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## Screening of the antimicrobial activity of some extracts of edible wild plants in Morocco

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### ABSTRACT

**Background:** Despite the availability of cultivated food crops and processed food, a large part of the Moroccan population, more particularly the populations of rural areas, still depend on the traditional use of wild plants, which constitute an important component of their food system. However, there is a lack of information on these plants and their medicinal and pharmacological properties, this is why our study aims to detect the antimicrobial activity of certain wild edible plants.

**Methods:** disc diffusion method was used to evaluate the antimicrobial activities of extracts of *Mercurialis annua* L, *Papaver rhoeas* L, *Foeniculum vulgare* Mill, *Chenopodium mural* L, and *Scolymus hispanicus* L against

the bacterial species *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas* sp, and against the yeasts *Cryptococcus neoformans*, and *Candida albicans*

**Results:** The results showed that the crude extracts from all the plants studied showed more or less important antimicrobial activities on one or other of the pathogenic microorganisms tested, except for the extract of *M. annua*, which showed no activity against all microbial strains. The highest antibacterial activity was observed for *Scolymus hispanicus* L extract against *Escherichia coli* (diameter of the inhibition zone:  $\varnothing=9\text{mm}$ ), the highest antifungal activity was marked for *Foeniculum vulgare* Mill extract against *Candida albicans* ( $\varnothing=8\text{mm}$ ), and the extract of *Scolymus hispanicus* L against *Cryptococcus neoformans* ( $\varnothing=8\text{mm}$ ).

**Conclusion:** These results reveal that, in addition to the role they play in the diet, the food plants studied have an additional biological value due to their bioactive compounds.

**Keywords:** In vitro antibacterial activity; Antifungal activity; Food wild plants; crude extract, Morocco

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**INTRODUCTION:** Wild plants are neither cultivated nor managed [1]. They are often associated with periods of famine or food shortages. They have contributed to biodiversity, and their consumption makes it possible to combat malnutrition and diversify food thanks to their traditional knowledge by local populations. However, these non-cultivated plants have not received much attention due to the abundance of other foods and changes in eating habits [2, 3]. Moreover, the lack of information on their nutritional content and their potential to serve as safe food means that they are underutilized, and their virtues are not valued.[4].The nutritional properties of wild plants have been reported in several studies[5-9], and in several countries : in Turkey[5], India[6], Greece[10],Ethiopia[11], Bangladesh[12], Morocco[13]and Spain [14].In Morocco, wild food plants had several uses but were neglected by the scientific community, which focused its research on medicinal plants. Therefore, few articles have studied pharmacological activities,

including the antimicrobial activity that these food plants may have.

This study aims to fill this gap by assessing the antimicrobial activity of five wild food plants commonly consumed by the local community in the Sidi Bennour region of Morocco, which are: *Mercurialis annua* L, *Papaver rhoeas* L, *Foeniculum vulgare* Mill, *Chenopodium mural* L and *Scolymus hispanicus* L, against bacterial species: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas* sp, and yeasts *Cryptococcus neoformans*, and *Candida albicans*.

#### **MATERIAL AND METHODS:**

**Plant material:** The plants used for the tests were collected in the Sidi Bennour region (central Morocco) in April 2019.the taxonomic identification of plants was carried out. The food plants, local names, family, and part used in this study were grouped in Table 1.

**Bioassay of extracts:** After collection, the fresh plant material was dried in an oven at 37°C, and the

powder material was then weighed. A mass of 100 g of powder is soaked for 48 hours in a mixture of two solvents, a polar solvent (ethanol) and an apolar solvent (dichloromethane) with a proportion of 50%:50%. The mixture was then filtered using Whatman

filter paper, and the filtrate was concentrated under reduced pressure (at 68°C) using a rotary evaporator until the solvent was removed entirely and the crude extract dried. The extracts were stored at 4°C until testing for antimicrobial activities.

