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Phytochemical analysis, antioxidant and photoprotective activities of aqueous extract of *Euphorbia retusa* Forssk. different parts from Algeria

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Abstract: *Euphorbia retusa* is an endemic medicinal plant of Sahara. This study aimed to determine the total phenolic and flavonoid contents of *Euphorbia retusa* seed, capsule and leaves aqueous extracts as well as to evaluate the antioxidant and photoprotective activities. The correlations between these activities and the different contents were also performed. The antioxidant activity was estimated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulfonic (ABTS) scavenging, β-carotene bleaching, cupric-reducing activity (CUPRAC) and reducing power essays. In addition, the sun protection factor (SPF) was reported for the first time and measured according to the Mansur equation. Results showed that, seeds exhibit a higher total phenolics and flavonoids contents. This organ showed the highest capacity in DPPH (IC_{50} = 50.79 ± 1.87 μg ml^{-1}), ABTS (IC_{50} < 6.25 μg ml^{-1}), β-carotene bleaching (IC_{50} < 6.25 μg ml^{-1}), reducing power ($A_{0.50}$ = 6.97 ± 0.75 μg ml^{-1}) and CUPRAC ($A_{0.50}$ = 7.64 ± 0.30 μg ml^{-1}) essays. Accordingly, seed extracts characterized by a high sun protection factor (SPF = 38.26 ± 0.07). Nevertheless, the Pearson correlation coefficients calculated show the highest positive correlation between total phenolic and flavonoids contents and photoprotective activity, while no correlations were found between SPF and other antioxidant activity. This plant could be used as alternative adjuncts in sunscreen product preparation.

Key words: *Euphorbia retusa* Forssk.; polyphenols; antioxidant activity; sun protection factor; Pearson correlation

Kemična analiza, antioksidacijska in fotoprotektivna aktivnost vodnih izvlečkov iz različnih delov vrste mlečka *Euphorbia retusa* Forssk. iz Alžirije

Izvleček: Vrsta *Euphorbia retusa* Forssk. je endemična zdravilna rastlina iz Sahare. Namen raziskave je bil določiti vsebnost celokupnih fenolov in flavonoidov v vodnih izvlečkih semen, glavic in listov te rastline kot tudi ovrednotiti njihovo antioksidacijsko in fotoprotektivno aktivnost. Pokazale so se povezave med različnimi aktivnostmi in vsebnostmi analiziranih sestavin. Antioksidacijska aktivnost je bila ocenjena na osnovi redukcijske moči snovi kot so DPPH, ABTS, bledenja β-karotena in redukcijske aktivnosti bakra (CUPRAC). Dodatno je bil prvič izmerjen zaščitni faktor pred soncem po Mansurjevi enačbi. Rezultati so pokazali, da imajo semena veliko vsebnost celokupnih fenolov in flavonoidov. Izvlečki iz semen so pokazali tudi največjo sposobnost pri uporabi DPPH (IC_{50} = 50,79 ± 1,87 μg ml^{-1}), ABTS (IC_{50} < 6,25 μg ml^{-1}), bledenju β-karotena (IC_{50} < 6,25 μg ml^{-1}), redukcijski moči ($A_{0.50}$ = 6,97 ± 0,75 μg ml^{-1}) in pri preiskusu CUPRAC ($A_{0.50}$ = 7,64 ± 0,30 μg ml^{-1}). Sorazmerno temu je bil za izvlečke semen značilen velik zaščitni faktor pred soncem. (SPF = 38,26 ± 0,07). Kljub temu, da je izračunani Pearsonov koeficient korelacije pokazal največjo pozitivno korelacijo med vsebnostjo celokupnih fenolov in flavonoidov ter aktivnostjo zaščite pred soncem ni bilo nobene korelacije med zaščito pred soncem in drugimi antioksidacijskimi aktivnostmi. Iz izledkov sledi, da bi se ta rastlina lahko uporabljala kot alternativni dodatek pri izdelavi zaščitnih pripravkov pred soncem.

