Total phenolic, total flavonoids contents and antioxidant and antibacterial activity of seeds and leaves extracts of Lawsonia alba from Algeria

R. Cherbi[1,*], A. Ouakkaf[2], S. Djaadi[3], F. Chelgham[1], Y. Benkrima[4]

[1] Laboratory for Valorization and Promotion of Saharan Resources (VPRS), Kasdi Merbah University, Ouargla - 30000, Algeria,

[2]Department of Matter Sciences, University Mohamed Khider 07000 Biskra , Algeria.

[3]Développement des énergies nouvelles et renouvelables dans les zones arides et sahariennes, LENREZA, P.O. Box 511, Ouargla 30 000, Algeria

[4]Ecole Normale Supérieure de Ouargla, 30000 Ouargla, Algeria

Received: 04/07/2023; Accepted: 25/10/2023; Published: 30/10/2023

Abstract

Lawsonia alba (Henna) is widely used in folkloric medicinal for a 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 and leaves of Lawsonia alba grown in Algeria and selected from two different regions (Adrar and Biskra). Total phenolics content ranged from 27.48 to 90.60mg gallic acid equivalents (GAE)/g dry weight, the flavonoids content varied from 1.457 to 6.267 mg quercetin equivalents (Q)/ g dry weight. The antioxidant activities of the extracts were evaluated by DPPH assay and potassium ferricyanide complex as reducing power assay. The results showed that all extracts from the seeds and leaves of Lawsonia alba seem to be good trappers of radicals, the IC50 values of the extracts ranged between 0.0019 and 0.014 g/l. All extracts showed very good activity of ferric reducing power.

The antibacterial activities of Lawsonia alba seeds and leaves extracts, determined by disk diffusion method (zone of inhibition), were compared to antibiotics (TM, CS, OXA, VA, C, AMX and AMC). The pathogenic bacterial strains used were Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (MTCC 424).

The antibacterial assay of the extracts revealed no inhibition zones with the Gram-negative bacteria tested. However, the extracts demonstrated activity against *S. aureus*. The zones of inhibition due to the extracts ranged from 9.5 – 17.5 mm.

Keywords: - Antioxidant activity; Antibacterial activity; Lawsonia alba; phenolic compounds; seeds; leaves.

Tob Regul Sci. ™ 2023;9(2): 510-525
DOI: doi.org/10.18001/TRS.9.2.33

1.Introduction

Medicinal plants represent the main source of natural bioactive compounds. Their use as a remedy for treating or preventing several illnesses has been known since antiquity.

In spite of the efforts of chemists (synthesizing new molecules), more than 50% of medicine prescribed in developed countries derive directly or indirectly from plants [1].

Lawsonia alba is a medicinal plant belonging to the Lythraceae family, commonly called "henna" by local people. It self-seeds and is widespread in north Africa and particularly in Algeria.

Lawsonia alba (henna) is well known as a colourant both for skin and hair but is less well known for its anti fungal and anhidrotic properties. Its use is therefore very varied.

Its medicinal properties are due, among other factors, to its tannin content. Henna can be taken orally with no known undesirable effects. It is used widely for its astringent, antiseptic and healing properties. As a poultice, it can be used to treat eczema, fungal infections, boils, abscesses, whitlows, chapped skin, inflammation, pains from sprains or fractures. It is also possible to treat burns and some haemorrhages and to encourage wounds to heal. As an infusion it helps combat ulcers, diarrhoea and renal stones. It can also be used an eye lotion in ophthalmic treatment [2-4].

Several studies have demonstrated the importance of phenolic compounds (flavonoids and tannins) as a source of anti-oxidation and chelation of free radicals; these latter being the origin of several cardiovascular illnesses and the development of several cancers [5].

In spite of the biological and medicinal importance of Lawsonia alba, this species has not been deeply studied. To date there has been no research on the constituents of the fruit of this plant.

The aim of the present study of the present study is to evaluate the phytochemical, content of phenolic, and flavonoid compounds of seeds and leaves extracts from Lawsonia alba from the tow regions of Algeria, and to examine their antioxidant activity (DPPH• and reducing power assays) compared to that of BHA, BHT, Gallic acid and VC standards, and to estimate their antibacterial effects against reference pathogenic strains: Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aerugenosa (P. aerugenosa), compared to that of seven antibiotics (TM, CS, OXA, VA, C, AMX and AMC).

