Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(6): 197-201
Received: 26-02-2015
Accepted: 29-03-2015

Abdoulraouf A. Habibi
Department of Microbiology, Biotechnology Research Center, Tripoli, Libya

Sadek A. Zubek
Department of Microbiology, Biotechnology Research Center, Tripoli, Libya

Mohamed A. Abushhiwa
Department of Surgery and Theriogenology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Mohamed O. Ahmed
Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Sabry A. El-Khodery
Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Hamdy Y. Osman
Department of Biological Sciences, Faculty of Arts and Science, EL-Mergheb University, Mislata, Libya

Emad M. Bennour
Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Correspondence:
Emad M. Bennour
Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Antibacterial activity of selected Libyan medicinal plants against *Pseudomonas aeruginosa* and *Escherichia coli*

Abdoulraouf A. Habibi, Sadek A. Zubek, Mohamed A. Abushhiwa, Mohamed O. Ahmed, Sabry A. El-Khodery, Hamdy Y. Osman, Emad M. Bennour

Abstract
The aim of the present study was to assess the antibacterial activity of *Zizyphus Vulgaris, Laurus nobilis, Thymus capitatus, Cistus salvifolius, Arbutus pavarii, Rhus tripartata, Pistacia atlantica* against reference strains of *Pseudomonas aeruginosa* and *Escherichia coli.* For this purpose, extract of leaves was obtained by 96% chloroform, 95% ethanol or petroleum ether. Antibacterial activity and minimum inhibitory concentration (MIC) for different extracts of each plant was evaluated. *Cistus salvifolius* and *Arbutus pavarii* were the most effective, as the values of inhibition zones were 9.0 ± 0.89 mm and 8.0 ± 1.7 mm for *E. coli*, and 14.0 ± 0.84 and 10.0 ± 0.81 mm for *P. aeruginosa,* respectively. *Zizyphus Vulgaris and Laurus nobilis* showed no effect on *E. coli* and *Thymus capitatus* showed no effect on *P. aeruginosa. Rhus tripartata* plant extract caused an inhibition of *E. coli* growth at 50 mg/ml, while *Zizyphus Vulgaris, Thymus capitatus* caused this inhibition at 100 mg/ml. However, the minimal inhibitory concentration was 200 mg/ml for other plant extract. The minimal inhibitory concentration for *P. aeruginosa* was 50 mg/ml for *Rhus tripartata* extract, 100 mg/ml for *Thymus capitatus,* and 200 mg/ml for other tested plant extracts. The petroleum ether extracts of the plants did not exhibit any effect on the growth of *P. aeruginosa and E. coli*. However, the chloroform extract of *Thymus capitatus* has inhibited the growth of *E. coli* (7 mm). The results of the present study indicate that ethanolic extract of *Cistus salvifolius* and *Arbutus pavarii, Rhus tripartata* has the most effective and potent antibacterial activity.

Keywords: Medicinal plants, Extract, Antibacterial activity, Libya

1. Introduction
Synthetic drugs used currently in the developing countries are expensive and inadequate for the treatment of diseases [31]. Furthermore, development of antimicrobial resistance by microorganisms has increased. In general, bacteria have the genetic ability to acquire and transmit resistance to drugs, which are utilized as therapeutic agents [12]. Therefore, alternative antimicrobial agents of herbal origin may be of interest.
Escherichia coli and *Pseudomonas aeruginosa* cause a wide range of diseases in both human and animals [15, 22]. Development of antimicrobial resistance in such bacteria has been documented extensively and has become common finding [5, 33].
Recently, an extensive work has been done to examine *in vitro* antimicrobial effects of many herbal plants [16, 18]. Plant extracts may include roots, stem, leaves or flowers [11, 18]. Additional antioxidant and food preservative effect of herbal plant extracts have also been documented [11]. The extraction of herbal plants is carried out by different methods and chemicals [24].
Several studies reported that extract of *Laurus nobilis* leaves has not only antibacterial and antioxidant effects [19, 24] but also has antiproliferative activity against human breast adenocarcinoma cells [3].
Rhus tripartitum has been found to have antioxidant and antifungal activity [1, 6]. Polymers from *Pistacia atlantica* were screened against Helicobacter pylori and other Gram-negative and Gram-positive bacteria to evaluate their antimicrobial action [32]. However, a study on *Zizyphus* spp. documented its antimicrobial activity [4].
To the best of author's knowledge, much less attention, however, has been given to the studies on the antibacterial effect of medicinal plants in Libya, especially on pathogenic bacteria. Therefore, the objective of the present study was to assess the antibacterial effect of ethanolic and ether extract of seven plants on *Pseudomonas aeruginosa* and *Escherichia coli.*

