https://doi.org/10.31925/farmacia.2023.5.21 **ORIGINAL ARTICLE**

STUDY OF THE CHEMICAL COMPOSITION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF THE AERIAL PART OF *LEONURUS CARDIACA*

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Abstract

The effect of various solvents (hexane, diethyl ether, ethyl acetate, isopropyl alcohol) on extraction of phytocomponents of *Leonurus cardiaca* leaf was studied a long with the antioxidant and antibacterial activity of the obtained extracts. The chemical composition of the obtained extracts was studied by the chemical and spectrophotometric methods. The results showed the presence of various phytocompounds in the extracts. It was found that the non-polar solvent hexane extracted a large amount of esters compared to all selected solvents. It was also established that the most polar solvent, isopropanol, extracted large amounts of fatty acids. More extractives (8.5% yield) were extracted with isopropanol and this extract contained the highest amounts of phenolic compounds (68.8 mg GAE/g) and flavonoids (22.7 mg RE/g). The analysis of the antioxidant activity showed that the isopropanol extract had the best result. The antibacterial analysis showed that all investigated conditionally pathogenic bacteria (*E. Coli*, *B. subtilis* and *Salmonella typhimurium*) were sensitive to all extracts.

Rezumat

Prezentul studiu a evaluat efectul unor solvenți (hexan, dietil eter, acetat de etil, alcool izopropilic) asupra procesului de extracție al fitocomponentelor din frunzele de *Leonurus cardiaca*, precum și activitățile antioxidantă și antibacteriană ale extractelor obținute. Compoziția chimică a acestora a fost studiată prin metode chimice și spectrofotometrice. S-a observat că hexanul a extras o cantitate mare de esteri în comparație cu restul solvenților. S-a stabilit, de asemenea, că solventul cel mai polar, izopropanolul, extrage cantități mari de acizi grași. Analiza activității antioxidante a arătat că extractul izopropanolic a avut cel mai bun rezultat. Analiza antibacteriană a arătat că toate bacteriile patogene condiționat investigate (*E. coli, B. subtilis* și *Salmonella typhimurium*) au fost sensibile la toate extractele.

Keywords: flavonoid, phenolic content, antioxidant, antibacterial activity

Introduction

In recent years, despite a great number of synthetic drugs used in modern veterinary and medicine, the interest in remedies of folk medicine has not disappeared, but rather revived. To a certain extent, it is explained by the increase in allergic reactions to the use of synthetic medicines.

The prospects for creating new drugs are no less complex than solving the problem of reducing their negative side effects. Therefore, currently, based on biochemical and pharmacological studies and in-depth study of the achievements of folk medicine, native plants are increasingly being used in the healing process. *Leonurus cardiaca L.* is currently distributed worldwide due to its medicinal uses [12, 36]. Its potential applications in the treatment of several diseases have made *L. cardiaca* an excellent candidate for the development of alternative therapies in both traditional oriental and modern medicine [12, 13]. The plant is rich in biologically active substances that determine its wide range of biological action [14]. In addition to its traditional medical use, *L. cardiaca* is used in some cuisines as a seasoning for various vegetable soups, as well as for seasoning beer and tea [37]. Therefore, the potential consumption of this medicinal plant increases and its deep study becomes relevant.

Physiological and biochemical studies of plants require knowledge of their qualitative and quantitative content. *L. cardiaca* is a perennial herbaceous plant. Several varieties of the plant are known that differ from each other in morphological features, however, their pharmacological activity is generally similar. *L. cardiaca* leaf is used for curing purposes. On the basis of the herb, an aqueous infusion and an alcoholic tincture are prepared, in folk medicine fresh juice is used prepared by squeezing the collected herb [16, 21, 22].

