



Preliminary phytochemical and antimicrobial investigations of extracts of *Haloxylon scoparium*

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Abstract

Although pharmacological industries have produced a number of new antibiotics, resistance of microorganisms to these drugs has increased. Some antibiotics have become almost obsolete because of drug resistance. In the course of our research program on medicinal plants, we investigated *Haloxylon scoparium* a species in Moroccan Sahara. In the present study, five crude extracts, including methanol, chloroform, ethyl acetate, petrol ether and water extracts from the aerial parts of *H. scoparium* at different doses of 500, 300 and 50 mg/ml were tested against fungi and bacteria using the diffusion method. Experimental work showed that only the concentration of 500 mg/ml of ethyl acetate extract have an antibacterial activity against *Staphylococcus aureus* a Gram-positive bacteria.

Keywords : *Haloxylon scoparium*; Phytochemical, Crude extracts; Antimicrobial activity

1. Introduction

Pharmaceutical and scientific communities have recently received the attention of the medicinal plants and various publications have documented the therapeutic worth of natural compounds to validate the claims of their biological activity [1]. Herbal remedies used in the traditional medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help to overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. In contrast to other types of medication, antibiotics ultimately lose their effectiveness as they are used over time and resistant strains of bacteria develop [2]. There is thus an urgent need to identify novel, active chemotypes as leads for drug development [3]. Natural products could play a crucial role in meeting this demand. The medicinal plants and phytotherapy occupy a dominating place in the Moroccan system of health [4]. This study deals with the valorisation of medicinal plants of the Moroccan flora, in order to find new bioactive natural products. We were interested to study the antimicrobial activity of *Haloxylon scoparium*, an ethnomedicinal Moroccan plant.

H. scoparium Pomel [= *Hammada scoparia* (Pomel) Iljin., *Arthrophytum scoparium* (Pomel) Iljin., *Salsola articulata* Cav., *Haloxylon articulatum* (Cav.)] [5, 6, 7], belongs to the family chenopodiaceae, which has 120 genera and more than 1300 species. They are worldwide distributed especially in desert and semi desert areas in soils containing much salt. The plants are herbs, shrubs, subshrubs and rarely small trees. The genus *Haloxylon* Bunge (Incl. *Hammada*) comprises of about 25 species. It is distributed from Western Mediterranean region to Arabia, Iran, Mongolia, Burma and Southwest of China [8]. Two species of the genus were recorded in the literature to have folkloric uses. *H. salicornicum* is reported to be used for diabetes [9], as antiseptic and anti-inflammatory [10]. In Oman the stems of this species are used as a mordant for dyeing wool in traditional weaving. In addition, *Haloxylon scoparium* (=Haloxylon

articulatum} is used to treat eye disorders [11]. Infusion and powder infusion of aerial part of *H. scoparium* are used in Morocco for their antidiabetic effects [12, 13].

On the other hand, few species of the genus *Haloxylon* (seven species) have been chemically investigated, which resulted in the isolation of the several alkaloids belonging to mainly seven classes of alkaloids. These classes are: aliphatic quaternary alkaloids, pyridine alkaloids, indole alkaloids, isoquinoline alkaloids, isoquinolone alkaloids, β -carboline alkaloids and phenylethylamine alkaloids [14, 15]. The crude extracts from certain *Haloxylon* species were biologically evaluated. The ethanol extract of *H. salicornicum* was found to have antidiabetic [9] and anticoagulant activity in experimental animals [16]. The aqueous extract of *Hammada scoparia* has been found to show anticancer and antiplasmodial [17] and larvicidal activity [18]. Furthermore, the volatile oil of *H. schmittiana* was also studied and showed to exhibit antimicrobial activities against *Bacillus subtilis* and *Staphylococcus aureus* [19].

We carried out a screening of aerial parts extract of *H. scoparium* against pathogenic bacteria and fungi in order to detect new sources of antimicrobial agents. In this study, we report the results of the antibacterial and antifungal activity of different solvent extract oh *Haloxylon scoparium* aerial part.

2. Materials and methods

2.1. Plant material

The plant material was collected from March to April 2010 in Taza region of Eastern Morocco. A voucher specimen is deposited at the herbarium of the Multidisciplinary Faculty of Taza. Aerial parts of *Haloxylon scoparium* were air-dried at room temperature for 30 days and then used for antimicrobial assay.



Fig.1. *Haloxylon scoparium*

2.2. Preparation of extracts

2.2.1. Aqueous extract

The powdered aerial parts of *Haloxylon scoparium* (20g) was extracted with boiling water (250 ml) for 20 min. After this step, the decoction was filtered using Watman N°1 filter paper.

2.2.2. Organic extracts

① Petroleum ether, chloroform, ethyl acetate and methanol extracts were obtained by Soxhlet extraction of 20g of aerial parts for 6h in about 250 ml of each solvent used separately. These four types of organic

extracts, with different polarities, were concentrated in a rotary evaporator completely. Residues were kept in a hermetically-closed colour glass vial at 4°C until using.