Ključne besede: *Euphorbia retusa* Forssk.; polifenoli; antioksidacijska aktivnost; zaščitni faktor pred soncem; Pearsonova korelacija

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1 INTRODUCTION

Recently, a high demand of natural antioxidants has increased to replace synthetic antioxidants that are known by their undesirable side effects on human health (Megdiche et al., 2013). In addition, the demand for herbal cosmetics used in sunscreens has grown rapidly also to give better protection against UV radiations which can provoke more damages and develop a number of skin diseases (Napagoda et al., 2016).

However, plants are an important source for the development of new chemotherapeutic including antioxidant agents, which can be protect cell constituents against oxidative damage and withstand the risk of various diseases associated with oxidative stress (Herlina et al., 2018). Furthermore, phenolic compounds are known for their potential antioxidant to eliminate toxic reactive oxygen species (ROS) as well as flavonoids, which were characterized by a strong potential protection against UV radiations (Hopkins, 2003; Macheix et al., 2005). The usage of plant species in the traditional medicine for the treatment of a variety of diseases (El-haj et al., 2014; Nematy et al., 2015) may be an important way to facilitate research on the sources of natural additives. Besides, medicinal plants of Sahara have higher secondary metabolites contents including phenolic compounds (Trabelsi et al., 2010; Gasmi et al., 2019).

Euphorbia retusa Forssk. is an endemic species of northern and central Sahara. It is an annual plant which grows naturally up to 30 cm high in hard climatic conditions of Sahara (Quezel and Santa, 1962; Ozenda, 2004). This plant is known for its use in folk medicine particularly as a treatment of dermatosis in the central Algerian Sahara (Ghareeb et al., 2018; Abdallah, 2014; Sdayria et al., 2019; Hammiche and Maiza, 2005).

Thus, the aim of this study was to investigate the antioxidant and photoprotective activities of the selected plant and to provide also the relationship between these activities and total phenolics and flavonoids contents of the aqueous extracts of *E. retusa* seed, capsule and leaves.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL COLLECTION

Euphorbia retusa Forssk. plant parts were collected from the South-East arid region of Algeria (34°54'21.751"N, 005°38'27"E) in June 2016. The plant samples were identified based on the flora of Ozenda (2004). The plant samples were separated into different parts: seeds, capsules and leaves. Then, samples were cleaned, dried in shade and grounded to powder.

2.2 EXTRACTION METHODS

In order to extract the phenolic compounds present in our plant, 10 g of each part of plant (seeds, capsules or leaves) were extracted separately with 100 ml of distilled water using Soxhlet apparatus at 40 °C for 8 hours. After extraction, the solvent of each part extracts was evaporated using a rotary vacuum evaporator until dryness.

2.3 TOTAL PHENOLICS AND FLAVONOIDS CONTENTS DETERMINATION

The total phenolics content of aqueous extracts was determined using Folin-Ciocalteu method following the protocol of Singleton et al. (1999) with slight modification. Briefly, 20 µl of each sample was mixed with 100 µl Folin–Ciocalteu reagent (10 fold diluted) and 75 µl of 7.5 % sodium carbonate solution. This mixture was incubated for 2 h at room temperature and the absorbance was measured at 765 nm using a 96-well microplate multimode plate reader (En Spire, PerkinElmer, MA, USA). The phenolic compounds concentrations were expressed as gallic acid equivalents/mg solid dry extract (µg GAE/mg DE) and calibration equation was found as: $y = 0.002\times + 0.010$, ($R^2 = 0.989$).

The total flavonoids content of aqueous extracts was quantified according to Moreno et al. (2000) method. 20 µl of each diluted extract solution was mixed with 10 µl of 10 % aluminium nitrate, 10 µl of potassium acetate (1 M) and 130 µl of methanol. After 40 min incubation at room temperature, the absorbance was measured at 415 nm. The total flavonoid content concentrations were expressed as Quercetin equivalents/ mg solid dry extract (µg QE/mg DE) and calibration equation was determined as: $y\ 0.006\times - 0.006$, ($r^2 = 0.998$).

