ORIGINAL PAPER

CARDUUS ACANTHOIDES FOLIUM TINCTURE: CHEMICAL COMPOSITION, ANTIBACTERIAL ACTIVITY AND SYNERGISTIC / ANTAGONIST EFFECT IN COMBINATION WITH ANTIBIOTICS

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Abstract. *Analysis of the Carduus acanthoides folium (CAF) tincture in UV-Vis spectra shows the presence of the maximum absorbance for chlorophyll a (i.e., λ = 640-670 nm) and two maximum absorbances at wavelengths 530-540 nm and 600-610 nm, probably due to flavonoids. The vibrations in the Fourier-transform infrared spectroscopy (FTIR) spectra of the tincture are attributed to the polyphenol compounds (flavonoids, polyphenolcarboxylic acids), carotenoids (β-carotene, carotenoid esters), triglycerides, phytosterols, and amino acids. The current study aimed, also, to investigate the antimicrobial potential of CAF tincture against five bacterial strains. The synergistic / antagonistic effect on the association between the tincture and the antibiotics of choice was tested. Following the research it was observed that the tincture has antibacterial effect against Staphylococcus aureus and Escherichia coli, the bacterias being classified as sensitive. The combination of tincture with the antibiotics of choice leads to the reduction of the antibacterial effect, and their association is not recommended.*

Keywords: *Carduus acanthoides folium, antibacterial effect, tincture, FTIR, UV-Vis*

1. INTRODUCTION

Nowadays according to the World Health Organization, natural therapies are used by 80% of the world's population [1]. *In vitro* studies have shown that plants have antibacterial efficacy against many bacterial species, discovering the importance of natural resources, little well studied in this regard, in the fight against bacterial agents. Antimicrobial activity of plant extracts and active principles of plants can be determined using antibacterial screening protocols [2, 3].

*Carduus acanthoides (*family *Asteraceae)* may exceed two meters high, the stalk and leaves are thorny, sometimes woolly. The plant has a basal rosette, at the end of the stems are found one or more inflorescences with a rounded purple disk appearance [4]. It is a biennial plant species that grows spontaneously in Europe, found in many parts of the world, including North and South America, New Zealand and Australia, being known as a harmful weed. It is an invasive species in many regions of Canada and the United States, California and West Virginia [4].

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In pharmaceutical purpose the chemical composition of plant extracts was usualy highlighted by Raman, FTIR and UV-Vis spectroscopies, often involving Ultra Performance Liquid Chromatography-Mass Spectrometry technique (UPLC-MS). *Carduus acanthoides* contains flavones (apigenin and luteolin) and saturated fatty acids [5]. The pharmacological effects of the plant species, according to literature data [5], are antiviral, being comparable to those of acyclovir and oseltamivir.

The CAF tincture were characterized, for the first time, both in terms of UV-Vis absorption spectra, and FTIR specific vibration. The current study aimed to investigate the antimicrobial potential of CAF tincture, taking as reference the antibacterial effect of antibiotics (control +) on five bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603). The synergistic / antagonistic effect on the association between the tincture and the antibiotics of choice was tested as well.

2. MATERIALS AND METHODS

2.1. MATERIALS

CAF was collected from "Alexandru Buia" University Botanical Garden (Craiova), in the period 2018-2019. The tincture was obtained by simple percolation, in a vegetable / solvent (70^o ethanol) of 1: 5 ratio (F.R. X, *Farmacopeea Romana, ed. X*).

2.2. SAMPLE PREPARATION AND ANALYTICAL TECHNIQUES

The tincture obtained by simple percolation [6] was characterized by UV-Vis spectroscopy. The tincture analyzed did not show UV absorption, the analysis was performed in Vis, using a Jasco V-530 UV – Vis spectrophotometer, working range (400-800 nm), deuterium lamp (UV emission, with λ 190-350 nm), halogen lamp (UV – VIS emission, with λ 330–1100 nm), single beam (single monochromator). A silicone photodiode detector (S1337), quartz cuvette (path length = 1 cm) and the sensitivity was ± 0.5 nm was used. The absorbance measurement was achieved automatically, at λ_{max}, by coupling the device with a computer. The subsequent processing of the absorption spectra in the visible was performed by software J-V-500W (V-500 / FP-750).

