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A Comparative Study of the Antibacterial Activity of *Cyndon dactylon* (L) pers ; its Synergic Effect with Some of the Standard Antimicrobs and Extracts of Some Medicinal Plants

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ABSTRACT

The antibacterial effect of some selected algerian plants like Cyndon dactylon (L) pers were evaluated on several bacterial strains : Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATTC 27853, Staphylococcus Coagulasse (ATTC 5118), Staphylococcus aureus ATCC 25923, *Klebcsiella pneumonie* and *Enterococcus faecalis*.The in vitro antibacterial activity was performed by agar disc diffusion method. The combination of Cyndon dactylon (L) pers with each of the standard antimicrobs E (Erythromycine), C (Chloramphenicol), CTX (Cefotaxime), AMX (Amoxicillin), CZN : (Cefazoline), CXN (Cefalexine) were most active and showed significant synergic effects. Moreover, Cyndon dactylon (L)/ other extracts of screened medicinal plants showed also high synergic effects. The results obtained in the present study suggest that Cyndon dactylon (L) pers can be used in treating deseases 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.

Key words : Cyndon dactylon (L) pers, bacterial strains, synergic effect, extract.

INTRODUCTION

Since the 1940's, chemists have developped all sorts of highly effective antibiotics (Sulfa drugs, penicillins, tetracyclines, and others that are effective) against bacterial and viral infections. In recent years there has been a flood of papers describing the synthesis of new antibacterial compounds and isolation of some natural products and study of their biological antimicrobial activities.[1-6]

Today there is an imperative necessity to find out new antibacterial compounds with various chemical structures and new mecanisms of action for new and re-emerging contagious syndoms.[7] Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs that are effective against bacterial infections and viral infections from infuenza and the common cold and even the more serious herbs infections and AIDS (Aquired Immune Defectious Syndrome). The viral infectious account for about 60% ilnesses, contrasted with about 15% for bacterial infections. 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.[8-10]

The medicinal plants have been used for ages as remedies for human diseases. Plant derived compounds are getting more and more

interest owing to their adaptable applications. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available.[11-14]

The aim of of this study was to evaluate the activity of extracts from 9 plants against several Gram-positive and Gram-negative bacterial strains in vitro.

EXPERIMENTAL

Materials and methods

Fresh plant/plant parts : Cynodon dactylon (L) Pers, Malva parviflora, Mentha viridis Hort, Mentha pulegium L, Artemisia, Rosmarinus Officinalis were collected randomly from the mountain of Batna-Algeria in November 2013. The plants were deposited at Lab. Dynamique, Interaction et Réactivité des Systèmes, Department of Process engeneering, Faculty of Applied Sciences, University of Kasdi Merbah-Ouargla, Algeria. Fresh plant material was washed under running tap water, air dried under dark and then homoggenized to fine powder and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

Qualitative Phytochemical analysis of the crude powder of th 9 plants collected was determined as follows :Resins,[15] Coumarins,[16] Terpenes and Steroids (Liebermann-Burchard reaction),[17] Phenols,[18] Tannins, Alkaloids, Saponins, Cardiac glycosides, Flavonoids.[19]

Extraction of plant material

The extracts were prepared by soaking 200g of the plant powder in a mixture of EtOH/H_2O (70/30) evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left overnight. The filtrates were subjected to extraction by various solvents with increasing polarity (petroleum ether, dichloromethane, ethyl acetate , and butanol). The organic phases were separated and evaporated. The rsulting residue was stored at 4°C.

Microorganisms

All bacterial standard strains : Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATTC 27853, Staphylococcus Coagulasse (ATTC 5118), Staphylococcus aureus ATCC 25923, Klebcsiella pneumonie, Enterococcus faecalis.

Preparation of the bacterial culture media

3.7 of muller Hilton agar was mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 minutes. After autoclavingit was allowed to cool to 45°C in a water bath. Then the medium was poured into sterillized petri dishes with a uniform depth of approximately 5 mm.[20]

Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterillized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants.[21]

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 antibecterial activities of plant extracts.[22] A bacterial suspension adjusted to 0.5 McFarland standard ($1.5x10^8$ 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 disces 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 rsulting residue of all extracts stored at 4°C were tested at a concentration of 10^{-3} g/ml and were prepared in DMSO.

RESULTS AND DISCUSSION

The antibacterial activity of 9 species extract tested in vitro by agar disc difusion against

Table 1: Antibacterial activity of extracts of screened medicinal plants

Bacteria strains / Plant extracts	Diameter of inhibition zone (mm)					
	Escherichia coli (ATTC 25922)	*Pseudomonas aeruginosa* (ATTC 27853)	*Staphylococcus aureus* (ATTC 25923)	*Staphylococcus coagulasse* (ATTC 5118)	*Klebcsiella pneumonie*	*Enterococcus faecale*
Cynodon dactylon (L) Pers	11	7	16	12	10	8
Juncus.maritimus ,Asch	11	12	13	10	10	7
Nigella sativa	9	7	16	10	11	7
camellia,sinensis	8	15	16	12	14	12
Malva parviflora	10	9	5	10	10	8
Mentha viridis Hort	5	9	11	11	8	8
Mentha,pulegiumL	6	7	9	9	8	7
Artemisia	8	11	11	12	10	8
Rosmarinus officinalis	9	12	14	9	8	7

Table 2: Antibacterial activity of some antibiotics (standard antimicrobs)

