



Phytochemical Screening and Antibacterial Activity of Extracts of Medicinal Plants of Southwest Algeria

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ABSTRACT

Background: The aim of this study was characterized to investigate the phytochemical compositions and antibacterial activity of the aerials parts extracts of *Launaea arborescens* and *Limonium sp* commonly used in Southeastern Algeria for the treatments of various ailments.

Methods: Antibacterial activities were tested against four pathogenic bacteria and fungi such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albican*.

Result: Ethanolic and aqueous extracts of *Limonium sp* (40 mg/ml) were highly effective against *E. coli* with 82 and 85% and 78 and 57% respectively and for chlorid acid extract against *Staphylococcus aureus* with 84.21% of *Limonium sp*. The extracted aqueous and methanolic extract of *Launaea arborescens* at 20 mg/ml were highly effective against *E. coli* (90% inhibition) and *Pseudomonas aeruginosa* (64.7% inhibition). Furthermore, the methanol extract of *Launaea arborescens* had the maximum antibacterial activity against *Pseudomonas aeruginosa*. For antifungal properties, ethanolic and chlorid acid extracts showed the inhibition activity against fungi tested. The study has shown that *Launaea arborescens* and *Limonium sp* are an excellent source of bioactive substances against a wide range of various microbial pathogens.

Key words: Antimicrobial activity, *Launaea arborescens*, *Limonium sp*, Medicinal plant, Phytochemical analysis.

INTRODUCTION

The world health organisation has advocated the use of traditional medicine as a safe remedy for ailments of microbial and non-microbial origin (Organization 2001). Medicinal plants produce a wide variety of secondary metabolites (Beniaich *et al.*, 2022). Traditional medical practices that involve the use of herbs are viewed as an integral part of the culture in those communities (Roucham and Cheriti, 2023). Plants produce several secondary metabolites that present many important biological activities (Lapikova *et al.*, 2008). Biological cost or metabolic price is a measure of the increased energy metabolism required to achieve a function. Drug resistance has a high metabolic price (Steven and Timothy, 2010). Some flavonoids are formed as antimicrobial barriers in plants response to microbial infection. Therefore, it should not be surprising that they have been found *in vitro* to be effective antimicrobial compounds against a wide array of microorganisms. Antimicrobial activities of apigenin and other derivatives have also been previously reported (El-Bassuony and Abdel-Hamid, 2006).

Saharan plants are known for their resistance to various stress factors. They contain several nutrients and phytochemicals and have been used to treat many human diseases (Berreguioua and Ziane, 2024). Algeria with its large area and diversified climate has a varied flora, which is a source of rich and abundant medical matter and in particular Sahara part constitutes an important reservoir of many plants which have not been investigated until today. Among this flora, species from family have been used in the local traditional ethnopharmacopeae. Because of their therapeutic properties, medicinal plants are one way of

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investigation the most interesting image of the discovery of new drugs from plants used in traditional medicine for the treatment of discharged diseases. *Limonium sp* and *Launaea arborescens* are among the most used plants in traditional medicine in the northern Sahara.

Our laboratory (LPSO) is among the many research laboratories which have taken charge of the valorization of some endemic plants of the South West region. Algeria from an ethnopharmacological, phytochemical and biological activities point of view.

Launaea arborescens, its vernacular name is mol-albina belonging to Asteraceae family origin of the southwest of Algeria (Seddiki *et al.*, 2021). It has a limited distribution in the north-western part of the Algerian Sahara and has its southerly limit in the Béni-Abbès region (Garbari and Bedini, 2009; Cheriti *et al.*, 2012). The aerial part of *Launaea arborescens* is used in Saharan folk medicine for the treatment of anti-inflammatory like antibacterial and for treating diarrhea, gastrointestinal (Seddiki *et al.*, 2021).

The genus *Limonium* includes important halophyte plants containing a variety of bioactive compounds of therapeutic interest (Senizza *et al.*, 2021). *Limonium* is present in the Sahara *Limonium sp* is a species endemic to Algeria which can resist to extremely arid conditions (Ozenda 2004 ; Akat *et al.*, 2022). *Limonium sinuatum* is a perennial, herbaceous plant, which is marketed both as a potted plant and as a specialty cut flower (Sheikh *et al.*, 2015; E Ibrahim *et al.*, 2016; Gandolfa *et al.*, 2023).

