

Comparative Study of Inhibitory Activity of Extracts of Some Medicinal Plants Towards Some Pathogenic Bacterial Species

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Abstract

The study aimed to identify the sensitivity of some bacteria (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25293), *Staphylococcus coagulase* (ATCC 5118), *Klebsiella pneumoniae*, *Enterococcus faecalis*), towards some plant extracts (roots of *Juncus maritimus* Asch. & Buschen, leaves of *Cynodon dactylon* (L) Pers., *Nigella sativa*, *Malva parviflora*, *Mentha viridis* Hort., *Mentha pulegium* L., *Artemisia*, *Rosmarinus officinalis*, and *Camellia sinensis*). The *in vitro* antibacterial activity was performed by agar disc diffusion method. The results showed that all studied bacterial isolates were sensitive to plant extracts at varying rates, and the combination of each extract with each other were most active and showed significant synergic effects.

Keywords

Juncus maritimus Asch., *Cynodon dactylon* (L) Pers., bacterial strains, synergic effect, ethanolic extract.

Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically and sometimes without resorting to drugs and synthetic materials. However, due to the appearance of new strains of the bacteria and the weakness of chemotherapeutics and antibiotic resistance exhibited by pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity [1-2]. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available [3-4].

At this study we try to measure the biological activity of some famous plants which are defined below, combined with two medicinal plants, its growth on mountains of Algeria (Batna Area).

Cynodon dactylon (L) Pers., is a herb that consists of creeping, well-developed wiry stolons (horizontal stem growing at ground level) from which arise, roots and up to 40 cm tall flowering stems. It is used in medicine against various ailments and diseases.

The plant is used as forage grass for feeding livestock, especially in wetter areas where it is more abundant [5].

Juncus maritimus Asch. & Buschen, is a plant perennial and grassy, but it is gradually expanding to a height of one meter. His leaves are very long and rough when touching. Flower stalks rise to about two meters above ground. Its flowers resemble the flowers of the Allies in shape. Active ingredients are Soapins, alkaloids, flavonoids and phenols [6].

Nigella sativa, or black bean, is an herbaceous plant with leafy leaves and star flowers. Its fruit contains a small black seed that contains aromatic

aroma and is used in the treatment of hair loss, gastrointestinal disorders, hepatitis, spleen etc. [7-8-9], its therapeutic effect is due to Thymol, thymoquinone and phenolic compounds that have an antagonistic effect on microorganisms [10].

Camellia Sinensis is one of the members of the family of the *Camellia Theaceae*, which is characterized by its evergreen, and its leaves shape, containing many of the oil glands. With pink flowers. Green tea consists of fluorine, manganese, vitamins (A, B, K, F and P) and contains phenols and flavonoids [10].

Malvaparviflor, is a herb with a height of 30 to 70 cm. Its leaves are round and winged, with long legs covered with fine hairs, violet flowers. Its medicinal benefits are soothing to cough, having a calming effect on inflammation and narrowing of the esophagus [11].

Mint is considered one of the finest oils. Peppermint is used in the treatment of many diseases, especially gastrointestinal diseases. It is used in the treatment of headache. Mint oil has some disinfectant properties and is therefore used in the preparation of toothpaste and soap making. The active ingredients of mint are generally volatile oils, menthol, lemon, pineoprene, ucalyptol and tannic acid [13].

Menthapulegium L or the wild Mint, is abundant in the mountains and is very similar to mint. The extract of this plant is in the form of oils used against colds, as well as relax the nerves and regulate blood sugar [12].

Artemisia is an aromatic plant containing active santoyen in the expulsion of worms from the stomach; it also treats colic [12].

Rosmarinus officinalis, is a semi-arid herbaceous herb that is an evergreen, anti-diarrheal, anti-diarrheal, menstrual, wound and scabies. The Rosemary contains phenolic acids, the most important of which are Rosemarinic acid, acid cafeique, and some flavonoids Epinephrine, dicosamine, oxytin, jinquin, and hespedyline [14].

Materials And Methods

Fresh *Juncus maritimus* Asch & Buschen plant and other plants were collected from the mountains of Arris-Batna- East of Algeria. The plants were deposited at Laboratory of "Dynamique Interaction et Réactivité des Systems", Department of Process engineering, Faculty of Applied Sciences, University of KasdiMerbah - Ouargla,

Algeria. Fresh material was washed under running tap water, air dried under dark and then homogenized to fine powder and stored in closed container away from light and moisture.

