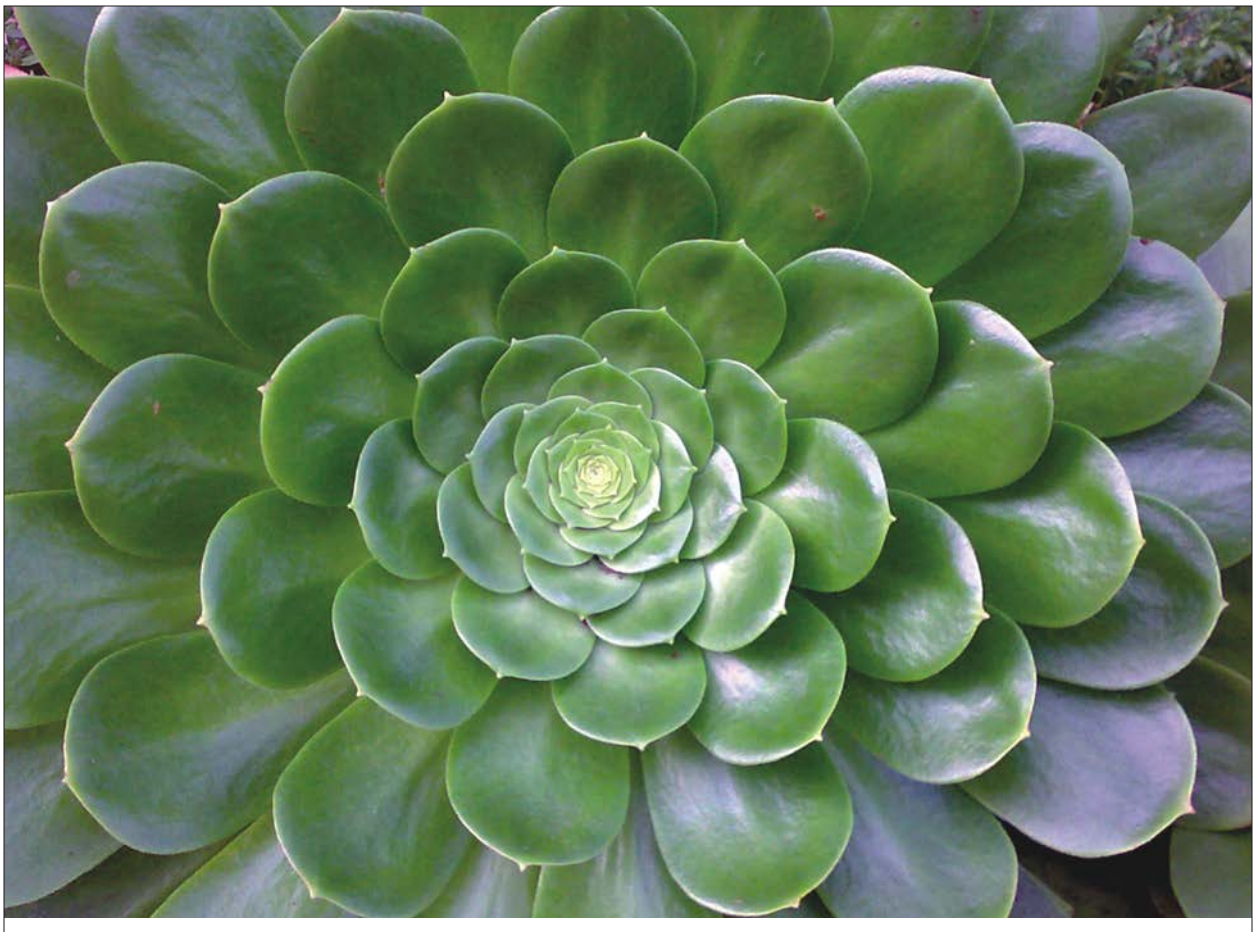

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Research Article
Antimicrobial Activity of *Lawsonia inermis* Leaf Extract Collected from South of Algeria Touat (Adrar) and Tidikelt (In Salah)

