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Chemical Profile and Antioxidant Power of Secondary Metabolites Extracted from Green, Brown, and Red Seaweeds Harvested from Algerian Coast

Imad Abdelhamid El Haci[1, 2*], Zahia Ghouila[1], Khadidja Dechira[1], Wacila Benguedda-Rahal[3], Meriem Belarbi[2]

[1]Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques CRAPC, Bou-Ismail, BP 384, 42004 Tipaza, Algérie
[2]Laboratoire des Produits Naturels, Département de Biologie, Faculté SNV-STU, Université de Tlemcen, B.P.119, 13000 Tlemcen, Algérie
[3]Laboratoire de Valorisation des Actions de l'Homme pour la Protection de l'Environnement et Application en Santé Publique, Faculté SNV-STU, Université de Tlemcen, B.P.119, 13000 Tlemcen, Algérie
[*]**Corresponding Author: imad.elhaci@crapc.dz**

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ABSTRACT

The growing trend toward natural products in pharmaceuticals has fueled a surge in interest for exploring bioactive compounds from marine algae. Phenolic compounds, a vast and ubiquitous class of plant chemicals, have become a major focus of research due to their diverse pharmacological properties and potential health benefits. Seaweeds hold an immense potential due to their vast array of versatile molecules with diverse properties and multiple biological activities. Three algal samples from the Chlorophyta (*Ulva lactuca*), Phaeophyta (*Sargassum vulgare*) and Rhodophyta (*Corallina officinalis*) were assessed for phenolic compounds content and antioxidant power, in addition of a chemical characterization of the most active fractions using RP-HPLC technique. Fifteen extracts were obtained and phenolics and flavonoids were quantified. *C. officinalis* ethyle acetate and butanolic fractions were the most potent extracts. Gallic, chlorogenic, and p-coumaric acids were identified in the both extracts in addition to rutin, catechin and naringenin compounds. Our findings highlighted the significant potential of *C. officinalis* as a powerful alga tested. Future research will delve deeper into this promising species to fully explore its bioactive potential.

INTRODUCTION

Algae, a diverse and often overlooked group of aquatic organisms, outshine their terrestrial counterparts in sheer abundance **(Baweja *et al.*, 2016; Peñalver *et al.*, 2020; Lomartire *et al.*, 2021)**. Contributing a significant 10% to the plant kingdom's biomass, they thrive in a multitude of aquatic environments (freshwater, harsh marine ecosystems, etc.) **(Alssali *et al.*, 2016; Pereira, 2020; Al-Adilah *et al.*, 2022; Zhao *et al.*, 2022)**. This diversity, coupled with their intricate cellular structures, presents a fascinating challenge for comprehensive study.

Algae, classified by size and structure, encompass two main categories: macroalgae, which include familiar seaweeds such as red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyceae) varieties, and microalgae, typically single-celled organisms, including blue-green algae **(Hernandez-Ledesma & Miguel Herrero, 2014; Beaumont *et al.*, 2021; Abdel-Kareem & El-Saied, 2022)**.

Living in a constant struggle for survival, marine algae have evolved a remarkable arsenal of unique, biologically active metabolites **(Filote *et al.*, 2021; Naiel *et al.*, 2021; Vijay Sankar *et al.*, 2023)**. These specialized molecules serve as a sophisticated defense system, allowing algae to flourish in competitive and often harsh environments **(Abdelfattah *et al.*, 2023; Singh *et al.*, 2023; Tan, 2023)**.

Notably, phenolic compounds represent a rich source of these secondary metabolites within marine algae **(Gager *et al.*, 2021; Jimenez-Lopez *et al.*, 2021)**. In fact, the content and the nature of these phenolic compounds exhibit significant variation not only between different algal species (interspecific) but also within the same species depending on factors like seasonality (intraspecific) **(Lomartire *et al.*, 2021; Qui-Minet *et al.*, 2021; Coaten *et al.*, 2023)**.

