Published online 2020 October 31.

**Research Article** 

# Antibacterial Effects of Hydroalcoholic and Aqueous Extracts of Two Medicinal Plants in Comparison with Popular Antibiotics: An In Vitro Study

## Sadra Ansaripour <sup>1</sup>, Niloofar Safaei <sup>1</sup> and Najme Bagheri <sup>1</sup>

<sup>1</sup>Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran

<sup>\*</sup> Corresponding author: Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran. Tel: +98-9130576733, Email: naba2021@yahoo.com **Received** 2019 September 08; **Revised** 2019 November 11; **Accepted** 2019 December 02.

## Abstract

**Background:** Acinetobacter baumannii is considered a dangerous and drug-resistant hospital-acquired infection. Nowadays, there has been an increasing interest in the use of herbal drugs.

**Objectives:** This in vitro study was conducted to determine the antimicrobial effects of *Rumex acetosella* L. and *Cucurbita maxima* L. on *Acinetobacter baumannii* in comparison with popular antibiotics.

**Methods:** In this experimental study, after extraction, the antibacterial effects of extracts were determined based on MIC and MBC using broth microdilution. The effects of different concentrations of the extracts on *A. baumannii* growth were also investigated by the disk diffusion method. The results were compared with choice antibiotics.

**Results:** The results of the study indicated that in broth microdilution, the MIC and MBC of the hydroalcoholic extract of *C. maxima* and the aqueous extract of *R. acetosella* were equal (64 and 128  $\mu$ g/mL, respectively). The MIC and MBC of the hydroalcoholic extract of *R. acetosella* and the aqueous extract of *C. maxima* were 128 and 256  $\mu$ g/mL, respectively, which indicated the weaker effects of these extracts. In the disk diffusion method, the greatest mean diameter of inhibition zone was obtained for *R. acetosella* extracts (24.83  $\pm$  0.29 and 21.83  $\pm$  0.29 mm for hydroalcoholic and aqueous extracts, respectively). Also, the lowest mean diameter was obtained for *C. maxima* extracts (10.33  $\pm$  0.58 and 8 mm for hydroalcoholic and aqueous extracts, respectively).

**Conclusions:** This study showed the potent antibacterial effects of *R. acetosella* and *C. maxima*. They were even more potent than commonly used antibiotics. Therefore, the plants can be used as antimicrobial agents, as well as pharmaceutical supplements and alternative therapies.

Keywords: Acinetobacter baumannii, Herbal Extracts, Antibacterial Agents, Antibiotics

## 1. Background

Infectious diseases caused by various microorganisms are very common worldwide (1). *Acinetobacter* spp. are hospital pathogens that have spread due to the widespread use of antibiotics. These bacteria are Gram-negative, oxidase-negative, obligately aerobic, and non-motile coccobacilli and a common cause of nosocomial infections (2-4). The bacteria from this genus are also resistant to disinfectants and can survive in natural environments such as water and soil for a long time and lead to infections, especially in immunodeficient individuals (5). *Acinetobacter baumannii*, the most important species of this genus, is responsible for respiratory, blood, urinary tract, and wound infections (6). The ICU environment and ICU patients can contribute to the spread of this microorganism. Generally, this bacterium is harmless to healthy people, but in some cases, is strongly resistant to antibiotics and can spread to patients. In addition, the treatment of such organisms is difficult and can lead to increased mortality (7). Infectious disease specialists have intended to use herbal medicines and natural compounds in recent years due to the numerous side effects of chemical drugs, as well as significant advances in the production of herbal medicines (7).

Nowadays, to discover new therapeutic methods that are more economical with fewer side effects, the study of medicinal plants and their properties is particularly important so that over 30% of medicinal plants are currently being used in hospitals and clinics (8, 9). Herbal drugs are usually a complex of hundreds of different chemical compounds, but only a few of them are responsible for the beneficial or adverse effects of these drugs (10).

Rumex acetosella L. is a perennial herb that widely ger-

Copyright © 2020, Zahedan Journal of Research in Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

minates in East Asia (11). This plant grows mainly in wet valleys and can reach up to one meter in height. Its leaves are green and fleshy and contain large amounts of chlorophyll. The chemical compounds isolated from it include flavonoids, phenolic compounds, and terpenoids (12). *Rumex acetosella* extract has diuretic, insecticidal, antiinflammatory, antioxidant, antimicrobial, and anticancer properties, and its aqueous extract is used for gastritis and gastric ulcers in traditional medicine (12-15). Various species of the *Rumex* genus have been studied, and it has been observed that different extracts of the aerial parts and root of this plant have antimicrobial effects on certain bacteria such as *Staphylococcus aureus*, *Pseudomonas aerug-inosa*, *Escherichia coli*, and *Bacillus cereus* (16, 17).