2. Materials and Methods

2.1. *Medicinal plants*

Seven medicinal plants, namely *Zizyphus Vulgaris, Laurus nobilis, Thymus capitatus, Cistus salvifolius, Arbutus pavarii, Rhus tripartata* and *Pistacia atlantica* were randomly selected and collected from eastern regions of Libya.

2.2. *Preparation of the plant materials*

The collected plant samples were cleaned using tap water to remove the dusts and then dried in an oven at 60 °C for 8 hr.

2.3. *Preparation of different plant extracts*

Extractions procedures were carried out at the Medicinal Plants Laboratory at the Biotechnology Research Centre, Tripoli. Leaves of plant samples were separately grounded in a blender. Ten grams of each grounded, tested plant were separately dissolved in a flask containing 100 ml of either 96% chloroform, 95% ethanol or petroleum ether for 72 hr. The samples were filtrated using Whatman No1 filter paper and the residue was re-extracted with the same used solvent. The filtrates were collected together and evaporated to dryness at 40 °C under reduced pressure. The residue was kept in the refrigerator at 4 °C until use according to previous methods [2].

2.4. *Bacterial isolates*

Bacterial isolates, *Escherichia coli* (ATTC10412) and *Pseudomonas aeruginosa* (ATTC27853), were obtained from the Department of Microbiology at the Biotechnology Research Center. The bacterial isolates were grown on nutrient agar slants for 24 hr at 37 °C then used in the experiment.

2.5. *Antibacterial activity assay*

The antibacterial activity of different extracts (Ethanol, petroleum ether and chloroform) was evaluated by disc diffusion method [9]. Briefly, this method is based on the diffusion of an antibiotic from a filter paper disc through the solidified culture medium (Muller- Hinton agar) of Petri dishes. Whatman filter paper (No2) was used to prepare small discs (6 mm in diameter), then sterilized at 121 °C for 15 min in an autoclave. The discs were separately impregnated with the previous extracts for 1-4 hrs at room temperature and allowed to dry. A swap of bacterial broth (previously prepared and adjusted to contain 108 cfu was dispersed on the agar plate surface. After drying, the previous discs were placed on the Petri dishes and then incubated at 37 °C for 24 hrs. Three replicated plates were used for each treatment. The diameter of inhibition zone created by each disc was measured (in mm) using a ruler.

2.6. *Determination of the minimum inhibitory concentration (MIC)*

The MIC of the plant extracts was estimated on solid medium (nutrient agar) using previously described method [12]. Six concentrations, i.e. 6.25, 12.5, 25, 50, 100 and 200 mg/ml were tested. Briefly, a stock solution was prepared by dissolving 200 mg of each extract in one ml of the solvent containing dimethyl sulfoxide and water in a ratio of 2:4 v/v, respectively.

One hundred microliter of nutrient broth medium was dispensed into one well for each treatment to be a first control. The second control consists of other well contained only 100 µl of the extract. Another 100 µl of stock solution was transferred to a third well. A serial dilutions were performed by taking 100 µl from the third well to the fourth well and this procedure was repeated with other 5 wells until reaching the desired concentration, i.e. (6.25 mg/ml). Aliquot of 100 µl of previously prepared bacterial broth were added to each well except the control well. All the plates were incubated at 37 °C for 24 hr. The MIC was detected by the lack of turbidity in the wells. For the confirmations of growth inhibition, the re-cultures plates were incubated at 37 °C for 24 hr.

