Anti-bacterial activity of methanolic extracts of the aerial parts of *Inulaviscosa* l., from Guelma (Algeria)

KarimaOunaissia[1,a],Asma Nassar[1], Bouthaina Soltani[1], Salima Bennadja[2] and Abdelghani Djahoudi[3]

[1]Laboratory of Medical Botany, Faculty of Medicine.University of Annaba. Algeria

[2]Laboratory of Biochemistry and Environmental Toxicology.University of Annaba. Algeria

[3]Laboratory of Microbiology, Faculty of Medicine, University of Annaba, Algeria

Abstract: the objective assigned to the present study isthe evaluation of the antibacterialactivity of the methanolic extracts of the aerial parts of *Inulaviscosa* L. The antibacterial activity of the extracts is carried out by the diffusion method on agar medium vis-à-vis twenty three bacterial strains, chosen according to the traditional use of this species in Algeria: *Bacillus cereus, Escherichia coli* (ATCC22), *Escherichia coli* (BLSE), *Escherichia coli* (ciproR), *Escherichia coli* (mcr1), *Klebsiellapneumoniae* (C+), *Klebsiellapneumoniae* (C-), *Klebsiellapneumoniae*Nassey Marseille, *Serratiasp, Salmonella sp, Pseudomonasaeruginosa* (ATCC53), *Pseudomonas sp, Pseudomonas aeruginosa* (VIM21), *Pseudomonas aeruginosa* (VIM22), *Acinetobacter* (NDM1), *Acinetobacter* (OXA23), *Staphylococcus aureus, Staphylococcus aureus* (23), *Staphylococcus aureus* (13), *Enterobacter cloacae* (FOSR1), *Enterobacter cloacae* (FOSR2), *Enterococcus faecalis*(Vanc R), *Enterococcus faecalis* (ATCC12). *Pseudomonas aeruginosa* (ATCC53) was the most sensitive species followed by *Pseudomonas sp*. significant sensitivity was observed for the *Klebsiellapneumoniae* isolated in Marseille. However, *Staphylococcus aureus* was the most resistant to the extracts tested.

Keywords: *Inula viscosa*L., Guelma, aerial part, methanolic extract, antibacterial effect.

[*]*Corresponding author e-mail address: ounaissia_k@yahoo.fr*

1. Introduction

Currently, several questions have arisen regarding the safety and efficacy of chemicals used in medicine. Indeed, over the last 20 years, it has been proven that the effectiveness of antibiotics has decreased significantly. Bacteria have become increasingly resistant to them due to the widespread use of these molecules and their large-scale and sometimes inappropriate prescription (Kheyar et al. 2014).

The therapeutic limits of classical antibiotics have pushed scientiststodirect research towards new avenues and especially the use of active plant ingredients (phenolic compounds, alkaloids, essential oils...) as antibacterial agents.

The Algerian flora is characterized by its floral diversity: Mediterranean, Saharan and a Paleo Tropical flora, estimated at more than 3000 species belonging to several botanical families, of which 15% are endemic (Gaussen et al. 1982). This has given the traditional pharmacopoeia an inestimable richness. Among these, *Inulaviscosa* L., subject of this article, this species is widely used in traditional medicine, such as treatment of skin irritations, diabetes, diuretic, antiseptic, anti-inflammatory and antipyretic.Commonly called in Eastern Algeria "Magramen", in order to confirm or deny these therapeutic virtues, we evaluated in vitro its inhibitory power against twenty-three pathogenic and multi-resistant strains.

2. Materials and methods

2.1. Plant Material

The species *Inulaviscosa*L. was harvested in the region of Guelma (Eastern Algeria) in December 2019.

The identification of the plant was done with the key of determination of the flora of Quezeland Santa (1963). Specimens were kept at the Laboratory of Cryptogamy and Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

2.2. Preparation of Methanolic Extracts

Dry aerial parts (stem and leaf) of *Inulaviscosa*L. have been ground and stored in glass bottles, hermetically sealed at low temperatures. 3 g of the vegetable powder was macerated in 60 ml of methanol with stirring for 24 hours at a temperature of 25 ± 2° C. The extract obtained was filtered and evaporated to dryness under reduced pressure at 50°C using a rotavapor.

The dry residue is solubilized in 3 ml of methanol and stored at -18 ° C until it is used(Falleh et al. 2008).