**Table 2:** Name, family, part used and mode of consumption of the wild food plants studied

<b>Scientific name</b>	<b>Family</b>	<b>Local name</b>	<b>Used part</b>	<b>Consumption mode</b>
<i>Mercurialis annua</i> L	Euphorbiacées	Horrigalmalssa	Aerial parts	The aerial parts of the plant are used for the preparation of traditional dishes, or they are used as a garnish for soups. [15]
<i>Papaver rhoeas</i> L	Papaveraceae	Belaaman	Leaves and flowers	The leaves of this plant are used to prepare traditional dishes, the flowers are used as spices or as garnish for traditional bread [15, 16]
<i>Foeniculum vulgare</i> Mill	Apiaceae	Besbasbeldi	Aerial parts	The leaves and stems are used as vegetables to prepare traditional dishes or to be eaten raw, the roots are used as spices and the seeds are used as condiments. [15, 16]
<i>Chenopodium mural</i> L	Chenopodiaceae	Berremram	Aerial parts	The aerial parts of the plant are used for the preparation of traditional dishes, or they are used as spices. [15]
<i>Scolymus hispanicus</i> L	Astéracées	El guernina	Stem and leaves	The stems are eaten raw, the leaves are cooked as a traditional vegetable, and the roots are used as spices or garnish of traditional dishes as they can be eaten raw.[15, 16]

**Test strains:** Six microbial species have been used, four of which are bacteria, and two are yeasts obtained from the Institute Pasteur Paris Collection (CIP) and the ATCC (American Type Culture Collection (ATCC). The species of the microbial strains are two Gram-negative bacteria: *Escherichia coli* CIP54127, and *Pseudomonas sp*, two Gram-positive bacteria: *Enterococcus faecalis* ATCC19433 and *Staphylococcus aureus* CIP 209 (ATCC 25923) and two yeasts: *Candida albicans* 48.72, and *Cryptococcus neoformans* CIP 960.

These microorganisms have been chosen being considered as pathogenic microorganisms that cause several infections and diseases in different beings, including humans. Among the infections caused by these microorganisms we quote: hospital-acquired

pneumonia and meningitis caused by *S. aureus*[17], Hemolytic uremic syndrome caused by *E.coli*[18], endocarditis caused by *E. faecalis*[19], urinary tract infections caused by *Pseudomonas sp*[20], blood stream infections caused by *C. albicans*[21] and cryptococcosis caused by *C. neoformans*[22].

**Antibacterial activity:** A bacterial suspension ( $10^6$  cells.ml<sup>-1</sup>), was prepared from 24-hour culture on Mueller-Hinton (MH). The screening for antibacterial activities was carried out by using the paper disc method as previously described by [23, 24]. 20 µl of crude extract was added to a sterile paper disc (diameter: 6mm) placed on a nutrient agar plate containing a bacterium. After 3 hours at 4°C, the Petri dishes were incubated at 37°C for 24 hours at

72 hours. The antibacterial activity of each extract was evaluated by measuring the diameters of the inhibition zones around the discs. Each test was carried out in three copies

**Antifungal activity:** The disc diffusion method was used for the determination of antifungal activity of the plant extracts [23, 24]. The inoculum of yeast was prepared to give a concentration of  $10^4$  cells.ml<sup>-1</sup>, from 24-hour culture on Sabouraud dextrose agar (SDA). The inoculated plates were kept at 4°C for 3 h and incubated at 28°C for 24 at 72 hours. 20 µl of crude extract was added to a sterile paper disc (diameter: 6mm) placed on a nutrient agar plate containing a yeast. The antifungal activity of each extract was evaluated by measuring the diameters of

the inhibition zones around the discs. Each test was carried out in three copies.

Ampicillin (30 µg) was used as positive control for the tested bacteria and Fluconazole (30 µg) for yeast. The same solvents (ethanol- dichloromethane) were used as a negative control for both types of strains.

**RESULTS:** The antimicrobial test results are presented in Table 2. The data reported in this table correspond to the inhibition diameter of each test strains pathogen . As can be seen, except *M. annua*, all the plant extracts studied exhibited varying degrees of antimicrobial activity against any of the microorganisms tested. The inhibition zone of the tested bacteria is 4 mm to 9 mm without counting the diameter of the disc (6mm).

