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Original Article

Antibacterial and Antioxidant Activities of Essential Oil of Inula Viscosa *L.* from Northwest of Algeria

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ABSTRACT

Inula viscosa has been used for years in folk medicine for its anti-inflammatory, antipyretic, antiseptic, and paper antiphlogistic activities. The aim of the present study is to investigate the antibacterial and antioxidant activities of the essential oils extracted by hydrodistillation from the leaves of *Inula viscosa* Ait., which was collected from Mohammadia region near Mascara city in the Northwest of Algeria. Antibacterial activity of the essential oils against *Escherichiacoli* and *Staphylococcus aureus*, was tested using the agar well-diffusion method and disc diffusion technique by determining the inhibition zone, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results of the diffusion methods showed that the oils had strongest antibacterial activity with inhibition zones ranged between 14-33 mm against *S. aureus*. The MIC and MBC were respectively 4.17 µl /ml and 8.32 µl /ml on *S. aureus*, in contrast, *Escherichiacoli* was resistant to the essential oil. Antioxidant activity was determined by in vitro tests using a quantitative DPPH (1,1-diphenyl-2-picryl hydrazyl) assay. *I. viscosa* oil exhibited effective radical scavenging capacity compared to the positive control (ascorbic acid) with 50% inhibitory concentration (IC_{50}) of 0.16 mg/mL. These results could support the use of plant by traditional healers to treat various infective diseases. These properties indicate the possibility of exploitation of essential oil of *I. viscosa* for food and pharmaceutical industries.

Keywords: Antibacterial activity, Antioxidant activity, *Inula viscosa* Ait. Essential oil.

INTRODUCTION

Resistance of microorganisms to antibiotics has created an immense clinical problem in the treatment of infectious diseases and has complicated the treatment of infectious diseases in immuno-compromised patients and nosocomial infections. This has rekindled the search for novel antibiotics (Ezeronye *et al*., 2005). In other hand, it has been found that free radicals and other reactive oxygen species play a cardinal role in oxidative damage to cellular constituents which leads to cell injury and death. This has been associated with pathogenesis of various chronic disease, e.g. carcinomas, coronary heart disease, and many other hearth

problems related to advancing age (Nantitanon *et al*., 2007). Therefore, there is a need to develop new and safe antioxidants from natural sources to minimize the oxidative damages in living cells.

Essential oil has a wide spectrum of different impressive qualities. Due to their multifunctional, it found a huge application area in medicine and aromatherapy. EOsshow significant antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria, fungi, yeasts and viruses. Therefore, plants were used for the treatment of infectious illnesses since ancient times even though (Al Yousef, 2014).

Currently, the use of plant-based natural antioxidants, such as those of phenolic substances like flavonoids and phenolic acids and tocopherols in foods, as well as preventive and therapeutic medicine, is gaining much recognition. Such natural substances are believed to exhibit anticarcinogenic potential and offer diverse health-promoting effects because of their antioxidant attributes (Anwar *et al*., 2008).

Asteraceae is the largest family of flowering plants in the world. The family includes over 1,600 genera and 23,000 individual species. Many members of the *Asteraceae* family are important for medicinal, ornamental, and economic purposes (Gao *et al*., 2010). *Inula* is a large genus in the tribe *Inuleae* with more than one hundred species. It comprises several species of reputed medicinal value (Seca *et al*., 2013). As far as ethnopharmacologic background is concerned, *Inula viscosa*is a well-known species amongst the members of *Inula* in Mediterranean region. It is a herbaceous perennial plant which occurs from southern Europe and Turkey, to the Middle East and northern Africa in the coastal range of the Mediterranean Sea without a clear microhabitat restriction (Parolin *et al*., 2013). In folklore medicine, the plant has many uses, including anthelmintic, lung disorders, antipyretic, antiphlogistic activities, to treat tuberculosis, anemia and as cataplasm for rheumatic pain, and it has been used for its antiseptic, skin inflammations properties and gastroduodenal disorder treatment (Chahmi *et al*., 2015; Talib *et al*., 2012).

