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Research Article
Valorisation of Algerian Medicinal Plants: *Inula viscosa* L. A Future Source of Antibacterial Drugs

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Abstract

Background and Objective: During this last century we observed the diminution of antibiotics efficacy. For this reason the development of new molecules is becoming a necessity to address with the threats of bacterial infections. In this study, we tried to evaluate the antibacterial effect of *Inula viscosa* L. on 5 clinical strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Morganella morganii* in addition to 2 reference strains: *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922. **Materials and Methods:** The polyphenolic compounds were extracted from the leaves by maceration in methanol and hexane fractionation. The antibacterial activity was determined by the solid-state disk diffusion method and the minimal inhibitory concentration by dilution in solid medium. **Results:** All the tested strains were sensitive to the polyphenolic extract with inhibition zones 10.8-21 mm. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* proved to be more sensitive to the polyphenolic extract in comparison with the tested antibiotics. The lowest minimal inhibitory concentration was recorded with *Staphylococcus aureus* and *Pseudomonas aeruginosa* (0.39 mg mL^{-1}). The highest minimal inhibitory concentration was found in both strains of *Escherichia coli*. The polyphenolic extract showed a bacteriostatic effect on *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* ATCC29213. It had however, a bactericidal effect on *Staphylococcus aureus*, *Morganella morganii* and *Escherichia coli* ATCC25922. **Conclusion:** *Inula viscosa* L. represents a natural and a promising source of chemical molecules which have important antibacterial activities on Gram positive and Gram negative bacteria.

Key words: Antibacterial activity, medicinal plants, minimal inhibitory concentration, *Inula viscosa* L., polyphenolic extract

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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

Global prevalence of infectious diseases caused by bacteria is a major public health problem[1,2]. Antibiotics are powerful drugs which help in reducing bacterial infections. They are not totally inoffensive for the organism. In addition to their antimicrobial activity, they may provoke undesirable effects[3]. Antibiotics are also treated by the appearance and spread of multi-resistant bacteria[4]. Faced with this problem, the use of herbal medicine is more and more relevant[5]. Plants are prospective source of antimicrobial agents in different countries[6]. Herbal products have been used in folk medicine since ancient times, in both eastern and western medical traditions[7]. Over the past decades, pharmaceutical companies have increased their interest in investigating plants as sources for new phytotherapeutic agents with proven efficacy, quality and safety[8]. *Inula viscosa* L. (Magramane) is a widespread plant in the Mediterranean basin[9,10]. Ethnobotanical study showed that the plant is very used in traditional medicine as an antiviral[11], antiseptic, antibacterial, healing[12,13] and antifungal[14,15]. In order to continue to exploit the plants growing in Algeria and famous for their antimicrobial phytopharmaceutical properties, we tried to detect and highlight the antibacterial power of polyphenolic extract of *Inula viscosa* L. harvested from the region of El Harrouch, the state of Skikda (Algeria) and to compare the effect of the extract with the tested antibiotics in order to better valorise this plant.

MATERIAL AND METHODS

The study was carried out in 2 sites during a period of 5 months, from February, 2019 until the end of June, 2019. The extraction of polyphenols was realised in the laboratory of soil chemical, department of agronomy, Skikda University. The isolation and identification of clinical strains was performed in the bacteriological analysis laboratory, the hospital of Laib Derradji, El Harrouch, the state of Skikda.

Isolation and identification of bacterial strains: Gram negative bacteria were isolated on Hektoen medium (Bio-Rad, 3bd Raymond Poincaré 92430 Marnes la Coquette, France) while Gram positive bacteria were isolated on Chapman medium (Bio-Rad, 3bd Raymond Poincaré 92430 Marnes, La Coquette, France). Negative bacteria were identified using the test kits API 20E system (Biomerieux, 5, rue des Aqueducs, France.). Gram positive bacteria were identified using the catalase and coagulase tests. The reference strains *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 were provided by Dr. Becheker Imane, the department of sciences of life and of nature, the University of Skikda.

Extraction of polyphenols

Maceration (solid/liquid extraction): The plant leaves previously washed and dried in the temperature room were grinded. About 10 g of the plant powder were then added to methanol (70%) and well shaked for 24 h.

Extraction liquid/liquid: The obtained solution was filtered towards filter paper. The obtained filtrate was measured and added to the same volume of hexane. The obtained solution was decanted and the polar part in the bottom was recovered. The extract was then underwent a fractionation to eliminate the non flavonoid fraction using the solvent ethyl acetate. It was then poured in petri dishes and dried at 30°C for 48 h. The extraction yield was determined according to the following equation:

$$R = (Me \times Mv)/100$$

Where:
R = Yield
Me = Weight of the extract
Mv = Weight of the vegetal material[16]

Evaluation of antibacterial activity of *Inula viscosa* L.: Antibacterial activity of *Inula viscosa* L. was determined using the method of diffusion in solid medium.

