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In vivo antidiarrheal and *in vitro* antimicrobial activities of the aerial part extracts of *Waronia saharae* Benthem ex Benth. & Coss.

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ABSTRACT

A North African endemic plant known as *Warionia saharae* Benth. & Coss is frequently used in Morocco for treating digestive problems. In this work, the possible *in vivo* antidiarrheal effect of the aqueous extract of aerial part of this plant was evaluated to confirm its traditional use, also, the organic fractions were tested *in vitro* on antimicrobial activity. The results obtained from the *in vivo* study of the antidiarrheal activity of the aqueous extract showed significant inhibition against the diarrheal effect induced by castor oil with 75.07% at a 400 mg/kg dose. Additionally, this extract significantly inhibited the amount of fluid that accumulated in the intestinal lumen with 38.45% at 400 mg/kg, as well as the intestinal transit of activated charcoal. The antibacterial activities were tested for the aqueous extract and four organic fractions against five

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strains for the Gram-negative and the Gram-positive bacteria. Also, these extracts were tested for antifungal activity. The results of the study reveal that the aqueous extract and the dichloromethane fraction demonstrated the greatest levels of activity against the tested bacteria. This work provides a possible strategy for treating bacterial infections, which can be used to take advantage of this antimicrobial activities promising in the treatment of diarrhea caused primarily by these bacteria.

Keywords: *Warionia saharae,* antidiarrheal effect, antibacterial effect, antifungal effect

INTRODUCTION

Diarrheal diseases are the fourth leading cause of death among children under five, accounting for more than one million deaths per year globally[1].

Pharmacologic agents used to treat diarrhea includes several categories. Anti-inflammatory agents, such as glucocorticoids, salicylates, and indomethacin, aim to reduce inflammation. Organic anions, including gallic acid (tannin), d-galacturonic acid (pectin), and nicotinic acid. Neuroactive drugs, such as catecholamines, somatostatin, propranolol, phenothiazines, local anesthetics, and opiates, including derivatives like synthetic opiates (e.g., diphenoxylate and loperamide) and enkephalins, also contribute to managing diarrhea[2], in addition, diarrhea is a very common symptom of gastrointestinal infections, which can be caused by a huge variety of pathogens, with microorganisms accounting for the majority of the causes. Humans with fungus infections have also been known to develop diarrhea. However, many antibiotics are developed to treat diarrhea due to microbial infections.

Several drugs on the above list have posed toxicity risks and undesirable effects, and the misuse of antibiotics is also the root cause of the emergence of multidrug-resistant bacteria or superbugs. To stop this process of developing toxicity or resistance, it is essential to explore natural alternative approaches to reduce or eliminate it without relying on synthetic products.

Medicine plants are an alternative in primary care systems, and as a result, a promising pathway for drug development has historically improved. *Cotula cinerea* Del.[3], *Artemisia campestris* L. subsp. *glutinosa*[4], *Dissotis thollonii* Cogn.[5], *Zygophyllum gaetulum* Emberger[6], and *Calpurnia aurea*[7] are examples of plants herbs used in tradionnal medicine for treating diarrhea. In this study, we came to choose *Warionia saharae* Benthem ex Benth. & Coss., a

plant species mentioned in the world of traditional pharmacopeia as a treatment for digestive problems[8].

W. saharae is a North African endemic shrub. This plant is native to Morocco and Algeria, and it is the only species in the genus *Warionia* (Asteraceae)[9]. The leaves infusion of *W. saharae* are used in Moroccan pharmacopeia for their gastrointestinal properties, inflammatory diseases, and epilepsy problems[8]. Previous studies on specific parts of this plant have shown their immense strength as an antioxidant[10], anti-inflammatory, and cytotoxic properties against the cancer cell line "KB cells"[11,12].