The yield of the methanolic extracts was calculated by the following formula: R (%) = (M/M_0) ×100. With: R (%): yield expressed in%; M: mass in grams of the resulting dry extract; M_0: mass in grams of the plant material to be treated.

2.3. Antibacterial test

The test of the sensitivity of the bacteria is carried out by the diffusion method in agar medium (the disk method). It is a method similar to that of the antibiogram which consists in determining the sensitivity of a bacterial strain vis-à-vis one or more substances(Falleh et al. 2008).

The antibacterial activityof the methanolic extracts of the aerial parts (stem and leaf) of *Inulaviscosa*L. is evaluated vis-à-vis twenty three bacterial strains, chosen according to the traditional use of this species in Algeria:*Bacillus cereus, Escherichia coli* (ATCC22), *Escherichia coli* (BLSE), *Escherichia coli* (ciproR), *Escherichia coli* (mcr1), *Klebsiellapneumoniae* (C+), *Klebsiellapneumoniae* (C-), *Klebsiellapneumoniae*Nassey Marseille, *Serratiasp, Salmonella sp, Pseudomonasaeruginosa* (ATCC53), *Pseudomonas sp, Pseudomonas aeruginosa* (VIM21), *Pseudomonas aeruginosa* (VIM22), *Acinetobacter* (NDM1), *Acinetobacter* (OXA23), *Staphylococcus aureus, Staphylococcus aureus* (23), *Staphylococcus aureus* (13), *Enterobacter cloacae* (FOSR1), *Enterobacter cloacae* (FOSR2), *Enterococcus faecalis*(Vanc R), *Enterococcus faecalis* (ATCC12). These strains were kindly provided by the Microbiology Laboratory Manager at Annaba Medical School, Algeria.

2.4. Preparation of the Inoculum

The inoculum of each strain is prepared by inserting colonies of the strain to be studied with a platinum loop into a tube containing 5 ml of sterile physiological serum.

For the preparation of the different concentrations of extracts, 2, 5 mg of each freeze-dried extract (methanolic extract of leaf and stem), are introduced into a labeled tube, in which we added 1 ml of dimethylsulfoxide (DMSO), solvent without any antibacterial effect.

The tubes are vortexed until complete dissolution of the extract, and the dilutions are prepared to obtain 1/3, 1/6 and 1/12 concentrations from the stock solution.

Seeding should be done within 15 minutes after the preparation of the inoculum. In 23 sterile Petri® dishes, 20 ml of agar are poured. After solidification of the medium, the latter is inoculated with 1 ml of bacteria to be studied.Then, it is spread on the surface using a glass rake.

Sterile 5 mm diameter disks prepared in Whatman® n°1 papers are impregnated using a micropipette with x volume of each concentration and placed on the surface of the solidified medium (Mueller Hinton) at the rate of 6 discs per box (3 leaf discs, 3 stem discs).

The dishes were incubated for half an hour at room temperature, then for 24 to 48 hours in an oven at 37 °C.

The reading is carried out by measuring the diameter of the inhibition zone (Ø), which translates into a translucent halo around each disc; the presence or absence of a halo would explain the sensitivity or the resistance of the germs vis-a-vis extracts tested; according to a symbolic notation scale from - to +++ (Ponce et al. 2003).

Table 1:Sensitivity of microbial strains according to zones of inhibition.

Sensitivity	Inhibition zone
Not sensitive or resistant (-)	Diameter <10 mm
Sensitive (+)	Diameter between 10 to 16 mm
Very sensitive (++)	Diameter between 16 to 25 mm
Extremely sensitive (+++)	Diameter> 25 mm

3. Results

3.1. Extraction yield

The yield of the methanolic extract of the stem is the highest, with a percentage of 22.3 %, and the yield of the methanolic extract of the leaf is 20.73 %.

3.2. Reading Antibiograms

The results of the antibacterial activity are shown in the Table (2).

Table 2: Inhibition Diameter (mm) of methanolic extracts of *Inulaviscosa* L.

