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Preliminary phytochemical investigation of antibacterial extracts from *Moricandia arvensis* growing in Algerian Sahara

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ABSTRACT

An investigation by disc diffusion method of antibacterial activities made it possible to know that the aqueous extract from *Moricandia arvensis* leaves showed a significant inhibition against all bacteria tested. Diethyl ether and Chloroform exracts had only one activity against *Enterococcus faecalis* and *Listeria monocytogenes* respectively, whereas the methanolic and water extracts exhibited the highest inhibition of *Pseudomonas aereginosa*. The activity seems to be due to the presence of flavonoids, tannins, cardinolids, saponins and alkaloids in the extracts.

KEYWORDS

Moricandia arvensis;
Brassicaceae;
Disc diffusion;
Phytochemical.

INTRODUCTION

Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global healthcare problem in the 21st century[1].

Saharan plants are known by their resistance to several stress factors. Under extreme climatic conditions, Saharan plants could constitute a large reservoir of new natural, safe and effective structural moieties which work together exhibiting a wide range of biological activities[2-5]. Over 75% of the antibacterial drugs in clinical use are of natural origin[6].

Like other vegetables, species from Brassicaceae family contain a number of phytochemicals and have been used to treat a wide range of human diseases [7-9].

In the aim for valorization of medicinal plants growing in Algerian Sahara and exploration of new bioactive natural products[10,11], it was considered of interest to carry out an antimicrobial investigation of crude extracts from *M. arvensis*. This last, known by the common name "krom jmal", is widely distributed in Algerian Sahara[12]. This specie provides food for camels[13] and it is used for treating various diseases as rheumatism, syphilis and scorbut[14,15].

Previous investigations on the chemical composition of this specie have led to the isolation of several phenolic glycosides, an indole derivative, glucosinolates, fatty acids and essential oil[16,17]. No significant antibacterial activity was observed in the study of the effects of essential oils from *M. arvensis* on the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*[17].

MATERIALS AND METHODS

Plant material

Moricandia arvensis (syn. *M. suffruticosa*) was collected during full blossom (February-March 2009) from Oued zouzfana (North of Bechar, Algeria) and

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identified by Pr A. Maarouf. A voucher specimen is deposited in the herbarium of Phytochemical and Organic Synthesis Laboratory (LPSO) of Bechar University under the number CA02/32

The leaves were dried and grounded into powder before to an extraction using reflux, during two hours, by several solvents with different polarities. The extracts were filtered and evaporated using a vacuum rotary evaporator.

Microorganisms

Pure cultures of the following microorganisms were used: *Escherichia coli* (ATCC 25922), *Pseudomonas aereginosa (*ATCC 27853), *Enterococcus faecalis (*ATCC 29212), *Listeria monocytogenes* (ATCC 19115), *Klebsiella pneumoniae (*Isolated), *Bacillus stearothermophilus (*ATCC 11778) and *Staphylococcus aureus* (ATCC 25923) which were obtained from Pasteur institute (Algiers, Algeria). The bacteria were maintained by frequent sub-culturing on Mueller Hinton agar plates (pH 7.4) and stored at 4°C.

Antibacterial test

The antibacterial essay was carried out by discs diffusion method on solid medium; the culture medium used is Muller Hinton[18,19]. The agar gel is treated with the appropriate microorganism suspension where each microorganism was inoculated at a concentration of 10^8 colony forming units per mL (CFU/mL).

Petri box (9 cm in diameter) were filled with 10 ml of the medium Muller Hinton, sterile discs filter paper of 6 mm in diameter charged with 3µl of extract were deposited on the medium surface. Because of the non miscibility of the majority of the investigated extracts, to water and therefore in the medium of culture, a dilution has been achieved by a solution of dimethylsulfoxide (DMSO).

A sterile disc impregnated with DMSO was used as negative control while Chloramphenicol (10µg/ml) was included in the test as reference (positive control). Petri box were incubated at 37°C during 24^h to 48^h[20]. All plates were observed for zone of growth inhibition. Zones are measured using sliding calipers or a ruler, which is held on the back of the inverted petri plate. Each experiment was carried out in triplicate.

RESULTS AND DISCUSSION

The results of the *in vitro* screening of different extracts of *M. arvensis* tested against pathogenic bacteria are shown in TABLE1.