According to the literature, these biological effects are mainly due to the presence in the *W. saharae* plant of several varieties of substances called secondary metabolites belonging to several classes such as phenolic compounds (flavonoids, chlorogenic acids), terpenes, and alkaloids, depending on the extract used; the hydrometanolic extract contains hydroxycinnamic acid derivatives and flavonoids[13], The chloroform extract contains the β-sitosterol as a major component, The ethyl acetate extract contains also the esculetin and cirsimaritin[14], Hilmi in 2002 reported the first-time isolation of 12 sesquiterpene lactones (SLs) of the guaianolide type from this plant. These SLs include dehydroleucodin, reynosin, 1,2-didehydro-3-oxo-costic acid, along with the flavonoid hispidulin[15], the essential oil contains in increasing order, nerolidyl acetate (21.44%), β-eudesmol (19.47%), and linalool (16.48%), which account for 57.39% of the total composition[16]. The primary goal of this study was to learn for the first time about the antidiarrheal and antimicrobial properties of *W. saharae* from southern Morocco.

METHODOLOGY

Plant material

W. saharae aerial parts were collected from the southeastern region of Morocco (Errachidia: 31°55′53 N′′, 4°25′35′′ W) on November 2020, identified by Professor Mostafa Elachouri and a specimen was registered in the herbarium of the Faculty of Sciences, Mohammed First University Oujda, Morocco, with the number UHPOM 450. Before the extraction process, the dried plant material is stored in a laboratory at 25° C in a dry and dark environment.

Preparation of the aqueous extract and the organic fractions

A quantity of 28 g of *W. saharae* aerial parts was infused in boiled distilled water (100 mL) for 1 hour, the aqueous extract (AEWS) was filtered, dried using a rotary evaporator (Buchi B-480, Switzerland), and then kept in a freezer until needed (-20°C). The various fractions of *W. saharae* were obtained following filho method[17] using a Soxhlet extractor, which dissolves the chemical compounds contained in a solid powder (100 mg) into a liquid or organic solvent (500 mL); the extraction takes place over a series of 6 h of cycles. The different solvents used have an increasing order of polarity, starting with hexane, followed by dichloromethane, ethyl acetate, and ending with methanol. The following formula employed to determine the extraction yield is:

$$\textbf{Extraction Yield}\ (\%) = \left(\frac{\text{Weight of extracted compounds}}{\text{Weight of dry plant material}}\right) \text{x } 100$$

Antidiarrheal effect of the aqueous extract

Animals

Albino mice weighing 25 to 30 g of both sexes were used in these experiments of antidiarrheal effect. They were placed under standard conditions in the animal house of the Faculty of Sciences, Oujda, Morocco, with free access to drinking water ad libitum. Maintained at controlled lighting (12h - 12h light-darkness cycle), humidity, and temperature. All the animals were fasted 18 hours before the day of the experiment with free access to the water. All animals were treated following the US National Institutes of Health's Guide for the Care and Use of Experimental Animals[18].

Castor oil-induced diarrhea

The protocol of Degu et al.,[19] was applied. Five groups of six mice each were treated according to the following repartition: Negative control: a group that received distilled water orally (1 mL/100g of body weight). The Positive control group received the loperamide hydrochloride (10 mg/kg) and the other groups received 50, 200, and 400 mg/kg of AEWS. For all groups, castor oil (0.5 mL) was administered 1 h after the first treatment, and finally, each animal was individually placed in cages with the ground coated with transparent paper and changed every 30 min with 4 h of observation. The parameters studied were the time of onset of first diarrhea, the total number of wet feces for 4 h, the total number of solid feces for 4 h, the total number of defecations for 4 h, and the number of mice with wet feces.

The percentage (%) of diarrhea inhibition is calculated as follows:

$$\% \text{ of diarrhea inhibition} = \left[\frac{(\text{WFC} - \text{WFT})}{\text{WFC}}\right] \times 100$$

WFC: average of wet feces in the negative control group.

WFT: average of wet feces in the treated group.

Small intestinal transit study

The protocol of Karim et al.[20] was applied. The following four groups of six mice each were randomly to perform the intestinal transit, treatment is carried out orally by gavage for all groups. A group that was given distilled water (1 mL/100g, body weight) served as the negative control. A group that received Loperamide hydrochloride (10 mg/kg) served as the positive control. The AEWS doses for the other groups were 200 and 400 mg/kg.

Fifteen min after this treatment, the mice in each group received 0.2 mL of an activated charcoal solution (3%) suspended in 0.5% methyl cellulose. After 30 min, all the mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the entire intestine was rapidly and carefully removed from the beginning from the duodenum to the end of the ileum. The results were expressed as a percentage of the distance traveled by the activated charcoal over the total length of the intestine:

$$\% \text{ of IP} = \frac{\text{Distance of intestine traveled by the activated charcoal (cm)}}{\text{The whole length of the intestine(cm)}} \times 100$$

With IP: Percentage of the intestinal propulsion.

