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ORIGINAL ARTICLE

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF MOROCCAN WILD EDIBLE PLANTS SELECTED BASED ON ETHNOBOTANICAL EVIDENCE

Abdelghani Aboukhalaf[1], *Youssef Lahlou*[2], *Adil Kalili*[1], *Sara Moujabbir*[1], *Jamila Elbiyad*[1], *Belkassem El Amraoui*[3], *Rekia Belahsen*[1]

[1]Laboratory of Anthropogenetic, Biotechnologies and Health, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco
[2]Laboratory of Control Quality in Bio-control Industry & Bioactive Molecules, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco
[3]Department of Biology, Laboratory of Biotechnology, Materials and Environment, Faculty Polydisciplinary of Taroudant, Ibn Zohr University, Agadir, Morocco

ABSTRACT

Background. Despite the extensive literature focusing on identifying novel antimicrobials of plant origin, little work has been undertaken to examine the antimicrobial activity of wild edible plants.

Objective. The current research aimed to determine the *in vitro* antimicrobial activity of methanolic extract of some common wild edible plants.

Material and Methods. Disc diffusion and broth micro dilution methods were used to evaluate the antimicrobial activity of extracts of *Mercurialis annua, Ziziphys lotus, Rubia peregrina, Origanum vulgare, Papaver rhoeas, Foeniculum vulgare*, and *Dysphania ambrosioides* against known human microorganisms' pathogens.

Results. The result indicated that most of the extracts exhibited a range of *in vitro* growth inhibitory action against all bacterial strains and yeasts tested with inhibition zones ranging from 11 mm to 32 mm, MIC value ranging from 0.048 to 50 mg/ml and MBC and MFC values ranging from 0.048 to 100 mg/ml. Among the seven plant extracts tested, *O. vulgare* was the most effective showing high antimicrobial activity against all tested microbial strains. All plant extracts exhibited bactericidal activities against all the tested bacteria strains except for those of *R. peregrina, P. rhoeas* and *F. vulgare* which showed a bacteriostatic activity against *E. coli* and *Pseudomonas* sp. Antifungal activity was shown only by *O. vulgare, F. vulgare* and *D. ambrosioides* against both *C. albicans* and *C. neoformans*.

Conclusion. These findings highlight the potential of wild edible plants to control human pathogenic microbes and demonstrate that these plants could be used as starting points for the development of novel antimicrobial compounds.

Keywords: *antibacterial activity, antifungal activity, wild edible plants, MIC, MBC, MFC, Morocco*

INTRODUCTION

The most relevant approach known to combat microbial diseases is the use of antibiotics. The latter are a class of chemicals with potent antimicrobial effects used to treat diseases related to pathogenic infections [1]. However, recent decades have seen indiscriminate use, overuse, and unnecessary prescription of antibiotics leading to antibiotic resistance and the spread of microorganisms [2]. As resistance to many synthetic antibiotics increases, a need to find effective, newer, less toxic antimicrobial drugs is of paramount importance. Plants have been recognized since ancient times, as valuable sources of natural chemicals with antimicrobial activities, which can be used as a cheaper alternative to expensive synthetic antibiotics [3, 4]. Morocco ranks among the countries with one of the richest plant diversities in the Mediterranean area [5]. Wild edible plants (WEPs) are a crucial element of this diversity. These plants are rich in protein, fiber, minerals and vitamins and their consumption helps fight malnutrition and food insecurity [6]. Additionally, WEPs are considered an excellent natural source of bioactive molecules associated with positive effects against various chronic diseases such as oxidative stress, cancer,

Corresponding author: Abdelghani Aboukhalaf, Laboratory of Anthropogenetic, Biotechnologies and Health, Training and Research Unit on Nutrition & Food Sciences, Chouaib Doukkali University, Faculty of Sciences, El Jadida 24000, Morocco; phone: +212 708279583; e-mail: aboukhalaf.a@ucd.ac.ma

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cardiovascular diseases, immune dysfunctions and other diseases [7-9]. Nevertheless, most studies in Morocco and other parts of the world focused on medicinal and aromatic plants. On the other hand, studies on WEPs and their biological properties have been somewhat neglected by the scientific community. To this end, the aim of this study was to evaluate *in vitro* antibacterial and antifungal activities against common microbial pathogens of methanolic extracts of some selected wild edible plants (WEPs). The selection of plants was based on their traditional use in the treatment of several types of infectious diseases including diarrhea, respiratory tract infections, skin and wound infections, etc.

