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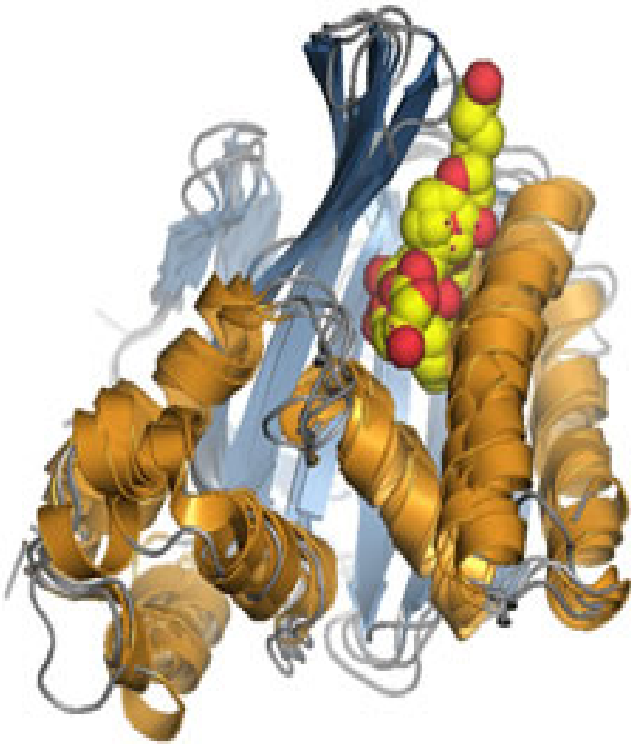

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Antibacterial activity of *Zilla macroptera* extracts from Algerian Sahara

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Abstract. *The study reported the antibacterial activity of extracts from aerial parts (without fruits) of Zilla macroptera (Brassicaceae) against seven bacterial strains (Escherichia coli, Pseudomonas aereginosa, Enterococcus faecalis, Listeria monocytogenes, Klebsiella pneumoniae, Bacillus stearothermophilus and Staphylococcus aureus) chosen for their high pathogenicity, shows that the strain of Pseudomonas aeruginosa was the most sensitive to the majority of extracts. The antibacterial activity of the ethanol extract is more important compared to other extracts.*

***Key Words*:** *Zlla macroptera*, Brassicaceae, antimicrobial activity, ethnopharmacology, phytochemical screening, Sahara

Introduction

Natural products possess interesting biological activities which attracted several researchers to their elucidation to provide knowledge that will lead to advancement medicine. Brassicaceae is one of the 10 most economically important plant families in the world, it contain a number of nutrients and phytochemicals and have been used to treat a wide range of human diseases [1-3].

Recently, researchers have reported the antimicrobial activity of traditional medicinal plants worldwide. So,it is of great interest to screen a wider range of cruciferous species from Sahara for production of antimicrobial metabolites.

The present investigations have focused on the *in vitro* screening of various extracts of *Zilla macroptera* tested against bacteria.

Materials and Methods

Vegetal product

Zilla macroptera, known by the common name "Boukhlala", is widely distributed in Algerian Sahara [4]. It was collected during the period of (February-March 2009) from Bechar (South Algeria). A voucher specimen is deposited in the herbarium of Phytochemistry and Organic Synthesis Laboratory (LPSO) under the number CA00/71 [5].

Zilla macroptera is a Saharan medicinal plant used by local population for various diseases. According to our previous works [2, 6 ,7], we summarized in table 1 and 2 the information collected from the ethnomedical survey on the traditional uses *of Zilla macroptera* and the phytochemical screening.

Table 1: Usage rate and diseases treated by *Zilla macroptera*

Diseases	(%)
gastric disorders and stomach pain	47
diarrhea	21
kidneys, liver and pancreas pain	11
Nausea and colds	7
Respiratory ailments	6
Eczema	3

Table 2 : Results of phytochemical screening

Alkaloïds	Saponosids	Tanins	Insatured sterols	Steroids	Cardinolids	Flavonoïds	Free Flavonoids	Glycosids flavonoïds.
+	++	++	-	+	-	++	+	+

Microorganisms

Pure cultures of the following microorganisms were used : (*Bacillus stearothermophilus* (*B.s*) (ATCC 11778), *Enterococcus faecalis* (*E.f*) (ATCC 29212), *Escherichia coli* (*E.c*) (ATCC 25922), *Klebsiella pneumoniae* (*K.p*), *Listeria monocytogenes* (*L.m*) (ATCC 19115), *Pseudomonas aereginosa* (*P.a*) (ATCC 27853) and *Staphylococcus aureus* (S.a) (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

Aerial part of *Zilla macroptera* was dried at room temperature for two weeks and it was cut into small pieces before to an extraction using reflux, during four hours (4h), by several solvents with different polarities. The extracts were filtered and evaporated using a vacuum rotary evaporator.