From this formula, we will calculate the percentage of inhibition of intestinal transit (IT):

$$\% \text{ of IT} = \frac{\% \text{ of IP (test)} - \% \text{ of IP (negative control)}}{\% \text{ of IP (negative control)}} \times 100$$

Enteropooling essay

This study was conducted on the same groups described previously; treatment is carried out orally by gavage for all groups. A group that was given distilled water (1 mL/100g, body weight) served as the negative control. A group that received Loperamide hydrochloride (10 mg/kg) served as the positive control. The AEWS doses for the other groups were 200 and 400 mg/kg. One hour after the treatment, all these animals received orally 0.2 mL of castor oil. After

30 min, the abdominal cavity was opened and two ligatures were performed, one at the level of the pylorus and the other at the level of the cecum. We took the entire intestine from these two nodes and weighed respectively with (W1) and without (W2) its intestinal liquid. We measured also the total length of intestine (L). The desired parameter in this study is the ability of the extract to inhibit intestinal secretions caused by the castor oil (Enteropooling)[21]. This parameter can be calculated from the following formula:

$$\text{Enteropooling} = \frac{\text{W1} - \text{W2}}{\text{L}} \times 100$$

Antimicrobial assay of the aqueous extract and organics fractions

Inoculums standardization

The antibacterial activities of the AEWS, hexane fraction (HFWS), dichloromethane fraction (DFWS), ethyl acetate fraction (EaFWS), and methanol fraction (MFWS) of *W. saharae* were examined against five strains: *Escherichia coli* (ATB:57) B6N; *Escherichia coli* (ATB:97) BGM; *Pseudomonas aeruginosa; Klebsiella pneumonia* for the Gram-negative bacteria and the strains: *Staphylococcus aureus* for the Gram-positive bacteria. These extracts were tested for their antifungal activity against *Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* ATCC9763. These different strains were obtained from the Microbiology Laboratory, Faculty of Medicine and Pharmacy Fez, and Hassan II Hospital Fez, and were preserved in Muller-Hinton agar under refrigeration (4°C). The antibiotic Streptomycin and the antifungal: Fluconazole have been used as positive controls.

Preparation of the microbial suspension

The microbial inoculum was prepared by the direct suspension method from 2 to 3 colonies of a fresh culture aged 24 hours which were collected aseptically and suspended in 0.9% sterile physiological saline solution (NaCl), turbidity was adjusted to 0.5 McFarland[22t]. Bacterial suspensions contain approximately 1-2 x 10^8 CFU/mL, while the yeast suspension contains approximately 1-5 x 10^6 CFU/mL. The McFarland standard was prepared with a mixture of 99.5 mL of a sulfuric acid solution (H_2SO_4, 0.36 N) with 0.5 mL of a dehydrated barium chloride solution (0.048 M).

Disc diffusion method

The sensitivity was tested by disc diffusion method in accordance to the standard method by Bauer et al.[23]. Mueller Hinton agar plates were inoculated by the standardized suspensions. Whatman paper discs (6 mm) were placed on

the surface of preinoculated agar, which had been impregnated with 10 µL of the test compound extracts (1 mg/disc). All plates were incubated at 37° C for the bacteria and 30°C for the yeasts for 24 hours. After incubation, the growth inhibition zones were measured in mm. The test was repeated three times to ensure reliability[24].

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined by the broth microdilution method following the guideline of the National Committee for Clinical and Laboratory Standards Institute[25]. Successive dilutions (dilution of factor ½ in each well) of the test compound (1 mg/mL) were prepared directly in a 96-well microplate containing a Muller Hinton (MH) used for bacteria or Yeast Peptone Glucose (YPG) used for yeasts to obtain various concentrations. The different fractions of *W. saharae* were diluted in 10% dimethyl sulfoxide (DMSO) in such a way, these later did not exceed 1% in the wells. On the other hand, the control positives and microbial suspensions were diluted in a culture medium. The microplate was incubated under agitation for 24 hours at 37°C for the bacteria and 30°C for the yeasts. To read the results, 20 µL of 2,3,5-triphenyltetrazolium chloride (TTC) (1%) purchased from the company BIOKAR, was added to all the wells, the wells containing bacterial growth became pink due to the activity of the dehydrogenases, while the well without bacterial growth remained color less after 2 h incubation[26].