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Abstract

Background and Objectives: The leaves of *Lawsonia inermis* (henna) are widely used as agent to have antimicrobial properties. It is now considered as a valuable source of natural products with pharmaceutical properties. In this study, an attempt was made to find out the antibacterial potential of *Lawsonia inermis* leaves from south of Algeria, commonly used in traditional medicine to treat various ailments. **Materials and Methods:** Cold maceration method was used for the extraction of the natural components from the leaves of the plant grown in two different regions. Different concentrations of ethanolic and aqueous extracts have been prepared for the antimicrobial activity (extract). The antimicrobial activity was performed against the different strains. **Results:** The results indicated that the yield of crude extracts of henna plant from Tidikelt is more active than Touat's plant. Its antibacterial potential displayed more antimicrobial activity as compared to Touat extracts. TiEth extracts have shown excellent antimicrobial potency against the majority of strains tested even with moderate concentrations of extracts (1.5 mg mL^{-1}). Aqueous extract does show a low activity against the majority of strains including: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC25922 and *Salmonella enteritidis* ATCC 2453 at all concentrations tested. *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 were sensitive toward of *Lawsonia inermis's* leaf extract, while others strains show activity average. **Conclusion:** The present study helped in identifying the phytochemical constituents present in the extract and its fractions which are responsible for various biological and antibacterial activities.

Key words: *Lawsonia inermis*, ethanolic extract, antimicrobial activity, inhibition zone, aqueous extract, plants, optimization

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Since time immemorial plants have been used in almost all cultures as a source of medicine and beauty. The herbal remedies have become more popular in the treatment of minor ailments. They are often cheaper, locally available, easily consumable (raw) and has simple medicinal preparations[1]. The secondary metabolites of these plants have been considered to be an essential source of bioactive principles because of their extensive biological activities and antioxidant properties[2]. Given its geographical and climatic situation, the southern part of Algeria has a long history of traditional treatment based on using plants as an alternative for medical drugs. Among the most used plants in the world and precisely in the southern part of Algeria are *Lawsonia inermis*[3-5]. It is a small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. Henna is a natural red colouring agent, used to dye skin and hair[6,7] and as tattooing agent in various civilizations and cultures. *L. inermis* contains high number of phenolic antioxidants such as lawsone, flavonoids, tannins, coumarins, sterols, gallic acid, cyclic derivatives of benzene and mannitol[1] molecules to treat rheumatoid arthritis, headache, ulcers, diarrhea, leprosy, fever, leucorrhoea, diabetes, cardiac disease and colouring agent to food additives[3,8,9]. Therefore, the objective of this study was to enhance the plant *L. inermis* from the Algerian Northern Sahara. Firstly, through a phytochemical analysis of the extracts from the leaves using different ethanolic and aqueous solvents of two samples originating from the Touat (Adrar) and Tidikelt (In Salah) regions in order to find the antimicrobial activity and antifungal of these natural extracts against certain pathogenic strains.

MATERIALS AND METHODS

Plant material: The leaves of *L. inermis* were collected from two different regions from southern Algeria. The first sample was collected during December, 2017, from the palm grove of Beni Ouazel, commune of Touat, Adrar. The second sample was collected in the Ingher palm grove, Tidikelt region, In Salah, during the month of January, 2018. The plant material was identified and authenticated at the level of the herbarium room, Department of Botany at the Ecole Nationale des Sciences Agronomique-El Harrach Alger (No. 38). The harvested plant material was dried away from moisture and light and then powdered with a crusher and stored in a brown bottle until use.

Extraction of crude drug: Ethanolic compounds extraction and fractionation was carried out according to Al-Edany *et al.*[10] and Basli *et al.*[11]. A total of 100 g of *L. inermis* dried leaves powder was macerated in 70% ethanol (Scharlau®, Spain) for 48 h at room temperature. The extracts obtained were filtered under vacuum and the various filtrates were concentrated to dryness by evaporation in a rotary evaporator (Heidolph instruments, Germany). The residues obtained were stored at 4°C. Various concentrations were prepared out using different ethanolic and aqueous solvents of two samples originating in the Touat region and another from the Tidikelt. Extracts obtained of the two regions were subjected to preliminary antimicrobial investigation.

Determination of phenolic compounds

Determination of total phenol content: The total phenol content of both extracts (ethanolic and aqueous) was determined spectrophotometrically (with a maximum absorption at 760 nm) using the Folin-Ciocalteu reagent, a method adopted by Qusti *et al.*[12] and Waterhouse *et al.*[13]. The technique consists of mixing 0.2 mL of each diluted extract with 1 mL of Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na_2CO_3. After 30 min of incubation, the absorbance was measured. The standard curve was drawn. The results were expressed in milligrams equivalent gallic acid per gram dry weight of henna (EAG mg g^{-1}).