2.7. *Statistical analysis*

Data analysis was performed using a statistical software program (Graphpad Prism for Windows Version 5.0, GraphPad Software, Inc., Sandiego, CA, USA). Data were assessed for normal distribution using D'Agostino and Pearson omnibus normality test. Data were normally distributed; consequently mean and standard deviation for each assessed variable was calculated. Two-way ANOVA with Duncan *post-hoc* multiple comparison tests was used to identify which group was statistically different from the rest. Differences between means at $p < 0.05$ were considered significant.

3. Results

The efficacy of ethanolic plant extracts varied significantly on growth of *P. aeruginosa* ($p<0.05$). *Cistus salvifolius* and *Arbutus pavarii* were the most effective for the inhibition of *P. aeruginosa*. Thus, the values of inhibition zones were 14.0 ±0.84 mm and 10.0±0.81 mm, respectively. Other tested extracts of medicinal plants were of the least effect (7-9 mm). Similarly, the efficacy of plant extracts varied significantly on *E. coli* growth ($p<0.05$). *Cistus salvifolius* and *Arbutus pavarii* were the most effective in the inhibition. Thus, the values of inhibition zones were 9.0±0.89mm and 8.0±1.7 mm, respectively (Table 1). *Zizyphus Vulgaris and Laurus nobilis* showed no effect on *E. coli* and *Thymus capitatus* showed no effect on *P. aeruginosa*

Table 1: The inhibitory effect of ethanolic extracts of selected medicinal plants on the growth of *E. coli* and *P. aeruginosa*

Plant extract	*E. coli*	*P. aeruginosa*
Zizyphus Vulgaris	0.0 [a]*	7.0±0.0[a]
Laurus nobilis	0.0 [a]*	9.0 ±0.8 [b]
Thymus capitatus	6.0.±0.0 [b]*	0.0 [c]
Cistus salvifolius	9.0 ±0.89 [c]*	14.0 ±0.84 [d]
Arbutus pavarii	8.0 ± 1.7 [c]*	10.0± 0.81 [b]
Rhus tripartata	6.0 ± 0.89 [b]	7.0± 0.1 [a]
Pistacia atlantica	6.0 ±0.9 [b]	7.0±0.2 [a]

[a,b,c]: Means with different superscript letters in the same column are significantly different at p< 0.05. Each column represents the comparison between different plant extracts on each bacteria

*: Means with different superscript letters in the same raw are significantly different at p< 0.05. Each raw represent comparative effect of each plant extract on both bacteria.

Table 2 shows that *Rhus tripartata* plant extract caused an inhibition of *E. coli* growth at 50 mg/ml, while *Zizyphus Vulgaris, Thymus capitatus* caused this inhibition at 100 mg/ml. On the other hand, the minimal inhibitory concentration was 200 mg/ml for other plant extracts. Table 3 shows that the minimal inhibitory concentration for *P. aeruginosa* was 50 mg/ml for *Rhus tripartita* extract, 100 mg/ml for *Thymus capitatus* and 200 mg/ml for other tested plant extracts.

The petroleum ether extracts of the plants did not exhibit any effect on the growth of *P. aeruginosa* and *E. coli*. However, the chloroform extract of *Thymus capitatus* has inhibited the growth of *E. coli* (7 mm).

Table 2: Minimum inhibitory concentration exhibited by ethanolic extract of tested plants on growth of *E. coli*

Tested plant	Tested concentrations (mg/ml)					
	6.25	12.5	25	50	100	200
Zizyphus Vulgaris	+	+	+	+	-	-
Laurus nobilis	+	+	+	+	+	-
Thymus capitatus	+	+	+	+	-	-
Cistus salvifolius	+	+	+	+	+	-
Arbutus pavarii	+	+	+	+	+	-
Rhus tripartata	+	+	+	-	-	-
Pistacia atlantica	+	+	+	+	+	-

Table 3: Minimum inhibitory concentration exhibited by ethanolic extract of tested plants on growth of *P. aeruginosa*

Tested plant	Tested concentrations (mg/ml)					
	6.25	12.5	25	50	100	200
Zizyphus Vulgaris	+	+	+	+	+	-
Laurus nobilis	+	+	+	+	+	-
Thymus capitatus	+	+	+	+	-	-
Cistus salvifolius	+	+	+	+	+	-
Arbutus pavarii	+	+	+	+	+	-
Rhus tripartata	+	+	+	-	-	-
Pistacia atlantica	+	+	+	+	+	-

4. Discussion

Over the past few decades, a number of publications have been reported on the antibacterial activities of extracts from medicinal plants [17, 36]. However, in Libya, the effect of different extracts of local medicinal plants on bacteria isolated from human patients has not been documented. Thus, in the current work, we determined the MIC and effect of extracts of seven medicinal plant leaves on *E. coli and P. aeruginosa*.

