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RESEARCH ARTICLE

Phenolic Content, Antioxidant Properties, Key Enzyme Inhibitory Potential and Photoprotective Activity of *Lawsonia inermis* L

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Abstract: ***Background***: *Lawsonia inermis* Linn (Lythraceae), commonly known as “Henna” is a medicinal plant, which is widely used as a folk remedy and for its cosmetic properties.

Objective: The objective of this present work was to evaluate biological activities and to quantify phenolics in extracts.

Methods: The extracts were obtained from seeds of *L. inermis* by increasing the polarity of the solvent. The content of total phenolics, flavonoids, flavonols and tannins was determined using colorimetric methods. Also, to evaluate the antioxidant activity, six different assays, DPPH, ABTS, superoxide radical scavenging, inhibition of β-carotene bleaching, ferric reducing antioxidant power and phenanthroline assays were used. Enzyme inhibition activity was evaluated by acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibition assays. Furthermore, photoprotective activity was determined by measuring sun protection factor.

Results: The extraction efficiency of phenolics, as well as the biological activities of plant extracts, were affected by solvent polarity. The highest content of phenolics was determined in methanol extract (786.54 ± 0.00 mg $GAEg^{-1}$ DW), followed by the aqueous and ethyl acetate extracts (526.48 ± 0.40 and 331.25 ± 0.00 $GAEg^{-1}$ DW, respectively).The above extracts also exhibited the highest antioxidant activity, while low polar extracts were characterized with the lowest content of phenolics, as well as the lowest antioxidant capacity. The highest enzyme inhibition activities were found in ethyl acetate extract. Moreover, the methanol extract showed the best photoprotective activity with sun protection factor of 43.05 ± 0.37.

Conclusion: These findings suggest a possible use of Henna seeds as a potential source of bioactive molecules with antioxidant, enzyme inhibition and skin protection properties.

Keywords: *Lawsonia inermis*,antioxidant activity, cholinesterase inhibition, tyrosinase inhibition, photoprotective activity, phenolic compounds.

1. INTRODUCTION

It is well known that free radicals and reactive oxygen species (ROS) which include: singlet oxygen (O_2), superoxide anion radical (O_2^-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) result from biotransformation of the oxygen [1]. These can have damaging effects directly on healthy cells of the body by alteration of DNA which causes mutation, protein denaturation, and lipid peroxidation, inducing loss of the fluidity of unsaturated lipid membrane, and leading to apoptosis and cell death [2, 3]. Antioxidants were reported to affect the oxidation process by scavenging free radicals and ROS or by chelating oxidation-catalytic metals.

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Acetylcholinesterase (AChE) is known as specific cholinesterase and it is usually present in the brain, while butyrylcholinesterase (BChE) is known as pseudocholinesterase and it is present in the peripheral tissues [4]. However, inhibitors of AChE and BChE increase the levels of acetylcholine in the brain and are used as therapeutic molecules for Alzheimer's disease (AD) [5].

Tyrosinase is an enzyme implicated in the biosynthesis of a dark biological pigment produced by epidermal melanocyte named "melanin" [6, 7]. Nevertheless, an abnormal melanogenesis causes several hyperpigmentation diseases. Tyrosinase inhibitors have several applications, including food, cosmetic and pharmaceutical fields [8].

Lawsonia inermis L. (synonym: *L. alba* Lam., *L. speciosa* L., *L. spinosa* L.) is the scientific name of the most famous flowering perennial shrub in the *Lythraceae* family. It has been widely used in traditional medicine with religious associations and commonly called "Henna" or "Mehndi". This small shrub (2-6 m in high) is a native plant of India, North Africa, Asia, Australia and many other parts of the world. *L. inermis* is the sole species in the genus and it is well known around the world for cosmetic use of the coloring material that is present in the leaves, and to treat many skin ailments [9-11]. In folk medicine, seeds have also been used in the treatment of ailments [10]. Several plants belonging to *Lythraceae* family have shown biological activities, for example, *Lythrum salicaria* L. has exhibited antioxidant and enzymatic inhibition properties [12], while *Punica granatum* L. has displayed photoprotective effect [13].

To the best of our knowledge, most of the previous biological activities focused on the leaves of henna. Therefore, the present research is an attempt to assess the antioxidant, anticholinesterase, anti-tyrosinase, and photoprotective activities of *L. inermis* growing locally in Algeria and to determine the amount of some phenolic compounds in extracts from its seeds.

2. MATERIALS AND METHODS

2.1. Chemical Reagents and Instruments

All tests were realized on 96-well microplate, and all spectrophotometric determinations were carried out on multimode plate reader (EnSpire, Perkin Elmer, Waltham, MA, USA). Rotary evaporator (Rotavapor R-210, Buchi, Switzerland) and freeze-dryer (Christ Beta 2-8 LD, Martin Christ, Germany) were used for the evaporation and the lyophilization of the extracts. Petroleum ether, n-hexane, chloroform, ethyl acetate, methanol and dimethylsulfoxide (DMSO) were of RPE analytical grade and were purchased from Sigma-Aldrich (St. Louis, USA). All the other chemicals were again RPE analytical grade and were also purchased from Sigma-Aldrich.