Medicinal raw material of *L. cardiaca* contains many biologically active substances (BAS). The pharmacological studies have confirmed the antibacterial, antioxidant,

anti-inflammatory and analgesic effects of the herb, as well as its effect on the heart and cardio-vascular system. The chloroform extract of *L. cardiaca* leaves, rich in diterpenes, inhibits the growth of *Staphylococcus aureus* [34], while the chloroform faction of the methanol extract of its aerial parts showed the activity against multi resistant *Plasmodium falciparum* strains [39]. Extracts of *L. cardiaca* have also exhibited the antioxidant activity in several *in vitro* studies. Studies of polyphenolic compounds have shown that mainly flavonoids (rutin) and hydroxy-cinnamic acid derivatives [5, 17, 19, 25, 26, 27] may be responsible for this effect.

Sedative and hypotensive effects have been proven in clinical trials [41]. It was confirmed that *L. cardiaca* preparations had sedative, anticonvulsant, cardiotonic and diuretic effects. Such pharmacological activity is directly related to the BAS complex contained in the plant [21].

To explore the potential advantages of *L. cardiaca* for the treatment of various diseases, additional research is undoubtedly required. Therefore, this study was aimed at determining the phytochemical constituents and the antioxidant and antimicrobial activity of various extracts of *L. cardiaca* herb growing in territory of Aparan (Armenia).

Materials and Methods

Collection of L. cardiaca

L. cardiaca leaf was harvested in Aparan (Armenia) in the flowering phase in early June 2021. The raw material was dried in shade at 25 - 30ºC to an air-dry state until moisture content was less than 10% and crushed to a particle size of 1 - 2 mm.

Preparation of extracts

Extraction was carried out in a flat-bottomed 250 mL flask. To carry out extraction, we took a sample of crushed raw material weighing 5 g with an accuracy of 0.0001 g, to which 100 mL of various organic solvents was added and the whole was thoroughly shaken. Organic solvents with increasing polarity were used as extractants – hexane, diethyl ether, ethyl acetate, isopropyl alcohol. All solvents have been purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the purity of these solvents is ≥ 98.5 - 99%. Later, the mixture was left to stand at room temperature for 48 hours and stirred 2 - 3 times a day [4, 15]. After filtering the mixture, the filtrate was evaporated to dryness using a Rotavapor vacuum evaporator. The final extracts were weighed to determine the yield (%) and the dried extracts were stored at 4ºC in a refrigerator for further studies.

Phytochemical screening

The presence of various phytochemical components, such as alkaloids, saponins, flavonoids, phenols, tannins, was determined by qualitative screening using standard methods [29]. The qualitative determination of the extracts composition was also carried out by the method of spectrophotometry in the ultraviolet and visible regions using UV 1800 spectrophotometer. The amount of fatty acids both free and bound in the form of triglycerides, as well as the physical parameters of the extracts, were studied by the generally accepted methods [1].

Determination of the total content of phenols and flavonoids

The total content of phenols in various solvent extracts was determined by the FC colorimetric method (Folin-Ciocâlteu) [35]. About 0.1 g of the dried extract was suspended in 1 mL of methanol. 0.1 mL of this solution was thoroughly mixed with 1 mL of sodium carbonate solution (20%) and 2 mL of 10% FC reagent. After that, the solution was left to stand at room temperature for about 20 minutes to observe the change of the colour. Absorbance was measured against water at 730 nm. The standard calibration curve was constructed. The total content of phenols was presented in mg of equivalents of gallic acid (GAE) per gram of dry extract.

Total content of flavonoids in different solvents

To quantify the amount of flavonoids in different extracts, a colorimetric method with aluminium chloride with modifications was applied [8]. For this purpose, to 0.5 mL of the extract 2 mL of distilled water was added, followed by 150 µL of $NaNO_2$ solution (5%) and the mixture was kept for 5 min at room temperature. Then 600 µL of $AlCl_3$ solution (10%) and 2 mL of NaOH solution (4%) were added. After thorough mixing, the volume of the solution was adjusted to 5 mL with distilled water and left to stand for 15 min at room temperature. Water was used as a standard solution. The optical density was measured at 510 nm wavelength. A standard calibration curve was constructed using various concentrations of rutin. The total content of flavonoids is expressed as mg of rutin equivalent (RE) per gram of the dried extract.

