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ORIGINAL ARTICLE

Determination Antimicrobial Activity from Ethanolic Extract of *Mentha piperita L.* (peppermint)

Amer Ali Hammadi [1] and **Hadeel Adnan** [2]

[1,2] Department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, Karbala, Iraq.

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Corresponding author:
Amer Ali Hammadi
Email: amer.a@uokerbala.edu.iq
Department of Clinical Laboratories
College of Applied Medical Sciences
University of Kerbala
Karbala
Iraq

ABSTRACT

Objectives: The current study aims to determine the inhibitory efficiency of alcoholic *Mentha piperita L.* extracts and investigate their effectiveness antimicrobial activities against different pathogenic bacteria.

Methods: One solvent used is, ethanol to obtain of cured extract of *M. piperita* extraction ,which tested the effectiveness on four type of bacteria are (*Klebsiella pneumonia* , *Escherichia coli*, *staphylococcus aureus* and *Streptococcus pneumonia*) to determine the most efficient concentration of solvent optimization, and then was determined minimum inhibitory concentration (MIC) of the extract more efficient.

Results: The extract showed activity in vitro against four species of pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumonia* , *Staphylococcus aureus* and *Streptococcus pneumonia*) compared with the antibiotic this appear in inhibition zone diameter for these four pathogenic bacteria (23,18, 9)mm, (12, 12, 10)mm,(17, 11)mm and (10, 8)mm in arrangement .All isolates were sensitive to ethanolic extracts of *M. piperita* and gram's negative bacteria more effectiveness than gram's positive.

Conclusion: The current report will help in the isolation of new products/drugs. The results of this work have shown that *M. piperita* extracts possess compounds with antimicrobial and antioxidant properties.

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INTRODUCTION

The substances from natural sources Biologically action had been always a great interest for scientists worked on infectious diseases [1]. The medicinal plants therapy value established the relationship among the chemically structure of the active pharmacodynamic and action phytocompounds they expand the reaction substance of the body [2]. Most medicin plants had complex chemical compounds from 2-3 to 10 substances or may be hundreds elucidate the multiple pharmacodynamic property of Mentha piperita L [3].

Mentha is a genus of plants in the family Lamiaceae (mint family) [4]. It is estimated that 13 to 18 species exist, and the exact distinction between species is still unclear [5]. Its composed primarily of menthol (29–48%), menthone (20–31%), menthofuran (6.8%) and menthyl acetate (3–10%). Other pharmacologically active ingredients include bitter substances, caffeic acid, flavonoids (12%), polymerized polyphenols (19%), carotenes, tocopherols, betaine, choline and tannins [6]. The main active ingredient in

Peppermint is *Menthol*, which is an organic compound that produces a cooling sensation when applied to the mouth or skin. It also acts as a mild anesthetic (which means a compound creating a reversible loss of sensation). Peppermint also contains vitamins A and C as well as a number of minerals [7].

M. piperita crude extract used to treatment several pathogenic cases involving several diseases like respiratory system infection and digestive system, migraines, nervous irritability, overload, neurasthenia, liver illnesses and depression and kidney infection [8]. *M. piperita* extraction used to treatment rheumatic problems , circulatory and had also antipyretic labor [9]. *M. piperita* was used also as adjuvant with digestive disorders and gastrointestinal infections like intestinal colic, enterocolitis, and meteorism. Respiratory infection like bronchitis, laryngitis, tracheobronchitis, and convulsive cough, pharyngitis, analgesic, mouth hygiene, nausea and nervous or mental fatigue [10].

The leaf extract (Methanolic) shown marked antibacterial action versus methicillin resistant *Staph. aureus* and *strept. pyogene* [11]. The developed plant sources might have antibacterial activity against many gram's negative pathogens [12].

MATERIALS AND METHODS

Plant Material

M. piperita getting from local market and dried then remove stem to get leaf as it can. Then placed in neat piles to dry out for a number of weeks before they may be transported and crushed by hand or by machine to get powder of *menthe* leaf [13].

Bacterial isolation

The bacterial genus collected from (AL-Hussainy hospital) and central health laboratory, four pathogenic genius *Staph. aureus*, *Strept. pneumonia*, *E. coli* and *K. pneumonia* isolated from patients. All bacterial identification by bacteriological and biochemical test.

