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In vitro Investigation of Antimicrobial Activity of *Artemisia herba-alba* Seeds Extracts against Some Clinical Pathogenic Microbes

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Abstract: This investigation was carried out to examine the antimicrobial activities of essential oil types extracted by hydro-distillation from the seeds of *Artemisia herba-alba* (Ah) purchased in Taif Governorate. Eleven Identified microbial strains, i.e., *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *shigella dysenteriae*, *Sallmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *acinetobacter*, *Bacillus cereus*, *Fusarium solani* and *Candida albicans* were subjected to Tests. The antimicrobial activities of different oils were tested using the diffusion method and by determining the inhibition zone. The results revealed that all examined oil extracts of *artemisia herba-alba* Seeds had great potential of antimicrobial activity against microbial strains mentioned above.

Key words: *Artemisia herba alba* · Seeds · Antimicrobial · Activity · Microbes · Extracts

INTRODUCTION

In this world, heavily populated with bacteria, viruses and fungi, infections are the major cause of diseases in human beings. In factious diseases arise from the organisms that are pathogenic and which harbor either in the external environment or in our body itself. Bacterial world is greatly congregated with too many species which produce infections, like tetanus, gas gangrene, syphilis, gonorrhea, diphtheria, leprosy, tuberculosis and urinary infections etc.

Health care management programmes include plants and their products for treatment and antibiotics for four basic aspects i. e. prevention, diagnosis, treatment and cure of human diseases. Application of plant drugs and their products for treatment of various human ailments has been made by man since ancient times.

Artemisia is one of the diverse genera of Asteraceae family with many important medicinally valuable essential oils and secondary metabolites. Essential oils of Artemisia spp. have been widely used for a variety of medicinal purposes for many years. Artemisia nilagirica (Clarke) pamp commonly called Indian wormwood, is widely found in the hilly areas of India. A. nilagirica has been reported to exhibit insecticidal activities [1]. Around 59 compounds were identified from essential oil of A. nilagirica which showed an inhibitory activity on Phytophthora capsici, causing "foot rot" in pepper [2].

Various species of Artemisia have been characterized for their biological activities. It is considered to produce most medicinally important secondary metabolites [3, 4]. Several interesting studies using Artemisia spp. showed a series of antimicrobial and antioxidant activities [5-6]. The qualitative determination of various secondary metabolites like flavonoids, terpenoids, saponins and polysaccharides of Artemisia spp. were detected by HPLC, GC-MS and NMR [7, 8]. Few considerable secondary metabolites were successfully isolated and used in food industry as an alternative to synthetic antimicrobials [9, 10]. Furthermore, extracts of Artemisia spp. were used as natural pesticide and also in the treatment of few human diseases [11-12]. The determination of potential antimicrobial activity of Artemisia nilagirica extracts could be more informative for the future use in controlling phytopathogens and also in clinical treatment as natural antimicrobial agents.

The organisms like Escherichia, Enterobacter, Klebsiella, Proteus, Shigella and Staphylococcusspecies are implicated to cause severe infections in human, as

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they are found in multiple environmental habitats [13, 14]. Erwinia spp., Clavibacter michiganense, Pseudomonas syringaeand Xanthomonas campestris were reported to be severe phytopathogens, causing damage in carrot, potato, tomato, leafy greens, onion, green pepper, squash and other cucurbits. Furthermore, these phytopathogens cause disease in any plant tissue it invades [15].

In the present study, the antimicrobial potency of chloroform, diethyl ether, ethanol, hexane, methanol and petroleum ether extracts of Artemisia nilagirica was investigated. The antibacterial activity was determined by disk diffusion method and minimum inhibitory concentration (MIC) test. Four plant pathogens and 11 clinically important CLSI [16] reference bacterial strains from American Type Culture Collection (ATCC), Microbial Type Culture Collection (MTCC) and a local isolate from SPIC Science Foundation Patholab (SSFP) were used as test cultures. The preliminary phytochemical screening was carried out to identify the derivatives in the extracts.

