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THE EFFECT OF SOLVENTS AND EXTRACTION PROCEDURE ON THE RECOVERY OF PHENOLIC COMPOUNDS AND THE ANTIOXIDANT CAPACITY OF ALGERIAN *BASSIA MURICATA* L. EXTRACTS

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Abstract. This paper focuses on the study of the effect of extraction solvent choice on phenolic compounds contents and antioxidant activity of *Bassia muricata.* In this study, five different solvents namely: water, acetone, ethanol, methanol and hexane, and three extraction techniques were used to extract phenolic compounds: microwave-assisted extraction, Soxhlet and maceration. Total phenolics (TPC), total flavonoids (TFC) and condensed tannins contents (CTC) were determined. The results showed that different solvents with different polarity had a major effect on polyphenolic contents and antioxidant activity; the highest TPC (122.15-144.82 mg GAE/g), TFC (64.12-70.32 mg QE/g) and CTC (30.38-36.09 mg CE/g) were obtained with methanol. However, different extraction methods gave comparable results. *In vitro* antioxidant activities were evaluated using the DPPH radical scavenging ability, reducing capacity and *β*-carotene bleaching assay. The methanolic extract showed the highest scavenging abilities on DPPH radicals and lipid peroxidation, while the aqueous extract exhibited the strongest reducing power. Microwave-assisted extraction was the best suited for the extraction of antioxidant molecules when compared to Soxhlet and maceration.

Keywords: polyphenol, flavonoid, condensed tannin, antioxidant, *Bassia muricata*.

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Introduction

Oxidation processes are considered harmful to human health, because they stimulate tissue damage responsible for many diseases. The use of synthetic antioxidants can prevent food oxidation or cell damage; however, these substances provide some toxicity [1]. For this reason, there has been an interest to the natural antioxidants aiming to replace the synthetic substances [2]. The plant world with about 350,000 species of plants is the source of a formidable diversity of molecules, possessing therapeutic properties and only a handful of those have been explored [3].

The Chenopodiaceae family comprises 1700 species distributed in about a hundred genera [4]. The members of Chenopodiaceae are mostly adapted for arid to semiarid and/or saline habitats. This family has a cosmopolitan distribution, and comprises herbs or shrubs, rarely small-trees or lianas [5]. The presence of various alkaloids, flavonols, flavonoids and triterpenoid saponins has been reported in the Chenopodiaceae [6,7]. The species *Bassia muricata* L. (Chenopodiaceae) is a plant, rower, with lying rod and grey leaves. It is a sandy grass, growing in the desert, common throughout the Sahara and especially in clay soils after the rain [8]. *Bassia muricata* L. Murr. is a common sandy herb growing in Egyptian and Algerian deserts, known locally as Ghobaira [9]. *B. muricata* can be used for different medical purposes such as anti-rheumatic, diuretic, antipyretic, analgesic and against spasticity, hypotension and kidney disease [10,11].

B. muricata is the source of diverse classes of natural substances such as phenols and flavonoids, with biological and pharmacological activities, including antimicrobial, antioxidant and anti-inflammatory compounds [10-16]. Reviewing current literature showed that the species *B. muricata* have not been well investigated with the purpose of extracting phytochemicals [10-15].

The first objective of this study was to evaluate several types of phytochemicals that are present in the dried powder of *B. muricata* aerial parts. The second objective was set to select the pair extraction method/solvent that led to the extracts with the highest antioxidant capacity. The extracts were obtained from the dried powder of *B. muricata* aerial parts using three different extraction methods: maceration, Soxhlet extraction and microwave assisted extraction (MAE); and five organic solvents: acetone, ethanol, hexane, methanol and water. Efficiency of extraction was evaluated by determination of the total phenols, flavonoids, tannins and the antioxidant activity.

Experimental

Plant material

The aerial parts of *Bassia muricata* were collected from Taleb Elarbi, region of El-Oued, south-east of Algeria in April 2014. Authentication was performed by a botanist in the National Institute of Agronomy, El-Harrach, Algiers. The samples were air-dried at room temperature and ground into fine powder using an electrical grinder.

Methods of extraction

Different extraction methods used in this study included maceration at room temperature, Soxhlet at hot temperature and microwave-assisted extraction (MAE). Five absolute solvents with different polarity were used (hexane, acetone, ethanol, methanol and water). The ratio used to extract polyphenols and tannins in this study was 1/20 (m/v) (1 g sample with 20 mL of solvent).

Microwave-assisted extraction

Phenolic compounds from powders of *B. muricata* were extracted using a domestic microwave oven system Milestone Ethos 1600 system (Sorisole, Italy). The oven was modified so that vapours generated during extraction were condensed and directed back into the sample. For each experiment, 1 g of *B. muricata* powder was stirred into the appropriate extraction solvent using MAE system. The effect of solvent type was studied by keeping the MAE extraction parameters constant during all experiments; the microwave power was set to 600 W and the extraction time was 90 s. Each extraction was carried out in triplicate. Afterwards, the extract was filtered through a Whatman No. 1 filter paper lined in a Büchner funnel and the supernatant was collected in a volumetric flask. The extract was stored at 4°C until further use.

Soxhlet extraction

The powder of *B. muricata* aerial parts was placed inside a thimble loaded into the Soxhlet extractor. The total extracting time was 6 h and the solvent was maintained continuously refluxing over the sample [17]. The solvent assays were performed at solvent boiling temperature.

Maceration extraction

Maceration was carried out for 24 h in a glass crystallizer entirely covered with aluminium foil using moderate mechanical agitation, at room temperature (25°C). Aluminum foil was used to preserve phenolic compound against reaction with light. Additionally, during extraction, the flasks had a plastic cap and paraffin film, to prevent solvent evaporation.

Extraction yield

The yield of the extraction was calculated using the Eq.(1).

$$\text{Extraction yield (\%)} = \frac{m_1}{m_2} \times 100\% \quad (1)$$

where, m_1- sample extract weight, g;
m_2- sample weight, g;

Determination of total phenolic content

Total phenolic compounds (TPC) from ethanol extract were determined using the Folin-Ciocalteu method described by Singleton, V.L. *et al.* [18]. Gallic acid was used as standard for the calibration curve to express the TPC concentration of the sample as mg/g of gallic acid equivalents (GAE). The calibration curve was drawn and the equation of linear regression was obtained: $y = 7.1471x + 0.0275$, $R^2 = 0.972$.