Pumpkin (*Cucurbita maxima* L.) is an herbaceous and annual plant that is used for treating earache, fever, and bronchitis, as well as diseases and infections of the urinary tract and prostate due to its antibacterial properties (18, 19). Anti-diabetic, antioxidant, anticancer, and antiinflammatory effects of *C. maxima* have also been demonstrated (20). In a study that examined the oil derived from *C. maxima* seeds, the oil was observed to contain essential fatty acids that could maintain the health of blood vessels, nerves, and tissues (21).

## 2. Objectives

Concerning the effective antibacterial properties of *R. acetosella* and *C. maxima*, lower costs, and fewer complications of herbal drugs, and recent advances in the production of plant-derived natural compounds in the treatment and prevention of bacterial infections, the present study was conducted to investigate the antimicrobial effects of these two plants on *A. baumannii* and compare them with conventional antibiotics in vitro.

## 3. Methods

An experimental study was carried out to investigate the antimicrobial effects of hydroalcoholic and aqueous extracts of *R. acetosella* and *C. maxima* on *A. baumannii*. To achieve this purpose, maceration was used for extraction; in addition, to measure the extracts' antibacterial effects, the lyophilized *A. baumannii* standard strain (ATCC no.: 747) was obtained from the Iranian Research Organization for Science and Technology (IROST). Then, the antibacterial effects of the hydroalcoholic and aqueous extracts of the plants on *A. baumannii* were investigated by broth microdilution and agar disk diffusion.

#### 3.1. Determination of Sample Size

We investigated the effects of two different extracts from two plants and five antibiotics on one bacterium, and each experiment was conducted in triplicate. Thus, the total number of experiments (sample size) was 60 (7).

### 3.2. Collection and Authentication of Studied Species

The plants were purchased from groceries and fruit stores and identified as the plants of interest by botanists according to the botanical and floral keys of Iran and available resources on plants. Also, they were adapted to the herbarium specimens available at the Medical Plants Research Center of Shahrekord University of Medical Sciences and Shahrekord Research Center for Agriculture and Natural Resources and assigned the herbarium vouchers of 184 (*R. acetosella*) and 1400 (*C. maxima*).

#### 3.3. Extraction Method

For the preparation of hydroalcoholic extracts, 300 g of each plant was ground in a mill, and extraction was conducted by the maceration method. For this purpose, 70% alcohol was added to 300 g of each plant to a final volume of one liter; the resulting solution was stored for four days and then filtered through a Whatman filter paper. The filtrate was then concentrated in a rotary evaporator at 40°C. After distillation, the concentrated filtrate was incubated at 37°C to obtain the extract, and a stock solution was prepared by dimethyl sulfoxide (DMSO). The aqueous extracts were prepared by boiling. For this purpose, 200 g of powder of each plant was mixed with 300 mL of distilled water, and the mixture was heated for 20 min while it was constantly being stirred. The resulting extract was filtered through a filter paper (7).

## 3.4. Preparation of Microbial Suspension

To prepare a microbial suspension, 24 hours before the experiments, a fresh (24-h) culture medium was prepared using culture media. Before starting the inoculation, some colonies were transferred from the surface of the Mueller-Hinton broth culture medium to the tube containing the physiological serum by using a sterile swab, and then the turbidity of microbial suspension was measured and compared with that of 0.5 McFarland standard (McFarland standard is a chemical solution with turbidity comparable to that of microbial suspension)(7).

#### 3.5. Determining Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined in a 96-well sterile plate by using the broth microdilution method and Mueller-Hinton broth culture medium according to specific instructions for the use of Merck (Germany) products. In this method, the first well was selected as a negative control, and the second well was selected as the positive control. After adding culture media, stock solution, and bacterial solution to the microplate wells and diluting them, the samples were incubated at 37°C for 24 h. The first well in which the turbidity was absent was considered for MIC determination (7).