Brown macroalgae, for instance, demonstrate a particular abundance of phlorotannins, a subclass of phenolics. Conversely, red and green algae generally exhibit lower levels and diversity of these compounds **(Del Mondo *et al.*, 2021; Torres *et al.*, 2024)**.

However, regardless of the vast potential of algal metabolites, particularly those with antioxidant properties, data on Algerian seaweeds remain scarce. There is a scarcity of data on the biological activities of algal extracts specific to this region **(Belalia *et al.*, 2020; Kerzabi-Kanoun *et al.*, 2021; Kord *et al.*, 2021; Benmahdjoub *et al.*, 2022; Saidani *et al.*, 2022; Mazouzi *et al.*, 2023)**.

This study aimed to bridge the knowledge gap regarding the antioxidant potential of Algerian seaweeds. The phenolic compound and antioxidant activities of algal extracts from the Algerian coast were investigated. Specifically, the study focused on abundant species in the study area with the potential to serve as a natural source of antioxidant metabolites for the pharmaceutical and medical sectors. Additionally, chemical characterization was carried out using the RP-HPLC-PDA technique to explore some of the obtained organic fractions.

The research sites were strategically selected along the western Algerian coast in the Tlemcen region. The algal species collected and investigated include *Ulva lactuca*, *Sargassum vulgare*, and *Corallina officinalis*.

MATERIALS AND METHODS

1. Biomass harvesting and processing

Chlorophyceae; *Ulva lactuca*, Phaeophyceae; *Sargassum vulgare*, and Rhodophyceae; *Corallina officinalis* were collected along the coast of Tafssout and from Elouardanya (Daira of Honaine) Tlemcen, Algeria during winter season (February 2024) (Table 1 & Fig. 1). Algae collected were extensively rinsed with tap water to remove adhering organisms and debris (epiphytes, barnacles, and gastropods). The algae samples were air dried and, after that, were powdered and stored at -23°C for further uses. Algal herbarium specimens were identified according to **Benmahdjoub (2022)**.

Table 1. List of the species studies, corresponding code names and harvesting regions (all samples were harvested in February 2024)

Species	Code	Harvest region	Coordinates
Ulva lactuca L.	ULEXT	Tafsout-Tlemcen	35°11′14.015″N 1°38′51.969″W
Sargassum vulgare C. Agardh.	SVEXT		
Corallina officinalis L.	COEXT	Elouardanya -Tlemcen	35°14′8.396″N 1°35′18.863″W