Determination of total flavonoid content

The total flavonoid content (TFC) of the solvent extracts was measured spectrophotometrically using the method developed by Jia, Z. *et al.* [19]. The TFC was expressed as mg quercetin equivalents (QE)/g of extract. The calibration curve was drawn and the equation of linear regression was obtained: $y = 33.731x + 0.0092$, $R^2 = 0.9992$.

Determination of condensed tannins content

The condensed tannin content (CTC) in extracts was determined using a method proposed by Swain, T. and Hillis, W.E. [20]. A volume of 2 mL of vanillin reagent (1 g of vanillin dissolved in 70% of sulphuric acid) was mixed with 1 mL of extract. After incubation at 50°C for 20 min, the absorbance was measured at 500 nm. Results were expressed as mg of catechin equivalent (CE)/g of extract.

Antioxidant activity evaluation

DPPH radical scavenging assay

The extract ability to inhibit DPPH free radicals was evaluated by the method of Amensour, M. *et al.* with some modifications [21]. A volume of 1 mL of sample at various concentrations was mixed with 1 mL of 0.2 mmol/L solution of DPPH in methanol. After incubation during 20 min in the dark and ambient temperature, the absorbance was measured at 517 nm. Methanol was used as control. All analyses were carried out in triplicate. The inhibition percentage was calculated according to the Eq.(2).

$$\text{Inhibition } (\%) = \frac{(\text{Abs}_{\text{Contr}} - \text{Abs}_{\text{Extr}})}{\text{Abs}_{\text{Contr}}} \times 100 \qquad (2)$$

where, $\text{Abs}_{\text{Contr}}$- absorbance of the control, a.u.;

Abs_{Extr}- absorbance of the extract, a.u.;

The effective concentration of sample required to scavenge DPPH radical by 50% (EC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between inhibition (%) and concentrations.

Reducing capacity

The reducing capacity was assayed according to the method reported by Oyaizu, M. [22] with some modifications. Briefly, 0.125 mL different concentration extract was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide (1.0%, w/v) in different test tubes. The mixtures were incubated for 20 min at 50°C. Then, 2.5 mL trichloroacetic acid in water (10%, w/v) was added to the mixtures and centrifuged at 5000 rpm for 10 min. A volume of 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of aqueous ferric chloride solution (0.1%, w/v), and the absorbance was measured at 700 nm.

The EC_{50} value derived from the plot, was expressed as the effective concentration for which the absorbance at 700 nm is 0.5.

β-Carotene bleaching assay

The *β*-carotene bleaching assay was done according to the method reported by Gursoy, N. *et al.* with some modifications [23]. A solution of *β*-carotene was prepared by dissolving *β*-carotene (2 mg) in chloroform (10 mL). A volume of 2 mL of this solution were pipetted into a round-bottom flask. After removal of solvent (evaporation at 40°C under vacuum), 400 mg of Tween 80 emulsifier, 40 mg of linoleic acid and 100 mL of distilled water were added under agitation. A 4.8 mL aliquot of the emulsion was transferred into test tubes containing 0.2 mL of sample solutions. After the emulsion was added to each tube, the absorbance was measured (λ= 470 nm) at t= 0 min (initial absorbance) and after 2 h incubation at 50°C against a blank consisting of an emulsion without *β*-carotene. *β*-Carotene bleaching inhibition was calculated using Eq. (3).

$$\text{AA}(\%) = \frac{\text{Abs}_{\beta-\text{carotene}}}{\text{Abs}_{\text{Initial}}} \times 100 \qquad (3)$$

where, $\text{Abs}_{\beta\text{-carotene}}$ - absorbance of *β*-carotene after 2 h, a.u.;

$\text{Abs}_{\text{Initial}}$- initial absorbance of *β*-carotene at t= 0 min, a.u.;

The effective concentration of sample that can inhibit the peroxidation of *β*-carotene linoleic acid by 50% (EC_{50} value) was deduced graphically by plotting inhibition percentage against concentration.

Statistical analysis

All determinations were carried out in triplicate. Data were expressed as mean ±S.D. Statistical differences were assessed using one-way ANOVA. A value of $P< 0.05$ was considered significant. Hierarchical cluster analysis (*HCA*) was used as multivariate statistical analyses to investigate the variability between the antioxidant activities according to the solvent and the extraction methods. HCA was performed using SPSS software (SPSS Inc., Chicago, USA).

Results and discussion

This study is one of the first investigating the effect of solvent type and extraction techniques on the recovery of phenolic compounds from *B. muricata* aerial parts.

Extract yield

The extraction yields could be influenced by many factors such as the extraction method, the extraction time and temperature and the extraction solvent [24-28]. In this work, different solvents were assayed for the extraction of *B. muricata* aerial parts (acetone, ethanol, hexane, methanol and water) and their effect on the extraction yield was determined using maceration, Soxhlet and MAE (Table 1).

The extraction yields varied according to the used solvents. Ethanol and water proved more efficiency with higher yields than those of other solvents considered in this study and for each extraction technique. The extraction yield using ethanol and water ranged from 12.69±0.63% and 12.51±0.22% to 34.30±0.58% and 33.30±1.24%

by maceration and MAE, respectively (Table 1). Extraction using methanol and acetone provided lower yet similar yields with better results recorded when using MAE (26.10±0.61% and 25.30±0.21%, respectively).

On the other hand, hexane gave the lowest yields and was significantly less effective than other solvents for each extraction technique with the lowest result when coupled with Soxhlet (8.71±0.89%). This trend remained the same for each extraction technique and the statistical analysis would allow denoting the solvent efficiency as follows (ethanol> water> methanol> acetone> hexane). These results showed a clear correlation between extraction yield and solvent polarity, where increasing in solvent polarity led to an increase in extraction yield. Almost, the same arrangement of solvent efficacy was previously reports on other species [25-27].

