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Original Article

In vitro Study of Antibacterial Activity of Hydro-Alcohol Morrocan Plants Extracts

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ABSTRACT

Context: Several aromatic and medicinal Morrocan plants have been used traditionally in pharmaceutical products and traditional medicine for the treatment of several pathologies. **Objective:** Evaluation of the protective power of nine Moroccan plants ethanol extracts against some strains of bacteria. **Method:** The antibacterial activities of ethanolic extracts (EE) were evaluated using agar-well diffusion method, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and IC_{50} against nine foodborne bacteria [*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus (PN15 and 25923)*, *Escherichia coli (TF2 and ATCC 25929)*, *Pseudomonas aeruginosa (P116 and 195)* and *Salmonella enterica*]. Screening of chemical constituents was carried out as well. **Results:** We noted the presence of leuco-anthocyanins, anthocyanins, essential oils, alkaloids, and aldehydes in the extracts. The strains of *S. enterica* followed by *S. aureus* and *P. aeruginosa* were the most resistants to the extracts effect. The principal component analysis (APC) demonstrated that the highest antibacterial activity was that of *L. nobilis* and *O. europaea* ethanol extract (EE), which was directly bactericidal on all the strains tested with the exception of *P. aeruginosa*. While, *R. tinctorum*, *S. indicum* and *L. sativum* were characterized by the lowest activity. **Statistical analysis:** Analysis of variance was performed by uni-varied ANOVA in the software SPSS 22 Fr. **Conclusion:** The active compounds were soluble in ethanol. The antimicrobial activities of *L. nobilis* and *O. europaea* may contribute to understand their involvement in pharmaceutical products and traditional medicine against many microbial infections.
Key words: Antibacterial activity, Chemical composition, Hydro-alcohol extract.
Key Message: The antibacterial effect of *L. nobilis* and *O. europaea* support their medicinal usage and the identification and isolation of the therapeutic antimicrobials.

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INTRODUCTION

In Morocco, as for developing countries, most people, especially in rural areas, use medicinal plants to treat infectious diseases.[1] These infectious diseases are a major cause of morbidity and mortality worldwide, but especially in developing countries.[2] This situation is aggravated by the high cost of available medicines and the growing number of resistant pathogenic micro-organisms. Few new classes of anti-bacterials are released when older classes lose their effectiveness; as a result, antibiotic resistance has become a growing public health problem. Therefore, the discovery and development of new antimicrobial agents is of crucial priority.[3] Thus, our goal is to study the antibacterial properties of crude ethanolic extracts of some Morrocan plant species belonging to different families.

MATERIALS AND METHODS

Chemicals

All reagents (PCA, MH, Agarose, Resazurin, Ethanol, Folin–Ciocalteau reagent, Folin–Denis reagentsodium carbonate, Gallic acid, potassium acetate, Aluminum trichloride, Quercetin), unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant collection and extract preparation

Plants were collected in March, May and Jun 2015 from different region of Morocco (Table 1). The selected parts were dried at 40°C for 15 h. All samples were then ground into a fine powder, which was passed through an 80-mesh sieve. Aqueous extracts were obtained by extraction of samples (30 g) with distilled water (300 ml), for 60 min at 80°C (HAE) or 24 h min at 25°C (CAE). Hydro-alcohol extracts were obtained by extraction of samples (20 g) with 200 ml of ethanol solution (70%) for 24 h. The extractions were performed three times. After evaporation, the extracts obtained were autoclaved at 121°C for 15 min and stored at 4°C away from light until use. The extracts yield was determined by the following formula.[4]

$$R = 1 + \frac{P_X}{P_Y} \times 100$$

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Table 1: Plant species description.

Botanical name	Vernacular name	Family	Origin	Harvest period	Part used	Wild / cultivated
Geranium roseum	Laatarcha	Geraniaceae	Khmiss anjra	February	Leafed stems	Wild
Aloysia triphylla Cav	Louiza	Verbenaceae	Khmiss anjra	February	Leaves	Wild
Laurus nobilis	Awrak seydna moussa	Lauraceae	Khmiss anjra	March	Leaves	Wild
Lepidium sativum	Habb rchad	Brassicaceae	Marrakech	July	Seeds	Cultivated
Nigella sativa .L	Chanouj	Ranunculaceae	Aghraisse	August	Seeds	Cultivated
Olea europaea. L	Zaytûn	Oleaceae	Aîn Bayda	April	Leaves	Wild
Rubia tinctorum L	Fewwa	Rubiaceae	Meknès	March	Roots	Cultivated
Sesamum indicum. L	Zenjlane	*Pedaliaceae*	Beni Mellal	November	Seeds	Cultivated
Trigonella foenum graecum	Halba	Fabaceae	Gharb	June	Seeds	Cultivated

Table 2: The bacterial strains used.