The genus of Artemisia (*Asteraceae* family) has ben used extensively in folk medicine by many cultures since ancient times (European medicine, North Africa and Arabic medicine) [17]. Several studies have reported many pharmacological activities of *Artemisia herba alba* (Ah), the main medicinal properties of Ah include anti-diabetic [18], anti-spasmodic [19], antimicrobial [20], antimalarial and antioxidant effects (21). Many of the claimed folk medicine uses of this plant have been tested., but there are no investigations available with respect to *Artemisia herba alba* antimicrobial activities of seeds. This study tries to present a brief overview of the potential antimicrobial activities of *Artemisia herba alba* seeds oil extracts against some clinical pathogenic microbes that may be infected for humans and animals.

MATERIALS AND METHODS

Plant Material: Seeds of *Artemisia herba-alba* were purchased from the local market of Taif Governorate in Saudi Arabia.

Essential Oil Extraction: The method of Alade and Irobi [22] and the modified approach advised by Albashan [23] was used for preparation of plant extract (seed oil) with little modification. 20 grams of dried plant material (seeds) was soaked in 200 ml of acetone for 3 days. The soaked material was stirred after every 24 hours. After 3 days the extract was filtered using Whatman filter paper. The filtrate obtained was concentrated at 39°C under reduced pressure.

Antimicrobial Activity: The antimicrobial activity tests were carried out according to the method described by Albashan [23] and technique recommended by Zouari *et al.* [24].

Test Microorganisms and Screening for Antimicrobial Activity: Antibacterial activities of *Artemisia herba-alba* essential oil were tested against 11 identified strains of gram-positive and gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *shigella dysenteriae*, *Sallmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *acinetobacter* and *Bacillus cereus*. Antifungal activities were tested using *Fusarium solani* and *candida albicans*. Microorganisms were obtained from the culture collection of the Department of Microbiology, Central Laboratory, Al-Hada Military Hospital, Taif Governorate, K.S.A). Agar diffusion method Antibacterial activities of *A. herba-alba* essential oil were assessed using the paper disk agar diffusion method according to Freney *et al.* [4, 5, 10, 20, 21, 24]. Culture suspension (200 µl) of the tested bacteria (2 x 106 CFU/ml) was spread on the Mueller-Hinton broth medium. For fungal strains, spore suspension (200 µl) containing 108 spores/ml was spread on the potato dextrose agar medium. Then, absorbent disk (Whatman disk No. 3 of 6 mm diameter) containing 10 µl of essential oil were applied on the surface of the plate (90 mm) inoculated with different microbial strains. Negative control was prepared using a disk impregnated with sterile water. After that, the Petri dishes were kept for 1h at 4°C before incubation at 37°C for 24 h (bacteria strains) or at 30°C for 72 h (fungal strains). Finally, antimicrobial activity was evaluated by measuring the diameter (mm) of the growth inhibition zones including the 6 mm disk. Where there was no inhibition, the value 0 mm was assigned to the tested sample. The measurements of inhibition zones were carried out for three sample replications and values were the average of three replicates.

RESULTS

Results Are Presented in Table (1) below.

Table 1: Antimicrobial activity of *Artemisia herb alba* seeds essential oils (Extracts) from the Taif Governorate of Saudi Arabia

Microorganism	Inhibition zone diameter (mm) of EO* activity
Gram-negative bacteria:	
Escherichia coli	11.0
Klebsiella pneumonia	15.5
shigella dysenteriae	14.0
Sallmonella typhimurium	17.5
Pseudomonas aeruginosa	13.0
Acinetobacter	12.5
Gram-positive bacteria:	
Enterococcus faecalis	18.5
Staphylococcus aureus	13.5
Bacillus cereus	15.0
Fungal Strains;	
Fusarium solani	7.5
Candida albicans	9.0

**E.O:Essential oil (Extract) [10 µl/disc] of *Artemisia herba alba*

DISCUSSION

Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contributions towards human health. Phyto-medicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic systems of medicine or it can be the base for the development of new drugs. Much of the exploration and utilization of natural products as antimicrobial agents arise from microbial sources. Though soil micro-organisms or fungi produce most of the clinically used antibiotics, higher plants involving Artemisia herba alba can be a very good source of antibiotics [23]. Present study was conducted to investigate the antimicrobial potential of the extract of *Artemisia herba alba* seeds used commonly in traditional medicine in Taif governorate, Saudi Arabia.