The organic functional groups of the residue obtained by tincture evaporation was determined by FTIR, using a Nicolet – Avatar ESP 360 FTIR spectrometer, with IR radiation source in the range 4000-400 cm^{-1}. The spectra of the sample was recorded in the area of 4000-800 cm^{-1}, using a DTGS KBr detector. Conditioned sample was realized in KBr pill, made by compression until a translucent film was obtained and was analyzed with the EZ Omnic E.S.P. 5.1 / 32 bit software for computerized data analysis.

2.3. ANTIBACTERIAL ACTIVITY

The antibacterial activity of the CAF tincture was tested by using the diffusimetric method on nutritional agar (*Kirby-Bauer*), in accordance with the provisions of F.R. X

(*Farmacopeea Romana, ed. X*). This is a very simple and fast method, which allows to determine the sensitivity spectrum of the microorganism. Rounds filter paper was impregnated with tincture and was deposited on the surface of the agarized medium sown "in cloth" with the standardized inoculum (obtained from the reference strain).The procedure is based on the radial diffusion in agar gel of the antimicrobial substances from the tincture. The active principles will diffuse into the environment, achieving a concentration gradient inversely proportional to the diameter of the diffusion zone, so with the distance to the disk. If the strain is sensitive to a particular antibiotic / plant extract, the microbial growth will be inhibited on a certain surface around the disk impregnated with the respective sample, a surface called a bacterial growth inhibition zone. In practice, is used the technique of discs impregnated with standardized antibiotics (as control +), recommended by NCCLS (National Committee for Clinical Laboratory Standardization). Procedure for the antibiogram has been described previously in other studies [7, 8].

Table 1. Sensitivity of tested germs

Microorganism test	***Chemotherapeutic agent (control +)***	***R****	***IS****	***S*****
Staphylococcus aureus	*Amoxicillin*	*≤15*	*16-19*	*≥20*
Escherichia coli	*Levofloxacin*	*≤13*	*14-16*	*≥17*
Proteus vulgaris	*Amikacin*	*≤14*	*15-16*	*≥17*
Pseudomonas aeruginosa	*ceftazidime*	*≤14*	*15-17*	*≥18*
Klebsiella pneumoniae	*cefotaxime*	*≤14*	*15-22*	*≥23*

R - resistant; IS** - intermediate sensitive; S*** - sensitive*

3. RESULTS AND DISCUSSION

The experimental data regarding the spectrophotometric characterization in Vis of the tincture are shown in Fig. 1. From the analysis of UV-Vis spectrum it can see the presence of the maximum absorption for chlorophyll *a* (λ = 640-670 nm) and two absorption maxima at wavelength 530-540 nm and 600-610 nm, assigned to the presence of flavonoids. Secondary metabolites in plants can be identified by FTIR due to the characteristic vibrations of certain functional groups in their structure (Table 2). Thus, by this method can be identified compounds of different classes: phenolic acids, flavonoids, terpenoids, alkaloids, iridoids, chlorophylls, phytosterols, fatty acids [9-18].