Different techniques gave significantly different extraction yields with MAE emerging as the most efficient. For each solvent, the latter technique provided the higher results than Soxhlet and maceration (Table 1). The use of microwaves as heat source improved the recovery of extracts with the highest result, and the best result was recorded for ethanol (34.30±0.58%). Soxhlet and maceration gave lower yields, however their results were in line of solvent efficiency stated above. The extraction using conventional heating source Soxhlet was more suitable than that performed at room temperature (maceration). The extraction yield with Soxhlet ranged from 10.30±0.10 to 21.12±0.76% using hexane and methanol respectively, while maceration yields ranged from 8.71±0.89% to 12.69±0.63%, respectively for hexane and ethanol.

In previous reports on *B. muricata*, Chemsa, A.E. *et al.* reported an extraction yield of 15.01% when using maceration with ethanol [12]. A similar yield was obtained from the same species from Egypt using the conventional technique (cold maceration) with ethanol (11%) [14], while a much lower yield of extract was reported in the species collected from Saudi Arabia (5.13%) [16]. Bouaziz, M. *et al.* working on several wild plants from Tunisia, reported lower extraction yields using maceration on the aerial parts of *B. muricata*, with hexane (2.45±0.10%) and methanol (5.15±0.21%) [15]. These, last values are significantly lower than those obtained in this study. The differences in yields might be due to the geographic region of the collected plant material.

Generally, the findings showed that the yield of *B. muricata* extract was highly affected by the extraction method, as well as by the solvent type. The use of MAE method with ethanol or water is recommended for better extraction yields of phytochemicals from *B. muricata.* Compared to other species, Dhanani, T. *et al.* indicated that the highest extraction yield of *Withnaia somnifera* was obtained using MAE with ethanol [27]. Nguyena, V.T. *et al.* investigations also showed that the combination of MAE with water is the most effective to extract phytochemicals from *Paramignya trimera* [28].

Total phenolic content

All the extracts obtained from *B. muricata* exhibited important variations in their total phenolic contents as presented in Figure 1. The highest levels have been detected in methanolic extracts (Figure 1) ranging from 122.15±1.73 to 144.82±3.21 mg GAE/g, using Soxhlet and MAE respectively; followed by aqueous (100.12±0.88 mg GAE/g using Soxhlet, and 98.58±1.26 mg GAE/g using MAE) and ethanolic extracts (120.94±1.03 mg GAE/g by Soxhlet and 111.13±1.78 mg GAE/g by MAE). The TPC of the hexane extract was significantly lower than those obtained from other solvents ($p< 0.05$).

Table 1

The extraction yield of phenolic compounds from *B. murricata* by various solvents and extraction methods.

Solvent	*Extraction yield (%)*		
	MAE	*Soxhlet*	*Maceration*
Acetone	$25.3\pm0.21^{a, A}$	$14.33\pm0.12^{b, A}$	$9.93\pm0.51^{b, A}$
Ethanol	$34.3\pm0.58^{a, B}$	$21.12\pm0.76^{b, A}$	$12.69\pm0.63^{c, A}$
Hexane	$15.2\pm1.22^{a, A}$	$10.30\pm0.10^{a, A}$	$8.71\pm0.89^{a, A}$
Methanol	$26.1\pm0.61^{a, A}$	$16.09\pm1.05^{b, A}$	$10.87\pm0.41^{b, A}$
Water	$33.3\pm1.24^{a, B}$	$20.91\pm1.09^{b, A}$	$12.51\pm0.22^{c, A}$

Values are averages ± standard deviation of triplicate analysis.

Data in the same row having different lower-case letters are significantly different ($P< 0.05$) among different extraction methods.

Data in the same column having different capital letters are significantly different ($P< 0.05$) among different essential oil harvesting sites.

Water seemed to be a very settle choice for extracting phenolic compounds from *B. muricata* aerial parts, as it provided high phenolic contents with advantages of being a cheap, available and non-toxic material when compared to other solvents. It should be noted that the solubility of the phenolic compounds is influenced by the nature of the used solvent and their polarity [29].

In comparison with other plants, methanol extracted the highest TPC from *P. trimera* roots compared with water (33.36 and 25.06 mg GAE/g dried sample, respectively), while the lowest level of TPC was obtained with hexane (4.58 mg GAE/g dried sample) [28]. Otherwise, Tiffany, L.K. *et al.* mentioned that the TPC of *Davidsonia pruriens* F. increased according to the used solvent in the order: acetone, water, methanol and ethanol (35.17, 45.14, 73.13 and 94.13 mg GAE/g, respectively) [30]. However, Vuong, Q.V. *et al.* reported that the ethanolic extract of *Carica papaya* had lower TPC than acetone, methanol and water extracts (9.43, 10.71, 15.03 and 23.06 mg GAE/g, respectively) [31]. By contrast, in a large study performed by Koffi, E. *et al.* on twenty-three Ivorian plants, the highest TPCs were obtained in ethanolic extracts for all samples [32].

The extraction technique had a major effect on the phenolic compounds' recovery, as the contents varied significantly for MAE, Soxhlet and maceration. Results from Figure 1 revealed that the highest contents of phenols were obtained using MAE by which results varied from 78.95±0.36 to 144.82±3.21 mg GAE/g for hexane and methanol, respectively). Soxhlet and maceration were most efficient when methanol was used (122.15±1.73 to 125.27±1.57 mg GAE/g, respectively). However, while these two extraction methods gave comparable TPCs for each solvent, they did not follow the same trend as that of MAE. Maceration provided higher levels of phenolics than those of Soxhlet using methanol, acetone and water, while Soxhlet was more efficient with ethanol and hexane. Similar results were reported by Nguyen, V.T. *et al.* who indicated that the level of TPC obtained by MAE from *P. trimera* was greater than those obtained with maceration and ultrasound-assisted extraction [28]. The higher yield of TPC using MAE could be attributed to the microwaves ability to penetrate cell matrix and interact with polar molecules resulting in volumetric heating of biomaterial, consequently leading to a pressure increase inside the plant cell [32].

