; Brand-Williams et al., 1995). The measurement of the decrease in DPPH uptake allows to determine the free radical scavenging capacity of the tested extracts. DPPH, which is purple in solution, has a characteristic absorbance at 517 nm. This color disappears rapidly when DPPH is reduced to diphenyl picryl hydrazine by a compound with antiradical properties, thus causing a discoloration. The intensity of the coloration is inversely proportional to the capacity of the antioxidants present in the medium to donate protons (Sanchez-Moreno 2002). This reaction can be summarized as follows:

DPPH + AH → DPPH-H + A

Where (AH) represents a compound capable of donating a hydrogen to the DPPH radical (purple) to convert it to diphenyl picryl hydrazine. This method is used in this experiment because it is simple, fast and inexpensive.

950 µl of a methanolic solution of DPPH (0.1 mM) are added to 50 µl of methanolic extract of the sample to be analyzed. After 30 min, the absorbance of the mixture is measured at 517 nm. The ability to trap the DPPH radical is calculated according to the following formula (Loo et al., 2008).

$$P = (A1\text{-}A2)/A1X100$$

P : Percentage of radical trapping.

A1 : absorbance of the control (DPPH solution without extract).

A2 : absorbance in presence of extract.

The DPPH- test is not quantitative, it allows to compare different extracts according to their capacity to trap DPPH- and thus, to appreciate the qualitative variations of phenolic compounds. The evaluation of the anti-free radical activity must be interpreted with precaution because the absorbance of DPPH- at 515-520 nm decreases under the action of light, oxygen, according to the pH and the type of solvent added to the antioxidant.

II - 7 - Statistical analysis

For each analysis three repetitions are carried out. The data are processed by the STATISTICA software, version 6.0. The analysis of variance (ANOVA) is used to determine the degree of significance. Means are compared using Duncan's tests at the probability threshold ($P < 0.05$).

III - RESULTS

III - 1 - Determination of the mineral elements of the algae

The results obtained from the mineral analysis of the three brown algae (*C. gibraltarica*, *B. bifurcata* and) showed the presence of various micro and macro elements (tab. 1). In general the nitrogen, calcium and potassium contents of *B. bifurcata* are significantly higher than those of *F. spiralis* and *C. gibraltarica*. The alga *F. spiralis* has significantly higher contents of magnesium, phosphorus and sodium than the other two algae *B. bifurcata* and *C. gibraltarica*. Similarly, the iron and copper contents of *F. spiralis* and *C. gibraltarica* are significantly equivalent and higher compared to the alga *B. bifurcata*. The manganese contents of *B. bifurcata* and *C. gibraltarica* are significantly equivalent and higher compared to *F. spiralis*. While the zinc content of *B. bifurcata* is significantly high compared to the other two algae *F. spiralis* and *C. gibraltarica* which have equivalent content.

The concentration of N and Ca is higher in *B. bifurcata* (1.75 and 3.24 g/100g DM, respectively), while P and Na are rather high in the extract of *F. spiralis* (0.2 and 1.85 g/100g DM, respectively). As for K and Ca are well represented in the extracts of the three algae with a higher content in *B. bifurcata* (5.02 g/100g DM) followed by *F. spiralis* (4.96 g/100g DM) and *C. gibraltarica* with a value of 4.12 g/100g DM. The concentration of Fe and Cu is high in the extracts of the two algae *F. spiralis* and *C. gibraltarica* (tab. 1). The Zn content is higher in the extract of *B. bifurcata* (13.6 mg/kg DM). As for Mn, it is well represented in the extracts of *B. bifurcata* and *C. gibraltarica* (18.44 and 18.45 mg/kg DM, respectively). The same remark for Mg which represents a high concentration in *F. spiralis* (1.03 g/100g DM).

Table 1: The content of macro and microelements in the extract of the three brown algae.

The values represent the mean ± SD (n = 3). *B.B: B. bifurcata*, *C.G*: *C. gibraltarica*, and *F.S*: *F. spiralis.* Values indicated by a different letter are significantly different $P \leq 0.05$.

III - 2 - Content of total sugars

The total sugar content of *C. gibraltarica* shows a significant difference with the two other algae *B. bifurcata* and *F. spiralis* and has the highest content. Also *B. bifurcata* has a significantly lower total sugar content than the alga *F. spiralis.*

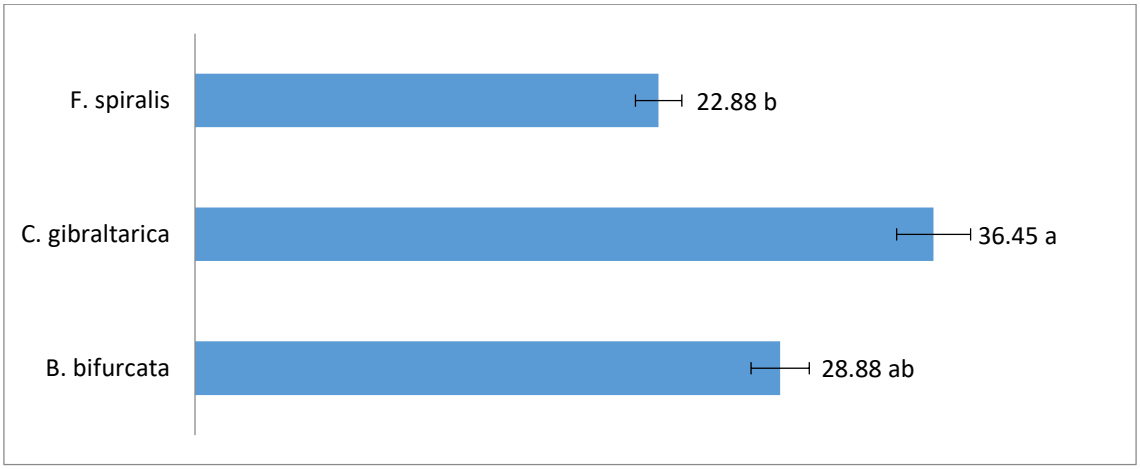

Figure 1: Total sugar contents of aqueous extracts of the three algae: *B. bifurcata, C. gibraltarica* and *F. spiralis* in mg/g DM. Values indicated by a different letter are significantly different $P \leq 0.05$.