2.2. Plant Material

The seeds of *L. inermis* were collected from Zribet El-Oued region, Biskra, Algeria (N 34°42'46.9", E 6°30'01.3" with altitude of 48 m) in October 2016. Plant material was identified by Professor Bachir Oudjehih, Agronomic and Veterinary Institute, University of Batna1, Algeria. A voucher specimen of the plant (AIMU.2016.C1) was deposited in the collections of the laboratory of the first author. Seeds were dried in shade at room temperature and kept in closed glass flask until extraction process.

2.3. Preparation of the Extracts

2.3.1. Preparation of Organic Extracts

Dried seeds were powdered with kitchen mortar to avoid the elevation of temperature that could alter the bioactive molecules. Powdered seeds (100 g) were first extracted by 300 mL of n-hexane at room temperature. After 24 h, the mixture was filtered with Whatman N°1 filter paper, the residual material from the filtration was extracted again twice using the same procedure. The residual material was then extracted with chloroform, ethyl acetate, and methanol sequentially in the same conditions. All extracts were evaporated until dryness to obtain finally hexane, chloroform, ethyl acetate, and methanol extracts, which were stored in dark at 4 °C until the assays were performed.

2.3.2. Preparation of Aqueous Extract

Powdered seeds (50 g) were added to 500 mL of distilled water, the mixture was left 24 h at room temperature, and then filtered by Whatman N°1 filter paper. After filtration, the same procedure was repeated twice in the same conditions, then the water was eliminated by lyophilization to obtain a brownish powder that was refrigerated at 4 °C until further uses.

2.4. Determination of Total Phenolic Content

The total phenolic content (TPC) of all extracts was determined by microplate assay described by Müller *et al.* [14]. A concentration of 1 $mgmL^{-1}$ was prepared from each extract in methanol or DMSO, then 20 µL of each sample was mixed with 100 µL of Folin-Ciocalteu reagent (10%), then 75 µL of sodium carbonate solution (7.5%) was added, and the mixture was allowed in dark at room temperature for 2 h. The absorbance was measured by a microplate reader at 765 nm against a blank prepared by replacing the extract with the solvent. The amount of total phenolic was calculated from the calibration curve of gallic acid and was expressed as mg of gallic acid equivalents (GAE) per g dry weight (DW) of extract.

2.5. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was estimated according to the method described by Topçu *et al.* [15] with minor modifications. Briefly, 50 µL of extract with a concentration of 1 $mgmL^{-1}$was reacted with 130 µL of methanol, 10 µL of potassium acetate (1 M), and 10 µL of 10% aluminium nitrate. The microplate was kept in darkness for 40 min at room temperature. The absorbance was recorded at 415 nm against a blank prepared by replacing the extract with the solvent. TFC was expressed as mg of quercetin equivalents

(QE) per g dry weight (DW) of extract according to a calibration curve prepared with quercetin solution (0.01 to 1 mgmL^{-1}) as standard.

2.6. Determination of Total Flavonol Content

The total flavonol content (TFLC) of extracts was determined by the colorimetric method previously described by Kumaran and Karunakaran [16]. To 50 µL of extract (1 mgmL^{-1}) in methanol or DMSO, 50 µL of aluminium trichloride (2%), and 150 µL of sodium acetate (5%) were added. The absorbance was measured at 440 nm after 2 h and 30 min in dark and at room temperature. The blank was prepared by replacing the extract with the solvent. A calibration curve with quercetin (0.01 to 1 mgmL^{-1}) as standard was used to estimate the amount of total flavonol that was expressed as mg of quercetin equivalents (QE) per g dry weight (DW) of extract.

2.7. Determination of Total Condensed Tannins Content

The total condensed tannin content (TCTC) was estimated using the protocol described by Julkunen-Tiitto *et al.* [17] with slight modifications. A calibration curve was plotted using catechin as standard with concentration range between 0 and 0.3 mgmL^{-1} in methanol. For all extracts, 25 µL (10 mgmL^{-1}) was mixed with 50 µL of methanolic solution of vanillin (4%) followed by 75 µL of 30% HCl in methanol and the mixture was incubated at 20 °C for 15 min. The blank was prepared by replacing the extract with the solvent and the absorbance was recorded at 500 nm. The results were expressed as mg equivalents of catechin (CE) per g dry weight (DW) of extract.

2.8. Assessment of Antioxidant Activity

2.8.1. DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity was assessed by the method of Blois [18] with some modifications. α-tocopherol, BHA, and BHT were used as antioxidant references. The absorbance of a methanolic solution of DPPH (0.004%) freshly prepared was adjusted to the absorbance of 0.500 ± 0.005 at 517 nm, then in each well of microplate, 160 µL of this later were added to 40 µL of different concentrations of extract and the microplate was allowed in dark at room temperature. Thirty minutes later, the absorbance was measured again at 517 nm against a reagent blank. The DPPH solution without sample solution was used as control. The ability to scavenge the DPPH radical was calculated by using the following equation:

$$\%\ scavenging\ activity = [(A_0 - A_1)/A_0]x100 \quad (1)$$

Where A is the absorbance of the control and A_1 is the absorbance of the extract.

