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ANTIMICROBIAL ACTIVITY AND HEAVY METALS FINDINGS OF *Euryops arabicus* (JABUR) LEAVES AGAINST PLANT AND HUMAN PATHOGENS

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Plant-pathogenic fungi and public human health are subject of utmost importance to the researchers; therefore, it is necessary to find alternative potent antimicrobial agent. Effectiveness of seven extracts from *Euryops arabicus* plants were tested against twelve of plant and human pathogens using well diffusion methods. Herbal plants are the main sources of many useful chemicals compounds, therefore, chemicals profiling by GC-MS and heavy metals were estimated for avoiding the risk of health hazards. Plants pathogens include *Alternaria alternata*, *Drechslera halodes*, *Fusarium oxysporum* F. sp. *lycopersici*, *Penicillium expansum*, *Pythium ultimum* and *Rhizoctonia solani* and human pathogens include *Staphylococcus aureus*, *Shigella flexneri*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Candida albicans* were inhibited to various degrees by chemicals extract from *Euryops arabicus* plants. Aqueous extraction by cold water did not show any activity against all tested microbes while hot water extract had an activity only against human pathogens. In all cases the effectiveness of *Euryops arabicus* extract against human pathogens more potent than plant pathogens also fresh extraction had an activity more than dry extractions. Chloroform and diethyl ether extracts had the highest activities against *Klebsiella oxytoca* and *Candida albicans* while *Staphylococcus aureus* is the least pathogenic bacterial strains inhibited by *Euryops arabicus* solvent extracts. Diethyl ether extracts had the maximum affect on the growth of all tested plant pathogens, while acetone extract had the minimum affect. Methanol and chloroform extracts exhibited almost the same trends of inhibition activities against *Drechslera halodes* and *Pythium ultimum.* GC-MS showed that *Euryops arabicus* leaves contains chemicals exhibit important biological activities as antimicrobials and antifungal. Trace amount of heavy metal was found in *E. arabicus* leaves. Therefore, *E. arabicus* leaves could be used as an important source to get natural antimicrobial agents for both plant and human pathogens.

Keywords: Antimicrobial, dry extract, *Euryops arabicus*, fresh extract, heavy metals, human pathogens, plant pathogens.

INTRODUCTION

Asteraceae or compositae is one of the major family in vascular plants including shrubs and herbaceous plants comprises of 1100 genera and 25,000 species famous in the tropical and subtropical regions (Underberg *et al*., 2007; Arekemase *et al*., 2013). Among 100 species of the genus *Euryops*, only *Euryops arabicus* is well known in Saudi Arabia country *E. arabicus* is small, erect undershrub reaching about 1 meter with intriguing 2 inches in long slender shape with lobed green rugged leaves grouped at the tips of each branch. In the Arabian Peninsula the warmed leaves and stems usually utilized for the treatment and mending the wounds (Abulafatih, 1987; Mossallam and zaid, 2000). Previous research showed that genus *Euryops* contains many chemicals such as secofuroermophilanes, flavonoids, furoermophilanes and eremophilanolides (Ozcelik *et al*., 2006). The extract of *E. arabicus* collected from Al-Taeif, KSA, was investigated for its anti-oxidant and protective activities against renal injuries and paracetamol-induced hepatic in rats and found that it could protect the kidney and liver from the toxicity ((Hafez *et al*., 1989). Recent research by Moustafa *et al.* (2016), found that *E. arabicus* extracts had a significant cytological and physiological effects on the growth of Chinese faba bean (*Vicia faba* L.) seedlings. Surprisingly, many scientists found a lot of novel antimicrobial chemicals however the resistance of the pathogenic microbial strains to these drugs was also increased (Adwan and Mhanna, 2008). At present natural plant extract enthusiasm many researchers all over the world to do their best to find an alternative antimicrobial agent for their safety and low prices. For example, Khan *et al*. (2017) and Alamri and Moustafa (2010) evaluated the antimicrobial properties of some medicinal plant growing in various localities. It was found that many plants had potent antimicrobial activities against human pathogenic microorganism and provided significant biological activities. Because there was strong correlation between plant chemicals composition and geographic plant distribution (Yavari *et al*., 2010), the need to find save and innovative antimicrobial drugs for infected