Determination of total flavonoids: The determination of total flavonoids was made by spectrophotometry (with a absorption at 510 nm) according to the method[14,15]. About 0.5 mL of each extract diluted with 1.5 mL of distilled water was mixed with 0.3 mL of 5% $NaNO_2$. Three milliliter of 10% $AlCl_3$ was added 5 min later. Afterward, 1 mL of 4% NaOH was added. Catechin was used as the standard for the calibration curve. The total flavonoid contents were expressed in mg catechin equivalent/g dry matter (mg EC g^{-1} MS).

Determination of tannins: About 0.1-0.5 mL of extract of the leaves of *L. inermis* were added to 3 mL of the vanillin/methanol solution of a 4% (w/v) and then vortexed. About 1.5 mL hydrochloric acid was added with stirring. Absorbance was measured at 550 nm after 20 min incubation[14]. The calibration curve was prepared under the same conditions using catechin as standard and the results were expressed in mg catechin equivalent/g dry matter (mg EC g^{-1} MS).

Microorganisms and media

Microbial strains: In order to assess the antimicrobial potential of *L. inermis* extracts, reference strains provided by LAMAABE laboratory were used in this study. These pathogenic species represent various infection sources. Evaluation of the antimicrobial activity was performed against four strains Gram positive, namely *Bacillus cereus* ATCC 11778 (*B. cereus*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Enterococcus faecalis* ATCC 29212 (*E. faecalis*) and *Listeria monocytogenes* ATCC 19115 (*L. monocytogenes*). Four Gram negative strains which *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Escherichia coli* ATCC25922 (*E. coli*), *Salmonella enteritidis* ATCC 2453 (*S. enteritidis*) and *Klebsiella pneumonia* (*K. pneumoniae*) ATCC 700603. And one fungal strains (yeast), *Candida albicans* ATCC 10231 (*C. albicans*).

Determination of antimicrobial activity: Antibacterial activity was determined by cup-plate agar diffusion method based on the recommendations of the Cos *et al.*[16], NCCLS[17]. In this, different dilutions of extracts were prepared. The disc diffusion methodology yields a quantitative result. The plates were inoculated by microorganisms subsequently bores were made in the solidified agar plate by using a sterile borer. About 6.00, 4.50, 3,00 and 1.50 mg mL^{-1} concentration of ethanolic and aqueous extracts was added in the bore and the plates were incubated. The activity evaluation was thus determined by measurement of the inhibitory zone diameter after incubation at 37°C during 24 h using vernier caliper[18,19]. Ciprofloxacin (CIP) and gentamicin (GEN) were used as positive controls. The choice of these drugs was based on the broad range of antibiotic activity with respect to the strains used[20]. Antifungal activity against *Candida albicans* ATCC 10231 was measured according to a standardized disc diffusion method for yeasts. Amphotericin B (AMB) was used as positive control[21,22].

MIC determination: To estimate the minimal inhibitory concentration (MIC), the broth microdilution method was using. The extracts were subjected to an evaluation based on the recommendations of the NCCLS. Series of dilutions of the plant extracts on DMSO were prepared. All tests were performed in triplicate and the data points constitute the mean of three replicates[16,23].

Statistical analysis: The results are given as Mean±SD. The statistical evaluation was carried out using the StatView software with an ANOVA analysis followed by the t-test. The value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Determination of polyphenolic compounds: The filtrates obtained showed a distinct difference of dark green color for extraction with ethanol and orange for extraction with distilled water. The extraction yield was estimated in relation to the weight of the crude extract and the mass of dry vegetable matter. It is expressed as a percentage and calculated according to the following equation:

$$\text{RE}\ (\%) = \frac{\text{PBE} - \text{PBV}}{\text{PP}} \times 100$$

where, RE is the extraction yield in percentage, BE is the weight of cans after drying which contains the crude extract (g), PBV is the weight of empty cans in gram and PP is the weight of dried plants (g).

Yield calculations show that the ethanol extract of henna from Tidikelt gave a good yield with a 29.08%, the Touat sample of the same solvent gave a reduced yield of 13.70%. The yield of the aqueous extract is less important, it is 26.68% for the Tidikelt sample and 24.76% for Touat. The result of polyphenols content is given in Table 1.

Flavonoid content: EthTi and AqTi were the richest in flavonoids with levels of 26.81±0.30 and 20.86±0.79 mg EC g^{-1}, respectively, whereas the EthTo was only 14.49±0.42 mg EC g^{-1}. The result of flavonoid content is given in Table 1.

