Evaluation of Aqueous and Ethanolic Extraction for Coriander Seeds, Leaves and Stems and Studying their Antibacterial Activity

Intisar M. Oudah, M.Sc.*
Yasmine H. Ali, M.Sc.**

المستخلص

الهدف: يهدف البحث إلى تقييم نوعين من الاستخلاص المائي والكحولي لنبات الكزبرة باستعمال البذور، السيقان والأوراق ودراسة الفعالية المضادة للبكتريا لهذه المستخلصات باختبار تسعة أنواع من البكتريا.

المنهجيّة: تمّ اختيار نبات الكزبرة لإجراء هذه الدراسة حيث جمعت البذور، الأوراق والسيقان من الأسواق المحلية في بغداد وجففت في الظل لمدة ١٠ أيام ثم طحنت إلى مسحوق ناعم وتمّ تحضير مستخلص مائي حار لمُدّة ساعة عند درجة حرارة (٥٠°c) وآخر بارد لمُدّة ٢٤ ساعة عند درجة حرارة (٤°c) لبذور، سيقان وأوراق الكزبرة،كذلك تمّ تحضير مستخلص مائي بارد لبذور الكزبرة لمُدّة ٤٨ و ٧٢ ساعة،كما تمّ إجراء استخلاص كحولي لمُدّة ٤٨ ساعة عند درجة حرارة (٤°c) لبذور، سيقان وأوراق النبات وتمّ دراسة فعالية هذه المستخلصات كمادة مضادة للبكتريا على تسعة أنواع من البكتيريا المعزولة.

النتائج: أظهرت النتائج أنّ المستخلص المائي الحار لمُدّة ساعة لكل أجزاء نبات الكزبرة لم يشير إلى أي تأثير، بينما المستخلص المائي البارد لمُدّة ٢٤ ساعة لبذور الكزبرة أعطى تأثيراً مضاداً لبعض أنواع البكتريا المستعملة وليس جميعها ولم يُلاحَظ ذلك بالنسبة للأوراق والسيقان. أعطتمُدّة الاستخلاص المائي البارد لمُدّة ٤٨ ساعة لبذور الكزبرة تأثيراً مضاداً لكل أنواع البكتريا المستعملة، أمّا عند مُدّة ٧٢ ساعة، فلم يظهر أي تأثير مثبط للبكتريا. فيما يتعلق بالمستخلص الكحولي للبذور، السيقان والأوراق لمُدّة ٤٨ ساعةK أظهرت النتائج أنّ هذا المستخلص له تأثيرٌ واضحٌ كمضاد للبكتريا ولوحظ أعلى قيمة لمنطقة التثبيط عند نوعي بكتريا *Proteus mirabilis* و *Klebsiella pneumoniae*

التوصيات: توصي الدراسةبضرورة اعتماد مستخلص بذور الكزبرة كعلاجٍ بديلٍ مُضادٍ لأنواع من البكتريا التي تظهر مقاومة للعلاج التقليدي. تعدّ هذه الدراسة خطوة أوليّة لدراسات لاحقة. لذا، من الضروري استعمال طرقاستخلاصٍ أخرى ممكن أن تستخلص المادّة الفعّالة بنسبة أكثر،فضلاً عن الاستعانة بمُذيبات عُضويّة مُختلفة وتطبيق المستخلصات ضدّ أنواع بكتيريا أخرى ذات مقاومة عالية للعلاج التقليدي.

Abstract:

Objective: To evaluate two kinds of extraction (aqueous and ethanolic) for coriander using seeds, leaves and stems and studying their antibacterial activity against nine different microorganisms.

Methodology: Coriander was selected to carry out this study. Seeds, leaves and stems were collected from local markets in Baghdad then dried in shade for at least 10 days and grinded to fine powder. Aqueous hot extracts for 1hr. at (50°c) and cold extracts for 24 hrs at (4°c) were performed by using seeds, leaves and stems then studied antibacterial effect against nine different microorganisms by using well diffusion technique. Cold aqueous extracts of coriander seeds for 48 hrs. and 72 hrs and ethanolic extraction for 48hrs of seed, leaves and stems also performed.