The genus *Artemisia* L. (family Asteraceae, tribe Anthemideae), comprises a variable number of species (from 200 to over 400, depending on the authors) found throughout the northern half of the world. The genus may be divided into sections *Artemisia and Dracunculus* [25]. The genus *Artemisia* is known to contain many bioactive compounds; artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells [26] and arglabin is employed for treating certain types of cancer in the former USSR [27]. Over the past decade *Artemisia* species have been used traditionally in varies populations, thus; A. *keiskeana* Miq has been used as a Traditional Chinese drug for the treatment of gynaecepathy, amenerrhea, bruise and rheumatic disease [28], A. vestita Wallex DC. hasbeen utilized for the treatment of fungal infections such as tinea, tympanitis and thrush [29], A. *abrotanum* L. was found to possess spasmolyticactivity on the carbacholine induced contraction of guinea-pig trachea [30]. Artemisia species are popular plants which are used for the treatment of diseases such as hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses [31].

The antimicrobial activities of *Artemisia herba alba* essential oils originating from the Taif Governorate of Saudi Arabia were evaluated by paper disc diffusion method against 11 identified microbes.

Table (1) declared that these oils have variable antimicrobial activity against all tested strains. From these results, the variation in quantities of different components might be responsible for the different antimicrobial activities [32]. The inhibition zones were in the range of 7 to 18.5 mm. All microbial strains showed variable susceptibility to the Artemisia herba alba. *Fusarium solani* and *Candida albicans* strains consecutively, revealed less susceptibility to the effects of *Artemisia herba alba*. This may be attributed to their biological resistibility. Among all gram-positive bacteria growths, the maximum zone of inhibition was recorded against *E. faecalis*; 18.5 mm, followed by *B. cereus*: 15.0 mm and *Staphylococcus aureus*: 13.5 mm. On the other hand, six different gram-negative bacterial strains were tested and among these microorganisms, *S. typhimurium* showed maximum zone of inhibition: 17.5 mm, followed by *K. pneumoniae*: 15.5 mm, *Shigella dysenteriae*: 14.0 mm, *Pseudomonas aeruginosa*: 13.0 mm, *Acinetobacter*: 12.5 mm and *Escherichia coli*: 11.0 mm respectively. In regard to fungal strains tested, the maximal inhibition zone was observed against *Candida albicans*: 9.0 mm, followed by *Fusarium solani*: 7.5 mm. In contrast of antimicrobial activities of Artemisia herba alba extracts, the oils did show much more maximal bacterial inhibitory effects on the strains of bacteria subjected to tests than the fungal isolates examined. These finding can be supported by those obtained by Ahmeethunisa and Hopper [33], Abou El-Hamd *et al*. [34], Mighri *et al.* [35, 36] and Zouari *et al*. [20] who found antimicrobial activities of *Artemisia herba alba* against several pathogenic microbes including gram-positive bacteria (*Streptococcus hemolyticus* and *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus*) and gram-negative bacteria involving : *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Sallmonella typhimurium*, *S. typhi*, *Pseudomonas aeruginosa* and *Acinetobacter*. The results assure the importance of using *Artemisia herba alba* oil extracts in the therapeutic programmes of microbial infections for humans.

The antimicrobial activity of Ah has been tested. for example, it has been noted that all examined essential oils of Ah had a great potential on antimicrobial activity against strains of *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus* and *Enterococcus faecalis*. In addition, the antimicrobial activity of Ah has been confirmed in some yeast strains of *Candida* (*C. albicans*, *C. globrata*, *C. tropicalis* and *C. sake*) [35, 36]. The antimicrobial and antifungal activities of Ah have been demonstrated by measuring the diameter of the zones inhibition of several microorganisms, the results obtained were significant and comparable to the Gentamicin antibiotics [36]. Furthermore, a stronger growth inhibitory activity of the plant on many fungi has been established; the antifungal activities were tested using *Fusarium solani*, *Fusarium sp*, *Aspergillus oxysporum* and *Candida albicans* [20].

Plants often contain wide variety of antioxidant molecules, such as phenolic acids, flavonoids and other natural antioxidants. Generation of free radicals may be, at least partially, the basis of many human diseases and conditions. Therefore, the antioxidant action of Ah may explain its claimed usefulness in folk medicine. Ah yields an aromatic essential oil which is rich in monoterpenes and seqquiterpene lactones widely distributed in plants and possess anti-inflammatory and anti-carcinogenic activities.