Extraction of Plant Material

Each extract was prepared by soaking 200 g of the plant powder in a mixture of EtOH/H₂O (70/30) evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left overnight. The resulting residue was stored at 4°C.

Microorganisms all Bacterial Standard Strains

Escherichia coli (ATCC25922), *Pseudomonas aeruginosa* (ATTC27853), *Staphylococcus Coagulase* (ATTC5118), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae*, and *Enterococcus faecalis* were obtained and diagnosed in Microbiology Laboratory, Arris-Batna Hospital, Algeria.

Preparation of the Bacterial Culture Media

3.7 g of Muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 min. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 5 mm [16].

Preparation of Plant Extract Impregnated Discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants [15].

Disc Diffusion Method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antibacterial activities of plant extracts. A bacterial suspension adjusted to 0.5 McFarland standard (1.5x10⁸ CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the petri dishes and overlapping rings of inhibition. The plate was then incubated at 37°C for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms

were demarcated by a circular area of clearing around the plant extract impregnated discs. The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter.

The resulting residue of all extracts stored at 4°C was tested at a concentration of 10–3 g/ml and were prepared

in DMSO.

Results

The results are summarized in the following table

Table 1. Antibacterial activity of extracts of screened medicinal plants.

Diameter of inhibition zone (mm)						Bacteria Strains Plant extracts
Enterococcus faecale	Klebsiellapneum onie	Staphylococcus coagulase (ATTC 5118)	Staphylo ccus aureus (ATTC 25923)	Pseudomonas aeruginosa (ATTC 27853)	Escherichia coli (ATTC 25922)	
08	1.	12	14	. 5	1/	Cynodon
. 5	1.	1.	11	12	1/	Juncus
15	07	12	11	01	13	Cynodon-Juncus
+05	-03	00	-05	+/1	+02	$\Delta R_1 = R_{Cy-J} - R_{(Cy*J)}$
Cynodon-Juncus	Cynod ,Juncu	Cynodon	Cynodon	Cynodon-Juncu s	Cynodon-Juncus	
07	11	10	16	07	09	Nigella sativa
08	13	10	08	13	11	Cynodon-Nigella
..	+02	-02	-08	+06	00	$\Delta R_0 = R_{Cy-N} - R_{(Cy*N)}$
14	18	14	15	12	12	Juncus-Nigella
+07	+07	+04	-1	00	+01	$\Delta R_0 = R_{J-N} - R_{(J*N)}$
Juncus-Nigella	Juncus-Nigella	Juncus-Nigella	Nigella	Cynodon-Nigell a	Juncus- Nigella	
10	/2	12	16	/3	08	Camellia *sinensis
12	09	11	07	15	11	Cynodon-Camellia
..	-. 3	-01	-09	00	00	$\Delta R_1 = R_{Cy-C} - R_{(Cy*C)}$
23	12	13	11	12	11	Juncus-Camellia
+11	-2	+1	-05	-3	00	$\Delta R_1 = R_{J-C} - R_{(J*C)}$
Juncus-Camellia	Camellia	Juncus-Camellia	Camellia	Camellia	Cynod ,Juncu	
08	10	10	05	09	10	Malvaparfiflora
09	10	07	08	10	12	Cynodon-Malva
+01	00	-05	-08	+01	+01	$\Delta R_2 = R_{Cy-M} - R_{(Cy*M)}$
05	21	13	11	12	13	Juncus-Malva
-2	+11	+03	-2	00	+02	$\Delta R_1 = R_{J-M} - R_{(J*M)}$
Cynodon-Malva	Juncus-Malva	Juncus-Malva	Juncus	Juncus	Juncus-Malva	
08	08	11	11	09	05	MenthaviridisHort
11	09	08	05	10	10	Cynodon-Mentha
+ . 1	-01	-04	-11	+01	-01	$\Delta R_3 = R_{Cy-M} - R_{(Cy*M)}$
23	15	15	25	08	12	Juncus-Mentha
+15	+05	+05	+12	-04	+01	$\Delta R_3 = R_{j-M} - R_{(j*M)}$
Juncus-Mentha	Juncus-Mentha	Juncus-Mentha	Juncus-Me ntha	Juncus	Juncus-Mentha	
07	08	09	09	07	06	MenthapulegiumL
07	09	07	06	09	05	Cynodon-Mentha *p