2.8.2. ABTS Cation Radical Decolorization Assay

The ABTS radical cation scavenging activity was evaluated by the method of Re *et al.* [19] with minor modifications. Before the test, the $ABTS^{\cdot+}$ was generated by a reaction between aqueous solution of ABTS (7 mM) and potassium persulfate (2.45 mM) 1:1 ratio and kept in the dark at 4 °C. Sixteen hours later the $ABTS^{\cdot+}$ solution was diluted with distilled water until an absorbance of 0.700 ± 0.002 at 734 nm was achieved. Then, in each well 160 µL of this solution was added to 40 µL of sample solution with different concentrations (0.01 to 1 mgmL^{-1}). After 10 min in the dark at room temperature, the absorbance was recorded at 734 nm against a blank. The control was prepared by replacing the sample with methanol. The $ABTS^{\cdot+}$ scavenging capability was expressed as percentage inhibition calculated using the above equation (1). BHA and BHT were used as antioxidant standards.

2.8.3. Scavenging of Superoxide Radical by Alkaline DMSO Method

Superoxide (SO) radical scavenging ability was determined by alkaline DMSO method [20]. Briefly, in each well 40 µL of extract with various concentrations was reacted with 130 µL of alkaline DMSO freshly prepared (1 mL of 5 mM NaOH completed with DMSO at 100 mL) and 30 µL of nitrotetrazolium blue chloride (0.1%). A control was prepared with reagent and methanol without extract, and the absorbance was recorded at 560 nm. The inhibition percentage of SO radical was calculated using the above formula (1). BHA and BHT were used as positive controls in the same conditions.

2.8.4. Ferric-Reducing Antioxidant Power

Ferric-reducing antioxidant power (FRAP) was determined by the previously described method of Oyaizu [21] with modifications. The reaction mixture containing 10 µL of various concentrations of plant extract, 40 µL of phosphate buffer (pH 6.6), and 50 µL of potassium ferricyanide (1%) was incubated at 50 °C for 20 min. Then, 50 µL of trichloroacetic acid (10%) was added to stop the reaction followed by 10 µL of ferric chloride (0.1%) and the microplate was agitated with microplate agitator to homogenate the mixture of green coloration. The absorbance of reaction mixture was measured at 700 nm. The sample concentration having 0.50 absorbance (A0.5) was calculated from the plot of absorbance against sample concentration. α-tocopherol, ascorbic acid, and tannic acid were used as standard antioxidants.

2.8.5. Phenanthroline Assay

Phenanthroline activity was performed according to a previously reported method with slight modifications [22]. BHA and BHT were used as standard antioxidants. 10 µL of various concentrations of extract or standard, 50 µL of 0.2% of ferric chloride, 30 µL of 0.5% of 1,10-phenanthroline in methanol, and 110 µL of methanol were placed in each well. The mixture was incubated for 20 min in the dark at 30 °C and the absorbance of an orange-red solution was recorded at 510 nm against a blank where the extract was replaced by the methanol. $A_{0.5}$ values were calculated from the absorbance curves.

2.8.6. β-carotene/Linoleic Acid Bleaching Activity

The β-carotene/linoleic acid test system was used to evaluate the inhibition of linoleic acid oxidation of extracts according to the method described earlier [23]. β-carotene solution was freshly prepared by dissolving 0.5 mg of β-carotene in 1 mL of chloroform. Then, 200 mg of Tween 40 and 25 µL of linoleic acid were added successively. The chloroform was evaporated totally from the reaction mixture in a reduced pressure. The absorbance of this solution was adjusted to 0.800-0.900 at 470 nm by the addition of approximately 50 mL of pure oxygenate water to the mixture. To 40 µL of various concentrations of extract, 160 µL of β-carotene solution was added in the dark. The absorbance was taken at 470 nm at 0 min and after incubation in 45 °C for 120 min. The control was prepared without extract. BHA and BHT were used as standard antioxidants. The percentage of antioxidant activity (AA) was calculated according to the following equation:

$$AA\,(\%) = [1 - (A_{H0} - A_{H120})/(A_{C0} - A_{C120})]x100$$

Where A_{H0} and A_{H120} are the absorbencies of the extract at t=0 and t=120 min, respectively, A_{C0} and A_{C120} are the absorbencies of the control at t=0 and t=120 min, respectively.

2.9. Acetylcholinesterase and Butyrylcholinesterase Inhibition Assay

The AChE and BChE inhibitory activities for various extracts were screened by the slightly modified spectrophotometric Ellman's assay [24]. Initially, 150 µL of sodium phosphate buffer (SPB) (100 mM, pH 8.0) was placed in each well of microplate, then 10 µL of various concentrations of extracts or galantamine as standard and 20 µL of AChE (5.32 x 10^{-3} UmL^{-1}) or BChE (6.85 x 10^{-3} UmL^{-1}) solution were added. After incubation of the mixture for 15 min at 25 °C, 10 µL of 5,5'-dithiobis[2-nitrobenzoic acid] (0.5 mM) and 10 µL of acetylthiocholine iodide (0.71 mM) or 10 µL of butyrylthiocholine chloride (0.2 mM) were added. The hydrolysis of these substrates was monitored at 412 nm at 0 min and after 15 min of incubation at 25 °C. The percentage of inhibition activity (IA) was calculated using the following formula:

$$IA\,(\%) = [(E - S)/E]x100 \quad (2)$$

Where *E*is the enzyme activity without test sample and *S* is the enzyme activity in the presence of the test sample.