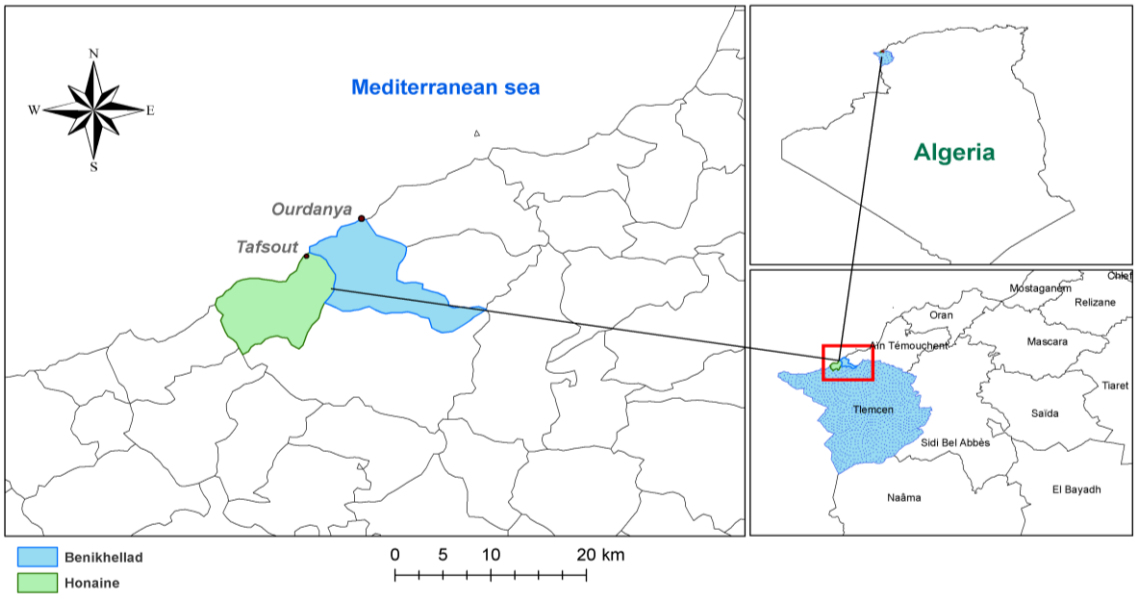

Fig. 1. Sampling locations of the algal materials studied

2. Secondary metabolites extraction

A total of 30g of each algae powder were mixed with 80% hydromethanolic solution and macerated at +4°C for 72h. After filtration, the mixture was evaporated to dryness under vacuum at +50°C. The resulting dry residue was weighed to calculate yield of extraction and was then dissolved in methanol to obtain crude extract. This operation was repeated to obtain a new dry residue, which was then subjected to successive liquid-liquid extractions using different solvents: dichloromethane, ethyl acetate, and butanol. After each fractionation, the organic phase was evaporated and a dry residue was obtained and recovered in methanol.

3. Phenolic compound contents

Total phenolic contents were estimated by using the Folin-Ciocalteu method described in **Vermerris and Nicholson (2006)**. Briefly, 100µL of a different extract was added to 2mL of 2% (w/w) sodium carbonate, followed by vigorous mixing for 5 minutes. Then, 0.1mL of Folin-Ciocalteu reagent was added, and incubated for 30 minutes. Absorbances were recorded at 750nm against a blank. Gallic acid calibration curve was used to express results as mg gallic acid equivalents per gram of dry extract (mg GAE/g).

4. Flavonoid compound contents

Total flavonoid content was measured using the method adapted from **Barros *et al.* (2011)**. 0.5mL of different extracts were mixed with 2mL of dH_2O, followed by 0.15mL of 15% (w/w) of sodium nitrate solution. Subsequently, 0.15mL of 10% (w/w) aluminum chloride solution was added. 2mL of 4% (w/w) sodium hydroxide solution was added. Immediately, the final volume was adjusted to 5mL with dH_2O. After incubation for 15 minutes, the absorbances were measured (510nm). The results were expressed as mg of catechin equivalents per gram of dry extract (mg CEQ/g).

5. DPPH scavenging activity

1950µL of $DPPH^{\bullet}$ solution (6×10^{-5} M in methanol) was added to each sample (50µL). After incubation at room temperature (30 minutes, dark conditions), absorbances were measured (517nm) **(Barros *et al.*, 2011)**. The DPPH radical scavenging activity (SA) was then calculated using the following equation:

$$SA\ \% = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Inhibition concentration of 50 % was recorded graphically from regression analysis.

6. RP-HPLC-PDA analysis

Separation and identification of phenolics contained in the most effective organic fractions were achieved using RP-HPLC-PDA technique. Analysis was conducted on a Perkin Elmer Flexar system (C18: 150 x 4.6 mm, 5µm). Mobile phase: A (ultra pure H_2O/acetic acid, 98:2) and acetonitrile as solvent B. Gradient elution program was employed, starting with 90% A for 5 minutes, followed by a decrease in A to 10% over 15 minutes. The mobile phase was then held at 100% B for 15 minutes, before returning to 90% A for a final 20-minute equilibration step. A flow rate of 1mL/ min was used.

Detection mode of separated compounds was achieved and recorded at 280nm **(El Haci *et al.*, 2020)**. Peak assignment was achieved by comparing retention times and UV spectra with those of standards already used.

7. Statistical analysis

All the reported data are shown in means ± S.D (standard deviation) (triplicate). Statistical analysis was conducted with 3 groups using one-way ANOVA. $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

1. Extraction yield, total phenolic and total flavonoid contents

Table (2) exhibits the results obtained for extraction yields, phenolic and flavonoid contents. As shown in this table, phenolic compounds, in the three studied seaweeds, varied from 0.13 to 3.47mg GAE/g, and 0.19 to 3.88mg CEQ/g, for phenolic and flavonoid, respectively. The obtained yields were arranged from 3.96 to 6%.