-0/	-01	-05	-10	+02	-06	$\Delta R_4 = R_{Cy-M} - R_{(Cy^*M)}$
25	10	14	24	10	12	Juncus-Mentha* _p
+18	00	+04	+11	-2	+01	$\Delta R_4 = R_{j-M} - R_{(j^*M)}$
Juncus-Mentha* _p	Juncus	Juncus-Mentha* _p	Juncus-Mentha* _p	Juncus	Juncus-Mentha* _p	
.6	1.	10	1/	1/	.6	Artemisia
09	10	09	09	10	05	Cynodon-Artemisia
-01	00	-03	-07	-01	-06	$\Delta R_5 = R_{Cy-R} - R_{(Cy^*R)}$
23	16	16	20	07	11	Juncus-Artemisia
+16	+06	+04	+07	-05	00	$\Delta R_5 = R_{j-A} - R_{(j^*A)}$
Juncus-Artemisia	Juncus-Artemisia	Juncus-Artemisia	Juncus-Artemisia	Juncus	Juncus	
07	.6	.7	/2	12	09	Rosmarinusofficinalis
10	09	12	10	13	10	Cynodon-Rosmarinus
+00	-01	00	-06	+01	-01	$\Delta R_6 = R_{Cy-R} - R_{(Cy^*R)}$
11	18	12	19	12	15	Juncus-Rosmarinus
+04	+08	+02	+05	00	+04	$\Delta R_6 = R_{j-R} - R_{(j^*R)}$
Juncus-Rosmarin	Juncus-Rosmarin	Juncus-Rosmarin	Juncus-Rosmarin	Cynodon-Rosmarin	Juncus-Rosmarin	
Juncus-Mentha* _p $R_{j-M} = 25$	Juncus-Malva $R_{j-M} = 21$	Juncus-Artemisia $R_{j-A} = 16$	Juncus-Mentha $R_{j-M} = 25$	Cynodon-Juncus $R_{Cy-j} = 23$	Juncus-Rosmarinus $R_{j-R} = /3$	Maximum Antibacterial Activity

Discussion

The table (1) showed that:

The microbial growth inhibition of nine species extract tested in vitro by agar disc diffusion against 6 bacterial species, showed significant bacterial activity against all the bacteria tested (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus Coagulase, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis).

Antibacterial activity of mixture of Juncus.maritimus,Asch/Cynodondactylon(L)Pers extracts, achieved synergic effect against Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis.

Antibacterial activity of mixture of Juncus.maritimus,Asch/Nigella sativa extracts, achieved synergic effect against Escherichia coli, Staphylococcus Coagulase, Klebsiella pneumoniae, and Enterococcus faecalis.

Antibacterial activity of mixture of Cynodondactylon(L)Pers/Nigella sativa extracts,

achieved synergic effect against Escherichia coli, Staphylococcus coagulase, Klebsiella pneumonia and Enterococcus faecalis.

Antibacterial activity of mixture of Juncus.maritimus,Asch/Camellia sinensis extracts, achieved synergic effect against Staphylococcus aureus, and Enterococcus faecalis.

Antibacterial activity of mixture of Cynodondactylon(L)Pers/Camellia sinensis extracts, didn't achieve any synergic effect against studied bacteria.

Antibacterial activity of mixture of Juncus.maritimus,Asch/Malva parviflora extracts, achieved synergic effect against Escherichia coli, Staphylococcus coagulase and Klebsiella pneumoniae.

Antibacterial activity of mixture of Cynodondactylon(L)Pers/Malva parviflora extracts, achieved synergic effect against Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis.

Antibacterial activity of mixture of Juncus.maritimus,Asch/Mentha viridis extracts, achieved synergic effect against all studied bacteria except Pseudomonas aeruginosa.

Antibacterial activity of mixture of *Cynodondactylon*(L)Pers/*Menthaviridis*Hort extracts, achieved synergic effect against *Pseudomonas aeruginosa*, and *Enterococcus faecalis*.