The present results revealed that ethanolic extract of selected plants was more effective compared to chloroform or petroleum ether solvents. There was a significant variation of the effect of different ethanolic plant extracts on the inhibition of selected bacteria. *Cistus salvifolius* was the most effective, where its ethanolic extract produced an inhibition zone of 14.0 ±0.84 and no bacterial growth was detected at 200 mg/ml for *E. coli and P. aeruginosa*. The antibacterial activity of the plant may be attributed to the various phytochemical constituents present in the crude extract. Similar results was reported on *Cistus ladanifer*, which has been found effective on five Gram-negative bacteria and Gram-positive bacteria including antibiotic resistant *Staphylococcus spp*. [18]. However, *Cistus salvifolius* was found to have antioxidant effect [27]. Water extract of *Cistus salvifolius* has been found to have potent inhibitory effect against COX-1 and COX-2, which are mediators of inflammation [26].

Ethanolic extract of *Ziziphus vulgaris* was found effective only on *P. aeruginosa*. However, it has been found also effective on Gram-negative bacteria and has antioxidant effect [7], suggesting that it may have broad spectrum antibacterial activity. *Laurus nobilis* was found effective only on *P. aeruginosa*. Similar results was previously reported [21]. On the other hand *Laurus nobilis* has not been found effective against bacteria [11].

Thymus capitatus provided an inhibitory effect on the growth of *E. coli* only. Similar result was previously reported on an ethanolic extract of this plant leaves [28]. Other studies indicated that *Thymus capitatus* has an antifungal and antioxidant effects [34], as well as an anthelmintic effect [10]. On the other hand, *Arbutus pavarii* produced significant inhibitory effect on *P. aeruginosa* compared with *E. coli*. Studies on other plant species *Arbutus unedo* extracts indicated that it has an antimicrobial [18], and antioxidant effect [35].

Rhus tripartata extract provided inhibitory effect on growth of *E. coli and P. aeruginosa* with no significant variation. *Rhus tripartitum* root bark extract could protect against ulcer due to its antioxidant and antisecretory effect [6]. On the contrary, it was reported that ingestion of *Rhus tripartitum* could produce acute toxic encephalopathy. Similar to *Rhus tripartata, Pistacia atlantica* was found effective against both bacterial isolates. In a study carried out on dental plaque bacteria and subgingival microorganisms, *Pistacia atlantica* could do significant decreases in the aerobic bacteria [8]. Moreover, *Pistacia atlantica subsp. kurdica* was included as an antioxidant [20].

Alcoholic extracts of tested plants provided better antibacterial effect than other solvents. The effectiveness of the extracts largely depends on the type of solvent used, where the organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts [29]. Cowan [14] mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents. Similar results showing that the alcoholic extract having the best antimicrobial activity of *Leucas aspera and Holarrhena antidysenterica* [25], *M. azedarach* [29] and *Callistemon citrinus* and *Albizia lebbeck* [30].

The limitation of present study is the use of only two species of Gram-negative bacteria. Another limitation is that the tested bacteria was not examined for antibiotic resistance based on molecular bases. In addition, we used only the leaves of tested plants. Therefore, all these shortcomings should be considered in further studies.

5. Conclusion

The results of the present study indicate that ethanolic extract of *Cistus salvifolius, Arbutus pavarii, Rhus tripartata, Pistacia atlantica* have potent effect on both *E. coli and P. aeruginosa* isolated from human patients. *Rhus tripartata* has been found highly effective with MIC of 50 mg/ml. This study indicated that these plants could be a potential source of effective antibacterial agents. further investigations are needed to be done on a wide range of bacteria and fungi to assess the spectrum of such plant extracts. Moreover, other parts of the examined plants are also needed to be assessed for their antibacterial activity.