2.4 ANTIOXIDANT ACTIVITY EVALUATION

2.4.1 Antiradical activity

The free radical scavenging activity of the aqueous extracts of each part was evaluated using DPPH assay described by Bloi (1958). 40 µl of the each extract concentrations (6.25, 12.5, 25, 50,100, 200 and 400 $\mu g ml^{-1}$) was mixed with 160 µl of a methanolic DPPH solution. The mixture was incubated at room temperature for 30 min. Then, the absorbance was measured at 517 nm using 96 well microplate reader. Results were expressed as % inhibition and as IC_{50} values in $\mu g\ ml^{-1}$. Butylhydroxytoluène (BHT) was used as a positive control. The inhibi-

tion percentage was calculated according to the following formula;

% *Inhibition* = [(*A0* – *A1* / *A0*)] × 100

Where; *A0* is the absorbance of the negative control, and *A1* is the absorbance of the sample at 30 min.

2.4.2 ABTS$^{\bullet+}$ scavenging activity

ABTS radical-scavenging activity of aqueous extracts was assessed according to the method developed by Re et al. (1999). 40 µl of extract at different concentrations (6.25, 12.5, 25, 50,100, 200 and 400 µg ml^{-1}) were mixed with to 160 µl of ABTS$^{\bullet+}$ solution in micro plate 96 wells. After 10 min of incubation, the absorbance was recorded at 734 nm. Butylhydroxytoluène (BHT) was used as a positive control and the inhibition percentage was calculated.

% *Inhibition* = [(*A0* – *A1* / *A0*)] × 100

Where; *A0* is the absorbance of the negative control, and *A1* is the absorbance of the sample at 10 min.

2.4.3 β-carotene–linoleic acid bleaching assay

β-carotene–linoleic acid bleaching assay of seeds, capsules and leaves aqueous extracts of *E. retusa* Forssk was measured following the method of Marco (1968). 40 µl of each sample at seven different concentrations was added to 160 µl of the β-carotene–linoleic acid emulsion. The first absorbance was measured in the zero-time at 470 nm and the second absorbance was recorded after 120 min of incubation on the same wavelength. Butylhydroxytoluène (BHT) was used as a positive control and the inhibition percentage as measured as following:

$$\%\ Inhibition = [1-(A0_{Extract} - At_{Extract})/(A0_{Control} - At_{Control})] \times 100$$

Where; $A_{0\ Control}$ is the absorbance of the negative control at 0 min. $At_{Control}$ is the absorbance of the negative control at 120 min. $A0_{Extract}$ is the absorbance of the sample at0 min. $A_{t\ Extract}$ is the absorbance of the sample at 0 min.

2.4.4 Reducing power assay

The reducing power of studied extracts was determined following the method of Bouratoua et al. (2017). 10 µl of extract were added to 40 µl of phosphate buffer (0.2 M, pH 6.6) and 50 µl of potassium ferricyanide (1%). The plate was incubated at 50 °C for 20 min. Then, 50 µl of tricarboxylic acid (10 %), 40 µl of distilled water and 10 µl of ferric chloride (0.1%) were added to mixture. Butylhydroxytoluène (BHT) was used as a positive control and the absorbance was measured at 700 nm. Results were expressed as absorbance against reagent blank and as $A_{0.50}$ values (µg ml^{-1}) corresponding the concentration indicating 0.50 absorbance intensity.

2.4.5 Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity of aqueous extracts was determined according to the method of Apak et al. (2004). 40 µl of the extracts were added to 50 µl of copper (II) chloride (10 mM), 50 µl of neocuproine at 7.5 mM, and 60 µl of ammonium acetate (NH4Ac) buffer (1 M, pH = 7.0) solutions. After 1 hour of incubation, the absorbance was measured at 450 nm and butylhydroxytoluène (BHT) was used as a positive control.

2.5 *IN VITRO* SUN PROTECTION FACTOR (SPF) DETERMINATION

In order to evaluate ultraviolet (UV) absorption ability of the aqueous extracts, the *in vitro* SPF is determined according to the spectrophotometric method of Mansur *et al.* (1986).