Results: This study showed that hot aqueous extracts for 1hr. to all parts of coriander indicated no antibacterial activity, while cold aqueous extract for 24hrs of coriander seeds had inhibitory effect for some tested bacteria, but leaves and stems had not. Cold aqueous extract of seeds for 48hrs showed antibacterial activity for all tested bacteria but in 72hrs there was no inhibitory effect. On the other hand, ethanolic extracts of seeds, leaves and stems for 48hrs had antibacterial activity and the highest values for inhibition zone shown in *Klebsiella pneumoniae* and *Proteus mirabilis.*

Recommendations: The study recommends using coriander seeds extract as alternative medical therapy for microorganisms which may resist conventional treatment. This study is a first step for further studies. It is necessary to use various extraction methods to give active materials with high percentage, although different organic solvents to be used with coriander plant to obtain extracts used for testing different kinds of microorganisms which have highly resistance to conventional treatment.

Introduction

Various medical plants have been used for years in daily life to treat disease all over the world. Herbs and spices are the most important part of human diet. Besides boosting flavor, herbs and spices are also known for their preservative and medicinal values [1, 2] which form one of the oldest sciences. It is only in recent years that modern science has started paying attention to the properties of spices [3]. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades [4]. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus [5-8].

Keywords: Coriander Extracts, Antibacterial Activity

* **Assistant Instructor, Basic Medical Sciences Department, College of Nursing, University of Baghdad**
** **Assistant Instructor, Basic Medical Sciences Department, College of Nursing, University of Baghdad**

The antibacterial activities of different medicinal plants have been known for a long time and a number of researches on the antibacterial effect of spices, essential oils and their derivatives have been reported. For example, the crude methanolic extracts of neem plant shown to have strong antibacterial activity [9]. Water extract of garlic and clove possesses antimicrobial activity. Some bacteria showing resistance to certain antibiotics were sensitive to extracts of both garlic and clove [10]. Aqueous extracts of sage and thyme had action against most of the tested microorganisms, while the ethanolic extract of parsley was more affected on *E. coli* [11].

One of the widely used medicinal plants is Coriander (Coriandrum sativum L). It is considered both as an herb and a spice since both of its leaves and seeds are used as a seasoning condiment. The name coriander is derived from the Greek word koris which means bug and commonly famous as Cilantro. Coriander seeds have a health-supporting reputation that is high on the list of the healing spices. It has traditionally been referred to as an anti-diabetic [12], anti-inflammatory and recently been studied for its cholesterol-lowering effects [13]. In addition, its leave has memory-enhancing effect, therefore appears to be a promising candidate for improving memory [14]. Antioxidant activity has been studied by using ethanolic extract of coriander fruits [15].

The present study was planned to assess the antibacterial activity of coriander by using aqueous and ethanolic extracts against nine pathogenic microorganisms.

Methodology

Plant Materials

Seeds, leaves and stems of coriander were collected from local markets in Baghdad. Leaves and stems were washed with distilled water, dried in shade for at least 10 days, and then all parts of the plant were grinded to fine powder and stored in airtight container at room temperature in the dark until used [16].

Extraction of plant materials

The powdered samples were prepared to extract using 10g dissolved in 100 ml of distilled water in 250 ml conical flask and start to heat at 50°c for 1hr. Another extraction were performed using 10 g of each samples in 100 ml distilled water in sterile wide-mouthed screw-capped bottles of 250 ml volume then allowed to soak for 24 hrs at 4°c. Ethanolic extraction also studied by using 10 g of powdered samples in 100 ml absolute ethanol (99%) and soaked for 48 hrs at 4°c. all the samples were filtered using what man filter paper No.2 on a buchner funnel.