Bacterial strains	Characteristics of bacteria		
	According to the requirement	According to Gram	According to the profile of sensitivity towards antibiotics
Staphylococcus aureus (PN15)	Non-demanding bacteria	Positive	Food isolates
Staphylococcus aureus 25923			Reference strains
Listeria monocytogenes 4032			
Bacillus cereus ATCC 14579			
Escherichia coli ATCC 25929			
Escherichia coli (TF2)		Negative	Food isolates
Pseudomonas aeruginosa (P116)			
Pseudomonas aeruginosa 195			
Salmonella enterica			

ATCC: American type culture collection

Table 3: Détection qualitative des groupes chimiques dans les extraits *éthanoliques* bruts.

Species	Alcaloïds	Leuco-anthocyanins	Iridoïds	Saponins	Anthra-quinons	Antho-cyanins	Deoxyoses	Aldehyds	Essential oils
G. roseum	-	+++	-	+	+	+	+	+	+
A. citriodora	-	-	-	-	-	-	-	-	+
L. nobilis	+	++	+	++	+	++	-	+	+
L. sativum	-	+	-	-	-	+	-	+	-
N. sativa	+	-	-	-	-	-	-	-	-
O. europaea	++	+	+	-	-	+	-	++	++
R. tinctorum	+	-	-	-	+	+	+	+	+
S. indicum	+	-	-	+	-	-	-	++	-
T. foenum graecum	++	+	+	+	-	-	-	-	++
Total (N)	6/9	5/9	3/9	4/9	2/9	5/9	2/9	5/9	6/9
Total (%)	**66**	**55**	**33**	**44**	**22**	**55**	**22**	**55**	**66**

+++ : Very abundant ; ++ : abundant ; + Presence of the metabolite ; - Absence of the metabolite

R: Extract yield (%), P_x: Extract weight (g), P_y: Plant weight (g).

Qualitative analysis of phytochemicals

Different groups of secondary metabolites such as aldehydes, terpenoids, polyphenols including flavonoids and tannins, alkaloids, saponins and quinone substances were investigated as used by.[5]

Evaluation of antibacterial activity

Antibacterial activity was evaluated at Laboratory of Microbiology of hygiene and food safety department of the Institute Pasteur Tangier – Morocco.

Microbial Strains and Growth Conditions

Six different reference strains and food-borne isolates were used for assessing the plant antimicrobial properties; including Gram-positive and Gram-negative bacteria (Table 2). Fresh cultures were prepared by

Table 4: The antimicrobial activity of Ees.