Total flavonoid content

The result of total flavonoid contents (TFC) of the extracts of *B. muricata* is given in (Figure 2). The TFC varied in different extracts according to the used solvent as follows: methanol> water> ethanol≈ acetone> hexane; being of 27.29±2.30 mg QE/g (hexane/MAE) and 70.32±1.02 mg QE/g (methanol/Soxhlet). The most efficient solvent for TFC extraction was methanol (70.32±1.02, 68.65±1.57 and 64.12±0.88 mg QE/g), when used in Soxhlet, maceration and MAE, respectively. Water/maceration gave higher TFC values in extracts (61.10±1.09 mg QE/g), while acetone and ethanol extracts exhibited lower but significantly comparable TFC values. Hexane was less efficient when used to extract flavonoids from *B. muricata* aerial parts powder and that for each extraction technique. Most of previous studies carried out on different plants, have suggested that absolute methanol is recommended for extraction of flavonoids [15,20,24,26,28].

Various extraction techniques were used for flavonoid compounds; the flavonoid contents determined in extracts also depended significantly ($p< 0.05$) on the used extraction technique.

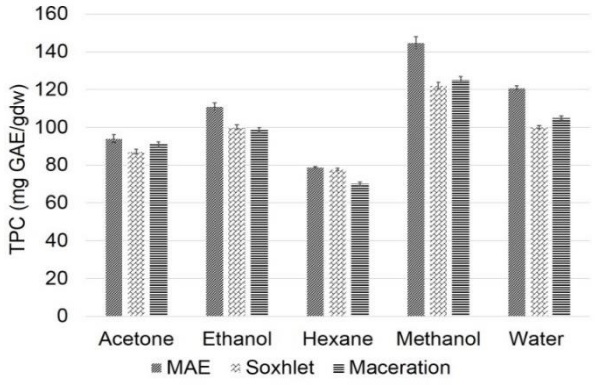

Figure 1. Effect of method and extraction solvent on the total phenolic content of *B. muricata.*

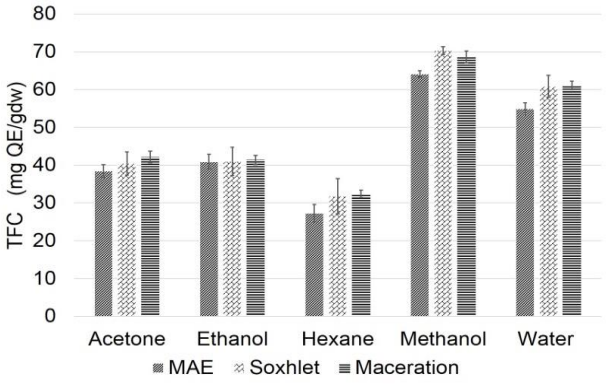

Figure 2. Effect of method and extraction solvent on the total flavonoid content of *B. muricata.*

Except methanol, maceration extracts presented the highest TFC, being of 32.40±0.94 mg QE/g (for hexane) and 61.10±1.09 mg QE/g (for water). There was no significant difference ($p< 0.05$) between the TFC obtained by Soxhlet and MAE techniques and that for ethanol and acetone. However, a significant difference ($p> 0.05$) was observed for the other solvents (hexane, methanol and water) when Soxhlet and MAE techniques are used. These results show that the TFC from *B. muricata* was highly affected by solvent type as well as extraction methods. The highest levels were generally obtained by Soxhlet with methanol.

Condensed tannins content

The obtained results showed that the condensed tannins contents (CTC) in *B. muricata* extracts presented clear differences ($p< 0.05$) according to the solvent used (Figure 3). The highest CTC was obtained by methanol when used in MAE with a rate of 36.09±1.04 mg CE/g, which could be due to their high molecular weights. The obtained CTC extract was comparable for water, ethanol and hexane, while the acetone extract presented the lowest CTC ranging from 9.52±1.07 mg CE/g (with maceration) to 12.21±2.01 mg CE/g (with MAE).

Regarding the influence of extraction method, the results show that the three investigated extraction techniques gave statistically comparable CTC values only for hexane extracts (18.85±2.04, 18.95±2.10 and 18.93±2.83 mg CE/g for maceration, Soxhlet and MAE, respectively). However, the CTC was significantly different for all the three processes and higher with MAE/acetone (CTC values 12.21±2.01 mg CE/g) and MAE/methanol (36.09±1.04 mg CE/g) than with Soxhlet/acetone (10.00±1.48 mg CE/g) and Soxhlet/methanol (30.38±1.43 mg CE/g).

Also, maceration was less effective in extraction of condensed tannins than MAE and Soxhlet (the lowest CT content recorded for acetone was 9.52±1.07 mg CE/g). These results show extraction method (except for hexane) and solvent type affect highly the CTC of *B. muricata.*

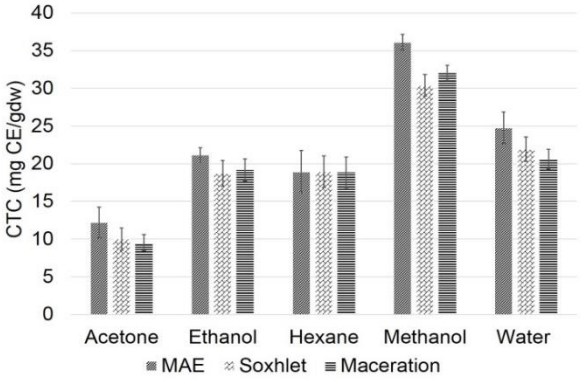

Figure 3. Effect of method and extraction solvent on the condensed tannins content of *B. muricata.*

Antioxidant activity

The antioxidant activity of *B. muricata* was determined using three different assays: DPPH radical scavenging assay, ferric reducing capacity and *β*-carotene bleaching test. The results of antioxidant activity values are given in (Table 2).

The model of scavenging stable DPPH-free radicals is used to evaluate the antioxidant activity in relatively short time [33]. The reduction of stable free DPPH radical to 1,1-diphenyl 2-picrylhydrazin results in colour change from purple to yellow. This leads to a decrease of absorbance associated with the ability of sample to donate hydrogen/electron [34].

Table 2

Effect of method and extraction solvent on the antioxidant activity of *B. muricata* extracts.