Present study was aimed at evaluating the crude extracts of *Limonium sp* and *Launaea arborescens* with different chemicals for antimicrobial (fungal and bacterial) potential and phytochemical screening of extracts by using various solvent by reflux extraction.

MATERIALS AND METHODS

Plant material

The aerial parts of plants were collected in Spring season 2022, from Bechar region (Southwest of Algeria).

Extraction method

The leaves were dried and grounded into powder before to an extraction using reflux during three hours. The dried powdered plants (50 g) were extracted using 300 ml of different solvents like, Petroleum ether (1), Ethanol (2), Dichloro methane (3), Chloroform (4), Methanol (5), Chloride Acid (6) and Aqueous (7). The extracts were filtered and evaporated using a vacuum rotary evaporator.

Phytochemical screening

The extracts of *Launaea arborescens* and *Limonium sp* were analyzed for the presence of secondary metabolites according to standard methods:

Detection of alkaloids

2 ml of extract was added to Hager reagent (Saturated solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids (Krishnaiah *et al.*, 2009).

Detection of flavonoids

About 7.5 mg of each dry extract was dissolved in 0.5 ml of ethanol, concentrated HCl and magnesium turnings. A yellowish coloration indicates the presence of flavonoids (Kebede *et al.*, 2021).

Detection of tannins

0.5 g of plant extract was stirred with 1 milliliter of distilled water, filtered and few drops of ferric chloride were added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins (Kokate *et al.*, 2005; Khandelwal, 2008; De *et al.*, 2010; Trease, 2010).

Detection of saponins

0.1 g of extract was shaken with 5 ml of distilled water in a test tube. Honey comb frothing that persisted for five minutes is taken as a preliminary evidence for the presence of saponins (Trease, 2010).

Detection of terpenoids

5 ml extract of each plant sample is mixed with 2 ml of CHCl_3 in a test tube. 3 ml of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present (Krishnaiah *et al.*, 2009).

Detection of steroids

2 ml of all the extracts were added to 5ml of chloroform. A volume of 1 ml of H_2SO_4 was added carefully along the walls of the tube containing the mixture (chloroform + H_2SO_4). The formation of reddish color in the lower layer indicates the presence of steroids (Kokate *et al.*, 2005; Khandelwal, 2008; De *et al.*, 2010; Trease, 2010).

Antibacterial activity

The antibacterial activity of the extracts of the aerial part of *Launaea arborescens* and *Limonium sp* were evaluated using three bacterial and one fungal strains, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albican*.

Disc diffusion method

Screening of plant extract for antibacterial activity was done by the Disc Diffusion Method. The organic extracts are solubilized in 10% dimethyl sulfoxide (DMSO). For each organic extract, we prepared the concentrations of 40 mg/ml, 20 mg/ml and then the different solutions prepared were sterilized by filtration on 0.45 μm filters. Petri dishes containing Mueller Hinton agar are swabbed with a suspension on a Mac Farland scale, which comes from a young culture of bacteria. (Shariffar *et al.* 2007). After drying the dishes, the discs (6 mm in diameter), soaked in 40 mg/ml, 20 mg/ml of organic extracts were deposited on the surface of an agar medium (Mueller–Hinton) after incubation for 24 h at 37°C.

Reading the results

The results were read after seven days of incubation at 25°C by the measurement of the diameter of the zone of fungi growth. In parallel, we determined the diameter of the fungal strain in the absence of the extract plant. The antibacterial effect is determined by measuring the percentage diametral growth inhibition using the following formula :

$$\% \text{ Inhibition of growth PI} = \frac{(D_T - D_C)}{D_T} \times 100$$

D_C : Diameter growth and test (mm) .

D_T : Diameter growth in control (mm).

Statistical analysis

All experiments were spotted three times. Ms Excel 2007 was used to express the values as the mean $\pm$ deviation.