2. 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 (Na2CO3), FeCl3,trichloroacetic acid, K3Fe(CN)6, aluminium chloride (AlCl3), ammonium sulphate, orthophosphoric acid, methanol, ethyl acetate, Folin–Ciocalteu reagent. All chemicals were purchased from Sigma-Aldrich.

2.2. Collection of plant material

The seeds and leaves of Lawsonia alba were harvested in the month of October 2011 from the regions of Zrebat Hamed (Biskra) and in the region of Bordj Badji Mokhtar (Adrar). Seeds and leaves were dried under the shade at room temperature. The dried seeds and leaves were ground using kitchen blender to obtain the course powder and stored in the dark at a dry place until further use.

2.3. Determining phytochemical compounds

2.3.1. Phytochemical screening of Lawsonia alba seeds

Phytochemical screening of Lawsonia alba seeds were tested for their presence or absence of alkaloids, saponins, glycosides, phenolic compounds, flavonoids, tannins, sterols and terpenoids, steroids and proteins according to methods described by N. Raaman (2006) [6].

2.4. Preparation of plant extracts

25 g of seeds and leaves of Lawsonia alba were macerated at room temperature with Methanol/H2O (80:20, 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 in the presence of an aqueous solution containing 20% ammonium sulphate and 2% of orthophosphoric acid solution. Then the ethyl acetate was concentrated under reduced pressure and re-dissolved with minimum of methanol and kept at 4°C [7].

2.4.1. Total phenolic content

Total phenol was estimated by the Folin–Ciocalteu method [8]. Briefly, 100µl of ethyl acetate extract was added to 500µl of Folin–Ciocalteu reagent (freshly prepared dilute (1:10)). The mixture was incubated in the dark for 5 min and then 2 mL of sodium carbonate 20% was added. After 30 min in the dark, the absorbance was measured at 760 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).

2.4.2. Total flavonoid content

The total flavonoid content of each extract was determined by the aluminium chloride method colorimetric [9]. Briefly, 1 mL of 10% (w/v) $AlCl_3$ in ethanol solution was added to 1 mL of extract and 1 ml of (0.1 N) sodium acetate solution. The absorbance was read at 410 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).

2.5. Determining antioxidant activity

2.5.1. Free radical scavenging activity on DPPH•

The free radical scavenging activity of the seeds and leaves extracts of Lawsonia alba was determined by using DPPH method [10, 11]. Aliquots (1mL) of different concentrations of seeds and leaves extract diluted in Tris buffer solution (100 mM; pH 7.4) were added to 1mL of a 500 mM 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:

Free radical scavenging activity (%) = [(A0 - AS)/ A0] × 100

Where:

A0 is the absorbance of the negative control.

AS 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 and Ascorbic acid were used as a reference compounds.

2.5.2. Reducing Power Assay

Reducing power of the seeds and leaves extracts was determined by the method of Oyaizu [12]. Different concentrations of the sample (1 ml) were mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The resulting solutions were incubated at 50°C for 20 minutes. After incubation, the reaction mixture mixed with 2.5 ml of 10% TCA. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v). The absorbance was measured at 700 nm, using ascorbic acid as a positive control. Increased absorbance of reaction mixture indicated reducing power. Reducing power was expressed mM as ascorbic acid equivalents antioxidant capacity (AEAC).

2.6. Antibacterial activity

2.6.1 Microorganisms

The antibacterial activity was evaluated using Three bacteria (reference strains) including one Gram-positive (Staphylococcus aureus ATCC 25923) and two Gram-negative (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) were used. The bacterial strains were provided by Institute Pasteur, Algiers. Bacterial strains were maintained by subculture on nutrient agar favorable to their growth for 24 h in the dark at 37 °C.

2.6. 2. Antibiotics

Standard antibiotics Tobramycin (TM) (10 µg/disk), Colistin (CS) (10 µg/disk), Oxacillin (OXA) (1 µg/disk), Vancomycin (VA) (30 µg/disk), Chloramphenical (C) (30 µg/disk), Amoxicillin (AMX) (20 µg/disk) and Amoxicillin + Clavulanic acid (AMC) (20/10 µg/disk) were used.