Test	*Extraction method*	*Solvent*				
		Acetone	*Ethanol*	*Hexane*	*Methanol*	*Water*
DPPH EC_{50} *(mg/L)**	*MAE*	6.70±0.04 [a]	6.12±1.12[a]	7.42±0.92 [a]	5.00±0.97 [a]	5.86±1.19 [a]
	Soxhlet	7.14±0.14 [b]	6.40±1.87[a]	8.03±1.69 [b]	5.09±0.12 [a]	5.98±0.69 [a]
	Maceration	7.00±0.90 [a]	6.20±0.23 [a]	7.51±0.71 [a]	5.03±0.89 [a]	5.90±1.93 [a]
Reducing capacity EC_{50} *(mg/L)***	*MAE*	3.87±1.73 [a]	2.29±0.80 [a]	4.49±0.19 [a]	1.63±0.33[a]	1.39±0.37 [a]
	Soxhlet	4.00±0.57 [a]	3.26±0.41 [a]	5.20±1.25 [a]	2.89±0.63 [a]	2.54±0.46 [a]
	Maceration	4.60±0.29 [a]	4.11±0.35 [a]	4.85±1.28 [a]	2.92±0.81 [a]	2.60±0.67 [a]
β-carotene EC_{50} *(mg/L)****	*MAE*	6.29±0.07 [c]	6.93±0.05 [d]	7.38±0.07 [e]	5.80±0.05 [b]	5.31±0.18 [a]
	Soxhlet	6.74±0.99 [a]	7.32±1.07 [a]	7.34±1.50 [a]	5.96±1.66 [a]	5.70±1.48 [a]
	Maceration	6.98±0.97 [a]	7.50±0.98 [a]	7.31±1.06 [a]	6.41±1.09 [a]	6.00±1.06 [a]

Values are averages ± standard deviation of triplicate analysis. Data in the same row having different lower case indicate significant difference ($p< 0.05$). Results are ranked in ascending order; a> b> c> d> e.

** EC_{50}: effective concentration of sample that can scavenge 50% of DPPH free radicals.*

*** EC_{50}: effective concentration of sample for which the absorbance at 700 nm is 0.5.*

**** EC_{50}: effective concentration of sample that can inhibit the peroxidation of β-carotene with linoleic acid by 50%.*

The necessary concentration of an antioxidant to decrease the initial DPPH concentration by 50% (EC_{50}) is widely used to evaluate the antioxidant activity [35]. In this study, solvents used for polyphenol extraction had significant effects on DPPH scavenging capacity determination for *B. muricata* extracts (Table 2).

Results presented in Table 2 showed that methanol extracts present strong antioxidant activity as they did not require a high concentration to inhibit 50% of DPPH (5.00±0.97, 5.03±0.89 and 5.09±0.12 mg/L for MAE, maceration and Soxhlet, respectively). This could be explained by the high phenolic and flavonoid contents in methanol extracts. While the hexane and acetone extracts presented statistically higher EC_{50} values when used in maceration (EC_{50} of 7.51±0.71 mg/L) and Soxhlet (EC_{50} of 8.03±1.69 mg/L); no significant differences ($p< 0.05$) in EC_{50} values were detected when comparing the DPPH inhibition ability of aqueous and ethanol extracts obtained with MAE and maceration, this is understandable considering the comparable TPC and TFC values recorded for those extracts.

The effect of the three extraction methods (MAE, Soxhlet and maceration) on the DPPH inhibition activity of *B. muricata* extracts was investigated (Figure 4*(a)*). The selected techniques did not have a major effect on extracts DPPH radicals scavenging capacity, and the EC_{50} values for each solvent extract were not highly influenced by the changing of extraction process ($p> 0.01$). Extracts from MAE showed lower EC_{50} values compared to other extraction methods, indicating stronger antioxidant activities of MAE with higher scavenging of DPPH radicals compared to maceration and Soxhlet methods. This is in accordance with the high TPC yields in *B. muricata* extracts obtained by MAE (Figure 1). In the case of the aqueous extract that displayed a high antioxidant activity, EC_{50} values for MAE were 5.86±1.19 mg/L (MAE), 5.90±1.93 mg/L (maceration) and 5.98±0.69 mg/L (Soxhlet). It was observed that Soxhlet extracts had the lowest fluorescence intensity during the test compared to other extraction methods (EC_{50} were between 5.09±0.12 mg/L for methanol and 8.03±1.69 mg/L for hexane).

The reducing capacity method reflects the electron donation ability of antioxidants present in the extracts to convert Fe^{3+} into Fe^{2+}. The amount of the Fe^{2+} complex was followed by measuring the formation of Perls' Prussian blue at the absorbance of 700 nm [36]. Table 2 depicts the reducing capacity EC_{50} values of *B. muricata* extracts. While the use of different solvents provided extracts with a relatively small range of reducing capacity; the aqueous extracts were the most effective (EC_{50} values ranging from 1.39±0.37 to 2.60±0.67 mg/L using MAE and maceration, respectively). On the other hand, hexane extracts showed the weakest reducing capacity for all three techniques (EC50 values of 4.49±0.19, 4.85±1.28 and 5.20±1.25 mg/L by MAE, maceration and Soxhlet respectively).

Extracts of *B. muricata* obtained by MAE showed the highest reducing capacity, except when water was used as solvent having the lowest EC_{50} values (1.39±0.37 mg/L). Maceration and Soxhlet methods gave extracts of very similar reducing capacity with EC_{50} values ranging from 2.54±0.46 and 2.60±0.67 mg/L using water to 5.20±1.25 and 4.85±1.28 mg/L for Soxhlet and maceration respectively when using hexane.

The lipid peroxidation inhibitory activities of *B. muricata* were assessed by *β*-carotene bleaching tests. This is an important test in food industry because the medium is an emulsion resembling to food, thus allowing the investigation of new alternatives to synthetic antioxidants. The lipid peroxidation inhibitory activity of *B. muricata* extracts varied according to the used solvent (Table 2). Aqueous and methanolic extracts exhibited strong peroxidation inhibitory activity, with EC_{50} values between 5.31±0.18 (MAE) and 6.00±1.06 mg/L (maceration) using water, while the use of methanol provided extracts with EC_{50} values of 5.80±0.05 and 6.41±1.09 mg/L for MAE and maceration, respectively. The extraction technique had a minor effect on the peroxidation inhibitory activity the extracts.