2.10. Tyrosinase Inhibition Assay

Anti-tyrosinase activity was evaluated according to the method described before by Ngoc *et al.* [25]. A serial of two-fold dilution of each extract ranging between 0.062 and 4 $mgmL^{-1}$ was prepared, and 10 µL of each concentration and 20 µL of mushroom tyrosinase (150 UmL^{-1}) were added to 150 µL of SPB (50 mM, pH 6.8). After pre-incubation at 37 °C for 10 min, 20 µL of L-DOPA (0.1%) were added to each well. The 96-well microplate was incubated again at 37 °C for 10 min and the dopachrome formation in the mixture was measured at 475 nm against a blank prepared without extract. Kojic acid was used as positive control. The tyrosinase inhibition activity was calculated according to the above equation (2).

2.11. Sun Protection Factor Determination

To determine the *in vitro* sun protection effect of extracts, the method of Mansur *et al.* [26] was followed. Briefly, 200 µL of extract (dissolved in ethanol, homogenized with sonication and filtered) with a concentration of 2 $mgmL^{-1}$was placed in microplate. The absorbance of samples was measured in the wavelength range of 290-320 nm at every 5 nm interval and the solvent (ethanol) was used as blank. Nivea© and Vichy© were used in the same conditions as a standard sun protection creams to compare the photoprotective effect of extracts. Sun protection factor (SPF) value was calculated according to following equation:

$$SPF_{spectrophotometric} = CF\ x \sum_{290}^{320} EE\ (\lambda)\ x\ I(\lambda)\ x\ Abs(\lambda)$$

Where $EE(\lambda)$ is the erythemal effect spectrum; $I(\lambda)$ is the solar intensity spectrum; $Abs(\lambda)$ is the absorbance; and CF is the correction factor (= 10).

2.12. Statistical Analysis

All data are the average of triplicate analyses and results were expressed as means ± standard deviation (SD). Statistical analysis was carried out with GraphPad Prism 7 software. One-way analysis of variance was performed by ANOVA procedures, significant differences between means were determined by Tukey and Student-Newman-Keul'smultiple range tests, p values <0.05 were regarded as significant.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield and Total Bioactive Compound Contents

The yields of different extracts, TPC, TFC, TFLC, and TCTC are reported in Table **1**. The extract yields varied between 0.72 and 28.23%. The highest yield was obtained with water (28.23%), followed by methanol (15.85%), while acetate ethyl showed low yield (0.72%). On the other hand, the methanol and aqueous extracts were rich in bioactive compounds while the lowest contents were found in the hexane extract. The aqueous extract showed the highest value of TFC, TFLC, and TCTC (60.09 ± 0.01 mg of QEg^{-1} DW, 24.69 ± 0.00 QEg^{-1} DW and 101.66 ± 0.01 mg of CEg^{-1} DW, respectively), while the higher TPC was observed for the methanol extract (786.55 ± 0.00 mg $GAEg^{-1}$ DW). As a result, phenolic compounds possess very low antioxidant activities (Table **5**). Our results are in agreement with that reported in some previous studies, where the solvent with high polarity was more effective for extraction of bioactive compounds [27]. Consequently, these differences in total amounts of bioactive compounds in various extracts may be explained by the solvent polarity effect and the ability of the solvent to penetrate the plant matrix during an extraction pr-

Table 1. Yield and total phenolic, flavonoid, flavonol, and tannin contents of different extracts of *L. inermis*.

Plant Extracts	Yield (%)	TPC (mg GAEg^{-1} DW)^A	TFC (mg QEg^{-1} DW)^A	TFLC (mg QEg^{-1} DW)^A	TCTC (mg CEg^{-1} DW)^A
Hexane	1.67	7.36±0.00^a	1.64±0.00^a	ND	0.93±0.00^a
Chloroform	2.71	40.56±0.00^b	6.59±0.00^b	ND	2.84±0.02^b
Ethyl acetate	0.72	331.25±0.00^c	21.82±0.01^c	ND	38.20±0.00^c
Methanol	15.85	786.54±0.00^d	34.15±0.02^d	17.80±0.01^a	59.43±0.58^d
Aqueous	28.23	526.48±0.40^e	60.09±0.01^e	24.69±0.00^b	101.66±0.01^e

^{A} Standard deviation of the mean of three assays. $^{a-e}$ Differences within columns (samples not connected by the same letter are statistically different at $p< 0.05$ as determined by Tukey and Student-Newman-Keul'smultiple range tests).ND: not determined. mg gallic acid equivalentsg^{-1}dried weight (mg GAEg^{-1} DW), mg quercetin equivalentsg^{-1}dried weight (mg QEg^{-1} DW), mg catechin equivalentsg^{-1} dried weight (mg CEg^{-1} DW).

Table 2. Antioxidant activities of different extracts of *L. inermis*.