RESULTS AND DISCUSSION

Phytochemical screening of extracts

Thus we think that carrying out a phytochemical screening on the bioactive extracts for this plant is necessary to

conducting a future works on the isolation and identification of natural compounds (Sheikh *et al.*, 2015). Phenolic compounds like sterols, carotenoids, terpenes, glucosinolates, alkaloids and various sulfur containing compounds are bioactive phytochemicals richly found in herbs (Islam *et al.*, 2024). Phytochemical screening shows that *Limonium sp* contains tannins (cathechic), saponins and sterols. The extracts of *Limonium sp* were screened for the presence of secondary metabolites by using standard procedures to identify the constituents as described in literature (Cheriti, 2000). These secondary metabolites have various applications due to their antibacterial properties. The results of phytochemical screening were given in the (Table 1).

Phytochemical Screening in *Limonium sp* and *Launaea arborescens* plants based on tests of colouration and precipitation was undertaken by the bioactive extracts (Methanol, ethanol, dicloromethane, chloroform, aqueous, petroleum ether and chlorid acid).

According to this results, the plants have an extensive variety of phytochemicals, which may account for its historical therapeutic application and potential health advantages. The tests carried out on aerial parts of plants showed presence of flavonoids (free and glycosides), saponins, terpens, tannins. However we observed less presence of tanins and absence of alkaloids. From the

results it is evident that all parts of the *Limonium sp* gave a positive test for a tested class of natural compounds whereas we noted the absence of alkaloids and steroids.

All the extracts produced positive reactions as follows: water extract (tanins, saponins, flavonoids, steroids, terpenoids, alkaloids and Glycosids flavonoïds and Flavonoïds aglycon), Methanol extract (tannins, saponins, terpenoids and flavonoids) and ethanol extract (tannins, flavonoids, saponins and terpenoids). However, the majority of the extracts gave positive results for flavonoids, saponins, terpenoids and tannins. Water, methanol and Methanol solvents are currently used for the extraction of polyphenols and flavonoids; these metabolites are considered as potent antioxidants, anti-inflammatory, antiviral and antibacterial.

Antibacterial activity

This work consisted in the evaluation of the antifungal activities and antibacterial properties of *Launaea arborescens* and *Limonium sp* extracts to verify the virtues advocated by the traditional use of this plant. The results and discussion focused on the dried extracts (leaves) are indicated in (Table 2) presents the activity of the extracts of *Launaea arborescens* on the germs subjected to the tests. The results of the antibacterial test of the extracts showed a high degree of inhibition all molds compared to the petroleum ether extracts. variable effects of the extracts

Table 1: Phytochemical screening of the extracts from *Limonium sp* and *Launaea arborescens*.

Extracts	Petroleum ether		Ethanol		Diclorom ethane		Chloroform		Methanol		Chlorid acid		Aqueous	
	LA	LS	LA	LS	LA	LS	LA	LS	LA	LS	LA	LS	LA	LS
Plant														
Alkaloids	-	-	+	-	+	+	-	-	+	-	+	+	+	+
Saponins	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenes	+	-	+	+	+	-	+	+	+	+	+	+	+	+
Tanins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoïds	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Flavonoïds aglycon	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosids flavonoïds	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	-	-	+	-	-	-	-	-	+	-	+	-	+	-

LA: *Launaea arborescens*; LS: *Limonium sp*.

Table 2: Result of antimicrobial and antifungal activity of *Launaea arborescens* (mm).

Extracts	Microbial strains							
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Candida albican</i>	
	20	40	20	40	20	40	20	40
Concentration (mg/ml)								
Petroleum ether	11±0.5	9±0.5	10±0.33	9±0.5	11±0.5	12±0.44	-	-
Ethanol	9±0.33	9±0.37	10±0.5	10±0.33	14±0.33	14±0.33	-	-
Dicloromethane	-	-	-	-	-	-	-	-
Chloroform	10±0.44	11±0.5	12±0.1	10±0.5	9±0.33	9±0.33	11±0.44	11±0.5
Methanol	10±0.44	10±0.33	11±0.44	12±0.44	12±0.44	17±0.01	10± 0.33	9±0.33
Chlorid acid	11±0.33	12±0.1	13±0.5	16±0.33	13±0.1	16±0.33	-	10±0.33
Aqueous	14±0.33	12±0.33	9±0.33	12±0.01	14±0.5	15±0.33	-	-

tested against bacterial and fungal strains and inhibition zones ranged from 9 to 17 mm (Bansemir *et al.*, 2006).