plant and humans has also increased. El Soda Mountains, Abha region, Saudi Arabia is characterized by an amazing natural environmental condition such as humidity, rainfall and elevation (about 2900 meter height), subsequently supporting a large number of diverse plant groups. Since members of Asteraceae showed a significant antimicrobial effect against various strains of bacteria, fungi and against human tumor cell lines (Edris, 2007; Yaser *et al*., 2014). Therefore, this study aimed to investigate antimicrobial inhibition activities of some solvents extract of dry and fresh leaves of *E. arabicus* growing in El Souad Mountain (2750 meters height), KSA, against some plant and human pathogens. For validation to use the *E. arabicus* plants as save antimicrobial agents, the amounts of heavy metals in leaves have been evaluated.

MATERIALS AND METHODS

***Extraction of bioactive substances*:** 249 grams from fresh leaves of *E. arabicus* plants were collected from El-Souda Mountains, KSA. Half of the sample (124.5 g) was dried in shadow at room temperature (23°C) and the rest was ground in a grinding apparatus for 20 minutes, then filtered using double-layered muslin cloth. Dry extraction was done by adding seven grams of grinded powder to 20 milliliters to solvent extract including acetone, chloroform, diethyl ether, methanol, petro ether, hot water and cold water. Fresh extraction was accomplished by adding seven milliliters of fresh filtrate to 20 milliliters of solvent extract. All samples were kept in incubator shaker for 72 hours at 170 rpm in a room temperature. The solvent was evaporated totally from the sample using air-drying oven at 49°C (Salvat *et al*., 2004). All solvents extract was weighted and saved in a refrigerator at 4°C for additional use.

***Inoculums*:** Bacteria, fungi and yeast cultures were obtained from the Biology Department and Microbiology Department, Faculty of Science and Faculty of Medicine, King Khalid University, Saudi Arabia. Human pathogenic bacteria and Candida strains include *S. aureus*, *S. flexneri*, *M. luteus*, *P. aeruginosa*, *K. oxytoca*, *P. mirabilis* and *C. albicans*. Plant pathogenic fungal include *A. alternata*, *D. halodes*, *F. oxysporum* F. sp. *lycopersici*, *P. expansum*, *P. ultimum* and *R. solani*. Old strains from bacteria were first subcultured on nutrient agar broth (NAB) for 24 h while fungal strain subcultured on Potato dextrose broth (PDB) for 48 h and placed in a rotary shaker at 37 °C and spectrophotometrically the cells density was standardized at OD 600 nm (Mahesh and Satish, 2008).

***Antimicrobial assay*:** The Well Diffusion Method (WDM) with little modification was used to examine pathogens susceptibility to the *E. arabicus* extracts. The surface of solidified nutrient agar and Potato dextrose agar (oxoid) were first swabbed equally with pathogenic bacterial strains about (5×105 cfu/ml) and with fungal strains (2.5×103 cfu/ml). One well of ring-shape (6.0 mm) in diameter was made in solidified agar plate using sterilized cork borer. *E. arabicus* extracts were dissolved in sterile dimethyl sulfoxide (DMSO). 150 µl volume of extract was inoculated in triplicate wells then kept at room temperature (24°C) for 60 minutes. Cefotoxin (30 µg/disc) and Fluconazole (30 µg/disc) were applied as positive control for pathogenic bacteria and fungi and dimethyl sulfoxide (DMSO) as negative control (Efstratiou *et al*., 2012). The assayed plates were incubated at 31°C for 24 h and 48 h for tested pathogenic bacterial and fungal strains. All examination was carried out in triplicates and the antimicrobial inhibition activity determined based on diameter size of clear zone in agar plate. Clear zone is expressed as mean of the three measurements ± standard deviation.