MATERIAL AND METHODS

Collection of plant material

The different parts of wild edible plants selected (Table 1) were collected in March 2022, from Sidi Bennour province (32° 39' 8.50" N, 8° 25' 39.68" W) in central Morocco. The plants were identified by a taxonomist in Department of Biology, Chouaïb Doukkali University, (El Jadida, Morocco) and voucher specimens of plants have been deposited in herbarium of the same department. Table 1 shows the botanical name, family, parts used, food use, and ethnomedicinal use of plants under this study.

Preparation of crude extracts

After collection, the samples were washed with distilled water and dried in an oven at 37°C for one week. The completely dried materials were ground into fine powder, and then extracted by maceration method with methanol. The obtained extract was subsequently filtered through a Whatman No. 1 filter paper, and the filtrate was evaporated under reduced pressure using a Rota evaporator. Dried extract was stored in refrigerator at 4°C till further use.

Antimicrobial activity

Microorganism strains

Extracts obtained from different plants were tested for antimicrobial activity against six strains of bacteria (three strains of Gram-positive bacteria and three strains of Gram-negative bacteria) and two strains of fungi provided from the Institute Pasteur Paris Collection (CIP) and the American Type Culture Collection (ATCC): *Enterococcus faecalis* (ATCC19433), *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC 66331), *Escherichia coli* (CIP54127), *Citrobacter freundii* (ATCC8090), *Pseudomonas* sp., *Candida albicans* (48.72), and *Cryptococcus neoformans* (CIP 960).

Disk Diffusion Assay

Screenings of extract for antimicrobial activity was done by the disc diffusion method [18]. In order to do this, bacterial and fungal sterile physiological saline suspension was prepared to 0.5 of the McFarland standards (1.5×10^8 CFU/mL) from bacterial colonies grown on nutrient agar overnight at 37°C and yeast grown on Sabouraud at 37°C for 48 h. Then the bacterial suspensions were spread on Mueller Hinton Agar and the yeast suspensions were spread on Sabouraud Dextrose Agar. A paper discs (6 mm

Table 1. Wild edible plants tested for their antimicrobial activity in the study

Botanical name (family)	Common name	Parts used	Food uses	Ethnomedicinal uses
Origanum vulgare L. (*Lamiaceae*)	Zaatar	Leaves	Arome/Spices/ Drink [10]	Cold-respiratory problems, antiseptic, diarrhea, influenza, cough, intestinal parasites [10, 11]
Papaver rhoeas L. (*Papaveraceae*)	Belaaman	Aerial parts	Vegetables, Spices [12, 13]	Urogenital disorder-cough-rheumatism-dermatological problems, pulmonary infection [14]
Foeniculum vulgare Mill (*Apiaceae*)	Besbas Beldi	Aerial parts	Vegetables/Arome/ Snack [10, 12, 13]	Asthma, renal disease, eczema, cough, parasitic disease [15]
Dysphania ambrosioide L. (*Amaranthaceae*)	Mkhinza	Leaves	Vegetables/Arome [13]	Anthelmintic, antidiarrheic, colds, detersive, gynecological disorders, influenza [10, 11, 14]
Mercurialis annua L. (*Euphorbiaceae*)	Horriga lmelssa	Aerial parts	Vegetables/Spices [13]	Internal parasitosis, wound healing, rheumatism [12, 16]
Rubia peregrina L. (*Rubiaceae*)	ELfowa	Roots	Spices, Drinks [10, 12, 13]	Hepatitis, liver problems [17]
Ziziphus lotus L. Lam (*Rhamnaceae*)	Nbeg/Sedra	Fruits	Snack [10, 12]	Anthelmintic, wounds healing, urinary tract infections, pulmonary infection [10, 14]

diameter) that were impregnated with 60 µL of extract at concentration of 100 mg/ml, were placed on the inoculated agar surface. Petri dishes were left for 2 h at 4°C to allow the diffusion of the extract before incubation at 37±2°C for 18-24 h for bacteria and at 28±2°C for 48 h for the yeast activity. After incubation, the diameters of the inhibition zones were measured in mm. Fluconazole and Ampicillin were used respectively as positive controls and methanol as negative control.

Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of *F. vulgare* extract was determined by quantitative the micro-dilution method using resazurin as viability indicator [19]. Volume of 100 µl aliquots of Mueller-Hinton broth were placed in sterile 96-microwell plates and 50 µL of each extract, were adjusted to (50; 25; 12.5; 6.25; 3.12; 1.56; 0.8; 0.4; 0.2 and 0.1 mg/ml), and added to the wells. To each well containing the mixture, was added 50 µl of the Microbial suspension (1×10^7 CFU/ml) prepared in Mueller-Hinton broth for bacteria and prepared in Sabouraud broth for yeast. The plates were incubated for 24-48 h at 37±2°C and for 48-96 h at 27±2°C respectively. After incubation, 5 µl of resazurin (1 mg/ml) was added to each well and the incubation continued for 45 min. Finally, the MIC was recorded.

Minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) determinations

All the well plate showing no growth after MIC tests were reinoculated for the determination of the MBC and MFC. The broths were incubated according to growth requirement of each microorganism. The absence of growth in the recovery medium was evidence of bactericidal and fungicidal activities. Moreover, the ratio MBC/MCI and MFC/MCI of each sample was calculated to assess the antimicrobial power. If the ratio ≤4, the effect is bactericidal/fungicidal and when the ratio >4, it's bacteriostatic/fungistatic [20].

RESULTS

Antimicrobial activity

Screening of the antimicrobial activity of methanolic extracts of the studied plants was performed initially by the disc diffusion method against known human microorganisms' pathogens. These microorganisms were frequently encountered in infectious diseases. The results were summarized in Table 2. It was observed that except *Z. lotus* all plant extracts studied exhibited varying degrees of antimicrobial activity against all bacterial strains and yeasts tested with inhibition zones ranging from 11 mm to 32 mm. According to Aldoweriej et al., 2016 [21], the crude extracts tested have high antimicrobial activity when the inhibition zone is >15 mm; moderate antimicrobial activity if it is of 10-14 mm; low antimicrobial activity if the zone is 7-9 mm.

As can be seen, *O. vulgare* was the most effective among the seven plant extracts tested. It showed high antibacterial activity against all Gram-negative bacteria, Gram-positive bacteria tested with zones ranging from 22 mm to 32 mm.

Table 2. Zone of inhibition (mm) of plant part extracts against microorganisms

Microbial control strains	Gram-positive bacteria			Gram-negative bacteria			Yeasts	
	S. aureus	*E. feacalis*	*Bacillus* sp.	*C. freundii*	*E. coli*	*Pseudomonas* sp.	*C. albicans*	*C. neoformans*
F. vulgare	19±1.72	20±1.03	13±1.00	17±0.56	16±1.65	17±1.03	12±1.23	14±0.63
P. rhoeas	15±0.5	14±1.00	13±1.45	15±0.23	13±0/33	11±1.17	NI	NI
O. vulgare	31±0.00	30±1.03	32±0.37	32±0.55	22±0.65	28±1.00	16±0.17	18±0.55
M. annua	17±1.13	18±0.34	17±0.19	17±1.52	18±1.03	16±0.39	NI	NI
Z. lotus	NI	NI	NI	12	NI	NI	NI	NI
D. ambrosioides	15±0.3	14±0.52	18±0.13	11±0.22	12±0.52	12±0.12	16±0.45	14±0.78
R. peregrina	16±0.5	14±0.7	13±0.85	14±0.1	16±0.38	14±0.12	NI	NI
Amplicillin	26±0.00	27±0.0	27±0.00	25±0.00	28±0.00	24±0.00	ND	ND
Fluconazol	ND	ND	ND	ND	ND	ND	26±0.00	27±0.00

NI – no inhibition; ND – not determined

The highest antibacterial activity of *F. vulgare* was against *E. faecalis* (20 mm) and the highest antibacterial activity of *P. rhoeas* and *R. peregrina* was against *S. aureus* (15 mm, 16 mm respectively). *M. annua* displayed also a high antibacterial activity against *E. faecalis* as well as *E. coli* (18 mm). *D. ambrosioides* exhibited high antibacterial activity against *Bacillus* sp. (18 mm). Antifungal activity was shown only by *O. vulgare, F. vulgare* and *D. ambrosioides* against both *C. albicans* and *C. neoformans*. None of the other plant extracts showed antifungal activity.