The evaluation extract of antibacterial activity was carried out by disk diffusion methods in solid medium. This method has shown that all extracts (Ethanol, methanol, petrol ether, chloroform and aqueous solvent extracts) of *Launaea arborescens* used with the concentration 40 mg/ml and 20 mg/ml are active, compared to other extracts. The extract aqueous and methanolic extract of *Launaea arborescens* at (20 mg/ml) were highly effective against *E. Coli* at (90%) and *Pseudomonas aeruginosa* (64,7%) inhibition. Ethanol extract of *Launaea arborescens* had the maximum antibacterial activity against *Pseudomonas aeruginosa* (64.28%) as seen in (Fig 1). For antifungal properties ethanolic and chlorid acid extracts showed the inhibition activity against fungi tested.

However, no effect was obtained for the dichloromethane extract there was no activity against all strains.

The studies on the antibacterial of different extracts of the aerial part of Algerian *Launaea arborescens* plant indicate variability in the biological activity of plant extracts which is attributed to differences in the content of polyphenolic compounds. The mechanisms of action of

natural compounds are explained in different ways by the authors.

According to Belboukhari and Cheriti, (2006), In addition, very interesting antifungal, antibacterial and insecticidal activities have been reported for the methanol extract of the plant (Belboukhari and Cheriti., 2006 ; Jbilou *et al.*, 2008, Belboukhari *et al.*, 2016).

The highest inhibition showed in *S. aureus*, a human pathogen, very interesting antifungal activity against *Candida albicans* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* have been reported for the methanol extract of *Launaea arborescens* (Lampe and Chang, 2007; Cheriti *et al.*, 2012, Rahmani *et al.*, 2012, Belboukhari *et al.*, 2014, Smahia *et al.*, 2016, Smahia *et al.*, 2016; Islam *et al.*, 2024). (Table 3) present the activity of the extracts of *Limonium sp* on the germs subjected to the tests.

The results obtained show that the methanolic, ethanol, aqueuse and chlorid acid extracts of *Limonium sp* used with the concentration (40 mg/ml and 20 mg/ml) are active compared to other extracts.

E. coli (28, 26 mm for extract aqueuse and ethanol), *Staphylococcus aureus* (38, 26 mm) for extract chlorid acid

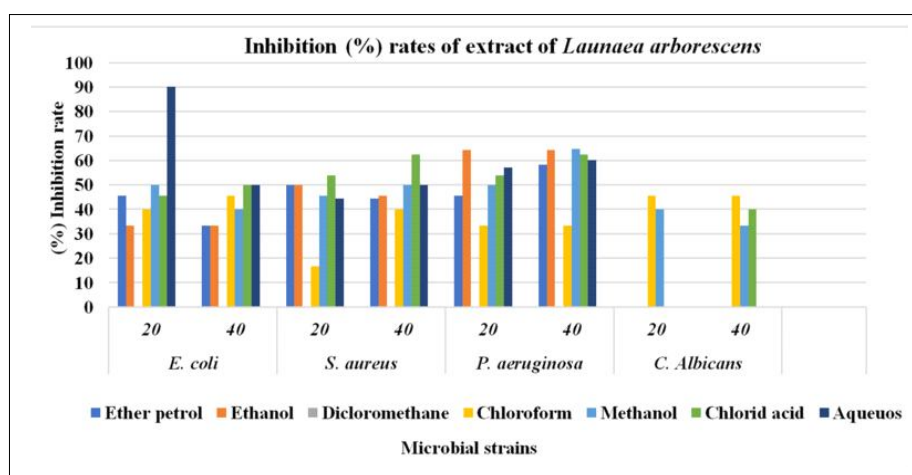


Fig 1: Inhibition rate (%) of *Launaea arborescens*.

Table 3: Result of Antimicrobial and antifungal activity of *Limonium sp* (mm).