The success of chemical extractions for bioactive compounds, like phenolics from seaweeds, is highly dependent on several factors. While time and temperature play a role, the solvent choice and the sample chemical composition are mainly considered critical determinants of extraction yield. Traditionally, researchers have utilized a range of solvents (methanol, ethanol, butanol, chloroform, water, etc.) for these extractions **(Lopez *et al.*, 2011; Osorio-Tobón, 2020; Ruth Alara *et al.*, 2021; Mir-Cerdà *et al.*, 2023)**.

Even the choice of solvent was made for methanol, the yields obtained and phenolic contents were low. This fact was reported in many studies undertaken on various seaweeds **(Abd El-Baky *et al.*, 2009; Abd Elmegeed *et al.*, 2014; Kim *et al.*, 2016; Anjali *et al.*, 2019; Generalić Mekinić *et al.*, 2019; Jimenez-Lopez *et al.*, 2021; Aissaoui *et al.*, 2022; Carpena *et al.*, 2023; Duan *et al.*, 2023)**.

The low phenolic compound contents in seaweeds is a complex issue influenced by multiple factors. These include species composition, environmental conditions (climate change, seasonal variations), harvesting time, and the reproductive stage **(Connan *et al.*, 2004; Fellah *et al.*, 2017)**. Additionally, physical factors like light intensity and quality, photoperiod, and temperature play a role. Notably, algae possess unique components not found in plants, and their abundance varies with species. Importantly, these factors are interconnected, not isolated, and likely work together to influence algal secondary metabolism, impacting phenolic contents **(Zhao *et al.*, 2023; Sadeghi *et al.*, 2024)**.

2. Antioxidant activity

Antioxidant activity assessment of the studied extracts is shown in Table (3). The most significant activity was recorded in *C. officinalis*, with an interesting IC_{50} found for

COEXT-13-EHI, followed by butanolic fraction and crude extract. *S. vulgare* presented a moderate activity especially for butanolic fraction with an IC_{50} of 680µg/ mL. *U. lactuca* crude and fractions presented a weak inhibitory effect on DPPH scavenging (Fig. 2). To explore the potential role of phenolic compounds in the extracts antioxidant activity, their concentrations were correlated with DPPH radical scavenging values (Table 4). Pearson's correlation demonstrated a strong correlation (r = 0.94) between TPC and DPPH assay results for *C. officinalis*. This finding suggests that phenolics significantly contribute to the antioxidant power of these red algae. These promising results support the potential of red algae as a source of natural antioxidants for the food manufacturing, with the potential to develop novel antioxidant products.

However, *U. lactuca* extracts exhibited weak negative correlations, indicating a different mechanism for their antioxidant activity. Conversely, *S. vulgare* extracts showed good correlations between TPC, TFC, and DPPH assay results, suggesting that phenolics play a key function in this species antioxidant properties.

Table 2. Extraction yields, total phenolic and flavonoid contents, DPPH IC_{50} of the different organic extracts