Statistical analysis

The results are presented on the average plus or minus the standard error expressed SEM, the different tests were carried out using the GraphPad Prism software (5 Software, San Diego, CA, USA). To compare the different means with each other and with the control, Tukey's post-test was used. A difference is considered significant if the probability P is less than 5% with * $p \leq 0.05$; ** $p \leq 0.01$, ***: $p<0.001$.

RESULTS and DISCUSSION

Yield of extraction

For the aqueous extract, a yield of 8.10% was obtained from the extraction of the aerial parts of *W. saharae*. The extraction processes made by Soxhlet apparatus starting with hexane and finishing with methanol, made it possible to reap four organic fractions of *W. saharae*. A fraction received by way of hexane whose yield is 3.41%, a fragment acquired by means of exhaustion with dichloromethane whose yield is 5.12%, a fragment of ethyl acetate received with 2.38% of yield and in the end a methanolic fraction of 8.8%.

Antidiarrheal effect of the aqueous extract

Castor oil-induced diarrhea

The effect of the AEWS (50, 200, 400 mg/kg) on castor oil-induced diarrhea in mice caused an extension of latency time, reduced defecation frequency with 21.76%, 40.52% and 49.9% respectively, as well as the number of wet feces in comparison with the untreated group (distilled water) with 34.83%, 69.96% and 75.07% respectively. The AEWS therefore exerted significant antidiarrheal activity in a dose-dependent manner with a maximum effect at 400 mg/kg (Table 1).

Table 1. Effect of the aqueous extract of *Warionia saharae* and loperamide (positive control) on castor oil-induced diarrhea in mice

Treatment	Onset of diarrhea (min)	Total number of defecations	Inhibition (%)	Total of wet feces (g)	Inhibition (%)
Distilled water	33.81 ± 3.81	5.33 ± 0.27	-	3.33 ± 0.18	-
Loperamide (10 mg/kg)	148 ± 5.01	0.83 ± 0.23***	84.42	0.33 ± 0.18**	90.09
AEWS (50 mg/ kg)	52.83 ± 5.53	4.17 ± 0.61NS	21.76	2.17 ± 0.61NS	34.83
AEWS (200 mg/kg)	85.50 ± 10.41	3.17 ± 0.64*	40.52	1 ± 0.27*	69.96
AEWS (400 mg/kg)	126.50 ± 2.76	2.67 ± 0.41**	49.9	0.83 ± 0.34**	75.07

AEWS: Aqueous extract of *Warionia saharae*. NS not significant; * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.001. The difference is statistically significant at the control. Results are presented as mean ± SEM with n=6.

The castor oil used in the present study is a trigger for diarrhea thanks to its inflammatory action on the intestinal mucosa, release of prostaglandins, nitric oxide, cyclic adenosine monophosphate, and platelet activating factors[27]. The prostaglandin synthesis inhibitors are considered antidiarrheal agent[7]. Pretreatment of mice with the AEWS elicited a significantly prolonged latency time, reduced defecation frequency, and decreased number of wet feces in comparison with the untreated group. However, the AEWS (200 mg/kg) reduced the number of fecal episodes by 40.52%, while the 400 mg/kg dose reduced the number of animals suffering from diarrhea by reducing the defecation frequency by 49.9%. Loperamide (10 mg/kg) profoundly reduced the

occurrence of castor oil-induced diarrhea and the number of diarrheal episodes by 84.42%. This latter was employed as a positive control, known for its antidiarrheal properties[28], and inhibitors of muscarinic and calcium receptors[29]. In terms of protection against diarrhea induced by castor oil, the AEWS (400 mg/kg) is capable of causing about half the protection induced by Loperamide. This could be due to the raw nature of the *W. saharae* extract as opposed to the pure form of Loperamide.

Small intestinal transit study

According to the results of this study (Table 2), we note that the AEWS has significantly reduced the distance traveled by activated charcoal by comparison to control and therefore bowel propulsion with 47.93 ± 1.98% at 400 mg/Kg. In the presence of Loperamide, the latter was reduced twice compared to the control.