The total sugar content in the aqueous extract of *C. gibraltarica* reached an average of 36.45 mg/g DM, while in the aqueous extract of *B. bifurcata*, this level reached an average of 28.8 mg/g DM. The lowest value is recorded in the aqueous extract of *F. spiralis* with an average of 22.88 mg/g DM (Fig. 1).

III - 3 - Protein content

In general no significant difference was recorded between the three algal extracts (Fig. 2). Indeed, the aqueous extract of *C. gibraltarica* showed a protein content of 48.44 mg/g DM, the aqueous extract of *F. spiralis* showed an average protein content of 45.55 mg/g DM, and that of the aqueous extract of *B. bifurcata* is 44.03 mg/g DM (Fig. 2).

Figure 2: Protein contents of aqueous extracts of the three algae: *B. bifurcata, C. gibraltarica* and *F. spiralis* in mg/g DM. Values indicated by a different letter are significantly different P≤0.05.

III - 4 - Organic matter content

The three brown algae *C. gibraltarica*, *B. bifurcata* and *F. spiralis* present consequent contents in organic matter. *F. spiralis* and *C. gibraltarica* have significantly equivalent organic matter contents (73.74 and 73.44 g/100g DM, respectively). *B. bifurcata*, which has the lowest value among the three algae, shows on the other hand a significantly different average organic matter content from the other two algae *F. spiralis* and *C. gibraltarica* (69.68 g/100g DM) (Fig. 3).

	g/100g							mg/kg			
species	**MO**	**N**	**Ca**	**K**	**Mg**	**P**	**Na**	**Fe**	**Mn**	**Cu**	**Zn**
B.B	**69,68b**	**1,75 ±0,1a**	**3,24±0,3a**	**5,02±0,02a**	**0,66±0,06c**	**0,16±0,01b**	**1,65±0,4b**	**37,28±0,4b**	**18,44±0,1a**	**12,87±008b**	**13,6±0,1a**
C.G	**73,44a**	**1,38±0,1b**	**2,46±0,1b**	**4,12±0,03c**	**0,74±0,1b**	**0,12±0,01c**	**0,98±0,2c**	**55,92±0,5a**	**18,45±0,1a**	**25,76±0,09a**	**5,45±0,1b**
F.S	**73,74a**	**1,33±0,8c**	**2,34±0,2c**	**4,96±0,01b**	**1,03±0,08a**	**0,20±0,02a**	**1,85±0,3a**	**55,94±0,9a**	**9,22±0,1b**	**25,76±0,08a**	**5,46±0,2b**

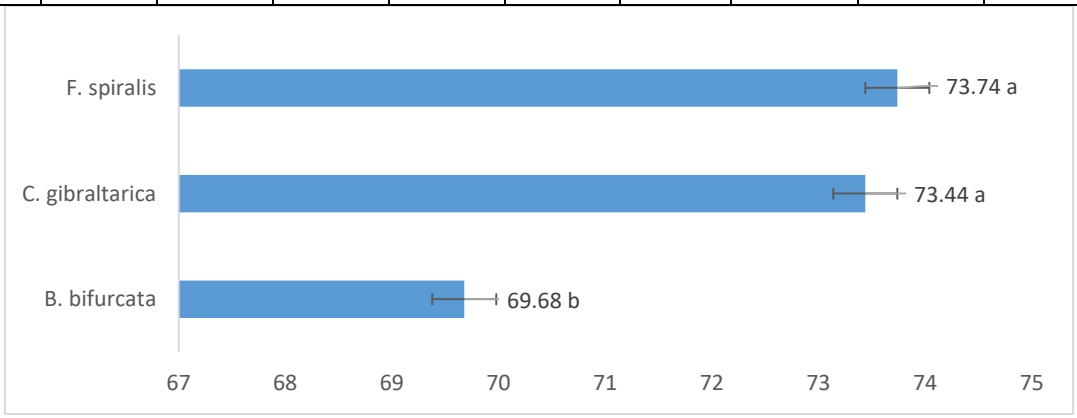

Figure 3: Organic matter contents of three algal species: *B. bifurcata, C. gibraltarica* and *F. spiralis* in g/100g DM. Values indicated by a different letter are significantly different P≤0.05.

III - 5 - Total phenol content

The results of the determination of the total phenols content are expressed in µg gallic acid equivalent (GAE) per mg DM.