In order to interpret the obtained results, the variability of the antioxidant activity of *B. muricata* extracts using different extracting methods, and different solvents was studied by using HCA based on matrix linking EC_{50} values of the antioxidant activity (Figure 4).

The obtained results have shown that the antioxidant activity of the extracts greatly depend to the extraction solvent, where the three different testing systems showed the same behaviour.

The reducing capacity and the DPPH results were in accordance (Figure 4*(a)* and *(b)*). In both tests, two groups were discriminated: the first group (cluster I) contained hexane and acetone extracts due to similarly high EC_{50} values indicating low antioxidant activity. The second group (cluster II) contained aqueous, ethanol and methanol extracts; this group was characterized by showing the lowest antioxidant activities.

Two groups were also observed when using the β-carotene bleaching test (Figure 4*(c)*). The first one (cluster II) containing ethanol, hexane and acetone extracts was characterized by the lowest activity; however, the second group (cluster I) presented the highest antioxidant capacity by the β-carotene bleaching test.

In conclusion, this variability of antioxidant capacity among extracts of *B. muricata* obtained with different solvents and using different extraction methods led to conclude that we should select the method of extraction and the used solvent carefully in order to have extract with the highest effectiveness in terms of biological activities.

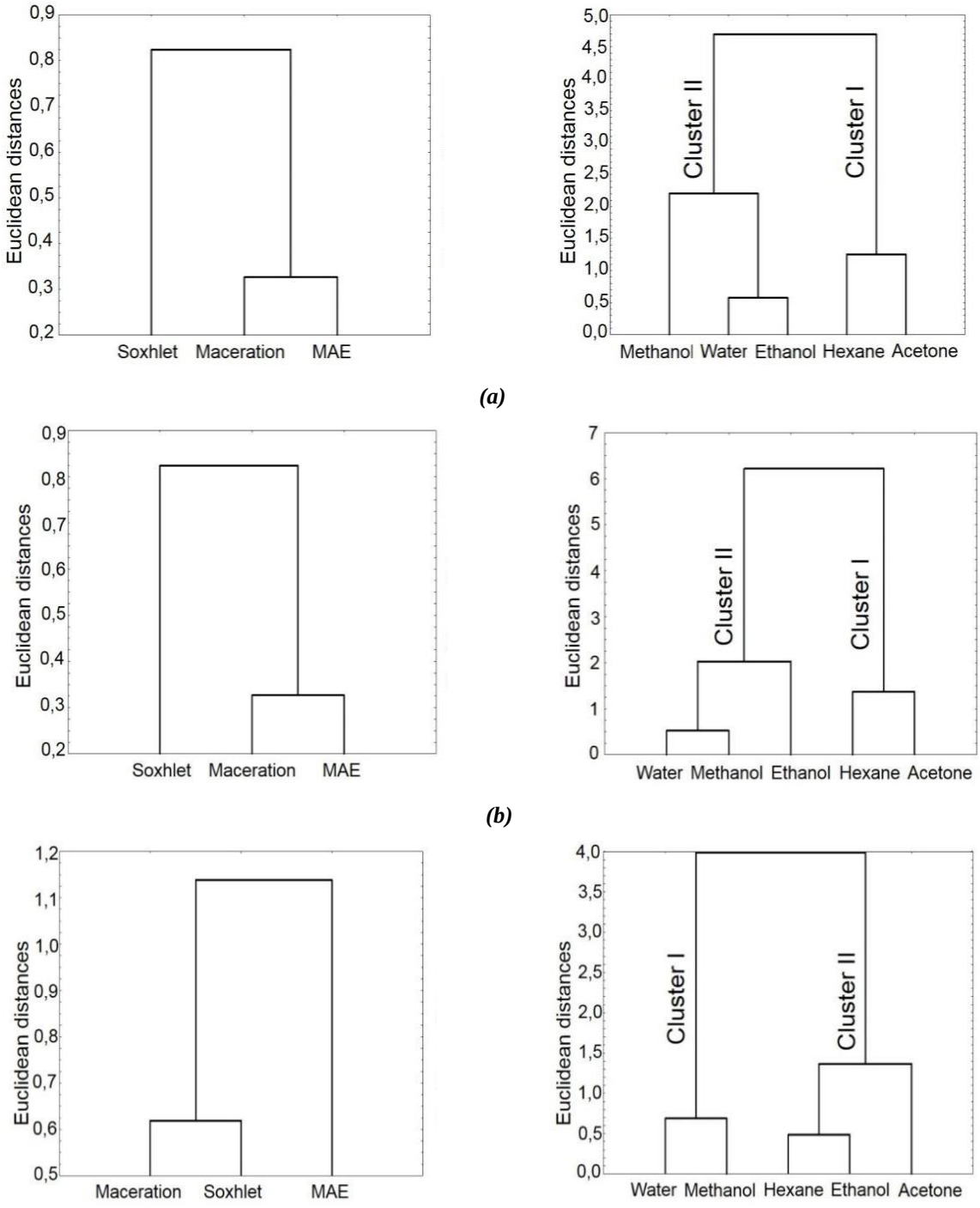

Figure 4. HCA for the antioxidant capacity of *B. muricata* extracts obtained using different extracting methods and different solvents (HCA performed on EC_{50} values of the antioxidant capacity assessed by: DPPH radical scavenging capacity test *(a)*, ferric reducing capacity test *(b)* and β-carotene bleaching test *(c)*).

Correlation between antioxidant activities and phytochemical compounds

The correlation coefficients between the antioxidant capacities and the total phenolic, flavonoid and condensed tannin contents for all extracts, prepared using different solvent and with three different techniques were determined (Tables 3). For DPPH, reducing power and *β*-carotene bleaching test, the EC_{50} values showed parallelism with antioxidant activities, it was therefore calculated and used for evaluating the correlations (Table 3).

Table 3

Correlation matrix between antioxidant contents and antioxidant capacity of extracts from *B. muricata*.