Species	Code	Extract	Yield (%)	TPC	TFC	DPPH IC_{50} (mg/mL)
U. lactuca	ULEXT-1-EHI	Crude extract	3.96 ± 0.92	0,43±0.00^{a}	0,65±0.02^{a}	6.66±0.23
	ULEXT-2-EHI	DCM fraction	2.16 ± 0.02	1,33±0.02^{b}	2,44±0.19^{b}	Nd
	ULEXT-3-EHI	EtOAc fraction	0.91 ± 0.00	1,77±0.04^{c}	2,97±0.12^{b}	Nd
	ULEXT-4-EHI	BuOH fraction	2.42 ± 0.01	1,48±0.02^{d}	1,45±0.04^{c}	Nd
	ULEXT-5-EHI	AQres fraction	72.59 ± 0.90	0,39±0.00^{e}	0,67±0.03^{a}	Nd
S. vulgare	SVEXT-6-EHI	Crude extract	4.00 ± 0.33	0,62±0.02^{a}	0,73±0.01^{a}	2.38±0.00
	SVEXT-7-EHI	DCM fraction	29.55 ± 0.12	0,60±0.03^{a}	1,25±0.01^{b}	Nd
	SVEXT-8-EHI	EtOAc fraction	5.6 ± 0.90	1,57±0.04^{b}	3,88±0.08^{c}	Nd
	SVEXT-9-EHI	Butanol fraction	19.67 ± 0.82	1,65±0.02^{b}	2,40±0.02^{d}	0.68±0.03
	SVEXT-10-EHI	AQres fraction	50.65 ± 0.98	0,13±0.02^{c}	0,19±0.01^{e}	Nd
C. officinalis	COEXT-11-EHI	Crude extract	6.11 ± 0.49	0,49±0.01^{a}	0,45±0.00^{a}	0.42±0.00
	COEXT-12-EHI	DCM fraction	0.98 ± 0.02	2,02±0.05^{b}	2,87±0.09^{b}	Nd
	COEXT-13-EHI	EtOAc fraction	0.91 ± 0.00	3,47±0.05^{c}	2,02±0.01^{c}	0.06±0.00
	COEXT-14-EHI	BuOH fraction	6.98 ± 0.00	1,34±0.01^{d}	1,11±0.01^{d}	0.37 ± 0.00
	COEXT-15-EHI	AQres fraction	38.78 ± 0.93	0,90±0.03^{e}	0,73±0.02^{e}	Nd

Each value represents the mean ± SD (n= 3). Total phenolic content (TPC) was expressed as mg gallic acid equivalents/g dried extract. Total flavonoid content (TFC) was expressed as mg catechin equivalents/g dried extract. IC_{50} values were expressed as final concentrations. Nd: not determined. DCM: dichloromethane extract, EtOAc: ethyl acetate extract, butanol: butanolic extract, AQres: residual aqueous phase. Within the same column, means followed by different letters are significantly different at $P< 0.05$.

Table 3. Antioxidant activity of the different extracts of seaweeds studied

Species		Inhibition percentages of DPPH at 1mg/ mL
U. lactuca	ULEXT-1-EHI	8.02 ± 0.10^{a}
	ULEXT-2-EHI	5.13 ± 0.38^{b}
	ULEXT-3-EHI	2.91 ± 0.56^{c}
	ULEXT-4-EHI	$4.39 \pm 0.51^{d,b,c}$
	ULEXT-5-EHI	$3.39 \pm 0.31^{e,c,d}$
S. vulgare	SVEXT-6-EHI	4.56 ± 0.44^{a}
	SVEXT-7-EHI	$3.90 \pm 0.01^{a,d}$
	SVEXT-8-EHI	8.38 ± 0.03^{b}
	SVEXT-9-EHI	5.77 ± 0.03^{c}
	SVEXT-10-EHI	$4.17 \pm 0.04^{a,e}$
C. officinalis	COEXT-11-EHI	4.54 ± 0.05^{a}
	COEXT-12-EHI	8.39 ± 0.06^{b}
	COEXT-13-EHI	24.85 ± 0.99^{c}
	COEXT-14-EHI	7.30 ± 0.04^{d}
	COEXT-15-EHI	5.90 ± 0.07^{e}
BHA	///	95.49 ± 0.04
Quercetin	///	96.05 ± 0.09

ULEXT-1-EHI: crude extract, ULEXT-2-EHI: dichloromethane fraction, ULEXT-3-EHI: ethyl acetate fraction, ULEXT-4-EHI: butanolic fraction, ULEXT-5-EHI: residual aqueous fraction, SVEXT-6-EHI: crude extract, SVEXT-7-EHI: dichloromethane fraction, SVEXT-8-EHI: ethyl acetate fraction, SVEXT-9-EHI: butanolic fraction, SVEXT-10-EHI: residual aqueous fraction, COEXT-11-EHI: crude extract, COEXT-12-EHI: dichloromethane fraction, COEXT-13-EHI: ethyl acetate fraction, COEXT-14-EHI: butanolic fraction, COEXT-15-EHI: residual aqueous fraction.

