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Total phenols, flavonoid content and antioxidant activity of seeds extracts of *Lawsonia alba* (henna) from Algeria

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ABSTRACT

Lawsonia alba (Henna) is widely used in folk medicine for treatment of various skin diseases such as Eczema (atopic dermatitis), boils and sores. The aim of the present study is to determine the antioxidant activity, total phenolics and flavonoids content of extracts from the seeds of Lawsonia alba grown in Algeria and selected from three different regions (Adrar, Biskra, and Ouargla). Total phenolics content ranged from 70.64 ± 0.42 to 87.88 ± 0.15 mg gallic acid equivalents (GAE)/g dry weight, the flavonoids content varied from 0.847 ± 0.00 to 1.10 ± 0.006 mg quercetin equivalents (QE)/ g dry weight. The antioxidant activities of the extracts were evaluated by DPPH and phosphomolybdenum assays. The results showed that all extracts from the seeds of Lawsonia alba seem to be good trappers of radicals, the IC50 values of the extracts ranged between 0.0084 and 0.0099 mg/mL. Total antioxidant capacity of the extracts decreased in the following order: SEA >SEB> SEO.

Keywords*:* Antioxidant activity, *Lawsonia alba*, Phenolic compounds, Flavonoids, Seeds.

INTRODUCTION

Reactive oxygen species (ROS) and nitrogen species (RNS) such as nitric oxide, singlet oxygen, hydrogen peroxide, superoxide, hydroxyl and peroxyl free radicals are produced in cells by the mitochondrial respiratory chain and known to play an obvious role in a wide variety of pathological manifestations [1-4]. Free radicals are very reactive with proteins, lipids, enzymes, carbohydrates and DNA and can cause cellular damage [5]. Oxidative stress resulting in the appearance of the free radicals and causing diseases in human organism like cancer, atherosclerosis, hypertension, atherogenesis, inflammatory injury, diabetes, atherogenesis, Alzheimers and Parkinson [6-8].

Medicinal plants are a main source of natural antioxidants, such as polyphenols (flavonoids, anthocyanins, tannins and phenolic acids), ascorbic acid, tocopherols, tocotrienols, cinnamic acids, benzoic acids, folic acid, selenium, ß-carotene, lycopene, lutein, and other carotenoids [9].

Antioxidants have capacity to scavenge the free radicals and reactive oxygen species and protect us from various diseases in man such as atherosclerosis, diabetes mellitus, arthritis, ischemia heart disease, gastritis, neurodegenerative diseases, ageing and cancer [2, 5].

As a medicinal plant, *Lawsonia alba* (Lythraceae), commonly called henna in North Africa, is widely used in traditional medicine for treatment of various skin diseases such as Eczema (atopic dermatitis), boils and sores [10, 11]. Seeds, leaves, stem bark, roots and flowers of *L. alba* have been reported to contain various compounds

including aromatic compounds, saponins, triterpenoids, dioxin derivatives [12],β-sitosterolglucosides, alkaloids, flavonoids, tannins, steroids, glycosides, quinonoids, naphthalene derivatives, luteolin, betulin, lupeol, garlic acid, coumarins, xanthones, lawsone and phenolic glycosides [13, 14].

Lawsonia alba exhibits a variety of biological activities such as hepatoprotective [15], antimicrobial [16], antifungal [17], antiviral [18], antiparasitic [19], antitrypanosomal, antimalarial [20], hypoglycemic [21], anti-inflammatory, analgesic and antipyretic [22, 23], tuberculostatic [24], anticomplementary, antidermatophytic, cytotoxic [25, 26], antioxidant [27], immunomodulatory [28], protein glycation inhibitory [29], trypsin inhibitory [30], antitumor [31], nootropics [32], Wound Healing Activity [33] and anticorrosion properties [34].

The objective of the present work is to evaluate the content of phenolic and flavonoid compounds of *Lawsonia alba*'s seed extract from three regions of Algeria, and also to examine their antioxidant activity by the DPPH$^{\bullet}$ and reducing power (PPM) assays compared to that of BHA, BHT, VE, VC standards.

MATERIALS AND METHODS

2.1. Chemicals and solvents

Ascorbic acid (VC), 1,1-diphenyl,2-picryl hydrazyl (DPPH), gallic acid, quercetin, Butylated Hydroxy Toluene (BHT), Butylated hydroxyanisole (BHA), α-tocopherol (Vitamin E) (VE), sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), sulphuric acid, sodium phosphate, ammonium molybdate, acetone, ethanol, methanol, ethyl acetate, Folin–Ciocalteu reagent. All chemicals were purchased from Sigma-Aldrich.

2.2. Collection of plant material

The seeds of *Lawsonia alba* was collected in October 2011 from the following regions in Algeria: Adrar (A), Biskra (B) and Ouargla (O). Seeds were dried under the shade at room temperature. The dried seeds were ground using kitchen blender to obtain the course powder and stored in the dark at a dry place until further use.