2.6.3. Antibacterial activity (assay disk)

The antibacterial activity of the seeds extracts was (determined) investigated by the disk diffusion method [13]. The Mueller– Hinton agar was poured in sterile petri dishes (90 mm diameter). The sterile filter paper discs (Whatman N° 3 disk 6-mm diameter) that were impregnated with 10 µL of each extracts (5, 10 and 15 mg/mL) (diluted with DMSO) were placed on the inoculated agar surface. Petri dishes were allowed to stand for 30 min at room temperature before incubation at 37 °C for 24 h. The DMSO solvent was used as the negative control. The effect of extracts was reflected by the appearance around disc with a transparent circular zone corresponding to the absence of growth. The diameter of inhibition zone was measured in mm. The obtained results were compared with the antibiotics tested on the same strains and by the same method. The larger the diameter of the inhibition zones of the growth bacteria the more susceptible the strain.

2.7. Statistical Analysis

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

3. Results and discussion

3.1. Phytochemical screening of Lawsonia alba seeds

The results of phytochemical screening indicate the presence of various classes of bioactive chemical constituents including phenolic compounds, tannins, flavonoids and glycosides. Whereas absence of alkaloids, terpenoids, proteins, steroids and sterols in all seeds and leaves Table 1. However, the presence of saponins in leaves. These results agree with those obtained by

other authors whose phytochemical analysis gave positive results for the flavonoids and tannins and negative results for alkaloids and steroids [14, 15].

Table 1: Preliminary phytochemical screening of seeds extracts of Lawsonia alba

	Seeds of Adrar	Seeds of BIskra	Leaves of Adrar	leaves of BIskra
Phenolic compounds and Tannins	+	+	+	+
Glycosides	+	+	+	+
Saponins	-	-	+	+
Flavonoids	+	+	+	+
Alkaloids	-	-	-	-
Terpenoids	-	-	-	-
Steroids and Sterols	-	-	-	-
Proteins	-	-	-	-

3.2. Total phenolics and flavonoid content

The levels of total phenolic compounds in L. alba seeds and leaves extract determined by Folin-Ciocalteu method. The total phenolic content of the extracts was expressed as mg gallic acid equivalent per gram of the plant dry weight. Table 2 lists the results obtained from total polyphenols and flavonoids measurements for each seeds and leaves extract. The total phenolic content of seeds extract was significantly higher than the leaves extract.

The concentrations of phenols in the seeds and leaves extract range from 27.48 to 90.60 mg GA/g.

The results indicated that the seeds extract Biskra (90.60) had highest total phenolic compounds, followed by seeds extract Adrar (70.38). While leaves extract are with small amounts of phenols 46.75 and 27.48 of Biskra and Adrar, respectively.

The obtained results showed a high concentration of phenolic compounds in the samples of seeds, corroborating the numerous literature data that report high levels of these compounds in the seeds [16].

The high amounts of phenolic compounds of the seeds and leaves extract were as follows; seeds extract Biskra > seeds extract Adrar > leaves extract Biskra > leaves extract Adrar (Fig 1).

The content of total flavonoid of the different L. alba seeds and leaves extract measured spectrophotometrically by using the aluminium chloride colorimetric assay is shown in Table 2. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the plant dry weight. The concentrations of flavonoids in seeds and leaves extract range from 1.457 to 6.267 mg QE /g.

The amounts of total flavonoids found in leaves extract Biskra were 6.267 mg QE /g, 3.215 mg QE /g for seeds extract Biskra and 2.093 mg QE /g and 1.457 mg QE /g for leaves extract Adrar and seeds extract Adrar, respectively.

The results indicated that the seeds and leaves extract Biskra have highest total flavonoids (Fig1).

Plants are important source of bioactive secondary metabolites such as phenols, phenolic acid, flavonoids, tannins, saponins, alkaloids, essential oils. They are known to reduce the risk and progression of diseases such as cancer, cardiovascular, neurodegenerative diseases and many others. These potential biological activities include antioxidant, antibacterial, antiviral, anti inflammatory anti-artherogenic, and anti-allergenic activities [17-19].