#### 3.6. Determining Minimum Bactericidal Concentration (MBC)

To determine the minimum bactericidal concentration (MBC), all wells without turbidity were cultured separately on blood agar, and after 24 hours, the lowest concentration of extract at which the bacterium could not grow was considered for MBC determination (7).

## 3.7. Assessment of Antimicrobial Effects

To perform the disk diffusion test, after isolating the bacteria, some colonies of the bacteria were dissolved in sterile physiological serum by using an inoculating needle. After preparing the homogeneous solution and shaking it with a sterile swab, it was transferred to Mueller-Hinton agar. Then, the sterile blank paper disks (Padtan Teb Co., Iran) were left in the extracts at prepared concentrations for 24 h so that the extracts were completely absorbed into the disks. Then, the discs containing the extracts at different concentrations were incubated at 37°C for one hour to dry, and then placed on the plates and cultivated at appropriate distances. They were incubated at 37°C for one hour to examine the antibacterial properties of the extracts. The diameters of the inhibition zones were measured with a ruler. For this purpose, the zero of the ruler was placed next to a diameter of the inhibition zone, and the distance between zero and the end of the inhibition zone was measured and expressed in mm. Then, the results were compared with standard disks containing amikacin, imipenem, ceftazidime, gentamicin, and ciprofloxacin (Padtan Co., Iran) in tables provided by the Clinical and Laboratory Standards Institute (CLSI) (7).

#### 3.8. Statistical Analysis

The tests were repeated in triplicate, and the mean and standard deviation (SD) of the growth inhibition zone diameters in the cup-plate method and the MIC and MBC of the extracts were determined.

## 4. Results

This study was carried out using the broth microdilution method to determine the antibacterial effects of hydroalcoholic and aqueous extracts of *R. acetosella* and *C.*  *maxima*. The results obtained from the hydroalcoholic extracts indicated the higher levels of MIC and MBC of *R. acetosella* extract (Table 1).

The findings on the aqueous extract of the plants showed that the MIC and MBC of *C. maxima* extract were higher. The highest MICs against *A. baumannii* were obtained for the *C. maxima* aqueous extract and *R. acetosella* hydroalcoholic extract, and the lowest MICs were obtained for *C. maxima* hydroalcoholic extract and *R. acetosella* aqueous extract.

The highest MBCs were obtained for *C. maxima* aqueous extract and *R. acetosella* hydroalcoholic extract, and the lowest MBCs were obtained for *C. maxima* hydroalcoholic extract and *R. acetosella* aqueous extract. The MICs and MBCs of the *C. maxima* hydroalcoholic extract were lower than those of its aqueous extract (Table 1).

In the disk diffusion method, the diameters of the inhibition zone in different groups are shown in Tables 2 and 3. The results showed that the *R. acetosella* hydroalcoholic extract exhibited the strongest antibacterial effect with a mean inhibition zone diameter of  $24.83 \pm 0.29$  mm and *C. maxima* aqueous extract at  $512 \ \mu g/mL$  concentration exhibited the lowest antibacterial effect with a mean inhibition zone diameter of 8 mm. There was a direct correlation between the inhibition zone and antibacterial activity of the extracts. No inhibition zone was observed around the blank disk and ethanol disk as negative controls.

## 5. Discussion

Acinetobacter baumannii is highly resistant to antimicrobial agents, which can be inherited or acquired through genetic resistance factors. Resistance to antimicrobial agents among clinical isolates may make it difficult to treat infections and may negatively affect clinical outcomes and treatment costs (22). Nowadays, due to changes in the pattern of resistance of bacteria and their resistance to common antibiotics, there is a tendency to replace them with new antibiotics. Meanwhile, the products of plant origin with antimicrobial activity have recently attracted special attention (7). In this study, the hydroalcoholic extracts of C. maxima and R. acetosella had the highest inhibitory effects on A. baumannii, which prevented the growth of this bacterium at a concentration of 64  $\mu$ g/mL. The study of Qian showed that C. maxima polysaccharides had a very strong antibacterial effect against Bacillus subtilis, S. aureus, and E. coli at a concentration of 100 mg/mL (23).