***Crude extract GC-MS analysis*:** Gas chromatography-mass spectrometry analysis the chemicals that present in the crude extract of *E. arabicus* by using a Perkin–Elmer GC Clarus 500 system as described previously (Ezhilan and Neelamegam, 2012). The mass spectrums of unknown chemicals were identified by sing the data base of NIST library (The National Institute of Standards and Technology). The chemicals name, molecular weight and molecular formula were characterized.

***Determination of heavy metals*:** After the plants were collected from the growing sites, its leaves were subjected to airdrying at room temperature (24°C). Then, the dried leaves were subjected to acid digestion by Nitric acid and Perchloric Acid (3:1v/v) in microwave (Allen 1989). Ferrous, aluminum, manganese, zinc, copper, nickel, lead and cobalt were evaluated, in the 0.5 g of *E. arabicus* leaves and in blank sample by using an atomic absorption spectrophotometer (Shimadzu Co. Ltd., Japan).

RESULTS

Antimicrobial inhibition activity of methanol, chloroform, petroleum ether, acetone, diethyl ether from succulent leaves of *E. arabicus* at 9 mg/ml revealed that all of them had variable effects against fungal and human pathogens as shown in Figure 1 and 2. Aqueous extraction by hot water had activity against *S. aureus*, *S. flexneri*, *M. luteus*, *P. aeruginosa*, *K. pneumonia*, *P. mirabilis* and *C. albicans* while no any activity against tested plant pathogens. The results also showed that cold extract either from dry or fresh leaves had no any antimicrobial inhibition activities against all tested pathogens. *K. oxytoca* and *C. albicans* are the most susceptible microbes by the chloroform and diethyl ether fresh leaves extracts with inhibition zone about 31.83±0.11 to (31.57±0.58 mm. The second highest activity achieved against *P. mirabilis* gained from fresh leaves methanol extract with an inhibition zone of (30.77±0.28 mm) followed by petroleum ether extract (29.13±0.0197 mm) while hot water extract found to have the least activity (13.97±0.23 mm).