Mueller-Hinton agar

Has been prepared by weighting 40 gm from media and solved in 140 L of Distal water based on method of company **(**Himedia company**).**

Ethanol extracted method

Mentha 50 mg of plant leaf powder has been mixed with 25ml of 70% ethanol and put in the shaker for overnight. Then filtration the suspension by filter paper and then put it in the Petri dish for evaporated the alcohol and become powder [14].

Well method procedure

Pour Muller Hinton Agar Medium in plates at depth 4mm. Spread a broth culture of an isolated bacterium in to an ager plates by cotton swab. After drying the plate at 37 ºC for 30 minutes, make a well in the plate (several well when used many concentration of a certain antibiotic) by using a sterile crock borer in appropriate diameter 10 mm under aseptic condition. Fill the well with a tested antibiotic. Incubation the plates at 37 ºC for 18-24 hr. Measure the inhibition zone around the well [14].

RESULTS

The current study used ethanol for the extraction of active components from the leaves of *Mentha* plant. Defferent dilutions had been used on four types of pathogenic bacteria *Staph. aureus, Strept. pneumonia, E.Coli* and *K. pneumoniae*. The antibacterial activity of the *Mentha* was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated. In first case *E.Coli* The results shown that the *Mentha* extracts possessed strong antibacterial activity in the (0.25mg/5 ml), (0.25mg/10ml) and(0.25mg/15ml) The inhibition zone for *E.Coli* in 21mm, 17mm and 9mm in diameter, through the LSD value there is significance differences between the first three dilution as shown in Table 1. We found that the *Mentha* ethanol extraction were successful in killing the bacteria in a dose dependent manner.

In Table 2 the result has been shown antibacterial activity against *K. pneumonia* in the first three dilution (0.25mg/5 ml), (0.25mg/10ml) and(0.25mg/15ml) The inhibition zone for *K. pneumonia* in 12mm, 12mm and 0mm in diameter, through the LSD value there is significance differences between five dilution in arrangement and by the shown result in both Table 1 and Table 2 there is effectiveness of *M. piperita* crude extract on pathogenic gram's negative bacteria.

In Table 3 the result showed effectiveness of *M. piperita* extraction on *Strept. pneumonia* pathogenic bacteria in two dilution (0.25mg/5 ml) (0.25mg/10ml) this appeared in diameter of inhibition zone for growth bacteria plate 17mm, 11mm reach near the inhibition value of antibiotic . Also there is significance differences on effect of crude extract of plant this appeared by LSD value in the first two dilution.

In Table 4 the result showed there is effectiveness of *M. piperita* crude extracted in the first three dilution (0.25mg/5 ml), (0.25mg/10ml) and (0.25mg/15ml) during inhibition zone 10mm, 8mm and 6mm in diameter the first dilution inhibition reach near inhibition zone of antibiotic. Also distinguish that through LSD value three first dilution by appear significance differences on the *Staph. aureus* pathogenic bacteria.

Table 1. **Inhibition zone(mm)of *Mentha* extract against *E. Coli*.**

Concentration of extract (mg/ml)	Alcoholic extract			Mean of concentration	$LSD_{0.05}$ conc.
	1	2	3		
Antibiotic control (tetracycline) 0.25mg/10ml	30 ± 0.0	28 ± 0.0	35 ± 0.0	31.00 A	**1.44**
0.25mg/5ml	27 ± 0.0	23 ± 0.0	21± 0.0	23.66 B	
0.25mg/10ml	19 ± 0.0	18 ± 0.0	17 ± 0.0	18.00 C	
0.25mg/15ml	11 ± 0.0	7 ± 0.0	9 ± 0.0	9.00 D	
0.25mg/20ml	9 ± 0.0	6 ± 0.0	6 ± 0.0	7.00 E	
0.25mg/25ml	1 ± 0.0	2 ± 0.0	1 ± 0.0	1.33 F	
0.25mg/30ml	1.5 ± 0.0	0.75 ± 0.0	1 ± 0.0	1.08 F	
0.25mg/35ml	0 ± 0.0	0 ± 0.0	1 ± 0.0	0.33 F	
0.25mg/40ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 F	
0.25mg/45ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 F	
Mean of extract Solvent	7.61 c	6.30 b	6.22 a	**$LSD_{0.05}$ Interference**	
$LSD_{0.05}$ Solvent	**0.41**			**0.83**	

* The numbers refer to mean ± Standard error.
* Various vertically capital letters indicate significant differences (P<0.05) between the concentrations.
*Various Horizontally small letters indicate significant differences(P<0.05) between Solvents.