Plant extracts	DPPH$^·$ IC$_{50}$ (µgmL^{-1})^{B,C}	ABTS$^{·+}$ IC$_{50}$ (µgmL^{-1})^{B,C}	SO IC$_{50}$ (µgmL^{-1})^{B,C}	FRAP A$_{0.50}$ (µgmL^{-1})^{B,D}	Phenanthroline A$_{0.50}$ (µgmL^{-1})^{B,D}	β-carotene IC$_{50}$ (µgmL^{-1})^{B,C}
Hexane	>200	>200	>200	>200	>200	>800
Chloroform	140.19±0.46^a	128.54±0.73^a	161.88±0.03^a	>200	>200	560.54±2.05^a
Ethyl acetate	18.22±0.25^b	0.76±0.02^{b,e}	1.29±0.05^b	5.07±0.12^a	0.87±0.02^a	72.11±0.10^b
Methanol	7.95±0.31^c	0.29±0.01^b	1.44±0.03^c	61.99±0.95^b	3.17±0.01^b	31.29±0.14^c
Aqueous	18.32±0.12^b	7.42±0.04^c	8.89±0.10^d	9.01±0.26^c	0.84±0.08^a	20.62±0.76^d
Ascorbic acid^A	NT	NT	NT	6.77±1.15^d	NT	NT
BHA^A	6.14±0.41^d	1.81±0.10^d	86.33±8.53^e	NT	0.93±0.07^a	1.05±0.03^e
BHT^A	12.99±0.41^e	1.29±0.30^{d,e}	85.30±2.08^f	NT	2.24±0.17^c	0.91±0.01^e
α-tocopherol^A	13.02±5.17^e	NT	NT	5.39±0.91^a	NT	NT
Tannic acid^A	NT	NT	NT	34.93±2.38^e	NT	NT

^{A} Compounds used as positive control. ^{B} Standard deviation of the mean of three assays. ^{C}Concentration that shows 50% activity. ^{D} The effective concentration at which the absorbance is 0.5 for FRAP and phenanthroline. $^{a-f}$ Differences within columns (samples not connected by the same letter are statistically different at $p< 0.05$ as determined by Tukey and Student-Newman-Keul'smultiple range tests). NT: not tested.

ocess [28]. One study has established the amounts of bioactive compounds of Algerian henna seeds [29], which reported 70.64 mg GAEg^{-1} DW and 1.10 mg QEg^{-1} DW for TPC and TFC, respectively. Concerning TPC, TFC and TCTC, lower values have been reported previously in studies carried out on seeds of *L. inermis* from Tunisia (457.50 mg GAEg^{-1} DW, 199.90 QEg^{-1} DW and 28.00 CEg^{-1} DW, respectively) [30]. These high amounts of bioactive compounds found in Algerian henna may be due to diverse factors such as extraction method, genetic variability of the plant, geographical region, and the Saharan climate conditions like short growing season, high solar exposure, hot temperature, and dryness [31, 32].

3.2. Antioxidant Activities

The results of antioxidant capacities are tabulated in Table **2**. All tested extracts exhibited antioxidant activity with various effectiveness with exception of hexane extract that was inactive. Strongest scavenging effects were exhibited by the methanol extract against DPPH and ABTS radicals with an IC$_{50}$ of 7.95 ± 0.31 and 0.29 ± 0.01 µgmL^{-1}, respectively. These activities were higher than the standard antioxidants used (Table **2**). The weakest scavenging activities were recorded for the chloroform extract (IC$_{50}$ of 140.19 ± 0.46 and 128.54 ± 0.73 µgmL^{-1}, respectively), while the hexane extract was inactive. Our results are in agreement with the results obtained by Chaibi *et al.* [30] where the methanol extract of *L. inermis* showed the highest capacity both in DPPH and ABTS radical scavenging activities (IC$_{50}$ of 4.6 and 3.0 µgmL^{-1}, respectively) while the hexane and chloroform extracts were inactive. Also, Cherbi *et al.* [29], Philip *et al.* [33], and Wiem *et al.* [34] have found that the aqueous extract exhibited a strong activity comparatively to the standards for the DPPH$^·$ scavenger activity (9.90-24.00 µgmL^{-1}). The results displayed in Table **2**, showed clearly that the polar extracts ethyl acetate, methanol, and aqueous extracts were efficient and strong scavengers of SO radicals generated in DMSO alkaline system. A low IC$_{50}$ values comparatively to the standards BHA and BHT were found. To the best of our knowledge, Wiem *et al.* [34] reported the first and single previous work on SO radical scavenging activity of seeds of *L. inermis*. However, very low activity was recorded for aqueous extract (IC$_{50}$ of 274 µgmL^{-1}) compared to the present result, which can be explained by the difference in the extraction method. In the literature, phenolic acids, flavonoids, and tannins have been proven to possess DPPH, ABTS and SO radical scavenging abilities [35, 36]. Phenolic compounds have been widely studied for their ability to react with DPPH or ABTS free radicals by transfer of electron

Table 3. Enzyme inhibition activities of different extracts of *L. inermis*.