Revivification of bacterial strains: The bacterial strains were cultivated in nutrient agar and incubated at 37°C for 24 h to obtain young cultures. *Klebsiella pneumoniae* however, was previously cultivated in brain heart infusion broth (BHIB) and incubated at 37°C for 18 h. A drop of the cultivated medium was then added to nutrient agar and incubated at 37°C for 24 h.

Preparation of the inoculums: Some well isolated bacterial colonies were added to 5 mL of sterile physiological water. The suspension was well homogenised to obtain an opacity of 0.5 MacFarland (density of 0.08-0.1 read at 625 nm). Mother solution of the tested plant was prepare by diluting the dried extract of the plant in dimethyl sulfoxide (DMSO) at a concentration of 100 mg mL^{-1}. The 300 UL of the bacterial suspension were inoculated to Muller-Hinton medium using swab method. Sterilised discs (6 mm of diameter) soaked with the extract were added to the petri dishes previously inoculated. A disk soaked in DMSO was used as control disk. Petri dishes were then incubated at 37°C for 24 h.

Evaluation of inhibition zones: Inhibition zones were determined after 24 h of incubation by measuring the average of 3 diameters passing from the center of the disc. Three repetitions were carried out for each strain. The results were explained according to Ponce *et al.*[17]:

Non sensitive: Diameter <8 mm, Sensitive: Diameter 9-14 mm, Very sensitive: Diameter 15-19 mm, Extremely sensitive: Diameter >20 mm

Determination of minimal inhibitory concentration (MIC) by the method of dilution in solid medium: This technique is the most economic. Four milliliters of the mother solution were diluted in 2 mL of distilled water. A series of dilutions was then prepared: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256[18]. Two milliliters of each dilution were put in petri dishes and 18 mL of Muller Hinton medium were added. The mixture was well homogenized. After solidification of the medium, petri dishes were divided according to the number of strains and inoculated by the steaks method. Control dish was inoculated on DMSO. The cultures were then incubated at 37°C for 24 h.

Determination of bactericide/bacteriostatic activity: We distinguish 2 types of the activity of the extract on the bacterial strains, a bactericide activity which exercises a lethal effect and a bacteriostatic effect which provokes an inhibition of the growth[19,20]. To confirm the effect of the extract, the transplantation of the strains from the inhibition zones on nutrient agar was preceded. The cultures were then incubated at 37°C for 24 h. The presence of a bacterial growth indicates that the extract has a bacteriostatic effect however, the absence of the growth means that the extract exercises a bactericide effect.

Test of bacterial antibioresistance: The antibioresistance of the identified bacterial strains was determined by the standard susceptibility method by diffusion on Muller Hinton medium according to the recommendations of the French Committee of the Antibiogram of the French Society of Microbiology[21]. A bacterial suspension of 0.5 MacFarland (optical density of 0.08-0.1 read at 625 nm) was prepared. After solidification of Muller Hinton medium, petri dishes were inoculated using swab method. Antibiotics disks were then deposited. The cultures were incubated at 37°C for 24 h. The diameters of the inhibition zones around the discs were translated according to the standards of CA-SFM[21]: Sensitive (S), resistant (R).

RESULTS AND DISCUSSION

Microbiological analysis: During this study we worked on 5 clinical strains *Staphylococcus aureus*, *Morganella morganii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* obtained from Laib Derradji Hospital, El-Harrouch, the state of Skikda, which were identified according to their morphological, physiological and biochemical characters (Table 1) in addition to 2 reference strains *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213. *Staphylococcus aureus* was identified by the tests of catalase and coagulase enzymes which were positive.

Yield of polyphenols extraction: The obtained extract was characterised by a dry, pasty aspect and a greenish colour. The yield of polyphenols explained by the percentage of the extract weight to the powder weight was 18.03%. The obtained results were less than those obtained by Chebouti-Meziou[22], who reported her work on the same species obtained from Boumerdes , the North of Algeria where she reported a yield of 37.41%. This difference is due to the nature of the plant organ, the period and the harvest mode as well as the soil and the edaphoclimatic conditions of the environment[22]. The conditioning mode also influences the extract yield[23].