The aliquots prepared were scanned between 290 and 320 nm, and the obtained absorbance values were multiplied with the respective EE (λ) values. Then, their summation was taken and multiplied with the correction factor. Methanol was taking as blank.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs\ (\lambda)$$

Where; *EE*: erythemal effect spectrum, *I*: solar intensity spectrum, *Abs*: absorbance of sunscreen product, *CF*: correction factor (= 10). The value of (EE × *I*) is constant and determined by Sayre et al. (1979) (Table 1).

2.6 STATISTICAL ANALYSIS

All values were expressed as the mean ± SD (standard deviation). Analysis of variance (ANOVA) test followed by Newman–Keuls test were performed to check significant differences between the studied samples using the statistical software Statistica version 6.0. $p < 0.05$ compared to control was considered to be statistically significant.

Table 1: Correlation between the erythemogenic effect (EE) and the radiation intensity at each wavelength (I)

Wavelength (λ nm)	EExI (λ) (Normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

3 RESULTS AND DISCUSSION

3.1 TOTAL PHENOLICS AND FLAVONOIDS CONTENTS

Total phenolics and flavonoids contents of *E. retusa* different parts were described in Table 2. The results revealed that the seeds aqueous extract exhibited the highest amount of total phenolic contents (356.83 ± 3.69 µg GAE/mg DE) followed by the capsules (114.25 ± 0.35 µg GAE/mg DE) and the leaves (75.83 ± 8.96 µg GAE/mg DE) extracts. According to Öztürk et al. (2006) and Karoune et al. (2015), secondary metabolites inter-organs as well as phenolic compounds are more variable in plant organs. Moreover, this variability may be dependent on the endogenous and exogenous factors (Oueslati et al., 2012). Furthermore, the aqueous extracts of *E. retusa* capsule registered the highest content on total phenolics than the methanolic extracts (105.33 ± 7.75 µg GAE/mg DE) reported by Lahmadi et al. (2020*)*. Thus, the phenolic compounds of *E. retusa* capsules are very soluble in water in distilled water than methanol, which means that this organ is rich in polar polyphenol (Baldosano et al., 2015).

Flavonoids contents were higher in seeds aqueous extract followed by capsules and leaves extracts (194.38 ± 8.31, 30.7 ± 0.4 and 44.25 ± 5.9 µg QE/mg DE respectively). However, flavonoids contents in leaves reported in the present work were higher than that reported by Sdayria et al. (2019) (20.50 ± 0.107 µg QE/mg DE) extracted by the maceration method which suggest that extraction with water was more effective than with 96 % ethanol.

3.2 DPPH SCAVENGING ACTIVITY

DPPH free radical scavenging activity of aqueous extracts of *E. retusa* seed, capsule and leaves is shown in Table 3. The results were expressed as inhibition percentage at different concentrations (6.25, 12.5, 25, 50, 100, 200 and 400 µg ml^{-1}) and as IC_{50} values in µg ml^{-1}. Data revealed that DPPH scavenging capacity increases with the raise in concentration of each extract. Furthermore, our findings showed that seeds aqueous extract exhibited a high activity competing with the both other extracts at all concentrations. Likewise, research reports found that seeds phenolic compounds are capable more for donating hydrogen to a free radical to scavenge the potential damage (Ksouri et al., 2009; Saeed et al., 2012). Ashraf et al. (2015) reported also the DPPH free radical scavenging activity of roots aqueous extract of *E. royleana*. The comparing of our results with this study showed that our samples (seeds, capsule and leaves) at 100 µg ml^{-1}present a higher effective scavenger of hydroxyl radical (79.67 % ± 1.44, 57.87 % ± 0.76 and 36.09 % ± 1.11 respectively) than their samples (20.18 % ± 0.96).

3.3 ABTS SCAVENGING ACTIVITY

For the ABTS radical-scavenging activity, seed extracts have a stronger capacity to quench $ABTS^{\bullet+}$ at concentrations ≥ 50 µg ml^{-1}as well as BHT (Table 4). The inhibition percentage of seed, capsule and leaves aqueous extracts was significantly important (92.01 % ± 1.64, 92.44 % ± 0.25 and 52.63 % ± 0.09 respectively) compared to those reported by Alaklabi et al. (2018) for root aqueous extracts of *Saururus chinensis* (Lour.) Baill. with 19.07 % ± 0.12 at same concentration (100 µg ml^{-1}).