In this study, the analysis of total phenols shows that *F. spiralis* presents a significant difference with the two other algae (Fig 4) and has the highest content (7.2 µg/mg DM). The contents of total phenols of *B. bifurcata* and *C. gibraltarica* are significantly equivalent with 3.6 µg/mg dry matter for *B. bifurcata* and 2.32 µg/mg dry matter for *C. gibraltarica.*

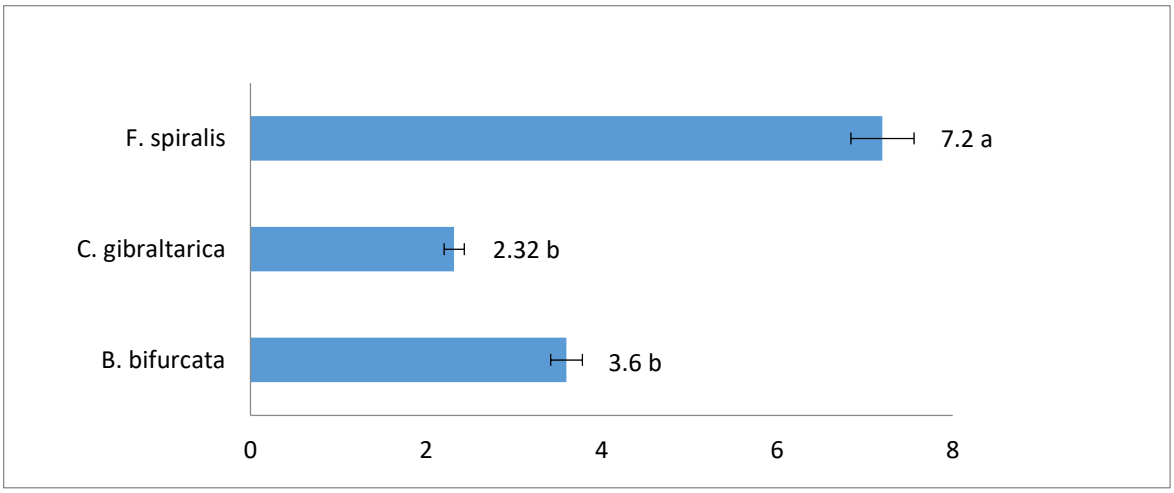

Figure 4: Total phenol contents of methanolic extracts of three algal species: *B. bifurcata, C. gibraltarica* and *F. spiralis* in µg/mg DM. Values indicated by a different letter are significantly different P≤0.05.

III - 6 - Flavonoid content

The flavonoid content expressed in µg quercetin equivalent/mg DM, varies according to the species. The results below indicate the flavonoid content of methanolic extracts of three brown algae by two different methods: Andary's method (Andary, 1990), using the NEU reagent (Neu, 1956) and Jay's method (Jay et al., 1975) using $AlCl_3$.

In general and regardless of the flavonoid assay method, the flavonoid content of the three algae is significantly different. The methanolic extract of *F. spiralis* shows the highest flavonoid content, followed by *B. bifurcata*. While the lowest content is presented with the methanolic extract of *C. gibraltarica* (Fig. 5).

We notice that for all three algal extracts, the determination with $AlCl_3$ gives higher flavonoid contents than the determination with Neu's reagent.

According to Andary's method, the highest flavonoid content was presented by the methanolic extract of *F. spiralis* 3.88 µg/mg dry matter, followed by the alga *B. bifurcata* 3.17 µg/mg dry matter. While the lowest flavonoid value was recorded by the methanolic extract of *C. gibraltarica* 2.61 µg/mg dry matter (Fig. 5). Similar results were obtained by Jay's method (flavonoid determination by $AlCl_3$). The highest value was also presented by the methanolic extract of *F. spiralis* 5.59 µg/mg DM., followed by *B. bifurcata* 3.77 µg/mg DM. While the lowest flavonoid content was recorded by the methanolic extract of *C. gibraltarica* with 3.12 µg/mg DM. (fig. 5).

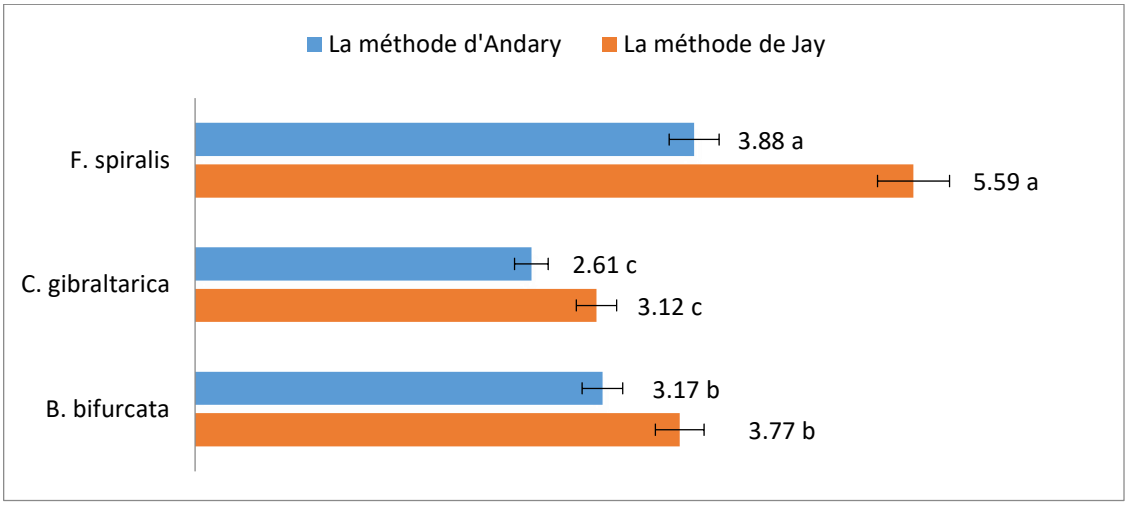

Figure 5: Flavonoid contents of methanolic extracts of three algal species: *B. bifurcata, C. gibraltarica* and *F. spiralis* by two methods (Andary and Jay). Values indicated by a different letter are significantly different P≤0.05.