**Table 2.** *In vitro* screening of antimicrobial activity of crude extracts from wild food plants.

Diameters of the zones of inhibition (mm)						
Microorganisms	Bacteria				Yeasts	
	Gram-positive bacteria		Gram-negative bacteria			
Extracts	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>Pseudomonas sp</i>	<i>C. neoformans</i>	<i>C. albicans</i>
<i>Mercurialis annua</i>	NI	NI	NI	NI	NI	NI
<i>Papaver rhoeas</i>	8±2,00	6±2,08	6±2,51	4±1,52	NI	6±0,3
<i>Foeniculum vulgare</i>	8±0,57	6±0,55	8±0,7	NI	NI	8±0,15
<i>Chenopodium mural</i>	NI	5±1,52	5±2,42	NI	NI	NI
<i>Scolymus hispanicus</i>	NI	8±1,52	9±0,00	NI	8±1,52	NI
Ampicillin	16	17	16	15		
Fluconazole					20	18

NI: No inhibition

According to Ali et al (Ali et al., 2001), the crude extracts tested have good antimicrobial activity when the inhibition zone is 8 mm or more; moderate antimicrobial activity if it is of 6–7 mm; low antimicrobial activity if the zone is 4–5 mm; very low

antimicrobial activity if it is 2–3mm, or without antimicrobial activity

As can be seen, except *M. annua*, all the plant extracts studied exhibited varying degrees of antimicrobial activity against any of the microorganisms tested. The inhibition zone of the

tested bacteria is 4 mm to 9 mm without counting the diameter of the disc (6mm).

According to the results of Table 2, the *M. annua* crude extract showed no antibacterial activity against all strains. *P. rhoeas* extract has inhibited all bacterial strains, with good antibacterial activity against *S. aureus*, moderate antimicrobial activity against *E. coli*, and *E. faecalis*, and low antibacterial activity against *Pseudomonas sp.* The *F.vulgare* extract showed good antibacterial activity against the strains *S. aureus* and *E. coli*, moderate activity against *E. faecalis*, and zero antibacterial activity against *Pseudomonas sp.* The extract of *C.mural* showed no antibacterial activity against the strains *S. aureus* and *Pseudomonas Sp*, but it showed low antibacterial activity against *E. faecalis* and *E. coli*. Finally, the extract of *S.hispanicus* has good antibacterial activity against *E. coli* and *E. faecalis*; in contrast, it has no effect on *S. aureus* and *Pseudomonas sp.* Regarding antifungal activity, most of the extracts showed no antifungal activity against *C. neoformans* and *C.albicans* strains except for *P. rhoeas* extract which showed moderate antifungal activity against *C. albicans*, the extract of *F.vulgare* which showed good antifungal activity against the strain *C. albicans* and finally the extract of *S. hispanicus* which showed good antifungal activity against *C.neoformans*.

Table 2 also shows that the crude extracts tested in this study exhibit antimicrobial activities, which are low compared to the standard antibiotics used. However, these activities remain considerable as crude extracts, and not their pure compounds are used.

**DISCUSSION:** The data found in this study reveal that *M. annua* extract has no antimicrobial activity

against all strains tested. This finding is consistent with previous studies that reported none antimicrobial activity of the extract of this species against *S.aureus*, *Pseudomonas aeruginosa*, *Propionibacteriumacne*, and *C.albicans*[25]. This result is also confirmed by other studies that report that *M.annua* extract does not affect several strains, including *S.aureus* and *E.coli* tested in this study [26, 27]Our results are also consistent with the literature regarding the antimicrobial activity of *P. rhoeas* extract against all microbial strains tested except *C. neoformans*. Indeed, many publications that have documented the antibacterial activity of *P.rhoeas* report the same effects of its extract against various microbial strains, namely *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *C. albicans* [28-31]. Other research on di-ethyl ether, chloroform, and *P.rhoeas* acetone extracts also revealed antibacterial activity against *S.aureus*[32]. Also, Coban et al. showed a positive association of antimicrobial activity of *P.rhoeas* extracts with the alkaloid composition of these extracts and mainly with a major alkaloid, roemerin, which has an aporphine alkaloid skeleton and a methylenedioxy fragment[29].