6. Acknowledgement

Authors would like to thank the staff of Department of Microbiology, Biotechnology Research Center, Libya for providing the bacterial isolates.

7. Conflict of interest

The authors declare that they have no conflict of interest.

8. References

1. Abbassi F, Hani K. *In vitro* antibacterial and antifungal activities of Rhus tripartitum used as antidiarrhoeal in Tunisian folk medicine. Nat Prod Res 2012; 26(23):2215-8.
2. Abu-Shanab B, Adwan D, Abu-Safiya D, Jarrar N, Adwan K. Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. Turk J Biol 2004; 28:99-102.
3. Al-Kalaldeh JZ, Abu-Dahab R, Afifi FU. Volatile oil composition and antiproliferative activity of *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells. Nutr Res 2010; 30(4):271-8.
4. Ali-Shtayeh MS, Yaghmour RM, Faidi YR, Salem K, Al-Nuri MA. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J Ethnopharmacol 1998; 60(3):265-71.
5. Ali MM, Ahmed SF, Klena JD, Mohamed ZK, Moussa TA, Ghenghesh KS. Enteroaggregative *Escherichia coli* in diarrheic children in Egypt: molecular characterization and antimicrobial susceptibility. J Infect Dev Ctries 2014; 8(5):589-96.
6. Alimi H, Mbarki S, Barka ZB, Feriani A, Bouoni Z, Hfaeidh N *et al.* Phytochemical, antioxidant and protective effect of *Rhus tripartitum* root bark extract against ethanol-induced ulcer in rats. Gen Physiol Biophys 2013; 32(1):115-27.
7. Amooaghaie R. Evaluation of antibacterial and antioxidant activities of *Ziziphus vulgaris* (Rhamnaceae). Planta Medica 2011, 77, 1.
8. Arami S, Mojaddadi MA, Pourabbas R, Chitsaz MT, Delazar A, Mobayen H. The Effect of *Pistacia atlantica* Var. mutica Mouthwash on Dental Plaque Bacteria and Subgingival Microorganisms: A Randomized and Controlled Triple-blind Study. Drug Res 2014; 14:14.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45(4):493-6.
10. Boubaker Elandalousi R, Akkari H, B'Chir F, Gharbi M, Mhadhbi M, Awadi S *et al. Thymus capitatus* from Tunisian arid zone: chemical composition and *in vitro* anthelmintic effects on *Haemonchus contortus*. Vet Parasitol 2013; 197(1-2):374-8.
11. Cherrat L, Espina L, Bakkali M, Garcia-Gonzalo D, Pagan R, Laglaoui A. Chemical composition and antioxidant properties of *Laurus nobilis* L. and *Myrtus communis* L. essential oils from Morocco and evaluation of their antimicrobial activity acting alone or in combined processes for food preservation. J Sci Food Agric 2014; 94(6):1197-204.
12. Cohen ML. Epidemiology of drug resistance: implications for a postantimicrobial era. Science 1992; 257:1050-1055.
13. Collins C, Grange J, Lyne P, Falkinham J. *Microbiological methods*, CRC Press: UK, 2004.
14. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4):564-82.
15. Czechowska K, McKeithen-Mead S, Al Moussawi K, Kazmierczak BI. Cheating by type 3 secretion system-negative *Pseudomonas aeruginosa* during pulmonary infection. Proc Natl Acad Sci USA 2014; 12:12.
16. Diba MA, Paolini J, Bendahou M, Varesi L, Allali H, Desjobert JM *et al.* Chemical composition of fatty acid and unsaponifiable fractions of leaves, stems and roots of Arbutus unedo and *in vitro* antimicrobial activity of unsaponifiable extracts. Nat Prod Commun 2010; 5(7):1085-90.
17. Fankam AG, Kuiate JR, Kuete V. Antibacterial activities of Beilschmiedia obscura and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. BMC Complement Altern Med 2014; 14(241):1472-6882.
18. Ferreira S, Santos J, Duarte A, Duarte AP, Queiroz JA, Domingues FC. Screening of antimicrobial activity of Cistus ladanifer and Arbutus unedo extracts. Nat Prod Res 2012; 26(16):1558-60.
19. Fukuyama N, Ino C, Suzuki Y, Kobayashi N, Hamamoto H, Sekimizu K *et al.* Antimicrobial sesquiterpenoids from Laurus nobilis L. Nat Prod Res 2011; 25(14):1295-303.
20. Hatamnia AA, Abbaspour N, Darvishzadeh R. Antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica* subsp. kurdica) fruits. Food Chem 2014; 145:306-11.
21. Lee S, Chung SC, Lee SH, Park W, Oh I, Mar W *et al.* Acylated kaempferol glycosides from Laurus nobilis leaves and their inhibitory effects on Na+/K+-adenosine triphosphatase. Biol Pharm Bull 2012; 35(3):428-32.
22. Li Bassi G, Rigol M, Marti JD, Saucedo L, Ranzani OT, Roca I *et al.* A Novel Porcine Model of Ventilator-associated Pneumonia Caused by Oropharyngeal Challenge with *Pseudomonas aeruginosa*. Anesthesiology 2014; 120(5):1205-15.
23. Nam TS, Choi SM, Kang KW, Kim JT, Lee SH, Park MS *et al.* Acute toxic encephalopathy due to the ingestion of Rhus extract. J Clin Neurosci 2009; 16(10):1377-8.
24. Ozcan B, Esen M, Sangun MK, Coleri A, Caliskan M. Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. J Environ Biol 2010; 31(5):637-41.
25. Preethi R, Devanathan VV, Loganathan M. Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens. Advances in Biological Research 2010; 4(2):4.
26. Qa'dan F, Nahrstedt A, Schmidt M. Isolation of two new bioactive proanthocyanidins from *Cistus salvifolius* herb extract. Pharmazie 2011; 66(6):454-7.
27. Qa'Dan F, Petereit F, Mansoor K, Nahrstedt A. Antioxidant oligomeric proanthocyanidins from *Cistus salvifolius*. Nat Prod Res 2006; 20(13):1216-24.
28. Qaralleh HN, Abboud MM, Khleifat KM, Tarawneh KA, Althunibat OY. Antibacterial activity *in vitro* of Thymus capitatus from Jordan. Pak J Pharm Sci 2009; 22(3):247-51.
29. Sen A, Batra A. Evaluation of Antimicrobial Activity of Different Solvent Extracts Of Medicinal Plant: Melia Azedarach L. International Journal of Current Pharmaceutical Research 2012; 4(2):7.
30. Seyydnejad SM, Niknejad M, Darabpoor I, Motamedi H. Antibacterial Activity of Hydroalcoholic Extract of *Callistemon citrinus* and Albizia lebbeck. American. Journal of Applied Science 2010; 7(1):4.
31. Shariff ZU. Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series (Volume 1), Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, 2001, 9-84.