III - 7 - Antioxidant activity of the studied algae

The results of the antioxidant potential using the DPPH reveal a significant difference between the three algae. Indeed, the extracts of the analyzed algae present an antioxidant activity varying between 33.61 and 88.3% (Fig. 6), with a high antioxidant potential (88.3%) for *F. spiralis* and thus an important capacity to trap the DPPH radical. While an average power of antioxidant activity is

recorded by the extract of *B. bifurcata* 64.3%. Like total phenols and total flavonoids, *C. gibraltarica* has the lowest antioxidant potential (33.61%) among the three analyzed algae (Fig. 6).

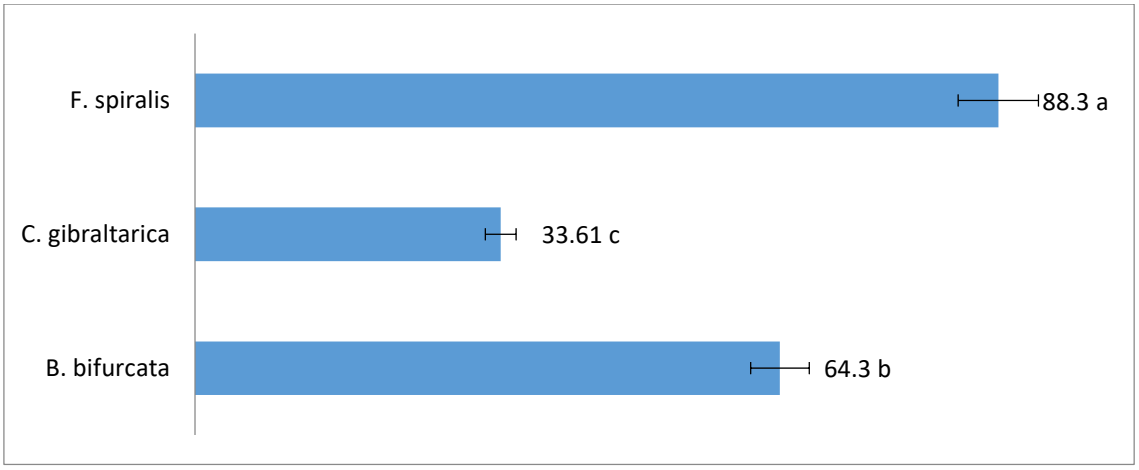

Figure 6: Antioxidant activity of methanolic extracts of three algal species: *B. bifurcata, C. gibraltarica* and *F. spiralis* using the scavenger method (DPPH). Values indicated by a different letter are significantly different P≤0.05.

IV - DISCUSSION

Mineral analyses of the three brown algae *F. spiralis, B. bifurcata* and *C. gibraltarica* highlighted the richness of these brown algae in macroelements (Ca, K, P, Na, N), which is in agreement with the work of Hong et al., (2007); Kalaivanan and Venkatesalu, (2012); Hernandez-Herrera et al., (2013). Mineral element contents of algae can vary depending on season, location and analytical methods (Castro-Gonzalez et al., 1996).

The alga *C. gibraltarica* shows a relatively high content of total sugars followed by *B. bifurcata*, while *F. spiralis* records the lowest value. Similar results showed a difference in the total sugar content of the three algal species *Ascophyllum nodosum* (63.6 g/100g), *Bifurcaria bifurcata* (15.71 g/100g) and *Fucus vesiculosus* (34.53 g/100g) (Agregan et al., 2016). In our study the total sugar contents of the three algae are low compared to those in the study by Agregan et al., (2016). It should be noted that the extract of *F. spiralis* is very viscous and difficult to filter, due to the presence of mucilaginous substances. This could explain the lower total sugar content of this species (Agregan et al., 2016).

C. gibraltarica also has the highest protein content followed by *F. spiralis* and *B. bifurcata*. The results obtained by Agregan et al, (2016) show high protein contents by *F. vesiculosus* followed by *B. bifurcata* while *A. nodosum* records the lowest content. On the other hand, the protein contents obtained from the study of the three algae (*C. gibraltarica, F. spiralis* and *B. bifurcata*) are low compared to those of the study by Agregan et al., (2016). The values of protein content and total sugars vary considerably among species.

Phenolic compounds play an important role as protective factors. They can delay or inhibit lipid oxidation by inhibiting the initiation or propagation of oxidative chain reactions, and are also involved in free radical scavenging (Piccolella et al., 2008).

Our study shows that the analysis of phenolic compounds of the methanolic extract obtained from the brown alga *F. spiralis* presents the highest content of total phenols and total flavonoids followed by *B. bifurcata* and *C. gibraltarica*. These results are relatively high compared to the values found by Yuan and Walsh (2006) who showed a very low content of total phenols in other algal species *Palmaria palmata* with 0.128 mg GAE/g dry matter and *Nereocystis leutkeana* with 0.039 mg GAE /g dry matter. On the other hand, the extract of the alga *Macrocystis integrifolia* (3.95 mg GAE/g DM) shows a higher content compared to the two brown algae *B. bifurcata* and *C. gibraltarica*, while it shows a low content compared to the extract of *F. spiralis*. The contents of total phenols obtained from the different algal species ranged from 2.32 to 7.2 µgGAE/mg. The values of total phenols obtained in our study are lower than those reported by Wang et al. (2009) (*Ascophyllum nodosum* 13.8 g/100g, *Fucus serratus* 16.9 g/100g and *Fucus vesiculosus* 17.6 g/100g), while these values are higher than those reported by Farvin and Jacobsen, (2013) who showed total phenols contents ranging from 0.011 to 0.61 g/100g for different algal species. On the other hand, other research reported that methanolic extracts are the richest in total phenols (Abdille et al., 2005). Indeed, the amount of phenolic compounds is different from one species to another because of genetic, physiological, abiotic factors... (Maisuthisakul et al., 2007; Ksouri et al., 2009). In general, higher phenolic content leads to higher antioxidant capacity.