	TPC	*TFC*	*CTE*	*DPPH*	*RP*	*Car*
TPC	1	-	-	-	-	-
TFC	0.826**	1	-	-	-	-
CTE	0.780**	0.722*	1	-	-	-
DPPH	-0.619**	-0.588*	-0.422*	1	-	-
RP	-0.382**	-0.273	-0.159	0.769*	1	-
Car	-0.413**	-0.424*	-0.200	0.572*	0.818*	1

**The correlation is significant at the level 0.01 (bilateral).*

Conclusions

This is the first report which evaluates the effects of both solvent and extraction method on various phenolic extracts of *Bassia muricata* aerial parts. The extraction method, as well as the extracting solvent, significantly affected the extraction yield, total polyphenols, flavonoid and condensed tannins content and the antioxidant activity of studied extracts.

The highest extraction yield was obtained using ethanol by microwave-assisted extraction (MAE) (34.30±0.58%) and the lowest one using hexane by maceration (8.71±0.89%). On the other hand, the highest contents of phenols were obtained using MAE varying for different solvents (78.95±0.36 and 144.82±3.21 mg GAE/g for hexane and methanol, respectively). In addition, the maceration extracts presented the highest flavonoid contents (32.40±0.94 for hexane and 68.85±1.57 mg QE/g for methanol). The condensed tannins content was significantly different for all the three methods, and the higher with MAE (condensed tannin content values of 12.21±2.01 mg CE/ g for acetone and 36.09±1.04 mg CE/ g for methanol).

The study of the antioxidant activity showed that the selected techniques did not have a major effect on antioxidant capacity. The methanol extracts presented the strongest DPPH radicals scavenging activity, however, the aqueous extracts were the most effective in reducing capacity and *β*-carotene bleaching test.

It is important to continue this work to optimize the couple solvent/method and determine the parameters which will allow to recover effectively the antioxidant molecules from *B. muricata*, a rare and an unexplored plant.

References

1. Kahl, R. Synthetic antioxidants: Biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. Toxicology, 1984, 33(3-4), pp. 185-228. DOI: https://doi.org/10.1016/0300-483X(84)90038-6
2. Pokorný, J. Are natural antioxidants better – and safer – than synthetic antioxidants? European journal of lipid science and technology, 2007, 109(6), pp. 629-642. DOI: https://doi.org/10.1002/ejlt.200700064
3. Frankel, O.H.; Brown, A.H.D.; Burdon, J.J. The Conservation of Plant Biodiversity. Cambridge University Press: New York, 1995, 316 p. https://www.cambridge.org/md/academic/subjects/life-sciences/ecology-and-conservation/conservation-plant-biodiversity?format=PB
4. Kadereit, G.; Borsch, T.; Weising, K.; Freitag, H. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C4 photosynthesis. International Journal of Plant Sciences, 2003, 164(6), pp. 959-986. DOI: https://doi.org/10.1086/378649
5. Aronson, J.A.; Whitehead, E.E. HALOPH: a database of salt tolerant plants of the world. Office of Arid Lands Studies. Tucson, university of Arizona: Arizona, 1989. https://alrs.arizona.edu/
6. Davis, P.H. Flora of Turkey and the East Aegean Islands. Edinburg University Press: Edinburg, 1967, 2, 567 p. https://edinburghuniversitypress.com/
7. Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala, A.; Yildirim, A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisiaabsinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. Journal of Agricultural and Food Chemistry, 2005, 53(24), pp. 9452-9458. DOI: https://doi.org/10.1021/jf0516538