Plant species	Bacterial strains	Ø of the inhibition zone for 50 mg/ mlof extract (mm)	Qualitative decision	MIC (mg/ ml)	MBC (mg/ ml)	Report MBC/ MIC	Decision according to Oursou et al., (2008)
Laurus nobilis (Awrak seydna moussa)	***E. coli***	6	No inhibitory	25	50	2	Bactericidal
	TF 2	6	No inhibitory	50	100	2	Bactericidal
	S. aureus	9	No inhibitory	1,56	3,12	2	Bactericidal
	PN 15	6	No inhibitory	3,12	6,25	2	Bactericidal
	L. monocytogenes	11±0.22*	Slight inhibitory	6,25	12,5	2	Bactericidal
	P 116	5	No inhibitory	50	>100	>2	Bacteriostatic
	P. aeruginosa	5	No inhibitory	100	>100	>1	Bacteriostatic
	B. cereus	15±0.13*	Slight inhibitory	0.5	0.5	1	Bactericidal
	S. enterica	6	No inhibitory	6.25	6.25	1	Bactericidal
Lepidium sativum (Habb Rchad)	***E. coli***	6	No inhibitory	25	>100	>4	Bacteriostatic
	TF 2	6	No inhibitory	50	>100	>2	Bacteriostatic
	S. aureus	6	No inhibitory	25	100	4	Bacteriostatic
	PN 15	6	No inhibitory	50	>100	>2	Bacteriostatic
	L. monocytogenes	6	No inhibitory	50	>100	>2	Bacteriostatic
	P 116	6	No inhibitory	50	>100	>2	Bacteriostatic
	P. aeruginosa	6	No inhibitory	50	>100	>2	Bacteriostatic
	B. cereus	6	No inhibitory	25	100	4	Bacteriostatic
	S. enterica	6	No inhibitory	100	>100	>1	Bacteriostatic
Nigella sativa. L (Chanouj)	***E. coli***	6	No inhibitory	25	>100	>4	Bacteriostatic
	TF 2	6	No inhibitory	50	>100	>2	Bacteriostatic
	S. aureus	9	No inhibitory	12,5	12,5	1	Bactericidal
	PN 15	12±0.15*	Slight inhibitory	25	50	1	Bactericidal
	L. monocytogenes	6	No inhibitory	25	100	4	Bacteriostatic
	P 116	6	No inhibitory	100	>100	>1	Bacteriostatic
	P. aeruginosa	6	No inhibitory	>100	>100	>1	Bacteriostatic
	B. cereus	17±0.23**	Moderate inhibitory	6,25	12.5	2	Bactericidal
	S. enterica	6	No inhibitory	50	100	2	Bactericidal
Olea. europaea.L (Zaytûn)	***E. coli***	6	No inhibitory	25	100	4	Bacteriostatic
	TF 2	6	No inhibitory	25	100	4	Bacteriostatic
	S. aureus	6	No inhibitory	100	>100	>1	Bacteriostatic
	PN 15	6	No inhibitory	>100	>100	>2	Bacteriostatic
	L. monocytogenes	6	No inhibitory	25	100	4	Bacteriostatic
	P 116	6	No inhibitory	25	100	4	Bacteriostatic
	P. aeruginosa	6	No inhibitory	25	>100	>4	Bacteriostatic
	B. cereus	8±0.2	No inhibitory	100	>100	1	Bacteriostatic
	S. enterica	6	No inhibitory	100	>100	>1	Bacteriostatic
Rubia tinctorum.L (Fewwa)	***E. coli***	6	No inhibitory	25	100	4	Bacteriostatic
	TF 2	6	No inhibitory	50	>100	>4	Bacteriostatic
	S. aureus	6	No inhibitory	6,25	12,5	2	Bactericidal
	PN 15	6	No inhibitory	12,5	100	4	Bactericidal
	L. monocytogenes	6	No inhibitory	6,25	12,5	8	Bacteriostatic
	P 116	6	No inhibitory	100	>100	>1	Bacteriostatic
	P. aeruginosa	6	No inhibitory	>100	>100	>1	Bacteriostatic
	B. cereus	9	No inhibitory	25	25	1	Bactericidal
	S. enterica	6	No inhibitory	12,5	50	2	Bactericidal

Continued...

Table 4: Cont'd.

	E. coli	6	No inhibitory	50	>100	>2	Bacteriostatic
	TF 2	6	No inhibitory	100	>100	>1	Bacteriostatic
	S. aureus	6	No inhibitory	50	>100	>2	Bacteriostatic
	PN 15	6	No inhibitory	50	>100	>2	Bacteriostatic
Sesamum indicum.L (Zenjlane)	***L. monocytogenes***	7	No inhibitory	25	100	4	Bacteriostatic
	P 116	6	No inhibitory	50	>100	>2	Bacteriostatic
	P. aeruginosa	6	No inhibitory	100	>100	>1	Bacteriostatic
	B. cereus	6	No inhibitory	50	>100	>2	Bacteriostatic
	S. enterica	6	No inhibitory	100	>100	>1	Bacteriostatic
	E. coli	6	No inhibitory	50	>100	>2	Bacteriostatic
	TF 2	6	No inhibitory	100	>100	>1	Bacteriostatic
	S. aureus	6	No inhibitory	50	>100	>2	Bacteriostatic
	PN 15	6	No inhibitory	100	>100	>1	Bacteriostatic
Trigonella. foenum grecum (Halba)	***L. monocytogenes***	6	No inhibitory	25	100	4	Bacteriostatic
	P 116	6	No inhibitory	100	>100	>1	Bacteriostatic
	P. aeruginosa	6	No inhibitory	>100	>100	>1	Bacteriostatic
	B. cereus	6	No inhibitory	25	100	4	Bacteriostatic
	S. enterica	6	No inhibitory	50	>100	>1	Bacteriostatic
	E. coli	6	No inhibitory	25	50	2	Bactericidal
	TF 2	6	No inhibitory	50	100	2	Bactericidal
	S. aureus	6	No inhibitory	25	50	2	Bactericidal
	PN 15	6	No inhibitory	25	50	2	Bactericidal
Géranium roseum (Laatarcha)	***L. monocytogenes***	10±0.31*	No inhibitory	12,5	25	2	Bactericidal
	P 116	14±0.21**	Slight inhibitory	12,5	100	8	Bacteriostatic
	P. aeruginosa	8±0.11*	No inhibitory	25	100	4	Bacteriostatic
	B. cereus	7±0.5	No inhibitory	25	50	2	Bactericidal
	S. enterica	6	No inhibitory	25	50	2	Bactericidal
	E. coli	6	No inhibitory	100	>100	>1	Bacteriostatic
	TF 2	6	No inhibitory	>100	>100	>1	Bacteriostatic
	S. aureus	6	No inhibitory	25	>100	>4	Bacteriostatic
	PN 15	6	No inhibitory	50	>100	>2	Bacteriostatic
Aloysia triphylla Cav (Louiza)	***L. monocytogenes***	6	No inhibitory	25	>100	>4	Bacteriostatic
	P 116	7±0.12	No inhibitory	25	>100	>4	Bacteriostatic
	P. aeruginosa	7±0.09	No inhibitory	50	>100	>2	Bacteriostatic
	B. cereus	8±0.15*	No inhibitory	50	>100	>2	Bacteriostatic
	S. enterica	6	No inhibitory	50	>100	>2	Bacteriostatic

