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ANTIMICROBIAL ACTIVITY OF SOME HYDROALCOHOLIC EXTRACTS OF ARTICHOKE (*CYNARA SCOLYMUS*), BURDOCK (*ARCTIUM LAPPA*) AND DANDELION (*TARAXACUM OFFICINALE*)

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Abstract: *The study aimed to determine the antimicrobial activity of some hydroalcoholic extracts obtained from three vegetable species with choleretic-cholagogue action: artichoke (Cynara scolymus), dandelion (Taraxacum officinale) and burdock (Arctium lappa). The antimicrobial activity of these products was tested by serial dilution method against bacterial strains (Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739 and Salmonella abony NCTC 6017). The hydroalcoholic extracts obtained from the studied vegetable species, have shown an antimicrobial activity against the bacterial strains of Escherichia coli and Salmonella abony, but they have not shown any antimicrobial activity against Staphylococcus aureus.*

Key words: *antimicrobial activity, Arctium lappa, Cynara scolymus, Taraxacum officinale.*

1. Introduction

Over time, medicinal plants and especially the naturals products derived from them, have been used in the prophylactic or curative scopes for maintaining the health. In fact, the plants are not universal remedies or a last resort refuge that they are often used, but they have a well-defined place in the therapeutic arsenal.

Since ancient times and Middle Ages, the plants were considered the only medicines for the treatment of diseases, because the bodies were programmed to accept those substances which the nature produces for the benefit of living beings. It is now increasingly accepted that medicinal plants can be successfully used in the treatment of various diseases.

The increased incidence of bacteria resistance to many antibacterial drugs is of great concern and medicinal plants have proven as an alternative source of antibacterial agents.

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In the attention of many researchers is the antibacterial potential of various plants such as: *Thymus capitatus* [1], *Piper longum* and *Taxus baccata* [2], *Taxus wallichiana* [3] or plants used as traditional remedies in Mexico [4] and Guinea-Bissau [28].Other researchers deal only with some parts of the plant: root of *Heranthus pectranthus* [5], leaves of *Luffa operculated* and *Peltophorum pterocarpum* [6], *Phyllanthus acidus* [7] and *Cassia fistula* [8].

The previous studies undertaken have proved the antimicrobial activity of some extracts with hepatoprotective action, obtained from *Rosmarinus officinalis* and *Tamarix gallica* [9].

In the literature there are a few studies about the antimicrobial properties of the three plants taken by us in the study.

This study evaluates the antimicrobial activity of hydroalcoholic extracts obtained from three plant species that have a choleretic-cholagogue action: *Cynara scolymus*, *Taraxacum officinale and Arctium lappa.* Antimicrobial activity of these products was tested using the serial dilution method against bacterial strains *Staphylococcus aureus* ATCC 6538 (gram-positive), *Escherichia coli* ATCC 8739 and *Salmonella abony entrica* NCTC 6017 (gram-negative).

Artichoke (*Cynara scolymus*) has been long known as plant food, but the research has also revealed medicinal qualities, such as positive effects on the liver and gallbladder, diuretic qualities and anti-dyspeptic [10]. Also the Artichoke leaf extract reduces the blood cholesterol and improves the symptoms of irritable bowel syndrome [11].

Eight phenolic compounds were isolated from the *n*-butanol soluble fraction of artichoke leaf extracts. The isolated compounds were examined for their antimicrobial activities on microorganisms, indicating that all compounds showed activity against most of the tested organisms. Among them, chlorogenic acid, cynarin, luteolin-7-rutinoside, and cynaroside exhibited a relatively higher activity than other compounds; in addition, they were more effective against fungi than bacteria [12].

Cardoon (*Cynara cardunculus L.*) is the designation of a group of Mediterranean species, traditionally used in Southwest Europe such as globe artichoke, cultivated cardoon and wild cardoon. Those applications consider the usage of the blanched leaves, fleshy leaf petioles and the receptacles in soups, stews and salads [13]. Cardoon leaves are used for their cholagogue, choleretic and choliokinetic actions, for treatment of dyspepsia and as anti-diabetics [14].

Several studies described numerous pharmacological activities associated to artichoke, such as hepatoprotective, antioxidative, anticarcinogenic, hypo-cholesterolemic, antiobacterial, anti-HIV, bile-expelling and urinative effects [15], [16].

The hydroalcoholic extracts obtained from *Cynara scolymus* have shown a significant inhibitory activity against the tested strains of *Listeria innocua* and *Baccillus cereus* [17].

The medicinal use of the roots of *Arctium lappa* (burdock) as done from ancient times, as an expectorant, property confirmed by Kardošová et al. [18]. Recent studies refer to hepato-protective effects [19] and gastro-protective activity [20] of the root but also at the antimicrobial properties of the extracts obtained from leaves and burdock root.

