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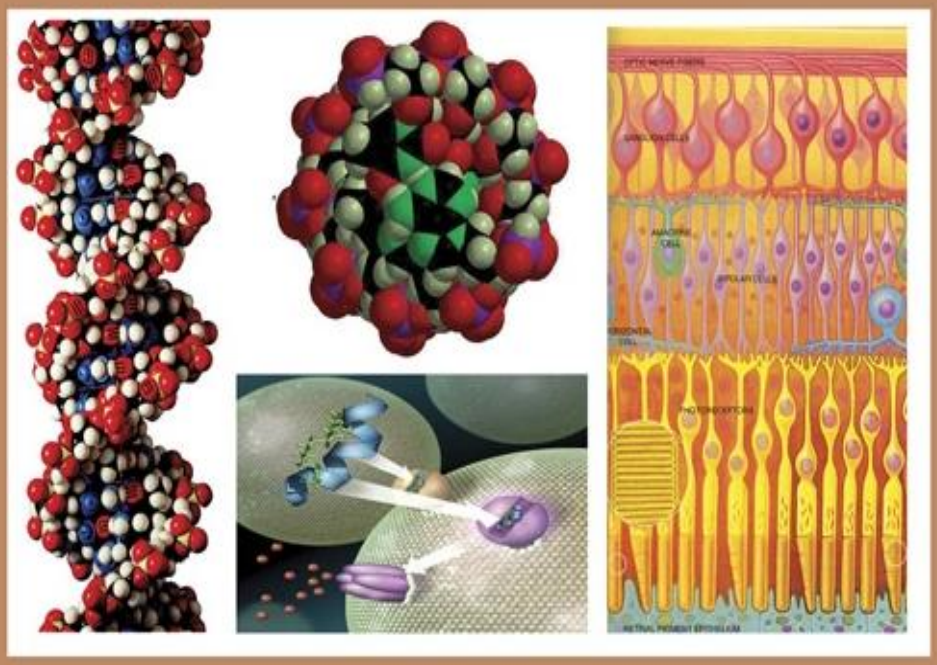

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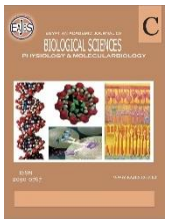

Antibacterial Activity of Essential Oils of *Inula viscosa* against Some Multi-Resistant *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923

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ABSTRACT

The essential oils of *Inula viscosa* have been commonly worldwide employed as a crude drug by people in Asia, the Middle East and Africa. Due to the development of antimicrobial resistance by the use of antibiotics among pathogenic bacteria, natural products extracted from the plants can be used as alternative strategies to reduce pathogenic bacteria from foods and patients.
The main aim of the present work was the extraction of essential oils from the leaves of *Inula viscosa*, an aromatic plant harvested in full bloom (March) from the mountainous region of Tessalah of Sidi Bel-Abbes (northwest Algeria) and the evaluation of their antibacterial properties against some bacteria such as *E. coli*, *Ps. aeruginosa* ATCC 27853, and *St. aureus* ATCC 25923, *B. subtilus* by using agar well diffusion method and by the study of bacterial growth in the absence and the presence of essential oils.

The quantitative analyzes of essential oils of *Inula viscosa* by hydrodistillation showed a low yield with a value of 0.23% and the antibacterial activity of essential oils of *Inula viscosa*, different concentrations (1, 2, 4, 6, 8, 12 µl), which was emulsified with Tween 80 and by using agar well diffusion method has indicated an excellent antibacterial activity by *E. coli*, *S. aureus* ATCC 25923, *Ps. aeruginosa* ATCC 27853 and *Ba. cereus* with a maximal diameter of inhibition zone 24, 13, 32, 22 mm respectively.

Furthermore, the study of *Ps. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 growth in the absence (Control) and in the presence of the essential oils of *Inula viscosa*, has manifested a considerable biomass reduction accompanied by unbalanced growth after adding of essential oils of *Inula viscosa*.

INTRODUCTION

The wide use of antibiotics in the treatment of bacterial infections has led to the appearance of resistant strains. The increase of this phenomenon threatens public health on a global scale as it reduces the effectiveness of treatments and increases morbidity, mortality and health care costs. Many plant extracts have been evaluated not only for direct antimicrobial activity but also as resistance-modifying agents with an indirect effect against many species of bacteria, by enhancing the activity of a specific antibiotic (Chekroud, 2020; Stefanović *et al.*, 2012).