TABLE 1 : Inhibition zone diameter (mm)

	Hex	Cyh	Et_2O	Dcm	Chl	EtOAc	MeOH	W	Ch 10 µg/ml
	Volume/disk (3µl)								
Bacillus stearothermophilus	-	08	09	08	-	10	10	12	25
Enterococcus faecalis	-	-	13	-	-	07	09	16	30
Escherichia coli	-	10	-	07	07	-	-	12	35
Klebsiella pneumoniae	09	-	-	12	08	11	12	11	15
Listeria monocytogenes	-	-	-	-	11	-	12	12	15
Pseudomonas aereginosa	08	08	10	12	10	12	15	15	28
Staphylococcus aureus	-	08	10	-	-	12	11	13	15

Hex: Hexane, Cyh: Cyclohexane, Et_2O: Diethyl ether, Dcm:Dichloromethane, Chl:Chloroform, EtOAc : Ethyl acetate, MeOH:Methanol, W: Water, Ch: Chloramphenical

The aqueous extract had a significant inhibitory of all bacteria tested, its highest activity is against *E. faecalis* and *P. aereginosa*. The activity seems to be due to the presence of polyphénolic compounds and antimicrobial agents in the aqueous extract[5,21]. The water and EtOAc extracts were most effective against *Staphylococcus aureus.* According to Ibiri[22], this last has the reputation to be in general very resistant to all sorts of antimicrobial agents and antibiotics. It is established that the inhibition of this bacterium by antimicrobial agents, require considerable concentrations. *S. aureus* is the cause of a number of diseases affecting humans and animals[23]. In spite of advances in medical science, epidemiology and the discovery of new antibiotics, *S. aureus* infections still present considerable morbidity and mortality[24].

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We remarked that the Dichloromethane extract is active against *K. pneumoniae* and *P. aereginosa*. Whereas the Chloroform extract exhibited the highest inhibition of *L. monocytogenes.* and *P. aereginosa*.

The crude extract of Methanol showed an important activity for *P. aereginosa*., *K. pneumoniae*, *L. monocytogenes* and *S. aureus* but it had no activity for *E. coli*.

The growth of *Pseudomonas aeriginosa* was not inhibited by the Cyclohexane, Diethyl ether and Chloroform extracts of *M. arvensis*. Infection caused by *P. aeriginosa* is among difficult to treat with conventional antibiotics[11].

Our present antimicrobial evaluation showed that, Hexane and Cyclohexane extracts had no activity for all bacteria tested. This result contrasts with the finding that the Hexane leaves extract of *M. arvensis* was to have antibacterial effect on the majority of the bacterial stumps used[25].

Water, Chloroform and Methanol extracts are the most active against *L. monocytogenes*. In another survey, the last extract showed important free radical scavenging activity toward the $DPPH^{\cdot}$ radical, whereas the Chloroform extract exhibited the highest value of TEAC against $ABTS^{+\cdot}$ radical[6].

According to our previous works on phytochemical screening[26] of *M. arvensis*, the family compounds detected in Methanol, Ethyl acetate, Petroleum ether, aqueous and Chloroform extracts of leaves were essentially flavonoids, tannins, cardinolids, saponins and alkaloids.

As shown in TABLE 2, the presence of flavonoïds, tannins, cardinolids, saponosids and alkaloids in water extracts, is responsible of its hight activity against the majority of bacteria.

Glucosinolates are found in the Brassicaceae and related families[27]. These secondary metabolites have various applications due to their antibacterial and antifungal properties[28].

In recent study, Skandrani et al.[29] indicate a significant presence of bioactive compounds in different extracts as: Chloroform extract (tannins 0.61% and sterols 12.5%), Ethyl acetate extract (flavonoids13.74%, sterols 0.25% and tannins 0.18%), Diethyl ether extract (sterols15%), Methanol extract (alkaloids 0.18%, flavonoids 8.83%, and tannins 0.10%).

TABLE 2 : Results of phytochemical screening

	Et_2O	Chl	EtOAc	MeOH	Water
Flavonoids	-	+	+	+	+
Tanins	-	+	+	+	+
Cardinolids	-	-	-	-	+
Saponosids	-	-	-	-	+
Alkaloids	+	-	-	+	+
Sterols	+	+	+	-	-

Et_2O: Diethyl ether, Chl: Chloroform, EtOAc: Ethyl acetate; MeOH: Methanol, W: Water; (+) Presence; (-) Absence

CONCLUSION

Moricandia arvensis is a dietetic species; it shows an important antioxidant activity and also serves as a source of various bioactive products, including polyphenols.

This present study evaluates antimicrobial effects of extracts from leaves of *Moricandia arvensis*. Extracts showed no effect when using *E. coli*, except for the water. Methanol and water extracts showed more effective antibacterial than the other extracts.

It appears that the majority of extracts from *M. arvensis* can inhibit the growth of some bacteria which causes different infections. This probably explains the use of this plant in traditional medicine against a number of human diseases for generations. Further chemical and pharmacological investigations should be carried out to isolate and identify bioactive compounds in the interesting extracts.

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