The purpose of the present study was to evaluate the antioxidant and antimicrobial effectiveness of the essential oil of *Inula viscosa* leaves grown in the north westregion of Algeria.

MATERIAL AND METHODS

Collection of Herb Material

Leaves of *I. viscosa* (*Asteraceae*) were collected in the northwest part of Algeria (Mohamadia, Mascara), in March to April 2015, from a single collection site. The leaves were air-dried in the shade at the ambient temperature. The identity of the plant specimen was confirmed at the Department of Biology, Faculty of Natural Sciences, University of Mascara.

Extraction of Essential Oil

The dried plant samples were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The oil recovered was stored in darkness at 4°C. The yield percentages, calculated as volume (ml) of EO per 100 g of plant dry matter, were 0.12%.

DPPH Scavenging Activity

Free radical scavenging activity was measured by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Molyneux (2004) with modifications. Briefly, a 1.0 ml aliquot of test sample was added to1.0 ml of 0,015 g/100mlDPPH methanolic solution. The mixture was shaken vigorously then left to stand at room temperature for one hour in darkness. Changes in the absorbance of the samples were measured at 517 nm using a spectrophotometer.
The percentage inhibition of activity was calculated as:

DPPH scavenging effect (%) = $[(A_0 - A_1 /A_0)]\times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the sample.

Ascorbic acid was used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the extract concentration.

Microbiological Screening

Antimicrobial activities of *I. viscosa* essential oils were evaluated by the agar well-diffusion method, Disc diffusion technique, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Microbial Strains

The In vitro antimicrobial studies were carried out against *Staphylococcus aureus* and *Escherichia coli*. The two microorganisms were clinical isolates obtained from the laboratory of Medical Analysis at Meslam Tayeb hospital in Mascara city situated in the North West of Algeria. The identity of the microorganisms used in this study was confirmed by standard biochemical tests and morphological studies.

The bacterial cultures were maintained on nutrient agar medium and was further maintained by sub-culturing regularly on the same medium and stored at 4°C, before use in experiments.

Determination of Antimicrobial Activity

Disc Diffusion Method

Antibacterial screening of the essential oils against test bacteria was done by disc diffusion method as reported by Alim *et al*., (2009) with modification. Mueller Hinton plates were seeded with bacterial strains and yeast, respectively. 1, 5, 10 and 15 µl of essential oils was applied on each sterilized paper disc. The plates were incubated at 37°C for overnight for bacterial pathogens to observe and measured the zone of inhibitions around the disc.

Agar-well diffusion method

The agar well-diffusion method was followed to determine the antimicrobial activity as reported by Archanaand Abraham (2011) with modification. Mueller Hinton plates were swabbed (sterile cotton swabs) with microorganisms cultures. Wells were made in each of these plates by using a sterile cork borer. Different volumes of the essential oils (1, 5, 10 and 15 µl) were added with a sterile syringe into the wells. The plates were incubated at 37°C for 24 hours. The inhibition zones formed around the wells were measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of the essential oils against the two test organisms was determined using the broth dilution method. About 50 µL of essential oil was added to test tube containing 2 750 µL of sterile nutrient agar.

A dilution series of the essential oil was obtained using Dimethylsulfoxide (DMSO) as the solvent. The final concentrations were 8.32, 4.17, 2.15, 1.04, 0.52, 0.26, and 0.13 mg/ml. The tubes were then incubated with 30 µL of the bacterial suspension and incubated at 37 °C for 24 h. The 8th test tube did not contain any essential oil, but a solution of pure solvent served as negative control. After incubation, the MIC of essential oil was determined by visual inspection of the tubes. The lowest concentration of the active ingredient that inhibited growth of the microorganism, as detected by lack of visual turbidity, was assigned to be the MIC. The MBC values were determined by making subcultures from the clear tubes which did not show any growth onto Mueller Hinton agar plates and incubated at 37 °C for 24 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC.

