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Antibacterial activity of phenolic compounds of *Pulicaria odora*, wild plant in northern Algeria

[1*]Touati, N., [1]Saidani, K., [2]Boudries, H., [1]Hammiche, H., [1]Ouazene, N. and [1]Bedjou, F.

[1]*Laboratoire de Biotechnologies Végétales et Ethnobotanique, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria.*
[2]*Laboratoire de Biochimie, Biophysique, Biomathématique et Scientometrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria.*

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Abstract

Pulicaria odora is an aromatic plant belonging to the Asteraceae family, tribe Inuleae with approximately 100 species, well known in morocco and traditionally used as a remedy for its anti-inflammatory activity. The aim of this work is to highlight the antibacterial activity of leaves and roots of this plant. Three different solvents namely methanol, acetone and chloroform were used for the extraction of phenolic compounds. The methanolic extract of leaves recorded the highest total polyphenols content (TFC) (90 µg CE/g of DM) and flavonoids (11.34 µg QE/g of DM). Antibacterial activity of the different extracts showed that most of the bacterial strains tested were sensitive to Pulicaria odora extracts. Gram positive bacteria (*B. subtilis* and *S. aureus*) are more sensitive than Gram negative bacteria (*E. coli* and *P. aeruginosa*). Results showed that *S.aureus* was the most sensitive, with the largest inhibition diameter (30.5 mm) obtained with the acetonic extract of roots, whereas *P. aeruginosa* was the most resistant (00 mm) for the chloroformic extract of leaves. Leaf extracts were the most actives against *S. aureus* and *P. aeruginosa,* with MIC/MBC equal to 1/1.4 mg/mL.

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Introduction

The use of natural resources in general, and medicinal plants in particular is one of the most important and interesting way to explore in order to find for the research of new antimicrobial agent.

Traditional medicine is the fundamental support for the medicinal practice in rural areas of Africa. The use of plant extracts containing bioactive components has become a very important approach in preventive medicine (Keita *et al.*, 2004). Indeed, in 2002 the WHO estimated that 80% of the African populations still refer to traditional medicine, for their cure. Therefore, these plant species, of great importance for the population health, should be studied scientifically for their better use (Karou *et al*., 2005).

Secondary metabolites, such as phenolic compounds and essential oils, to which an inhibitory effect against microorganisms was assigned, were the subject of many *in vivo* and *in vitro* works (Knobloch *et al.*, 1989; Cowan, 1999; Bylka *et al*., 2004; Cushnie and Lamb, 2005; Karabay-Yavasoglu *et al.*, 2007; Doss *et al*., 2009; Masibo and He, 2009).

The genus *Pulicaria,* belongs to the Asteraceae family (Compositae), tribe Inuleae, which includes 100 species (Quezel and Santa, 1963). Different *Pulicaria* species have been traditionally used in several countries; *Pulicaria jaubertii,* indigenous to Yemen, locally known as "Ansif" is used in folk medicine as diuretic, pyritic conditions in urogenic organs and to cure fever (Algabr *et al*., 2012). In Iran, *Pulicaria* species are known as "kak kosh" and "shebang" and are commonly used as herbal tea, flavoring agent, and medicinal plant (Kamkar *et al*., 2013). *Pulicaria odora* L. in Morocco is used in traditional medicine to treat back-pain, intestinal disorders and menstrual cramps. The plant is also a constituent of the traditional remedy called "Mssakhen", which is given to women after childbirth (Ezoubeiri *et al*., 2005). Various biological activities have been reported for some species of *Pulicaria* such as neuroprotective *in vivo* activity against neurodegenerative diseases of *Pulicaria glutinosa* (Farooq, 2015), analgesic, antipyritic and anti-inflammatory in hepatic and nephretic conditions of the aerial parts of *Pulicaria arabica* (Yusufoglu, 2014), anticonvulsant property of *Pulicaria gnaphalodes* (Zendehdel *et al*., 2013), antibacterial an antioxidant activities of *Pulicaria crispa* (Elshiekh and AbdElMoniem, 2015).

Pulicaria odora is a Mediterranean species

*Corresponding author.
Email: *nanout_82@yahoo.fr*

(Ezoubeiri *et al.*, 2005), it colonizes the bushes, maquis and clearings (Williams *et al.*, 2003). This plant is known in Morroco as "ouden elhallouf" traditionally used for its anti-inflammatory properties (Bellakhdar, 1997). To the best of our knowledge, essential oils composition of *Pulicaria odora* and their antibacterial activity were studied (Ezoubeiri *et al*., 2005; Hanbali *et al.*, 2005). No antibacterial activity of phenolic extracts have been studied, that's the main objective of our research on the leaf and root extracts of *Pulicaria odora* harvested in the region of Bejaia (Algeria).

Materials and Methods

Plant material

Pulicaria odora samples were collected during the month of March 2011, in the Ain skhoune region, city of Bejaia at 180 m altitude (36 ° 45 '3' 'N, 5 ° 00' 21 '' E) north of Algeria. These samples are transported in polyethylene bags. The plant was identified at the laboratory of ecology of Bejaia University.

The stems of *Pulicaria odora* samples were removed, while leaves and roots were cleaned with tape water. After drying at 40°C, leaves and roots were cut into small pieces, ground to a fine powder and then sieved to a particle size powder of less than 200 µm.

Extraction of phenolic compounds

Polyphenols were extracted by maceration. 5 g of *Pulicaria odora* leaf and root powder were put in 50 mL of each absolute solvent used: acetone, chloroform and methanol. After two hours of stirring, the solutions were centrifuged at 1500 x *g* for 10 minutes; supernatants were collected and filtered with standard filter paper. After solvent evaporation in rotary evaporator under vacuum at 40°C, the extract was redisolve in absolute methanol at a concentration of 100 mg/mL, then stored in dark vials at - 20°C (Cox *et al.*, 2010).