The low antibacterial activity was showed by the *C. murale* crude extract against *E. coli* and *E. faecalis* strains against *E. coli* and *E. faecalis* strains. These results are inconsistent with the work of Ali et al. They found that ethanolic extracts prepared from *C. murale* sheets showed no antibacterial activity against the strains *E. faecalis*, *E.coli* and *Pseudomonas aeruginosa* [33]. This difference between our results and those of the literature can be explained by the choice of solvents, the extraction method, the collection region and by collection period of plants. Besides, the absence of activity of *C.murale* extract on *S. aureus* is consistent

with the results of other studies showing that the extract alone from the leaves of this plant showed no antibacterial activity against this strain[34]. Our study also reported antimicrobial activity of the species *F.vulgare*. The latter is considered as an important medicinal and aromatic plant well known and widely used as carminative, digestive, lactagogue, and diuretic and in the treatment of respiratory and gastrointestinal disorders. Its seeds are used as flavorings in bakery products, meat and fish dishes, ice cream, alcoholic beverages, and herbal blends [35]. The extract of this species shows excellent activity against food-borne pathogens *E. coli* and *S. aureus*. This result is similar to those found by Kaur and Arora, which showed that the aqueous and organic extracts of *F.vulgare* seeds had effects against *E.faecalis* strains, *S.aureus*, *E. coli*, *P.aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri* and *Salmonella Typhi* [36]. In another study, *F. vulgare* fruit extract was shown to have an antibacterial effect against other germs, including *Campylobacter jejuni* and *Helicobacter pylori* [37]. Seed extracts from *F. vulgare* also have antifungal activity against *C. albicans* [38]. Several chemical constituents of *F. vulgare* have been identified as active antimicrobial principles. Among these, a derivative of phenylpropanoid – Dillapional has proven to be the active antimicrobial principle in the stem of *F. vulgare*. Another molecule – Scopoletin, derived from coumarin, was also isolated from this plant with a marginal antimicrobial effect[39].

*S. hispanicus* is a plant included in herbal remedies [40]. The leaves, stems, and flowers are traditionally used as a “bitter” tonic to stimulate appetite, improve bile secretion, reduce flatulence, and facilitate digestion[41]. It has also historically been used as a diuretic, diaphoretic, and

antipyretic[41]. The plant extract showed good antibacterial activity against *E. faecalis*, *E. coli*, and *C. neoformans*. Other recent studies have reported numerous antimicrobial activities for *S. hispanicus* against several strains, namely *E.coli*, *Bacillus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Penicillium italicum*, and *Penicillium cyclopium*[42]. Marmouzi et al. found very strong antibacterial activity from the roots of *S. hispanicus* against *E. coli*. Moreover, the flowers of this plant were also reported to be very active against *S. aureus* and *Bacillus subtilis*. Other research has also shown better leaf activity of *S. hispanicus* against *Salmonella enterica* and *P. aeruginosa* strains.[4]. The antimicrobial properties of this species could be attributed to its chemical composition. Indeed, the different parts of the plant have high levels of  $\alpha$ -tocopherol, flavonoids, and phenolic acids that have been associated with the control of foodborne pathogens [4, 43].

**CONCLUSION:** In addition to their use as food, edible wild plants studied in this work, except *M. annua*, exhibit significant antimicrobial activities that can be attributed to their phytochemical composition. Two of these plants, *Foeniculum vulgare* Mill and *Scolymus hispanicus* L present important activities both antibacterial and antifungal. Exploration of these potentially valuable sources is of great importance to the local population and the pharmaceutical industry.

As a perspective, fractionation and isolation of the bioactive compounds responsible for the significant antimicrobial activities of certain extracts such as *F.vulgare* and *S.hispanicus* are recommended. In addition, the development of

methods of cultivation and preservation of these promising plants is essential.

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**Abbreviations:** Institute Pasteur Paris Collection (CIP), American Type Culture Collection (ATCC),

Mueller-Hinton (MH), Sabouraud dextrose agar (SDA).

**Competing interests:** The authors declare that they have no financial interest or conflicts of interest.

**Author's contributions:** All authors contributed to this work. A. Aboukhalaf implemented the research and drafted the paper. B. El Amraoui and M. Tbatou participated to the design of the experimental protocol. J. M. Ferreira da Rocha participated to the results discussion and the paper editing. R. Belahsen supervised the research, contributed to the results discussion and to the paper writing.

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