Antioxidant activity

For this, 8 mL of freshly distilled boiled water of room temperature, 1 mL of a 20% sulfuric acid solution and 1 mL of a 0.05 N potassium permanganate solution were poured into a 50 mL beaker. The whole was stirred, and titrated with *L. cardiaca* extracts from a microburette (volume 1 mL with a 0.01 mL division) until the pink colour disappeared. For the control experiment, about 0.05 g (exact weight) of quercetin was dissolved in 40 mL of ethyl alcohol, transferred to a 100 mL volumetric flask, brought to the mark with an alcohol and stirred. 8 mL of freshly boiled and cooled distilled water, 1 mL of a 20% sulfuric acid solution, and 1 mL of a 0.05 N potassium permanganate solution were poured into a 50 mL titration beaker, stirred and titrated with a quercetin solution from a micro-burette (volume 1 mL with a division of 0.01 mL)

until the pink colour disappeared. 1 mL of 0.05 N potassium permanganate solution corresponds to 0.25 mg of quercetin.

The calculation of the antioxidant activity index (AOA), which corresponds to the BAS concentration of a reducing nature in terms of quercetin (in mg/g) was carried out according to the formula:

$$B = C_k \times V_k \times V_o / V_x \times m,$$

where, B is the concentration of BAS of a reducing nature of the object under study, consumed for titration of 1 mL of a 0.05 N potassium permanganate solution, mg/mL (0.5 mg/mL); V_k is the volume of a quercetin solution used for titration of 1 mL of 0.05 N potassium permanganate solution, mL (1.4 mL); V_o is the volume of the test solution, mL (50 mL); V_x is the volume of the test-object solution used for titration of 1 mL of a 0.05 N potassium permanganate solution, mL (0.4 mL); m is the mass of the exact weight of the object under study, g (1 g). The total amount of restorative BAS was determined in terms of quercetin per 1 g of the preparation.

Determination of antibacterial activity

To determine the antimicrobial activity, conditionally pathogenic Gram-positive *Bacillus subtilis 17-89* and Gram-negative *Salmonella typhimurium G-38* and *Escherichia coli K-12* bacteria maintained in the culture collection of the Laboratory of Probiotics Biotechnology of the SPC "Armbiotechnology" NAS RA, were used as test cultures. The test cultures were grown on the nutrient agar medium at 37ºC for 16 - 18 h.

The drop point method was used to evaluate the antimicrobial activity. Test cultures grown in nutrient broth (biomass density was 1 x 10^6 - 2 x10^6 GAM/ mL) were sown on Petri dishes at 37ºC for 16 - 18 h with appropriate solid media. 40 mg of each extract (dray weight) was dissolved in 1 mL of dimethyl-sulfoxide (DMSO). 20, 40 and 60 µL portions of each sample were dropped onto the surface of the test cultures. DMSO served as a negative control. Petri dishes were kept at 4ºC for 1 - 2 h to facilitate diffusion of the samples into the agar. Then the dishes were placed in a there most at 30°C for 24 - 48 h. The antimicrobial activity was evaluated by measuring the diameter (size, Ø, mm) of the grow the suppression zone of the test cultures after 24 - 48 h of incubation [32].

Results and Discussion

To determine the amount and composition of the main classes of extractives, extraction with hexane, diethyl ether, ethyl acetate and isopropanol was carried out.

Table I shows the data on the yield of extractives obtained by extracting the aerial part of *L. cardiaca*. Various solvents significantly affected the yield of extractives. In particular, the isopropanol extract resulted in an excellent extraction yield (8.5%). As can be seen from the table, these solvents can be compared as extractants in the following way: iso-propanol > ethyl acetate > diethyl ether > hexane. The presence of significant differences between the dry weight of extracts and the yields of extractive substances extracted by various organic solvents may be due to different polarities of the tested solvents [38].

Table I

Total yield of extractives of various solvent extracts *L. cardiaca* leaves

Solvent extracts	Yield of extractives (%)
Hexane	3.4
Diethyl ether	3.9
Ethyl acetate	4.1
Isopropyl alcohol	8.5

The results of qualitative study of phytochemicals present in various *L. cardiac*a extracts are presented in Table II. Various phytocompounds such as alkaloids, saponins, flavonoids, phenols, tannins were studied in extracts. Flavonoids, phenols were found in all extracts used. Isopropanol extracts with 5 phytocomponents turned out to be the best among the organic solvents evaluated in our study. The hexane extract showed the presence of 2 phytocomponents, while the diethyl ether and ethyl acetate extracts contained 4 phyto-compounds.