Polyphenols content

Determination of total phenolic content (TFC)

0.8 mL of Folin-Ciocalteu reagent at 10% was added to 0.4 mL of diluted extract in pure methanol. After 3 minutes, 1.6 mL of sodium carbonate solution (Na2CO3) at 10% was added. After incubation for one hour in the dark at room temperature, the absorbance is measured with a spectrophotometer (Shimadzu Uvmini.1240) at 750 nm (Kuda *et al.,* 2005).

The results ware expressed in equivalent microgram of catechin per gram of dry weight (µg CE / g DW).

Determination of flavonoid content

Flavonoid content of the extracts was determined by spectrophotometry according to the method established by Djeridane *et al.* (2006) based on the formation of a flavonoid-aluminum complex having a maximum absorption at 430 nm. 1.5 mL of diluted sample was mixed with 1.5 mL of a solution of aluminum chloride ($AlCl_3$) 2%. After incubation at room temperature for 15 mins, the absorbance of the mixture is measured at 430 nm with a UV-Vis spectrophotometer (Shimadzu Uvmini.1240).

Flavonoid contents were expressed in microgram equivalents of quercetin per gram of dry weight (µg QE / g DW).

Antibacterial activity

Four bacterial strains were used to assess the antibacterial properties of the test samples. Two Gram-positive bacteria: *Bacillus subtilis* ATCC 6633 an Methicillin-Resistant *Staphyloccocus aureus* ATCC 6538 (MRSA); the Gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, which were supplied by Laboratory of Applied Microbiology, University of Bejaia.

Screening of the antibacterial activity

The method of Daifas *et al.* (2004), adapted to the essential oils, was modified to assess the antibacterial activity of different extracts. Four dilutions ranging from 12.5 mg/mL to 100 mg/mL were prepared. 20 µL of each dilution was deposited on the surface of Mueller Hinton agar previously spread with 10^8 CFU/mL of bacterial suspension using a swab (Karabay-Yavasoglu *et al.*, 2007). The Petri dishes were placed in the refrigerator at 4°C for three hours to a pre-diffusion (Bansemir *et al*., 2006). After incubation at 37°C/24h, inhibition zones around the spot were measured in millimeters (Karabay-Yavasoglu *et al*., 2007).

Absolute methanol is used as a negative control. Standard polyphenols (gallic acid, catechin and quercetin) were also tested against all bacterial strains.

Determination of miminum inhibitory concentrations (MIC)

The MIC is defined as the lowest concentration that allows the inhibition of bacterial growth after 18 to 24 hours of incubation at 37°C (Caquet and Bru, 2008). The MIC of extracts was determined by the

solid medium dilution method described by (Tuncel and Nergiz, 1993).

For the MIC determination of the extracts, concentrations ranging from 0.1 to 2 mg/mL were used. spreading, by spot the different strains to be tested, was made from suspensions of 10^7 CFU/mL per sampling 1uL that to say 10^4 cells / spot (Committee, 2003). A negative control without extract and standards (gallic acid, catechin and quercetin) were also tested. After incubation at 37°C for 24 hours, the absence and the presence of bacterial growth, at the different concentrations was performed. The MIC is the lowest concentration for which there is no bacterial growth (Moroh *et al.*, 2008).

Determination of minimum bactericid concentration (MBC)

The MBC is defined as the lowest concentration resulting in a considerable killing of bacteria with a percentage of 0.01% of survivors (Meyer *et al.*, 2004).

Nutrient broth tubes are inoculated with agar pieces scraped from the deposited spots where no bacterial growth was observed. After incubation at 37°C for 24 hours, the presence or absence of turbidity was checked. The MBC is the lowest concentration where no trouble is observed.

Statistical analyses

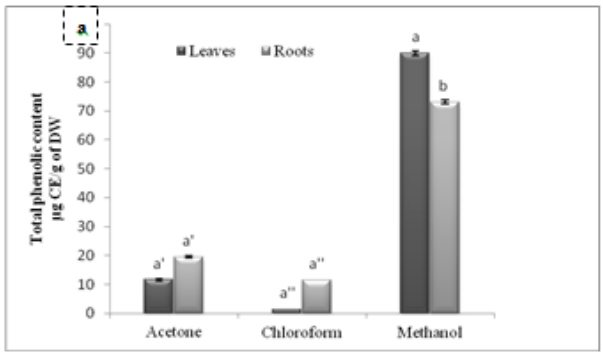
For statistical analyses, all the assays are carried out in triplicate. Results were expressed as mean ± standard deviation. Data were analyzed using the analysis of variance test (ANOVA). Significant differences ($p < 0.05$) between the averages were determined by the LSD test (Low Significant Difference) using STATISTICA 5.5 software.

Results and Discussion

Total phenolic content (TPC)

Methanol extracts of the leaves gave the best total polyphenol content with a value of 90 ± 0.63 µg CE / g DW, followed by the roots (73 ± 0.68 µg CE/g DW) with no significant difference ($p < 0.05$). Other extracts (chloroform and acetone) have low contents of total polyphenols with no significant difference ($p < 0.05$) as it is showed in Figure 1a. The lowest content was obtained by the chloroform extract of the leaves (1.36 ± 0.077 µg CE /g DW).

A total polyphenol content higher than those obtained in our study for the methanol extract of leaves, were observed by Bousselsela *et al.* (2012) for the methanol extract of *Hertia cheirifolia* leaves (30.33 ± 2.82 µg GAE / mg of extract). On the other hand, high concentrations were obtained by the same authors from various other solvents used for the same part of the plant as well. This difference may be due to the maturity of the plant. The distribution of secondary metabolites may change during the development of the plant (Falleh *et al*., 2008). Many factors can affect the total polyphenol content. Different studies have shown that extrinsic factors (such as geographic and climatic), genetic factors, the degree of maturation of the plant and the storage period have a strong influence on polyphenol content (Bouzid *et al.*, 2011).