In addition, the study by Ravishankar et al. showed that the ethanolic extract of *C. maxima* seed, due to active biological compounds such as carbohydrates, steroids, proteins, and amino acids, exhibited antibacterial activity

Plant	Hydroalcol	nolic Extract	Aqueous Extract		
Tant	MIC, $\mu \mathbf{g}/\mathbf{mL}$	MBC, $\mu \mathbf{g}/\mathbf{mL}$	MIC, $\mu \mathbf{g}/\mathbf{mL}$	MBC, $\mu \mathbf{g}/\mathbf{mL}$	
Rumexacetosella L.	128	256	64	128	
Cucurbita maxima L.	64	128	128	256	

Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

Table 2. Mean of A. baumannii Growth Inhibition Zone Diameters at Different Concentrations of Hydroalcoholic Extracts of R. acetosella and C. maxima with Disk Diffusion Assay (mm)<sup>a, b</sup>

Extract	Different Concentrations, $\mu g/mL$						
	8	16	32	64	128	256	512
Rumexacetosella L.	R	R	R	R	R	$21.5\pm0.5$	$24.83\pm0.29$
Cucurbita maxima L.	R	R	$1.67\pm0.58$	$4.17\pm0.29$	$5.5\pm0.5$	$7.67\pm0.58$	$10.33\pm0.58$

Abbreviation: R, resistant.

<sup>a</sup>Values are expressed as mean  $\pm$  SD.

 $^{b}$ Gentamicin: 14.5  $\pm$  0.5 mm, imipenem: 15.67  $\pm$  0.58 mm, amikacin: 16.83  $\pm$  0.29 mm, ceftazidime: 17.83  $\pm$  0.29 mm, ciprofloxacin: 21  $\pm$  0.5 mm.

Table 3. Mean of *A. baumannii* Growth Inhibition Zone Diameters at Different Concentrations of Aqueous Extracts of *R. acetosella* and *C. maxima* with Disk Diffusion Assay (mm)<sup>a, b</sup>

Extract	Different Concentrations, $\mu$ g/mL							
	8	16	32	64	128	256	512	
Rumexacetosella L.	R	R	R	R	$14.5\pm0.5$	$19.33 \pm 0.58$	$21.83 \pm 0.29$	
Cucurbita maxima L.	R	R	R	$2.83\pm0.29$	$4.83\pm0.29$	7	8	

Abbreviation: R, resistant.

<sup>a</sup>Values are expressed as mean  $\pm$  SD.

 $^{b}$ Gentamicin: 14.5  $\pm$  0.5 mm, imipenem: 15.67  $\pm$  0.58 mm, amikacin: 16.83  $\pm$  0.29 mm, ceftazidime: 17.83  $\pm$  0.29 mm, ciprofloxacin: 21  $\pm$  0.5 mm.

against S. aureus, Staphylococcus warneri, B. subtilis, P. aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, and E. coli (21). However, in the study by Elzaawely et al. (17), the antioxidant and antibacterial activities of aerial parts of a species from the *Rumex* genus was studied, showing that the ethyl acetate extract of aerial parts of the plant had the most potent antibacterial effect among the ethanol, hexane, chloroform, and aqueous extracts against Bacillus and E. coli. It has been reported that, in general, phenolic compounds in plant extracts act as active compounds against Gramnegative Acinetobacter bacteria (24). Many of the isolated compounds from plants whose activity has been demonstrated are secondary compounds that have been isolated by using certain solvents such as methanol, ethanol, water, and acetone by a variety of techniques. Antimicrobial agents are certain polyphenols, such as simple phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins. Besides, terpenoids, essential essences, alkaloids, lectins, polypeptides, and other compounds have also been reported. The action mechanism of these compounds varies depending on their type. These mechanisms include the enzymatic activity inhibition, the reaction of herbal extract active compounds with extracellular proteins, and the solution of microbial cell or bacterial cell wall (25). Some extracts can also interact with the microorganisms' DNA, which may create ionic channels in the microbial membrane, or may compete with microbial proteins to bind to host polysaccharide receptors (26). As known, *R. acetosella* and *C. maxima* are among the plants that have phenolic compounds.

In the present study, the inhibitory and bactericidal effects of *R. acetosella* aqueous extract were stronger than those of its hydroalcoholic extract, while in the study by Jimoh et al. (27) on the antibacterial effects and phenolic content of methanolic, aqueous, and acetonic extracts of a species from the *Rumex* genus, the phenolic content and antibacterial effects of the methanolic and acetonic extracts were stronger than those of the aqueous extract.

Therefore, essences and herbal extracts can be used in pharmacology, microbiology, phytopathology, and food preservation. Nowadays, due to chemical material misuse and acquisition of antibiotic resistance due to the improper use of antibiotics, it is proposed to replace these substances with natural compounds such as herbal extracts and essences, including the plants used in this study, to control and prevent diseases.