Inula viscosa has found several biological activities such as antifungal (Al-Masri *et al.,* 2015; Rhimi *et al.,* 2017, 2018; Sriti Eljazi *et al.,* 2018; Mohti *et al.,* 2019; Gharred *et al.,* 2019), antimicrobial (Larbi *et al.,* 2016; Rhimi *et al.,*2017, 2018; Aissa *et al.,*2019, Ounoughi *et al.,* 2020), antioxidant (Sriti Eljazi *et al.,* 2018; Mohti *et al.,* 2019; Gharred *et al.* 2019), anti-tumoral (Isil et al. 2018; Bar-Shalom et al. 2019; Hepokur *et al.,* 2019). Furthermore, Inula's essential oils are also used in the food industry to increase the shelf life of a large number.The chemical composition of essential oils of *Inula viscosa* growing in different countries has been investigated; in Algeria (Boudouda *et al.,* 2014; Madani *et al.,* 2014).

In an early study, Silva and co-workers reported that the tested essential oil of *I. viscosa* against *Listeria monocytogenes* has manifested a high resistance. On the other site, the essential oil showed excellent antibacterial activity against E. *coli, K. pneumoniae, L. innocua, S. aureus, Ps. aeruginosa* bacterial strains (Kheyar *et al.,* 2014; Chebouti-Meziou 2016). *Enterobacter sp., Bacillus thuringiensis, Micrococcus sp. and Aspergillus niger and Candida albicans* strains, were sensitive to *I. viscosa* oils (Chebouti-Meziou 2016).

The main aim of the present work was the extraction of essential oils from the leaves of *Inula viscosa*, an aromatic plant harvested in full bloom (March) from the mountainous region of Tessalah of Sidi Bel-Abbes (northwest Algeria) and the evaluation of the antibacterial properties of some pathogen bacteria such as *E. coli, Ps. aeruginosa* ATCC 27853, *St. aureus* ATCC 25923 and, *B. subtilus,* by using agar well diffusion method and by the study of bacterial growth *Ps. aeruginosa* ATCC 27853 and *St. aureus* ATCC 25923 in the absence and the presence of essential oils.

MATERIALS AND METHODS

Extraction of Essential Oils:

The samples of leaves and flowers of *I. viscosa* were collected during Mars and May (early dry season) of 2014 and 2015 and harvested from local regions of Sidi Bel Abbes (North West-Algeria). The extraction of essential oil of *Inula viscosa* was carried out by standard hydro distillation method from Cleavenger's apparatus and all operations have been achieved at room temperature. The crushed seed powder (200 g) was placed in a separate flask together with distilled water (1 L). After 5 to 6 h, the oil was collected from the apparatus and it was dehydrated by passing through anhydrous sodium sulphate for removal of water traces stored into a dark bottle and at 4°C until use, the yield was 0.35% (w/v). The essential oil was used for the disc diffusion test and determination of bacterial growth in the absence and the presence of essential oils of *Inula viscosa*. Essential oils yield was calculated as followed:

$$\text{Yield } (\%) = \frac{\text{Weight of EO recovered}}{\text{Weight of spices x 100}}$$

Each essential oil dilution (60 mg/mL) was prepared in (DMSO), followed by sterilization using a 0.45 ìm membrane filter.

Bacterial Strains:

The used bacterial strains in this work were *E. coli, ps. aeruginosa* ATCC 27853, *St. aureus* ATCC 25922 *and B. subtilus* were provided from the bacteriological laboratory of the hospital CHU of Sidi Bel Abbès, Algeria. They were subcultured and used throughout the studies. Each of the bacterial specimens was incubated in liquid culture dilutions in Mueller Hinton Agar (MHA Oxoid- CM337) and incubated at 37°C for 20 min to reach the logarithmic growth phase, then measured to a 0.5 McFarland dilution (Standard concentrations), which delivered a final concentration of approximately 10^5 CFU per ml. Then the agar plates were inoculated with the essential oils of *Inula viscosa* and incubated overnight at 37°C.