Minimum inhibitory and bactericidal/Fungicidal concentrations of extracts

The tests of the studied extracts efficacy on the microbial strains used, was determined by measuring the minimum inhibitory concentration, MIC was determined for only microorganisms which showed a zone of inhibition and were sensitive to the plant extracts in the preliminary test using the disc diffusion method. MIC values varied from 0.048-50 mg/ml. Most active plants against test microorganisms are shown in Table 3. The most active plant against *E. coli*, *E. faecalis*, *Bacillus* sp., *Pseudomonas* sp. and *C. neoformans* was *O. vulgare* (0.048, 0.048, 0.097, 0.097 and 6.25 mg/ml respectively), against *C. freundii* were *M. annua* and *O. vulgare* (0.39 mg/ml), against *E. coli* was *F. vulgare* (0.78 mg/ml), against *C. albicans* was *D. ambrosioides* (6.25 mg/ml).

More precise data on the antimicrobial properties were obtained through the determination of bacteriostatic/fungistatic and bactericidal/fungicidal concentrations. As reported in Table 3, MBC and MFC values ranged from 0.048 to 100 mg/ml. As can be also seen all plant extracts exhibited bactericidal activity against bacteria strains except for those of *R. peregrina*, *P. rhoeas* and *F. vulgare* which showed a bacteriostatic activity against *E. coli* and *Pseudomonas* sp. Antifungal activity was shown by *O. vulgare, F. vulgare* and *D. ambrosioides* against both *C. albicans* and *C. neoformans*.

DISCUSSION

The current problem associated with antibiotic resistance represents a serious threat to public health, requiring surveillance, which continuously challenges the healthcare sector in a large part of the world [22, 23]. This problem is compounded by the decreasing effectiveness and increasing toxicity of antibiotics; which confront the scientific community with the obligation to seek new alternatives. Wild edible plants could be those alternatives. In the present study, the antibacterial and antifungal activities of seven Moroccan wild edible plants were investigated against six bacterial and two fungal reference strains.

In accordance with previous findings, it appears that Gram-positive bacteria are more sensitive to the extracts examined in this study than Gram-negative bacteria [24]. This difference in sensitivity could be related to the different composition of the Gram-negative and Gram-positive bacteria cell walls [25].

Findings from the current study revealed that except *Z. lotus*, all plant extracts tested showed varying degrees of antimicrobial activity against any of the microorganisms tested. A maximum activity against all microorganism tested was also obtained with the extracts of the plant *O. vulgare*. In addition, high MIC, MBC and MFC values are obtained with these extracts as well as a bactericidal and fungicidal activities against all tested strains. In accordance with these results, other studies have reported an antimicrobial activity of methanolic extract of *O. vulgare* [26], while, contrary to the present study data, no antimicrobial activity against *E. coli* and *C. albicans* were found by these studies. These discrepancies could be related to strain specificity or to plant geographical variations. *O. vulgare* was also found to be significantly active against *S. aureus*, *P. aeruginosa* and *E. coli*. Likewise, Mehreen et al., 2016 [27]. Another investigation carried out in Peru, showed that ethanolic extract of *O. vulgare* has antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa*. [28]. The antimicrobial potential of *O. vulgare* could be explained by its high content in monoterpenic hydrocarbons and in phenolic compounds such as carvacrol, thymol, p-cymene and 1-octacosanol [29, 30].

The extract from the Plant *F. vulgare* was found to have antimicrobial activity against all bacteria and fungi, with the highest activity obtained against *E. faecalis*. In agreement with these results, Aboukhalaf et al., 2020 [24] and Zellagui et al., 2011 [31] are have previously reported an inhibitory effect of extracts from *F. vulgare aerial part* against *S. aureus*, *E. coli*, *E. faecalis* and, *C. albicans*. Antagonistic activities of aqueous and organic extracts of *F. vulgare* against some human pathogenic bacteria such as *E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa, Salmonella typhi*, and *Shigella flexneri* are also found by Kaur and Arora 2008 [32]. Another study showed the efficacy of *F. vulgare* extract against *C. albicans* [33]. Studies on *F. vulgare* identified the phenylpropanoid derivative-dillapional as the compound responsible for the observed antimicrobial activity, scopoletin which is a coumarin derivative was also identified [34].