RESULTS AND DISCUSSIONS

Antioxidant Activity

Antioxidant activity of essential oils extracted by hydrodistillation from leaves, of *I. viscosa* has been determined by the DPPH assay. All data are presented in Table 1.

Table 1: Antioxidant activity (IC50 (mg/mL)) of the leaves of *I. viscosa* essential oil compared to Ascorbic acid

Samples	IC50 (mg/mL)
I. viscosa oil	0.16
Ascorbic acid	0.35

According to the DPPH assay results, the essential oils of the leaves of *I. viscosa* showed good antioxidant activity.The concentrations that led to 50% inhibition (IC_{50}) for *I. viscosa* oil and Ascorbic acid are 0.16 mg/mL and 0.35 mg/mL respectively.

Our findings are strongly agreed with several other studies, which reported great antioxidants profile of *I.viscosa* essential oils or extracts (Schinella *et al*., 2002; Bekkara *et al*., 2008; Mahmoudi *et al*., 2015; Chahmi *et al*., 2015).

Antioxidant activity of *I. viscosa* essential oils was reported to be derived partly from the presence of phenolic groups (Bekkara *et al*., 2008; Schinella *et al*., 2002; Mahmoudi *et al*., 2015; Chahmi *et al*., 2015). Polyphenols are known to be antioxidants, considerable interest was given to these compounds recently because

of their potential beneficial effects on human health in fighting diseases such as cancer and cardiovascular disease (Scalbert *et al*., 2005; Shohaib *et al*., 2011) and the most common types of polyphenols are flavonoids which are known to present in this plant (Grande *et al*., 1985; Wollenweber *et al*., 1991; Benayache *et al*., 1991; Danino *et al*., 2009). On the other hand, the putative antioxidant activity of *I. viscosa*extract could justify its use for the treatment of oxidant related disease such as diabetes and skin inflammation (Mahmoudi *et al*., 2015).

Antibacterial activity of essential oils

The antibacterial activity of *I. viscosa* oil is summarized in the Table 2. The results proved that *I. viscosa* oil had significant activity against *S. aureus* with diameters of inhibition zones ranged between 16 to 33 mm by Disc diffusion method and 14 to 30 mm by Agar-well diffusion technique. Against E. coli total absence of activity was noticed in sensitivity *I. viscosa* oil, by the two methods.

Tableau 2: Diameters of inhibition zones of *I. viscosa* essential oils

		E. coli	*S. aureus*
Disc diffusion - method	1µl	NI	16
	5µl	NI	20
	10µl	NI	20
	15µl	NI	33
Agar-well diffusion method	1µl	NI	14
	5µl	NI	16
	10µl	NI	30
	15µl	NI	20

NI: No Inhibition

The broth dilution method demonstrated general agreement with disk diffusion and Agar-well methods. *I. viscosa* oil showed bacteriostatic and bactericidal effects. The results of MIC and MBC of *I. viscosa* oil were recorded as in Table 3.

The MIC of *I. viscosa* oil was found to be 4.17 µl /mL, but *E. coli* did not show any sensitivity to tested concentrations of this essential oil.

Tableau 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *I. viscosa* essential oils against *S. aureus* and *E. coli*

Strains	CMI	CMB	CMB /CMI
E. coli	NI	NI	-
S. aureus	4.17 µl /ml	8.32 µl /ml	1,99 bactericidal activity

NI: No Inhibition

Many authors conducted studies which support the finding of the present work in which the *I. viscosa* oil was able to have inhibitory effects against *Staphylococcus* and no effect on *Escherichia coli* (Ali-Shtayeh *et al*., 1998; Bensegueni, 2001; Bekkara *et al*., 2008; Ramli, 2013; Benyahia, 2014). On the other hand, and in contradiction to the results obtained by this study, Boumaza (2011) reported that *E.coli* and *S. aureus* were totally resistant to this oil in the well agar diffusion method, but by broth dilution method *S. aureus* presented a CMI of 2.5µg /ml. Berhail *et al*., (2012) found that these oils were more effective against the two bacteria. Kheyar *et al*., (2014) also examined the antibacterial properties of three essential oils at four dilutions (1/1, 1/2, 1/4 and 1/8) prepared with DMSO against five organisms.