transferring a loop of cells from the agar slant to a test tube containing 5 ml of brain heart infusion (BHI) (BioRad) and then incubated overnight at 37°C.

Disk Diffusion Assay

Disc-diffusion assay was used to determine growth inhibition caused by plant extracts.[14] For each strain, inoculums (10^6–10^8 CFU per milliliter), was spread on Mueller–Hinton Agar (MHA) (BioRad). Enumeration of bacteria was performed by measuring turbidity at 550 nm (VARIAN Cary 50 UV-Vis). Sterile Whitman's filter discs (N°40; Ø =6 mm), impregnated with 10 µ l of different extracts dilutions from the initial concentration of 50 mg/ml, were deposited on the surface of each petri dish. In parallel, an empty disc and an antibiotic disc were used as a negative and positive control respectively. The petri dishes were kept at 4°C for 15 to 20 min to allow the diffusion of the extract, then incubated at 37°C for 18 to 24 h, under normal atmosphere, after which, inhibition zones around each disc (> 6 mm) were measured (disc diameter included). Each test was performed in triplicate.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of ethanol extracts was determined by the method of Mann and Markham, (1998),[6] using resazurin as viability indicator. Different dilutions of the extracts (50; 25; 12.5; 6.25; 3.12; 1.56; 0.8; 0.4; 0.2 and 0.1 mg/ml) were prepared from a stock solution (100 mg/ml). To each well containing 50 µ l of the mixture, was added 50 µ l of the bacterial suspension (10^6 to 10^8 CFU/ml) prepared in Mueller-Hinton Broth medium (MHB). Plate was then incubated at 37°C for 18 to 20 h. After the first incubation step, 5 µ l of

Table 5: Study of the antibacterial effect by comparison of IC_{50} of EEs, between the nine plant species.