Determination of Antibacterial Activity:

The disc diffusion method described by (Modzelewska *et al*. 2005) was used for the investigation of the antibacterial activity.

For this purpose, the MHA plates were prepared by pouring 15 mL of molten media into sterile Petri dishes. Different concentrations of the essential oils of *Inula viscosa* were prepared by dissolving in DMSO to obtain the following concentrations (1, 2, 4, 6, 8, 12 µl). After that, a sterile paper disc (6 mm) was placed on the surface of the solid culture medium to allow the diffusion of the compounds, so that each disc was impregnated with 6 µL of residue and the plates were kept for incubation at 37°C for 24 hours. The sensitivity of different bacterial strains to the essential oils was calculated by measuring the diameter (In millimeters) of the inhibition zone. Readings were taken at the end of 24 hours and 48hours. Bacteria showing a clear zone of inhibition >6 mm were considered to be sensitive. Experiments have been achieved intriplicates for each combination of extract and the tested bacterial strain. Discs containing water and ethanol were used as controls. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the discs.

Disc Diffusion Assay:

Standardized inoculum of bacteria $0.1.10^8$ cells per mL of *Ps. aeruginosa* ATCC 27853, was spread on the solid media and nutrient Agar (NA) plates respectively with the help of a sterile spreader. The inoculated plates were allowed to dry and a sterile cork borer of diameter 8.0 mm was used to bore wells in the center of inoculated agar plates. Different concentrations of the essential oils of *Inula viscosa* were prepared by dissolving in DMSO. Subsequently, a 6µL volume of essentials oil of *Inula viscosa* was introduced in wells. Sterile DMSO served as the control. The plates were allowed to stand for 1 hour to diffuse and then incubated at 37°C for 24 h for bacteria. The zone of inhibition was recorded to the nearest size in mm.

Inoculation and Bacterial Growth:

In order to explore a bacterial growth, a volume of 1 ml of 18 hours old culture of *Ps. aeruginosa* ATCC 27853 *and St. aureus* ATCC 25923 was separately inoculated in an Erlenmeyer flask, which was contained a volume of 50 mL of culture medium, incubated by shaking at temperature 37°C for 24 hours. The contained 100 mL of culture medium in 2 Erlenmeyer flasks was inoculated with a volume of overnight bacterial suspension, which was adjusted to 0.5 corresponding to bacterial biomass of $4.5\text{-}5.10^7$ bacteria per 1 ml of the medium at 578nm, incubated at 37°C for 24 hours. A regular sample has been taken every hour. After that, a volume of 1 mL of essential oils of *Inula viscosa* was added 6 hours after the onset of the bacterial growth. The yield of the biomass production was evaluated by the measurement of optical density at 578 nm wavelength **(**Abbouni, *et al.,* 2003).

Protein Assay:

The protein concentration was determined according to the described method by Warburg and Christian as the followed reaction:

Protéines (mg/mL) = $1.55\ A_{280} - 0.76\ A_{260}$

RESULTS AND DISCUSSION

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including bacteria and fungi (Erdogrul, 2002).

In the present study, the antibacterial activity of the essential oils of *Inula viscosa* against various Gram-positive and Gram-negative bacteria has been investigated. For this purpose, the disc diffusion method, and the bacterial growth in the presence and the absence of essential oils of *Inula viscosa* against various Gram-positive and Gram-negative bacteria were used for the antibacterial evaluation of essential oils of *Inula viscosa.*

The illustrated results in (Figs. 1 and 2) revealed the essential oils of *Inula viscosa* in the dilution of 1 to 16 µl. The obtained results on the antibacterial activity of the essential oils of *Inula viscosa* have manifested a clear inhibition zone of at least 7 mm for all the strains tested. The tested

activity of essential oils of *Inula viscosa* at various concentrations against various Gram-positive (*S. aureus*, *Ba. cereus*) and Gram-negative bacteria such as *E. coli*, *Ps. aeruginosa* ATCC 27853, has manifested an excellent antibacterial activity by *E. coli*, *S. aureus* ATCC 25923, *Ps. aeruginosa* ATCC 27853 and *Ba. cereus* with a maximal diameter of inhibition zone 24, 13, 32, 22 mm respectively.