Table 2: Total phenolics and flavonoids contents of the aqueous extracts of *E. retusa* different parts

Extract	Seeds	Capsules	Leaves
Total phenolics (µg GAE/mg DE)	356.83 ± 3.69^{a}	114.25 ± 0.35^{b}	75.83 ± 8.96^{c}
Total flavonoids (µg QE/mg DE)	194.38 ± 8.31^{a}	30.7 ± 0.4^{b}	44.25 ± 5.9^{c}

Values expressed as mean ± SD (n = 3). Values in the same line followed by a different letter (a-c) are significantly different ($p < 0.05$). µg GAE/mg DE: microgram gallic acid equivalent per milligram of dry plant extract. *µg QE/mg DE*: microgram quercetin equivalent per milligram of dry plant extract

Table 3: Antioxidant activity of the aqueous extracts of *E. retusa* different parts by DPPH assay

Concentrations µg ml^{-1}	% Inhibition in DPPH scavenging assay			
	Seed	Capsule	Leaves	BHT
6.25	9.21 ± 1.25	1.44 ± 1.29	1.94 ± 1.80	18.55 ± 2.46
12.5	16.14 ± 2.34	4.60 ± 1.37	5.16 ± 0.35	32.60 ± 3.72
25	27.12 ± 1.10	11.47 ± 1.03	8.88 ± 1.15	53.80 ± 2.58
50	49.99 ± 1.62	28.19 ± 1.11	19.77 ± 0.35	74.97 ± 2.14
100	79.67 ± 1.44	57.87 ± 0.76	36.09 ± 1.11	83.41 ± 0.86
200	84.12 ± 0.34	82.20 ± 0.68	73.69 ± 0.95	84.59 ± 0.46
400	92.82 ± 0.41	92.83 ± 0.17	81.79 ± 0.42	85.76 ± 0.91
IC_{50} µg ml^{-1}	50.79 ± 1.87^{b}	87.38 ± 1.53^{c}	158.49 ± 3.24^{d}	23.54 ± 1.83^{a}

Values expressed as mean ± SD (n = 3). Values in the last line followed by a different letter (a-d) are significantly different ($p < 0.05$). BHT: butyl hydroxytoluene. IC50: half maximal inhibitory concentration expressed as the necessary concentration to decrease the initial absorbance of DPPH by 50 %

Table 4: Antioxidant activity of the aqueous extract of *E. retusa* different parts by ABTS assay

Concentrations µgml^{-1}	% Inhibition in ABTS assay			
	Seeds	Capsules	Leaves	BHT
6.25	69.10 ± 2.97	18.38 ± 1.51	-	61.38 ± 0.57
12.5	88.06 ± 2.60	33.88 ± 1.99	9.68 ± 2.78	62.02 ± 3.82
25	88.93 ± 1.51	56.27 ± 4.35	12.92 ± 3.96	76.50 ± 1.40
50	91.14 ± 0.37	86.05 ± 2.87	25.83 ± 1.78	82.55 ± 1.04
100	92.01 ± 1.64	92.44 ± 0.25	52.63 ± 0.09	88.60 ± 2.66
200	92.82 ± 0.41	92.83 ± 0.17	73.69 ± 0.95	90.38 ± 0.67
400	-	92.89 ± 0.19	90.33 ± 0.25	-
IC_{50} µg ml^{-1}	< 6.25^{a}	21.12 ± 0.76^{b}	95.92 ± 1.20^{c}	< 6.25^{a}

Values expressed as mean ± SD (n = 3). Values in the last line followed by a different letter (a-c) are significantly different ($p < 0.05$). BHT: butylhydroxytoluene. IC50: half maximal inhibitory concentration expressed as the necessary concentration to decrease the initial absorbance of DPPH by 50 %

Table 5: Antioxidant activity of the aqueous extract of *E. retusa* different parts by β-carotene–linoleic acid bleaching assay

