ANTIMICROBIAL ACTIVITIES OF TUNISIAN ARTICHOKE (*CYNARA SCOLYMUS* L.) LEAVES EXTRACTS
ACTIVITES ANTIMICROBIENNES DES EXTRAITS TUNISIENNE DE FEUILLES D'ARTICHAUT (*CYNARA SCOLYMUS* L.)

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Abstract

Cynara scolymus L. (Artichoke) was a medicinal plant used in traditional medicine for treatment of many diseases. The current study was carried out to determine the antimicrobial activity of artichoke. The antibacterial activity was evaluated by the disc diffusion method. The ethanol and ethyl acetate extract showed a bactericide activity against *Salmonella enteritidis*.
Artichoke leaves extract could be potentially useful as a natural antioxidant and antimicrobial agent in human diseases.

Key - words: Artichoke; Leaves; Ethanol; Extract.

Résumé

La *Cynara scolymus* L. (Artichaut) a été une plante médicinale utilisée en médecine traditionnelle pour le traitement de nombreuses maladies. La présente étude a été réalisée pour déterminer son activité antimicrobienne. Celle-ci a été évaluée par la méthode de diffusion de disque. L'extrait à l'éthanol et à l'acétate d'éthyle a montré une activité bactéricide contre *Salmonella enteritidis*.
L'extrait de feuilles d'artichaut pourrait être potentiellement utile comme antioxydant naturel et agents antimicrobiens dans les maladies humaines.

Mots - clés: Artichaut ; Feuilles ; Ethanol ; Extrait.

ملخص

الخرشوف أو الخرشف بلغتنا العامةهو نبات طبي يستخدم في الطب التقليدي لعلاج العديد من الأمراض. أجرينا الدراسة الحالية لتحديد النشاط المضاد للميكروبات لهته النبتة. تم تقييم ذلك من خلال طريقة انتشار القرص. أظهر مستخلص الإيثانول وخلات الإيثيل فاعلية مبيد للجراثيم و منها ضد جرثومة السالمونيلا المعوية. يمكن أن يكون عصارة أو مستخلص أوراق الخرشوف مفيدًا كعامل طبيعي مضاد للأكسدة ومضاد للميكروبات في الأمراض التي يمكن تصيب الإنسان.

الكلمات المفاتيح: الخرشوف ; الأوراق ; الإيثانول ; العصارة.

1. INTRODUCTION

Up to day, there are several drugs resistances in human pathogenic microorganisms have been advanced due to confusing the use of commercial antimicrobial drugs in chemotherapeutic agents [1]. Consequently, medicinal plants have been an important source of medicine for thousands of years, even today. The properties of medicinal plants essential source of medicine for many years, even today. Rojas et al. [2] showed that the properties of medicinal plants have been authorized in ancient Tunisian traditional populations and they have been to be ameliorating in the treatment of chronic diseases. Therefore, researchers are progressively going back to their retention to traditional medicine, since they develop higher quality drugs against these pathogens microorganisms. In these days, there are increasing to target on using natural antimicrobial compounds from plant extracts of herbs in order to decrease outbreaks of foodborne pathogenic microorganisms [3, 4].

Cynara scolymus, commonly known as artichoke, from traditional therapy, artichoke extract has been used as a drug in the treatment of several diseases effects of the biliary tract, digestive action, scurvy, and anemia effect [5,6].

Many of the studies using experimental models of rats have been identified various potential protective effects of *Cynara* extract as anti-inflammatory, hypoglycemic, cardioprotective, hepatoprotective ,and renoprotective effect according to Ben Salem et al. [6,7,8,9,10].

Many explorations *in vitro* disclosed fruitful *cynara* as an antioxidant agent thanks to phenolic compounds [11]. They have also been described to contain concentrations of phytochemical and nutrients and to be rich in carotenoids, inulin, fiber, minerals, Crude protein, and carbohydrates contents [11].

Soumaya *et al*. [12] have valued the leaf, flowers, and seeds of *cynara* from different regions of Tunisia such as the region of El Jem, El Kef, Wad Mliz, Zriba and Enfidha. However, no studies focused in the region of Bizerte which known as a successful and fruitful region of cultivated *cynara* in compared with the other regions of the governorate of Tunisia. Therefore, we focused in our study to evaluate the antibacterial activities of *cynara scolymus* leaves extract, to support their traditional claims.

2. MATERIALS AND METHODS

2.1. Plant Material

Freshly green Leaves around the stems of the *cynara* were collected during December 2015 from a culture area located in the governorate of Bizerte region (Altitude: 33m; Latitude: 37°16′28″ N; Longitude: 9°52′26″) Northwest of Tunisia characterize by a humid climate season.