Antibacterial activity of mixture of *Juncus.maritimus*,Asch/*Menthapulegium* L extracts, achieved synergic effect against all studied bacteria except *Klebsiellapneumoniae*, and *Pseudomonas aeruginosa*.

Antibacterial activity of mixture of *Cynodondactylon*(L)Pers/*Menthapulegium* L extracts, achieved synergic effect against only *Pseudomonas aeruginosa*.

Antibacterial activity of mixture of *Juncus.maritimus*,Asch/*Artemisia* extracts, achieved synergic effect against all studied bacteria except *Escherichia coli*, and *Pseudomonas aeruginosa*.

Antibacterial activity of mixture of *Cynodondactylon*(L)Pers/*Artemisia* extracts, didn't achieve any synergic effect against studied bacteria.

Antibacterial activity of mixture of *Juncus.maritimus*,Asch/*Rosmarinusofficinalis*, achieved synergic effect against all studied bacteria except *Pseudomonas aeruginosa*.

Antibacterial activity of mixture of *Cynodondactylon*(L)Pers/*Rosmarinusofficinalis* extract s, all studied bacteria except *Enterococcus faecalis*, and *Pseudomonas aeruginosa*.

The maximum antibacterial achieved by *Juncus/Rosmarinus* is against *E. coli* by rate 15 mm.

The maximum antibacterial achieved by *Juncus/Cynodon* is against *Pseudomonas* by rate 23 mm.

The maximum antibacterial achieved by *Juncus/Mentha* is against *Staphylococcus aureus* by rate 25 mm.

The maximum antibacterial achieved by *Juncus/Mentha* is against *Staphylococcus coagulase* by rate 16 mm.

The maximum antibacterial achieved by *Juncus/Malva* is against *Klebsiellapneumoniae* by rate 21 mm.

The maximum antibacterial achieved by *Juncus/Malva* is against *Enterococcus* by rate 25 mm.

Conclusion

The results obtained in the present study suggest that (roots of *Juncusmaritimus* Asch. &Buschen, leaves of

Cynodondactylon(L)Pers, *Nigella sativa*, *Malvaparfiflora*, *Menthaviridis*Hort, *Menthapulegium* L, *Artemisia*, , and, *Camellia Sinensis*) extracts, can be used *Rosmarinusofficinalis* separated or together, in treating diseases caused by the tested organisms. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plants responsible for the antimicrobial activity.

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References

- Winberg J. Urinary tract infections in children curr. Opin. Inf. Dis 1990 ; 3 : 55-61.
- Iwu MW, Ducan, A R, Okunji CO. New antimicrobials of plant origin. In; Janick J. ed. Perspectives on new Crops and new Uses. Alexandria, VA; ASHS Press 1999; 457-461.
- Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium stivum*). *Lebensm-Wiss u- Technol* 2004 ; 37: 263-267.
- Babamer ZY, Sekhri L, Al-Jaber H I, Al-Qudah MA, Abu Zarga, MH. *Journal of Asian Natural Products Research* 2012; 1-7.
- Identification and determination of flavonoids, carotenoids and chlorophyll concentration in *Cynodondactylon* (L.) by HPLC analysis article was downloaded by University of Saskatchewan Library.
- Global Biodiversity Information Facility in English.
- Medical Plants Cultivated by its Components Benefits of Madinah (1977) P-150-148, *Leukemiapolypeptdes. int. j. Pharmacog.* 33 (1): 16 -20 (1995).
- Ranunculaceae. J. Hand Medicus* ., Chaudhary.
- Seeds. *J. Ethnopharm* (2000), 70.
- Medical products for therapeutic prescriptions for aromatic medicinal plants Dar Al-Arabiya Publishers 2005 P-65,
- Khalaf, N. A.; Shakya, A. K.; Al- Othman, A.; El- Agbar, Z. & Farah, H. (2008). Various fields of Medicine Engineering Economics in Moscow, Belgorod, Perm.
- Qudama, Ahmed. Nutritional dictionary and herbal medicine. Fifth Edition, Dar Al-Nafais Publications, Beirut.
- Swarnamoni D, Mukundam B, Shagufa A. *Asian Pharm Clin Res* 2013; 6 (4) : 136-139.
- Cappuccino JG, Sherman N. *Micro-A Laboratory manual*. Addison Wesley Longman Inc 1999; 254-256.