Table 2. Effect of the aqueous extract of *Warionia saharae* (AEWS) and loperamide (positive control) on intestinal transit induced by castor oil in mice

Treatment	Length of the intestine (cm)	Distance covered by charcoal meal (cm)	IP (%)	(IT) (%)
Distilled water (Control)	32.58 ± 1.19	25.38 ± 0.92	77.9 ± 4.85	-
Loperamide (10 mg/ kg)	35.37 ± 1.71	10.50 ± 1.29***	29.97 ± 2.81***	61.53
AEWS (200 mg/kg)	31 ± 0.41	17.33 ± 0.52***	55.85 ± 1.20*	28.3
AEWS (400 mg/kg)	33.25 ± 1.46	15.94 ± 0.43***	47.93 ± 1.98**	38.45

AEWS: Aqueous extract of *Warionia saharae*. **IP:** intestinal propulsion, **IT**: inhibition of intestinal transit. *p≤ 0.05; ** p≤ 0.01; *** p≤ 0.001. The difference is statistically significant at the control. Results are presented as mean ± SEM with n=6.

In this intestinal motility test, we wanted to know the mechanism of action of the AEWS on castor oil-induced diarrhea. We notice that the AEWS (200 and 400 mg/kg) considerably delays the intestinal transit of activated charcoal in mice compared to the positive control. For mice that received these doses of the extract, intestinal propulsion was 55.85% and 47.93%, respectively, while in mice given just water, it was 77.9%. In the case of mice treated with Loperamide (10 mg/kg), only 29.97% of the entire intestine has been passed through by activated charcoal. It was also observed that the antidiarrheal effect of the extract was increased with increasing doses. These results allow us to suggest

that the AEWS might have antimotility properties, so we categorize it as an antidiarrheal agent. These results are in agreement with a previous work showing the antispasmodic activity of the aqueous extract of this same plant on rat and rabbit jejunums[30].

Enteropooling essay

Treatment of mice with castor oil resulted in an increase in the volume accumulation of fluids in the intestinal lumen (Figure 1); on the other hand, treatment with loperamide (10 mg/kg) and AEWS (200 - 400 mg/kg) significantly reduced this last with 13.17% and 7.47% respectively.

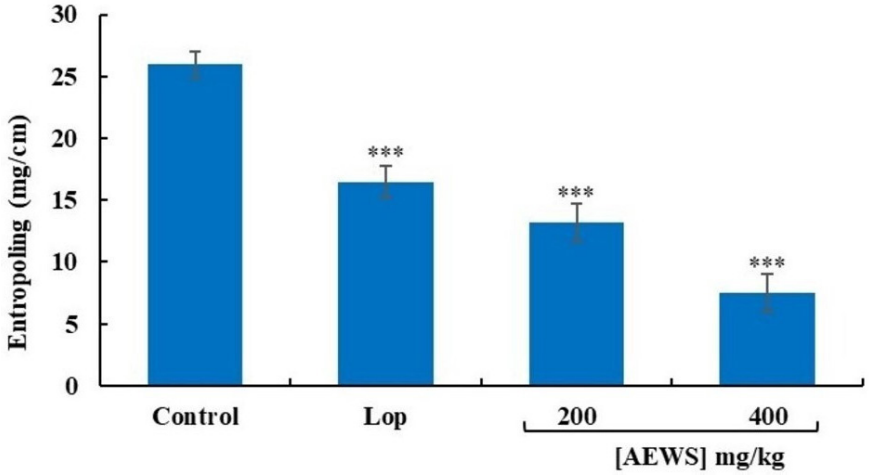

Figure 1. Effect of the AEWS on the accumulation of fluids in the intestinal lumen on castor oil-induced gut motility in mice

***: p≤0.001. The difference is statistically significant at the control. The results are presented in mean ± SEM with n=6. Lop: Loperamide.