The harvested plant was identified in the botany of the laboratory of the faculty of Sciences, Sfax University, Tunisia, by Professor Mohamed Chaeib and a voucher specimen was deposited at the Herbarium of the laboratory of the Biotechnology Centre of the Technopark of Borj-Cedria. The leaves were manually separated and dried in the shade.

2.2. Antimicrobial activity

2.2.1. Microbial strains and growth conditions

The antibacterial activity of *cynara* leave extracts was tested against seven strains of bacteria. These included Gram-positive bacteria: *Bacillus subtilis* (JN 934392), *Bacillus cereus* (JN 934390), *Micrococcus luteus* (ATCC 4698), *Listeria monocytogenes* (ATCC 43251) and Gram-negative bacteria included *Salmonella enteric serotype Enteritidis* (ATCC43972), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumonia* (WHO24)

Antifungal activities were tested using three fungal strains: *Fusarium* (*sp.*JX391934), *Fusarium oxysporum* (AB586994),a nd *Penicillium sp* (AY598675).

The test bacteria was cultured on Petri dishes containing Mueller Hinton Agar (MH) and incubated for 18-24 h. All Bacterial culture was prepared in 3 ml MH broth with agitation (200 rpm) for 24 h at 37°C, except for *Bacillus* species, which was incubated at 30°C. Optical density was determined at 625 nm ranged from 0.08 to 0.10 a density equivalent to 10^7 CFU/ml [13].

For fungal strains, growth was carried out at 30°C for 4 days on Sabouraud agar until mycelia growth covered the entire dishes from which a spore suspension was obtained in 10 ml sterile water containing (0.1%) of Tween 80. Optical density was also adjusted to 0.08-0.10 to obtain a solution equivalent to 10^6 spores/ml [14].

2.2.2. Test Microorganisms and Growth Media

Antibacterial and antifungal activities were detected by the agar well diffusion test using a slightly modified version of the method of Hsouna *et* al. [14].

In brief, a cavity (wells) of 6 mm was created in the MHA using a sterile Pasteur pipette. A freshly prepared bacterial suspension or spore solution (100 µl) adjusted to 10^7 CFU/ml for bacteria and 10^6 spores/ml for fungus was inoculated into the surface of agar plates using a sterile swab. Each well was then filled with 80 µl of each plant extract at the concentration of 125 mg/ml DMSO. Negative control was composed with only the DMSO. The plate was left at + 4°C for 2 h to facilitate the diffusion of each extract in the agar and then incubated at 37°C for 24 h for bacterial strains and 30°C for 4-7 h for fungal strains. Antimicrobial activity was determined by measuring the diameter of the inhibition zone (DDs) around the well.

2.2.3.Broth microdilution assay

MIC was defined as the lowest concentration of the plant extract to inhibit the growth of the microorganisms. MBC values were interpreted as the highest dilution (lowest concentration) of the plant extract which showed clear fluid with no turbidity development and without visible growth of microorganisms after incubation for 48 h at 37°C and MFC was considered as the lowest concentration that prevented mycelium growth Microorganism viability assays involved by the addition of 25 µl of MTT (3- (4.5 –dimethyl -2-thiazolyl) -2.5- diphenyl- 2 H- tetrazolium bromide) (0.5 mg/ml of sterile distilled water) as an oxidation-reduction indicator to each well and subsequent incubation of the mixture for 30 min at 37°C. In this assay, the wells involving microbial growth inhibition stay clear after incubation with MTT [15].

3. STATISTICAL ANALYSIS

Data are expressed as the mean and standard error of the mean (mean ± SD). The one-way analysis of variance (ANOVA) and the Tukey post hoc test were performed on the data for intergroup comparisons. Database management and statistical analysis were performed using SPSS (SPSS Inc. Chicago. IL. USA) statistical software package.
The nominal statistical significance level was set at $p< 0.05$.

4. RESULTS

4.1. Antimicrobial activity

Table I, showed that the ethyl acetate and ethanol extract were found to be the most effective against Gram-positive and Gram-negative bacteria.
The largest inhibition zone was produced by ethyl acetate extract with DDs values 28 ± 0.0 mm to *Listeria monocytogenes* and 29 ± 0.5 mm to *Micrococcus luteus* followed by ethanol extract with DDs values 20±1.5 mm to *Bacillus cereus* and 20± 2.0 mm to *Micrococcus luteus.*
For the Gram-negative bacteria, both ethanol and ethyl acetate extract exhibited the inhibition growth of *Salmonella Enteritidis* with DDs values 28 ± 1.0 and 26 ± 0.5 mm.
However, the aqueous and hexane extract didn't show any effect on almost all the tested bacteria. MIC and MBC results from leaves of artichoke were determined by the micro broth dilution assays respectively (Table II). Ethyl acetate extract was more active against *M. luteus* and *B.cereus* presenting an important growth inhibition at lower concentrations (MIC: 1.56 mg/ml and MBC: 6.25 mg/ml), besides the ethanol extract exhibited a moderate inhibition activity against *B. subtilis* (MIC: 6.25 mg/ml and MBC: 25 mg/ml).
The ethyl acetate and ethanol extract showed a bacteriostatic activity against Gram-positive bacteria as *M.luteus, B. subtilis* and *B. cereus* and bactericidal activity against Gram-negative Bacteria such as *S. Enteritidis* (Table III).