Microbiological tests of plant extracts

Nine different microorganisms were isolated from urine, blood, stool and cerebra-spinal fluid of different patients taken from the clinical laboratory of medical city hospital in Baghdad. Media: Mueller-Hinton agar (MHA) (Merck) was used as base medium for screening of antibacterial activity and Mueller-Hinton broth (MHB)(Merck) for preparation of Inoculum[17]. McFarland tube number 0.5 was prepared by mixing 9.95ml 1% Sulphuric acid in MHB and 0.05ml 1% Barium chloride in distilled water in order to estimate bacterial density [2].The tube was sealed and used for comparison of bacterial suspension with standard whenever required. To prepare inoculum, four to five colonies from pure growth of each test organism were transferred to 5ml of MHB. The broth was incubated at 35-37°c for 18-24 hrs. The turbidity of the culture was compared with 0.5 McFarland Nephelometer standards to get 150 x 10^6 CFU/ml. The standardized inoculum suspension was inoculated within 15-20 mins. The antibacterial activity was performed by well diffusion technique. The MHA plates were seeded with 0.1ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. A standard cork borer of 8mm diameter was used to cut uniform wells on the surface of the MHA and 100µl of plant extracts (100%) of coriander was introduced in the well, The inoculated plates were incubated at 35-37°c for 24 hrs and zone of inhibition was measured in triplicate. The antibacterial activity of the plant extracts were recorded as the mean diameter of the resulting inhibition zones of growth measured in millimeters.

Results

In this study as indicated in Table (1) the hot aqueous extract for seeds, leaves and stems showed no effect against the tested bacteria while, the cold aqueous extract for 24 hrs. of coriander seeds had inhibitory effect against *Burkhella capacia, Escherichi coli, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Proteus mirabilis*. The highest value of inhibition zone found with *Proteus mirabilis*,as seen in Fig1. The cold aqueous extract of leaves and stems had no effect. From this result, another cold extracts of coriander seeds for 48hrs and 72hrs were undertaken.

Table 1. Effect of aqueous extracts of coriander on different microorganisms

	Diameter of inhibition zone(mm) – mean zone of inhibition ± SE								
Extracts	**GM**	**BC**	**EC**	**ST**	**SP**	**KP**	**ECL**	**PM**	**HS**
S/aqueous/ hot	-	-	-	-	-	-	-	-	-
L/aqueous/ hot	-	-	-	-	-	-	-	-	-
T/aqueous/ hot	-	-	-	-	-	-	-	-	-
S/aqueous/ cold(24hr.)	-	16.1 ±0.1	13.27±0.25	-	13.33±0.15	18.03±0.15	-	20.23±0.15	-
L/aqueous/ cold(24hr.)	-	-	-	-	-	-	-	-	-
T/aqueous/ cold(24hr.)	-	-	-	-	-	-	-	-	-

BC=*Burkhella capacia*; EC= *Escherichia coli*; ECL=*Enterobacter cloacae*; GM= *Gamella morbillorum*; HS= *α-Haemolytic streptococci;* KP= *Klebsiella pneumonia*; L=leaves; PM= *Proteus mirabilis*; S= seeds;S P=*Streptococcus pneumonia*; ST=*Salmonella typhi*; T= stems (Values=mean of 3 readings); (-)= no activity

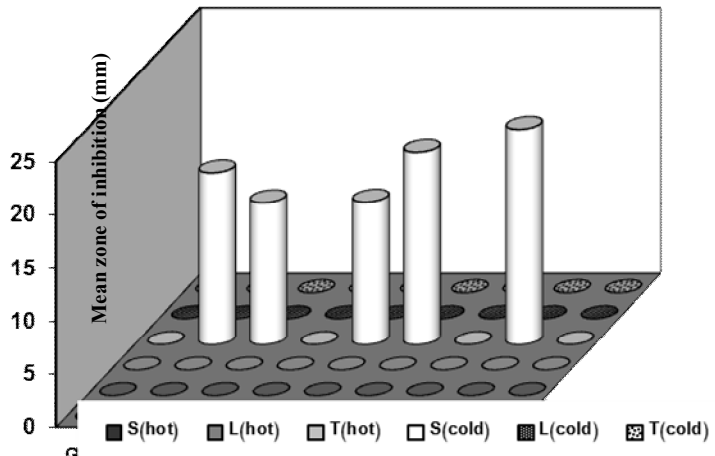

Figure 1: The inhibition levels on the microorganisms by using aqueous extracts
BC=*Burkhella capacia*; **EC**= *Escherichia col*; **ECL**=*Enterobacter cloacae*; **GM=** *Gamella morbillorum*; **HS**= *α-Haemolytic streptococci***; KP**= *Klebsiella pneumonia*; **L**=leaves**; PM**= *Proteus mirabilis*; **SP**=*Streptococcus pneumonia*; **S**= seeds**; ST**=*Salmonella typhi*; **T**= stems

The result shown in Table (2) Seeds extract for 48hrs had inhibitory effect against all microorganisms but 72 hrs. there was no effect. The highest value of inhibition zone found with *Escherichia coli,* as shown in Fig. 2.