Table 1: Illustration of the antibacterial activity by the produced zone inhibition (mm) in the presence of several concentrations of the essential oils of *Inula viscosa* (mg/mL) against pathogen bacteria.

Tested bacteria	**The produced zone inhibition (mm) by the using of increasing concentration of essentials oils of *Inula viscosa* (mg/mL).**						
	0	1	2	4	6	8	12
E. coli	0	1	7	8	12	21	24
Ps. aeruginosa	0	0	0	0	8	10	13
St. aureus	0	0	24	26	26	29	32
Ba. cereus	0	22	10	10	10	10	13

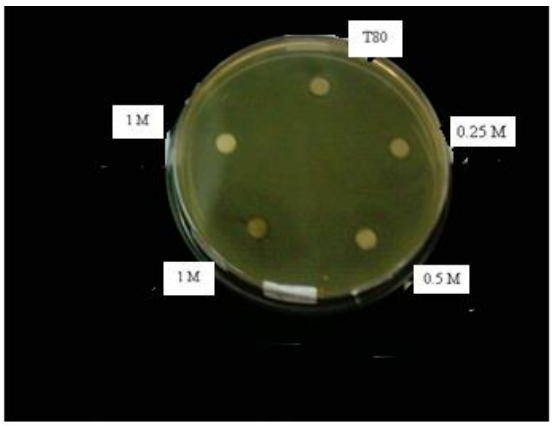

Fig. 1: Illustration of the antibacterial activity of essential oils of *Inula viscosa* at different concentrations (0, 25, 50, 100%) against *Staphylococcus aureus* ATCC 25923, inoculated on solid Mueller Hinton culture medium, incubated at 37°C for 24 hours by the produced inhibition zone (mm)

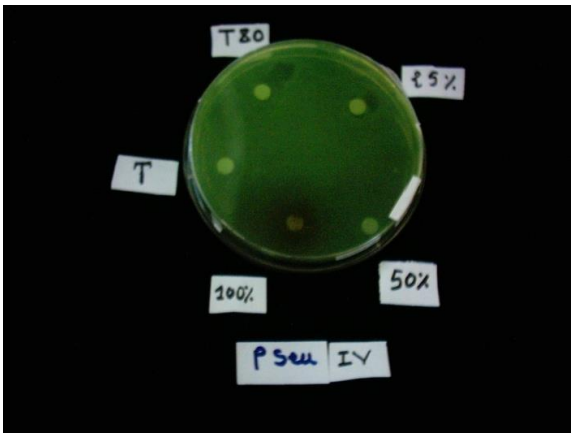

Fig. 2: Illustration of the antibacterial activity of essential oils of *Inula viscosa* at different concentrations (0, 25, 50, 100%) against *Pseudomonas aeruginosa* ATCC 27853, inoculated on solid Mueller Hinton culture medium, incubated at 37°C for 24 hours by the produced inhibition zone (mm).

The obtained results indicated that the essential oils of *Inula viscosa* possess a wide inhibition activity spectrum on pathogenic bacteria for humans. A more careful analysis should be performed *in vivo* in order to determine its real effects. In particular, the sesquiterpenes and their derivatives seem to be a promising class of natural compounds in the search for a new antibacterial agent (Modzelewska *et al.*, 2005, Agrawal *et al.*, 1979).

Topozada and their co-workers (1965) have reported that *Salmonella* and *Pseudomonas* bacteria are very sensitive to the essential oil of *Inula viscosa* and with the exception of *E. coli* ATCC 25922, other Gram-negative strains are less sensitive than Gram-positive strains.

Likewise, Larbi *et al.* (2016) reported that the essential of *Inula viscosa* showed complete growth inhibition against *B. cereus, B. subtilis* and *S. aureus, Ps. aeruginosa* by the agar well diffusion method. This resistance is due to the chemical composition of the wall which is rich in lipopolysaccharides not allowing the penetration of hydrophilic molecules.