Table 4. Pearson's correlation results for the antioxidant activity vs TPC, TFC

Antioxidant activity (DPPH scavenging)	TPC	TFC
U.lactuca	-0,49	-0,47
S.vulgare	0,80	0,93
C. officinalis	0,94	0,46

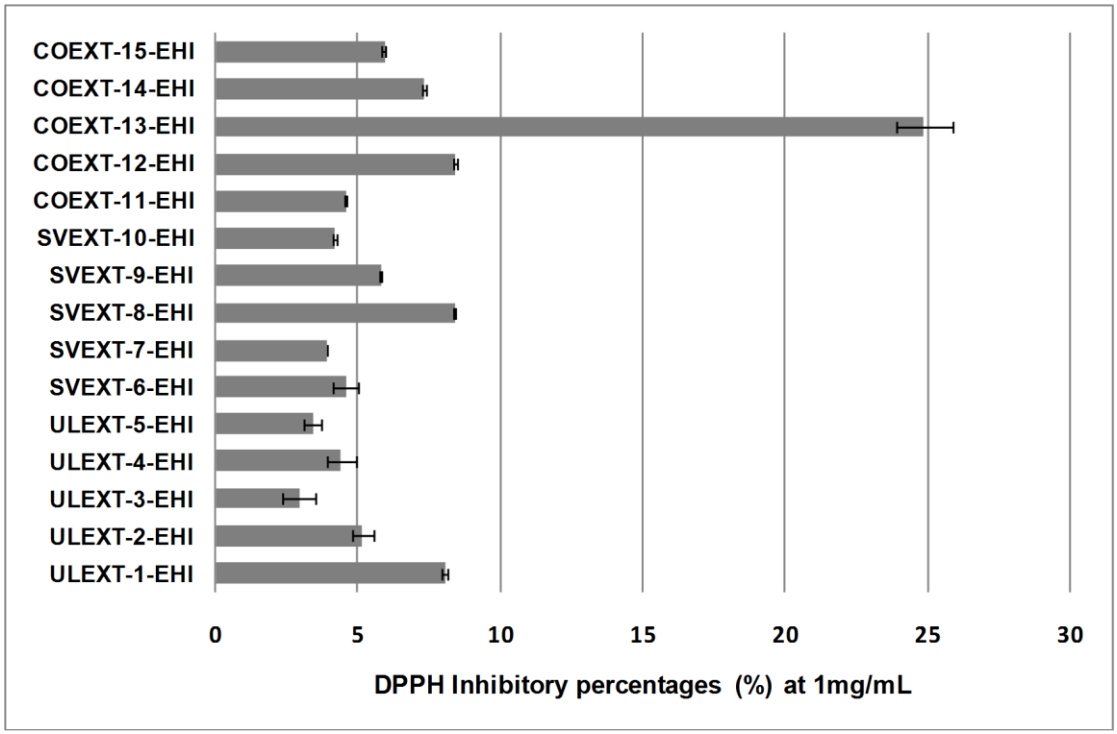

Fig. 2. DPPH inhibition percentages of the different organic extracts of the seaweeds studied

ULEXT-1-EHI: crude extract, ULEXT-2-EHI: dichloromethane fraction, ULEXT-3-EHI: ethyl acetate fraction, ULEXT-4-EHI: butanolic fraction, ULEXT-5-EHI: residual aqueous fraction, SVEXT-6-EHI: crude extract, SVEXT-7-EHI: dichloromethane fraction, SVEXT-8-EHI: ethyl acetate fraction, SVEXT-9-EHI: butanolic fraction, SVEXT-10-EHI: residual aqueous fraction, COEXT-11-EHI: crude extract, COEXT-12-EHI: dichloromethane fraction, COEXT-13-EHI: ethyl acetate fraction, COEXT-14-EHI: butanolic fraction, COEXT-15-EHI: residual aqueous fraction.