Table II

Qualitative screening of phytochemicals present in different solvent extracts of *L. cardiaca* leaves

Phytochemicals	Hexane	Ethyl acetate	Diethyl ether	Isopropil alcohol
Saponins	-	+	+	+
Tannins	-	+	-	+
Alkaloids	-	-	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	+

"+" = present; "–" = absent

To determine the main classes of compounds extracted with various solvents, the method of UV and visible spectrophotometry was also used (Figures 1, 2, 3 and 4).

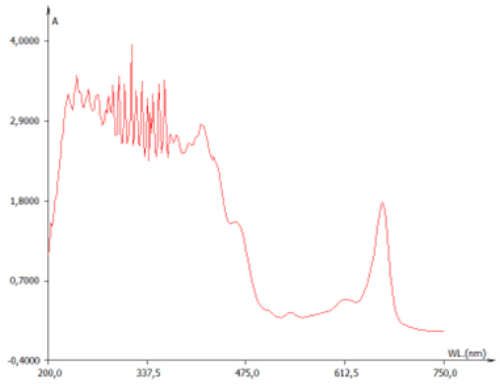

Figure 1.
UV spectrum of isopropyl alcohol extract

As can be seen from Figure 1, the spectrum of the isopropanol extract had an absorption band in the region of 230 - 470 nm, which indicates the presence of tannins, saponins, coumarins, flavanols [20]. In spectrum of the isopropyl extract, absorption bands were also found at λ = 415 and 670 nm that correspond to chlorophyll and chlorophyll-substituted compounds. Besides, the spectrum of the isopropyl extract also had bands at 505 nm and 535 nm, corresponding to anthocyanins.

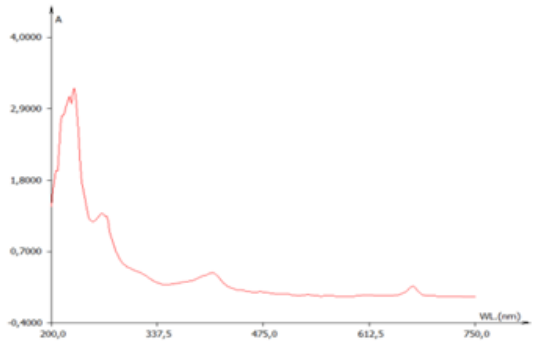

Figure 2.
UV spectrum of hexane extract

The non-polar solvent hexane extracts mainly substances of the lipid nature. As can be seen from Figure 2, in the hexane extract, the presence of the first maximum is caused by the presence of triglycerides and fatty acids that absorb in the UV-region at λ = 210 - 226 nm. The hexane extract also contains a small amount of saponins (250 nm), aurones (405 nm); a pick at 640 - 680 nm indicates the presence of exclusively chlorophylls (Figure 2) [6, 9, 30].

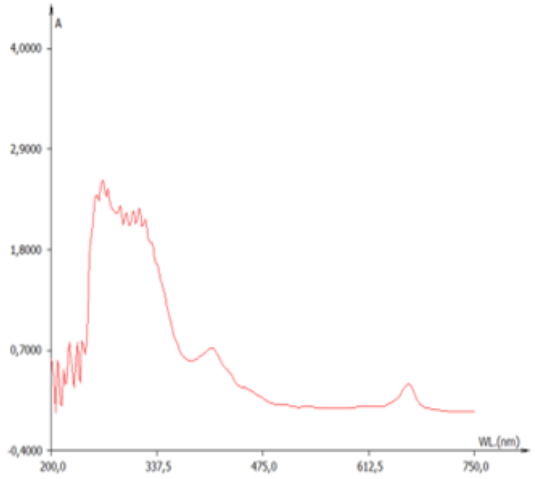

Figure 3.
UV spectrum of ethyl acetate extract

The extract isolated with ethyl acetate, contains flavanones, triterpene, saponins, chlorophyll, which confirms the presence of absorption bands in the spectrum at 250 - 350, 670 nm. The absorption band at 405 nm in the spectrum of the ethyl acetate extract indicates a low content of aurones (Figure 3). The extract isolated with diethyl ether, contains flavanones, triterpene saponins, aurones, chlorophyll, which confirms the presence of the absorption bands in the spectrum at 250 - 350, 670 nm (Figure 4) [28, 31].