Bacteria strains / Anti- biotics	Diameter of inhibition zone (mm)					
	Escherichia coli (ATTC 25922)	*Pseudomonas aeruginosa* (ATTC 27853)	*Staphylococcus aureus* (ATTC 25923)	*Staphylococcus coagulasse* (ATTC 5118)	*Klebcsiella pneumonie*	*Enterococcus faecale*
E (15µg)	_	_	26	_	_	_
C (30µg)	29	_	23	28	23	11
CTX (30µg)	30	20	25	24	27	_
CZN (30µg)	29	_	26	_	22	15
CXN (30µg)	24	_	27	_	26	_
AMX (25µg)	27	_	30	_	15	28

E : Erythromycine ; C : Chloramphenicol ; CTX : Cefotaxime ; AMX : Amoxicillin ; CZN : Cefazoline ; CXN : Cefalexine

Table 3: Antibacterial activity of of Cyndon dactylon (L) pers with some antibiotics

Bacteria strains / Plant extract/ Antibiotic	Diameter of inhibition zone (mm)					
	Escherichia coli (TTC25922)	*Pseudomonas aeruginosa* (TTC 27853)	*Staphylococcus aureus* (ATTC 25923)	*Staphylococcus coagulasse* (ATTC 5118)	*Klebcsiella pneumonie*	*Enterococcus faecale*
Cynodon dactylon (L) Pers/ E (15µg)	10	16	9	6	20	9
Cynodon dactylon (L) Pers/ C (30µg)	27	12	16	10	14	12
Cynodon dactylon (L) Pers/ CTX (30µg)	32	21	25	23	20	10
Cynodon dactylon (L) Pers/ CZN (30µg)	27	6	7	6	8	8
Cynodon dactylon (L) Pers/ CXN (30µg)	23	7	6	5	10	8
Cynodon dactylon (L) Pers/ AMX (25µg)	29	5	5	5	5	9

Table 4: Antibacterial activity of a mixture of Cynodon dactylon (L) Pers and the other extracts of screened medicinal plants

Bacteria strains / Plant extract/ Antibiotic	Diameter of inhibition zone (mm)					
	Escherichia coli (TTC25922)	*Pseudomonas aeruginosa* (TTC 27853)	*Staphylococcus aureus* (ATTC 25923)	*Staphylococcus coagulasse* (ATTC 5118)	*Klebcsiella pneumonie*	*Enterococcus faecale*
Cynodon dactylon (L) Pers/ Juncus.maritimus,Asch	1 3	23	11	12	7	15
Cynodon dactylon (L) Pers/ Nigella sativa	11	13	8	10	13	8
Cynodon dactylon (L) Pers/ camellia,sinensis	11	15	7	11	9	12
Cynodon dactylon (L) Pers/ Malva parviflora	12	10	8	7	10	9
Cynodon dactylon (L) Pers/ Mentha viridis Hort	10	10	5	8	9	11
Cynodon dactylon (L) Pers/ Mentha,pulegiumL	5	9	6	7	9	7
Cynodon dactylon (L) Pers/ Artemisia	5	10	9	9	10	9
Cynodon dactylon (L) Pers/ Rosmarinus officinalis	10	13	10	12	9	10

6 bacterial species. Table 1 summarizes the microbial growth inhibition of these extracts of the screened plant species.These extracts of nine plants showed significant bacterial activity against all the bacteria tested (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus Coagulasse, Staphylococcus aureus, Klebcsiella pneumonie, Enterococcus faecalis*). The maximum antibacterial activity was recorded against Staphylococus aureus and a maximum inhibition diameter of 16 mm with Cyndon dactylon (L) Pers, Nigella sativa and Camilia sinensis. Weak inhibition was recorded with the extract of medicinal plant Mentha viridis Hort against all the bacteria tested. As far as Rosmarinus officinalis is concerned, the maximum antibacterial activity was recorded against Staphylococus aureus and a maximum inhibition diameter of 14 mm. Similar results were obtained with Artemisia with a maximum inhibition diameter of 12 against Staphylococus Coagulasse.

As far as the synergic effect is concerned the combination of Cyndon dactylon (L) pers with each of the standard antimicrobics, E, C, CTX, CZN and AMX were most active and showed the synergic effect. The maximum antibacterial activity was recorded against Esherichia coli, Pseudomonas aeruginosa, Staphylococcus coagulasse and Enterococcos faecale with a maximum synergic effect of "R = 02-04 mm, whereas Cyndon dactylon (L)/CXN Showed no synergic effect against Pseudomonas aeruginosa, and Staphylococus coagulasse. **Table-3** summarizes the microbial growth inhibition of Cyndon dactylon (L)/standard antimicrobics.

The maximum antibacterial activity was recorded with Cyndon dactylon (L) Pers/ Nigella sativa and Cyndon dactylon (L) Pers/ Juncus maritimus asch against Pseudomonas aeruginosa and Enterococcos faecal with a maximum synergic effect of "R = 6 mm and 5 mm respectively. **Table-4** summarizes the microbial growth inhibition of Cyndon dactylon (L)/ other extracts of screened medicinal plants.

In conclusion, Cyndon dactylon (L) pers with each of the standard antimicrobics, E, C, CTX, CZN and AMX were most active and showed the synergic effect against Esherichia coli, Pseudomonas aeruginosa, Staphylococcus coagulasse and Enterococcos faecale. Antibacterial activity of mixture of Cynodon dactylon (L) Pers and other extracts of screened medicinal plants possess a broad spectrum of activity against a panel of bacteria responsible for the moste common bacterial diseases. These promisssory extracts open the possibility of finding new clinically effective antibacterial compounds. 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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