3. HPLC analysis

A performant and accurate method were carried to evaluate the chemical composition of the extracts presented the highest antioxidant activity, namely COEXT-13-EHI and COEXT-14-EHI, ethyl acetate and butanolic fractions of *C. officinalis*, respectively (Figs. 3 and 4).

Both extracts presented a good chromatographic signal of p-coumaric acid, gallic acid, and were well separated in COEXT-13-EHI. This fact explains the high amount of phenolics and flavonoids in this fraction compared to butanolic one. Chlorogenic acid, resorcinol, catechin, rutin and naringenin were also revealed.

Some studies reported the chemical characterization of phenolic compounds by using HPLC from seaweed samples to evaluate results as those present in the current study **(Sabeena Farvin & Jacobsen, 2013; Rajauria *et al.*, 2016; Agregán *et al.*, 2017; Olate-Gallegos *et al.*, 2019; Mazouzi *et al.*, 2023; El-Gammal *et al.*, 2024)**.

It is notable to highlight the importance of the point that the amounts of those compounds varied strongly in the same species in relation with vegetative state, season and environmental conditions.

It is not clear how to understand and explain the liaison (correlation) between different parameters (TPC, extraction yields, antioxidant activities), because of the diversity of phenolic compounds contained in extracts which certainly have different manner and ways to express antioxidant responses.

In our study, extreme variation in gallic acid concentration when solvent polarity increases were reported. In fact, solvent polarity does not modify the amounts of phenolic compounds, but not the phenolic profile.

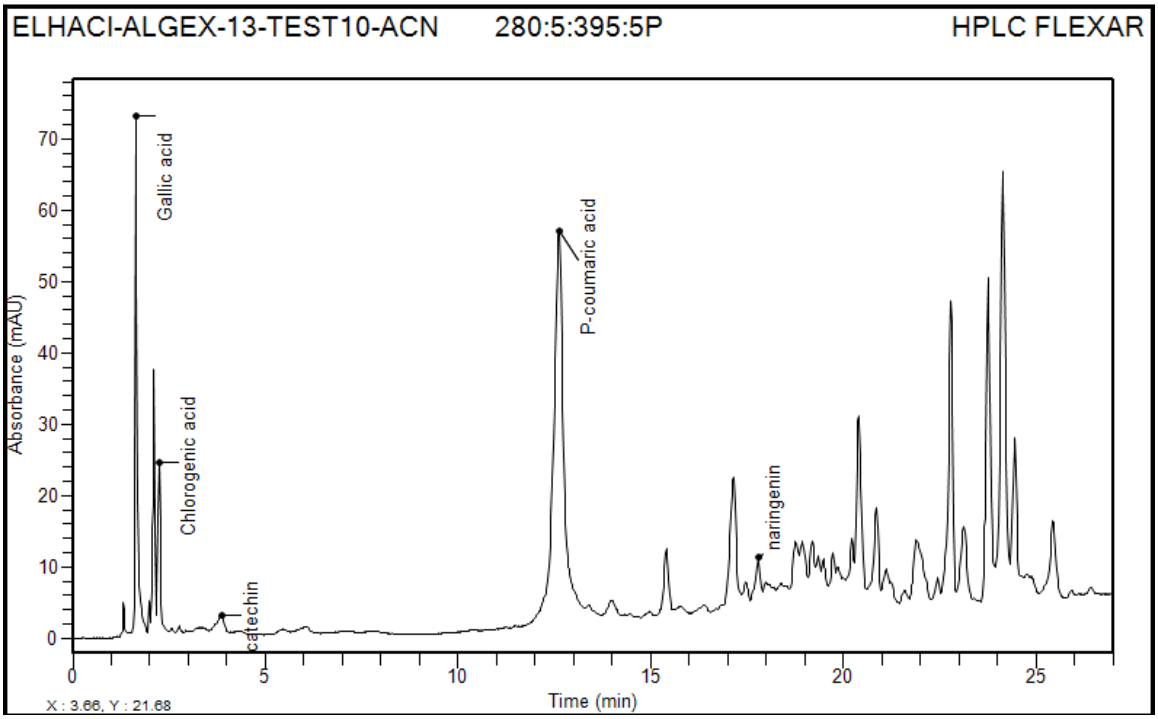

Fig. 3. RP-HPLC chromatogram of COEXT-13-EHI (ethyl acetate fraction of *C. officinalis*) obtained at 280nm