	Dilutions of leaf extract			Dilutions of stem extract			T
	X/3	X/6	X/12	X/3	X/6	X/12	
Bacillus cereus	8,1	8,2	8 ,5	9,5	8	7	6
Candida albicans	15,5	15,5	14	14	13	12,5	6
EscherichiaAcoli (ATCC22)	7	7	8,5	7,5	7	6,5	6
Escherichia coli (BLSE)	9,5	9,5	10	10	11	11	6
Escherichia coli (ciproR)	8,5	8,5	9	7,5	8,5	8	6
Escherichia coli (mcr1)	7,5	10,1	13	10	12	13,1	6
Klebsiellapneumoniae (C^+)	<6	7,9	8,5	9	6,5	7	6
Klebsiellapneumoniae (C^-)	6	8	8,9	8	7,5	8,9	6
KlebsiellapneumoniaeNassey Marseille	15,9	13,9	16, 3	16	15,5	15 ,9	6
Serratiasp	7,5	8	10	7	8	8,5	6
Salmonella sp	13,5	11,1	10	12	10	8	6
Pseudomonas aeruginosa (ATCC53)	21,2	13,2	12,1	14,1	9,6	13,2	6
Pseudomonas sp	19,7	17,2	15, 3	10,2	11,2	19,2	6
Pseudomonas aeruginosa(VIM21)	16,5	15,5	8,1	14,1	8,2	8,1	6
Pseudomonas aeruginosa (VIM22)	7	8	10,9	7	9	10	6
Acinetobacter (NDM1)	15	13	10	10	10,1	12	6
Acinetobacter (OXA23)	8,5	10,1	12	7,5	7	10	6
Staphylococcus aureus	<6	<6	<6	<6	<6	<6	6
Staphylococcus aureus (23)	13,1	13	10	9	9,9	<6	6
Staphylococcus aureus (13)	11,8	12,2	12,5	8	9,5	10	6
Enterobactercloacae (FOSR1)	8,7	8,5	9	7	7,1	7,3	6
Enterobactercloacae (FOSR2)	9,6	8	7,5	7	8,1	9	6
Enterococcusfaecalis(Vanc R)	9	9	11	10	9	11	6
Enterococcusfaecalis (ATCC12)	13 ,1	12,9	9	10	9,9	10	6

T: control (disc soaked with DMSO: dimethyl sulfoxide)

4. Discussion

By comparing our results with those of (Chahmi et al. 2015) who worked on the same species, originating from three Moroccan regions, we notice that the yield of our sample is close to their yields which are of the order of 23.90%, 20.08% and 13.35%. Indeed, the yield depends on the method and the conditions under which the extraction was made. Otherwise, this difference may be due to the nature of the plant material (Smith et al. 2001). It varies according to the organ harvested, the period and the method of harvesting. It is closely related to the climatic factors of the environment (rainfall, altitude, latitude and the nature of the soil). Storage and conditioning also affect yield (Lee et al. 2003).

On the other hand, (Lee et al. 2003) and (Athamena et al. 2010) note that the extraction method also affects the total polyphenol and flavonoid content. These same authors proved that ethanol is the best solvent for the extraction of phenolic compounds, followed by methanol and finally water.

The strain sensitivity test showed the presence of some antibacterial activity. According to Table (2),the most sensitive germ is *Pseudomonas aeruginosa* (ATCC53) with a diameter of 21.2 mm for the dilution of the leaf extract X/3; followed by *Pseudomonassp* with the diameters 19.7 / 17.2 / 15.3 mm for the dilutions of the leaf extract X/3, X/6 and X/12 respectively and the diameter 19.2 for the X/12 dilution of the stem extract, we also noticed an important sensitivity for all the used dilutions of the stem and leaf for the germ: *Klebsiellapneumoniae* isolated in Marseille. An absence of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (ATCC22) was observed.A low concentration gives more activity than a high concentration for several strains was noticed,a saturation of the molecular target by the active compounds could explain this effect(AboyaMoroh, 2013).

Comparing our results with the work of (Ramli 2013), he states that E. coli (ATCC22) is resistant to the lyophilized hydroalcoholic extract (methanol-water) of *InulaViscosa*. He also reports that no inhibition zones were observed, which would have allowed him to conclude that *E. coli* (ATCC22) is resistant to the molecules contained in the hydroalcoholic extract of *Inulaviscosa*. The work of (Laghrifi et al. 2013) shows that the methanolic and ethanolic extract of *Inulaviscosa* have a strong antibacterial power against Gram-positive and Gram-negative bacteria including *E.coli* and *K. pneumoniae*. The methanolic extract was found to be the most active against all bacterial strains tested. In addition, they also found that the ethanolic extract was more antibacterial than the aqueous extract.