Table 2**: Inhibition zone(mm)of *Mentha* extract against *K. pneumonia*.**

Concentration of extract (mg/ml)	Alcoholic extract			Mean of concentration	$LSD_{0.05}$ conc.
	1	2	3		
Antibiotic control (tetracycline) 0.25mg/10ml	24 ± 0.0	33 ± 0.0	31 ± 0.0	29.33 A	**1.86**
0.25mg/5ml	14 ± 0.0	12 ± 0.0	11 ± 0.0	12.33 B	
0.25mg/10ml	13 ± 0.0	12 ± 0.0	12 ± 0.0	12.33 C	
0.25mg/15ml	10 ± 0.0	11 ± 0.0	11 ± 0.0	10.66 D	
0.25mg/20ml	10 ± 0.0	6 ± 0.0	2 ± 0.0	6.00 D	
0.25mg/25ml	6.5 ± 0.0	4 ± 0.0	2 ± 0.0	3.50 E	
0.25mg/30ml	1 ± 0.0	1 ± 0.0	1 ± 0.0	1.00 EF	
0.25mg/35ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
0.25mg/40ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
0.25mg/45ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
Mean of extract Solvent	6.05 c	5.11 b	4.33 a	**$LSD_{0.05}$ Interference**	
$LSD_{0.05}$ Solvent	**0.53**			**1.07**	

* The numbers refer to mean ± Standard error.
* Various vertically capital letters indicate significant differences (P<0.05) between the concentrations.
*Various Horizontally small letters indicate significant differences(P<0.05) between Solvents.

Table 3**: Inhibition zone(mm)of *Mentha* extract against *Streptococcus pneumonia*.**

Concentration of extract (mg/ml)	Alcoholic extract			Mean of concentration	$LSD_{0.05}$ conc.
	1	2	3		
Antibiotic control (tetracycline) 0.25mg/10ml	26 ± 0.0	26.5 ± 0.0	26 ± 0.0	26.16 A	**1.84**
0.25mg/5ml	23 ± 0.0	16 ± 0.0	13 ± 0.0	17.33 B	
0.25mg/10ml	11 ± 0.0	7 ± 0.0	16 ± 0.0	11.33 C	
0.25mg/15ml	6.5 ± 0.0	3 ± 0.0	2 ± 0.0	3.83 D	
0.25mg/20ml	1 ± 0.0	2 ± 0.0	1 ± 0.0	1.33D	
0.25mg/25ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 E	
0.25mg/30ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
0.25mg/35ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
0.25mg/40ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 FG	
0.25mg/45ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 G	
Mean of extract Solvent	4.6 c	3.11 b	3.55 a	**$LSD_{0.05}$ Interference**	
$LSD_{0.05}$ Solvent	**0.53**			**1.06**	

* The numbers refer to mean ± Standard error.
* Various vertically capital letters indicate significant differences (P<0.05) between the concentrations.
*Various Horizontally small letters indicate significant differences(P<0.05) between Solvents.

Table 4: **Inhibition zone(mm)of *Mentha* extract against *Staphylococcus aureus*.**

Concentration of extract (mg/ml)	Alcoholic extract			Mean of concentration	$LSD_{0.05}$ conc.
	1	2	3		
Antibiotic control (tetracycline) 0.25mg/10ml	24 ± 0.0	26 ± 0.0	21 ± 0.0	23.66 A	
0.25mg/5ml	13 ± 0.0	10 ± 0.0	9 ± 0.0	10.66 B	
0.25mg/10ml	10 ± 0.0	6 ± 0.0	8 ± 0.0	8.00 C	
0.25mg/15ml	9 ± 0.0	4± 0.0	5 ± 0.0	6.00 D	
0.25mg/20ml	3 ± 0.0	3 ± 0.0	3 ± 0.0	3.00D	**1.80**
0.25mg/25ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 E	
0.25mg/30ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
0.25mg/35ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 EF	
0.25mg/40ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 FG	
0.25mg/45ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 G	
Mean of extract Solvent	4.22 c	2.55 b	2.77 a	**$LSD_{0.05}$ Interference**	
$LSD_{0.05}$ Solvent	**0.34**			**0.68**	

* The numbers refer to mean ± Standard error.
* Various vertically capital letters indicate significant differences (P<0.05) between the concentrations.
*Various Horizontally small letters indicate significant differences(P<0.05) between Solvents.