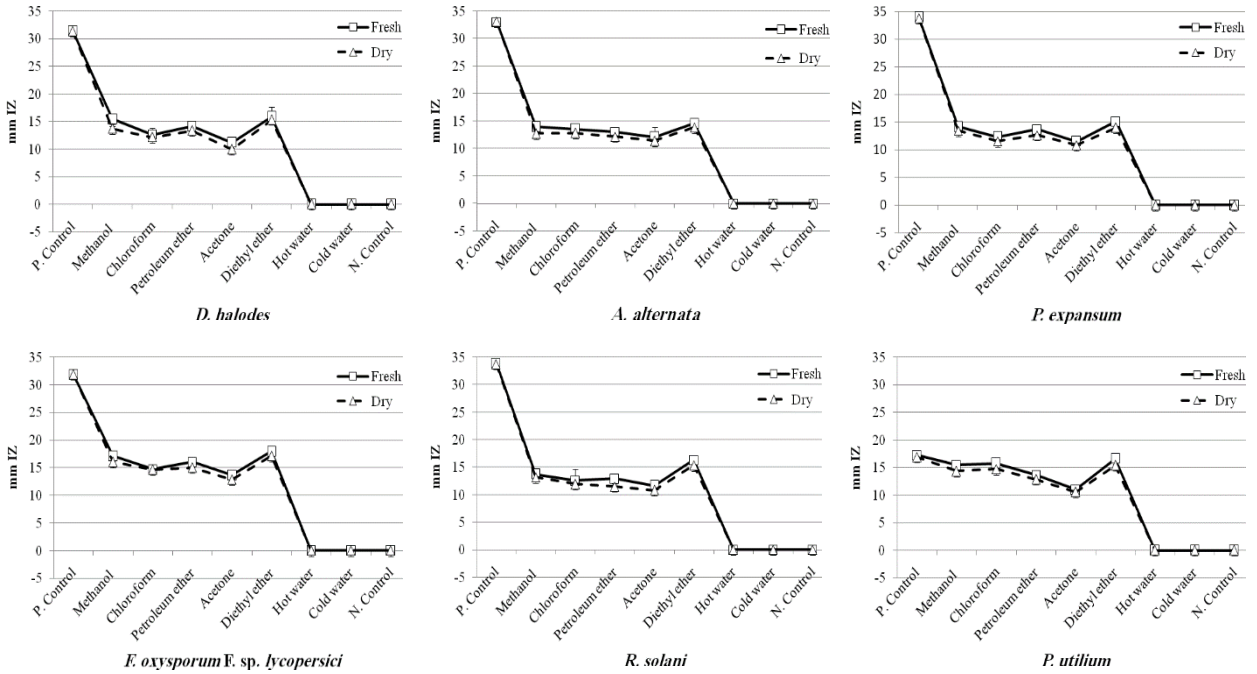

Figure 1. Antifungal activities of fresh and dry extract of *E. arabicus*. Bars (mean value ± SD, n= 3). Error bars (SD) in some measurements were smaller than the size of the symbols used. mm IZ; millimeters of inhibition zone.

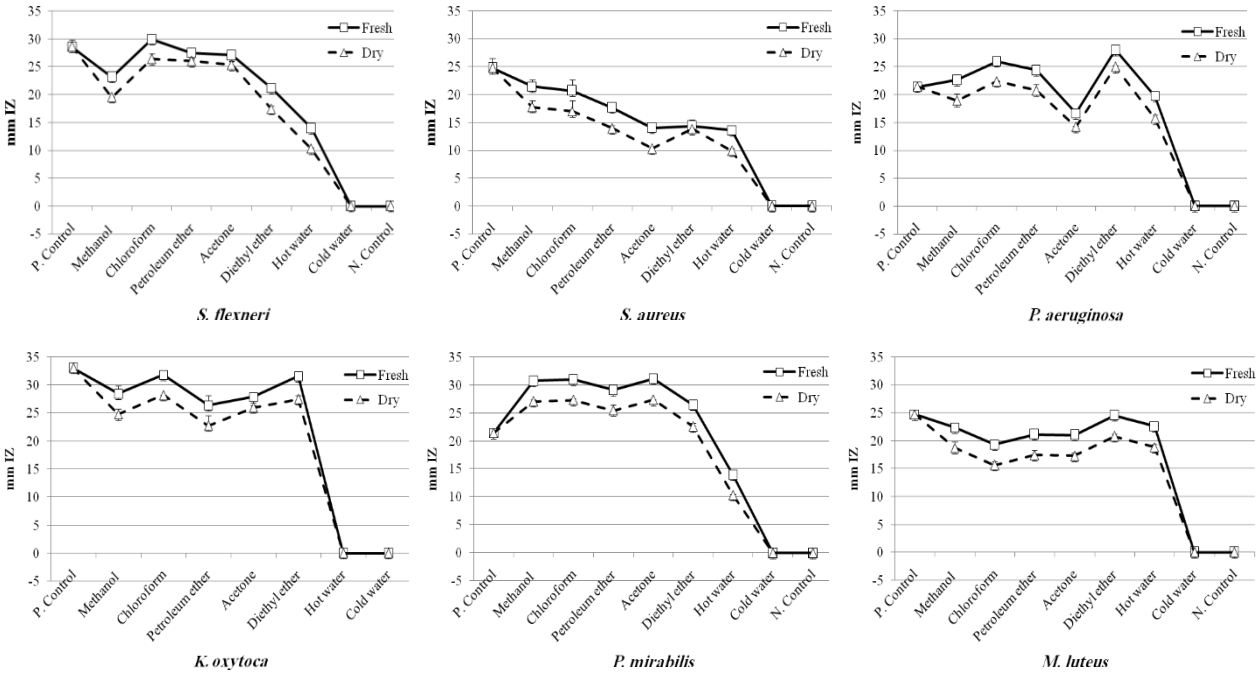

Figure 2. Antimicrobial activities of fresh and dry extract of *E. arabicus*. Bars (mean value ± SD, n= 3). Error bars (SD) in some measurements were smaller than the size of the symbols used. mm IZ; millimeters of inhibition zone.