Table 2: Total phenolic, flavonoid contents

Extraits	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
Seeds extract Adrar	70.38	1.457
Leaves extract Adrar	27.48	2.093
Seeds extract Biskra	90.60	3.215
Leaves extract Biskra	46.75	6.267

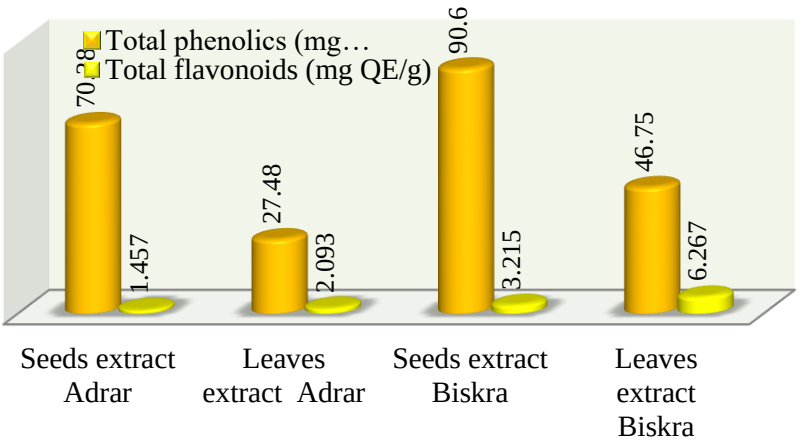

Figure 1: Total phenols and flavonoids contents in seeds and leaves extracts of Lawsonia alba

Table 3: reducing power and DPPH scavenging

Extraits	DPPH (IC50 in µg/mL)	Reducing power (mM)
Seeds extract Adrar	0.0075	452,68
Leaves extract Adrar	0.0019	66,199
Seeds extract Biskra	0.009	861,9
Leaves extract Biskra	0.014	294,99
Acide ascorbique	0.019	-
BHT	0.022	0.751
BHA	0.021	0.556
Gallic acid	-	1.122

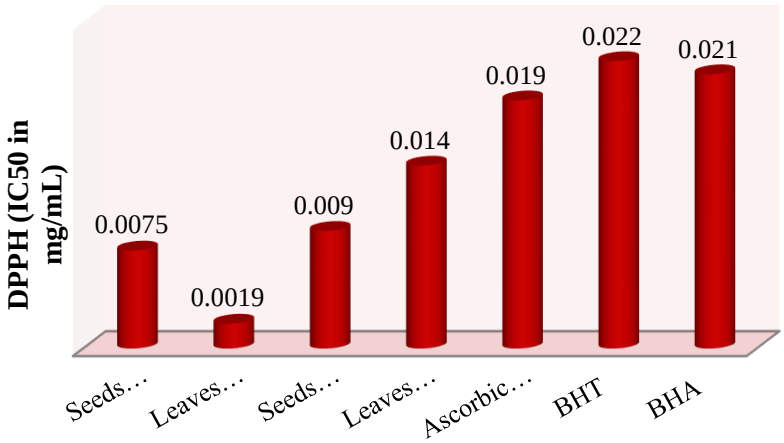

Figure 2: DPPH• radical scavenging activity of L. alba seeds and leaves extracts

3.3. Radical-scavenging activities on a,a-diphenyl-bpicrylhydrazyl (DPPH.)

Free radical scavenging capacity

DPPH is a stable free radical widely used to evaluate the antioxidant activity of a plant extracts.

The free radical-scavenging activities of Lawsonia alba seeds and leaves extracts with the reference standards such as ascorbic acid, BHT and BHA were determined by the DPPH. Method and the results are shown in Table 3 and Fig 2. A lower value of IC50 indicates a higher ability of the antioxidant to trap the radical.

The antioxidant activity of seeds and leaves extracts increased with an increase in their concentrations.

The leaves extract Adrar had the strongest (highest) radical scavenging activity with the lowest IC50 value of 0.0019mg/mL. This was higher than the seeds extract Adrar with an IC50 value of 0.0075mg/mL, seeds extract Biskra with an IC50 value of 0.009mg/mL and leaves extract Biskra with an IC50 value of 0.014mg/mL. In addition, DPPH scavenging abilities of all investigated extracts were higher than that of the standards (ascorbic acid with an IC50 value of 0.019mg/mL, BHA with an IC50 value of 0.021mg/mL and BHT with an IC50 value of 0.022mg/mL). The results indicated that seeds and leaves extract demonstrated significant antioxidant effects.

In the present study, the order of scavenging activity of extracts and standards were as follows: leaves extract Adrar > seeds extract Adrar > seeds extract Biskra > leaves extract Biskra > ascorbic acid > BHA > BHT.