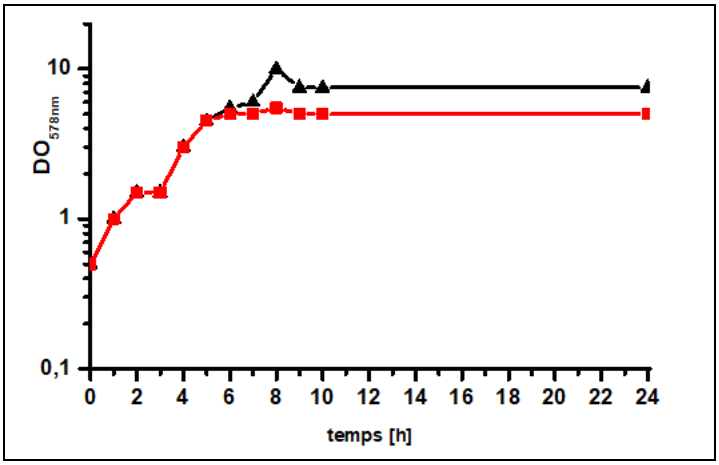

Fig. 3: The study of *Staphylococcus aureus* ATCC 25923 growth, inoculated in the seed liquid culture medium, incubated at 37°C for 24 hours, in the absence **(▲)** and the presence **(■)** of essential oils of *Inula viscosa.*

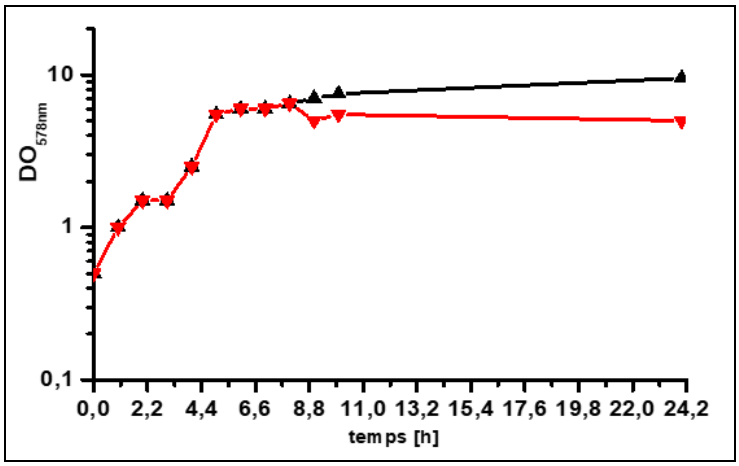

Fig. 4: The study of *Pseudomonas aeruginosa* ATCC 27853 growth, inoculated in the seed liquid culture medium, incubated at 37°C for 24 hours, in the absence **(▲)** and the presence (▼) of essential oils of *Inula viscosa.*

This antibacterial activity may be indicative of the presence of metabolic toxins or broad-spectrum antibacterial compounds. This is in agreement with previous reports by several researchers (Randhawa *et al.,* 2002). Furthermore, Ulubelen *et al*. (1987) has reported that the essential oils of *Inula viscosa* showed high inhibitory activity against a range of bacteria resistant to antibiotics.

It may be related to the fact that gram-positive bacteria such as t*S. aureus* ATCC 25923, *B. subtilus* are more sensitive against antibacterial agents compared to the tested gram-negative bacteria because of the difference in their cell wall structures (Kanoun *et al.,* 2012; Vishal *et al.,* 2012).

In order to explore the effect of the compounds present in the essential oils of *Inula viscosa* on tested pathogens bacteria, the bacterial growth of *St. aureus* ATCC 25923 and *Ps. aeruginosa* ATCC 27853 in the absence (control) and in the presence of the essential oils of *Inula viscosa* has been investigated. For this purpose, *St. aureus* ATCC 25923 and *Ps. aeruginosa* ATCC 27853 were inoculated in seed media with an initial optical density of 0.5 at 578 nm according to the protocol described by Abbouni and coworkers (2003)**,** in the absence and in the presence of the essential oils of *Inula viscosa*, which was added 6 hours after the onset of the bacterial growth. The obtained results manifested (Figs. 3&4) showed considerable inhibition of the growth of *St. aureus* ATCC 25923 and *Ps. aeruginosa* ATCC 27853, after the addition of the essential oils of *Inula viscosa* in the early exponential growth phase. In conclusion, the molecules present in the essential oils of *Inula viscosa* were able to induce unbalanced growth and further the arrest the cell cycle of *St. aureus* ATCC 25923 and *P. vulgaris* in comparison with the untreated biomass with the crude extract (balanced growth) **(**Abbouni, *et al.,* 2009; 2004; 2003; Den Blaauwen *et al.*,2014; Grenga, 2010).