32. Sharifi MS, Ebrahimi D, Hibbert DB, Hook J, Hazell SL. Bio-activity of natural polymers from the genus Pistacia: a validated model for their antimicrobial action. Glob J Health Sci 2011; 4(1):149-61.
33. Smith S, Wang J, Fanning S, McMahon BJ. Antimicrobial resistant bacteria in wild mammals and birds: a coincidence or cause for concern? Ir Vet J 2014; 67(1):2046-0481.
34. Tabti L, Dib Mel A, Gaouar N, Samira B, Tabti B. Antioxidant and Antifungal Activity of Extracts of the Aerial Parts of *Thymus capitatus* (L.) Hoffmanns against Four Phytopathogenic Fungi of Citrus sinensis. Jundishapur J Nat Pharm Prod 2014; 9(1):49-54.
35. Tuberoso CI, Boban M, Bifulco E, Budimir D, Pirisi FM. Antioxidant capacity and vasodilatory properties of Mediterranean food: the case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. Food Chem 2013; 140(4):686-91.
36. Zengin G, Uysal A, Gunes E, Aktumsek A. Survey of Phytochemical Composition and Biological Effects of Three Extracts from a Wild Plant (*Cotoneaster nummularia* Fisch. et Mey.): A Potential Source for Functional Food Ingredients and Drug Formulations. PLoS One 2014; 9(11).