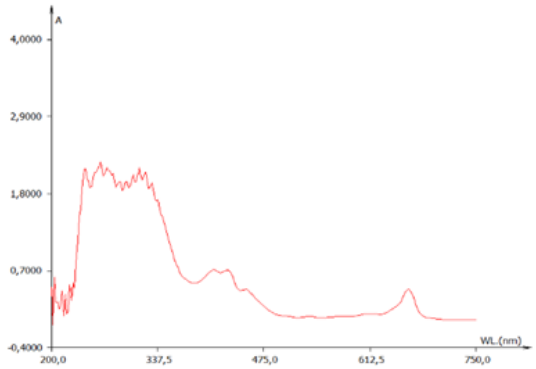

Figure 4.
UV spectrum of diethyl ether extract

All these identified phytochemicals are known to have a wide range of biological activities, including antibacterial, antifungal, antiviral, antioxidant, and cytotoxic properties [33]. Polyphenols and flavonoids are secondary plant metabolites and found in some medicinal plants known to have antimicrobial, anti-oxidant, antispasmodic, antidepressant, anti-tumour, antimutagenic, anti-inflammatory and many other kinds of biological activity [2, 33]. In plants, these phenolic compounds provide protection against various pathogens, regulate cell division and growth, help with pigmentation and many other metabolic processes [23]. Therefore, we investigated the presence of common phenolic and flavonoid compounds in various extracts of organic solvents (Table III). The results clearly indicated the existence of statistically significant differences between different extracts.

Table III
Total phenolics and flavonoids content of different solvent extracts of *L. cardiaca* leaves

Solvent extracts	Total phenolic content[a]	Flavonoid content[b]
Hexane	30.3	13.6
Ethyl acetate	55.6	11.8
Diethyl ether	48.2	14.4
Isopropyl alcohol	68.8	22.7

A = mg gallic acid equivalent, mg GAE/g DW (dry weight); b = mg rutin equivalent, mg RE/g DW

The highest content of phenols and flavonoids was observed in the isopropanol extract. Due to the high polarity of isopropanol, it was found to exhibit the best efficiency in extracting phytocompounds of

different polarities (phenols and flavonoids) from *L. cardiaca*.

Physicochemical parameters, such as acid number, which shows the presence of free organic acids, the saponification number, which shows the presence of the amount of esters, the ester number, which characterizes the total number of ester bonds, were also studied. Physicochemical parameters of extracts of *L. cardiaca* leaves are shown in Table IV.

Table IV

Physico-chemical parameters of extracts of *L. cardiaca* leaves

Name indicator	Hexane	Ethyl acetate	Diethyl ether	Isopropyl alcohol
Appearance	slightly turbid solution	clear solution	clear solution	clear solution
Colour	pale yellow	pale green	green	pale green
Smell	grassy	grassy	grassy	grassy
Density, g/cm^3	0.692	0.9006	0.7012	0.9184
Refractive index, n^{20}_D	1.377	1.373	1.355	1.378
Acid number, mg KOH/1 g	5.05	7.29	6.73	13.46
Ester number, mg KOH/1 g	107.17	15.154	35.3525	31.428
Saponification number, mg KOH/1 g	112.22	22.444	42.0825	44.888

Free organic acids are extracted with isopropanol to the greatest extent. The largest amount of esters is contained in the hexane extract of *L. cardiaca*, which is confirmed by the saponification number (Table IV**)**.