Plant Extracts	AChE IC_{50} (μgmL^{-1})[B,C]	BChE IC_{50} (μgmL^{-1})[B,C]	Tyrosinase IC_{50} (μgmL^{-1})[B,C]
Hexane	36.30±0.20[a]	188.32±1.44[a]	94.94±2.36[a]
Chloroform	30.57±1.13[b]	>200	>200
Ethyl acetate	8.84±0.15[c]	6.10±0.09[b]	5.07±0.01[b]
Methanol	70.17±0.51[d]	22.14±0.14[c]	62.00±0.03[c]
Aqueous	59.34±0.73[e]	15.93±0.08[d]	9.06±0.21[d]
Galantamine[A]	6.27±1.15[c]	34.75±1.99[e]	NT
Kojic acid[A]	NT	NT	25.23±0.78[e]

[A] Compounds used as positive control. [B] Standard deviation of the mean of three assays. [C] Concentration that shows 50% activity. [a-e] Differences within columns (samples not connected by the same letter are statistically different at $p< 0.05$ as determined by Tukey and Student-Newman-Keul'smultiple range tests). NT.: not tested.

or donation of hydrogen atom to neutralize them [37]. Furthermore, polyphenols and flavonoids have been reported to brought significant inhibition of SO radicals [38, 39]. So, the important antiradical activities observed in polar extracts like ethyl acetate, methanol, and aqueous extracts, can be related to the high amounts of total phenolics, total flavonoids, total flavonols, and total tannins. The FRAP and phenanthroline assays showed that ethyl acetate extract displayed the highest reducing power (IC_{50} of 5.07 ± 0.12 and 0.87 ± 0.02 μgmL^{-1}, respectively) which was quantitatively lower than all standards used as references, followed by the aqueous extract which also exhibited an important potential comparatively to the BHA and BHT in phenathroline assay. Methanol extract was found to be moderately active, whereas the hexane and chloroform extracts were inactive in both assays. In the study of Philip *et al.* [33], ethyl acetate extract has shown the best reducing capacity which is in agreement with the result of the present study. The β-carotene/linoleic acid bleaching results given in Table **2** indicated a moderate activity on lipid peroxidation inhibition for the tested extracts. The aqueous extract was found to be the most active one (IC_{50} of 20.62 ± 0.76 μgmL^{-1}), none of them showed better activity than the standards. The presence of antioxidants in plant extracts has been reported to inhibit the oxidation of β-carotene [40].

3.3. Acetylcholinesterase and Butyrylcholinesterase Inhibition

The results of AChE and BChE inhibition by the extracts are presented in Table **3**. All extracts showed inhibitory activities except chloroform extract which was inactive in the BChE assay. The ethyl acetate extract exhibited high anti-enzyme activities (IC_{50} of 8.84 ± 0.15 and 6.10 ± 0.09 μgmL^{-1} for the AChE and BChE inhibitory activity respectively). The low polar extracts like hexane and chloroform extracts showed moderate AChE inhibitory activity. The results recorded in the present study are in agreement with that of Chaibi *et al.* [30], where the polar extract was the most active, while the low polar extracts were revealed inactive for AChE inhibitory activity. The methanol and aqueous extracts exhibited also moderate inhibition against BChE. The differences observed in the anti-enzyme activities can be explained by the different phytochemical compositions of the plant extracts [41]. In previous studies, ethyl acetate, methanol, and water have been reported to extract molecules from plants with cholinesterase inhibitory activities [42]. Moreover, in previous study on seeds of Tunisian *L. inermis*, aqueous extract was appeared less effective against AChE (IC_{50} of 893 μgmL^{-1}) [34] compared to the result obtained in the present work, this difference might be related to the extraction technique or to the environmental conditions. A positive correlation between the TPC and AChE inhibitory activity was observed (Table **5**). This tends to suggest that *L. inermis* phenolic compounds were mainly responsible for the inhibition of AChE.

3.4. Tyrosinase Inhibitory Activity

The anti-tyrosinase capacity of all extracts was assessed in order to discover new skin-whitening agents. Based on IC_{50} presented in Table **3**, the most active extract was the ethyl acetate extract followed by the aqueous extract with IC_{50} equal to 5.07 ± 0.01 and 9.06 ± 0.21 μgmL^{-1}, respectively and lower than the IC_{50} of kojic acid, used as reference (IC_{50} of 25.23 ± 0.78 μgmL^{-1}). Methanol and hexane extracts exhibited a moderate activity, while the chloroform extract was appeared inactive. This high activity compared to the kojic acid can be associated with the presence of phenolic compounds, like flavonoids and flavonols. These classes of secondary metabolites have an inhibition mechanism very similar to kojic acid, related to the presence of their hydroxyl groups, which have the ability to inhibit dopachrome formation *via* chelating copper at the active site of enzyme by competitive way [43-45].

3.5. Sun Protection Factor

Today, the use of plant extracts in sunscreens, as sources of medicinal compounds, continues to grow, especially when they show antioxidant effect. Thus, phenolics are studied in relation to photoprotective power [46]. Indeed, the extracts of *L. inermis* with higher phenolic contents were tested for this power. The results recorded in the current study showed the capacity of all extracts to absorb UV radiations and hence demonstrated its UV protection ability. The calculated SPF given in Table **4** ranged between 2.23 ± 0.37

Table 4. Sun protection factor of different extracts of *L. inermis*.