Antibacterial activity of *Inula viscosa* L. extract: The presence of inhibition zones around all the discs soaked by the extract was observed, with all the strains, where as the control disks did not show any inhibition zone. Bakhta[24] showed however that this plant had no inhibition effect on *Pseudomonas aeruginosa* and *Escherichia coli*. According to Benhammou[25] the plant extracts slightly inhibited *Escherichia coli* and strongly inhibited *Pseudomonas aeruginosa.* The inhibition zones diameters differ according to the bacterial strain (Table 2). *Morganella morganii*, *Staphylococcus aureus* ATCC29213 and *Staphylococcus aureus* were extremely sensitive (21 and 20.5 mm) followed by *Klebsiella pneumoniae* (19 mm), *Escherichia coli* ATCC25922 (17 mm) and *Pseudomonas aeruginosa* (15 mm). *Escherichia coli* however, were less sensitive (10.8 mm). Excepting *Morganella morganii* (Gram-), Gram positive bacteria were more sensitive in comparison with Gram negative bacteria. This is due to the differences in the cell wall composition of Gram positive and Gram negative bacteria[26-28]. The cell wall of Gram negative bacteria contains an external membrane due to the presence of polysaccharides which acts as a barrier against biomolecules[29]. The phytochemical study of *Inula viscosa* L. brought out a series of important flavonoides[30,31]. The flavonoids containing 2 or 3 hydroxyl groups on the cycle A and B were more active against Gram positive bacteria.

Table 1: Biochemical characters of Gram negative strains

	Bacterial strains			
Biochemical characters	*Morganilla morganii*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
ONPG	Negative	Positive	Positive	Non feasible test
ADH	Non feasible test	Negative	Positive	Positive
LDH	Non feasible test	Positive	Positive	Non feasible test
ODH	Non feasible test	Positive	Positive	Non feasible test
CIT	Non feasible test	Negative	Positive	Positive
H2S	Negative	Negative	Negative	Non feasible test
URE	Positive	Negative	Positive	Negative
TDA	Positive	Negative	Negative	Non feasible test
IND	Non feasible test	Positive	Positive	Non feasible test
VP	Negative	Negative	Positive	Non feasible test
GEL	Non feasible test	Negative	Negative	Non feasible test
GLU	Non feasible test	Positive	Positive	Negative
MAN	Non feasible test	Positive	Positive	Positive
INO	Positive	Negative	Positive	Non feasible test
SOR	Non feasible test	Positive	Positive	Non feasible test
RHA	Non feasible test	Positive	Positive	Non feasible test
SAC	Non feasible test	Negative	Positive	Non feasible test
MEL	Non feasible test	Positive	Positive	Non feasible test
AMY	Non feasible test	Negative	Positive	Non feasible test
NO3	Non feasible test	Non feasible test	Non feasible test	Positive
TRP	Non feasible test	Non feasible test	Non feasible test	Negative
ESC	Non feasible test	Non feasible test	Non feasible test	Negative
GEL	Non feasible test	Non feasible test	Non feasible test	Positive
PNPG	Non feasible test	Non feasible test	Non feasible test	Negative
ARA	Non feasible test	Non feasible test	Non feasible test	Negative
MNE	Non feasible test	Non feasible test	Non feasible test	Negative
NAG	Non feasible test	Non feasible test	Non feasible test	Positive
MAL	Non feasible test	Non feasible test	Non feasible test	Negative
GNT	Non feasible test	Non feasible test	Non feasible test	Positive
CAP	Non feasible test	Non feasible test	Non feasible test	Negative
ADI	Non feasible test	Non feasible test	Non feasible test	Positive
MLT	Non feasible test	Non feasible test	Non feasible test	Positive
PAC	Non feasible test	Non feasible test	Non feasible test	Negative
Oxydase	Negative	Negative	Negative	Positive
Catalase	Positive	Positive	Positive	Positive
Lactose	Negative	Non feasible test	Non feasible test	Non feasible test

Table 2: Classification of the bacterial strains sensitivity towards *Inula viscosa* L. polyphenols

Bacterial strains	Inhibition zone (mm)	Sensitivity
Morganella morganii	21.0	+++
Staphylococcus aureus ATCC 29213	21.0	+++
Staphylococcus aureus	20.5	+++
Escherichia coli ATCC 25922	17.0	++
Klebsiella pneumoniae	19.0	++
Pseudomonas aeruginosa	15.0	++
Escherichia coli	10.8	+

+: Sensitive (9-14 mm), ++: Very sensitive (15-19 mm), +++: Extremely sensitive (>20 mm)

Comparison of antibacterial activity of antibiotics and polyphenols extract

Enterobacteria: The obtained results revealed that *Morganella morganii* was extremely sensitive towards the extract as well as towards the majority of the tested antibiotics IPM, CTX, CX, AMC and FOX except the antibiotic CZ (Table 3). *Escherichia coli* was sensitive to the polyphenolic extract and to the antibiotics CL, FOX, C, CTX, AZM, GEN, CIP and IPM. It was on the other hand resistant to AMC, AMX and DO. *Escherichia coli* ATCC259 presented a sensitivity towards the plant extract as well as the antibiotics AMX, DO, CL, FOX, C, CTX, AZM, GEN, CIP and IPM. *Klebsiella pneumoniae* was very sensitive to the polyphenolic extract. Excepting the antibiotics GEN, CIP and K, this strain was resistant to the majority of the tested antibiotics, it was by the way a multi-resistant bacteria.