② Air dried plant material (20g) was finely ground and macerated at room temperature in methanol for 48h. The extracted was subsequently filtered and concentrated in rotary evaporator completely at 69°C.

2.3. Microorganisms

We used for the preliminary test six strains isolated from clinical service of bacteriology and parasitology of university hospital Hassan II of Fez distributed as follows :

Bacteria, *Staphylococcus aureus*, *Staphylococcus xylosus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Branhamella catarrhalis* and fungi, *Candidas albicans*.

2.4. Studied activity

Antibacterial and antifungal activities of different extracts from aerial part of the plant were assessed using disc-diffusion method [20]. Petri plates were prepared with sterile Mueller Hinton Agar (MHA). The test cultures were swabbed on the top of the solidified media and allowed to dry for 30 min. The tests were conducted at three different concentrations of the crude extracts (500 mg/ml, 300 mg/ml and 50mg/ml per disc) with three replicates. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. The plates were incubated for 24 h at 37°C for bacteria and 48 h at 27°C for fungi.

Antimicrobial activity was assessed by measuring the inhibition zone. This was the diameter of the zone visibly showing the absence of growth, including the 6-mm disk. All the tests were performed in triplicate.

3. Results and discussion

Naturally occurring products from plants have played an important role in the discovery of new therapeutic agents since ancient times. The use of plant extracts and phytochemicals, with known antimicrobial properties, may be of immense importance in therapeutic treatments. Numerous studies have been conducted in the past few years to prove such efficiency [21-24].

In the current study, preliminary phytochemical investigations of the aerial parts of *H. scoparium* were conducted using four types of organic extracts, with different polarities. The results are summarised in **table 1**.

The five crude extracts, including methanol, chloroform, ethyl acetate, petrol ether and water extracts were tested at different doses of 500, 300 and 50 mg/ml against fungi and bacteria using the diffusion method (**Table 2**).

3.1. Extraction yield of aerial part

The aerial part extraction with different solvents showed the highest yields for water, and then for methanol (**Table 1**). The high yield of extraction in polar solvents exhibited rich polar constituents of the aerial part of the *H. scoparium*.

3.2. Antibacterial activity

The antibacterial activities of the different aerial extracts of *H. scoparium*, aqueous, methanolic, ethyl acetate, petroleum ether and chloroform, were determined against six bacterial strains. The inhibition zone, measured in millimetres, including the diameter of the paper disk, was used as the criterion for measuring the antibacterial activity of *H. scoparium* (**Table 2**) shows that none of the extracts showed activity against the *Staphylococcus xylosus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Branhamella catarrhalis*.

The ethyl acetate extract was active and specific against *Staphylococcus aureus* with the mean growth inhibition zone (7e12 mm). Our results are in agreement with previous reports showing that *Staphylococcus aureus* exhibited good sensitivity to the volatile oil of *Haloxylon schmittiana* [19].

Table 1 Yield of extracts from aerial parts of *Haloxylon scoparium*.

Extracted fractions	Yield (%)
Aqueous extract	10.50
Methanolic extract obtained by Soxhlet	10.47
Macerated méthanol extract	10
Chloroform extract	3.09
Ethyl acetate extract	2.44
Petroleum ether extract	1.00

Table 2 Antibacterial activity of different solvent extracts of *Haloxylon scoparium* aerial parts

Extracts	Concentration (mg/ml)	Inhibition zones (mm) against					
		E.c	S.a	S.x	K.p	Bra	P.a
Petroleum ether extract	500	-	-	-	-	-	-
	300	-	-	-	-	-	-
	50	-	-	-	-	-	-
Chloroform extract	500	-	-	-	-	-	-
	300	-	-	-	-	-	-
	50	-	-	-	-	-	-
Ethyl acetate extract	500	-	12	-	-	-	-
	300	-	7	-	-	-	-
	50	-	-	-	-	-	-
Methanolic extract	500	-	-	-	-	-	-
	300	-	-	-	-	-	-
	50	-	-	-	-	-	-
Aqueous extract	500	-	-	-	-	-	-
	300	-	-	-	-	-	-
	50	-	-	-	-	-	-

-, no activity;

Values are inhibition zone diameter (mm)

Negative controls did not show any activity

S.a- *Staphylococcus aureus*; *S.x*-*Staphylococcus xylosus* ; *E.c*.- *Escherichia coli*; *P.a*-*Pseudomonas aeruginosa* ; *K.p*-*Klebsiella pneumoniae*; *bra*.- *Branhamella catarrhalis* ;

3.3. Antifungal activity

None of different solvent extracts of *Haloxylon scoparium* aerial parts showed activity against the *Candidas albican fungi*.

Conclusion

The result of this work shows that among the bacterial strains tested only *Staphylococcus aureus* exhibited good sensitivity (7-12mm) to *H. scoparium* ethylacetate extract.

No antifungal activity could be found in our study for the different solvent extracts prepared from aerial parts of *Haloxylon scoparium* collected from Taza, region of Eastern Morocco.

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