The antimicrobial activities were carried out by the disc diffusion method of Müller-Hinton on solid medium; the strains were reactivated using an 20^h culture growth at 37°C and adjusted to 10^8 CFU/mL.

Petri boxes (9 cm in diameter) were filled with 10 ml of the medium Muller Hinton. The bacterial strains was sowed on the surface of the agar plates in radial spots form by means of

swab and suspensions of young bacterial cultures prepared according to the committee for laboratory standards institute (CLSI) [8]; 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). The application is made by sterile filters paper discs (6 diameter) which were placed on the inoculated agar surfaces and impregnated with 3 µL of each extract; One disc impregnated with DMSO was used as negative control while chloramphenicol (10 µg/ml) was included in the test as reference (positive control). A gentle downward pressure should be applied to each disc before incubation to ensure complete contact between the disc and the agar surface. Petri box were incubated at 37°C during 24h to 48h [9,10] and the reading of the results was made by the measurement of the inhibition diameter around the disc, 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 experimental results on of phytochemical screening revealed the presence of flavonoids, saponosids and tannins. The presence of tannins is confirmed by positive reaction with ferric chloride ($FeCl_3$). Flavonoids test results showed positive reaction in the presence of magnesium and HCl. In contrast, the study indicated that insatured sterols and cardinolids were absent in aerial part extracts of our studied plant. However we observed less presence of steroids and alkaloids.

The antibacterial activity is summarized in table 3. The acetone extract of leaves had a significant inhibitory of all bacteria tested, except *E.coli*; its highest activity is against *B.s* and *P. aereginosa.*. The growth of S.a was inhibited by all extracts except EtAcO, which justifies the use of this plant in traditional medicine for treating respiratory diseases.

Table 3: Inhibition zone diameter (mm)

	Hexane	DEt	Acetone	EtOAc	Ethanol	Methanol	Water	Chl.10 µg/ml
	Volume/disk (3µl)							
Enterococcus faecalis	-	-	12	-	10	Nt	Nt	30
Listeria monocytogenes	-	15	10	10	6	Nt	Nt	15
Staphylocoque aureus	14	12	12	-	12	14	11	15
Klebsiella pneumoniae	-	10	10	10	10	13	14	15
Bacillus steariothermophilus	10	10	15	14	14	Nt	Nt	25
Escherichia coli	12	7	6	10	6	15	12	35
Pseudomonas aeruginosa	-	-	15	-	20	-	13	28

DEt: diethyl ether, EtOAc : ethyl acetate, Chl: chloramphenical,
Nt : not tested.

The MeOH and hexane extracts were most effective against *S. aureus.* [11], this last has the reputation to be in general very resistant to all sorts of antimicrobial agents and antibiotics. It is the cause of a number of diseases affecting humans and animals [12-14].
We remarked that Et extract is active against four bacteria, its highest activity is against *L. monocytogenes.* Whereas the MeOH extract exhibited the highest inhibition of *E. coli* and showed an important activity for *K. pneumoniae*, but it had no activity for *P. aereginosa*
The growth of *pseudomonas aeriginosa* was inhibited by EtOH, buy it was not inhibited by EtOAc, Et and hexane extracts of aerial part of *Z. macroptera*. Infection caused by *P. aeriginosa* is among difficult to treat with conventional antibiotics [15].
Our present antimicrobial evaluation showed that hexane,DEt and EtOAc extracts had no activity for E f and P a bacteria tested.
Water and methanol extracts are the most active against *K. pneumoniae*. The activity seems to be due to the presence of polyphénolic compounds and antimicrobial agents in the aqueous and methanol extracts [16]. The water, methanol and ethyl acetate solvents beeing currently used for the extraction of polyphenols and flavonoids ; theese last are considered as potent antioxidants, anti-inflamatory, antiviral and anti-bacterial [17, 18].
The family compounds detected, after the phytochemical screening [7] of *Z.macroptera* were essentially flavonoids, saponosids and tannins.
It was reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues[25]. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea. Glucosinolates are found in the Brassicaceae and related families. These secondary metabolites have various applications due to their antibacterial properties [19-22]. These observations therefore support the use of *Zilla macroptera* in herbal cure remedies.

Conclusion

According to the test results of antibacterial activity, it appears that some extracts of this plant were found to be active with a different degree, related to the content of the extracts and interactions of substances with antibacterial activity of extraction solvents.
Traditionally, *Zilla macroptera* plant is used to treat rheumatism, gastric disorders, diarrhea, Respiratory ailments and other diseases; the inhibition of the growth of bacteria responsible for these diseases can at least partially justify the ethno pharmacological uses of this plant.
The plant studied here can be seen as a potential source of useful drugs. Further studies are going in order to isolate, identify, characterize and elucidate structures of the bioactive compounds.

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