Another selected plant examined in this study is *D. ambrosioide*. It is considered as an important wild edible plant well known and widely used for the treatment of respiratory, urogenital, gastrointestinal, vascular and nervous disorders, and for metabolic disturbances

Table 3. The antimicrobial activity parameters

Plant species / Microbial strains		*F. vulgare*	*P. rhoeas*	*O. vulgare*	*M. annua*	*R. peregrina*	*D. ambrosioides*	*Z. lotus*
S. aureus	MIC	0.39	3.125	0.048	0.097	25	25	ND
	MBC	0.39	6.25	0.048	0.097	100	50	ND
	MBC/MIC	1	2	1	1	4	2	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
E. feacalis	MIC	0.097	6.25	0.048	0.097	12.5	12.5	ND
	MBC	0.097	12.5	0.048	0.097	25	12.5	ND
	MBC/MIC	1	2	1	1	2	1	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
Bacillus sp.	MIC	6.25	12.5	0.097	0.39	25	12.5	ND
	MBC	6.25	25	0.097	0.78	50	12.5	ND
	MBC/MIC	1	2	1	2	2	1	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
C. freundii	MIC	0.78	6.25	0.39	0.39	1.56	12.5	ND
	MBC	0.78	12.5	0.39	0.78	6.25	12.5	ND
	MBC/MIC	1	2	1	2	4	1	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
E. coli	MIC	0.78	6.25	1.56	3.125	12.5	25	ND
	MBC	3.125	50	3.125	6.25	100	50	ND
	MBC/MIC	2	8	2	2	8	2	ND
	Decision	Bc	Bs	Bc	Bc	Bs	Bc	ND
Pseudomonas sp.	MIC	0.39	12.5	0.097	0.39	12.5	12.5	ND
	MBC	6.25	100	0.197	0.78	100	12.5	ND
	MBC/MIC	16	8	2	2	8	1	ND
	Decision	Bs	Bs	Bc	Bc	Bs	Bc	ND
C. albicans	MIC	25	ND	25	ND	ND	12.5	ND
	MBC	50	ND	25	ND	ND	25	ND
	MBC/MIC	2	ND	1	ND	ND	2	ND
	Decision	Fs	ND	Fs	ND	ND	Fs	ND
C. neoformans	MIC	12.5	ND	6	ND	ND	25	ND
	MBC	50	ND	12.5	ND	ND	50	ND
	MBC/MIC	4	ND	2	ND	ND	2	ND
	Decision	Fs	ND	Fs	ND	ND	Fs	ND

Bc – bactericidal; Bs – bacteriostatic; Fs – fungistatic; ND – not determined

such as diabetes and hypercholesterolemia [35]. Its leaves are used in the preparation of traditional dishes as dietary condiment [35]. The results concerning the antimicrobial activity against Gram-positive, Gram-negative bacteria and yeasts observed in this study are contradictory to those of another study reporting no antimicrobial activity of *D. ambrosioides* against a wide range of microbial strains, namely *S. aureus*, *E. coli, P. aeruginosa* and *C. albicans* [35].

P. rhoeas was reported to produce diverse chemicals including fatty acids, hydroxyl-phenol groups, anthocyanin, flavonoid (flavon-3-ols, flavones and tannins), alkaloids typical rhoeadine groups that show antimicrobial activity [36, 37]. The extract of *P. rhoeas* was found to have moderate antimicrobial potential against both Gram-positive and Gram-negative bacteria, this result is in accordance with those found by Aboukhalaf et al., 2020 [24]. However, the lack of sensitivity of *C. albicans* and *C. neoformans* to *P. rhoeas* observed in the present study is in disagreement with other literature findings that showed an antifungal activity of *P. rhoeas* against *C. albicans* [24, 38, 39].

Concerning *M. annua*, bactericidal activities against all tested bacteria, are obtained with the extract of this plant. The present study results are different from those found in other researchers reports on antimicrobial activity of *M. annua* [24, 40]. This difference could be due to different factors related to the climate, the season, the geographical location, the time of the plant harvest, the part of the plant used for analysis, as well as the solvents chosen and the extraction procedure used.

For *R. peregrina*, there is only one report on antimicrobial activity by Ozgen et al., 2003 [41], indicating a potential inhibitory effect of this species against *Bacillus subtilis*, *S aureus* and *E. coli* and a limited inhibitory effect on *C. albicans,* this result is similar to our finding.

CONCLUSIONS

In conclusion, there is a strong antimicrobial activity against bacteria and fungi of wild edible plants such as, *O. vulgare*, *F. vulgare*, *D. ambrosioides*, *M. annua*, *P. rhoeas* and *R. peregrina* that deserve to be considered as a prospective tool in the treatment of diseases related to pathogenic infections. These plants could, in addition, be used in food industry, as a new natural additive to maintain the quality and shelf life of food products. Further investigations are however required, to demonstrate the *in vivo* efficacy, stability, and ability of these studied extracts to control microbial strains.

Conflict of interest

The authors declare no conflict of interest.

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