Concentrations µg ml^{-1}	% Inhibition in β-carotene–linoleic acid bleaching assay			
	Seeds	Capsules	Leaves	BHT
6.25	97.83 ± 0.38	96.14 ± 0.43	92.94 ± 0.50	57.25 ± 3.1
12.5	97.42 ± 0.22	96.02 ± 0.37	89.37 ± 0.95	82.39 ± 2.79
25	96.29 ± 0.20	94.21 ± 0.41	82.29 ± 0.30	83.12 ± 2.82
50	94.67 ± 0.39	93.77 ± 1.52	72.53 ± 1.72	92.99 ± 3.26
100	93.07 ± 0.18	92.01 ± 0.14	57.08 ± 2.74	92.65 ± 3.19
200	89.6 ± 0.04	89.28 ± 0.23	30.34 ± 1.36	93.52 ± 0.00
400	79.83 ± 0.32	84.44 ± 0.18	14.24 ± 0.91	94.22 ± 0.30
IC_{50} µg ml^{-1}	< 6.25 ± 0.00^{a}	< 6.25 ± 0.00^{a}	23.82 ± 0.95^{b}	< 6.250 ± 00 ^{a}

Values expressed as mean ± SD (n = 3). Values in the last line followed by a different letter (a-b) are significantly different ($p < 0.05$). BHT: butylhydroxytoluene. IC50: half maximal inhibitory concentration expressed as the necessary concentration to decrease the initial absorbance of DPPH by 50 %

Table 6: Antioxidant activity of the aqueous extracts of *E. retusa* different parts by reducing power assay

Concentrations µg ml^{-1}	Absorbance in reducing power assay			
	Seeds	Capsules	Leaves	BHT
6.25	0.22 ± 0.01	0.08 ± 0.01	-	0.05 ± 0.02
12.5	0.31 ± 0.02	0.1 ± 0.00	-	0.07 ± 0.02
25	0.46 ± 0.03	0.14 ± 0.00	-	0.11 ± 0.03
50	0.7 ± 0.04	0.19 ± 0.02	-	0.19 ± 0.02
100	0.85 ± 0.11	0.29 ± 0.01	0.05 ± 0.00	0.30 ± 0.03
200	1.29 ± 0.09	0.35 ± 0.01	0.05 ± 0.00	0.74 ± 0.18
400	2.14 ± 0.00	0.57 ± 0.01	0.06 ± 0.02	1.07 ± 0.17
$A_{0.50}$ µg ml^{-1}	6.97 ± 0.75^{a}	84.49 ± 2.38^{c}	> 100	37.41 ± 3.89^{b}

Values expressed as mean ± SD (n = 3). Values in the last line followed by a different letter (a-c) are significantly different ($p < 0.05$). BHT**:** butyl-4-methylphenol ou butylhydroxytoluene. $A_{0.50}$: corresponding the concentration indicating 0.50 absorbance intensity

3.4 B-CAROTENE–LINOLEIC ACID BLEACHING ASSAY

The bleaching of β-carotene assay was used to evaluate the ability of the antioxidants to inhibit lipid peroxidation (Moualek et al., 2016). Furthermore, up to our knowledge, there are no reports on the bleaching of β-carotene assay of *E. retusa* organs. So this is the first report which deals with this effect. The results of this activity were expressed as inhibition percentage and as IC_{50} µg ml^{-1} (Table 5). Results showed that seed, capsule aqueous extracts and BHT as a standard have a stronger capacity to inhibit the coupled oxidation of β-carotene and linoleic acid (IC_{50} < 6.25 µg ml^{-1}).

3.5 REDUCING POWER ASSAY

For reducing power activity (Table 6), the absorbance of samples and BHT as a standard were increased by the rising of concentrations. However, the results showed that seeds aqueous extract had the strongest capacity to reduce ion at all concentrations compared with BHT or stems and leaves aqueous extracts. Moreover, seeds aqueous extract ($A_{0.50\ =}$ 6.97 ± 0.75 µg ml^{-1}) indicates a high $A_{0.50}$ value as compared with seeds methanolic extract ($A_{0.50\ =}$ 11.84 ± 1.72 µg ml^{-1}) reported by Lahmadi et al. (2019).