Regardless of the flavonoid assay method, the methanolic extract of *F. spiralis* showed the highest flavonoid content, followed by *B. bifurcata* and *C. gibraltarica*. The highest flavonoid values are presented by Jay's method (flavonoid determination by $AlCl_3$) (El Guiche et al., 2015). This difference is probably due to the reagent used, indeed the Neu reagent is more specific than $AlCl_3$. On the other hand, the flavonoid content in the three algal species *F. spiralis, B. bifurcata* and *C. gibraltarica* is relatively low compared to other algae such as *Ulva intestinalis* (8.04 mg/g DM) and *Ulva clathrata* (33.09 mg/g DM) (Farasat et al., 2014). Research has

shown marked changes in the chemical constituents of algae with season and environmental conditions (Manivannan et al., 2009). This variation in flavonoid content may be due to variation in physicochemical parameters such as salinity at harvesting stations (Farasat et al., 2014). Flavonoids remain a very important class of phenolic compounds, these bioactive molecules have effective antioxidant properties. They have a primary role in neutralizing free radicals (Zaragozá et al., 2008).

The present study shows values of antioxidant activity of the three studied brown algae *F. spiralis, B. bifurcata,* and *C. gibraltarica* (88.3%, 64.3%, and 33.61%, respectively) that are higher compared to those found by Agregan et al., (2016) for the three algae *A. nodosum, F. vesiculosus*, and *B. bifurcata*. (4.34%, 38.88%, and 23.58%, respectively). Antioxidant activity values are proportional to phenolic compound contents (total phenols and flavonoids) in the three brown algae (*F. spiralis, B. bifurcata*, and *C. gibraltarica*). A higher antioxidant power is recorded by *F. spiralis* as well as a maximum content of phenolic compounds. While, *C. gibraltarica* presents the lowest antioxidant power with a low content of phenolic compounds. These results obtained are confirmed by the correlation test, since there is a strong positive correlation between DPPH and phenolic compounds (total phenols and flavonoids). Most of the results previously reported are expressed as percentage of inhibition of DPPH scavenging activity. The values recorded in terrestrial plant extracts are generally above 50% (Ali et al., 2016). On the other hand the values of antioxidant activity cannot exceed 50% in most of the algal extracts, the case of our *C. gibraltarica* extract and the three algal extracts in the study by Agregan et al., (2016).

V - CONCLUSION

The results of our study showed that the three algal species *F. spiralis, B. bifurcata* and *C. gibraltarica* are rich in active molecules that can influence several parameters at the level of the studied algae. The alga *F. spiralis* presents a high content of phenolic compounds with a high antioxidant power, and a high content of organic matter. While *C. gibraltarica* shows a high content of proteins and total sugars. The mineral analysis of the extracts of *F. spiralis, B. bifurcata* and *C. gibraltarica* highlighted the richness of these brown algae in macroelements (Ca, K, P, Na, N).

These three studied brown algae can play an important role as biostimulants in the agricultural field. They represent a promising source of biologically active molecules that can be used in organic agriculture.

REFERENCES

1. Abdille, M. H., Singh, R., Jayaprakasha, G., & Jena, B. (2005). Antioxidant activity of the extracts from Dillenia indica fruits. *Food chemistry*, *90*(4), 891-896.
2. Agregan, R. n., Munekata, P. E., Domanguez, R., Carballo, J., Franco, D., & Lorenzo, J. M. (2016). Proximate composition, phenolic content and in vitro antioxidant activity of aqueous extracts of the seaweeds *Ascophyllum nodosum, Bifurcaria bifurcata* and *Fucus vesiculosus*. Effect of addition of the extracts on the oxidative stability of canola oil under accelerated storage conditions. *Food Research International*, *99*, 986-994.
3. Águila-Ramírez, R. N., Arenas-González, A., Hernández-Guerrero, C. J., González-Acosta, B., Borges-Souza, J. M., Véron, B., (2017). Antimicrobial and antifouling activities achieved by extracts of seaweeds from Gulf of California, Mexico. *Hidrobiológica*, *22*(1), 8-15.
4. Ahn. (2007). A Study on the Growth and Management Status of Mansongjung forest in Hahoe, Andong (10.7229/jkn.2011.4.3.161).
5. Ali, N., A. Farrell, A. Ramsubhag and J. Jayaraman (2015). "The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato grown under tropical conditions." *Journal of Applied Phycology* 28(2): 1353-1362.
6. Andary, C. 1990. Documentation chimique et pharmaceutique pour l'AMM du MERALOPS comprimés. Laboratoire Allergan-Dulcis, Monaco, France.
7. Ben Ahmed, Z., Yousfi, M, Viaene, J, Dejaegher, B, Demeyer, K, Mangelings, D, Heyden, YV. (2016). "Determination of optimal extraction conditions for phenolic compounds from Pistacia atlantica leaves using the response surface methodology." Analytical Methods 8(31): 6107-6114.
8. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199.
9. Boizot, N. and Charpentier, J.-P. 2006. Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. *Le Cahier des Techniques de l'NRA, Numéro spécial 2006: Méthodes et outils pour l'observation et l'évaluation des milieux forestiers, prairiaux et aquatiques*: 79-82.
10. Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.
11. Bruneton, J. (1999). Pharmacognosy. Phytochemistry. Medicinal plants (second ed.). Paris: Lavoisier. Publishing: 1095-1119.
12. Castro-Gonzalez, M. I., Pérez-Gil, F., Pérez-Estrella, S., & Carrillo-Doma-nguez, S. (1996). Chemical composition of the green alga Ulva lactuca. *Ciencias Marinas*, *22*(2), 205-213.

13. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356.
14. El Guiche, R., S. Tahrouch, O. Amri, K. El Mehrach and A. Hatimie (2015). "Antioxidant activity and total phenolic and flavonoid contents of 30 medicinal and aromatic plants located in the South of Morocco." *International Journal of New Technology and Research* 1(3).
15. Farasat, M., Khavari-Nejad, R.-A., Nabavi, S. M. B., & Namjooyan, F. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian journal of pharmaceutical research: IJPR*, *13*(1), 163.
16. Farvin, K. S. and C. Jacobsen (2013). "Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast." *Food chemistry* 138(2-3): 1670-1681.
17. Frolund, B., Griebe, T., & Nielsen, P. H. (1995). Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology*, *43*(4), 755-761.
18. Harnafi, H., el Houda Bouanani, N., Aziz, M., Caid, H. S., Ghalim, N., & Amrani, S. (2007). The hypolipidaemic activity of aqueous Erica multiflora flowers extract in Triton WR-1339 induced hyperlipidaemic rats: a comparison with fenofibrate. *Journal of Ethnopharmacology*, *109*(1), 156-160.
19. Hernandez-Herrera, Mireya, R., Santacruz-Ruvalcaba, F., Ruiz-Lopez, M. A., Norrie, J., & Hernandez-Carmona, G. (2013). Effect of liquid seaweed extracts on growth of tomato seedlings (Solanum lycopersicum L.). *Journal of applied phycology*, *26*(1), 619-628.
20. Hong, D. D., Hien, H. M., & Son, P. N. (2007). Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology*, *19*(6), 817-826.
21. Jay, M., Gonnet, J.-F. o., Wollenweber, E., & Voirin, B. (1975). Sur l'analyse qualitative des aglycones flavoniques dans une optique chimiotaxinomique. *Phytochemistry*, *14*(7), 1605-1612.
22. Kalaivanan, C., & Venkatesalu, V. (2012). Utilization of seaweed Sargassum myriocystum extracts as a stimulant of seedlings of Vigna mungo (L.) Hepper. *Spanish Journal of Agricultural Research*, *10*(2), 466-470.
23. Khan,W., Rayirath, U.P., Subramanian, S., Mundaya, N., Jithesh, M.N., Rayirath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J., Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul., 28:386–399.
24. Kindleysides, S., Quek, S.-Y., & Miller, M. R. (2012). Inhibition of fish oil oxidation and the radical scavenging activity of New Zealand seaweed extracts. *Food Chemistry*, *133*(4), 1624-1631.
25. Ksouri, R., Falleh, H., Megdiche, W., Trabelsi, N., Mhamdi, B., Chaieb, K., et al. (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. *Food and Chemical toxicology*, *47*(8), 2083-2091.
26. Loo, A., Jain, K. and Darah, I. 2008. Antioxidant activity of compounds isolated from the pyroligneous acid, Rhizophora apiculata. *Food Chemistry*, 107(3): 1151-1160.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol chem*, *193*(1), 265-275.
28. Maisuthisakul, P., Suttajit, M., & Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food chemistry*, *100*(4), 1409-1418.
29. Makkar, H. P. (2003). Quantification of tannins in tree and shrub foliage: a laboratory manual, Springer Science & Business Media. 4-6.
30. Manivannan, S., Balamurugan, M., Parthasarathi, K., Gunasekaran, G., & Ranganathan, L. S. (2009). Effect of vermicompost on soil fertility and crop productivity-beans (Phaseolus vulgaris). *Journal of Environmental Biology*, 30(2): 275-281.
31. Neu, R. 1956. A new reagent for differentiating and determining flavones on paper chromatograms. *Naturwissenschaften*, 43(82): 10.1007.
32. Olsen, S. L. Dean (1965). Phosphorus. Chemical and microbiological properties. *Methods of Soil Analysis*, Part 2: 1035-1048.
33. Page, Miller RH, Keeney DR, Baker D, Ellis R and R. J (1982). "Methods of soil analysis. eds." 631.41: 9-2.
34. Pérez, M. J., Falqué, E., & Domínguez, H. (2016). Antimicrobial action of compounds from marine seaweed. *Marine drugs*, 14(3): 52-57.
35. Piccolella, S., A. Fiorentino, S. Pacifico, B. D'Abrosca, P. Uzzo and P. Monaco (2008). "Antioxidant properties of sour cherries (Prunus cerasus L.): role of colorless phytochemicals from the methanolic extract of ripe fruits." *Journal of agricultural and food chemistry* 56(6): 1928-1935.
36. Riadi, H. (1998). Biodiversité des algues marines. *Rapport étude nationale de biodiversité Projet GEF/6105*, 96.