8. Ozenda, P. Flora of the Sahara, CNRS, 1977, 226 p.
9. Täckholm, V. Students Flora of Egypt. Cairo University: Cairo, 1974, 888 p.
10. Al-Yahya, M.A.; Al-Meshal, I.A.R.; Mossa, J.S.; Al-Badr, A.A.; Tariq, M. Saudi Plants: A Phytochemical and Biological approach. King Saud University Press: Riyadh, 64, 1990, 523 p.
11. El-Khatib, A.S.; Khaleel, A.E. Evaluation of some pharmacological properties of different extracts of *Bauhinia racemosa* Lam. Leaf and *Bassia muricata* L. whole plant. Bulletin of Faculty of Pharmacy, Cairo University, 1995, 33(2), pp. 59-65.
12. Chemsa, A.E.; Derdouri, S.; Labbi, Z.; Acila, S.; Amara, D.G.; Chouikh, A.; Kherraz, K.; Allali, A.; Zellagui, A. Total phenolic and total flavonoid contents of different solvent extracts of *Bassia muricata* (L.) Asch. and evaluation of antibacterial and antioxidant activities. Journal of Chemical and Pharmaceutical Research, 2016, 8(4), pp. 1317-1321. http://www.jocpr.com/abstract/total-phenolic-and-total-flavonoid-contents-of-different-solvent-extracts-of-bassia-muricatal-asch-and-evaluation-of-ant-8009.html
13. Sadeek, A.M.M.; Abdallah, E.M. Antimicrobial properties of methanol extract of *Bassia muricata* growing in arid zones in Qassim, Saudi Arabia. Indian Journal of Fundamental and Applied Life Sciences, 2018, 8(4), pp. 1-5. http://www.cibtech.org/J-LIFE-SCIENCES/PUBLICATIONS/2018/VOL-8-NO-4/JLS-08-04-Contents.htm
14. Kamel, M.S.; Mohamed, K.M.; Hassanean, H.A.; Ohtani, K.; Kasai, R.; Yamasaki, K. Acylated flavonoid glycosides from *Bassia muricata*. Phytochemistry, 2001, 57(8), pp. 1259-1262. DOI: https://doi.org/10.1016/S0031-9422(01)00240-0
15. Bouaziz, M.; Dhouib, A.; Loukil, S.; Boukhris, M.; Sayadi, S. Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. African Journal of Biotechnology, 2009, 8(24), pp. 7017-7027. DOI: https://doi.org/10.5897/AJB2009.000-9545
16. Al-Sehemi, A.G.; Irfan, A.; Aljubiri, S.M.; Shaker, K.H. Density functional theory investigations of radical scavenging activity of 3'-methyl-quercetin. Journal of Saudi Chemical Society, 2016 20, Supplement 1, pp. S21-S28. DOI: http://doi.org/10.1016/j.jscs.2012.08.004
17. Michielin, E.M.Z.; Salvador, A.A.; Riehl, C.A.S.; Smania-Jr, A.; Smania, E.F.A.; Ferreira, S.R.S. Chemical composition and antibacterial activity of *Cordia verbenacea* extracts obtained by different methods. Bioresource Technology, 2009, 100(24), pp. 6615-6623. DOI: https://doi.org/10.1016/j.biortech.2009.07.061
18. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 1999, 299, pp. 152-178. DOI: https://doi.org/10.1016/S0076-6879(99)99017-1
19. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 1999, 64(4), pp. 555-559. DOI: https://doi.org/10.1016/S0308-8146(98)00102-2
20. Swain, T.; Hillis, W.E. The phenolic constituents of *prunus domestica*. I. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 1959, 10(1), pp. 63-68. DOI: https://doi.org/10.1002/jsfa.2740100110
21. Amensour, M.; Sendra, E.; Pérez-Alvarez, J.A.; Skali-Senhaji, N.; Abrini, J.; Fernández-López, J. Antioxidant activity and chemical content of methanol and ethanol extracts from leaves of rockrose (*Cistus ladaniferus*). Plant Foods for Human Nutrition, 2010, 65(2), pp. 170-178. DOI: https://doi.org/10.1007/s11130-010-0168-2
22. Oyaizu, M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics, 1986, 44(6), pp. 307-315. DOI: https://doi.org/10.5264/eiyogakuzashi.44.307
23. Gursoy, N.; Sarikurkcu, C.; Cengiz, M.; Solak, M.H. Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. Food and Chemical Toxicology, 2009, 47(9), pp. 2381-2388. DOI: https://doi.org/10.1016/j.fct.2009.06.032
24. Hayta, M.; İşçimen, E.M. Antidiabetic, antihypertensive and antioxidant properties of grapevine leaf extracts obtained by ultrasound, microwave assisted, and classical solvent extraction. Erwerbs-Obstbau, 2018, 60, Supplement 1, pp. 79-85. DOI: https://doi.org/10.1007/s10341-018-0406-6
25. Do, Q.D.; Angkawijaya, A.E.; Tran-Nguyen, P.L.; Huynh, L.H.; Soetaredjo, F.E.; Ismadji, S.; Ju, Y.H. Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis, 2014, 22(3), pp. 296-302. DOI: http://doi.org/10.1016/j.jfda.2013.11.001
26. Sultana, B.; Anwar, F.; Ashraf, M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 2009, 14(6), pp. 2167-2180. DOI: https://doi.org/10.3390/molecules14062167
27. Sultana, B.; Anwar, F.; Ashraf, M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 2009, 14(6), pp. 2167-2180. DOI: https://doi.org/10.3390/molecules14062167
28. Nguyen, V.T.; Bowyer, M.C.; Vuong, Q.V.; Altena, I.A.V.; Scarlett, C.J. Phytochemicals and antioxidant capacity of Xao tam phan (*Paramignya trimera*) root as affected by various solvents and extraction methods. Industrial Crops and Products, 2015, 67, pp. 192-200. DOI: http://doi.org/10.1016/j.indcrop.2015.01.051

29. Naczk, M.; Shahidi, F. Extraction and analysis of phenolics in food. Journal of Chromatography A, 2004, 1054(1-2), pp. 95-111. DOI: https://doi.org/10.1016/j.chroma.2004.08.059
30. Chuen, T.L.K.; Vuong, Q.V.; Hirun, S.; Bowyer, M.C.; Predebon, M.J.; Goldsmith, C.D.; Sakoff, J.A.; Scarlett, C.J. Antioxidant and anti-proliferative properties of Davidson's plum (*Davidsonia pruriens* F. Muell) phenolic-enriched extracts as affected by different extraction solvents. Journal of Herbal Medicine, 2016, 6(4), pp. 187-192. DOI: http://doi.org/10.1016/j.hermed.2016.08.005
31. Vuong, Q.V.; Hirun, S.; Roach, P.D.; Bowyer, M.C.; Phillips, P.A.; Scarlett, C.J. Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica* papaya leaf aqueous extracts. Journal of Herbal Medicine, 2013, 3(3), pp. 104-111. DOI: https://doi.org/10.1016/j.hermed.2013.04.004
32. Koffi, E.; Sea, T.; Dodehe, Y.; Soro, S. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. Journal of Animal and Plant Science, 2010, 5(3), pp. 550-558. http://www.m.elewa.org/JAPS/2010/5.3/Abstract3-koffi.html
33. Nayak, B.; Dahmoune, F.; Moussi, K.; Remini, H.; Dairi, S.; Aoun, O.; Khodir M. Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from *Citrus sinensis* peels. Food Chemistry, 2015, 187, pp. 507-516. DOI: https://doi.org/10.1016/j.foodchem.2015.04.081
34. Conforti, F.; Statti, G.; Uzunov, D.; Menichini, F. Comparative chemical composition and antioxidant activities of wild and cultivated *Laurus nobilis* L. leaves and *Foeniculum vulgare* subsp. *piperitum* (Ucria) coutinho seeds. Biological and Pharmaceutical Bulletin, 2006, 29(10), pp. 2056-2064. DOI: https://doi.org/10.1248/bpb.29.2056
35. Gutierrez, L.; Conejero, G.; Castelain, M.; Guénin, S.; Verdeil, J.L.; Thomasset, B.; Van Wuytswinkel, O. Identification of new gene expression regulators specifically expressed during plant seed maturation. Journal of Experimental Botany, 2006, 57(9), pp. 1919-1932. DOI: https://doi.org/10.1093/jxb/erj138
36. Atoui, A.K.; Mansouri, A.; Boskou, G.; Kefalas, P. Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chemistry, 2005, 89(1), pp. 27-36. DOI: https://doi.org/10.1016/j.foodchem.2004.01.075
37. Amarowicz, R.; Estrella, I.; Hernandez, T.; Robredo, S.; Troszynska, A.; Kosinska, A.; Pegg, R.B. Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). Food Chemistry, 2010, 121(3), pp. 705-711. DOI: https://doi.org/10.1016/j.foodchem.2010.01.009