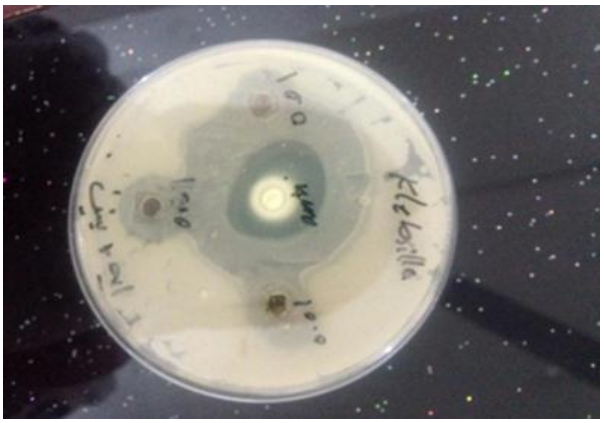

Figure1. ***Klebsiella pneumoniae***

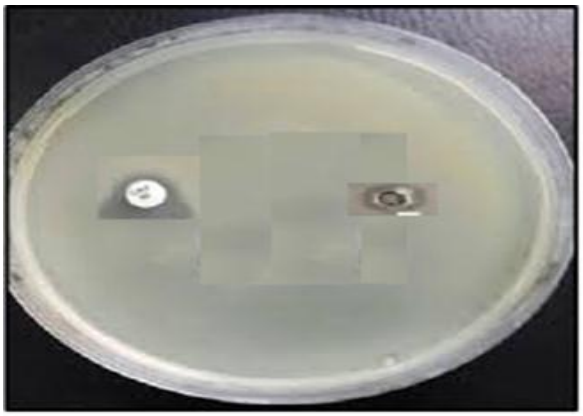

Figure 3. ***Streptococcus pneumoniae***

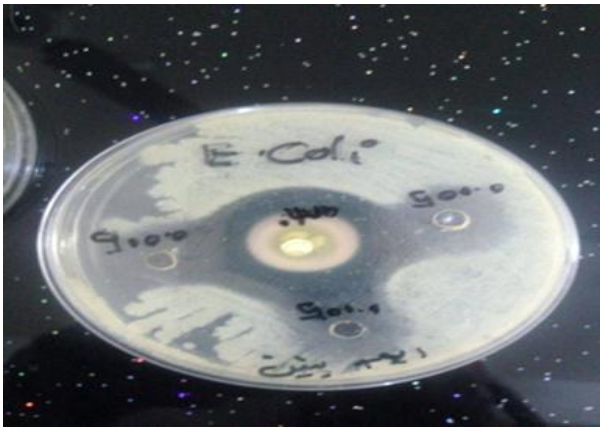

Figure 2. ***Escherichia coli***

Figure 4 ***Staphylococcus aureus***

DISCUSSION

Multidrug resistant strains they had been an tremendous increased in many clinical pathogens bacteria pertinent which was create major challenged to the scientists. The multidrug resistance increased reckon engender because of indistinctive used of antibiotics. An addition, the increased cost of new generation of antibiotics non-available coupled with exponential arising the deaths number occurred by infectious diseases cause. As result, they need squeezing for cheap, effectiveness and novel anti infection cured. They search for effectiveness antimicrobial agents from plants, with objective for find out find out benefit antimicrobial substance could act as origin and model for the created a new antimicrobial cured [15].