Plant Extracts	SPF[B]
Hexane	2.23±0.37[a]
Chloroform	25.97±0.60[b]
Ethyl acetate	38.24±0.05[c]
Methanol	43.05±0.37[d]
Aqueous	42.53±0.66[d]
Nivea ©[A]	50.11±0.53[e]
Vichy ©[A]	44.22±0.35[f]

[A] Standard sun protection creams. [B] Standard deviation of the mean of three assays. [a-f] Differences within columns (samples not connected by the same letter are statistically different at $p< 0.05$ as determined by Tukey and Student-Newman-Keul'smultiple range tests).

Table 5. Correlation between TPC, TFC, TFLC, TCTC and biological activities.

SPF	Tyrosinase	BChE	AChE	β-carotene	Phenanthroline	FRAP	SO	$ABTS^{+}$	$DPPH^{\cdot}$	TCTC	TFLC	TFC	TPC	Assays	
0.82	-0.30	-0.67	0.67	-0.69	0.44	0.46	-0.67	-0.67	-0.70	0.80	-0.28	0.77	1.00	TPC	Correlation R^2
0.78	-0.60	-0.66	0.58	-0.63	-0.13	-0.08	-0.57	-0.56	-0.58	1.00	0.39	1.00	0.77	TFC	
-0.01	-0.50	-0.03	-0.16	-0.02	-0.87	-0.83	0.04	0.05	0.07	0.35	1.00	0.39	-0.28	TFLC	
0.78	-0.58	-0.65	0.57	-0.69	-0.11	-0.07	-0.63	-0.63	-0.64	1.00	0.35	1.00	0.80	TCTC	
-0.39	0.15	0.24	-0.32	1.00	-0.08	-0.08	1.00	1.00	1.00	-0.64	0.07	-0.58	-0.70	$DPPH^{\cdot}$	
-0.38	0.18	0.25	-0.26	0.99	-0.03	-0.03	1.00	1.00	1.00	-0.63	0.05	-0.56	-0.67	$ABTS^{+}$	
-0.38	0.19	0.25	-0.26	0.99	-0.02	-0.02	1.00	1.00	1.00	-0.63	0.04	-0.57	-0.67	SO	
0.07	0.60	0.06	0.65	-0.03	0.99	1.00	-0.02	-0.03	-0.08	-0.07	-0.83	-0.08	0.46	FRAP	
0.06	0.60	0.06	0.60	-0.03	1.00	0.99	-0.02	-0.03	-0.08	-0.11	-0.87	-0.13	0.44	Phenanthroline	
-0.39	0.18	0.24	-0.35	1.00	-0.03	-0.03	0.99	0.99	1.00	-0.69	-0.02	-0.63	-0.69	β-carotene	
0.31	0.25	-0.10	1.00	-0.35	0.60	0.65	-0.26	-0.26	-0.32	0.57	-0.16	0.58	0.67	AChE	
-0.97	0.84	1.00	-0.10	0.24	0.06	0.06	0.25	0.25	0.24	-0.65	-0.03	-0.66	-0.67	BChE	
-0.75	1.00	0.84	0.25	0.18	0.60	0.60	0.19	0.18	0.15	-0.58	-0.50	-0.60	-0.30	Tyrosinase	
1.00	-0.75	-0.97	0.31	-0.39	0.06	0.07	-0.38	-0.38	-0.39	0.78	-0.01	0.78	0.82	SPF	

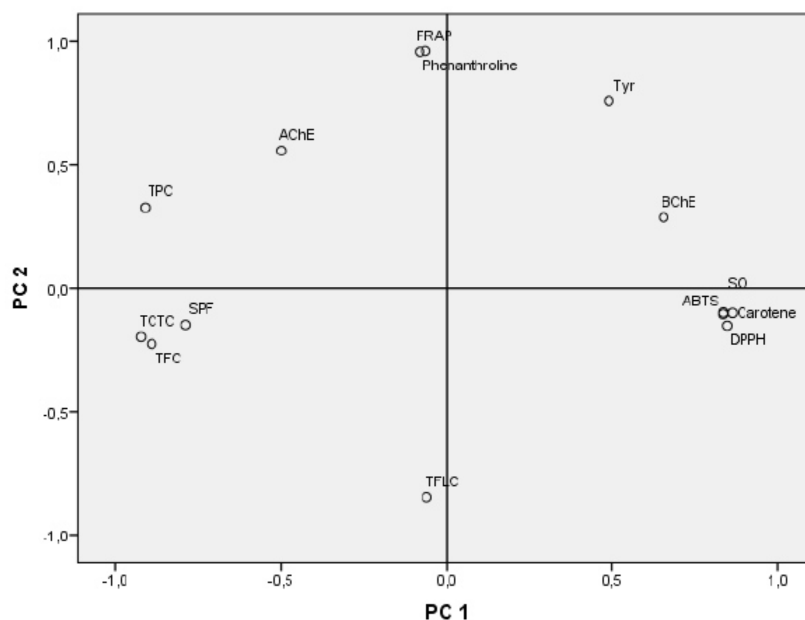

Fig. (1). PCA of TPC, TFC, TFLC, TCTC and biological activities.