37. Sabeena, F., Jacobsen (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. (10.1016/j.foodchem.2012.10.078).
38. Sanchez-Moreno, C. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food science and technology international*, *8*(3), 121-137.
39. Sharma, H. S. S., C. Fleming, C. Selby, J. R. Rao, T. Martin (2014). Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *Journal of Applied Phycology*, 26(1): 465-490.
40. Singleton, V. L., R. Orthofer and R. M. Lamuela-Raventós (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, 299: 152-178.
41. Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of applied phycology*, 16(4): 245-262.
42. Wang, T., R. Jonsdottir and G. Ólafsdóttir (2009). Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food chemistry*, 116(1): 240-248.
43. Yuan, Y. V., & Walsh, N. A. (2006). Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food and chemical toxicology*, *44*(7) : 1144-1150.
44. Zaragozá, M., López, D., P. Sáiz, M., Poquet, M., Pérez, J., Puig-Parellada, P., et al. (2008). Toxicity and antioxidant activity in vitro and in vivo of two Fucus vesiculosus extracts. *Journal of agricultural and food chemistry*, *56*(17), 7773-7780.
45. Zodape, S., S. Mukhopadhyay, K. Eswaran, M. Reddy and J. Chikara (2010). Enhanced yield and nutritional quality in green gram (Phaseolus radiata L) treated with seaweed (Kappaphycus alvarezii) extract. *Journal of scientific and industrial research*, 69: 468-471.