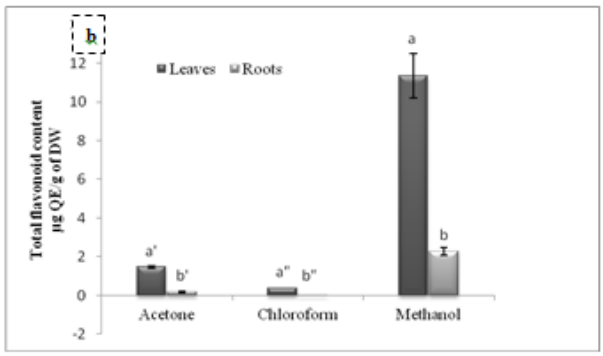


Figure 1. (a) Total phenolic (b) Total flavonoid contents of leaf and root extracts of Pulicaria odora of different solvents used
Vertical bars represent standard deviation..
Values bearing the same letters show no significant differences ($p<0,05$

Total flavonoid content (TFC)

A significant difference was recorded between the flavonoid contents of leaves and roots of *Pulicaria odora* ($p < 0.05$). As it is showed in Figure 1b the highest content was obtained for the methanol extract of leaves (11.34 ± 3.15 µg QE/g DW), followed by the acetone extract (1.47 ± 0.05 µg QE/g DW). Low levels were obtained in acetone and methanol extracts of roots (0.18 ± 0.04 and 2.28 ± 0.19 µg QE/g DW, respectively). Negative values were recorded for the chloroform extract of the roots. This indicates that flavonoids have an unqualled distribution in the various parts of *Pulicaria odora,* and that the

Table 1. Diameters of the inhibition zones of P*ulicaria odora* extracts

Extract	Dilution (mg/20µL)	Diameter of inhibition zones (mm)			
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
Leaf acetone	2	16,50±00,38^{c}	14,50±00,38^{gh}	11,00±00,00^{gh}	07,50±00,38^{l}
	1	15,00±00,00^{de}	11,50±00,38^{kl}	12 ,50±00,38^{de}	07,00±00,00jk
	0,5	14,50±00,38^{fe}	13,50±00,38^{hl}	12,00±00,00^{ef}	08,50±00,38^{l}
	0,25	14,00±00,00^{g}	13,00±00,00^{ll}	12,00±00,00^{ef}	09,50±00,38^{gh}
leaf chloroform	2	15,50±00,38^{d}	15,50±00,38^{f}	09,50±00,38^{jk}	00,00±00,00^{l}
	1	13,50±00,38^{gh}	12,50±00,38^{jk}	10,50±00,38^{hl}	12,00±00,00^{bc}
	0,5	12,50±00,38^{ll}	12,00±00,00^{k}	13,50±00,38^{c}	10,50±00,38^{ef}
	0,25	14,50±00,38^{ef}	09,50±00,38^{m}	11,00±00,00^{gh}	14,50±00,38^{a}
leaf methanol	2	19,50±00,38^{a}	17,50±00,38^{e}	09,00±00,00^{k}	10,50±00,38^{ef}
	1	18,50±00,38^{b}	15,50±00,38^{f}	11,00±00,00^{gh}	10,00±00,00^{fg}
	0,5	11 ,50±00,38^{k}	14,50±00,38^{gh}	09,50±00,38^{jk}	09,50±00,38^{gh}
	0,25	10,50±00,38^{l}	15,50±00,38^{f}	10,50±00,38^{hl}	10,50±00,38^{ef}
Root acetone	2	16,50±00,38^{c}	19,50±00,38^{d}	14,50±00,38^{b}	06,50±00,38^{k}
	1	14,00±00,00^{fg}	19,50±00,38^{d}	15,00±00,00^{ab}	11,00±00,00^{ed}
	0,5	15,50±00,38^{d}	30,50±00,38^{a}	12,50±00,38^{de}	07,00±00,00^{kl}
	0,25	13,00±00,00^{hl}	25,00±00,00^{c}	13,00±00,38^{d}	07,50±00,38^{l}
Root	2	05,50±00,38^{m}	25,00±00,00^{c}	11,50±00,38^{fg}	09,00±00,00^{hl}
chloroform	1	11,50±00,38^{k}	14,00±00,00^{h}	12,50±00,38^{de}	09,50±00,38^{gh}
	0,5	12,00±00,00^{jk}	12,00±00,00^{k}	15,50±00,38^{a}	10,00±00,00^{fg}
	0,25	14,00±00,00^{fg}	10,00±00,00^{m}	12,50±00,38^{de}	08,50±00,3^{l}
Root methanol	2	12,50±00,38^{ll}	24,50±00,38^{c}	11,50±00,38^{fg}	12,00±00,00^{bc}
	1	11,50±00,38^{k}	27,50±00,38^{b}	11,00±00,00^{gh}	12,50±00,38^{b}
	0,5	14,50±00,38^{ef}	25,00±00,00^{c}	12,00±00,00^{ef}	11,50±00,38^{cd}
	0,25	15,50±00,38^{d}	19,50±00,38^{d}	10,00±00,00^{ll}	09,00±00,00d^{h}

Each value represents a mean ± standard deviation (n=3).
Values bearing the same letters show no significant differences (p<0,05).

compounds of this latter were probably hydrophilic, hence their insolubility in non-polar solvents.

Falleh *et al.* (2008) found that flavonoid contents higher than those obtained in our study were observed in methanol extract of *Cynara cardunculus* leaves (9.08 mg CE/g DW). High levels of flavonoids may be linked to ecological conditions of the *Asteraceae* growth (hot temperature, high solar exposure, drought and salinity) that stimulate the biosynthesis of secondary metabolites such as polyphenols.