It has also shown that Ah essential oils were found to have some antioxidant abilities for preventing the linoleic oxidation and to reduce DPPH radicals (2, 2-diphenyl-l-l-picrylhydrazil) and stable ABTS radicals (2, 20 azinobis-3ethylbenzthiazoline-6- sulphonic acid). This effect seems to be due to the rich phenolic compounds in Ah [21, 37, 38] Additional studies have confirmed the antioxidant effect of Ah [20, 39].

The essential oil of *Artemisia herba alba* showed weak to good fungal inhibitory effects on the test fungi named *Fusarium solani* and *Candida albicans* strains respectively. The obtained results in the present work were in unaccordance with Younes *et al*. [32] who detected that the essential oil exhibited a strong growth inhibitory activity against all the studied fungi of *Fusarium solani*, *Fusarium sp* and *Aspergillus oxysporum*. In general, the recorded results regarding the antibacterial activities of *Artemisia herba alba* oil extracts showed that these activities were higher than the antifungal effects respecting growth inhibition zones established.

As mentioned by Younes [32] and Abou El-Hamd [34], the antibacterial activity and/or antifungal activity of *Artemisia herba alba* essential oils as observed in *A. arborescens* would be related to their oxygenated monoterpenes components which constitute more than 50.9% of the oils. Indeed, in essential oils, it was shown that monoterpenes hydrocarbons and oxygenated monoterpenes are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration [24, 40]. However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antibacterial and/or microbial activity exhibited. In oils, the possible compounds synergistic and antagonistic effects would play an important role in bacterial inhibition and should also be taken into consideration. It has been recognized that many pathogenic microorganisms can be infected humans by various ways such as inhalation, direct contact, foodstuffs and drinking water etc., because of their apparently contagious ability. One of the problems limiting the prevention and controls programs of these pathogens, has been the lack of development of adequate laboratory microbial examinations, natural extraction of medical plants oils and therapeutic antibiotics and drugs [41, 42, 43, 44].

From these results, it is concluded that the essential oils of *Artemisia herba alba* have a capacity to inhibit the growth of both gram-positive and gram-negative bacterial strains and thriving of fungal isolates. Further, they showed an interesting activity for some tested strains. More studies must be done on the role of different antimicrobial plants extracts in relation to treatment and control of microbial diseases.

REFERENCES

1. Banerji, A., D.L. Luthriya and S.D. Kokate, 1990. Toxicity of capillin, the insecticidal principle of Artemisia nilagirica Clarke. Indian J. Exp. Biol., 28: 588-589.
2. Shafi, P.M., M.K.G. Nambiar, R.A. Clery, Y.R. Sarma and S.S. Veena, 2004.Composition and antifungal activity of the oil of Artemisia nilagirica (Clarke) Pamp. JEOR, 16:377-379.
3. Ambasta, S.P., 1986. The useful plants of India. Publications & Information Directorate.CSIR, New Delhi, pp: 55-56.
4. Priscila, I.U., T.N.S. Mariama, C.D.S. Luiz, B. Luciano and A.J. Fernandes, 2007. Antibacterial activity of medicinal plant extracts. Braz J. Microbiol., 38: 717-719.