This suggested that seeds and leaves extract contain compounds such as phenolic compounds that can donate electron/hydrogen easily. Numerous studies indicated that this phenolic compounds (phenolic acid, flavonoids and tannins) were a potent antioxidant agent [20].

The antioxidant activity of seeds and leaves extract of Lawsonia alba could be attributed to its relatively high content of the molecular structure of flavonoids and differences in number and position of the hydroxyl group on the aromatic ring [20].

Phenolic compounds including flavonoids (flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones), are very important antioxidant agents with scavenging of free radicals such as hydroxyl, superoxide, and peroxyl or primary oxidants, chelate pro-oxidant metals (mainly iron), inactivate microbial activity, cell envelope transport proteins and modify the activity of enzymes, reducing lowdensity lipoproteins in plasma [19, 21-24].

Phenolic compounds (phenolic acids, flavonoids and tannins) are aromatic secondary metabolites their antioxidant activity depends on the number and position of the hydroxyl groups in the

molecules. They are can donate hydrogen atoms from their hydroxyl groups to radicals and produce stable phenoxyl radicals [17, 23, 25].

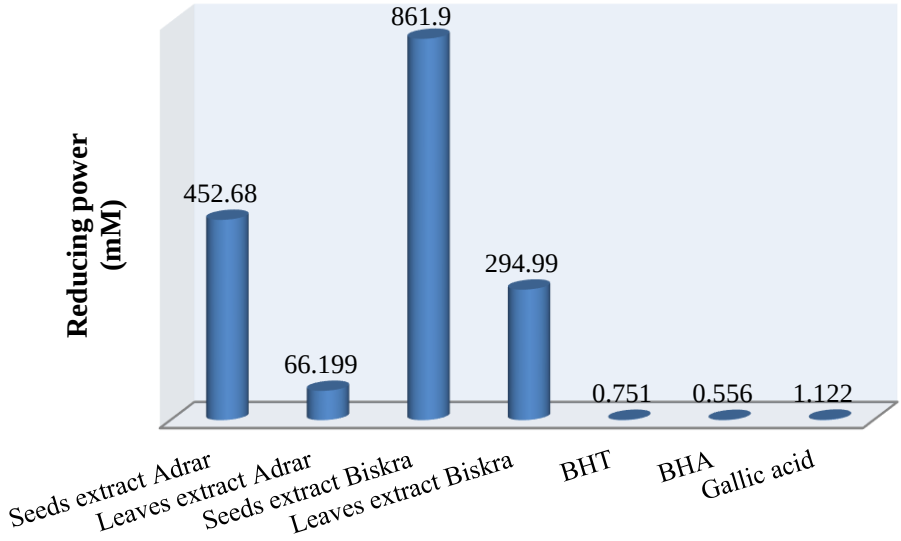

Figure 3: Reducing power activities of seeds and leaves extracts of Lawsona alba

3.4. Reducing power

The extracts obtained were determined for their antioxidant activities. The extracts were investigated for the reducing ability by using the potassium ferricyanide reduction method.

The reducing capacity of a plant extracts may serve as indicator of its potential antioxidant activity.

High values of AEAC indicated a stronger reducing power.

The AEAC value for reducing power by L. alba seeds and leaves extracts are summarized in Table 3 and Fig 3.

The reducing power of seeds and leaves extracts increased with an increase in their concentrations.

In general, the seeds extract of Biskra showed the highest activity (861,9), followed by the seeds extract of Adrar (452,68) and leaves extract of Biskra (294,99). The lowest reducing power was found in the leaves extract of Adrar (66,199). When compared to the Gallic acid (1.122), BHT (0.751), and BHA (0.556). The results indicated that seeds and leaves extract demonstrated significant reducing effect.

Reducing power of extracts increased in the order of seeds extract Biskra > seeds extract Adrar > leaves extract Biskra > leaves extract Adrar > Gallic acid > BHT > BHA.

Secondary metabolites, including phenolics, flavonoids and tannins have antioxidant activity due to their redox properties and chemical structures [21].

3.5. Antibacterial assay

The disc diffusion method was used to determine the inhibition zones of the different extracts from seeds and leaves samples. The antibacterial activity of the seeds and leaves extracts of *Lawsonia alba* were studied in different concentrations (5, 10 and 15 mg/mL) against three pathogenic bacterial strains, one Gram-positive (*Staphylococcus aureus* MTCC 25923) and two Gram-negative (*Escherichia coli* MTCC 25922, *Pseudomonas aeruginosa* MTCC 27853).