Antibacterial activity

The aim of this study is to evaluate the antibacterial activity of various extracts of *Pulicaria odora* leaves and roots.

Screaning of the antibacterial activity

In this study, leaf and root extracts of *Pulicaria odora* were tested against four bacterial strains, two Gram-positive and two Gram-negative. The results were expressed according to three levels of activity: Resistant: D <8mm ; intermediate 15mm ≥D≥8mm and sensitive: D> 15mm (Belaiche, 1979).

where D: diameter of the inhibition zones.

The antibacterial activity of the extracts studied is showed in Table 1. Some pictures of the inhibition zones of the different extracts on the four strains are also showed in Figure 2.

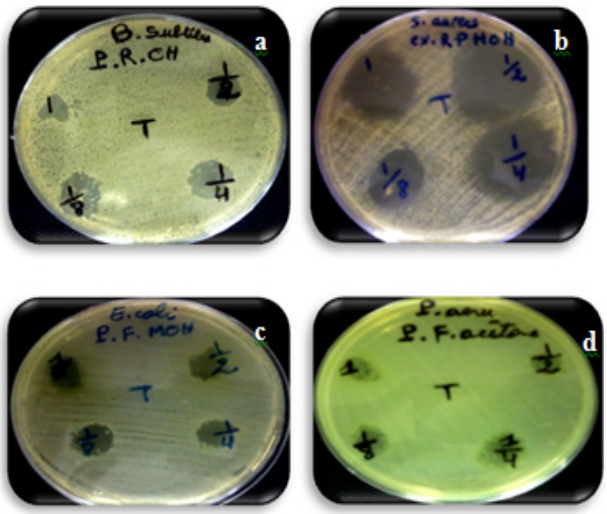


Figure 2. Pictures of some inhibition zones of the four strains obtained by different extracts of Pulicaria odora. (a) Chloroform roots on *B. subtilis* (b) : Methanol roots on *S aureus*, (c) : methanol leaves on *E. coli,* and (d) : acetone leaves on *P. aeruginosa.*

Antibacterial activity against S. aureus

All extracts (acetone, chloroform and methanolic) of leaves and roots of *Pulicaria odora* showed a good activity against *S.aureus* with inhibition zones ranging from 11.5 ± 0.38 to 30.5 ± 0.38 mm. The statistical analysis shows a significant difference (p <0.05) between leaves and roots. The widest inhibition zone was obtained with the acetone extract of roots (30.5 ± 0.38 mm) at a concentration of 0.5 mg / 20 µl, followed by methanol extract (27.5 ± 0.38 mm) at a concentration of 1 mg / 20 µl of the same

Table 2. Antibacterial activity of some phenolic standards

Phenolic Standards	Dilution (mg/20µL)	Diameter of inhibition zones (mm) *E. coli*	*P.aeruginosa*	*B. subtilis*	*S. aureus*
	1	20,00±00,00[a]	18,67±00,57[e]	20,67±00,57[b]	27,33±00,57[a]
Gallic acid	0,5	18,33±00,57[b]	21,33±00,57[b]	19,33±00,57[c]	24,33±00,57[b]
	0,25	18,33±00,57[b]	19,33±00,57[d]	18,33±00,57[d]	15,33±00,57[h]
	1	17,00±00,00[c]	17,33±00,57[f]	20,33±00,57[b]	20,33±00,57[d]
Catechin	0,5	09,33±00,57[e]	15,33±00,57[h]	19,33±00,57[c]	18,33±00,57f
	0,25	06,33±00,57[f]	16,67±00,57[g]	18,33±00,57[d]	18,00±00,00f
	1	17,33±00,57[c]	18,33±00,57[e]	17,33±00,57[e]	17,33±00,57[g]
Quercetin	0,5	15,33±00,57[d]	20,00±00,00[c]	20,33±00,57[b]	22,33±00,57[c]
	0,25	18,00±00,00[b]	23,33±00,57[a]	25,00±00,00[a]	19,33±00,57[e]

* note that negative controle (pure methanol) didn't give a result
Each value represents a mean ± standard deviation (n=3).
Values bearing the same letters show no significant differences (p<0,05).

portion of the plant. The lowest activity was observed with the acetone extract of the leaves (11.5 ± 0.38 mm) at a concentration of 1 mg / 20 µl.

Despite the low total polyphenol content of the chloroform extract of the roots, it presents a good antibacterial activity against this strain (25 ± 00 mm) at a concentration of 2 mg / 20 µl. This can be explained by the qualitative and quantitative character of different compounds present in the extract.

Nickavar and Mojab (2003) found that the methanol extract of the aerial parts of *Pulicaria dysenterica* showed an intermediate activity against *S. aureus* (13 mm) and the chloroform extract of the same parts had a low activity (8 mm), while our results showed that methanolic and chloroform extracts have a good activity against this strain (17.5 and 15.5 mm, respectively) at a concentration of 2mg / 20 µl.

Similar results have been found for methanolic extract of leaves (17.5 ± 0.38 mm) were obtained by Mothana and Lindequist (2005) with methanol extract of aerial parts (leaves and flowers) of *Pulicari stephanocarpa* (19 mm). (El-Kamali and Mahjoub, 2009) found almost the same result (19 mm) as well with ethyl acetate extract of the aerial part of *Pulicaria undulata.*

Falleh *et al*. (2008), showed that methanol extracts of *Cynara cardunculus* leaves had a good activity against *S. aureus* (25.7 ± 0.6 mm) compared to our results with the same leaf extract but with *Pulicaria odora* (17.5 ± 0.38 mm) at a concentration of 2 mg / 20 µl, this may be due to the disc method used.