Pseudomonas aeruginosa: The isolated strain was very sensitive to the polyphenolic extract. It was also sensitive to the antibiotics CIP, IPM, CL and GEN contrary to DO, FF, C, FOX, AMC, TIM and AMX to whom the strain was resistant. It was by the way a multi resistant bacterium (Table 4).

Table 3: Antibioresistance profile of the tested enterobacteriaceae

	Bacterial strains			
Antibiotics	*Morganella morganii*	*Escherichia coli* ATCC25922	*Escherichia coli*	*Klebsiella pneumoniae*
Amoxicillin+clavulanic acid	Sensitive	Resistant	Resistant	Resistant
Amoxicilline	Not tested	Sensitive	Resistant	Not tested
Doxycycline	Not tested	Sensitive	Resistant	Not tested
Colistine	Not tested	Sensitive	Sensitive	Not tested
Cefoxitin	Sensitive	Sensitive	Sensitive	Not tested
Chloramphenicol	Not tested	Sensitive	Sensitive	Not tested
Cefotaxime	Sensitive	Sensitive	Sensitive	Resistant
Azithromycine	Not tested	Sensitive	Sensitive	Not tested
Gentamicine	Not tested	Sensitive	Sensitive	Sensitive
Ciprofloxacine	Not tested	Sensitive	Sensitive	Sensitive
Imipenem	Sensitive	Sensitive	Sensitive	Not tested
Ticarcillin	Not tested	Not tested	Not tested	Resistant
Ofloxacine	Not tested	Not tested	Not tested	Resistant
Cephalexin	Not tested	Not tested	Not tested	Resistant
Aztreonam	Not tested	Not tested	Not tested	Resistant
Colistin	Not tested	Not tested	Not tested	Resistant
Tobramycin	Not tested	Not tested	Not tested	Resistant
Kanamycine	Not tested	Not tested	Not tested	Sensitive
Cefotaxime	Sensitive	Not tested	Not tested	Not tested
Cefazolin	Resistant	Not tested	Not tested	Not tested
Polyphenolic extract of *Inula viscosa* L.	+++	++	+	++

++: Very sensitive (15-19 mm), +++: Extremely sensitive (>20 mm), +: Sensitive (9-14 mm)

Table 4: Antibioresistance profile of the tested *Pseudomonas auruginosa*

Antibiotics	*Pseudomonas aeruginosa*
Imipenem	Sensitive
Ciprofloxacine	Sensitive
Gentamicine	Sensitive
Colistine	Sensitive
Doxycycline	Resistant
Fosfomycine	Resistant
Chloramphenicol	Resistant
Cefoxitin	Resistant
Amoxicilline+clavulanic acid	Resistant
Ticarcillin	Resistant
Amoxicilline	Resistant
Polyphenolic extract of *Inula viscosa* L.	+

+: Sensitive (9-14 mm)

Table 5: Antibioresistance profile of the tested *Staphylococcus aureus* strains

Antibiotics	*Staphylococcus aureus* ATCC29213	*Staphylococcus aureus*
Ampicilline	Sensitive	Resistant
Vancomycine	Sensitive	Sensitive
Gentamicine	Sensitive	Sensitive
Erythromycine	Sensitive	Sensitive
Pénicilline	Resistant	Resistant
Oxacilline	Sensitive	Resistant
Polyphenolic extract of *'Inula viscosa* L.	+++	+++

+++: Extremely sensitive (>20 mm)

***Staphylococcus aureus*:** The tested strain was extremely sensitive to the plant extract and sensitive to the antibiotics VA, GEN and E. It was on the other hand a multi-resistant bacteria to P, OX, AM and CL. The reference strain *Staphylococcus aureus* ATCC29213 was extremely sensitive to the polyphenolic extract and sensitive to all the tested antibiotics except the penicillin (Table 5).

The most resistant clinical strains develop mechanisms of adaptation to the tested antibiotics[32]. This resistance is due to chromosomal mutations or to the acquisition of resistance genes carried by genetic elements (Plasmids, phages, transposon and integrons). These resistances led to search new molecules antibacterial agents having a more important efficiency than synthetic drogues and more accepted by the organism[33,34].