Appendix

Appendix I

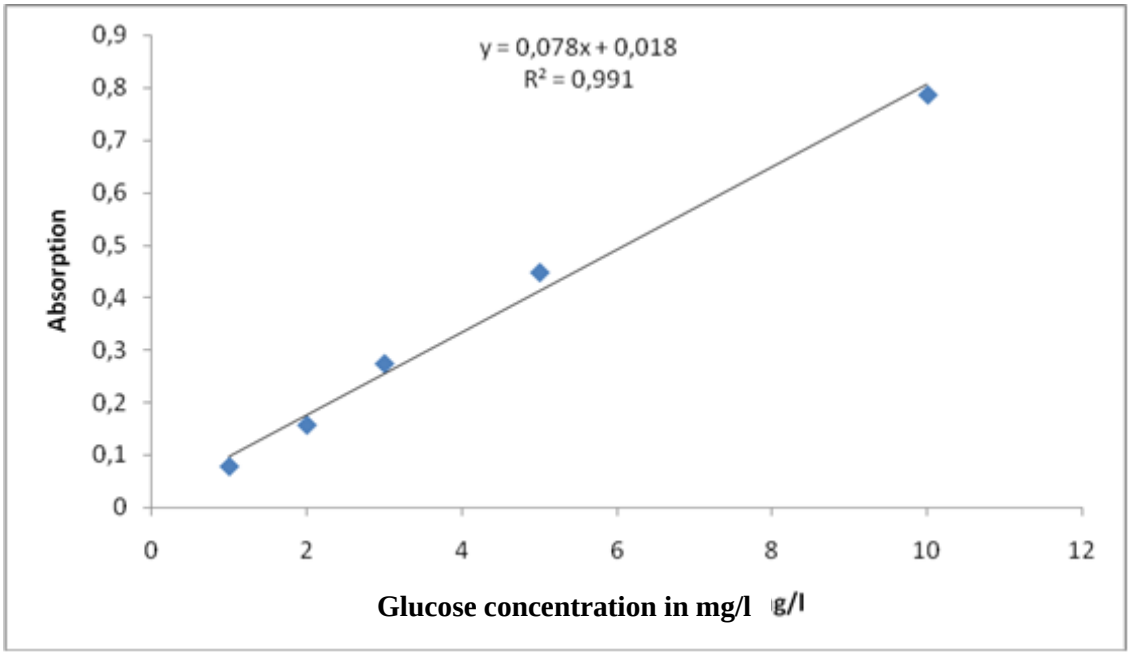

Figure 1: Calibration range for glucose in mg/l

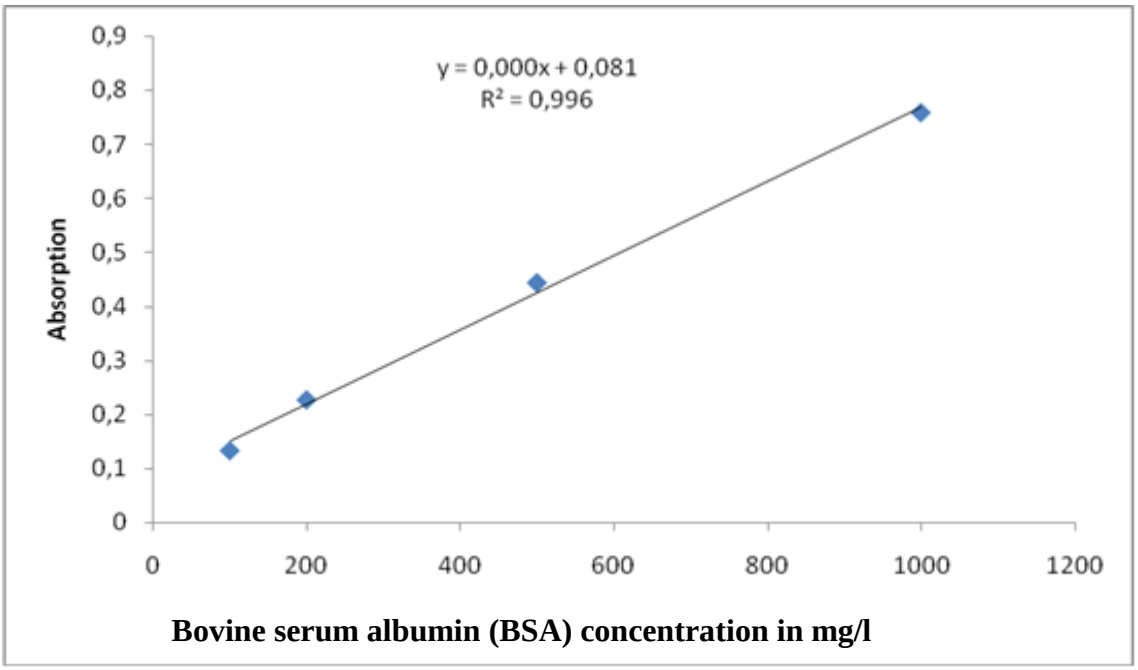

Figure 2: Calibration range of BSA in mg/l

Appendix II

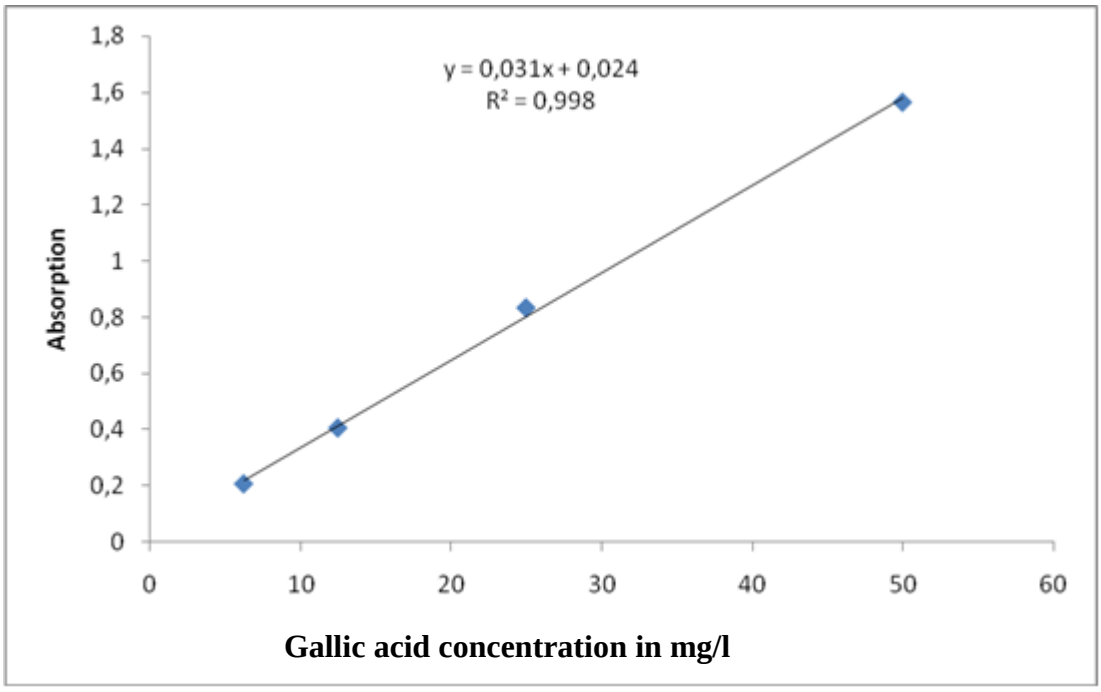

Figure 3: Calibration range of gallic acid in mg/l.

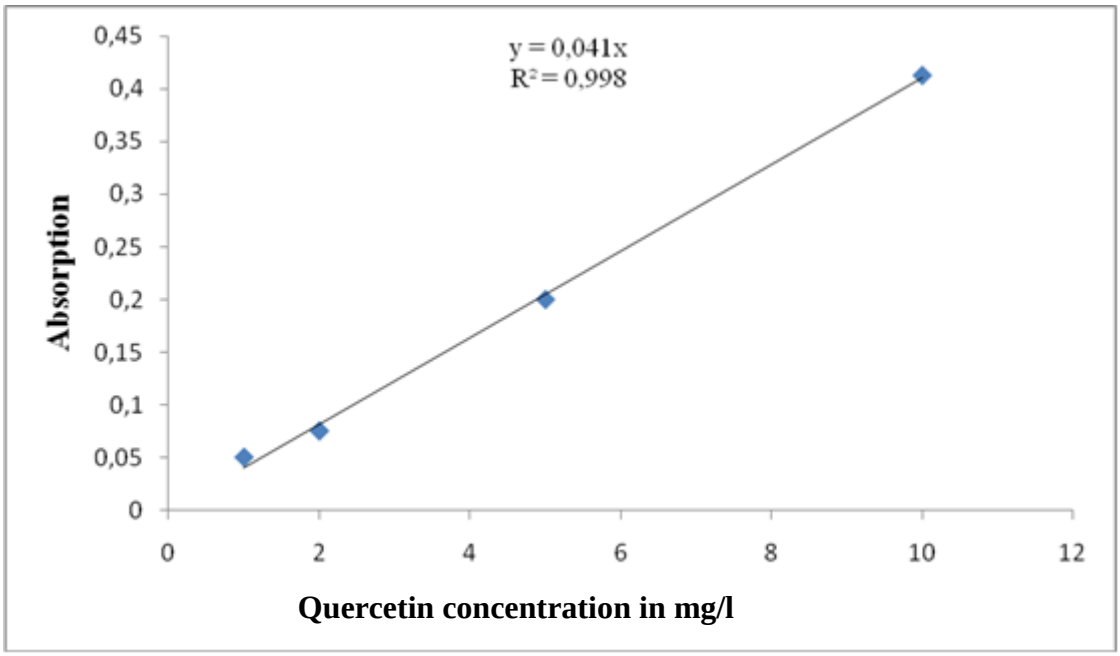

Figure 4: Calibration range of quercetin in mg/l.