Table 1: Secondary metabolites contents of leaves of *L. inermis* extracts

Extracts	Polyphenols (mg EAG g^{-1})	Flavonoids (mg EC g^{-1})	Tannins (mg EQ g^{-1})
Aqueous Touat (AqTo)	30.20±0.31	13.04±0.30	04.80±0.03
Ethanol Touat (EthTo)	48.99±0.55	14.49±0.42	03.20±0.12
Aqueous Tidikelt (AqTi)	76.89±0.69	20.86±0.79	08.46±0.27
Ethanol Tidikelt (EthTi)	96.89±0.22	26.81±0.30	03.46±0.18

Tannins content: AqTi and AqTo contain significant amounts of tannins, which were respectively 08.46±0.27 and 04.80±0.03 mg EQ g^{-1}, greater than those of Eth Ti and Eth To with 03.46±0.18 and 03.20±0.12 mg EQ g^{-1}, respectively. The result of tannins content is given in Table 1.

Microorganisms and media

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MIC determination: To estimate the minimal inhibitory concentration (MIC), the broth microdilution method was using. The extracts were subjected to an evaluation based on the recommendations of the NCCLS. Series of dilutions of the plant extracts on DMSO were prepared. All tests were performed in triplicate and the data points constitute the mean of three replicates[16,23].

Plants have always been considered a vital source of potentially useful products for the development of new therapeutic agents. Extraction of plants is a crucial step for the isolation and identification of active ingredients from the plant[24].

Tidikelt and Touat regions are known for their hot desert and hyper-arid climate (Köppen climate classification BWh). The coldest month is January (12-16°C) and the hottest is July (48.9°C). Precipitation range is 0 (on July), 3.6 mm (on October), with a annual precipitation of 10 mm. Oases have a microclimate specific in relation with the water presence and the vegetation. During the summer, the Sahara region of Algeria is the source of a scorching, sometimes dusty and southerly wind called the Sirocco[25].

The results of the different yields obtained from this case study demonstrate that the best yield was collected with the ethanol solvent from the Tidikelt sample (29.08%), followed by the aqueous solvent of the same sample (26.68%). The henna plant from the Touat region gave the lowest yield in terms of the aqueous extract. This is probably due to the geographical origin of the plant; it is an extrinsic factor that directly influences its development, the quantitative and qualitative composition as well as the raw component yield. Every plant species has requirements vis-à-vis the climate in which it evolves. These result in a certain number of thermal-thermal needs for the fulfillment of its development and the necessity of water for its growth. The moisture compensation factor can play a central role in maintaining vegetation cover. Drought can also significantly influence the plant's performance and can lead to major constraints in its functioning, particularly on its development, morphological, physiological and metabolic changes. Studies have also shown that drought can affect the growth of plant organs differently, which can lead to alteration of plant morphology[3,26,27].

Nevertheless, it is difficult to compare these results with those cited within the text, because the yield is relative and seems to be related not only to the geographical origin of our samples but also to the conditions of conduct of cultures, the duration of storage harvesting and the applied extraction methods. Hossain and Rahman[14] argue that henna is a plant that not only fears the presence of too frequent winds but is still sensitive to both strong sunstroke and shade, to the point of developing well, that in areas protected from the wind and insulation too brutal[14]. Plants under palms have minimal

development compared to that with a sufficiently sunny plant. In this study, this difference in yield between the extracts by the chemical components which differ from one extract to another. Numerous studies have shown the influence of composition chimique of extracts and different extraction conditions such as time, extraction temperature and solvent polarity on the extraction yields[28]. Extraction and quantification methods can also influence the estimation of the whole phenol content[24]. Alcoholic solvents are usually used to extract phenolics from natural sources where they gave quite a high yield of the total extract. The phytochemical study of extracts shows the presence of phenol, flavonoids and tannins. The activity antimicrobial may be due to these compounds. The phytochemical analyses realised, have revealed a high content of polyphenols in all extracts. Flavonoids represent more than 25% of the leaf polyphenols of TiEh (Table 1). The herbal plants such as henna (*L. inermis*) contains a high amount of flavanol and phenolic acid, also known as antioxidants[2,3,29].