Table 2. Effect of cold aqueous extracts of coriander seeds on different microorganisms

	Diameter of inhibition zone(mm) – mean zone of inhibition ± SE								
Extracts	**GM**	**BC**	**EC**	**ST**	**SP**	**KP**	**ECL**	**PM**	**HS**
S/aqueous/ cold(48hr.)	16.36 ±0.15	16.16 ±0.15	22.3 ±0.2	16.1 ±0.03	20.1 ±0.03	16.3 ±0.15	18.2 ±0.1	18.1 ±0.15	18.1± 0.1
S/aqueous/ cold(72hr.)	-	-	-	-	-	-	-	-	-

BC=*Burkhella capacia*; EC= *Escherichi coli*; ECL=*Enterobacter cloacae*; GM= *Gamella morbillorum;* HS= *α-Haemolytic streptococci;* KP= *Klebsiella pneumonia*; PM= *Proteus mirabilis*; (-)= no activity, S= seeds (Values=mean of 3 readings); SP=*Streptococcus pneumonia*; ST=*Salmonella typhi*

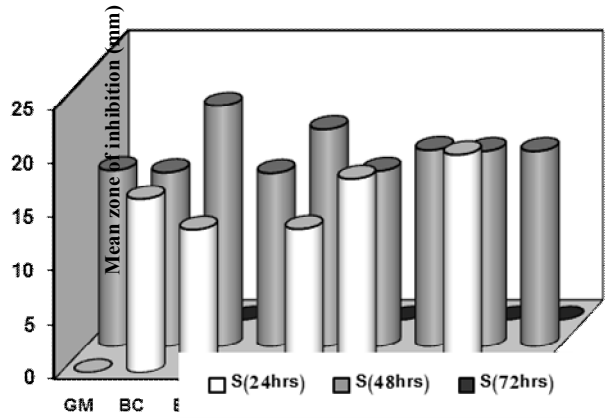

Figure 2. The inhibition levels on the microorganisms by using seed aqueous extracts

BC=*Burkhella capacia*; **EC**= *Escherichia coli*; **ECL**=*Enterobacter cloacae*; **GM=** *Gamella morbillorum;* **HS**= *α-Haemolytic streptococci;* **KP**= *Klebsiella pneumonia*; **PM**= *Proteus mirabilis*; **S**= seeds**; SP**=*Streptococcus pneumonia*; **ST**=*Salmonella typhi*

Table (3) showed the antibacterial activity with ethanolic extract of coriander seeds, leaves and stems for 48 hrs. The seeds extract inhibited all types of tested bacteria, the highest values for inhibition zone showed in *Klebsiella pneumoniae* and *Proteus mirabilis.* In leaves and stems extracts, the inhibition effect found in all testes bacteria except *Salmonella typhi and Klebsiella pneumoniae*. The highest value for inhibition zone of leaves and stems extracts belongs to *Proteus mirabilis,*as seen in Fig. 3.The control ethanol indicated no inhibition zone. Seeds extracts of coriander could be the best in medical treatment against these types of microorganisms.

Table 3. Effect of cold ethanolic extracts (48hrs.) of coriander on different microorganisms

	Diameter of inhibition zone(mm) – mean zone of inhibition ± SE								
Extracts	**GM**	**BC**	**EC**	**ST**	**SP**	**KP**	**ECL**	**PM**	**HS**
S/ethanol	20.2 ±0.13	16.3 ±0.10	20.1 ±0.25	20.32 ±0.11	18.16 ±0.13	30.2 ±0.05	18.15 ±0.12	30.33 ±0.20	18.22 ±0.14
L/ethanol	20.1 ±0.15	13.1 ±0.12	22.1 ±0.05	-	16.3 ±0.15	-	18.25 ±0.21	30.20 ±0.10	22.2 ±0.05
T/ethanol	16.3 ± 0,15	16.15 ±0.25	20.2 ±0.11	-	18.12 ±0.15	-	16.3 ±0.22	20.33 ±0.13	20.17 ±0.05
C/ethanol	-	-	-	-	-	-	-	-	-