S. flexneri and *P. aeruginosa* are greatly inhibited by chloroform extract (29.97±0.16 mm) and diethyl ether extract (28.03±0.91 mm) respectively. *S. aureus* is the least susceptible pathogenic bacterial strains whereas methanol, chloroform and petroleum ether fresh leaves extracts showed inhibition zone between (21.47±1.16 to 17.63±0.54 mm), and

the inhibition activity from acetone, diethyl ether and hot water fresh leaves extracts between (14.00±0.17 to 13.63±0.40 mm). The results showed that inhibition activity of solvent extract of *E. arabicus* against human pathogenic microbes more potent than plant pathogens in a range between (46.13 to 64.78%). Diethyl ether extract had the highest effect to all plant pathogens with inhibition zone between (17.93±1.85 to 14.53±0.57 mm) followed by methanol extract on the *F. oxysporum* F. sp. *lycopersici* (17.13±1.34 mm). Methanol and chloroform extracts exhibited almost the same inhibition activities on the growth of *D. halodes* and *P. utilium* with inhibition zone between (15.43±0.76 to 15.73 ±0.26 mm). The least activities in all plant pathogens gained from acetone extract with inhibition activities between (11.63±0.28 to 13.63±0.28 mm). The trend of activities obtained from dry leaves almost same as from fresh leaves against tested human and plant pathogens. However, the activities from fresh extraction had more antimicrobial activities than dry extraction in arrange between (3.64 to 17.71%). Very little amount of heavy metal was found in the leaves of *E. arabicus* as the concentration ranged between 0.05883 ppm for ferrous to 0.00008 ppm for cobalt in the following order: Fe > Al > Cr > Mn > Zn > Cu > Ni > Pb > Co (Table 1). The identified chemicals by GC-MS present in the crude extracts of *E. arabicus* are presented in the (Table 2). Five chemicals were identified in following order, Acetaldehyde (22.70%), H-1,2,4-Triazole (21.21%), Acetic acid (20.30%), Methylhippuric acid (18.68%) and Formamide (17.19%) (Table 2).

Table 1. Heavy metal in *E. arabicus* leaves.

Heavy metal	Concentration in ppm
Cr	4.8 ±0.005075E-17
Mn	1.4 ±0.003802E-15
Fe	3.2 ±0.058838E-15
Co	6.43 ±0.00008E-06
Ni	4.2 ±0.000160E-18
Cu	3.1 ±0.000490E-18
Zn	2.6 ±0.001799E-17
Pb	6.7 ±0.000125E-18
Al	2.2 ±0.047636E-16

Table 2. GC-MS analysis of extract of *E. arabicus* leaves.

Compounds	Rt Time	% Area	Molecular Weight	Chemical Formula
Acetaldehyde	3.225	22.70	44.053	C_2H_4O
1H-1,2,4-Triazole	27.56	21.12	69.067	$C_2H_3N_3$
Acetic acid	34.25	20.30	60.050	CH_3CO_2H
Methylhippuric acid	38.52	18.68	193.19	$C_{10}H_{11}NO_3$
Formamide	42.38	17.19	45.040	$HCONH_2$