	Mean Ps (S) ± Er.Std	Mean Ps (R) ± Er.Std	Mean EC (S) ± Er.Std	Mean EC (R) ± Er.Std	Mean SA (S) ± Er.Std	Mean SA (R) ± Er.Std	Mean Sal (S) ± Er.Std	Mean Bac (S) ± Er.Std	Mean Lis (S) ± Er.Std
G. roseum	19,47±0,28 (c)	68,58±0,21 (d)	27,61±0,27 (f)	33,98±0,45 (e)	29,71±0,33 (b)	29,55±0,44 (a)	10,28±0,17 (a)	5,86±0,24 (a) (b)	14,90±0,19 (e)
A. citrodora	15,24±0,23 (b)	23,50±0,34 (b)	22,15±0,29 (e)	24,54±0,21 (d)	58,39±0,22 (e)	131,45±0,29 (g)	121,41±0,34 (f)	17,56±0,31 (d)	13,26±0,25 (d)
L. nobilis	16,20±0,18 (b)	42,74±0,24 (c)	7,44±0,13 (b)	19,23±0,29 (b)	39,12±0,33 (c)	42,44±0,13 (b)	26,62±0,29 (b)	7,3±0,18 (b)	23,29±0,22 (f)
L. sativum	102,42±0,36 (e)	246,86±0,78 (g)	11,31±0,30 (c)	21,79±0,35 (c)	686,78±0,31 (i)	1536,27±0,79 (i)	1334,008±0,96 (h)	66,97±0,64 (g)	126,64±0,31 (i)
N. sativa	115,42±0,68 (f)	435,13±0,71 (h)	20,62±0,39 (d)	34,49±0,43 (e)	91,76±0,58 (g)	116,20±0,82 (f)	28,34±0,55 (b)	24,27±0,25 (f)	29,84±0,34 (g)
O. europaea	5,51±0,15 (a)	4,72±0,27 (a)	3,38±0,22 (a)	10,75±0,19 (a)	23,17±0,25 (a)	61,52±0,27 (d)	38,92±0,30 (c)	5,37±0,19 (a)	1,47±0,16 (a)
R. tinctorum	103,36±0,42 (e)	122,66±0,24 (f)	117,47±0,47 (h)	131,99±0,56 (h)	41,68±0,25 (d)	54,05±0,46 (c)	48,25±0,29 (d)	20,49±0,28 (e)	11,54±0,28 (c)
S. indicum	214,25±0,17 (g)	857,21±0,30 (i)	27,57±0,30 (f)	63,45±0,19 (f)	463,91±0,33 (h)	1213,65±0,55 (h)	435,94±0,45 (g)	12,03±0,43 (c)	8,97±0,18 (b)
T. foenum graecum	78,34±0,25 (d)	82,46±0,41 (e)	65,29±0,32 (g)	82,11±0,34 (g)	81,69±0,34 (f)	104,06±0,24 (e)	63,50±0,20 (e)	65,54±0,28 (g)	48,54±0,20 (h)
TOTAL	74,47±6,84	202,04±27,14	33,65±3,62	46,92±3,91	168,47±23,82	365,46±57,86	234,16±34,28	25,04±2,42	30,94±3,84
FISHER	39228,86 (p<0,000) **	399567,45 (p<0,000) **	13166,2 (p<0,000) **	11791,48 (p<0,000) **	471130,42 (p<0,000) **	1325772,26 (p<0,000) **	904313,22 (p<0,000) **	5000,34 (p<0,000) **	24019,31 (p<0,000) **

Groups with the same letters do not differ significantly by tukey test. Er.Std: standard error; R: Resistant ; S : Sensitive ; **: Very highly significant difference.

resazurin (1 mg/ml) was added to each well. Reading results was carried out after further incubation for 2 h at 37°C. The MIC corresponds to the lowest extract concentration, which does not produce change of resazurin staining. Then, the optical density at 550 nm was measured (Epoch BioTek UV-Vis) for IC_{50} determination. The following formula was used to calculate the survival germs percentage.[6]

$$S = \frac{d_f - d_1}{D_f - D_1} \times 100$$

S: survival percentage of germs, *di*: densimat value of experimental tube before incubation, *Di*: densimat value control tube before incubation, *Df*: densimat value after incubation control tube, *df*: densimat value of experimental tube after incubation.

Determination of the minimal bactericidal concentration (MBC)

Plate counting agar (PCA) (BioRad) was seeded with 10 µ l of samples from plate wells where there was no resazurin color change. Dishes were then incubated for 18 to 20 h at 37°C. The MBC corresponds to the lowest extract concentration that gives no growth. Moreover, the ratio MBC/CMI of each sample was calculated to assess the antibacterial power.

Statistical Analysis

All *in vitro* experiments were conducted in triplicate and results were expressed as mean ± SD. Analysis of variance was performed by uni-varied ANOVA for determination of phenolic, flavonoid and tannin contents. Statistical analysis of the antibacterial activity was performed by analysis of variance with two factors in the software SPSS 22 Fr. IC_{50} value were determined by regression analysis. The values $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Detection of chemical groups

The chemical groups screening showed the presence of essential oils, saponins, iridoïds, alkaloids, anthocyanins, and aldehydes (Table 3). In general, the distribution of secondary metabolites differs between species. *Laurus nobilis* and *G. roseum* have shown the presence of the majority of the screened chemical compounds.