5. Juteau, F., V. Masotti, J.M. Bessiere, M. Dherbomez and J. Viano, 2002. Antibacterial and antioxidant activities of Artemisia annua essential oil.Fitoterapia, 73:532-535.
6. Cha, J.D., E.K. Jung, B.S. Kil and K.Y. Lee, 2007. Chemical composition and antibacterial activity of essential oil from Artemisia feddei. J. Microbiol. Biotechnol., 17: 2061-2065.
7. Xie, G., I.A. Schepetkin, D.W. Siemsen, L.N. Kirpotina, J.A. Wiley and M.T. Quinn, 2008. Fractionation and Characterization of Biologically-active Polysaccharides from Artemisia tripartite. Phytochemistry, 69: 1359-1371.
8. Avula, B., Y.H. Wang, T.J. Smillie, W. Mabusela, L. Vincent, F. Weitz and I.A. Khan, 2009. Quantitative determination of flavonoids by column high-performance liquid chromatography with mass spectrometry and ultraviolet absorption detection in Artemisia afar and comparative studies with various species of Artemisia plants.J AOAC Int, 92: 633-44.
9. Ng, T.B., 2004. Antifungal proteins and peptides of leguminous and non-leguminous origins. Peptides, 25: 1215-22.
10. Pattison, L.T., D. Lindsay and A. Von Holy, 2004. Natural antimicrobial as potential replacements for calcium propionate in bread. S Afr. J. Sci., 100: 339-342.
11. Mueller, M.S., I.B. Karhagomba, H.M. Hirt and E. Wemakor, 2000. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. J. Ethnopharmacol., 73: 487-493.
12. Meneses, R., R.E. Ocazionez, J.R. Martínez and E.E.S. Tashenko, 2009. Inhibitory effect of essential oils obtained from plants grown in Colombia on yellow fever virus replication *in vitro*. Ann Clin Microbiol Antimicrob, 8: 8.
13. Emori, T.G. and R.P. Gaynes, 1993. An overview of nosocomial infections, including the role of the microbiology laboratory. Cllin Microbiol. Rev., 6: 428-442.
14. Maleki, S., S.M. Seyyednejad, N.M. Damabi and H. Motamedi, 2008. Antibacterial activity of the fruits of Iranian torilis leptophylla against some clinical pathogens. Pak J. Biol. Sci., 11: 1286-1289.
15. Setubal, J.C., L.M. Moreira and A.C. Da Silva, 2005. Bacterial phytopathogens and genome science. Curr. Opin Microbiol., 8: 595-600.
16. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Eighteenth informational supplement CLSI document M100-S18, Wayne, PA, USA 2008.
17. Moufid, A. and M. Eddouks, 2013. *Aretemisia herb alba*: A popular plant with potential medicinal properties. Pak J. Biol. Sci., 15: 1152-1159.
18. Al-Khazraji, SM., L.A. Al-Shamaony and H.A.A. Twaij, 1993. Hypoglycaemic effect of *Artemisia herba alba*. I. EEffect of different parts and influence of the solvent on hypoglycaemic activity. J. Ethnopharmacol., 40: 163-166.
19. Gose, I., A. Alim, S.A. CEtinus, N. Durmus, N. Vural and H.M. Goze, 2009. chemical composition and antioxidant, antimicrobial, antispasmodic activities of the essential oil of *Thymus Fallax Fisch*. J. Med Plants Res., 3: 174-178.
20. Zouari, S., N. Zouari, N. Fakhfakh, A. Bougatef, M.A. Ayadi and M. Neffati, 2010.Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba alba* Asso. J. Med. Plants Res., 4: 871-880.
21. Kadri, A., I.B. Chobba, Z. Zarai, A. Bekir, N. Gharsallah, M. Damak and R. Gdoura, 2011. Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba alba* grown in Tunisian semi-arid region. Afr. J. Biotechnol., 10: 2923-2929.
22. Alade, P.I. and O.N. Irobi, 1993. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana*. J. Ethanopharmacology, 39: 171-174.
23. Albashan, M.M., 2006. Studies on the antimicrobial activity of *Nigella sativa* Linn. Seeds (Black Cumin) Extract. Hamdard Medicus, Vol. XLIx, No. 1, pp: 169-179.
24. Zouari, S., N., Zouari, N. Fakhfakh, A. Bougatef, M.A. Ayadi and M. Neffati, 2010. Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba alba* Asso. Journal of Medicinal Plants Research, 4(10): 871-880.
25. Mohsen, H. and F. Ali, 2008. Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers, African J. Biotechnol., 7(1): 44-50.
26. Efferth, T., 2007. Antiplasmodial and antitumor activity of artemisinin-from bench to beside, Planta Med., 73: 299-309.