In addition, the same authors report that the ethanolic extract from the flowers of Inulaviscosa was found to be more active on K.pneumoniae than the ethanolic extract from the leaves of the same plant. Bssaibis et al. (2009) reported that *Inulaviscosa* leaf and flower extracts have antibacterial activities against *E.coli* and two other gram-positive bacteria. The results obtained show that the antibacterial activities are related to the organ used (flowers or leaves), the nature of the extraction solvent and the strain tested. Indeed, extracts with methanol are the most active, followed by those with ethanol and then those with acetone. The precise mechanisms involved in the antimicrobial action of extracts are still far from being fully elucidated given the richness of a plant extract in chemical components, it is obvious that the antibacterial activity cannot be due to a single specific mechanism of action but rather to various mechanisms. Recalling that *Inulaviscosa* L. contains flavonoids, saponins, tannins, tri-terpenes and other phenolic compounds, these compounds having known antibacterial properties, their presence could therefore explain the observed microbial properties (Scalbert 1991). Rojas et al (1992) and (Marjorie 1999) showed that flavonoids, tri terpinoids, tannins and other phenolic compounds or free hydroxyl groups are classified as highly active antibiotic compounds. Furthermore, it has been shown that the mechanism of toxicity of flavonoids on microorganisms is either by deprivation of metal ions such as iron, or by non-specific interactions such as the establishment of hydrogen bridges with microorganism cell wall proteins (Bruneton 1999).

Therefore flavonoids known for their antioxidant power could potentially have an effect in iron chelation and thus prevents intracellular penetration of the Ca+2 cofactor to the bacterial cell, which causes inhibition of their activity (Kokkini 1996; Raj et al. 2001), according to Paris and Moyse (1965) the antibacterial effectiveness of flavonoids results from the inhibition of bacterial enzymes due to the addition reaction with the thiol or amine group. Bezzaz (2014), found in his work that flavonoids can cause a leakage of potassium ions at the plasma membrane, resulting in major lesions leading to death.

Regarding the influence of the wall on the sensitivity to extracts; the gram (-) bacteria are rich in proteins assembled in a lipopolysacharride layer (LPS), which thanks to its negative surface charges prevent the diffusion of hydrophobic molecules, and the proteins exclude the passage of hydrophilic molecules of high molecular weight, the membrane therefore constitutes an effective permeability barrier, while the gram (+) bacteria are less protected, because peptidoglycan only hinders the diffusion of molecules higher than 50000 D (Hogan and Kolter 2002), therefore according to this theory gram (-) bacteria must present a strong resistance to

the extract used compared to gram (+) bacteria, our results obtained escape a little from this theory with a strong activity towards the *Pseudomonas aeruginosa* (ATCC53), *Pseudomonas sp*, *Klebsiellapneumoiae* isolated in Marseille, *Acinetobacter* (NDM1) and *Escherichia coli* (BLSE) (gram negative bacillus), a complete absence of antibacterial activity on *Staphylococcus aureus* (gram positive bacillus) and *Escherichia coli* (ATCC22) (gram negative bacillus), This can be explained by the variety of bacterial strains used (acquired resistance such as MRSA-like staphylococcus with high resistance), or because the discs applied do not contain the same concentration of the extract (accuracy problem due to handling) or this concentration has not reached the MIC (minimum inhibitory concentration) of the bacteria .

5. Conclusion

The search for new antimicrobial substances that are purely natural and effective is the main concern of most researchers today. The present work to develop a medicinal plant that is very abundant in the Algerian flora but little exploited in the scientific world despite its many therapeutic virtues of the family Asteraceae (*Inulaviscosa* L.).

The evaluation of the antibacterial activity by the method of diffusion in agar medium, showed that the two methanolic extracts have an inhibitory effect on the majority of the strains tested.

Our results contribute to the scientific validation of the massive traditional use of this species by Algerian populations. In perspective, it would be important to deepen research on a wide range of microbial strains and to identify the active constituents responsible for the antibacterial activity.

Acknowledgement

We have financial interests related to the material in the manuscript from Faculty of Medicine, university of Annaba - Algeria.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

References

AboyaMoroh, J.L. (2013). Résistance bactérienne et phytomolécules antimicrobiennes issues de *Morindamorindoides*. Sciences agricoles. Université de Bretagne occidentale - Brest.NNT: 2013BRES0028, 214p.

Athamena, I.S., chalgheman, A., Kassah, L.S., Laroui, S., khebri, S. (2010). Activité antioxydant et antimicrobienne d'extrait de *Cuminiumcyminium* L. Libanaise science Journal, 472-478.