4.2. Anti-fungal activity

The results of the anti-fungal activity indicates that artichoke extracts generally have showed no activity towards *Fusarium oxysporum, Fusarium sp , and Penicillium sp* (Table 1). However, the values for positive control (cycloheximide) were higher with DDs of 20 ± 2.0 mm and 18 ± 1.5 against *Fusarium oxysporum* and *Fusarium sp*.

Table I: *In vitro* anti-microbial activities of artichoke leaves extract

Bacterial strains	**Diameter of zone inhibition (mm)**					
	Hexane	***Butanol***	***Ethyl acetate***	***Ethanol***	***Aqueous***	***Chloram[a]***
Bacillus cereus	-	11±1.5	25 ± 1.0	20 ± 1.5	17 ± 0.5	26±1.00
Bacillus subtilis	15 ± 0.5	12 ± 0.5	25 ± 1.00	18 ± 1.00	-	24 ± 0.0
Micrococcus luteus	15 ± 1.0	19±1.5	29 ± 0.5	20 ± 2.0	10 ± 1.0	12 ± 1.0
Listeria monocytogenes	12 ± 0.0	14 ± 2.00	28 ± 0.0	15 ± 1.5	-	20 ± 2.0
Salmonella Enteritidis	15 ± 1.5	20 ± 0.0	26 ± 0.5	28 ± 1.0	10 ± 1.0	16 ± 0.0
Escherichia coli	-	-	-	-	-	-
Klebsiela pneumoniae	10 ± 0.5	18 ± 0.5	15 ± 1.5	15 ± 0.0	-	15 ± 0.0
Fungal strains						
	Hexane	***Butanol***	***Ethylacetate***	***Ethanol***	***Aqueous***	***Cyclo[b]***
Penicillium sp	-	-	9 ± 1.0	-	-	14 ± 1.0
Fusarium oxysporum	9 ± 0.0	11 ± 0.0	14 ± 0.5	10 ± 1.0	9 ± 0.0	20 ± 2.0
Fusarium sp	-	-	10 ± 1.5	8 ± 0.5	-	18 ± 1.5

Values are expressed as mean± SD (n=3).
-: no inhibition.
[a]: Chloramphenicol was used as a standard antibiotic at a concentration of 15 µg/well.
[b]: Cycloheximide was used as a standard antibiotic at a concentration of 20 µg/well.

Table II: Determination of MIC, MBC and MFC (mg/mL) of *artichoke* leaves extracts in bacterial and fungal strains

Bacterial strains	**Hexane**		**Butanol**		**Ethylacetate**		**Ethanol**		**Aqueous**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
B. subtilis	-	-	6.25	12.5	6.25	100	6.25	25	6.25	25
B. cereus	6.25	12.5	6.25	50	1.56	6.25	12.5	100	-	-
M.luteus	3.125	25	6.25	25	1.56	6.25	12.5	100	25	100
L. monocytoges	12.5	25	12.5	6.25	50	-	-	6.25	25	-
S. Enteritidis	6.25	25		-	25	6.25	25	6.25	25	100
E. coli	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	12.5	100	12.5	100	12.5	25	12.5	50	-	-
Fungal strains	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MIC**	**MIC**	**MFC**	**MIC**	**MFC**
Penicillium sp	-	-	-	-	25	100	-	-	-	-
Fusarium oxysporum	25	100	12.5	100	12.5	50	12.5	100	25	100
Fusarium sp	-	-	6.25	25	1.56	6.25	12.5	100	25	100

Table 3: Mechanism action bactericidal and bacteriostatic of artichoke leaves extract

Bacterial Strains			Ethyl acetate	Ethanol
Gram -	*Salmonella Enteritidis*	**CMI**	25	25
		CMB	6.25	6.25
		CMB/CMI	<4	<4
Gram +	*Bacillus subtilis*	**CMI**	6.25	6.25
		CMB	100	25
		CMB/CMI	≥ 4	≥ 4
	Micrococcus luteus	**CMI**	1.56	12.5
		CMB	6.25	100
		CMB/CMI	≥4	≥4
	Bacillus cereus	**CMI**	1.56	12.5
		CMB	6.25	100
		CMB/CMI	≥4	≥4

MBC/MIC≥4: Bacteriostatic
MBC/MIC<4: Bactericidal

5. DISCUSSION

The present study was conducted to evaluate in *vitro* the antibacterial activities of different extracts from leaves of *Cynara scolymus*.