## 5.1. Conclusions

This study, for the first time, showed the moderateto-strong antibacterial effect of hydroalcoholic and aqueous extracts of *R. acetosella* and *C. maxima* altering in a concentration-dependent manner. The aqueous extracts had the strongest effect and can be, therefore, used as antimicrobial agents, as well as complementary and alternative therapies. However, laboratory and clinical studies are necessary to investigate the inhibitory effect of these extracts against *A. baumannii*.

## Acknowledgments

Hereby, we gratefully thank the Deputy of Research and Technology of Shahrekord University of Medical Sciences for funding this work and the Medical Plants Research Center of this university for cooperating with it. This study was derived from a research project approved at the Students Research Committee (approval no.: 2140) in this deputy.

#### Footnotes

**Authors' Contribution:** SA developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and is a guarantor. NB and NS contributed to the development of the protocol, abstracted data, and prepared the manuscript.

**Conflict of Interests:** All authors declare that they do not have any conflict of interest associated with this study to disclose.

**Ethical Approval:** Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, and redundancy) have been completely observed by the authors. This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (code: IR.SKUMS.REC.1395.96).

**Funding/Support:** This study was financially supported by the Deputy of Research and Technology of Shahrekord University of Medical Sciences.

#### References

- Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12(4):564–82. [PubMed: 10515903]. [PubMed Central: PMC88925].
- Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. Salmonella nomenclature. *J Clin Microbiol*. 2000;**38**(7):2465-7. doi: 10.1128/JCM.38.7.2465-2467.2000. [PubMed: 10878026]. [PubMed Central: PMC86943].

- Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol. 2007;5(12):939–51. doi: 10.1038/nrmicro1789. [PubMed: 18007677].
- Khosrishahi N, Sharifi M. Isolation of carbapenem resistant Acinetobacter baumannii(CRAB) strains from patients and equipments of Intensive care units (ICUs) at Qazvin between 2005-2006. *Iran J Med Microbiol*. 2007;1(3):33–8.
- Joly-Guillou ML. Clinical impact and pathogenicity of Acinetobacter. *Clin Microbiol Infect.* 2005;11(11):868–73. doi: 10.1111/j.1469-0691.2005.01227.x. [PubMed: 16216100].
- Amini Rad S, Amani J, Imani Fooladi AA, Saeedi P, Moosazadeh Moghadam M. Detection of Acinetobacter baumannii by PCR-ELISA method. J Shahrekord Univ of Med Sci. 2017;19(2):6–16.
- Bagheri N, Safaei N, Aleebrahim-Dehkordy E, Khaledi M, Madmoli M, Ansaripour S. In vitro antibacterial activity of Bunium persicum and Rheum ribes on Acinetobacter baumanii. *Int J Ayurvedic Med.* 2019;**10**(1):47–51.
- Hamoonnavard S, Bahrami AM, Razmjo M, Asadi-Samani M, Hatami-Lak M. Evaluation of Nerium oleander aqueous extract effect on Staphylococcus aureus and Staphylococcus epidermis. J Shahrekord Univ Med Sci. 2013;15(1):46–56.
- Rafieian-Kopaei M, Aleebrahim-Dehkordy E, Ansaripour S, Saberianpour S. Effects of substances on plants' active compounds on changes in the hormone levels of the pituitary-thyroid axis in hyperthyroidism and hypothyroidism. *Pharmacogn Rev.* 2018;12(23):1. doi: 10.4103/phrev.phrev\_48\_17.
- Drasar P, Moravcova J. Recent advances in analysis of Chinese medical plants and traditional medicines. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2004;812(1-2):3-21. doi: 10.1016/j.jchromb.2004.09.037. [PubMed: 15556485].
- Bae JY, Lee YS, Han SY, Jeong EJ, Lee MK, Kong JY, et al. A comparison between water and ethanol extracts of rumex acetosa for protective effects on gastric ulcers in mice. *Biomol Ther (Seoul)*. 2012;**20**(4):425–30. doi: 10.4062/biomolther.2012.20.4.425. [PubMed: 24009831]. [PubMed Central: PMC3762272].
- 12. Baig H, Ahmed D, Zara S, Aujla MI, Asghar MN. In vitro evaluation of antioxidant properties of different solvent extracts of Rumex acetosella leaves. *Orient J Chem*. 2011;**27**(4):1509.
- Lee NJ, Choi JH, Koo BS, Ryu SY, Han YH, Lee SI, et al. Antimutagenicity and cytotoxicity of the constituents from the aerial parts of Rumex acetosa. *Biol Pharm Bull*. 2005;**28**(11):2158–61. doi: 10.1248/bpb.28.2158. [PubMed: 16272711].
- Gescher K, Hensel A, Hafezi W, Derksen A, Kuhn J. Oligomeric proanthocyanidins from Rumex acetosa L. inhibit the attachment of herpes simplex virus type-1. *Antiviral Res.* 2011;89(1):9-18. doi: 10.1016/j.antiviral.2010.10.007. [PubMed: 21070811].
- Wegiera M, Kosikowska U, Malm A, Smolarz H. Antimicrobial activity of the extracts from fruits of Rumex L. species. *Open Life Sciences*. 2011;6(6):1036–43. doi: 10.2478/s11535-011-0066-0.
- Mostafa HAM, Elbakry AA, Eman AA. Evaluation of antibacterial and antioxidant activities of different plant parts of Rumex vesicarius L.(Polygonaceae). Int J Pharm Pharm Sci. 2011;3(2):109–18.
- Elzaawely AA, Xuan TD, Tawata S. Antioxidant and antibacterial activities of Rumex japonicus HOUTT. Aerial parts. *Biol Pharm Bull*. 2005;**28**(12):2225–30. doi: 10.1248/bpb.28.2225. [PubMed: 16327154].
- Caili F, Huan S, Quanhong L. A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods Hum Nutr.* 2006;61(2):73–80. doi: 10.1007/s11130-006-0016-6. [PubMed: 16758316].
- Mayor L, Moreira R, Sereno AM. Shrinkage, density, porosity and shape changes during dehydration of pumpkin (Cucurbita pepo L.) fruits. J Food Eng. 2011;103(1):29–37. doi: 10.1016/j.jfoodeng.2010.08.031.
- Yadav M, Jain S, Tomar R, Prasad GB, Yadav H. Medicinal and biological potential of pumpkin: an updated review. *Nutr Res Rev.* 2010;23(2):184– 90. doi: 10.1017/S0954422410000107. [PubMed: 21110905].