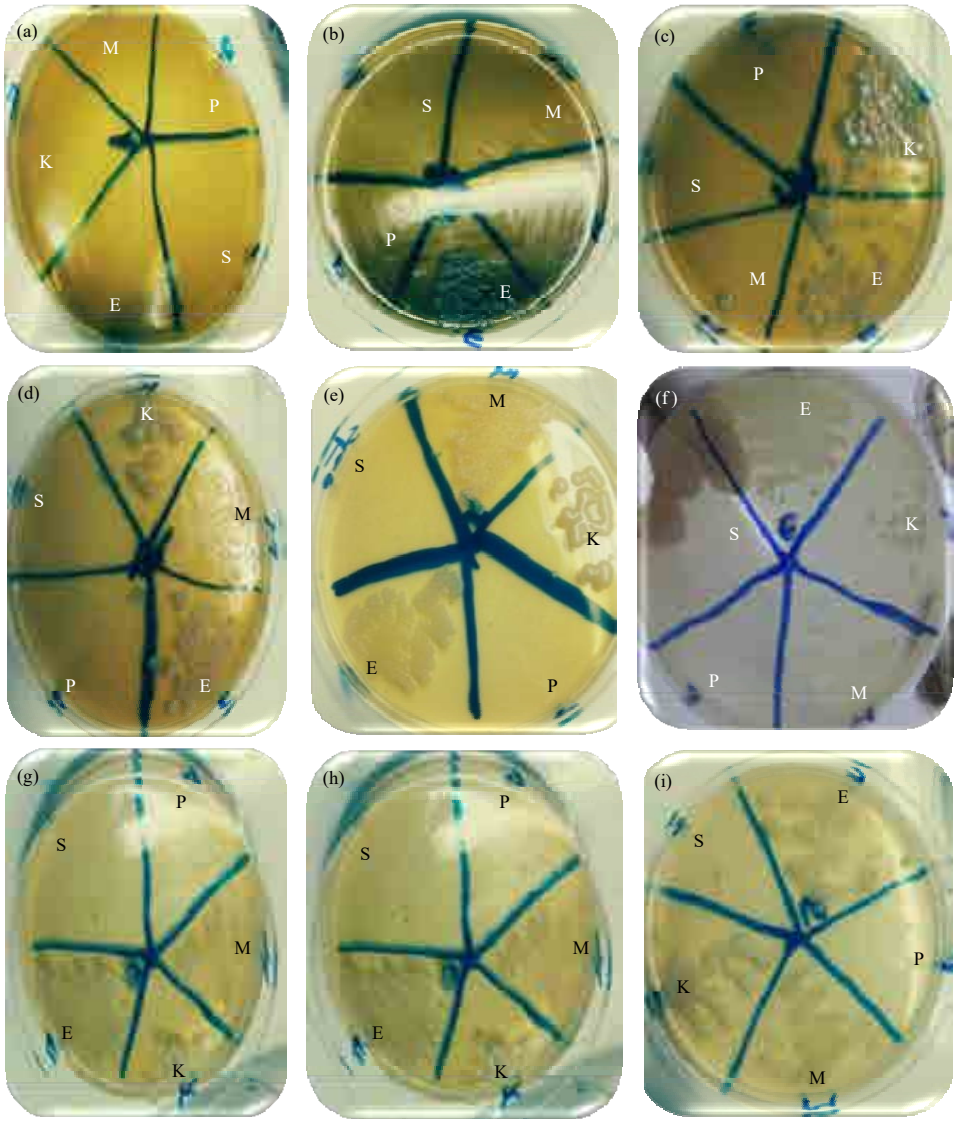

Fig. 1(a-i): Minimal inhibitory concentration of *Inula viscosa* L. extract towards the clinical strains, (a) 100 mg mL^{-1}, (b) 50 mg mL^{-1}, (c) 25 mg mL^{-1}, (d) 12.5 mg mL^{-1}, (e) 6.25 mg mL^{-1}, (f) 3.16 mg mL^{-1}, (g) 1.56 mg mL^{-1}, (h) 0.78 mg mL^{-1} and (i) 0.39 mg mL^{-1}

P: *Pseudomonas aeruginosa,* E: *Escherichia coli* , M: *Morganella morganii* , K: *Klebsiella pneumoniae,* S: *Staphylococcus aureus*

The advantage of natural antibiotics (plants extract), is that they don't push the microbes to develop resistance against them. They are composed of many different molecules so that the microbe needs to synthesise many enzymes to be able to neutralise all of these[35].

Determination of the minimal inhibitory concentration: The results obtained by the method of diffusion on solid medium were confirmed quantitatively by the method of dilution on solid medium. The obtained results (Fig. 1, 2) showed that the lowest inhibitory concentration was recorded with *Staphylococcus aureus* and *Pseudomonas aeruginosa* (0.39 mg mL^{-1}). The highest concentration was obtained with the strains of *Escherichia coli* (100 mg mL^{-1}) followed by *Klebsiella pneumoniae*, *Staphylococcus aureus* ATCC29213 (50 mg mL^{-1}) and *Morganilla morganii* (25 mg mL^{-1}). In deed the weak sensitivity of some strains towards the tested extract may be explained by the fact that the components with antibacterial activity are highly lipophilic[36]. By the way Hydrosoluble molecules exercise a weaker antibacterial effect in comparison with non hydrosoluble substances[33]. This refers probably to the capacity of liposoluble molecules to intercalate in the bacterial membranes and to damage them[37,38].