The harvest area and other parameters as the pH and its richness in organic matter, influence greatly the production of chemical compounds in the plant.[7]

Alkaloids play an important role in biological structures and well known for their high antibacterial power.[8] Antibiotic, antifungal, antiviral activities have been reported about saponins.[9,10] While for essential oils, their presence is in general equated with a bacteriostatic effect.[11,12]

Antibacterial activity

Among the EEs of the investigated species, only *L. nobilis* and *G. roseum* showed a bactericidal effect on all the strains, except those of *P. aeruginosa* for which the effect was bacteriostatic. In addition, there was a strong significant activity on the solid medium, with a mild to moderate inhibitory effect in the case of *B. cereus* and *L. monocytogenes* in *L. nobilis*; *P. aeruginosa* (S and R) and *L. monocytogenes* in *G. roseum*; *B. cereus* and *S. aureus* (R) in *N. sativa*. The other species showed a bacteriostatic effect with high MIC and MBC values (Table 4).

The IC_{50} analysis by the tukey test showed a strong antibacterial effect in *O. europaea* which, on the other hand, has no inhibitory effect on

Table 6: Comparison of two to two means of the EEs extracts on the different bacterial strains.

Paired samples		t student	Sig.
P. aeruginosa (S)	***P. aeruginosa (R)***	**6,22**	***p*<0,000 ****
E. coli (S)	*E. coli (R)*	13,66	***p*<0,000** **
S. aureus (S)	*S. aureus (R)*	5,74	***p*<0,000** **
	E. coli (S)	5,94	***p*<0,000** **
	E. coli (R)	4,48	***p*<0,000** **
	S. aureus (S)	4,62	***p*<0,000** **
P. aeruginosa (S)	*S. aureus (R)*	5,41	***p*<0,000** **
	S. enterica (S)	3,87	***p*<0,000** **
	B. cereus (S)	7,39	***p*<0,000** **
	L. monocytogenes (S)	5,98	***p*<0,000** **
	E. coli (R)	6,2	***p*<0,000** **
	S. aureus (S)	5,9	***p*<0,000** **
P. aeruginosa (R)	*S. aureus (R)*	1,7	***p*<0,091**
	S. enterica (S)	3,39	***p*<0,0001****
	B. cereus (S)	0,56	***p*<0,572**
	L. monocytogenes (S)	6,59	***p*<0,000** **
	S. aureus (S)	6,36	***p*<0,000** **
	S. aureus (R)	5,41	***p*<0,000** **
E. coli (S)	*S. enterica (S)*	5,63	***p*<0,000** **
	B. cereus (S)	4,52	***p*<0,000** **
	L. monocytogenes (S)	2,16	***p*<0,032**
	S. aureus (S)	0,47	***p*<0,633**
	S. aureus (R)	4,95	***p*<0,000** **
E. coli (R)	*S. enterica (S)*	5,45	***p*<0,000** **
	B. cereus (S)	4,23	***p*<0,000** **
	L. monocytogenes (S)	5,19	***p*<0,000** **
	S. aureus (R)	2,7	***p*<0,008** **
S. aureus (S)	*S. enterica (S)*	2,97	***p*<0,003** **
	B. cereus (S)	6,32	***p*<0,000** **
	L. monocytogenes (S)	6,49	***p*<0,000** **
	S. enterica (S)	5,24	***p*<0,000** **
S. aureus (R)	*B. cereus (S)*	5,98	***p*<0,000** **
	L. monocytogenes (S)	6,03	***p*<0,000** **
S. enterica (S)	*B. cereus (S)*	4,99	***p*<0,000** **
	L. monocytogenes (S)	5,07	***p*<0,000** **
B. cereus (S)	*L. monocytogenes (S)*	2,62	***p*<0,01** *

solid medium. The extract of this specie has a bacteriostatic effect on all strains, except for *S. aureus* (R) and *S. enterica* which were more susceptible to *G. roseum* effect. The highest values were those of *S. indicum* in the case of *P. aeruginosa* (S and R), *R. tinctorum* in the case of *E. coli* (S and R) and *L. sativum* in the case of other strains (Table 5).

In general, the lowest values of antibacterial parameters were obtained with *B. cereus*, *S. aureus* followed by *L. monocytogenes* and were therefore the most sensitive strains. However, strains of *P. aeruginosa*, *E. coli* and *S. enterica* remain the most resistant to the effect of extracts, with high MIC and IC_{50} values. This corroborates with the results of Sqalli *et al.,* (2008).[13]

In fact, some studies do not reveal any selective antimicrobial activity against Gram (+) or Gram (-) bacteria.[14] On the other hand, other studies have highlighted the high sensitivity of Gram (+) bacteria compared to Gram (-).[15,16] This can be attributed to the difference in the outer layers of Gram (-) and Gram (+) bacteria.