2.3. Preparation of plant extracts

25 g of seeds of *Lawsonia alba* were macerated at room temperature with Acetone/H_2O (70:30, v/v) for 24 h, three times. After filtration, the filtrate was evaporated till dryness and the residue was dissolved in water and partitioned using ethyl acetate for three times, then the ethyl acetate was concentrated under reduced pressure and re-dissolved with minimum of methanol and kept at 4°C.

2.3.1. Total phenolic content

Total phenol was estimated by the Folin–Ciocalteu method [35]. Briefly, 0.1 mL of ethyl acetate extract was added to 1.5 mL of Folin–Ciocalteu reagent. The mixture was incubated in the dark for 5 min and then 1.5 mL of sodium carbonate 6% was added. After 90 min in the dark, the absorbance was measured at 725 nm. Total phenolic content was calculated using a gallic acid and standard curve (30-300 µg/mL). Results were presented as milligrams of gallic acid equivalents per gram of plant dry weight (mg GAE/g DW).

2.3.2. Total flavonoid content

The total flavonoid content of each seed extract was determined by the aluminium chloride method colorimetric [36]. Briefly, 1 mL of 2% $AlCl_3$ ethanol solution was added to 1 mL of extract. The absorbance was read at 430 nm after 30 min of incubation at room temperature. Total flavonoids were estimated based on a standard calibration curve (concentration range: 5 - 50 µg/mL). Results were presented in milligrams of quercetin equivalents per gram of plant dry weight (mg QE/g DW).

2.4. Determining antioxidant activity

2.4.1. Free radical scavenging activity on DPPH$^{\bullet}$

The free radical scavenging activity of the seeds extracts of *Lawsonia alba* was determined by using DPPH method [37]. Aliquots (1mL) of different concentrations of seeds extract were added to 1mL of a 250 µM ethanol solution of DPPH. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm. The percentage of inhibition (I%) of free radical DPPH was calculated using the formula:

$$\text{Free radical scavenging activity (\%)} = [(A_0 - A_S)/ A_0] \times 100$$

where:
A_0 is the absorbance of the negative control.
A_S is the absorbance of the sample.

DPPH scavenging capacity is expressed as an IC50 value. IC50 is the concentration of the sample which possesses 50% inhibition of the free radicals present in the test solution. BHT, BHA, Vitamin E and Ascorbic acid were used as a reference compounds.

2.4.2. Phosphomolybdenum assay (Total antioxidant capacity) (TAC)

The total antioxidant capacity of extracts was estimated using the assay of a green phosphate/Mo (V) complex [38]. An aliquot 0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated for 90 min in a boiling water bath at 95°C. After the samples had cooled to room temperature, the absorbance was read at 695 nm. The antioxidant capacities were expressed as equivalents of gram Ascorbic acid (AEAC). BHA, Vitamin E and Gallic acid were used as a positive control.

2.5. Statistical Analysis

Data are reported as mean of three determinations. Origin (version 6.0) was used for graph plotting. The IC50 value was determined by linear regression.

RESULTS AND DISCUSSION

3.1. Total phenolics and flavonoid content

Phenolic compounds are widely distributed in nature, including flavonoids, phenolic acids and tannins. The total phenolics and flavonoid content of the extracts obtained from *L. alba* seeds are shown in Table 1 and Figure 1.

Total phenolic content of seed extract was estimated by Folin–Ciocalteu reagent (FCR), the results are expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW) (the standard curve equation: y = -0.0034 + 3.3321x, R = 0.999). Total phenolic content of the different extracts of *L. alba* seeds were ranging from 70.64 ± 0.42 and 87.88 ± 0.15mg/g GAE/g DW. The SO extract exhibited the highest total phenolic content (87.88 mg/g) compared to SA (84.14 mg/g) and SB (70.64 mg/g) extracts.

Total flavonoid content was determined using spectrophotometric method with aluminum chloride. The content of flavonoid expressed as quercetin equivalents per gram dry weight (mg QE/g DW) (the standard curve equation: y = 0.02517x + 35.3660, R = 0.999), varied from 0.847 ± 0.00 to 1.10 ± 0.006 mg QE/g DW. The highest amount of flavonoid contents was determined for seeds extract (B), followed by seeds extract (A) and seeds extract (O) with the values 1.10, 1.045 and 0.847 mg GAE/g, respectively.