In this study, various ethanolic and aqueous leaf extracts of *L. inermis* were assayed by agar disc diffusion method against: *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC25922, *Salmonella enteritidis* ATCC 2453, *Klebsiella pneumonia* ATCC 700603 and one fungal strains, *Candida albicans* ATCC 10231. Some work indicates that the primary screening for the antimicrobial activity must be initiated by the aqueous crude and ethanolic extract, followed by less polar solvents[30]. These extracts have efficiently proven their potential to inhibit microbial growth. Enneb *et al.*[3] report that almost all the active components identified from plants for their antimicrobial activity are part of the aromatic compounds or saturated organic compounds and that they are obtained by an initial extraction with ethanol or methanol[3]. According to the scale of the antimicrobial activity estimate, a bacterial strain is considered to be resistant to antibacterial agents when the inhibition diameter is <10 mm and >6 mm[31]. This leads us to deduce that the strains studied in this work revealed a notable antibacterial activity of plant extracts against the bacteria tested.

Among all the extracts, ethanolic demonstrates good activity. The results expo that Tidikelt extracts displayed more antimicrobial activity as compared to Touat extracts. And among all the extracts tested, ethanolic reveal good activity. TiEth extracts have unfold excellent antimicrobial potency against the majority of strains tested even with low concentrations of extract (1.5 mg mL^{-1} is sufficient to obtain an antimicrobial effect). The aqueous extract does show a moderate activity against the majority of strains, including *P. aeruginosa* ATCC 27853, *E. coli* ATCC25922 and *S. enteritidis* ATCC 2453 at all concentrations tested. *B. cereus* ATCC 11778, *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 were sensitive toward of *L. inermis* leaf extract while others strains show activity average.

The data of the antimicrobial screening exposed a various degree of activity between the different extracts (ethanolic and aqueous) of Tidikelt. They showed fascinating MIC against all strains used, especially for ethanol extracts (Table 2 and 3). In this study ethanolic extracts have high activity against *B. cereus*, *S. aureus*, *C. albicans* and a moderate activity against *K. pneumonia* ATCC 700603, *E. faecalis* ATCC 29212, *L. monocytogenes* ATCC 19115 and *E. coli* ATCC25922. This activity was regarded as being interesting. Similar results were reported by Ahmad and Beg[32]. They presented that the ethanolic extract of henna leaves has an antibacterial effect against a wide range of bacterial strains such as *S. aureus*, *B. subtilis*, *E. coli*, *S. paratyphi*, *S. dysenteriae* and *C. albicans*. Indeed, it is assumed that chemical components have a lower solubility in water and are soluble in alcohol. Antimicrobial substances from plants are usually insoluble in water. The study conducted by Al-Edany *et al.*[10] showed that the activity of ethanolic extract of *Lawsonia inermis* against *S. aureus* isolates, *E. coli* and *P. aeruginosa* was the most effective followed by aqueous extracts[10]. However, Saadabi[33] showed that the aqueous extract of *L. inermis* leaves was more find to be active in vitro and gives good reproducibility against the strains tested[33]. The antimicrobial activity returns to the presence of Lawsone compound. It is a heteroside form in the leaves[10]. Further studies are needed to identify compound/compounds responsible for the antibacterial activity of these extracts.

Several studies have been conducted to understand the mechanism of action of plant extracts. They attribute this function to phenolic compounds. These compounds can interfere with biomembranes by causing cellular damage and leakage of cellular material and eventually, the death of microorganisms[34]. Variations in chemical composition can probably explain the observed differences in the antimicrobial activity of extracts from the same plant species or from diverse plants. The optimal efficiency of an extract may not be due to a main active constituent, but to the combined action (synergy) of various compounds at the origin of this extract. Our study confirms the previous work on *L. inermis* leaves extracts and its fractions. According to our study, plant leaves extract and its fraction and especially the Tidikelt plant have various phytoconstituents exhibiting antimicrobial activity

Table 2: Antimicrobial activities of different *L. inermis* extracts of Touat and Tidikelt using the disc diffusion method