Antibacterial activity against B. subtilis

A significant difference (p <0.05) was recorded between the different extracts of leaves and roots of *Pulicaria odora* against *B. subtilis*. All leaf extracts (acetone, methanolic and chloroform extract) showed better activity with inhibition zones of 16.5, 15.5 and 19.5 mm respectively, at a concentration of 2 mg / 20 µL. The decrease in concentration of these extracts was accompanied by a decrease of the inhibition zone, which corresponds to a dose-dependent effect.

The lowest activity against *B. subtilis* was observed with the chloroform extract of the roots (11.5 ± 0.38 mm) at a concentration of 1 mg / 20 µL. Unlike the leaves, increasing of the concentration of the chloroform extract of the roots was accompanied by a decrease of the inhibition diameters where this strain showed a resistance against the chloroform extracts of the roots (5.5 ± 0.38 mm) at a concentration of 2 mg / 20 µL. This could be explained by the decreased activity of the extract that could be due to a decrease in the solubility of active substances. However, with higher concentrations of extract, their solubility could become a limiting factor (Lindberg *et al.*, 2004).

It is possible that the decrease of the activity was due to a modification of the substance properties in the presence of other compounds of the extract (Pereira *et al.,* 2007), resulting in a combination of two active components (major or minor) acting in synergy (Brijesh *et al*., 2006), or the minor extract components which are active at low concentrations (Lindberg *et al.*, 2004).

El-Kamali and Mahjoub (2009) found a good activity against *B. subtilis* with extracts prepared in ethanol and petroleum ether (23 and 30 mm, respectively) from the stem bark of *Pulicaria undulata.* These results are superior to those obtained in this study with the methanol extract of the leaves (19.5 ± 0.38). Similar results were obtained by the same authors with the methanol extracts and ethyl

Table 3. Minimum inhibitory and bactericid concentrations of different root and leaf extracts of *Pulicaria odora* and phenolic standards against the strains tested

Part of the plant	Solvent/ Standards	MIC/MBC (mg/mL)			
		E. coli	*P .aeroginosa*	*B. subtilis*	*S.aureus*
Leaf	Acetone	>2/>2	1/1,6	1/>2	1,6/>2
	Chloroform	>2/>2	>2/>2	2/>2	>2/>2
	Methanol	>2/>2	1/1,4	1,6/>2	1/1,4
Root	Acetone	>2/>2	1/1,8	1/>2	1/>2
	Chloroform	>2/>2	>2/>2	1/>2	2/>2
	Methanol	>2/>2	1,6/1,8	1/>2	1/1,6
	Gallic acid	0,5/0,7	0,1/0,1	0,4/0,8	0,1/0,2
	Catechin	0,7/0,8	>2/>2	0,7/0,9	0,7/0,7
	Quercetin	>2/>2	>2/>2	>2/>2	>2/>2

Each value represents a mean ± standard deviation (n=3).
Values bearing the same letters show no significant differences (p<0,05).

acetate (17 mm) of the aerial part of the plant, while their aqueous extract showed no activity against this strain. So the choice of the protocol and the solvent as well as part of the plant studied is very important.

Antibacterial activity against E. coli

The statistical analysis showed a significant difference ($p<0.05$) between the leaves and roots of *Pulicaria odora,* the best activity was exercised by the chloroform extract of the roots (15.5 ± 0.38 mm) at a concentration of 0.5 mg / 20 µl, followed by the acetone extract (15 mm) at a concentration of 1mg / 20 µl of the same portion of the plant. Extracts from the leaves exhibited an moderate antibacterial activity against this strain, the diameters of the inhibition zones were between 09 and 13.5 mm.

Inhibition zones similar to those we found for the methanol extract of leaves (11 ± 00 mm) at a concentration of 1 mg / 20 µl, were observed by (Karabegović *et al.,* 2011) for the methanolic extracts of the aerial parts of Artemisia vulgaris with an inhibition zone equal to 12.7 ± 0.3 mm. Whereas methanolic extracts of *Artemisia campestris* gave an better inhibition zone (20 ± 0.4 mm). This difference may be due to the difference between plant species, although they belong to the same family (Asteraceae).

Antibacterial activity against P. aeruginosa

Weak antibacterial activity was obtained with the extracts of leaves and roots of *Pulicaria odora* against *P. aeruginosa.* The statistical analysis showed no significant difference between leaves and roots ($p<0.05$).

The diameter of the inhibition zones varies between 00 and 14.5 ± 0.38 mm at different concentrations. The best activity against this bacterial strain was obtained for the chloroform extract of leaves (14.5 ± 0.38 mm) at a concentration of 0.25 mg / 20 µL, followed by root methanolic extract (12.5 ± 0.38 mm) at a concentration of 1 mg / 20 µL, with no significant difference ($p<0.05$). The lowest activity was obtained with the acetone extract of the leaves and root extracts of the chloroform (8.5 ± 0.38 mm) at concentrations of 0.5 and 0.25 mg / 20 µL, respectively. No inhibition zone was noticed with chloroformic leaf extract at a concentration of 2mg / 20 µl.

Falleh *et al*. (2008) obtained a larger area (13.7) with the methanol extract of the leaves of *Cynara cardunculus* compared with that obtained in this study (10.5 ± 0.38 mm) at a concentration of 2 and 0.25 mg / 20 µL.

Unlike our results for the chloroform extract of the leaves (14 ± 0.38 mm), El-Kamali and Mahjoub (2009) found that *P. aeruginosa* showed a sensitivity (20 mm) against ethyl acetate extract of the aerial part of *Pulicaria undulate.*

The difference between our results and those of others may be due to the method used to assess antibacterial activity, indeed Bousselsela *et al.* (2012) found that the well method is better than the disc test.