Table 1: Total phenolic and flavonoid contents

Extract	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
Seeds extract A	84.14 ± 1.20	1.045 ± 0.0035
Seeds extract B	70.64 ± 0.42	01.10 ± 0.006
Seeds extract O	87.88 ± 0.15	0.847 ± 0.00

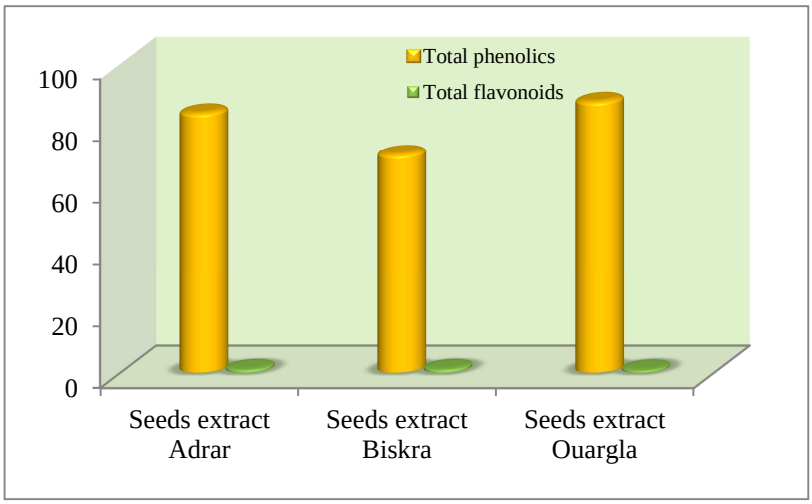

Figure 1: Total phenols and flavonoids contents in seeds extracts of *Lawsonia alba*

Phenolic compounds such as flavonoids, phenolic acids and tannins are known as antioxidants. Many antioxidant substances have important biological properties, such as anti-oxidative, anti-allergic, antibiotic, hypoglycaemic, anti-inflammatory, antiatherosclerotic and anti-carcinogenic [39].

Flavonoids are polyphenolic compounds accounting for majority of secondary metabolites of plants. They have long been recognized as possessing anti-inflammatory. antiallergic. Antimicrobial, anthelminthic, hepatoprotective, antihormonal, antithrombotic, antiviral activities. Moreover, they are able to inhibit cancer cell growth and lipid peroxidation. These activities might be related to their antioxidant activity [40, 41].

The phenolics or polyphenols can prevent injury caused by free radicals by chelating redox-active metal ions, inactivating lipid free radical chains, and avoiding the hydroperoxide conversions into reactive oxyradicals [41, 42].

3.2. Antioxidant capacity

3.2.1. Free radical scavenging activity on DPPH$^{\bullet}$

The free radical scavenging activity was evaluated by DPPH method. DPPH is very stable free radical and has been widely used for studying the free radical scavenging activity of several kinds of antioxidants [43].

The parameter used to measure the radical scavenging activity of extract evaluated is IC50 value. defined as the amount of antioxidant required for 50% scavenging of DPPH radicals [44]. Therefore a lower IC50 value indicates strong antioxidant activity [45]. IC50 values were calculated from the linear regression of the % inhibition versus extracts concentrations.

The results of the free radical scavenging activity are shown in Table 2 and graphically represented in Figure 2. Inhibition percentage of the seeds extracts increased with increasing concentration. The IC50 value for SO extract was 0.0084 mg/mL, which was comparatively lower than the IC50 of ascorbic acid (0.00957 mg/mL), BHA (0.0086 mg/mL), BHT (0.1 mg/mL) and of α-tocopherol (0.0188 mg/mL).

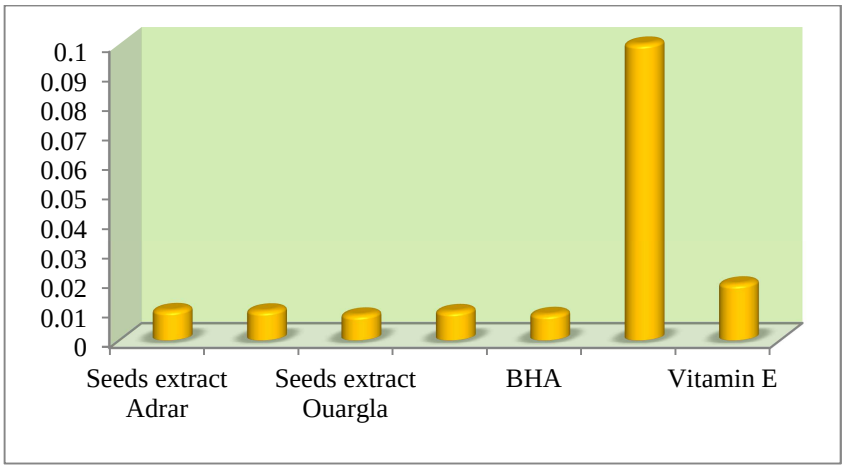

Figure 2: DPPH$^{\bullet}$ radical scavenging activity of *L. alba* seeds extracts

The IC50 values expressed in mg/mL of seed extracts and standards were in the following order: SOE > BHA > Vit C > SBE > SAE > αTC > BHT. The IC50 < 1 mg/mL indicates that the seeds possess significant antioxidant activity [46].