By the tukey test, it appeared that the difference was highly significant in most cases. The graphical IC_{50} means representation of these nine species EEs showed a remarkable susceptibility of *B. cereus*, followed by *L. monocytogenes*, which showed a non-significant difference to *E. coli* (S) (Table 5). Comparing IC_{50} of the other bacterial strains, paired two by two, showed a highly significant difference ($p < 0.000$). Also, for a threshold $\alpha = 5\%$, the Fisher Table provided large critical values, which means that the significant difference observed between IC_{50} means depended on the species used.

According to the graph of Figure 1, *S. aureus* (R) followed by *S. enterica* and *P. aeruginosa* ($p > 0.05$), showed a marked resistance to the effect of EEs used. Also, resistant strains had significantly higher IC_{50} than sensitive ones. However, there were high standard errors in the case of *S. aureus*, *P. aeruginosa* (R) and *S. enterica*, which means that there was a significant difference in the survival of these strains from one specie to the other. This can be explained by the difference in the phytochemical composition of each specie and the concentration in these compounds, since they belong to different plant families.

Analysis of the total variance showed that the percentage of inertia around axis 2 was 77.09% (Table 7). Projection of variables and active species of the PCA on the factorial graph showed the strains distribution into three groups. Groups 1 and 2 were on the positive side and strongly characterized *G. roseum*, *N. sativa* and *A. citrodora*. The species *O. europaea* and *L. nobilis* acted on strains of group 1, 2 and 3 at the same time.

The species *S. indicum*, *L. sativum*, *R. tinctorum* followed by *T. foenum graecum* remained the plants with the lowest growth inhibitory activity of all the bacteria tested (Figure 2).

Comparison of the numerical values of our study with other publications is often qualitative, since the authors express their results with different units making the quantitative comparison difficult.[17] Qualitatively, our results were correlated with those of literature.

Kroum., (2009).[18] study showed that methanolic and aqueous extracts of *T. foenum graecum* seeds were not good antibacterial agents. According

Table 7: Principal component analysis of the total variance explained.

Component	Initial values			Sum of factors squares selected for rotation		
	Total	% of variance	% cumulated	Total	% of variance	% cumulated
1	4,57	50,82	50,82	4,57	50,77	50,77
2	2,36	26,26	77,09	2,36	26,31	77,09

Extraction method: Principal component analysis.

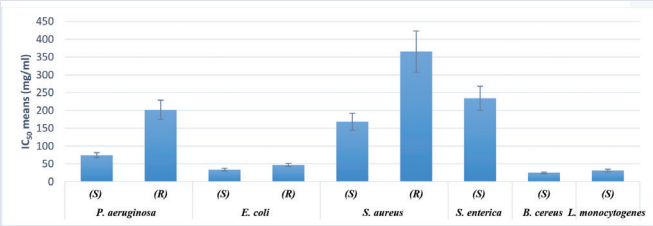

Figure 1: The IC_{50} means representation of EEs of the nine plant species for the nine strains studied. S : Sensitive ; R : Resistant.

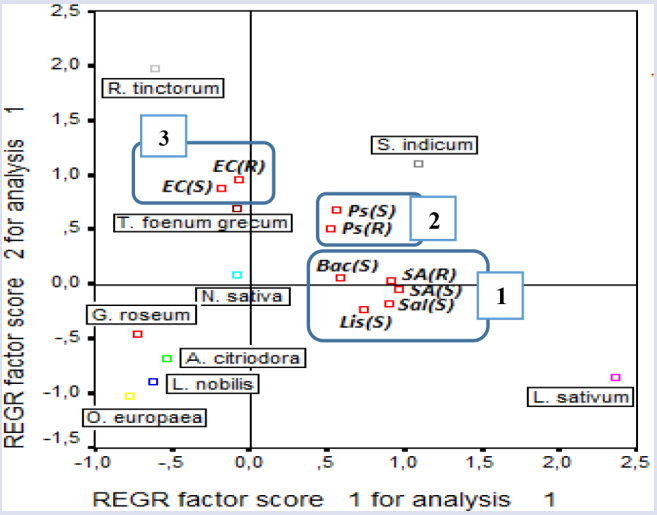

Figure 2: Projection of different variables of the Principal Component Analysis (PCA) on the factorial graph. S : Sensitive ; R : Resistant.

to Essawi et srour (2000),[19] the seeds of *N. sativa* have not demonstrated antibacterial activity. Ogunsola., (2014) [20] tested the antibacterial activity of aqueous and ethanolic extract of *S. indicum* seeds and as in our case, the aqueous extract was inactive on the bacteria tested ; the antibacterial activity of these species is may be in their aerial parts.