DISCUSSION

Plant and human diseases are still crucial problem throughout the world. Antibacterial activities gained from this study in agreements to some extent with the previous investigation from the oils of two Asteraceae genus including *Laggera decurrens* (Vahl) and *Euryops arabicus* (Steud) growing in Indonesia (Mothana *et al*., 2011). To the best of our information no references found matching either antifungal activities and the amount of heavy metal of *E. arabicus* plant. This research proofed that efficacy of *E. arabicus* plant towards plant pathogens is less than human pathogens probably due to the difference in the nature of cell wall of investigated microbes. Many studies simultaneously showed the differences between fungi and bacteria, especially in the their metabolic activity in response to the drugs (Meidute *et al*., 2008; Brandstätter *et al*., 2013; Fabian *et al*., 2017). It was interpreted that the outer layer of both fungal and bacterial cells differs in the structure of nucleotide peptidoglycan proteins, oligomerization domain proteins, ctypelectins and N- and O-linked mannans etc.) that ultimately affect the efflux of antimicrobial compounds (Sukhithasri *et al*., 2013). Although, the composition of cell wall of Gram-negative and Gram-positive and the surface charge differ greatly (Shrivastava *et al*., 2007; Tamboli *et al*., 2013), however, our results showed that the *E. arabicus* chemicals extracts were active against all tested pathogenic bacteria except for *S. aureus*. Paralleled to the earlier study that showed the same chemicals compound may inhibit specific bacterial strains (Wattanastcha *et al*., 2012), and this ensure the resistance of *S. aureus* to many classes of antibiotics through expression of a foreign substances causing fatal infection so why such strains need urgent research and manual therapists (Stapleton and Taylor, 2002; Green *et al*., 2012). In current study extract from cold water had no inhibition zone against all tested microbes in agreement with study by (Bojlul *et al*., 2016) who found that extraction by cold water gained from *Ficus vesiculosus* plant did not contain any bioactive compound/s. Studies about extraction by chemicals or by hot water tend to precipitate the actives chemical compound/s having the ability to cease the growth of pathogenic microorganism ((Bakht *et al*., 2011). In addition, it has been established that polar and no polar chemicals such as acetone, chloroform, petroleum ether, etc, could extract more antimicrobial agents than cold water (Moustafa and Alrumman, 2015). The higher sensitivity of *K. oxytoca* and *C. albicans* to the solvent extracts means there must be specific natural antimicrobial agent so it is worthwhile to identify and quantity them in *E. arabicus* plants. As it was reported that *C. albicans* can easily transform from peaceful fungi causing Candidiasis infections (Gozalbo *et al*., 2004) and *K. oxytoca* reported as a main cause for leukemia, solid tumours, etc (Watson *et al*., 2005). In all cases antimicrobial efficacy gained from fresh leaves against pathogens either to plant or human more potent than dry leaves in the form of inhibition zone. Usually drying causes the complete or partial lose of volatile oil, in agreement with previous study that fresh extraction gave excellent result than

dry extraction as in *Rosa abyssinica* (Moustafa and Alrumman, 2015), *Zingiber officinale* (Sasidharan *et al*., 2010) and *Lippia gracilis* (Bitu *et al*., 2012). The present study also showed that level of heavy metal including ferrous, aluminum, manganese, zinc, copper, nickel, lead and cobalt was very low as compared to that present in edible foods. For example, the estimated concentrations of heavy metal in some edible vegetables and fruits were between 0.5 and 100 ppm (Manahan, 2001; Lujain *et al*., 2017). In addition, this detected amount of heavy metal beyond the allowed limit of 2.0 ppm as evaluated by WHO (2007) for raw herbal materials. As it was reported that there was strong correlation between microbial growth and heavy metal concentration (Rathnayake *et al*., 2013) and this explain that antimicrobial properties of *E. arabicus* probably due to the specific certain chemical/s compound but not because of heavy metal. Characterized chemicals by GC-MS showed various biological effects. For example, acetaldehyde plays a crucial role of pest mortality (Simpson *et al*., 2003), triazoles used as antimicrobial, anticancer, anti-inflammatory, insecticidal, antimycobacterial and many else (Kumar and Kavitha, 2013).

***Conclusion*:** Therefore, it can be concluded that the succulent leaves of *E. arabicus* have antimicrobial properties against human microbes and plants pathogens, deserving further study for isolation and characterization the safe bioactive chemicals.

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REFERENCES

Abulafatih, H.A. 1987. Medicinal plants in Southwestern Saudi Arabia. Econ. Bot. 41:354-60.