A recent study evaluated the antibacterial activity of a phytotherapeutic agent prepared from an ethyl acetate fraction (AcOEt) extracted from *Arctium lappa*. This agent inhibited the growth of all tested microorganisms (*Pseudomonas aeruginosa*, *Escherichia coli*,

Lactobacillus acidophilus, *Streptococcus mutans* and *Candida albicans*) [21].

The *Taraxacum officinale* Weber (dandelion) considered as weed plant but also as valuable plant mellifera, is known as plant food (leaves and flowers in salads) and medicine (especially root and leaves) [22]. Modern research studies confirm the traditional use effects on digestion and for many hepatic biliary problems (is cholagogue and choleretic). The leaf extract has a diuretic effect [23].

In our country there have been pharmacognostical studies on the achenes of *Taraxacum* [24] and a pharmacognostical comparative analysis of the root, stem, leaf and flower of dandelion, analysis correlated with miscoscopica structure of plant parts [25].

Recent research has shown antibacterial activity of aqueous and ethanol extracts obtained from dandelion leaves, on the following microorganisms: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [26].

2. Material and Methods

2.1. Plant materials and preparation of the extracts

Plants used in the study are *Cynara scolymus* (leaves), *Taraxacum officinale* (leaves) and *Arctium lappa* (roots) from S.C.Hofigal S.A's own crops. Dried plant materials have been extracted with 40% ethanol. After filtration three hydroalcoholic extracts were obtained.

2.2. Bacterial cultures

Three bacterial strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Salmonella abony enterica* NCTC 6017) have been used for testing antimicrobial activity.

2.3. Culture media

In order to determine the antimicrobial activity, the following culture media were necessary: Casein soya bean digest agar; Casein soya bean digest broth; Mannitol salt agar; MacConkey agar; MacConkey broth; Xylose, lysine, deoxycholate agar; Sodium chloride buffered solution pH = 7.00.

2.4. Work method

The antimicrobial activity (bactericidal effect) was determined with the dilute solutions method [27].

Serial dilution method. The principle of this method consists in the contact of standard inoculants from the microorganism-test with decreasing concentrations of the test sample.

Microorganism-suspension test. In the buffered sodium chloride solution, suspensions have been prepared with a concentration of 10^7 UFC /ml micro-test, corresponding to the tube's turbidity of 0.5 Mac Farland.

The samples for analysis: 100% and 50 % dilution (1/2).

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the serial dilution method and by carrying out subcultures.

The microorganism suspensions and the dilutions of the test sample have been obtained according to the method described above. In 10/100 mm tubes, binary dilutions of the test sample in the broth medium for bacteria. Into each tube 0.1 ml of the standardised suspensions of microorganisms was placed. Concomitantly, duplicate samples were prepared for each microorganism in which the test sample was not added. The tubes were incubated in aero-biotic conditions, at 30 – 35 ºC for bacteria, for 18 – 24 hours.

The lowest concentration of the tested products that inhibited the development of microorganisms (i.e., lacking any trace of macroscopically visible growth) was the MIC.

In order to determine the MBC (minimum bactericidal concentration), 0.1 ml was transferred from tubes in which the inhibition of microorganism growth was found into big tubes where 5 ml of broth medium for bacteria had been distributed. The tubes were incubated at 30 – 35 ºC for bacteria for 18 – 24 hours.

The minimum bactericidal concentration (MBC) was represented by the dilution of the test sample in which the development of bacteria was not observed. The bactericidal effect was demonstrated by performing sub-cultures on solid medium.

All tested microorganism cultures were dispersed upon their respective Petri dishes media: Mannitol salt agar for *Staphylococcus aureus*, MacConkey agar for *Escherichia coli*, Xylose, lysine, deoxycholate agar for *Salmonella abony enterica.* The samples were incubated for 18 – 24 hours at 30 – 35 ºC for bacteria.

3. Results and Discussions

The descriptions of the three medicinal plants used in this study is shown in Table 1.

Table 1

Uses and properties of medicinal plants collected for antimicrobial screening ..

Botanical name	Family	Local name	Part of plant used
Cynara scolymus	Asteraceae	Articoke	leaves
Taraxacum officinale	Asteraceae	Dandelion	leaves
Arctium lappa	Asteraceae	Burdock	roots

As it was noted above, the determination of the bactericidal effect of the artichoke extracts, dandelion and burdock to the reference strains of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella abony,* was achieved by means of the dilution method MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration).

The minimum inhibitory concentration (MIC), namely the minimum amount of extract that inhibits the growing bacterial strain under study, is based on the property of antimicrobial substances (extracts analyzed) to diffuse in a solid culture medium that is seeding the bacterial culture test. The interpretation was based on the size of the inhibition zone or by the number and size of colonies developed, and an assessment as to whether the bacterial strain is considered susceptible, intermediate or resistant to the specific extract was conducted.