Still within the framework of the same objective of clarifying the mechanism of action of the AEWS on castor oil-induced diarrhea, we tried to see the effect of this extract on the accumulation of intestinal fluids. The AEWS (200 and 400 mg/kg) and the antidiarrheal agent Loperamide both reduced the volume of fluids accumulation in the intestinal lumen. It seems that the AEWS has antisecretory and antimotility activities, which are due to the presence of one or more relaxant components in this extract. Antimuscarinic drugs and calcium channel blockers may be antispasmodics, antimotilities, and antidiarrheals[29,31]. In addition, in our previous studies, we have discovered that the aqueous extract and essential oil of this plant possessed an antispasmodic activities[16,30], which contributes to its effectiveness in treating diarrhea and abdominal

spasms. Similar results with other extract plants have been found in aqueous and methanolic leaves extracts of *Dissotis thollonii* Cogn[5], *Rubia tinctorum* L.[20], and *Streblus asper*[32].

Antimicrobial assay of the aqueous extract and organics fractions

Antibacterial activity

The antibacterial activity tested in the present study has been assessed quantitatively and qualitatively based on the presence or absence of zones of inhibition compared to the streptomycin used as a reference antibiotic. These later values ranged from 9 ± 0.2 to 20 ± 0.03 mm for Gram-positive bacteria and from 7 ± 0.30 to 14 ± 0.60 mm for Gram-negative bacteria (Table 3).

Table 3. Diameters of the zones of inhibition (mm) of the aqueous and organic extracts of *Warionia saharae* and streptomycin (positive control) against Gram-positive and Gram-negative bacteria

	Gram (-) bacteria				Gram (+) bacteria
	Escherichia coli 57	*Escherichia coli 97*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
	Diameter (mm)				
AEWS (1 mg/disc)	10 ± 0.40	9 ± 0.11	11 ± 1.04	10 ± 0.60	16 ± 1.10
MFWS (1 mg/disc)	7 ± 0.80	11 ± 1.05	8 ± 1.60	7 ± 0.30	14 ± 1.02
DFWS (1 mg/disc)	14 ± 0.60	-	10 ± 0.24	11 ± 0.07	14 ± 2.40
EaFWS (1 mg/disc)	10 ± 0.60	13 ± 1.03	-	12 ± 0.50	20 ± 0.03
HFWS (1 mg/disc)	11 ± 0.60	13 ± 1.40	-	-	9 ± 0.2
Streptomycin (0,02 mg/disc)	-	-	-	-	9 ± 1.00

AEWS: aqueous extract, **MFWS**: Methanolic fraction, **DFWS**: Dichloromethanic fraction, **EaFWS**: Ethyl acetate fraction, **HFWS**: Hexanic fraction, of *Warionia saharae*. Data are expressed as mean ± SEM (n=3 trials for each sample). **-:** no activity.

The AEWS showed a satisfactory result in inhibiting all the microorganisms test (*Escherichia coli* 57, *Escherichia coli* 97, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) with an inhibition diameter around of 9 ± 0.11 to 16 ± 1.10 mm. The MFWS, showed the inhibition of these five bacteria but by an inhibition diameter lower than that due to the AEWS (7 ± 0.80 à 14 ± 1.02 mm). While the EaFWS and DFWS were only active against

four organisms test (*Escherichia coli* 57, *Escherichia coli* 97, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*) and (*Escherichia coli* 57, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*) respectively. The hexane extract was only active against three strains: *Escherichia coli* 57, *Escherichia coli* 97, and *Staphylococcus aureus*. Whereas, Streptomycin showed no activity for these microorganisms. The greatest and minimum inhibitory diameters of *W. saharae* extracts were from, 20 ± 0.03 mm with EaFWS against *Staphylococcus aureus* and 7 ± 0.30 mm with MFWS against *Pseudomonas aeruginosa* (Table 3).

The antibacterial activity of *W. saharae* was also assessed by the MIC assay. In this study, all the extracts displayed a broad spectrum of antimicrobial activity, with MIC values ranging from 0.009 to 0.625 mg/mL for Gram-positive bacteria and from 0.009 to 25 mg/mL for Gram-negative bacteria (Table 4). For gram (-) bacteria, the best activities were expressed by the DFWS (0.009 to 0.312 mg/mL) and AEWS (0.02 to 0.40 mg/mL) against all microorganisms tested. MFWS had only been active against *Pseudomonas aeruginosa* (0.09 mg/mL), while EaFWS and HFWS were weakly active (5 to 10 mg/mL). Streptomycin was active against all of these microorganisms except *Pseudomonas aeruginosa* (Table 4).