Kheyer *et al.,* 2004 and Ali-Shtayeh, 1998 reported that the essential oils of *Inula viscosa* showed more effective activity against Gram + bacteria, which was compared to the obtained results described by (Chaouki Selles ***et al.,*** 2013) applied to A. Pyrethrum. The biological activity of plant extracts against tested bacteria could be attributed to the presence of biologically active components such as flavonoïds; phenolic acids and terpenoïds (Laghrifi *et al.,* 2013).

Conclusion

A number of plants have been documented for their biological and antimicrobial properties. The most tested bacteria are characterized by the developing a resistance to commonly employed antibiotics and are a common cause of many enteric infections. Therefore, in the present study, the antibacterial activity of the essential oils of *Inula viscosa* has been investigated against a gram-positive bacterium (*St. aureus* ATCC 25923, *Ba. cereus*) and Gram-negative bacteria such as *E. coli*, *ps. aeruginosa* ATCC 27853, has manifested an excellent antibacterial activity by *E. coli*, *S. aureus* ATCC 25923, *Ps. aeruginosa* ATCC 27853 and *Ba. cereus* with a maximal diameter of inhibition zone 24, 13, 32, 22 mm respectively.

In conclusion, this work confirms the antibacterial activity of methanolic extracts and essential oils of *Inula viscosa*. It shows their potential use as agents which enhance antibiotic activity. This indicates that these plants may be useful for developing alternative compounds to treat bacterial infections. Therefore, further studies involving the purification of the chemical compounds of the essential oils of *Inula viscosa* by using modern techniques such as Gas chromatography, HPLC, and IRM, are required for the determination of the active metabolites present in the essential oils of *Inula viscosa.*

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REFERENCES

Abbouni, B.; Elhariry, H. and Auling, G. (2003). Arrest of cell cycle by inhibition of ribonucleotide reductase induces accumulation of NAD+ by Mn2+-supplemented growth of *Corynebacterium ammoniagenes*. *Biotechnology letters*, 25(2), 143-147.

Abbouni, B.; Oehlmann, W.; Stolle, P.; Pierik, A. and Auling, G. (2009). Electron paramagnetic resonance (EPR) spectroscopy of the stable-free radical in the native metallo-cofactor of the manganese-ribonucleotide reductase (Mn-RNR) of *Corynebacterium glutamicum*. *Free radical research*, 43, 943-950. doi: 10.1080/10715760903140568

Agarwal, R.; Kharya, M. D. and Shrivastava, R. (1979). Antimicrobial & anthelmintic activities of the essential oil of *Nigella sativa Linn*. *Indian journal of experimental biology*, 17(11), 1264-1265.

Aissa, I.; Znati, M.; Bergaoui, A.; Flamini, G.; Ascrizzi, R. and Ben Jannet, H. (2019). GC, GC–MS, and NMR spectroscopy integrated analyses and in vitro antibacterial, anticholinesterase, anti-tyrosinase, and anti-5-lipoxygenase potential of *Inula viscosa* root fractionated essential oil. *South African Journal of Botany*, 125, 386-392. doi: 10.1016/j.sajb.2019.08.019

Al-Masri, M.I., Sharawi, S.M. and Barakat, R.M. (2015). Effect of *Clammy Inula* (*Inula viscosa*) Plant Extract in Combination with a Low Dose of the Fungicide Iprodione on *Botrytis cinerea* in Vitro and in Vivo. *American Journal of Plant Sciences*, 1519-1526. doi: 10.4236/ajps.2015.69151

Ali-Shtayeh, M. S.; Yaghmour, R. M. R.; Faidi, Y. R.; Salem, K. and Al-Nuri, M. A. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *Journal of Ethnopharmacology*, 60(3), 265-271. doi: https://doi.org/10.1016/S0378-8741(97)00153-0

Bar-Shalom, R.; Bergman, M.; Grossman, S.; Azzam, N.; Sharvit, L. and Fares, F. (2019). *Inula Viscosa* Extract Inhibits Growth of Colorectal Cancer Cells in vitro and in vivo Through Induction of Apoptosis. [Original Research]. *Frontiers in Oncology*, 9.