According to,[21] the effect of infusion and decoction of *L. nobilis* on 6 strains was inactive on all strains tested, which is in contrast with our results where *L. nobilis* EE was active on *S. aureus* and on *B. cereus*. In a recent study, *L. nobilis* EE was highly active against Gram + and Gram- bacteria.[22] While, the study.[23] showed that EE of the same specie has a very low antibacterial activity against *E. coli* and *P. aeruginosa* (MIC = 100 mg/ml) in comparison with previous study. The antibacterial potential of *L. nobilis* was attributed to its constitutional richness in terpenoids, glycosides, anthocyanins and essential oils.[24]

In general, results revealed variable responses according to the strains and their resistance, the type of the extract and its concentration, which was in agreement with the results of.[25] The difference in the action between these EEs is probably due to the difference in the chemical composition, the nature and composition of the microorganism's membrane and the influence of the reaction medium.[26,27]

Several classes of polyphenols such as tannins and flavonoids such as epigallocatechin, catechin, myricetin, quercetin,[15] luteolin and flavanones,[28] are very active antibacterial substances. Their absence of an extract could justify its weak activity.

In addition, recent results have shown that saponins are the most remarkable antibacterial compounds compared to polyphenols and flavonoids. Alkaloids, in turn, are recognized for their high antibacterial potency.[29] These alkaloids concentrated in our EEs could be partly responsible for the antibacterial activity obtained. Oxygenated terpenes and especially terpene alcohols are also very active antimicrobial agents.[30]

CONCLUSION

The antimicrobial activity evaluation of the hydro-ethanolic extracts of nine plant species showed the presence of a moderate activity in all the investigated species. The best effect was noticed in *L. nobilis*. The most sensitive strains were *S. aureus* and *B. cereus* with a dose-response relationship, while the most resistant were *P. aeruginosa* and *E. coli* (R).

Comparison of our results with those of the literature showed that the antibacterial activity of the plant extracts was very variable depending on the phytochemical composition of the plant, the solvents used for the extraction, and the bacteria tested.

The susceptibility of germs to EEs may justify their use in the traditional treatment of some microbial diseases in different regions of Morocco. These plants seem to have a broad spectrum of antibacterial activity. As a result, these extracts would present major targets, safe and effective in antibacterial therapy and for the preservation of food, and can be used in antiseptic and disinfectant formulations, as well as in chemotherapy.

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ABBREVIATIONS USED

EE: Ethanolic Extract; **PCA:** Principal component analysis; **MIC:** minimum inhibitory concentration; **MBC:** minimum bactericidal concentration; **BHI:** brain heart infusion; **MH:** Mueller-Hinton; **PCA:** Plate Count Agar; **MAPs:** medicinal and aromatic plants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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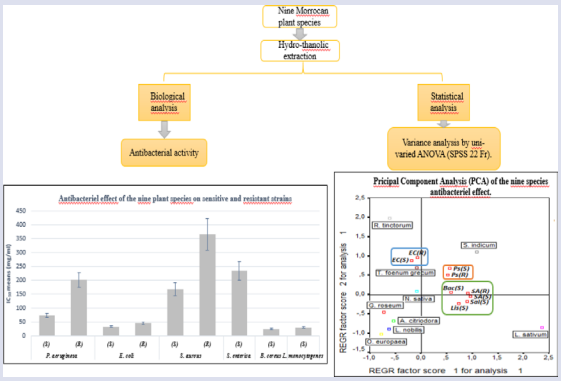
GRAPHICAL ABSTRACT



SUMMARY

- Ethanol extracts of the nine plant species were rich of leuco-anthocyanins, anthocyanins, essential oils, alkaloids, and aldehydes
- The ethanol extract of *L. nobilis* and *O. europaea* was directly bactericidal on all the strains tested with the exception of *P. aeruginosa*.
- The principal component analysis demonstrated that L. nobilis and *O. europaea* had the highest antibacterial activity. While, *R. tinctorum*, *S. indicum* and *L. sativum* were characterized by the lowest activity.

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