Table 4. MICs values of the aqueous and organic extracts of *Warionia saharae* and streptomycin (positive control) against Gram-positive and Gram-negative bacteria

	Gram (-) bacteria				Gram (+) bacteria
	Escherichia coli 57	Escherichia coli 97	Klebsiella pneumoniae	Pseudomonas aeruginosa	Staphylococcus aureus
	MIC (mg/mL)				
AEWS	0.20	0.02	0.20	0.40	0.009
MFWS	8	5	25	0.09	0.009
DFWS	0.156	0.039	0.009	0.312	0.009
EaFWS	5	5	2.5	10	0.078
HFWS	10	10	10	10	0.625
Streptomycin	0.25	0.5	0.003	-	0.062

AEWS: aqueous extract, **MFWS**: Methanolic fraction, **DFWS**: Dichloromethanic fraction, **EaFWS**: Ethyl acetate fraction, **HFWS**: Hexanic fraction, of *Warionia saharae*. Data are expressed as mean ± SEM (n=3 trials for each sample). **-:** no activity.

For the first time, Rechek et al.[13] allowed the identification of 24 compounds from the hydromethanolic extract of *W. saharae* including derivatives of hydroxycinnamic acid and flavonoids. However, only ten compounds were isolated from the chloroform and ethyl acetate extracts from the aerial sections of the Algerian plant *W. saharae* using UV, IR, and NMR (1 H and 13 C) spectroscopies. These isolated compounds are β-sitosterol, stigmasterol, scopoletin, esculetin, hispidulin, cirsimaritin, chryseriol, luteolin, taxifolin, and quercetin[14]. Flavonoids are recognized for their antidiarrheal activity, which can be attributed to various mechanisms including antimicrobial action, inhibition of intestinal motility, and an antisecretory effect[7]. For instance, quercetin has demonstrated a significant decline in the Na^+/K^+–ATPase activity of enteropathogenic *Escherichia coli*-induced infectious diarrhoea, potentially leading to a notable reduction in the reabsorption of ions and water[33]. Additionally, this antidiarrheal effect may be associated with the presence of caffeic acid[34] and thymol[35]therefore, used for standardization in the German pharmacopoeia (0.03% phenols calculated as thymol in their composition. This activity can also be linked to the chemical components found in the essential oil, which possess spasmolytic properties. Examples includes cineol, 1-terpinen-4-ol[36], terpineol acetate[37] and linalool[38].

The different fractions of *W. saharae* and the aqueous extract demonstrated a broad spectrum of antimicrobial activity against the bacteria tested; Gram-positive and Gram-negative, with microbial growth inhibition ranging from 7 ± 0.30 to 20 ± 0.03 mm. Those extracts have also displayed a large spectrum of antimicrobial activity, with MIC values starting from 0.009 to 0.625 mg/mL for Gram-positive bacteria and from 0.009 to 25 mg/mL for Gram-negative bacteria. In the literature, numerous studies have been conducted to classify the distinct plant extracts consistent with their MIC value[39,40]. Kuete[41] classified crude extract activity as significant if the MIC is less than 100 µg/mL, moderate if the MIC is between 100 and 625 µg/mL, and low if the MIC is greater than 625 µg/mL. In this study, extracts with MIC values ranging from 0.009-0.4 mg/mL were considered to have good activity against the bacterial strains tested.

The best inhibitory activities were exerted by the DFWS and the AEWS, while the MFWS, EaFWS, and HFWS showed weak activity when considered with them. The DFWS showed a high level of antibacterial activity. It has been reported that the phenolic diterpenoids, which can be the primary compounds of the apolar fraction of plant extracts, are responsible for the antibacterial action[42]. These compounds are particularly lipophilic in nature and are extracted with low-polarity solvents such as hexane. However, the hexane fraction of this study showed antibacterial activity that was relatively low compared to other

extracts. Therefore, the size and load of the particles present in such an extract influence this activity. When compared to the fractions (MFWS, EaFWS, and HFWS) and streptomycin, the AEWS was also active against the five bacterial strains tested. It could be due to secondary metabolites (polyphenols and flavonoids) found in many crude extracts, which have a variety of pharmacological activities, including antibacterial activity[43]. It has also been demonstrated that these secondary metabolites have a large site capable of causing the rupture of the lipopolysaccharide layer from the plasma membrane of Gram-negative bacteria such as *Escherichia coli*, allowing for the alteration of vital intracellular enzyme systems in bacteria[44].