27. Wong, H. and G.D. Brown, 2002, Germacranolides from Artemisia myriantha and their conformation, Phytochemistry, 59: 529-536.
28. Kwak, J.H., W.Y.W.Y. Jang, O.P. Zee and R.K. Lee, 1997. A new Coumarin-Monoterpene ether from *Artemisia* keiskeana, Planta Med., 63: 474-476.
29. Tan, R.X., H. Lu, J.L Wolfender, T.T. Yu, W.F. Zheng, L.S. Yang, Gafner and K. Hostettmann, 1998. Mono-and sesquiterpenes and antifungal constituents from *Artemisia* species, Planta Med., 65: 64-67.
30. Bergendorff, O. and O. Sterner, 1995. Spasmolytic flavonols from *Artemisia abrotanum*, Planta Med., 61: 370-371.
31. Kim, J.H., H.K. Kim, S.B. Jeon, K.H. Son, E.H. Kim, S.K. Kang, N.D.B.M. Sung and B.M. Kwon, 2002. New sesquiterpene-monoterpene lactone, artemisolide, isolated from *Artemisia argyi*, Tetrahedron Lett., 43: 6205-6208.
32. Younes, K., S. Merghache, N. Djabou, D. Merghache, A. Muselli, B. Tabti and J. Costa, 2012. Chemical composition, antibacterial and antioxidant activities of a new essential oil chemotype of Algerian *Artemisia arborescence* L. African Journal of Pharmacy and Pharmacology, 6(42): 2912-2921.
33. Ahameethunisa, A.R. and W. Hopper, 2010. Antibacteria activity of *Artemisia nilagirica* leaf extracts against clinical and Phytopathogenic bacteria. BMComplementary and Alternative Medicine, 10:6.http://www.biomedcentral.com/1472-6882/10/6.
34. Abou El-Hamd, H.M., M.A. El-Sayed, M.E. Hegazy, S.E. Helaly, A.M. Esmail and N.S. Mohamed, 2010. Chemical constituents and biological activities of *Artemisia herba-alba*, Rec. Nat. Prod., 4(1): 1-25.
35. Mighri, H., H. Hajlaoui, A. Akrout, H. Najjaa and M. Neffati, 2010a. Antimicrobial and antioxidant of Artemisia herba alba essential oil cultivated in Tunisian arid zone. Comptes Rendus Chimie, 13(3): 380-386.
36. Mighri, H., A. Akrout, H. El-Jeni, S. Zaidi, F. Tomi, J. Casanova and M. Neffati, 2010b. Composition and intraspecific chemical variability of the essential oil from *Artemisia herba alba* growing wild in a Tunisian arid zone. Chem. Biodivers., 7: 2709-2717.
37. Al-Mustafa, A.H. and O.Y. Al-Thunibat, 2008. antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes. Pak. J. Biol. Sci., 11: 351-358.
38. Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohammad and T. El-Elimat, 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem., 04: 1372-1378.
39. Akrout, A., H. El-Jani, S. Amouri and M. Neffati, 2010. Screening of antiradical and antibacterial activities of essential oils of *Artemisia campestris*, *Artemisia herba alba* asso and *thymus capitatus* hoff. et link. Growing wild in southern of Tunisia. Recent Res. Sci. Technol., 2: 29-39.
40. Cox, S.D., C.M. Mann, J.L. Markham, H.C. Bell, J.E. Gustafson, J. R. Warmington and S.G. Wyllie, 2000. The mode of antimicrobial action of the essential oil of *Melaleuce altemifolia* (tea tree oil). J. Appl. Microbiol., 88: 170-175.
41. Al-Bashan, M.M., 2011. *In vitro* assessment of the antimicrobial activity and biochemical properties of camel's urine against some human pathogenic microbes. Middle-East Journal of Scientific Research, 7(6): 947-958.
42. Al-Bashan, M.M., 2012. Influences of some physic-chemical stress conditions on the survivality and resistibility of *Shigella flexneri* and *shigella sonii.* Global Veterinaria, 9(6): 706-716.
43. Al-Bashan, M.M., 2009. Acid tolerance of *Escherichia coli* O157:H7 Serotype and *Salmonella typhi* (A Group D Serotype) and their survival in apple and orange juices., 2009. World Journal of Medical Sciences, 4(1): 33-40.
44. Al-Tayib, Y.A. and M.M. Al-Bashan, 2007. Experimental studies on the potential for acid tolerance, growth and survival of *Salmonella enteric* Serovar *Typhimurium* and *Escherichia coli* O157:H7 in orange Juices. Advances in Biological Research, 1(3-4): 99-107.