Taking in to account a rich composition of the extracts, the presence of phenolic compounds, as well as of flavonoids, the next stage was to study the antioxidant activity. A 0.05% quercetin solution was used as a control. According to the results obtained during titration, in the case of quercetin (1.4 mL) the consumption of *L. cardiaca* extracts was, respectively, isopropanol – 0.52 mL, diethyl ether – 1.25 mL, ethyl acetate – 1.1 mL, hexane – 1.8 mL, which indicates that the *L. cardiaca* extract can be considered as an effective prophylactic agent of antioxidant activity. The amount of antioxidant substances was determined by the following formula:

in the case of the isopropanol extract:

$$B = (0.5 \times 1.4 \times 100)/(0.52 \times 1) = 134.61 \text{ mg/g};$$

in the case of the diethyl ether extract:

$$B = (0.5 \times 1.4 \times 100)/(1.25 \times 1) = 56.0 \text{ mg/g};$$

in the case of the ethyl acetate extract:

$$B = (0.5 \times 1.4 \times 100)/(1.1 \times 1) = 63.63 \text{ mg/g};$$

in the case of the hexane extract:

$$B = (0.5 \times 1.4 \times 100)/(1.8 \times 1) = 38.38 \text{ mg/g}.$$

The antioxidant activity assessment method allows screening medicinal plant raw materials, phytopreparation and dietary supplements with high antioxidant activity (AOA) dependent on phenols, flavanoids, tanins, etc. [24]. The presence of antioxidant activity in extracts can be explained by the fact that the investigated extracts contained multifunctional BAS together with easily oxidized functional groups (for example, -SH, $(CH_3)_2CH$-), that relatively faster bind free radicals formed in living organisms.

Studies have shown that, as expected, the isopropanol extract has the most pronounced antioxidant activity compared to other studied extracts.

Currently, the prevalence of deadly bacterial diseases has increased. Microbes adapting to antibiotics are of great concern in the world [40]. There from is the greatest research interest of the medical community towards the development or discovery of new anti-microbial agents. Due to side effects of some synthetic antibiotics, research focusing on the discovery of natural plant-based drugs is preferred [3, 10]. Since *L. cardiaca* extracts are rich in numerous biologically active substances, we evaluated the effect of their various extracts against some common strains of bacteria pathogenic for human.

In the next stage the research of the antimicrobial activity of the synthesized compounds was carried out along with the evaluation of the resistance of the given test cultures to a number of antibiotics. The data are shown in Table V.

The results of the investigation of the antimicrobial activity of the different solvent extracts of *L. cardiaca* at different concentrations are presented in Table VI.

The results of our study showed that all studied ere effective against both gram-negative (*E. Coli K-12*, *Salmonella typhimurium G-38*) and gram-positive bacteria (*Bacillus subtilis 17-89*), their effectiveness being different (Table V). With the increase in the concentration, the increased antibacterial activity was observed irrespective of the solvent.

As can be seen from the Table VI, *Bacillus subtilis17-89* is more susceptible to all extracts compared to *E. Coli K-12* and *Salmonella typhimurium G-38*. Amongst all the extracts of *L. cardiaca*, the isopropyl alcohol extract exhibited maximum anti-bacterial activity against the studied gram positive and Gram-negative bacteria. This activity may be related to the presence in this extract of even more phytocomponents than in others (Figure 1, Tables I, II and III).

Thus, *L. cardiaca* extracts may be useful in the treatment of bacterial diseases.

Table V

Determination of resistance of *Salmonella typhimurium G 38, E. coli K-12* and *Bacillus subtilis* G17-89 test cultures to antibiotics