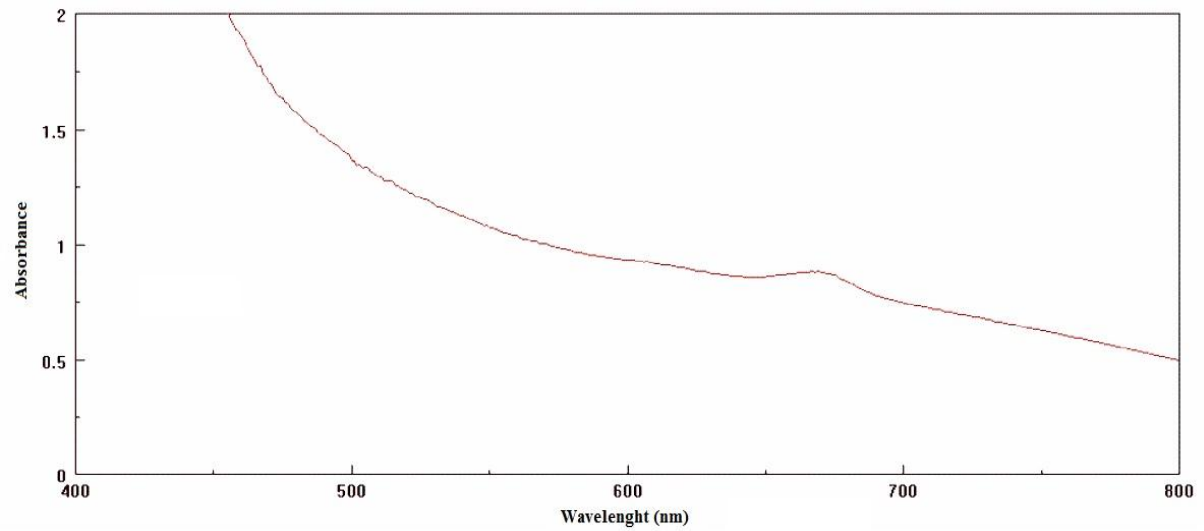

Figure 1. UV-VIS spectrum of CAF tincture

In Table 2 and Fig. 2 the vibrations obtained from the FTIR analysis of the tincture are compared to the extraction solvent (ethanol, Fig.3).

Table 2. Results of the CAF tincture FTIR analysis

Sample / Characteristic frequencies in IR [cm^{-1}]		**Assignment**
CAF	**Ethanol**	
3442	3432	Stretching vibration of O-H and N-H (amino acid, amines, amides).
2974		νC-H (asym.) (lipids, fatty acids).
2929		Asymmetrical stretching vibrations - CH_3, $–CH_2–$ (carboxylic acids).
	2355	C≡C triple bond. Stretching vibration of the P – H group (phosphoric compounds).
1647	1651, 1630	Stretching C = O group in the secondary amides.
1504		νC-H (bending) (the $–CH_2$ group in proteins).
1384	1347	Deformation (C – H) vibrations of the $–CH_3$, $–CH_2$ groups in aliphatic compounds.
1258	1257	νC-O, νO-H def. (polyphenols).
1042	1042	νC-O (stretch) polysaccharides.
878		νC-H (bending out of the plane).
	550	The vibration of the molecule's skeleton.

The bands from the region 750–3500 cm^{-1} were analyzed in particular, knowing that the biochemical composition of the plant products is reflected in this region [19]. The distinctive peak from 1630–1650 cm^{-1} was attributed to the stretching vibration of the C = O group of the secondary amides, while the peak from 1340–1350 cm^{-1} corresponds to the deformation vibrations (C – H) of the $–CH_3$ groups and $–CH_2–$ in aliphatic compounds.

The peak at 1723 cm^{-1} is due to the stretching vibration of the C = O group, which may be part of the molecules of secondary compounds, triglyceride classes or more likely carotenoid esters [20]. The peak at 1377 cm^{-1} appears in the literature as characteristic deformation vibration (C-H) of $-CH_3$ and $-CH_2$ groups from β-carotene [21].