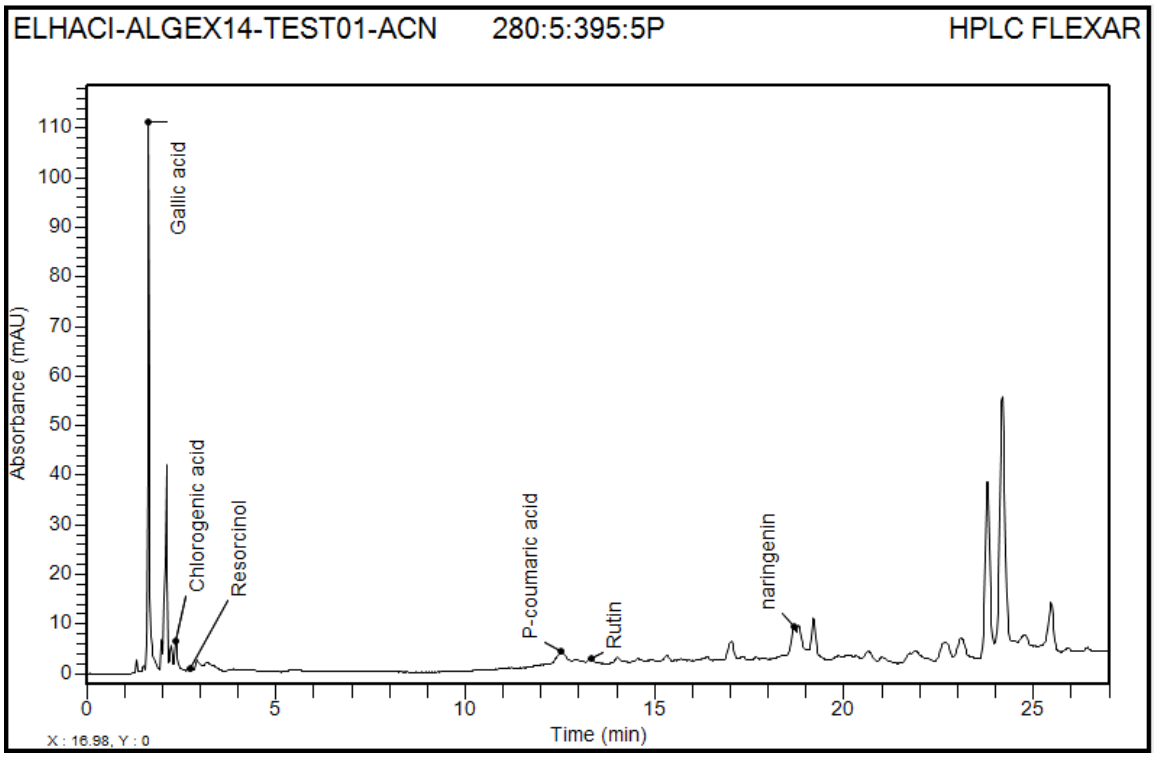

Fig. 4. RP-HPLC chromatogram of COEXT-14-EHI (butanolic fraction of *C. officinalis*) obtained at 280nm

CONCLUSION

Algae are certainly a source of interesting bioactive compounds. The obtained result may show that *C. officinalis* extracts presented a powerful antioxidant compounds such as catechin, gallic acid and p-coumaric acid.

By exploring these underexploited resources, this research contributes to a more comprehensive understanding of the bioactive potential of Algerian seaweeds. Additionally, the utilization of these valuable marine resources may, potentially, enrich the Algerian economy and align with the growing trend toward natural remedies and food sources.

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