Antibiotics (Antibiotics Sensitivity Discs)	Discs Dosage	Test cultures					
		Salmonella typhimurium G-38		*Bacillus subtilis 17-89*		*E. coli K-12*	
		Growth pressure zone, Ø, mm	Sensitivity	Growth pressure zone, Ø, mm	Sensitivity	Growth pressure zone, Ø, mm	Sensitivity
Clarithromycin(CLR)	**15 µg**	18 ± 2	+	28 ± 2	+	20 ± 2	+
Doxycyclin (DXT)	**30 µg**	14 ± 1	-	18 ± 2	+	25 ± 2	+
Azithromycin (AZM)	**15 µg**	25 ± 2	+	22 ± 2	+	30 ± 2	+
Tetracycline (TE)	**30 µg**	12 ± 1	-	16 ± 1	+	16 ± 2	-
Ampicillin (AMP)	**10 µg**	18 ± 2	+	18 ± 2	+	20 ± 2	+
Amicacin (AN)	**30 µg**	30 ± 2	+	28 ± 2	+	28 ± 2	+
Ofloxacin (OFX)	**5 µg**	0	-	24 ± 2	+	40 ± 2	+
Erythromycin (E)	**15 µg**	0	-	27 ± 2	+	20 ± 2	+
Vancomycin (VA)	**5 µg**	0	-	16 ± 1	+	0	-
Piperacillin (tazobactam, TZP)	**110 µg**	20 ± 2	+	26 ± 2	+	30 ± 2	+
Cefazolin (KZ)	**30 µg**	17 ± 2	+	30 ± 2	+	18 ± 2	-
Penicillin G (P)	**10 IU**	10 ± 1	-	22 ± 2	+	0	-
Amoxicillin (AML)	**10 µg**	28 ± 2	+	26 ± 2	+	25 ± 2	+
Rifampicin (RD)	**5 µg**	15 ± 1	-	16 ± 1	+	14 ± 2	-

"+" = sensitive (+) and "–" = stable

Table VI

Antimicrobial effect of the different solvent extracts on the growth of *Salmonella typhimurium G 38, E. Coli K-12* and *Bacillus subtilis G17-89*

Solvent extracts	Test cultures								
	Salmonella typhimurium G-38			*Bacillus subtilis 17-89*			*E. Coli K*		
	20 µL	40 µL	60 µL	20 µL	40 µL	60 µL	20 µL	40 µL	60 µL
Hexane	18 ± 1	19 ± 1	20 ± 1	20 ± 1	21 ± 1	22 ± 1	11 ± 1	12 ± 1	12 ± 1
Ethyl acetate	16 ± 2	17 ± 1	18 ± 1	20 ± 2	22 ± 1	23 ± 1	9 ± 1	10 ± 2	1 ± 2
Diethyl ether	15 ± 1	17 ± 1	19 ± 1	17 ± 1	20 ± 2	24 ± 1	13 ± 1	15 ± 1	15 ± 1
Isopropyl alcohol	19 ± 1	22 ± 1	25 ± 2	24 ± 1	28 ± 1	30 ± 1	11 ± 1	14 ± 2	16 ± 1
DMSO	5 ± 1	8 ± 1	10 ± 1	6 ± 1	9 ± 1	12 ± 1	3 ± 1	5 ± 1	8 ± 1

growth = growth pressure; negative = lack of growth pressure; µL = injected doses

These results suggest that *L. cardiaca* is a potential source of broad-spectrum antimicrobials. The antimicrobial activity of the extracts may be due to the high content of flavonoids, which are reported to be involved in the inhibition of biosynthesis of nucleic acid sand other metabolic processes [11]. Flavonoids have also been reported to inhibit spore germination of plant pathogens [18]. Moreover, flavonoids are synthesized by plants in response to microbial infection. Phenolic compounds are also often referred to as microbial agents [7].

Previously, researchers have reported the varied antimicrobial potential of this plant and thus our reports support these findings [41]. Our study clearly indicated the existence of considerable differences in the antibacterial activity among the various solvent extracts evaluated. This could be due to varied phytochemical constituents present in different solvent extracts.

Conclusions

In conclusion, the present study clearly showed that the phytochemical composition of *L. cardiaca* extract varied with different solvents. In different extracts, the content of phenols and flavonoids varies significantly due to the polarities of various solvents.

Isopropyl *L. cardiaca* extract contained more extractable metabolites than other solvents. Moreover, all extracts of *L. cardiaca* had significant antioxidant and antimicrobial activity with a difference. The differences are due to their different phytochemical composition. Thus, our study showed that the isopropanol extract of *L. cardiaca*, containing many biologically active compounds, could be used as a therapeutic source for the extraction of various biologically active substances.

Conflict of interest

The authors declare no conflict of interest.

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