According to our results, various extracts from leaves and roots of *Pulicaria odora* showed moderate antibacterial activities against the Gram negative bacteria (*E. coli* and *P. aeruginosa*), the wider area is obtained against *E. coli* (15.5 mm) and *P. aeruginosa* (14.5 mm), whereas the Gram-positive bacteria (*B. subtilis* and *S. aureus*) showed more sensitivity against these extracts, with wide inhibition zones (19.5 and 30.5 mm, respectively). These results could be explained by the activity of different components present in this plant. Indeed, Ezoubeiri *et al*. (2005) found the two major ones; the 2-isopropyl-4-methylphenol and the isobutyric acid 2-isopropyl-4-methylphenylester, tested against some bacteria. The first one showed a significant antibacterial activity against *E. coli* and *S. aureus* with diameters of inhibition zones about 10 and 20 mm respectively, whereas the second one was inactive against the two bacteria. The sensitivity of Gram-positive strains versus Gram negative could be due to the composition of the cell wall (Bouzid *et al*., 2011). Indeed, the resistance of gram-negative bacteria is linked to the lipopolysaccharide (LPS) (Alzoreky and Nakahara, 2003), which limits the permeability of the membrane to most bioatifs agents

(Bouzid *et al.*, 2011), whereas Gram-positive bacteria are less protected against antibacterial compounds, peptidoglycan can only prevents diffusion of molecules greater than 50,000Da (Basli *et al*., 2012).

Table 2 shows the antibacterial activity of some polyphenols standards, gallic acid, catechin and quercetin, used at various concentrations.

All polyphenol standards studied showed antibacterial activity against all bacterial strains tested. The best inhibition zone was developed by gallic acid (27.33 ± 00.57 mm) at 1 mg / 20µL against *S. aureus,* followed by quercetin (25.00 ± 00.00 mm) at 0.25 mg / 20 µL against *B. subtilis*. While Catechin showed low activity compared to other polyphenol standards (20.33 ± 00.57 mm) against both *S. aureus* and *B. subtilis* (1mg / 20 µL).

Methanolic extract of leaves at 2 mg / 20µL exhibited a similar inhibitory activity than gallic acid and catechin at 0.5 mg / 20µL against *B. subtilis,* with no significant difference ($p < 0.05$). whereas at concentrations of 1 and 0.5 mg / 20µL, extracts from leaves and roots showed low activity against this strain compared to gallic acid, catechin and quercetin 0.5 and 0.25 mg / 20µL.

A significant difference ($p < 0.05$) was recorded between the extracts of leaves and roots of *Pulicaria odora* and studied polyphenol standards. The latters exhibited a good activity against *P. aeruginosa,* with anexception of the chloroform extract of leaves at 0.25 mg / 20µL, which showed a similar inhibitory activity than catechin at 1 mg / 20µL, with a significant difference ($p < 0.05$). This difference between the extracts and polyphenol standards may be due to the purity of standards.

Acetone extract of the roots at 0.5 mg / 20µL showed a significant difference ($p<0.05$) with all polyphenol standards tested against *S. aureus,* it exhibited a better activity (30.5 ± 0.38 mm). This difference may be due to the high concentration of plant extracts. No significant difference ($p < 0.05$) was observed between methanolic extract of the roots at 1 mg / 20µL and gallic acid at the same concentration, and the methanolic, chloroform and acetone extracts (2 and 0.5 , 2 and 0.25 mg / 20µL respectively) of roots and gallic acid at 0.5 mg / 20µL . No significant difference was observed between methanolic extract of the leaves at 2 mg / 20µL with Catechin at 0.25 mg / 20µL and quercetin at 1 mg / 20µL, against the same bacterial strain.

Chloroform extract of the roots at 0.5 mg / 20µL showed a similar activity than quercetin at 0.5mg / 20 µL against *E. coli* strain, with no significant difference ($p < 0,05$). For other concentrations, gallic acid and quercetin exhibited a better activity than our extracts. Catechin at 0.5 mg / 20µL showed inhibition zones similar to those obtained with the methanol extract at 2 and 0.5 mg / 20µL and the chloroform extract of leaves at 2 mg / 20µL without any significant difference ($p < 0.05$). This standard at 0.25 mg / 20µl has no activity against this strain and revealed a significant difference ($p<0.05$) with the extracts of leaves and roots of *Pulicaria odora.*

Unlike our results, Rauha *et al*. (2000) did not observe inhibitory effects of gallic acid and catechin against *E. coli, S. aureus* and *B. subtilis.* This could be due to their low concentration (0.5 mg / 500µL). Quercetin inhibited the growth of all species studied by these authors (*S. aureus, S. epidermidis, M. luteus, B. subtilis, E. coli* and *P. aeruginosa)*. This result was in agreement with our study.

Determination of minimum inhibitory and bactericidal concentration (MIC/MBC)

Table 3 summarizes the MIC/MBC of all the extracts and standards. The best antibacterial activities are obtained with leaf methanolic extract of *Pulicaria odora* against *P. aeruginosa* and *S. aureus* with MIC/MBC 1/1.4 mg/mL, followed by the leaf acetone extracts against *P. aeruginosa* and the root methanolic extracts against *S. aureus* with MIC / MBC 1 / 1.6 mg / mL. The acetone and methanolic extracts of roots showed a bactericidal inhibitory effect at 1.8 mg / mL.

Leaf and root chloroform extracts showed no inhibitory effect on any of the bacterial strains tested except for *B. subtilis* where the chloroform extract of the roots exhibits inhibitory activity at 1 mg / mL. For *E. coli* strain, there is no inhibitory effect for the extracts of the leaves and roots at a concentration of 2 mg / mL. This agrees with the work done by Meyer and Afolayan (1995), which showed that the methanol and dichlorométhaniques extracts did not exert any antibacterial activity against *E. coli.*

Süzgeç-Selçuk and Birteksöz (2011) tested different flavonoid extracts of the aerial part of *Helichrysum chasmolycicum,* they found no inhibitory activity against *E. coli, P. aeruginosa* and *S. aureus,* only the ethyl acetate extract showed inhibitory activity (MIC = 625 mg / mL) against *P. aeruginosa* which was lower than the values obtained by the different leaf extracts of *Pulicaria odora.*

It was noted that catechin had the same inhibitory effect on *E. coli, B. subtilis* and *S. aureus* with a MIC of 0.7 mg / mL and MBC 0.8, 0.9 and 0.7 mg / mL respectively, no inhibitory activity was observed by catechin against *P. aeruginosa.* Gallic acid had an inhibitory activity against all tested bacterial strains, the MICs are between 0.1 and 0.5 and MBCs vary

from 0.1 to 0.8. Quercetin had no inhibitory activity against any tested bacterial strain up to 2mg / mL.