	ToAq (mg mL^{-1})				ToEth (mg mL^{-1})						
									CIP	GEN	AMB
Strains	06	4.5	03	1.5	06	4.5	03	1.5	5 µg	10 µg	100 µg
Touat extracts: Inhibition zone diameter (mm)											
B. cereus	25±1	23±1	21±1	20±0	32±1	30±1	25±1	23±1	36±0	20±1	na
S. aureus	23±1	22±1	20±1	19±1	25±1	22±1	21±1	20±1	28±1	24±1	
E. faecalis	10±1	08±1	07±1	06±0	12±1	11±1	10±1	09±1	30±1	28±1	
L. monocytogenes	11±1	09±1	07±1	06±0	12±1	10±1	08±1	06±1	na	20±1	
P. aeruginosa	07±0	06±0	06±0	06±0	08±1	07±1	06±0	06±0	30±0	20±1	
E. coli	06±1	06±1	06±1	06±0	11±1	08±1	07±1	06±1	32±1	24±0	
S. enteritidis	07±1	06±1	06±1	06±0	09±1	08±1	07±1	06±1	na	18±1	
K. pneumoniae	11±0	10±0	07±0	06±0	14±1	13±1	11±1	06±0	20±0	16±1	
C. albicans	18±1	16±1	14±0	10±1	20±1	19±0	18±1	16±1	na	na	21±1
	TiAq (mg mL^{-1})				TiEth (mg mL^{-1})						
									CIP	GEN	AMB
Strains	06	4.5	03	1.5	06	4.5	03	1.5	5 µg	10 µg	100 µg
Tidikelt extracts: Inhibition zone diameter (mm)											
B. cereus	28±0	24±1	21±1	19±2	36±2	34±2	27±2	25±1	36±0	20±1	na
S. aureus	26±1	24±1	21±1	20±1	28±0	25±1	23±1	20±1	28±1	24±1	
E. faecalis	12±1	10±1	09±1	07±1	14±1	12±1	11±1	10±1	30±1	28±1	
L. monocytogenes	11±1	10±1	07±1	06±1	14±1	12±2	10±1	09±0	na	20±1	
P. aeruginosa	08±0	07±1	06±1	06±1	11±0	09±1	08±1	07±1	30±0	18±1	
E. coli	10±1	08±1	07±1	06±0	13±1	11±1	09±1	06±2	32±1	24±0	
S. enteritidis	08±1	07±1	06±1	06±0	10±1	09±1	07±1	06±1	na	18±1	
K. pneumoniae	13±1	10±1	09±0	07±0	16±1	14±1	11±1	10±0	20±0	16±1	
C. albicans	21±1	19±1	18±1	16±1	24±1	22±1	21±1	20±1	na	na	21±1

na: Not applicable, values are expressed as Mean±SD

Table 3: Comparison of minimal inhibitory concentrations (MIC) of ethanol and aqueous tidikelt extracts

	Concentration 6 mg mL^{-1}		CIP 05 µg	GEN 10 µg	AMB 100 µg
Strains	AqMIC	EthMIC	MIC	MIC	MIC
B. cereus	2.40	3.50	2.00	0.40	na
S. aureus	2.00	2.50	0.30	0.50	
E. faecalis	2.30	2.50	1.20	8.00	
L. monocytogenes	2.00	2.50	na	2.00	
P. aeruginosa	2.50	3.50	0.45	2.00	
E. coli	1.25	2.40	0.01	0.50	
S. enteritidis	1.50	2.10	na	2.00	
K. pneumoniae	2.00	2.50	na	na	
C. albicans	8.20	10.40	na	na	0.01

MIC: Minimal inhibition concentration (mg mL^{-1}), na: Not applicable

against Gram positive, Gram negative bacteria and fungal strain. It gives an idea that *L. inermis* contain various phytoconstituents which have effective antimicrobial properties. Further studies are recommended to identify the molecule of interest and *in vivo* activity of this plant extract should be done, so that it can be used in human welfare as medicine for treating different diseases.

CONCLUSION

Multi-resistance microbial poses problems in public health, it always forces scientists to tap into the natural resources of the plant kingdom, a key source for the development of new therapeutic remedies. The present work focused on the plant, *Lawsonia inermis* recognized by its various therapeutic virtues. It highlight through a phytochemical analysis and the evaluation of the antimicrobial activity of these aqueous and ethanolic extracts the richness of this plant is natural component having the power to cure. The determination of the yield of crude extracts of the plant has shown that henna originating from Tidikelt has more activity than that of Touat. The results also illustrate the potential of the *Lawsonia inermis* plant from southwestern Algeria through the study of its antimicrobial activity.

SIGNIFICANCE STATEMENT

This study is an attempt was made to identify the phytochemical constituents responsible for biological role and antimicrobial activity in *Lawsonia inermis* leaf extract collected from south of Algeria Touat (Adrar) and Tidikelt (In Salah). The results confirmed the presence of various secondary metabolites such as flavonoids, Tannins and phenols which are responsible for various biological activities. The identification of phytoconstituents present in the extract and its fractions which are responsible for various biological and antimicrobial activities help the researchers to uncover the composition of the specific molecule responsible for this effect. The present research opens up vistas to carry other research to identify a new molecule on may be arrived with new structure in a hitherto unexplored area of utilizing the leaf of *Lawsonia inermis* in a novel way.

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