3.6 CUPRIC REDUCING ANTIOXIDANT CAPACITY (CUPRAC)

The CUPRIC reducing antioxidant capacity method described by Apak et al.(2004) measures the absorbance of Cu(II)- neocuproine (Nc) chelate formed by the redox reaction of chain-breaking antioxidants with the CUPRAC reagent. Cupric reducing antioxidant capacity

Table 7: Antioxidant activity of the aqueous extract of *E. retusa* different parts by CUPRAC assay

Concentrations µg ml^{-1}	Absorbance in CUPRAC assay			
	Seeds	Capsules	Leaves	BHT
6.25	0.44 ± 0.01	0.14 ± 0.01	-	0.44 ± 0.03
12.5	0.72 ± 0.03	0.20 ± 0.02	0.07 ± 0.00	1.32 ± 0.07
25	1.15 ± 0.05	0.30 ± 0.02	0.08 ± 0.00	1.80 ± 0.09
50	2.00 ± 0.19	0.53 ± 0.03	0.08 ± 0.00	1.82 ± 0.22
100	3.28 ± 0.03	0.81 ± 0.02	0.09 ± 0.00	2.39 ± 0.39
200	3.69 ± 0.12	0.98 ± 0.08	0.10 ± 0.00	2.71 ± 0.46
400	4.06 ± 0.02	1.45 ± 0.07	0.18 ± 0.05	2.76 ± 0.46
$A_{0.50}$ µg ml^{-1}	7.64 ± 0.30^{b}	49.50 ± 1.51^{c}	> 400	6.64 ± 0.18^{a}

Values expressed as mean ± SD (n = 3). Values in the last line followed by a different letter (a-c) are significantly different ($p < 0.05$). BHT: butyl-4-methylphenol ou butylhydroxytoluene. $A_{0.50}$: A0.5 (µg ml^{-1}) corresponding the concentration indicating 0.50 absorbance intensity

Table 8: SPF values of the aqueous extract of *E. retusa* different parts

Absorbance			CF× EE(λ)×I(λ)× Abs (λ)		
Seeds	Capsules	Leaves	Seeds	Capsules	Leaves
3.85	2.21	2.84	0.58	0.33	0 .43
3.86	1.85	2.48	3.15	1.51	2.02
3.85	1.61	2.26	11.07	4.62	6.48
3.82	1.50	2.17	12.53	4.90	7.10
3.80	1.46	2.15	7.08	2.71	4.00
3.78	1.46	2.14	3.17	1.22	1.79
3.75	1.50	2.12	0.68	0.27	0.38
Sun Protection Factor (SPF)			38.26 ± 0.07^{a}	15.57 ± 0.24^{b}	22.21 ± 0.56^{c}

Values expressed as mean ± SD (n = 3). Values in the last line followed by a different letter (a-c) are significantly different ($p < 0.05$). EE: erythemal effect spectrum, I: solar intensity spectrum, Abs: absorbance of sunscreen product, CF: correction factor

(CUPRAC) of the organ extracts and the BHT are shown in Table 7. The ranking order for CUPRAC test was BHT > seeds > capsules > leaves at all concentrations. Accordingly, the results of CUPRAC test showed that BHT have a higher activity ($A_{0.50}$ = 6.64 ± 0.18 µg ml^{-1}) followed by seeds ($A_{0.50}$ = 7.64 ± 0.30 µg ml^{-1}), capsule ($A_{0.50}$ = 49.50 ± 1.51 µg ml^{-1}) and leaves ($A_{0.50}$ = > 400 µg ml^{-1}) extracts.