Antibacterial potential of seeds and leaves extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Figure 4–7.

The antibacterial activities of the extracts increased with increase in concentration of seeds and leaves extract (mg/ml). All seeds extracts from the two regions showed antibacterial activity against S. aureus. As compared with standard antibiotic drugs, the results revealed that in the extracts for bacterial activity, S. aureus was shows good result as compare with E. coli and P. aeruginosa. The growth inhibition zone measured ranged from 9.5 to 17.5 mm for all the sensitive bacteria (Figures 4–7). The antibacterial activity of seeds extract of Biskra and seeds and leaves extract of Adrar showed the activity against S. aureus and didn't show any inhibitory activity against P. aeruginosa and E. coli.

The seeds extract of Biskra was shown to have the most effective antibacterial activity against S. aureus (17.5mm at 15 mg/ml). However, the leave and seed extracts from Adrar exhibited showed moderate inhibitory activity against S. aureus (12mm at 15mg/ml and 11.4mm at 15mg/ml, respectively). Among the four extracts, only leaves extract of Biskra showed no activity against all microorganisms tested.

The results of standard antibiotics sensitivity test on pathogens were given in Figure 3. Among the four extracts tested, the seeds and leaves extract of Adrar and seeds extract of Biskra exhibited significant antibacterial activity when compared with the standard antibiotics tested against S. aureus.

Antibacterial activity of seeds and leaves extract is reported to be due to the phenolic compounds, polysaccharides, flavonoids, tannins (condensed tannins and hydrolyzable tannins), aromatic acids, and its esters [26, 27].

The seeds and leaves extracts of Lawsonia alba had a highly significant antibacterial activity for Gram-positive bacteria comparatively to Gram-negative bacteria. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts [28].

Table 4. Antimicrobial activity of the extracts of Lawsonia alba leaves and seeds against the tested microorganisms based on disc diffusion method.

Plant extract	Zone of inhibition; mm								
	E. coli			S. aureus			P. aerogenosa		
	5 (mg/ml)	10 (mg/ml)	15 (mg/ml)	5 (mg/ml)	10 (mg/ml)	15 (mg/ml)	5 (mg/ml)	10 (mg/ml)	15 (mg/ml)
Seeds extract Adrar	NA	NA	NA	9.5	11	11.4	NA	NA	NA
Leaves extract Adrar	NA	NA	NA	NA	9.1	12	NA	NA	NA
Seeds extract Biskra	NA	NA	NA	11.5	14.9	17.5	NA	NA	NA
Leaves extract Biskra	NA	NA	NA	NA	NA	NA	NA	NA	NA
TM	ND			ND			21,5		
CS	ND			ND			16		
OXA	ND			15			ND		
VA	ND			20			ND		
C	ND			24			ND		
AMX	23			ND			ND		
AMC	22			ND			ND		

NA: No Activity observed

ND: No Determined

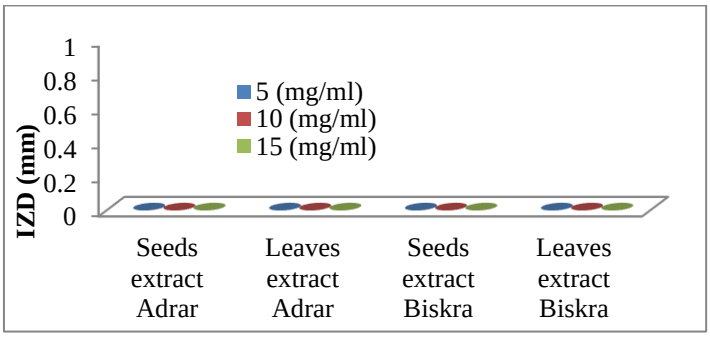

Figure 4: Antibacterial activity from seeds and leaves extracts of Lawsonia alba against E. coli (ATTCC25922)