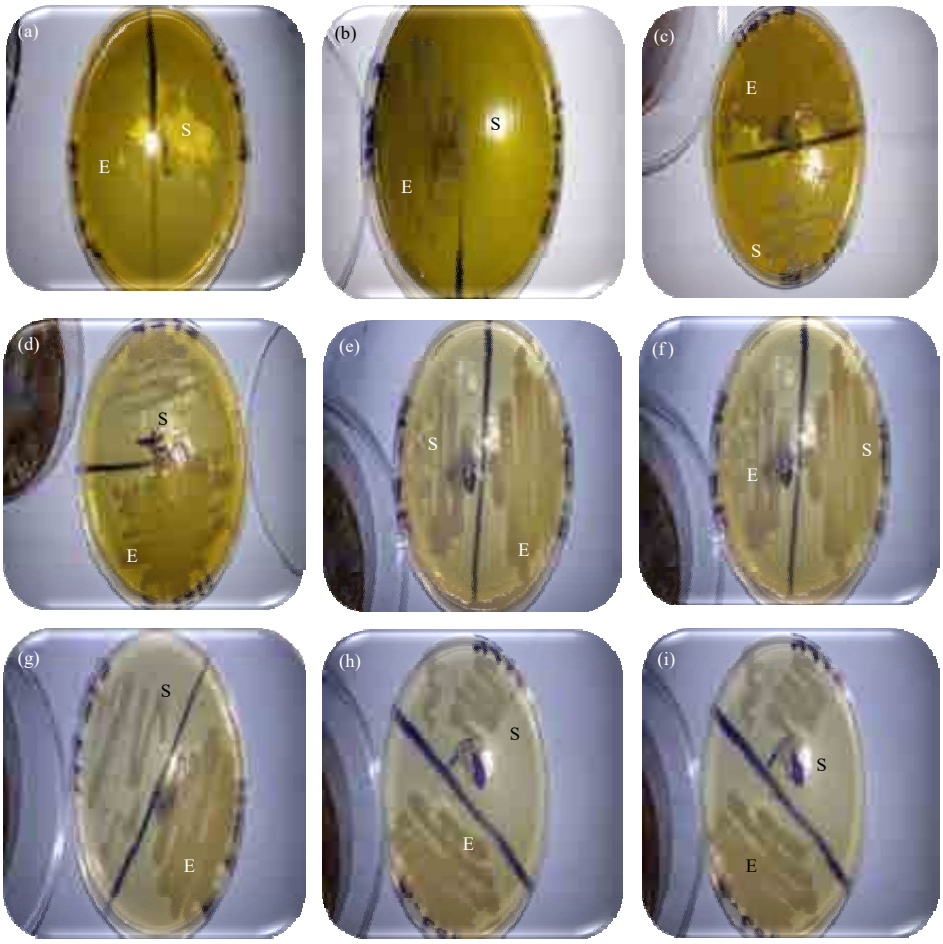

Fig. 2(a-i): Minimal inhibitory concentration of *Inula viscosa* L. extract towards the reference strains, (a) 100 mg mL^{-1}, (b) 50 mg mL^{-1}, (c) 25 mg mL^{-1}, (d) 12.5 mg mL^{-1}, (e) 6.25 mg mL^{-1}, (f) 3.16 mg mL^{-1}, (g) 1.56 mg mL^{-1}, (h) 0.78 mg mL^{-1} and (i) 0.39 mg mL^{-1}
E: *Escherichia coli* ATCC25922 S: *Staphylococcus aureus* ATCC29213

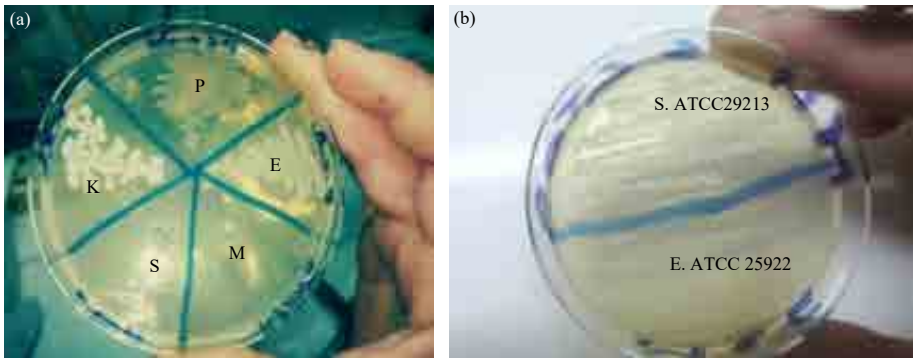

Fig. 3(a-b): Determination of bacteriostatic/bactericide effect of the polyphenolic extract
E: *Escherichia coli*, E. ATCC25922: *Escherichia coli* ATCC25922, K: *Klebsiella pneumoniae*, M: *Morganella morganii*, S: *Staphylococcus aureus*, P: *Pseudomonas aeruginosa*, S: ATCC29213: *Staphylococcus aureus* ATCC29213