They found that *I. viscosa* essential oils had an inhibitory activity against *S. aureus* and *E. coli* at all dilutions. Bssaibis *et al*. (2009) investigated the antibacterial activity of *I. viscosa* (*Dittrichia viscosa*) extracts obtained by three different solvents (ethanol, methanol and acetone) at different dilutions (Pure, 1/10, 1/20, 1/40, 1/80, 1/100 and 1 /200) against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. They found that these extracts were more effective against the tested bacteria in the high concentrations (Pure, 1/10 and 1/20) but inhibited slightly the growth of the three organisms at lower levels. Maozand Neeman (1998) screened 10 plant aqueous extracts (including *I. viscoa*) for their antibacterial properties against *Bacillus subtilis*, *Sarcina lutea* and *Staphylococcus aureus* by using the agar dilution method. They found that *I. viscosa* aqueous extracts showed maximal inhibitory effect against all three bacteria with MIC of 1.25%.

Blanc *et al*., (2006) had screened and tested acidic and neutral parts of *I. viscoa* essential oil and tested for their antibacterial activity against three Gram-positive and two Gram negative bacteria, two yeasts) and three filamentous fungi. The acidic and neutral parts appeared to be inactive against *Escherichia coli*, while only the acidic part was active against *Staphylococcus aureus* with 2.5 µl/ml.

Results of this study demonstrated that the gram-negative bacteria (*E. coli*) was more resistant to the *I. viscosa* essential oil but the gram-positive bacteria (*S. aureus*) was

susceptible when it was tested with the same extract. Generally, Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria (Nazzaro *et al*., 2013; Al Laham and Al Fadel, 2014).

Because lipopolysaccharide (LPS) layer of gram- negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules. Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram- negative bacteria because cell wall of the gram- positive bacteria contained only peptidoglycan (Ababutain, 2011). Cafarchia *et al*., (2002) attributed the greatest antimicrobial efficacy exhibited by leaf extract of *I. viscosa* to the high concentration of sesquiterpene (carboxyeudesmadiene).

Mahmoudi *et al*., (2015) and Bekkara *et al*., 2008 show that the antimicrobial activity of *I. viscosa* extractcould be associated with the presence of some phenolic compounds endowed with antimicrobial activity. Bssaibis *et al*., (2009) linked the presence of thephenolic compounds and terpenoids to the antimicrobial properties of leave extracts of *I. viscosa*. Kheyar *et al*., (2014) antibacterial activity major compounds thymol (6.93) and carvacrol (2.27%) are well-known substances with pronounced antimicrobial properties (Dorman et Deans, 2000).

The inhibitory effects of carvacrol have beenrecorded in a number of strains of bacteria and fungi, including *S. aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *E. coli*, *Bacillus cereus*, *Salmonella enterica*, *Clostridium jejuni* and *C. albicans*. Thymol, which has a similar structure to carvacrol, differingonly in the position of the hydroxyl group on the aromatic ring, has also been shown to be active against *E. coli*, *S. aureus*, *S. epidermidis*, *Listeria monocytogenes*, *C. jejuni*, and *S. enterica* (Cosentino *et al*., 1999; Friedman *et al*., 2002; Nostro *et al*., 2007; Rivas *et al*., 2010).

CONCLUSION

The essential oil of *I. viscosa* grown in North West part of Algeria was found to possess antioxidant and antibacterial activities. The in vitro antioxidant study shows that the oil has the ability to scavenge DPPH free radicals.The antibacterial activity study revealed that the oil inhibit only *S. aureus*. The results obtained in this study show that the Algerian essential oil from *I. viscosa* may be a new potential source of natural antioxidants and antimicrobial agents for food, pharmaceuticals and cosmetics industries. However, further studies need to be conducted to understand the mechanism of the activity and obtain more information on the safety and toxicity of the oil.

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