From Table 3, it was noticed that the two polyphenol standards, gallic acid and catechin, showed a better inhibitory activity against all tested bacterial strains, compared to the leaf and root extracts of *Pulicaria odora* with the exception of *P. aeruginosa* wherein the acetone and methanolic extracts of leaves and roots showed a better inhibitory effect against this strain compared to catechin.

Conclusion

Several studies were carried out on plants belonging to the Asteraceae family, they have shown their richness of bioactive compounds. However, there are few data and studies evaluating the antibacterial potential of *Pulicaria odora* (Asteraceae), hence this study was conducted to evaluate the antibacterial activity of this species, in the region of Bejaia (Algeria), against four bacterial strains, two Gram-positive and two Gram negative. The results of total polyphenol contents showed that leaf methanol extract was the richest, followed by methanol extract of roots, nevertheless, the chloroform extract of leaves recorded the lowest total polyphenol content. Regarding the total flavonoids content, the leaf methanol extracts gave the highest flavonoid contents while low levels were recorded in the other extracts. The evaluation of the antibacterial effect showed that most of the bacterial strains tested were sensitive to different leaf and root extracts of *Pulicaria odora.* A high sensitivity is observed in Gram-positive bacteria with inhibition zones between 11.5 ± 0.38 and 30.5 ± 0.38mm. The *S. aureus* strain has proved to be the most sensitive with an inhibition zone of 30.5 mm obtained for the root acetone extracts. concerning the minimal inhibitory concentrations (MIC) and bactericidal (MBC) of plant extracts, the results revealed that *P. aeruginosa* and *S. aureus* were the two most sensitive strains to the methanolic extract of leaves with values of MIC / MBC equal to 1/1.4mg / mL.

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References

Algabr, M., Ameddah, S., Menad, A., Mekkiou, R., Chalchat, J., Benayache, S. and Benayache, F. 2012. Essential oil composition of *Pulicaria jaubertii* from Yemen. International Journal of Medicinal and Aromatic Plants 2(4): 688-690.

Alzoreky, N. and Nakahara, K. 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology 80(3): 223-230.

Bansemir, A., Blume, M., Schröder, S. and Lindequist, U. 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 252(1): 79-84.

Basli, A., Chibane, M., Madani, K. and Oukil, N. 2012. Antibacterial activity of phenolics extracted from a medicinal plant of Algerian flora: *Origanum glandulosum* Desf. Phytotherapie 10(1): 2-9. (In french).

Belaiche, P. 1979. Treaty of phytotherapy and aromatherapy. Vol.1. The Aromatogram. Maloine, Paris, p. 125-127. (in french).

Bellakhdar, J. 1997. The traditional Moroccan pharmacopoeia: ancient arab medicine and popular knowledge. Ibis press, Paris. (In French).

Bousselsela, H., Benhouda, A., Yahia, M., Benbia, S., Ghecham, A. and Zidani, A. 2012. *In vitro* evaluation of antioxidant and antibacterial activities of extracts of Hertia cheirifolia leaves. Natural Science 04(11): 825-831.

Bouzid, W., Yahia, M., Abdeddaim, M., Aberkane, M. and Ayachi, A. 2011. Evaluation of the antioxidant and antimicrobial activity of hawthorn monogyne extracts. Lebanese Science Journal 12(1): 59. (In french)

Brijesh, S., Daswani, P., Tetali, P., Rojatkar, S., Antia, N. and Birdi, T. 2006. Studies on *Pongamia pinnata* (L.) Pierre leaves: understanding the mechanism (s) of action in infectious diarrhea. Journal of Zhejiang University Science B 7(8): 665-674.

Bylka, W., Matlawska, I. and Pilewski, N. 2004. Natural flavonoids as antimicrobial agents. Jana 7(2): 24-31.

Caquet, R. and Bru, A. 2008. Nurses'Guide for laboratiry exams: Elsevier-Masson. (In French).

Committee, S. A. 2003. Antibiogram Committee of the French Microbiology Society. International Journal of Antimicrobial Agents 21(4): 364-391.(in french).

Cowan, M. M. 1999. Plant products as antimicrobial agents. Clinical microbiology reviews 12(4): 564-582.

Cox, S., Abu-Ghannam, N. and Gupta, S. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal 17: 205-220.

Cushnie, T. T. and Lamb, A. J. 2005. Antimicrobial activity of flavonoids. International journal of antimicrobial agents 26(5): 343-356.

Daifas, D. P., Smith, J. P., Blanchfield, B., Sanders, G., Austin, J. W. and Koukoutisis, J. 2004. Effects of mastic resin and its essential oil on the growth of proteolytic *Clostridium botulinum*. International Journal of Food Microbiology 94(3): 313-322.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry 97(4): 654-660.

Doss, A., Mubarack, H. M. and Dhanabalan, R. 2009.

Antibacterial activity of tannins from the leaves of *Solanum trilobatum* L. Indian Journal of science and Technology 2(2): 41-43.

El-Kamali, H. H. and Mahjoub, S. A.-T. 2009. Antibacterial activity of Francoeuria crispa, *Pulicaria undulata, Ziziphus spina-christi* and *Cucurbita pepo* against seven standard pathogenic bacteria. Ethnobotanical Leaflets 2009(6): 6.