Phenolic compounds were widely distributed in medicinal plants and provided to the biological activity [11.] Several potential mechanisms are suggested for the antioxidant activity of bioactive compounds of *cynara* as follows as mentioned Valko *et al*. [16]: free radicals scavenging by acting as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators, as well as modulating of ROS-dependent cell functional signaling at several key sites. *Cynara* and its extracts could be powerful as an antioxidant in the treatment of disease associated with oxidative stress.

The increasing resistance to antibiotics constitues the main factor justifying the need to find and/or to develop new antimicrobial agents. Thus, many studies have been focused on antimicrobial agents and the antimicrobial properties of plant-derived active principles. Currently, much attention has been paid to health promotion related to the activity of novel bioactive compounds from the medicinal plant as an effective strategy for the treatment of different diseases [17]. Even though this large flow of background on the promising properties and attributes of *cynara scolymus* extracts, no studies have so far been performed to explore the antimicrobial properities of *cynara* in various extracts.

The antibacterial activity of *cynara scolymus* leaves extracts was diagnosed against eight microorganisms including Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes* and *Micrococcus luteus*), Gram-negative (*E. coli*, *K. pneumonieae* and *S. enteritidis*) and three fungi (*Penicillium sp, Fusarium oxysporum* and *Fusarium sp)*.

The result demonstrated different degrees of antibacterial activity against most of the Gram-positive, Gram-negative bacterial and fungal strains tested.

The highest antibacterial activity was observed by ethyl acetate followed by ethanol extract against Gram-positive (*Bacillus subtilis*, *Bacillus cerues*, *Micrococcus luteus*, *Listeria monocytogenes*) and Gram-negative bacteria (*Salmonella enteritidis*) at the concentration of 150 µL/mL. But , the aqueous and hexane extract had no antimicrobial activity against any of the bacterial or fungal isolates tested in the present study. From these results, the ethyl acetate and ethanol extract were more active than the other extracts.

This may be attributed to the presence of soluble phenolic compounds that possess antimicrobial activities that provide chemical barriers for invading microorganisms [18].

In contrast, Mossi and Echeverrigaray [19], found that dichloromethane extract of *cynara* could inhibit the growth of three bacteria; *S.aureus*, *B.cereus* , and *B. subtilis* in concentrations of 5 mg/mL.

The reason for the differential sensitivity of Gram-positive and Gram-negative bacteria to plant extracts may be clarified by the morphological of membranes differences between the Gram-positive and Gram-negative. Smith [20], explained that the significant resistance of Gram-negative bacteria could be due to the difference in their cell membranes since their outer phospholipidic membrane carries the structural lipopolysaccharide components and offers their surfaces highly hydrophilic. Meanwhile, the Gram-positive bacteria must be more susceptible since they have only an outer peptidoglycan layer, which is not an active permeability barrier and may facilitate the infiltration of hydrophobic compounds [21]. The reason for the differential sensitivity of Gram-positive and Gram-negative bacteria to plant extracts may be explained by the morphological of membranes differences between the Gram-positive and Gram-negative.

As Ben Salem et al. [7] study identified the phenolic compounds by HPLC. Among these flavonoids identified; quercetin and apigenin-7-glucoside have been previously reported mainly to offer promising antibacterial activity [22]. Quercetin acts as bacteriostatic due to its capacity to inhibit the D-Ala-D-Ala ligation in bacterial cells by inhibiting D-alanine ligase enzyme and preventing bacterial growth [23].

The inhibitory effect of *cynara* leaves extract against pathogenic bacterial strains can offer the plant as a potential applicant for drug development for the treatment of ailments caused by these pathogens. The inactivity of the aqueous and hexane extract against most bacterial strains studied in this research is in agreement with previous works which proved that these solvent extract of *cynara* generally showed little or no antibacterial activities [23] and this explained that the active chemical compounds was not soluble in water and hexane as confirmed by Nazi [24].

However, it is worth mentioning that the composition of phenolic compounds from a particular species of plant can widely differ based on the harvesting seasons, extraction method and geographical origin [25].

Tunisian *cynara scolymus* based antimicrobials have enormous therapeutic potential as it can act the purpose with lesser side effects that are often associated with synthetic antimicrobials. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy.

6. CONCLUSION

The use of natural sources of antimicrobial and antioxidant compounds is of benefit to the food industry. The antimicrobial effect of the *cynara* extracts was not in a large area of the spectrum, only a gram-positive bacterium was found to be sensitive against ethanol and ethyl acetate extracts. So, to obtain an excellent conclusion, *cynara* leaves extract could be a useful as tool for investigating the mechanistic effects of polyphenols released from the food matrix.

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