M. piperita shown significantly action because of leaf possessing numeral powerful substance like limnone, menthofuran , menthol, menthone, and menthyl acetate. These substance possessing highest medicine rate especial with treating the epigastric bloating, impaired digestion, dyspepsia, flatulence and eructions, tropical

utilized to alleviate nasal congested in the (common cold) and tropic protective agents itch alleviate used [16]. Antibacterial actions of mint oils had been also previous investigation through differences scientists. The existence of many secondary metabolites mention through the early scientists from the leaf like essential oils, glycosides, isoflavone and eudesmanoids possibly caused the antimicrobial activities of *M. piperita* [17]. The literature have been clear that flavonoid glycosides flavonoids, phenols, terpenoids, and tannins had action versus broad ambit of microbes [18, 19]. Several scientists had publishing that together gram's positive bacteria species examined were sensitivity to mint essential oil caused inhibition zone between 13-17 mm. Literature have proof that gram's negative bacteria are most sensitively to mint oil and their extracts than gram's positive bacteria [20, 21]. The effectiveness of substance like essential oil on cell membrane safety on both gram's negative and gram's positive bacteria has been formerly advertised. In gram's positive bacteria, hydrophobic substances and volatile oil may be contact immediately with the cell membrane phospholipid bilayer. Several scientists mention that these Substances caused arising in ion permeability, infiltration of pivotal intracellular component or bacterial enzyme systems feebleness [22, 23]. Previously mentions also discussion the antimicrobial action of the peppermint essential oils versus *S. aureus*, Strept. pneumonia, *E. coli* and *Klebsiella* spp. [24].

REFERENCES

1. Sujana P., SridharT.M., Josthna P. and Naidu C.V. "Antibacterial Activity and Phytochemical Analysis of Mentha piperita L. (Peppermint)—An Important Multipurpose Medicinal Plant". American J. of Plant Sc. 2013; 4(1): 77-83. DOI: 10.4236/ajps.2013.41012.
2. Mukul S., Surabhi K., Atul N. "Cosmeceuticals for the skin: An overview". Asian J. Pharm. Clin. Res. 2015; 4(2): 1–6.
3. Alexa E., Danciu C., Radulov I., Obistioiu D., Sumalan R.M. and Morar A. "Phytochemical Screening and Biological Activity of Mentha×piperita L. and Lavandula angustifolia Mill. Extracts". Hindawi Anal. Cellul. Path. 2018; 1(2018): 7. DOI: 10.1155/2018/2678924.
4. Harley R.M., Atkins S., Budantsev A.L., Cantino P.D. *et al.* "Labiatae". In Kubitzki K., Kadereit J.W. "The Families and Genera of Vascular Plants. VII". Berlin; Heidelberg, Germany: Springer-Verlag. 2004; pp. 167–275 ISBN 978-3-540-40593-1.
5. Bunsawat J., Elliott N.E., Hertweck K.L., Sproles E., Alice L.A. "Phylogenetics of Mentha (Lamiaceae): Evidence from Chloroplast DNA Sequences". Systematic Botany. 2004; 29 (4): 959-9 64. https://doi.org/10.1600/0363644042450973.
6. Karuza L., Blazevic N. and Soljic Z. "Isolation and structure of flavonoids from peppermint (Mentha piperita) leaves". Acta Pharm. 1996; 46 (1996): 315–320.
7. Taofiq O., González-Paramás A.M., Barreiro M.F. and Ferreira I.C. "Hydroxycinnamic acids and their derivatives: cosmeceutical significance, challenges and future perspectives". International Journal of Molecular Sciences. 2017; 22(2): 281. DOI: 10.3390/molecules22020281.
8. Pramila D.M., Xavier R., Marimuthu K., Kathiresan S., Khoo M. and Senthilkumar L. "Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita: Lamiaceae)". J. of Med. Plants Res. 2012; 6(2): 331-335. DOI: 10.5897/JMPR11.
9. Ekambaram S., Perumal S., Balakrishnan A., Marappan N., Gajendran S. and Viswanathan V. 2016. "Antibacterial synergy between rosmarinic acid and antibiotics against methicillinresistant Staphylococcus aureus". Journal of Intercultural Ethnopharmacology. 2016; 5 (4): 358–363. DOI: 10.5455/jice.20160906035020.
10. Blazeković B, Vladimir-Knezević S., Brantner A. and Stefan M.B. "Evaluation of antioxidant potential of Lavandula x intermedia Emeric ex Loisel. 'Budrovka': a comparative study with L. angustifolia Mill". Molecules. 2010; 15(9): 5971– 5987. DOI: 10.3390/molecules15095971.
11. Sahgal G., Sreeramanan S., Sasidharan S., Xavier R., Ong M.T. "Screening selected medicinal plants for antibacterial activity against Methicillin Resistant Staphylococcus aureus (MRSA)". Adv. Nat. Appl. Sci. 2009; 3(3): 330-338.
12. Thanes G., Xavier R., Marimuthu K., Kathiresan S., Sasidharan S., Sreeramanan S. "Isolation and identification of Salmonella from curry samples and its sensitivity to commercial antibiotics and aqueous extracts of Camelia sinensis (L) and Trachyspermum ammi (L)". Asian Pacific J. Trop. Biomedicine. 2011; 1(4): 261-269. DOI: 10.1016/S2221-1691(11)60040-3.
13. Hammadi A.A., Abdullah J.A. and Abed H.A. 2017. "Study of inhibition efficiency of peat against burn infection bacteria". Sc. J. of Med. Rese. 2017; 1 (2): 78 - 81. DOI: 10.37623/SJMR.2017.1209.
14. Abdullah J.A., Hammadi A.A., Hakem R., Hatef Z. and Hussein N. "Study effect of plant extraction for Cuscuta europaea (Dodder) against two species of bacteria Staphylococcus aureus and Escherichia coli". J Contemp. Med. Sci. 2016; 2(8): 133–137.
15. Zhang L., gen Xu S., Liang W., Mei J., Di Y. and Lan H. "Antibacterial Activity and Mode of Action of Mentha arvensis Ethanol Extract against Multidrug-Resistant Acinetobacter baumannii". Trop J Pharm Res. 2015; 14(11): 2099-2106. DOI: 10.4314/tjpr.v14i11.21.
16. Bupesh G., Amutha C., Nandagopal S., Ganeshkumar A., P. Sureshkumar P. and Murali K.S. "Antibacterial activity of Mentha piperita L. (peppermint) from leaf extracts – a medicinal plant". Acta. agricultural Slovenica. 2007; 89 (1): 73 – 79.
17. Okmen A.S., Okmen G., Arslan A. and Vurkun M. "Antibacterial Activities of Mentha piperita L. Extracts Against Bacteria Isolated from Soccer Player's Shoes and its Antioxidant Activities". Indian J. of Pharmaceu. Ed. and Res. 2017; 51(3): 163- 169.
18. Singh C.S. and Agarwal R. "Evaluation of antibacterial activity of volatile oil from Mentha spicata L". JDDT. 2013; 3(4):120-121. DOI: 10.22270/jddt.v3i4.580.
19. Singh R., Shushni M.A.M. and Belkheir A. "Antibacterial and antioxidant activities of Mentha piperita L". Arab J Chem. 2015; 8(3):322-8. https://doi.org/10.1016/j.arabjc.2011.01.019.
20. Jeyakumar E., Lawrence R. and Pal T. "Comparative evaluation in the efficacy of peppermint (Mentha piperita) oil with standards antibiotics against selected bacterial pathogens". Asian Pac J Trop Biomed. 2011; 1(2):253-7. https://doi.org/10.1016/S2221-1691(11)60165-2.