The results of this study suggest that the seeds extracts of *Lawsonia alba* have a strong antioxidant activity. Antioxidants fight against free radicals and prevent us from various diseases.

In the literature, the inhibition of DPPH radical scavenging by plant extracts can be attributed to the phenolic compounds (phenolic acids, flavonoids and tannins). The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations [47].

DPPH scavenging effect of seeds extracts (antioxidants) may be attributed to the phytochemical constituents with radical scavenging potential such as phenolic compounds, flavonoids and tannins, which can donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and to form nonradical products, resulting in the inhibition of propagating phase of lipid peroxidation according to the following equation [48, 49]:

$$ArOH + R^{\bullet} = ArO^{\bullet} + RH$$

Where:
$R^{\bullet}$ is a free radical.
Ar is an antioxidant

Antioxidant activity of phenolic compounds (phenolic acids, flavonoids and tannins) depends on the presence of free hydroxyl groups, especially 3-OH and 5-OH [3, 50].

Table 2: reducing power and DPPH scavenging

Extract	AEAC (mg/mL)	DPPH (IC50 in mg/mL)
Seeds extract A	1.62 ± 0.058	0.0099 ± 0.0003
Seeds extract B	1.37 ± 0.0497	0.00978 ± 0.0004
Seeds extract O	1.066 ± 0.0387	0.0084 ± 0.0001
Ascorbic acid	-	0.00957 ± 0.0001
BHA	1.64 ± 0.059	0.0086 ± 0.0001
BHT	-	0.1 ± 0.01
Vitamin E	1.135 ± 0.04	0.0188 ± 0.0008
Gallic acid	3.78 ± 0.137	-

3.2.2. Phosphomolybdenum assay

The antioxidant activity of the seeds extract was evaluated by the phosphomolybdenum method according to the procedure describe by Prieto et al. (1999). The assay was based on the reduction of Mo (VI) to Mo (V) by the extract (antioxidant compounds) and the formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695 nm. Higher absorbance indicates a higher total antioxidant capacity [45]. Total antioxidant capacity of seeds *Lawsonia alba* extracts, expressed as the number of gram equivalents of ascorbic acid (AEAC), are summarized is shown in Table 2.

The SA extract of *L. alba* showed higher phosphomolybdenum reduction (1.62 ± 0.058 mg/mL) followed by SB extract (1.37 ± 0.0497 mg/mL) and SO extract (1.066 ± 0.0387 mg/mL), respectively Figure 3. In brief, the antioxidative ability of the seeds extracts and standards decreased in the following order: Gallic acid > BHA > SA extract > SB extract > Vitamin E > SO extract.

According to the results of the antioxidant capacities, the seeds extracts were able to inhibit the Mo complex, and the phytochemical analysis showed the factors which are found in the form of secondary metabolites were responsible for antioxidant activity, this may be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants [51].

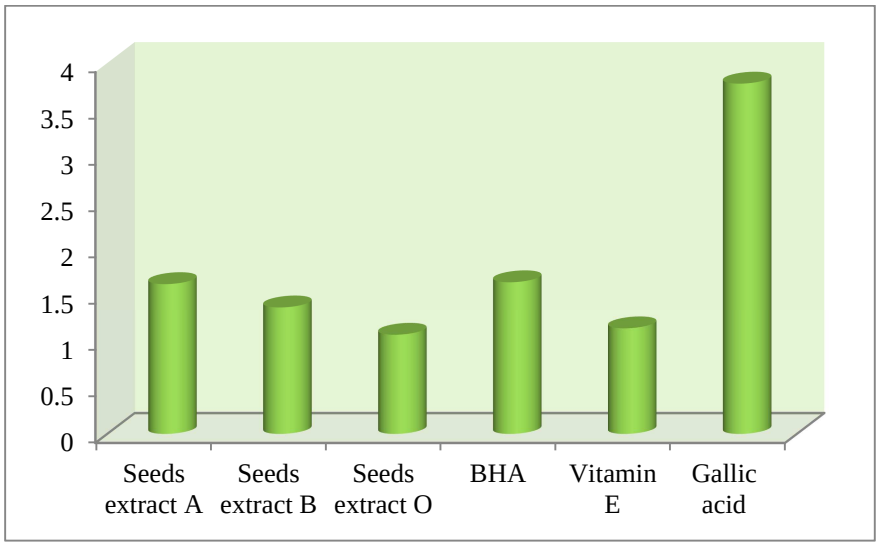

Fig 3: Total antioxidant activity of *L. alba* seeds extracts

CONCLUSION

According to our result, seeds extract of *Lawsonia alba* is an excellent source of polyphenols and flavonoids, which are natural antioxidants that can be used as a medicine for the treatment of different chronic diseases.

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