Bendahou, M., Benyoucef, M., Benkada, D., Soussa Elisa, M.B.D., Galvao, E.L., Marque M.M.O., Muselli A., DesjobertJ.M., BernardiniA.F., Costa J. (2007). Influence of the processus extraction on essential oil of *Origanumglandulosum*. *Journal of applied sciences*, 7(8): 1152-1157.

Bezzaz, N. (2014). Détermination structurale des métabolites secondaires, et extraction des huiles essentielles de *Mentharotundifolia*. Mémoire de magistère. Université de M'sila .Faculté des Sciences Département de Chimie, Algérie.

Bruneton, J. (1999). Pharmacognsie. Photochimie, plantes médicinales. Ed Lavoisier, 1120 p.

Bssaibis, F., Gmira, N., Meziane. (2009). Activité antibactérienne de *Ditrichiaviscosa*(L.)W Greuter. Rev. Microbial. Ind. San et Environn, 3(1): 44-45.

Chahmi, N., Anissi, J., Jennan, S., Farah, A., Sendide, K., ElHassouni, M. (2015). Antioxidant activities and total phenol content of *Inulaviscosa* extracts selected of Morocco. Asian Pacific Journal of Tropical Biomedicine, 5(3), 228-233.

Falleh H., Ksouri R., Chaieb K., Karray-Bouraoui N., Trabelsi N., Boulaaba M., Abdelly C. (2008). Phenolic composition of *Cynaracardunculus*L. organs, and their biological activities.*Comptes Rendus de Biologie*, 331: 372-379.

Gaussen, H., Deuroy, J.F., Ozenda, P. (1982). Précis de botanique II «les végétaux supérieurs ». Ed : Masson ,1982 ; pp 215-408.

Hogan, D.A., Kolter, R. (2002). Pseudomonas-Candida Interactions: An Ecological Role for Virulence Factors. *Science*, 296, 2229-2232.

Kheyar, N., Meridja, D., Belhamel, K. (2014). Etude de l'activité antibactérienne des huiles essentielles d'*Inulaviscosa*, *Salviaofficinalis*et *Laurusnobilis*de la région de Bejaia. *Algerian J. Nat. Products*, 2(1): 18-26.

Kokkini, S. (1996).Taxonomy, diversity and distribution of Oreganum species.Proceeding of the IPGRI International Workshop on Oregano. Bari, Italy.

Laghrifi, K., Lemrhari, A., Zouhair, R., El Idrissi, M., Makoudi, Y., Alnamer R. (2013). In vitro potential antibacterial activity of the dichloromethane and hexane extract of *Inulaviscosa* from Morocco. International journal of universal pharmacy and bio science, 2(6): 166- 178.

Lee, K.W., Kim, Y., Lee, H.J., Lee C.Y. (2003). Cocoa Has More PHENOLIC phytochemicals and a Higher Antioxidant Capacity than Teas and Red Wine.J.Agric.Food Chem., 51:7292-7295.

Marjorie, M.C. (1999). Plant products as antimicrobial agents. ClinicalMicrobiology, 12 (4) : 564 -582.

Paris, R.R., Moyse, H. (1965). Abrégé de matières médicales. Collection de pharmacie sous la direction de JAMOT, tome 1. Ed. Masson pp. 78-79-453.

Ponce A.G., Fritz R., Delvalle C., RouraS.I. (2003).Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *Lebensmittel-Wissenschaft and Technologic*, 36: 679-684.

Quezel, P., Santa, S. (1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales. Ed Centre national de la recherche scientifique, 1170 p.

Raj, N.K., Spiral, R.M., Chaluvadi, M.R., Krishna, D.R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential.Indian J Pharmacol, 33: 2–16.

Ramli B. (2013). Extraction flavonoïdes de la plante *Inulaviscosa* de la région d'Oran et mise en évidence de l'activité microbienne Mémoire du magistère en chimie. Universitéd'Oran, Algérie, 77p.

Rojas, A., Hernandez, L., Pereda-Miranda, R., Mata, R. (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants.Journal of ethnopharmacology, 35(3), 275-283.

Scalbert, A. (1991). Antimicrobial properties of tannins.*Phytochemistry*, 30: 3875-3883.Smith-Palmer, A., Stewart, J., Feyel, L. (2001).The potential application of plants essential oils as natural food perservative in soft cheese. Food Microbiology, 18: 463-470.

Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.info **ISSN 2458-5920**