Extracts	Microbial strains							
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Candida albican</i>	
Concentration (mg/ml)	20	40	20	40	20	40	20	40
Petroleum ether	14±0.33	19±0.5	16±0.33	27±0.01	-	-	-	-
Ethanol	26±0.33	35±0.01	20±0.44	26±0.5	13±0.5	18±0.3	-	14±0.33
Dicloromethane	-	-	-	-	-	-	-	-
Chloroform	-	15±0.37	-	14±0.33	-	-	-	-
Methanol	11±0.5	9±0.5	15±0.5	22±0.44	12±0.44	17±0.35	10±0.33	9±0.1
Chlorid acid	15±0.01	25±0.33	27±0.66	38±0.1	12±0.33	19±0.1	16±0.44	21±0.5
Aqueous	17±0.44	28±0.44	17±0.33	19±0.3	13±0.5	16±0.5	11±0.5	10±0.01

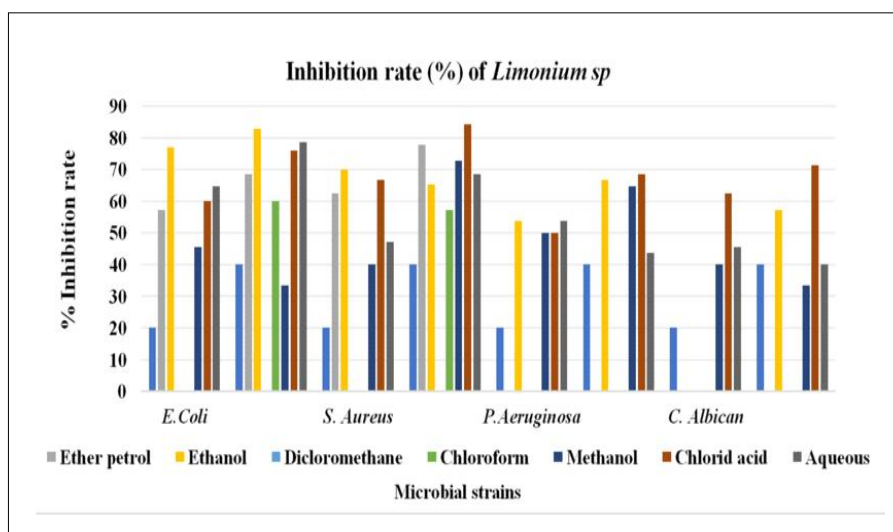


Fig 2: Inhibition rate (%) of *Limonium sp.*

and Ethanol), *Pseudomonas aeruginosa* (18,19,17,16 mm) for extracts ethanol, chlorid acid, methanol and aqueous, *Candida albican* (21 mm,14 mm) for extract chlorid acid and Ethanol). However, no effect was obtained for the dichloromethane and chloroform extracts there was no activity against all strains and ether petrol was no activity against *Pseudomonas aeruginosa* and *Candida albican*. The crude extract of methanol showed an important activity for *K. pneumoniae* and *P. aeruginosa*, this last has the reputation to be in general very resistant to all sorts of antimicrobial agents and antibiotics (Berregioua and Cheriti, 2018).

Methanol extract was the percentage inhibition zone against *Staphylococcus aureus* strain (72.72%).

For *Candida albican* no activity for extracts ether petrol and aqueous and the highest inhibition zone against *Candida albican* (57.42%) for ethanol extract (Fig 2).

The ethanolic and aqueous extracts of *Limonium sp* at (40 mg/ml) were highly effective against *E. coli* with (82.85%) and (78.57%) respectively and for chlorid Acid extract against *Staphylococcus aureus* with (84.21%) of *Limonium sp.* The extract Aqueous of *Launaea arborescens* at (20 mg/ml) were highly effective against *E. coli* at (78.57%) *Staphylococcus aureus* (68.42%) inhibition.

CONCLUSION

These results indicated that antibacterial effects of medicinal plants and their Phytochemical components to find better options against this pathogenic bacterium. Conclusively, methanolic, chlorid acid and ethanol extracts have showed a powerful antibacterial and antifungal activity of *Limonium sp.* This plants can be used as natural medicine in the treatments of various infectious diseases. Plant metabolites were found to possess antibacterial activity. Finally, we have evaluated the biological efficacy as good and this is confirmed by the investigations conducted

in the beginning of the study as an initial step on the traditional uses of the plants used.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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