Bavunoglu, I.; Turkmen, Z.; Serin, İ.; Kuloglu, M.; Tekin, T.; Mercan, S. and Açikkol, M. (2018). The Negative Effects of *Inula Viscosa* L. Use o n a Cancer Treatment. *Acta Scientific Medical Sciences*, 8(2), 19-24.

Berahail Boudouda, H. (2014). Etude phytochimique et biologique des espèces *Biscutella raphanifolia*, *Zilla macroptera*, *Inula graveolens* et *Inula viscosa*. Doctorat LMD Université de Constantine 1, Algerie.

Chebouti-Meziou, N. (2016). Contributions to Study of the Antimicrobial Activity of *Inula Viscosa* Harvested in Boumerdes (Algeria). *International Journal of Advances in Chemical Engg & Biological Sciences*,3(1), 1-4.

Chekroud, Z.; Kheffif , A.. and Bassout, R. (2020). Valorisation of Algerian Medicinal Plants: *Inula viscosa L*. A Future Source of Antibacterial Drugs. *Asian Journal of Scientific Research*, 13 (1), 58-66. doi: 10.3923/ajsr.2020.58.66

Den Blaauwen, T.; Andreu, J. M. and Monasterio, O. (2014). Bacterial cell division proteins as antibiotic targets. *Bioorganic Chemistry*, 55,

27-38. doi: 10.1016/j.bioorg.2014.03.007.

Erdogrul, Ö. T. (2002). Antibacterial Activities of Some Plant Extracts Used in Folk Medicine. *Pharmaceutical Biology,* 40(4), 269-273. doi: 10.1076/phbi.40.4.269.8474

Gharred, N.; Dbeibia, A.; Danilo, F.; Hammami, S.; Piras, A. and Dridi-Dhaouadi, S. (2019). Chemical composition, antibacterial and antioxidant activities of essential oils from flowers, leaves and aerial parts of Tunisian *Dittrichia Viscosa*. *Journal of Essential Oil Research,* 31, 1-8. doi: 10.1080/ 10412905. 2019.1612789

Grenga, L.; Guglielmi, G.; Melino, S.; Ghelardini, P. and Paolozzi, L. (2010). FtsQ interaction mutants: A way to identify new antibacterial targets. *New biotechnology,* 27, 870-881. doi: 10.1016/j.nbt. 2010. 05.002

Hepokur, C.; Budak, Y.; Karayel, H.; Selvi, B.. and Yaylim, I. (2019). Investigation of Cytotoxic Effects of *Inula Viscosa* Extract. *Cumhuriyet Science Journal,* 40, 578-582. doi: 10.17776/csj.437993

Kanoun, K.; Abbouni, B. and Bénine, M. L. (2014). Etude de l'efficacité de l'extrait éthanolique d'écorces de *Punica granatum*. *linn* sur deux souches phytopathogènes: *Ascocyhta rabiei* (pass.) labr. et *Fusarium oxysporum f.sp.radicis - lycopersici*. *European scientific journal*, 10.

Kheyar, N.; Meridja, D. and Belhamel, K. (2014). Etude de l'activité antibactérienne des huiles essentielles d'*inula viscosa*, Salvia officinalis et Laurus nobilis de la région de Bejaia. *Algerian Journal of Natural Products*, 2 (1), 18-26.

Laghrifi, K.; Lemrhari, A.; Rachid, Z.; Idrissi, M.; Makoudi, Y. and Alnamer, R. (2013). In vitro potential antibacterial activity of the dichloromethane and hexane extract of *inula viscosa* from morocco. *International Journal of Universal Pharmacy and Bio Sciences.*

Larbi, K. S.; Meddah, B.; Meddah, A. T. T. and Sonnet, P. (2016). The antibacterial effect of two medicinal plants Inula viscosa, Anacyclus valentinus (Asteraceae) and their synergistic interaction with antibiotic drugs. *Journal of Fundamental and Applied Sciences,* 8(2).