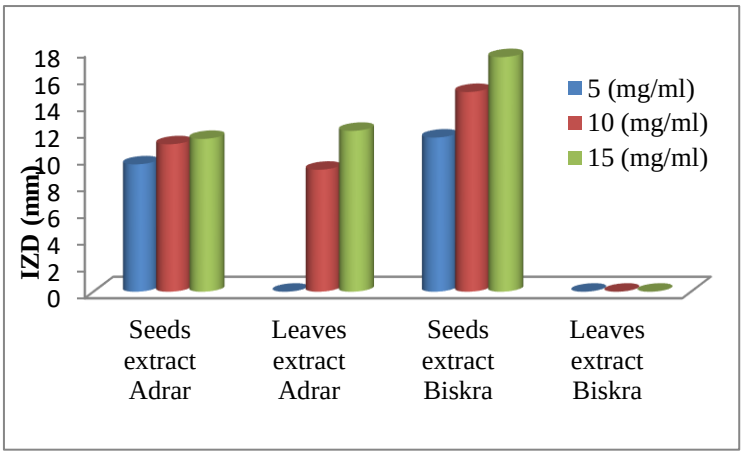

Figure 5: Antibacterial activity from seeds and leaves extracts of Lawsonia alba against S. aureus (ATTCC 25923)

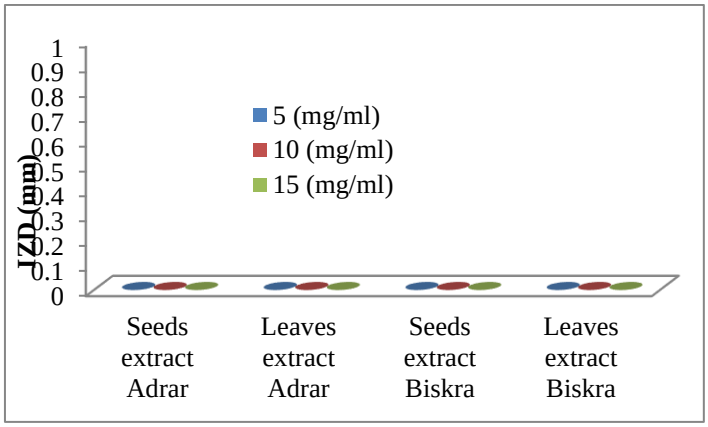

Figure 6: Antibacterial activity from seeds and leaves extracts of Lawsonia alba against P. aerogenosa (ATTCC 27853)

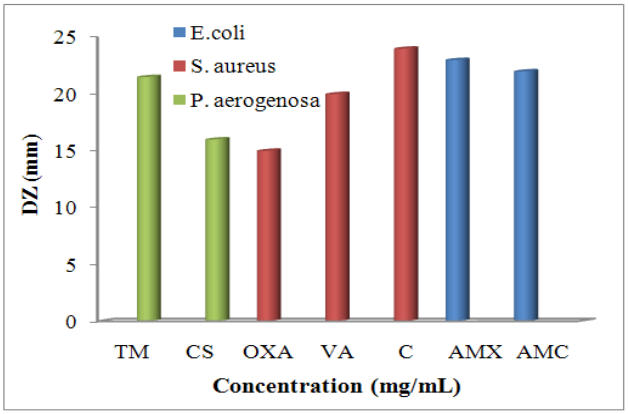

Figure 7: Antibacterial activity from standard antibiotic drugs against E. coli (ATTCC25922), S. aureus (ATTCC 25923) and P. aerogenosa (ATTCC 27853)

4. Conclusion

The results of preliminary phytochemical screening suggest that both seeds and leaves of L. alba are good sources of beneficial phytochemicals. The present investigation revealed that the seeds and leaves contain significant amount of phenols and flavonoids. Our results suggest that L. alba is a potential source of antioxidant and antibacterial agents against S. aureus and could be used as a natural antioxidant. Further work is needed towards isolation and identification of the structure of active principles (phenolics and flavonoids) component of the plant extracts and treatment of infectious diseases.