21. Sujana P., Sridhar T., Josthna P. and Naid C.V. "Antibacterial activity and phytochemical analysis of Mentha piperita L. (Peppermint) - An important multipurpose medicinal plant". AJPS. 2013; 4(01):77-83. DOI: 10.4236/ajps.2013.41012.
22. Alsabri S.G., El-Basir H.M., Rmeli N.B., Mohamed S.B., Allafi A.A., Zetrini A.A., Salem A.A., Mohamed S.S., Gbaj A. and El-Baseir M.M. "Phytochemical screening, antioxidant, antimicrobial and anti-proliferative activities study of Arbutus pavarii plant". J Chem Pharma Res. 2013; 5(1):32-36.
23. Rachitha P., Krupashree K., Jayashree G.V., Gopalan N. and Khanum F. "Growth inhibition and morphological alteration of Fusarium sporotrichioides by Mentha piperita essential oil". Pharmacognosy Magazine. 2017; 9(1):74-79. DOI: 10.4103/0974-8490.199771.
24. Naidu J.R., Ismail R.B., Yeng C., Sasidaran S. and Kumar P. "Chemical composition and antioxidant activity of the crude methanolic extracts of Mentha spicata". J.Phytol. 2012; 4(1):13-1. doi: 10.3390/molecules24152825.