Adwan, G. and M. Mhanna. 2008. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Middle East J. Sci. Res. 3:134-139.

Alamri, S.A. and M.F. Moustafa. 2010. Antibacterial activity of the latex of *Argemone ochroleuca* Sweet. Saudi Med. J. 31:1207-1210.

Allen, S. 1989. Chemical analysis of ecological materials. Blackwell Scientific Publications, London.

Arekemase, M.O., G.P. Oyeyiola, and K.I. Balogun. 2013. Assessment of *Vernonia amygdalina* on some selected pathogenic microorganisms from university of Ilorin teaching hospital. J. Microbiol. Biotechnol. Food Sci. 2:2360-2365.

Bakht, J., M. Tayyab, H. Ali, A. Islam and M. Shafi. 2011. Effect of different solvent extracted sample of *Allium sativum* (L.) on bacteria and fungi. Afr. J. Biotechnol. 10:5910-15.

Bitu, V., M.A. Botelho, J.G.M.d. Costa, F.F.G. Rodrigues, H.N.H. Veras, K.T. Martins, A. Lyra, G.G. Coluchi, R.S. Ruela, D.B. Queiroz, J.d.S. Siqueira and L.J. Quintans-Junior. 2012. Phythochemical screening and antimicrobial activity phythochemical of essential oil from *Lippia gracillis*. Rev. bras. Farmacogn. 22:69-75.

Bojlul, B., V.O. John, J.S. Thomas, M.A. Albin and S. Torres. 2016. A cold water extract of *Fucus vesiculosus* inhibits lipopolysaccharide (LPS) induced pro-inflammatory responses in the porcine colon ex-vivo model. Innov. Food Sci. Emerg. Technol. 37:229-236.

Brandstatter, C., K. Keiblinger, W. Wanek and S. Zechmeister-Boltenstern. 2013. A closeup study of early beech litter decomposition: Potential drivers and microbial interactions on a changing substrate. Plant Soil 371:139-154.

Edris, A.E. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytother. Res. 21:308-323.

Efstratiou, E., A. I. Hussain, P.S. Nigam, J.E. Moore, M.A. Ayub and J. R. Rao. 2012. Antimicrobial activity of *Calendula officinalis* petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. Complement Ther. Clin. Pract. 18:173-176.

Ezhilan, B. P. and R. Neelamegam. 2012. GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Pharmacogn. Res. 4:11-14.

Fabian, J., S. Zlatanovic, M. Mutz and K. Premke. 2017. Fungal-bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality. ISME J. 11:415-425.

Gozalbo, D., P. Roig, E. Villamón and M.L. Gil. 2004. Candida and candidiasis: the cell wall as a potential molecular target for antifungal therapy. Curr. Drug Targets 4:117-135.

Green, B.N, C.D. Johnson, J.T. Egan, M. Rosenthal, E.A. Griffith and M.W. Evans. 2012. Methicillin-resistant *Staphylococcus aureus*: an overview for manual therapists. J. Chiropr. Med. 11:64-76.

Hafez, S., J. Jakupovic, F. Bohlmann, T.M. Sarg and A.A. Omar. 1989. Transformation products of furoeremophilanes and other constituents from *Euryops arabicus*. Phytochemistry 28:843-847.

Khan, S., M. Imran, M. Imran and N. Pindari. 2017. Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant Candida spp. *Bioinformation* 13:67-72.

Kumar, S.S. and H. P. Kavitha. 2013. Synthesis and biological applications of triazole derivatives– a review. Mini Rev. Org. Chem. 5:40-65.

Lujain, H.I. and A.A. Salwan. 2017. Accumulation detection of some heavy metals in some types of fruits in the local

market of Al-diwaniyah city, Iraq, Rasayan. J. Chem. 10:339-343.

Mahesh, B. and S. Satish. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci. 4:839-843.

Manahan, S.E. 2001. Fundamental of environmental chemistry: Boca Raton: CRC Press, LLC.