Madani, L.; Derriche, R. and Haoui, I. E. (2014) Essential oil of Algerian *Inula viscosa* leaves. *Journal of Essential Oil-Bearing Plants*, 17, 164-168. doi: 10.1080/0972060x.2014.884778

Modzelewska, A.; Sur S Fau - Kumar, S. K.; Kumar Sk Fau - Khan, S. R. and Khan, S. R. (2005). Sesquiterpenes: natural products that decrease cancer growth. Cure Med Chem Anticancer Agents, 5(5), 477-499.

Mohti, H.; Taviano, M. F.; Cacciola, F.; Dugo, P.; Mondello, L.; Marino, A.; Crisafi, G., et al. (2020). *Inula viscosa* (L.) Aiton leaves and flower buds: Effect of extraction solvent/technique on their antioxidant ability, antimicrobial properties and phenolic profile. *Natural product research,* 34(1), 46-52. doi: 10.1080/14786419. 2019.1569659

Ounoughi, A.; Messaoud, R.; Lograda, T.; Chalard, P.. and Figueredo, G. (2020). Chemotypes and antibacterial activities of *Inula viscosa* essential oils from Algeria. *Biodiversitas Journal of Biological Diversity,* 21. doi: 10.13057/biodiv/ d210431

Randhawa, M. and Al-Ghamdi, M. (2002). A review of pharmaco-therapeutics effects of *Nigella sativa*. *Pakistan Journal of Medical Research,* 41(2).

Rhimi, W.; Ben Salem, I.; Iatta, R.;

Chaabane, H.; Saidi, M.; Boulila, A. and Cafarchia, C. (2018). *Dittrichia viscosa* L. leaves lipid extract: An unexploited source of essential fatty acids and tocopherols with antifungal and anti-inflammatory properties. *Industrial Crops and Products,* 113, 196-201. doi: 10.1016/j.indcrop.2018.01.032

Rhimi, W.; Ben Salem, I.; Immediato, D.; Saidi, M.; Boulila, A. and Cafarchia, C. (2017). Chemical Composition, Antibacterial and Antifungal Activities of Crude *Dittrichia viscosa (L.)* Greuter Leaf Extracts. *Molecules (Basel, Switzerland),* 22(7). doi: 10.3390/ molecules22070942.

Selles, C.; Dib Mel A Fau - Djabou, N.; Djabou N Fau - Beddou, F.; Beddou F Fau - Muselli, A.; Muselli A Fau - Tabti, B.; Tabti B Fau - Costa, J.; Costa J Fau - Hammouti, B., et al. (2013). Antimicrobial activity and evolution of the composition of essential oil from Algerian *Anacyclus pyrethrum L*. through the vegetative cycle. *Natural Product Research*, 27(23), 2231-2234.

Side Larb, K.; Meddah, B.; Tir Touil Meddah, A. and Sonnet, P. (2016). The antibacterial effect of two medicinal plants *Inula viscosa*, *Anacyclus valentinus* (Asteraceae) and their synergistic interaction with antibiotic drugs. *Journal of Fundamental and Applied Sciences*, 8(2), 244. doi: 10.4314/jfas. v8i2.5

Sriti, J.; Selmi, S.; Zarroug, Y.; Wesleti, I.; Aouini, B.; Jallouli, S. and Limam, F. (2018). Essential oil composition, phenolic compound, and antioxidant potential of *Inula viscosa* as affected by extraction process. *International Journal of Food Properties,* 21, 2309-2319. doi: 10.1080/10942912.2018.1517782

Stefanovic, O. and Čomić, L. (2012). Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics. *Journal of Applied Pharmaceutical Science*, 2, 1-5.

Toppozada, H. H.; Mazloum, H. A. and El-Dakhakhny, M. (1965). The antibacterial properties of the *Nigella sativa l.* seeds. Active principle with some clinical applications. *The Journal of the Egyptian Medical Association,* 48, 187-202.

Ulubelen, A.; Oksuz, S. and Goren, N. (1987). Sesquiterpene acids from *Inula viscosa*. *Phytochemistry,* 26, 1223-1224.

Vishal, J.; Viswanatha, G. L.; Manohar, D. and Shivaprasad, H. N. (2012). Isolation of Antidiabetic Principle from Fruit Rinds of "*Punica granatum*". *Evidence-Based Complementary and Alternative Medicine,* 2012, 11. doi: 10.1155/ 2012/147202