Our results are in agreement with those already mentioned. Gilbert et al.[5] for example, confirmed that the aqueous extract of *Dissotis thollonii* Cogn. exhibited a stronger antibacterial impact than the ones of hexane, methanol, and ethyl acetate from this plant. At the same time, Guadie et al.[45] proved the opposite case.

The EaWS recorded the highest levels of antibacterial activity for *Staphylococcus aureus*. This last one is a pathogenic Gram-positive bacterium that is found primarily in the intestinal lumen and can cause diarrhea[39]. Likewise, *Escherichia coli* and *Pseudomonas aeruginosa* are among the Gram-negative bacteria inhabiting the human gastrointestinal tract[46], and have presented a good range of sensitivity, specifically through the FDWS (MIC = 0.156-0.312 mg/ml) and the AEWS (MIC = 0.20-0.40 mg/ml) (Table 4). However, *Pseudomonas aeruginosa* is renowned for having an excessive level of intrinsic resistance, which was acquired against the majority of antibiotics[47].

Antifungal activity

Regarding the antifungal activity, the aqueous extract and the organic fractions of *W. saharae* all exhibited inhibitory activities towards *Candida albicans* and *Saccharomyces Cerevisiae* with inhibitory diameters ranging from 10 to 15 mm (Table 5), and MIC values ranging from 0.009 to 0.625 mg/mL (Table 6). In fact, the best antifungal activities detected as decreasing have been those of the DFWS, the MFWS, the AEWS, and subsequently the EaFWS, whose activities are greater than those of the HFWS and fluconazole.

Table 5. Diameters of the zones of inhibition (mm) of the aqueous and organic extracts of *Warionia saharae* and Fluconazole (positive control) against Gram-positive and Gram-negative bacteria

		Candida albicans	*Saccharomyces Cerevisiae*
	Dose	**Diameter (mm)**	
AEWS	1 mg/disc	10	11
MFWS	1 mg/disc	14	13
DFWS	1 mg/disc	15	12
EaFWS	1 mg/disc	12	11
HFWS	1 mg/disc	11	12
Fluconazole	5 mg/disc	21	27

AEWS: aqueous extract, **MFWS**: Methanolic fraction, **DFWS**: Dichloromethanic fraction, **EaFWS**: Ethyl acetate fraction, **HFWS**: Hexanic fraction, of *Warionia saharae*. Data are expressed as mean ± SEM (n=3 trials for each sample). -: no activity.

Table 6. MICs values of the aqueous and organic extracts of *Warionia saharae* and Fluconazole (positive control) against Gram-positive and Gram-negative bacteria

	Candida albicans	*Saccharomyces Cerevisiae*
	MIC (mg/mL)	
AEWS	0.3	0.1
MFWS	0.1	0.1
DFWS	0.018	0.009
EaFWS	0.312	0.312
HFWS	0.625	0.625
Fluconazole	0.4	0.2

AEWS: aqueous extract, **MFWS**: Methanolic fraction, **DFWS**: Dichloromethanic fraction, **EaFWS**: Ethyl acetate fraction, **HFWS**: Hexanic fraction, of *Warionia saharae*. Data are expressed as mean ± SEM (n=3 trials for each sample)

Each of these extracts demonstrated antifungal activity, as shown by the results of inhibition experiments using *W. saharae* aqueous extract and organic fractions on *Candida albicans* and *Saccharomyces cerevisiae*; no signs of resistance were found. Regarding the aqueous extract, we observe that it has very

strong antifungal activity. We list the dichloromethane fraction, the methanolic fraction, the ethyl acetate fraction, and lastly the hexanic fraction for the organic fractions in decreasing order of antifungal activity. This effectiveness may be connected to the extract's chemical composition[48], the solvent's ability to solubilize a number of substances found in the crushed plant, or the study itself[49]. Similar studies in the literature have proven that organic fractions and aqueous extracts of the plants showed good antifungal activity[50].

STATEMENT OF ETHICS

This study was approved by The Faculty of Sciences, Mohammed First University, Oujda (Morocco) under Trial Registration Number: 08/24-LBBEH-08 and 01/08/2024.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contribute the work equally throughout.

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