BC=*Burkhella capacia*; C=control (Values=mean of 3 readings); EC= *Escherichi coli*; ECL=*Enterobacter cloacae*; GM= *Gamella morbillorum*; HS= *α-Haemolytic streptococci*; KP= *Klebsiella pneumonia*; L=leaves; PM= *Proteus mirabilis*; S= seeds; SP=*Streptococcus pneumonia*; ST=*Salmonella typhi*, T= stems; (-)= no activity

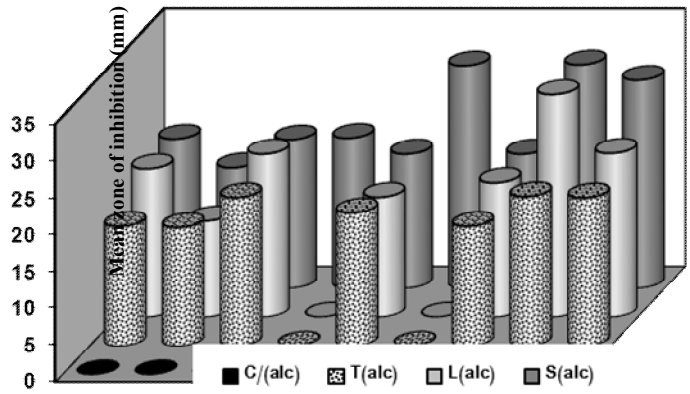

Figure 3. The inhibition levels on the microorganisms by using ethanolic extracts

BC=*Burkhella capacia*; **C**=control**; EC**= *Escherichia coli*; **ECL**=*Enterobacter cloacae*; **GM**= *Gamella morbillorum*; **HS**= *α-Haemolytic streptococci;* **KP**= *Klebsiella pneumonia*; **L**=leaves; **PM**= *Proteus mirabilis*; **S**= seeds; **SP**=*Streptococcus pneumonia*; **ST**=*Salmonella typhi*; **T**= stems

Discussion

Two studies have been performed [17, 18] which showed that the extracts of the plant Coriandrum sativum have no antibacterial activity. In contrary, some workers have found that seeds oil of coriander have a strong antibacterial activity [19].

The results in Table (1) have showed that the hot aqueous extract for seeds, leaves and stems have no effect against the tested bacteria, may be due to loss of some active compounds during extraction process of the sample. This finding is consistent with the study of Sampathkumar *et.al* [20]. The cold aqueous extract for 24 hrs. of coriander seeds had an inhibitory effect. While, the same extract and conditions used for leaves and stems, revealed no effect, this may be discussed as that the active materials in the plant may be present as a sufficient quantities in the seeds more than leaves and stems to show the inhibitory effect with the dose levels employed [16], the active materials could be concentrate in one part of plant, but not in other parts or may be excited with low quantity. So, the use of other types of extract for leave and stems could be given good results. Depending on the results which mentioned above, another conditions used as showed in Table (2), seeds extract for 48 hrs. had an inhibitory effect against all microorganisms. While, in extract for 72 hrs. there were no effects. In plants extraction, the period of soaking is an important factor to extract active materials, but sometimes long time is not necessary, because several unimportant substances may be released which give contrary results. So, the extract of 72 hrs. in this study may lead to extract more substances which resisted the inhibition of microorganisms. Therefore, the time 48 hrs. is suitable for future extracts by these methods. The ethanolic extract of seeds inhibited all types of tested bacteria. It could be the best extract comparing with the others, this occurs from the results in Table (3).

The extract of active constituents which have an inhibitory effect could be increased due to the kind of solvents used for extraction and these findings are consistent with another study [16].

These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. On the other hand, ethanol solution as a control is given no effect with these nine tested bacteria. This finding is congruent with the results of another study of [18].

The antibacterial effect of coriander appeared to be highly variable depending on plants conditions and species [19]. It is obvious that the present study has revealed the importance of medical plants to control resistant bacteria which are becoming a threat to human health. This scientific information can serve as an important platform for the development of inexpensive, safe and effective natural medicines.