Determination of the bacteriostatic/bactericide effect of the plant extract: The sub culturing of the strains from the inhibition zones was preceded. *Pseudomonas aeruginosa*, *Staphylococcus aureus* ATCC29213 and *Klebsiella pneumoniae* had grown up which confirms the bacteriostatic effect of the extract on these strains (Fig. 3). On the other

hand no growth of the strains of *Staphylococcus aureus* was observed, *Morganella morganii* and *Escherichia coli* ATCC25922 which confirms the bactericide effect of the extract on these strains. The bacteriostatic effect of the extract may be due to the interference of polyphenols with proteins synthesis, DNA production or with cellular metabolism. The bactericide effect of the polyphenolic is not well elucidated, it may be due to the capacity of polyphenols to intercalate with the cellular membranes and to deteriorate them[38].

CONCLUSION

The abusive use of antibiotics favourites the apparition of resistant bacterial strains which limits the number of used antibiotics. The assigned objective of this study is to evaluate in *vitro* the antibacterial activity of the polyphenolic extract of widespread plant in Algeria *Inula visocsa* L. which was harvested from the region of El Harrouch, the North East of Algeria. In light of the achieved results we can conclude that *Inula viscosa* L. is a natural promising source of chemical molecules which has a very important anti-bacterial activity. The obtained results *in vitro* seem to be interesting but it is only a 1st step in the research of active bio-molecules. Therefore, complementary essays are necessary to confirm the highlighted performances of the plant. This concerns mainly the characterisation of the active principles of the plant, to test *in vivo* the antibacterial activity of the plant and to evaluate the existence of a potential toxicity of the plant.

This study provides new and less expensive source of natural molecules which may be used as complementary drugs with antibiotics. As such, it helps in overcoming the problem of bacterial multi-resistance and finding effective treatments of infectious diseases.

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REFERENCES

1. Zhang, R., K. Eggleston, V. Rotimi and R.J. Zeckhauser, 2006. Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States. Globaliz. Health, Vol. 2. 10.1186/1744-8603-2-6.
2. Paterson, D.L., 2008. Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clin. Infect. Dis., 47: S14-S20.
3. Berche, P., J.L. Gaillard and M. Simonet, 1988. Bactériologie: Bactéries des Infections Humaines. Flammarion Médecine-Sciences, Paris, France, ISBN: 2257104897, Pages: 660.
4. WHO., 2002. Resistance to antimicrobes. Help-Memory No. 194, World Health Organization, Geneva, Switzerland.
5. Trojan-Rodrigues, M., T.L.S. Alves, G.L.G. Soares and M.R. Ritter, 2012. Plants used as antidiabetics in popular medicine in Rio Grande do Sul, Southern Brazil. J. Ethnopharmacol., 139: 155-163.
6. Alviano, D.S. and C.S. Alviano, 2009. Plant extracts: Search for new alternatives to treat microbial diseases. Curr. Pharm. Biotechnol., 10: 106-121.
7. Pai, P.G., M.M. Dayakar, A.R. Nath and G. Ashwini, 2019. Phytotherapeutics in the management of periodontal disease-a review. SRM J. Res. Dent. Sci., 10: 82-89.
8. Calixto, J.B., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (phytotherapeutic agents). Braz. J. Med. Biol. Res., 33: 179-189.
9. Oka, Y., B.H. Ben-Daniel and Y. Cohen, 2006. Control of *Meloidogyne javanica* by formulations of *Inula viscosa* leaf extracts. J. Nematol., 38: 46-51.
10. Parolin, P., M.I. Scotta and C.G. Bresch, 2016. Biology of *Dittrichia viscosa*, a Mediterranean ruderal plant: A review. Phyton: Int. J. Exp. Bot., 83: 251-262.
11. Sassi, A.B., F. Harzallah-Skhiri, N. Bourgougnon and M. Aouni, 2008. Antiviral activity of some Tunisian medicinal plants against *Herpes simplex* virus type 1. Nat. Prod. Res., 22: 53-65.
12. Benseguni-Tounsi, L., 2001. Etude *in vitro* de l'effet antibacterien et antifongique de *Inula viscosa*, *Lawsonia inermis*, *Asphodelus microcarpus*, *Aloe vera*, *Juniperus oxycedrus*. Master Thesis, Université de Constantine, Algeria.
13. Hmamouchi, M., J. Hamamouchi, M. Zouhdi and J.M. Bessiere, 2001. Chemical and antimicrobial properties of essential oils of five Moroccan Pinaceae. J. Essent. Oil Res., 13: 298-302.
14. Ibrahim, S.R.M., N.S.A.D.A. El-Shaer, H.Z. Asfour, K.Z. Elshali, M.I.A. Shaaban, A.A.M. Al-Attas and G.A.A. Mohamed, 2019. Antimicrobial, antiquorum sensing and antiproliferative activities of sesquiterpenes from *Costus speciosus* rhizomes. Pak. J. Pharmaceut. Sci., 32: 109-115.
15. Talib, W.H., M.H. Abu Zarga and A.M. Mahasneh, 2012. Antiproliferative, antimicrobial and apoptosis inducing effects of compounds isolated from *Inula viscosa*. Molecules, 17: 3291-3303.
16. Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, UK., ISBN-13: 9780412572708, pp: 203-214.