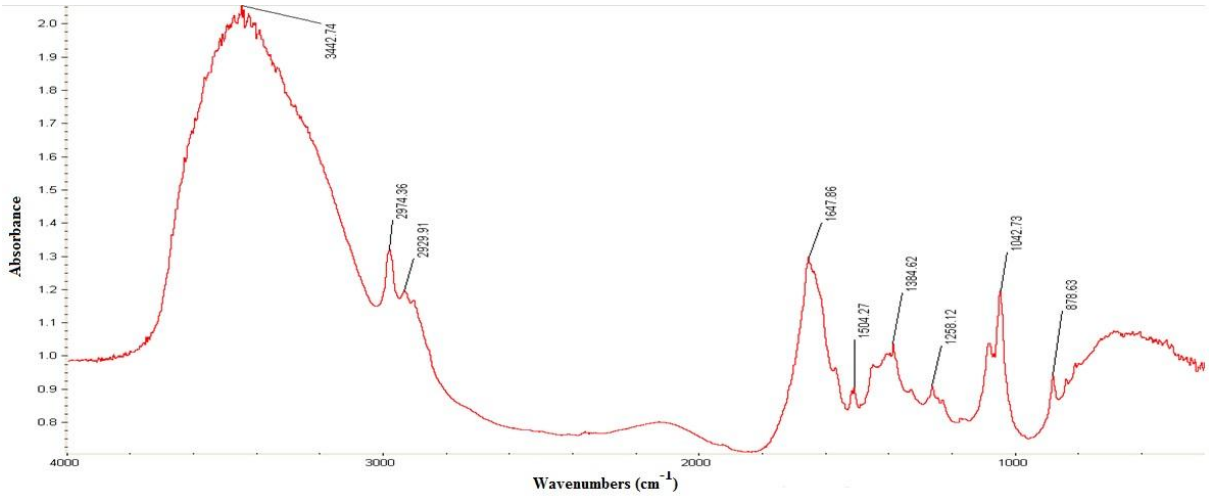

Figure 2. FTIR spectrum of CAF tincture.

In the region 3400–3450 cm^{-1} was observed the appearance of broad bands representative for the vibrations of the -OH or –NH groups, which could come from polyphenolic compounds, amino acids, amines, amides. The peaks in the range 1220–1260 cm^{-1} are representative for symmetrical C–O and O–H vibrations, which may also be due to the polyphenols in the tincture. At 2930–2980 cm^{-1} frequencies, characteristic peaks of a class more difficult to characterize by FTIR was observed, but whose presence has been previously identified, namely phytosterols.

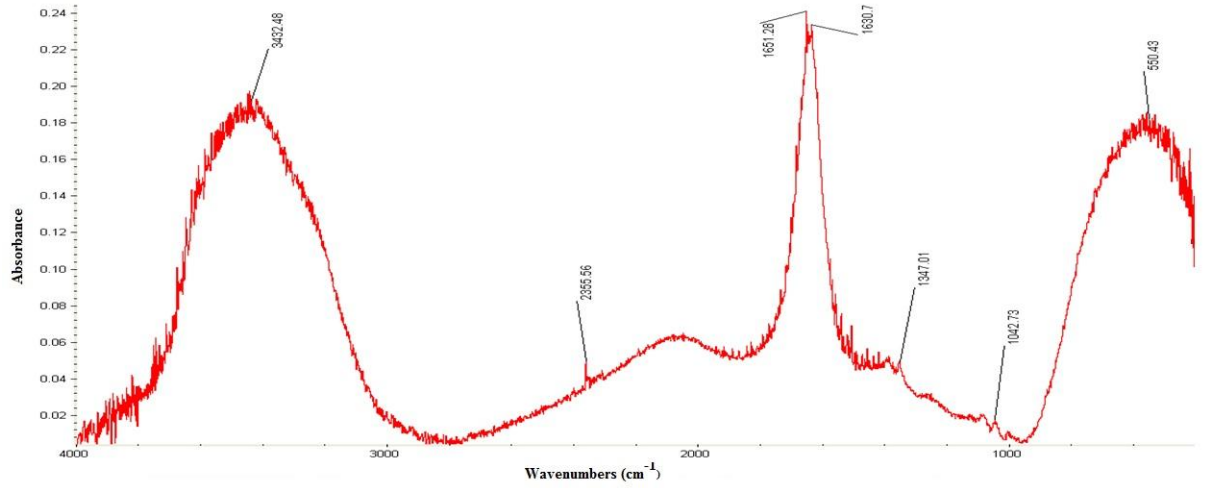

Figure 3. FTIR spectrum of ethanol.

Staphylococcus aureus is a gram-positive, round-shaped bacterium, frequently found in the upper respiratory tract and on the skin [22]. *S. Aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery [23]. Analysis of the antibacterial potential on the species *Staphylococcus aureus* showed the presence of the effect in the case of CAF tincture.