- 21. Ravishankar K, Kiranmayi GVN, Appa Reddy GV, Sowjanya VVL, Baba Sainadh V, Lakshmi VG, et al. Preliminary phytochemical screening and In-vitro antibacterial activity of Cucurbita maxima seed extract. *Int J Res Pharm Biomed Sci.* 2012;**2**(1):86–91.
- Landman D, Quale JM, Mayorga D, Adedeji A, Vangala K, Ravishankar J, et al. Citywide clonal outbreak of multiresistant Acinetobacter baumannii and Pseudomonas aeruginosa in Brooklyn, NY: the preantibiotic era has returned. Arch Intern Med. 2002;162(13):1515–20. doi: 10.1001/archinte.162.13.1515. [PubMed: 12090889].
- Qian ZG. Cellulase-assisted extraction of polysaccharides from Cucurbita moschata and their antibacterial activity. *Carbohydr Polym.* 2014;101:432-4. doi: 10.1016/j.carbpol.2013.09.071. [PubMed: 24299793].
- 24. Miyasaki Y, Rabenstein JD, Rhea J, Crouch ML, Mocek UM, Kittell PE,

et al. Isolation and characterization of antimicrobial compounds in plant extracts against multidrug-resistant Acinetobacter baumannii. *PLoS One.* 2013;8(4). e61594. doi: 10.1371/journal.pone.0061594. [PubMed: 23630600]. [PubMed Central: PMC3632535].

- Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. *Int J Food Microbiol*. 2004;94(3):223-53. doi: 10.1016/j.ijfoodmicro.2004.03.022. [PubMed: 15246235].
- Hili P, Evans CS, Veness RG. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. *Lett Appl Microbiol.* 1997;24(4):269–75. doi: 10.1046/j.1472-765x.1997.00073.x. [PubMed: 9134774].
- Jimoh FO, Adedapo AA, Aliero AA, Afolayan AJ. Polyphenolic Contents and Biological Activities ofRumex ecklonianus. *Pharm Biol.* 2008;46(5):333–40. doi: 10.1080/13880200801887765.