5. Referance

1. Gurib-Fakim, A., Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine, 2006. 27(1): p. 1-93.
2. Inder K. Makhija, D.D.R., Vijay Kumar S., Raviraj Devkar, Devang Khamar, Nishant Manglani et Sandeep Chandrakar Lawsonia inermis From Traditional Use to Scientific Assessment. African Journal of Pharmaceutical Sciences and Pharmacy, 2011. 2(1): p. 145-165.
3. Chaudhary, S.G., Priyanka Poonia, Lawsonia inermis Linnaeus: A Phytopharmacological Review Gagandeep. International Journal of Pharmaceutical Sciences and Drug Research, 2010. 2(2): p. 91-98.
4. Rekia CHERBI, M.Y., Mokhtar SAIDI and Mahdi BELGUIDOUM, Total phenols, flavonoid content and antioxidant activity of seeds extracts of Lawsonia alba (henna) from Algeria. Der Pharma Chemica, 2016. 8(11): p. 216-221.
5. Anthony Ojeil, N.E.D., Youssef El Hajj, Paulette Bou Mouncef, Toufic J. Rizk et Richard G. Maroun, Identification et caractérisation fonctionnelle de composés phénoliques extraits du raisin château KSARA. Lebanese Science Journal 2010. 11(2): p. 117-131.
6. Raaman, N., Phytochemical Techniques2006: New India Publishing Agency.
7. Djeridane A, Y.M., Nadjemi B, Boutassouna D, Stocker P, Vidal N Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006. 97: p. 654–660.
8. Singleton VL, R.J., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic, 1965. 16: p. 144–158.
9. Chang CC, Y.M., Wen HM, Chern JC Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal, 2002. 10: p. 178–182.
10. Brand-williams W, C.M., Berset C Use of a free radical method to evaluate antioxidant activity. LWT, 1995. 28: p. 25–30.
11. Djeridane A, Y.M., Nadjemi B, Maamrim S, Djireb F, Stocker P Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase. J Enzym Inhib Med Chem, 2006. 21: p. 719–726.

12. M, O., Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr, 1986. 44: p. 307–315.
13. Lesueur D, et al., Chemical composition and antibacterial activity of the essential oil of Michelia faveolata Meryll ex Dandy from Vietnam. Flavour Fragr. J, 2007. 22: p. 317-321.
14. Philip, J.P., G. Madhumitha, and S.A. Mary, Free radical scavenging and reducing power of Lawsonia inermis L. seeds. Asian Pacific Journal of Tropical Medicine, 2011. 4(6): p. 457-461.
15. Tadesse Eguale and M. Giday, In vitro anthelmintic activity of three medicinal plants against Haemonchus contortus. International Journal of Green Pharmacy, 2009
16. Philip, J.P., G. Madhumitha, et al., Free radical scavenging and reducing power of Lawsonia inermis L. seeds." Asian Pacific Journal of Tropical Medicine, 2011. 4(6): p. 457-461.
17. Bajalan, I., et al., Antioxidant activity and total phenolic and flavonoid content of the extract and chemical composition of the essential oil of Eremostachys laciniata collected from Zagros. Asian Pacific Journal of Tropical Biomedicine, 2017. 7(2): p. 144-146.
18. Chigayo, K., et al., Phytochemical and antioxidant properties of different solvent extracts of Kirkia wilmsii tubers. Asian Pacific Journal of Tropical Biomedicine, 2016. 6(12): p. 1037-1043.
19. Shahidi, F. and P. Ambigaipalan, Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. Journal of functional foods, 2015. 2015 v.18: p. pp. 820-897.
20. Bourgou, S., et al., Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots. Comptes Rendus Biologies, 2008. 331(1): p. 48-55.
21. Subedi, L., et al., Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. Journal of Traditional Chinese Medicine, 2014. 34(5): p. 584-590.
22. Alabri, T., et al., Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L. Vol. 26. 2014. 237-243.
23. Gawlik-Dziki, D.K.M.Ś.J.C.U., The phenolic content and antioxidant activity of the aqueous and hydroalcoholic extracts of hops and their pellets J. Inst. Brew, 2013. 119(3): p. 103-110.
24. Iuliana Spiridon, R.B., Carmen-Alice Teaca, Total phenolic content and antioxidant activity of plants used in traditional Romanian herbal medicine. Cent. Eur. J. Biol, 2012. 6(3): p. 388-396.
25. Shahidi, F. and P. Ambigaipalan, Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. Vol. 18. 2015.
26. Choi, Y.M., et al., Antioxidant and antimicrobial activities of propolis from several regions of Korea. LWT - Food Science and Technology, 2006. 39(7): p. 756-761.

27. Fadi Qa'dan, et al., The Antimicrobial Activities of Psidium guajava and Juglans regia Leaf Extracts to Acne-Developing Organisms. The American Journal of Chinese Medicine, 2005. 33(2): p. 197–204.
28. Bipul Biswas, et al., Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria. International Journal of Microbiology, 2013 2013 (7).