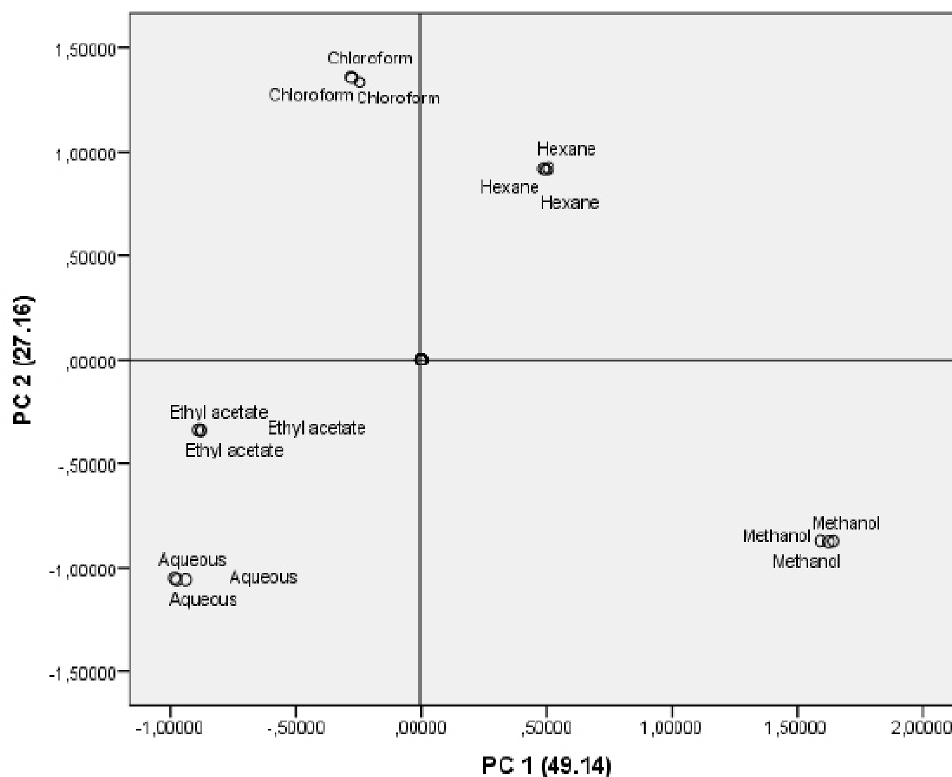

Fig. (2). PCAscore plot obtained from the TPC, TFC, TFLC, TCTC and biological activities.

and 43.05 ± 0.37; where the methanolic extract (SPF of 43.05 ± 0.37) exhibited the most sun protection effect followed by aqueous extract (SPF of 42.53 ± 0.66). This photoprotective activity is considered interesting compared to sunscreen protectors Nivea© and Vichy©. It has been reported that values of SPF ranging between 30-50 are considered as having high sun protective activity [47]. In particular, it has also been studied that high SPF values are correlated to phenolic contents, like flavonoids [48]. Univariate analysis (Table **5**) showed a high positive correlation between the TFC and photoprotective activity. This suggests that flavonoids were mainly responsible for observed activity.

3.6. Statistical Observations

After univariate analysis (Table **5**), the data were also further studied by using multivariate analysis tools. Firstly, principal component analysis (PCA) was performed to obtain an overview on relation between phenolic compounds (TPC, TFC, TFLC, and TCTC) and biological activities (Fig. **1**). On the basis of PCA approach there was good correlation between phenolic compounds, except TFLC. Regarding correlation between biological activities, high correlation was observed between FRAP and phenanthroline, also between all radical scavenging activities and inhibition of β-carotene bleaching activity. Concerning enzyme inhibition activities, high correlation was observed between BChE and tyrosinase inhibition activities. Additionally, a negative correlation between the AChE and BChE enzyme inhibitory activities was shown. In the PCAscore plot, the first two principal factors showed 76.3% of the total variance, signifying that the first two components could cover maximum information of the total data (Fig. **2**). A distinct separation of extract according to solvents was observed. As presented in (Fig. **2**), methanol extract showed a high DPPH, ABTS and SO radical scavenging and inhibition of β-carotene bleaching activities, which is in accordance with the observations reported from Table **2** after statistical analysis obtained by Tukey and Student-Newman-Keul'smultiple range tests. The same figure showed that the ethyl acetate and aqueous extracts demonstrated higher phenolic contents (TPC, TFC and TCTC) and photoprotective activity. Finally, the statistical analysis significantly showed the influence of solvents on *L.inermis* biological activities.

CONCLUSION

The results showed that the polar extracts from seeds of the Algerian *L. inermis* were a rich source of biomolecules with interesting biological activities. From this study, it was also seen that the extraction methods were efficient in extracting molecules from the plant matrix in comparison with other studies. The seeds of this plant rich in phenolics could be used as natural alternative in pharmaceutical, cosmetic, and food fields for improving the antiradical defenses, treatment of AD, and protection of skin. Further studies to iso-

late and characterize the active constituents and to identify their precise mechanism of action are required.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article are available in the [Supplementary material] at [https://drive.google.com/file/d/1Igl1UhLzld_Sksjnvy9eKsYCy2xt-pCMT/view?usp=sharing].

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None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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