Recommendations

The present study has been suggested to use coriander extracts as an alternative medical therapy for microorganisms which may resist conventional treatment. The future studies will be considered to evaluate some organic solvents extracts of coriander seeds. Also, different methods of extraction could be used for testing various kinds of microorganisms which have highly resistance against the conventional therapy. This will offer a great help in facing the emergence spread of bacteria.

References

1. DeSouza EL, Stamford TLM, Lima EO, Tarajano VN and Filho JBM. Antimicrobial effectiveness of spices: An approach for use in food conservation system. *Braz. Arch. Biol. Technol.*2005; 48(4): 1516-8913.

2. Saeed S, Tariq P. Effects of some seasonal vegetables and fruits on the growth of bacteria. *Pakistan Journal of Biological Sciences* 2006;9(8): 1547-1551.

3. Chaudhry NMA, Tariq P. Bactericidal activity of black peeper, bay leaf, aniseed and coriander against oral isolates. *Pak. J. Pharm. Sci.* 2006;19: 214-218.

4. Ansari MA, Ahmed SP, Haider S, Ansari NL. Nigella Sativa: A non-conventional herbal option for the management of seasonal allergic rhinitis. *Pakistan Journal of Pharmacology* 2006;23(2): 31-35.

5. Horvath G, Kocsis B, Botz L, Nemeth J, Szabo L. Antibacterial activity of thymus phenols by direct bioautography. *Acta Biol Szegediensis* 2002; 46: 145-6.

6. Jantan I, Yassin M, Chin C, Chen L, Sim N. Antifungal activity of the essential oils of nine zingiberaceae species. *Pharmaceut Biol*, 2003;41: 392-7.

7. Khan M, Kihara M, Omoloso A. Antimicrobial activity of the alkaloidal constituents of the root bark of eupomatia laurina. *Pharmaceut Biol.* 2003;41: 277-80.

8. Perez RM. Antiviral Activity of Compounds Isolated from Plants. *Pharmaceut Biol*. 2003;41: 107-57.

9. Okemo P, Mwatha W, Chhabra S, and Fabry W. The kill kinetics of azadirachta Indica A. juss. (meliaceae) extracts on staphylococcus aureus, E. coli, psedomonas aeruginosa and candida albicans. *Afr J of Sci Tech*. 2001;2: 113-8.

10. Arora D, Kaur J. Antimicrobial activity of spices. *Intern J Antimicrob Agents*, 1999;12: 257-62.

11. El-Astal ZY, Ashour AERA, Kerrit AAM. Antimicrobial activity of some medicinal plant extracts in Palestine. *Pak J Med Sci*. 2005; 21(2): 187-93.

12. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant coriandrum sativum (coriander). *Br. J. Nutr.* 1999;81(3): 203-9.

13. Chithra V, Leelamma S. Hypolipidemic effect of coriander seeds (coriandrum sativum): mechanism of action. *Plant Foods Hum. Nutr.* 1999; 51(2): 167-72.

14. Vasudevan Mani, Milind Parle. Memory-enhancing activity of coriandrum sativum in rats. *Pharmacologyonline* 2009;2: 827-839.

15. Nickavar Bahman, Abolhasani Farideh Al-Sadat. Screening of antioxidant properties of seven mbelliferae fruits from Iran. *Pak. J. of Pharm. Sci.* 2009; 22(1): 30-35.

16. Anjana Sharma, Rani Verma, Padmini Ramteke. Antibacterial activity of some medicinal plants used by tribals against uti causing pathogens. *World Appl. Sci. J.* 2009; 7(3): 332- 339.

17. Sabahat Saeed, Perween Tariq. Antimicrobial activities of emblica officinalis and coriandrum sativum against gram positive bacteria and candida albicans. *Pak. J. Bot.* 2007;39(3): 913-917.

18. Ayfer ATEÞ DD, zlem Turgay ERDOÚRUL. Antimicrobial activities of various medicinal and commercial plant extracts. *Turk. J. of Biol.* 2003;27: 157-162.

19. Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. of Food Prot.* 2001; 64(7): 1019-1024.

20. Sampathkumar P, Dheeba B, Vidhyasagar V, Arulprakash T, Vinothkannan R. Potential antimicrobial activity of various extracts of B. monnieri (Linn). *Int. J. Pharmacol.* 2008; 4(3): 230-232.