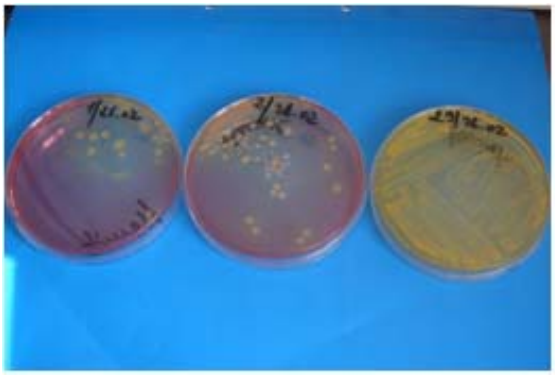
The minimum bactericidal concentration (MBC) has revealed the minimum amount of extract analyzed that kills 99.9% of inoculated strains after 18h – 24 h of incubation.

The results obtained for the dilute solution method are shown in Table 2.

Results obtained for dilute solutions method ..... Table 2

Test product	Bacterial cultures	Product concentration	Development the average macroscopic broth (the first passage of microbial strains)	Subcultures		
				The second passage on the broth medium /results	The third passage on special mediums	
					Culture medium	Results
Artichoke extract/ Dandelion extract/ Burdock extract	*Staphylococcus aureus* ATCC 6538	100%	++++	++++	Mannitol salt agar	Typical colonies of *S. aureus*
		50%	++++	++++		Typical colonies of *S. aureus*
		Control strain	++++	++++		Typical colonies of *S. aureus*
		Control product	visible development was not observed	visible development was not observed		Not observe colonies
	Escherichia coli ATCC 8739	100%	-	-	Mac Conkey agar	**Bactericidal effect**
		50%	-	-		
		Control strain	++++	++++		Typical colonies of *E coli* (they fermented the lactose within the culture medium)
		Control product	visible development was not observed	visible development was not observed		Not observe colonies
	Salmonella abony enterica NCTC 6017	100%	-	-	Xylose, lysine, deoxycholate agar	**Bactericidal effect**
		50%	-	-		
		Control strain	++++	++++		Typical colonies of *S. abony*, black center, similar to "cat eyes"
		Control product	visible development was not observed	visible development was not observed		Not observe colonies

Legend: ++++ = development microorganism culture medium opalescent.



- Petri dishe 1 – *S.aureus* + Artichoke extract (100 %)
- Petri dishe 2 – *S.aureus* + Artichoke extract (50 %)
- Petri dishe 29 – *S.aureus* – standard strain control

Without bactericidal effect

Fig. 1. *The bactericidal effect of the artichoke extract on the standard strain of S. aureus*

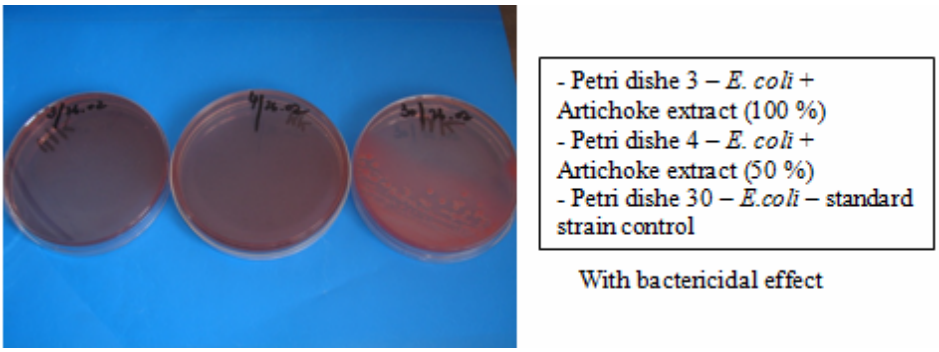


Fig. 2. *The bactericidal effect of the artichoke extract on the standard strain of E. coli*

4. Conclusions

The determination of the bactericidal effect of the extracts of artichoke, dandelion and burdock to the reference strains of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella abony enterica* was achieved by the dilution method MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration).

The products which have been tested, have not shown any microbial contamination, and they were within the limits specified by the European Pharmacopoeia edition in force.

The extracts of artichoke, burdock, dandelion, undiluted (100%) and diluted (50%), had no antimicrobial activity (bactericidal) against *Staphylococcus aureus*.

The extracts of artichoke, burdock, dandelion, undiluted (100%) and diluted (50%), exhibited antimicrobial activity (bactericidal) against the test microorganisms used: *Eschericha coli, Salmonella abony enterica.*

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