The measurement of the average diameters of the inhibition zones (mm) shows the superior therapeutic power of the elective's antibiotic compared with CAF tincture.

Table 3. Average diameters of bacterial growth inhibition, following simple tincture testing

Test Product	***S. aureus***	***E. coli***	***P. vulgaris***	***P. aeruginosa***	***K. pneumoniae***
CAF	18.6*	25.4***	0*	0*	0*
amoxicillin + clavulanic acid	32.6***	nt	nt	nt	nt
levofloxacin	nt	34.2***	nt	nt	nt
amikacin	nt	nt	33.6***	nt	nt
ceftazidime	nt	nt	nt	28.6***	nt
cefotaxime	nt	nt	nt	nt	35.8***
70° ethyl alcohol	16*	7.3*	0*	0*	0*

** resistant ** intermediate *** sensitive, nt-not tested*

Table 4. Average diameters of bacterial growth inhibition, following the testing of the tincture associated with the antibiotic of choice

Test Product	***S. aureus***	***E. coli***	***P. vulgaris***	***P. aeruginosa***	***K. pneumoniae***
(C+) + CAF	29.1***	28.4***	25.4***	26.9***	30,5***
amoxicillin + clavulanic acid (C +)	32.7***	nt	nt	nt	nt
levofloxacin (C +)	nt	33.4***	nt	nt	nt
amikacin (C +)	nt	nt	33.4***	nt	nt
ceftazidime (C +)	nt	nt	nt	29.1**	nt
cefotaxime (C +)	nt	nt	nt	nt	31.8***
(C+) + 70° ethyl alcohol	32***	32***	31*	28.4***	29.1***

** resistant ** intermediate *** sensitive, nt-not tested*

Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. Coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls [24]. The tincture of the species *Carduus acanthoides* has antibacterial effect on *E coli*, but has lower efficacy than levofloxacin (control +).

Proteus vulgaris is a rod-shaped, gram-negative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water, and fecal matter. It is known to cause wound infections and other species of its genera are known to cause urinary tract infections [25]. After performing the antibiogram was observed the lack of antibacterial effect

on the species *Proteus vulgaris* for tincture CAF. At the association between the reference antibiotic and CAF there is a decrease in the antibacterial effect of amikacin.

Pseudomonas aeruginosa is a common gram-negative bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. Aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes [25]. CAF tincture did not have therapeutic efficacy on *P aeruginosa*.

Klebsiella pneumoniae affects middle-aged and older men with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, glucocorticoid therapy, kidney failure, and certain occupational exposures (such as papermill workers). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). From the study was observed the lack of antibacterial effect on *Klebsiella pneumoniae* species for tincture CAF.

It can observe that CAF tincture has antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*. The combination of antibiotics with CAF tincture is not recommended, because the results showed that the antibacterial activity of the antibiotics is lower compared to the antibiotic alone (Table 4.).

4. CONCLUSIONS

Antibiotic resistance is considered to be one of the most pressing public health issues in the world, with the vast majority of bacteria becoming less sensitive to antibiotic treatment. The genetic ability of the bacteria to acquire multiple resistance to the chemotherapeutic agents used, leads to the unfavorable evolution of the infections and to the therapeutic failure. The development of bacterial resistance to classical antibiotics has led to the development of research in this field, in search of new, effective antibacterial agents.

Herbal extracts with antimicrobial potential represent an important research issue in the actual context of medical world, with the aim of isolating the active components, in order to develop new chemotherapeutic agents with applicability in the treatment or use as adjuvant therapy in infectious conditions. The antimicrobial properties of plants are conferred by their ability to synthesize certain secondary metabolites with relatively complex structures.

The CAF tincture is active against *Staphylococcus aureus* and *Escherichia coli* and has no antibacterial activity against *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The combination of the antibiotics of choice with the CAF tincture is not recommended, as there is a slight reduction of the antibacterial potential of the synthetic chemotherapies.

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