Meidute, S, F. Demoling and E. Baath. 2008. Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. Soil Biol. Biochem. 40:2334-2343.

Mossallam, H.A, and S.A. Ba zaid. 2000. An illustrated guide to the wild plants of Taif. Taif University, Botany Dept., Kingdom of Saudi Arabia.

Mothana, R.A, M.S. Alsaid and N.M. Al-Musayeib. 2011. Phytochemical analysis and *in vitro* antimicrobial and free-radical-scavenging activities of the essential oils from *Euryops arabicus* and *Laggera decurrens*. Molecules 16:5149-5158.

Moustafa, M.F., S.A. Alrumman, H. Zaki, N. Loutfy and M. Hashem. 2016. Cyto-physiological effects of aqueous extracts of some weeds and clove on the growth of Chinese faba bean (*Vicia Faba* L.). J. Adv. Biol. Biotechnol. 9:1-8.

Moustafa, M.F. and S.A. Alrumman. 2015. First report about pharmaceutical properties and phytochemical analysis of *Rosa abyssinica* R. Br. ex Lindl. (Rosaceae)". Pak. J. Pharm. Sci. 28:2009-2017.

Ozcelik, B., I. Orhan and G. Toker. 2006. Antiviral and antimicrobial assessment of some selected flavonoids. Z. Naturforsch C. 61:632-638.

Rathnayake I.V.N., M. Megharaj, G.S.R. Krishnamurti, N.S. Bolan and R. Naidu. 2013. Heavy metal toxicity to bacteria – Are the existing growth media accurate enough to determine heavy metal toxicity? Chemosphere 90:1195-1200.

Salvat, A., L. Antonacci, R.H. Fortunato, E.Y. Suárez and H. M. Godoy. 2004. Antimicrobial activity in methanolic extracts of several plant species from Northern Argentina. Phytomedicine 11:230-234.

Sasidharan, I. and N. Menon. 2010. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (*Zingiber officinale* Roscoe). Int. J. Curr. Pharm. Res. 2:40-43.

Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao and D. Dash. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 18:225103-225111.

Simpson, T., V. Bikoba and E. J. Mitcham. 2003. Effects of acetaldehyde on fruit quality and target pest mortality for harvested strawberries. Postharv. Biol. Technol. 28:405–416.

Stapleton, P.D. and P.W. Taylor. 2002. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. Sci. Prog. 85:57–72.

Sukhithasri, V., N. Nisha, L. Biswas, V. Anil Kumar and R. Biswas. 2013. Innate immune recognition of microbial cell wall components and microbial strategies to evade such recognitions. Microbiol. Res. 168:396-406.

Tamboli, D.P. and D.S. Lee. 2013. Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram-negative bacteria. J. Hazard Mater. 260:878-884.

Watson, J.T., R.C. Jones, A.M. Siston, J.R. Fernandez, K. Martin, E. Beck, S. Sokalski, B.J. Jensen, M.J. Arduino, A. Srinivasan and S.I. Gerber. 2005. Outbreak of catheter associated *Klebsiella oxytoca* and *Enterobacter cloacae* bloodstream infections in an oncology chemotherapy center. Arch. Intern. Med. 165:2639-2643.

Wattanasatcha, A., S. Rengpipat and S. Wanichwecharungruang. 2012. Thymol nanospheres as an effective anti-bacterial agent. Int. J. Pharm. 434:360-365.

WHO. 2007. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. World Health Organization, Geneva.

Yaser, G.G., M.A.A. Qasem, A.B. Abdulrahman, K.A. Adel, S.A. Ahmed and M.M. Khaled. 2014. Cytotoxic And antimicrobial activities of some Compositae plants growing in Taif area, Saudi Arabia. Int. J. Pharm. Sci. Invent. 3:43-48.

Yavari, A., V. Nazeri, F. Sefidkon and M.E. Hassani. 2010. Influence of some environmental factors on the essential oil variability of *Thymus migricus*. Nat. Prod. Commun. 5:943-948.