17. Ponce, A.G., R. Fritz, C. del Valle and S.I. Roura, 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. LWT-Food Sci. Technol., 36: 679-684.
18. Benzeggouta, N., 2015. Study of antibacterial activity of infused essential oils of four medicinal plants know as aliments. Master Thesis, University of Mentouri, Constantine, Algeria.
19. Kunle, O., J. Okogun, E. Egamana, E. Emojevwe and M. Shok, 2003. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. Phytomedicine, 10: 59-61.
20. Walsh, S.E., J.Y. Maillard, A.D. Russell, C.E. Catrenich, D.L. Charbonneau and R.G. Bartolo, 2003. Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J. Applied Microbiol., 94: 240-247.
21. CA-SFM., 2010. Comite de l'Antibiogramme de la Societe Francaise de Microbiologie recommandations 2010. Société Française de Microbiologie, Paris, France, January 2010.
22. Chebouti-Meziou, N., 2016. Contributions to study of the antimicrobial activity of Inula viscosa harvested in Boumerdes (Algeria). Int. J. Adv. Chem. Eng. Biol. Sci., 3: 159-162.
23. Lee, K.W., Y.J. Kim, H.J. Lee and C.Y. Lee, 2003. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J. Agric. Food Chem., 51: 7292-7295.
24. Ramli, B., 2013. Extraction of flavonoides of the plant *Inula viscosa*, the region of Oran and highlighting of antimicrobial activity. Master Thesis, University of Oran, Algeria.
25. Benhammou, N., 2005. Contribution to the study of antifongic power of *Inula viscosa* essential oil. Master Thesis, Department of Biology, University of Abou Bekr Belkaïd, Tlemcen, Algeria.
26. Inouye, S., T. Takizawa and H. Yamaguchi, 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrobiol. Chemother., 47: 565-573.
27. Lopez, V., S. Akerreta, E. Casanova, J.M. Garsia-Mina, R.Y. Cavero and M. Calvo, 2008. Screening of Spanish medicinal plants for antioxidant and antifungal activities Pharmaceut. Biol., 46: 602-609.
28. Bozin, B., N. Mimica-Dukic, N. Simin and G. Anackov, 2006. Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food Chem., 54: 1822-1828.
29. Bagamboula, C.F., M. Uyttendaele and J. Debevere, 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol., 21: 33-42.
30. Ulubelen, A., S. Oksuz and N. Goren, 1987. Sesquiterpene acids from *Inula viscosa*. Phytochemistry, 26: 1223-1224.
31. Wollenweber, E., K. Mayer and J.N. Roitman, 1991. Exudate flavonoids of *Inula viscosa*. Phytochemistry, 30: 2445-2446.
32. Silver, L.L., 2011. Challenges of antibacterial discovery. Clin. Microbiol. Rev., 24: 71-109.
33. Garcia-Ruiz, A., B. Bartolome, A.J. Martinez-Rodriguez, E. Pueyo, P.J. Martin-Alvarez and M.V. Moreno-Arribas, 2008. Potential of phenolic compounds for controlling lactic acid bacteria growth in wine. Food Control, 19: 835-841.
34. Kempf, I. and S. Zeitouni, 2012. [The cost of antibiotic resistance: Analysis and consequences]. Pathol. Biol., 60: e9-e14, (In French).
35. Vasey, C., 2014. Alternatives Naturelles aux Antibiotiques: Lutter Contre les Infections en Renforçant le Terrain. 2nd Edn., Editions Jouvence, France, ISBN: 978-2-88911-526-6, Pages: 222.
36. Albano, S.M. and M.G. Miguel, 2011. Biological activities of extracts of plants grown in Portugal. Ind. Crops Prod., 33: 338-343.
37. Candan, F., M. Unlu, B. Tepe, D. Daferera, M. Polissiou, A. Sokmen and H.A. Akpulat, 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J. Ethnopharmacol., 87: 215-220.
38. El Amri, J., K. Elbadaoui, T. Zair, H. Bouharb, S. Chakir and T.I. Alaoui, 2014. [Study the antibacterial activity of essential oils *Teucrium capitatium* L and *Silene vulgaris* extract on different strains tested]. J. Applied Biosci., 82: 7481-7492, (In French).