Elshiekh, Y. H. and AbdElMoniem, M. A. 2015. Phytochemical, antibacterial screening and antioxidant activity of *Pulicaria crispa* extracts. The Pharma Innovation Journal 3(12): 12-15

Ezoubeiri, A., Gadhi, C. A., Fdil, N., Benharref, A., Jana, M. and Vanhaelen, M. 2005. Isolation and antimicrobial activity of two phenolic compounds from *Pulicaria odora* L. Journal of Ethnopharmacology 99(2): 287-292.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M. and Abdelly, C. 2008. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. Comptes Rendus de Biologie 331(5): 372-379.

Farooq, M. 2015. Neuro-protective Activity of Pulicaria glutinosa in Oxidative Stress-induced Neurotoxicity in Zebrafish Embryos. International Journal of Agriculture and Biology 17(1): 193–198.

Hanbali, F. E., Akssira, M., Ezoubeiri, A., Gadhi, C. E., Mellouki, F., Benherraf, A. and Boira, H. 2005. Chemical composition and antibacterial activity of essential oil of *Pulicaria odora* L. Journal of Ethnopharmacology 99(3): 399-401.

Kamkar, A., Ardekani, M. R. S., Shariatifar, N., Misagi, A., Nejad, A. S. M. and Jamshidi, A. H. 2013. Antioxidative effect of Iranian *Pulicaria gnaphalodes* L. extracts in soybean oil. South African Journal of Botany 85: 39-43.

Karabay-Yavasoglu, N. U., Sukatar, A., Ozdemir, G. and Horzum, Z. 2007. Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. Phytotherapy Research 21(2): 153-156.

Karabay-Yavasoglu, N. U., Sukatar, A., Ozdemir, G. and Horzum, Z. 2007. Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. Phytotherapy Research 21(2): 153-156.

Karabegović, I., Nikolova, M., Veličković, D., Stojičević, S., Veljković, V. and Lazić, M. 2011. Comparison of antioxidant and antimicrobial activities of methanolic extracts of the *Artemisia* sp. recovered by different extraction techniques. Chinese Journal of Chemical Engineering 19(3): 504-511.

Karou, D., Dicko, M. H., Simpore, J. and Traore, A. S. 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of *Burkina Faso*. African Journal of Biotechnology 4(8): 823-828.

Keita, Y., Koné, O., Ly, A. K. and Häkkinen, V. 2004. Étude chimique et de l'activité antibactérienne des distillats de quelques variétés de mangue de Guinée. Comptes Rendus Chimie 7(10): 1095-1100.

Knobloch, K., Pauli, A., Iberl, B., Weigand, H. and Weis, N. 1989. Antibacterial and antifungal properties of essential oil components. Journal of Essential Oil Research. 1(3): 119-128.

Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y. 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. Journal of Food Composition and Analysis 18(7): 625-633.

Lindberg, L., Willför, S. and Holmbom, B. 2004. Antibacterial effects of knotwood extractives on paper mill bacteria. Journal of Industrial Microbiology and Biotechnology 31(3): 137-147.

Masibo, M. and He, Q. 2009. *In vitro* antimicrobial activity and the major polyphenol in leaf extract of *Mangifera indica* L. Malaysian Journal of Microbiology 5(2): 73-80.

Meyer, A., Deiana, J. and Bernard, A. 2004. Courses of general microbiology: corrected problems and exercices Wolters Kluwer France. (In french)

Meyer, J. and Afolayan, A. 1995. Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). Journal of Ethnopharmacology 47(2): 109-111.

Moroh, J., Bahi, C., Dje, K., Loukou, Y. and Guede-Guina, F. 2008. Study of th antibacterial actvity of acetatic extracts of *Morinda morindoides* (Baker) milne-redheat (rubiaceae) on *in vitro* growth of *Escherichia coli* strains. Bulletin of the royal society of sciences of Liege 77: 44-61. (In French).

Mothana, R. A. and Lindequist, U. 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of Ethnopharmacology 96(1): 177-181.

Nickavar, B. and Mojab, F. 2003. Antibacterial activity of *Pulicaria dysenterica* extracts. Fitoterapia 74(4): 390-393.

Pereira, A. P., Ferreira, I. C., Marcelino, F., Valentão, P., Andrade, P. B., Seabra, R. and Pereira, J. A. 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules 12(5): 1153-1162.

Quezel, P. and Santa, S. 1963. New flora of Algeria and southern desertic regions. National Research Science Center. Paris. (In French).

Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T. and Vuorela, P. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology 56(1): 3-12.

Süzgeç-Selçuk, S. and Birteksöz, A. 2011. Flavonoids of *Helichrysum chasmolycicum* and its antioxidant and antimicrobial activities. South African Journal of Botany 77(1): 170-174.

Tuncel, G. and Nergiz, C. 1993. Antimicrobial effect of some olive phenols in a laboratory medium. Letters in Applied Microbiology 17(6): 300-302.

Williams, C. A., Harborne, J. B., Greenham, J. R., Grayer, R. J., Kite, G. C. and Eagles, J. 2003. Variations in lipophilic and vacuolar flavonoids among European *Pulicaria* species. Phytochemistry 64(1): 275-283.

Yusufoglu, H. S. 2014. Analgesic, antipyretic, anti-inflammatory, hepatoprotective and nephritic effects of the aerial parts of *Pulicaria arabica* (Family:

Compositae) on rats. Asian Pacific Journal of Tropical Medicine 7: S583-S590.

Zendehdel, M., Fallah, R., Baghbanzadeh, A., Pourrahimi, M., Shariatifar, N. and Garavand, S. 2013. Effect of intracerebroventricular injection of aqueous extract and essential oil of *Pulicaria gnaphalode*s on PTZ-induced seizures in male rat. Physiology and Pharmacology 17(1): 94-100.