3.7 IN VITRO SUN PROTECTION FACTOR (SPF) DETERMINATION

The sun protection factor (SPF) values of different part samples were shown in the table 8. The seeds extract has the higher SPF values (38.26 ± 0.07) followed by leaves and capsules extracts (SPF = 22.21 ± 0.56, 15.57 ± 0.24 respectively). In literature, the data about photoprotective activity of this plant is not available. According to Afssaps (2011), SPF is generally divided into four protection classes; low (SPF values: 6-15), medium or moderate (SPF values: 15-30), high (SPF values: 30 - 50) and very high (SPF values > 50). Thus, seed aqueous extract belongs to the range of good sunscreen activity while leaves and capsules aqueous extracts were characterized by moderate sunscreen activity. In comparison to other works on SPF values, seed extracts of *E. retusa* presented a higher SPF than *Mentha spicata* L. aerial parts methanolic extract reported by El Aanachi et al. (2021) with SPF = 35.76 ± 0.21 and the aerial parts methanolic extract *of Capnophyllum peregrinum* (L.) Lange reported by Lefahal et al. (2018) with SPF = 35.21 ± 0.18. In fact, SPF result shows that the aqueous extracts of seed have a good sun protection activity against ultraviolet radiation.

In order to analyze the relationship between total phenolics and flavonoids contents, antioxidant and photoprotective activities of *E. retusa* seed, capsule and leaves aqueous extracts, Pearson's correlations were applied (Table 9). A statistically significant positive correlation between total phenolic and flavonoid contents and photoprotective activity with Pearson's correlation coefficients $r > 0.90$, suggesting that the SPF was dependent not only on the total phenolic but also on the total flavonoid contents, which may be attributed to their synergistic action. These results support the hypothesis that flavonoid contents contribute to photoprotective activity (Macheix et al., 2005). However, total phenolics contents showed a significant negative correlation with antioxidant activities (DPPH and ABTS radical-scavenging capacity, β -carotene bleaching and cupric-reducing antioxidant capacity (CUPRAC) and reducing power) with $r > - 0.59$. Similarity, a statistically significant negative correlation between total phenolic contents, DPPH free radical scavenging capacity and β carotene bleaching was supported by Terpinc et al. (2012). Also, Kainama et al. (2020) found the negative correlation between total phenolic content and ABTS scavenging activity in Garcinia stem and bark ethyl acetate extracts ($r = – 0.91$), indicating that the antioxidant activity may be linked to the structure and the nature of the phenolic compounds (Oueslati, 2013). However, negative correlation between flavonoid contents and antioxidant activities (DPPH and ABTS cation radical scavenging, cupric-reducing antioxidant capacity (CUPRAC) and reducing power) was shown. On the other hand, reducing power showed a significant negative correlation with β carotene bleaching inhibition and SPF values with Pearson's correlation coefficients of -0.56 and -0.67 respectively. These results indicated the antagonist effects of reducing power with β carotene bleaching inhibition and with SPF. Furthermore, no correlations were found between SPF and other antioxidant activity. Similar results were found by Ebrahimzadeh et

Table 9: Correlations among *E. retusa* total phenolics and flavonoids contents, antioxidant and photoprotective activities

	TPC	TFC	$DPPH^{+}$	$ABTS^{+}$	β-CLAB	RP	CUPRAC	SPF
TPC	1							
TFC	0.97	1						
DPPH	- 0.84	- 0.70	1					
ABTS	- 0.76	- 0.61	0.99	1				
β-CLAB	- 0.60	- 0.43	0.94	0.98	1			
RP	- 0.31	- 0.5	- 0.25	- 0.38	- 0.56	1		
CUPRAC	- 0.71	- 056	0.98	0.1	0.99	- 0.44	1	
SPF	0.91	0.975	- 0.54	- 0.43	- 0.23	- 0.67	- 0.37	1

Correlation is significant at $p < 0.05$. TPC: total phenolics content, TFC: total flavonoids content, DPPH: 2,2-diphenyl-1-picrylhydrazyl test, ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt cation radical scavenging, *β-CLAB: β*-Carotene–linoleic acid bleaching, RP: reducing power, CUPRAC: cupric-reducing activity, SPF: sun protection factor

al. (2014). Hence, our results indicated no correlations between SPF and DPPH radical-scavenging activity.

4 CONCLUSIONS

Our results revealed that seed extract demonstrates the best total phenolics and flavonoids contents and SPF value. Also, this extract showed a great potential for antioxidant activity. Furthermore, the correlation analysis revealed that SPF is positively correlated with total phenolics and flavonoids contents. But, generally no correlations were found between SPF and antioxidant activity. According to the obtained results, *E. retusa* may be considered as a remarkable antioxidant and pharmaceutical source.

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