International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-2, Issue-3, March 2016
ISSN: 2395-3470
www.ijseas.com

Antibacterial, antifungal, antitumor and toxicity of essential oils of *Salvia officinalis, Thymus vulgaris, Eugenia caryophyllata* and *Artemisia absinthium*

Dalal Hussien M. Alkhalifah, Associate Professor Microbiology of Biology Dept., Faculty of Sciences Princess Nourah bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia

Introduction

Antimicrobial chemotherapy has conferred huge benefits on human health. Many microorganisms that have acquired resistance to drugs through a variety of mechanisms have emerged and continue to plague human beings (Tomoo and Keizo, 2009). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Srour, 2000). Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity (Juven *et al*, 1994). The use of plant compounds to treat infection is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (Gangoue-pieboji, *et al*., 2006). Traditional healers have long used plants to prevent or cure infectious conditions and plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999).

Sage (*Salvia officinalis* L., Lamiaceae) has antimicrobial potential (Bozin *et al*., 2007) and is used as a medicinal plant to treat dental diseases in Brazil (Oliveira *et al*., 2007). The antimicrobial activity may probably present because the essential oils are

composed of several substances that can interact jointly in a synergic/potential form, giving them strong antimicrobial activity (Delamare *et al*., 2007; Faleiro *et al*., 2003).

The genus *Artemisia* consists of small herbs and shrubs (Baytop *et al*. 1984; Davis, 1982) and has much pharmaceutical interest (Baytop *et al*. 1984; Kalemba *et al*., 2002). *Artemisia* species have been used in folk remedies as an antipyretic, antiseptic, antihelminitic, tonic, diuretic and for the treatment of stomach ache (Baytop *et al*. 1984).

E. caryophyllata (clove) was found to be effective against egg and adult of *Pediculus capitis* (Yang *et al*., 2003). It has antiseptic as well as bacteriostatic and bactericidal activity against *E coli* and *S. aureus* (Burt and Reinders, 2003; Ernst, 2001). Growth of *Helicobacter pylori* being one of the major causes of peptic ulcer disease has been shown to be inhibited by *E. caryophyllata* (Bae *et al*., 1998). Several studies have demonstrated potent antifungal activity (Arina & Iqbal, 2002; Giordani *et al.,* 2004; Pawar & Thaker, 2006; Park *et al.,* 2007), antiviral (Chaieb *et al.,* 2007a) and antibacterial effects of clove (Cai & Wu, 1996; Lopez *et al.,* 2005; Li *et al.,* 2005; Betoni *et al.,* 2006).

The antimicrobial and fungicidal activities of *Thymus vulgaris* (thyme), were analyzed (Panizzi *et al*., 1993) against Gram negative and positive bacteria (Marino *et al*., 1999). Carvacrol was identified to be a predominant compound in thyme oil (Karaman *et al*., 2001). The composition of essential oils of some *Thymus* species was analyzed and *in vitro* antimicrobial activities of its components were reported and antibacterial activity of methanolic extract of *T. pubescense* was detected (Azaz *et al*., 2004; Mehrgan *et al*., 2008).

Materials and Methods

Plant materials

Aerial parts, fruits, roots or leaves of the plants were collected from local markets in Jeddah, Saudi Arabia during summer 2012. The collected plant materials were put in clean plastic bags and transferred directly to the lab. The plants under investigation were *Salvia officinalis, Thymus vulgaris, Eugenia caryophyllata* and *Artemisia absinthium*

Tested organisms

Bacterial and fungal isolates were obtained from Biology Department, Faculty of Science, King Abdul-Aziz University (KAU), Jeddah. The bacterial strains were *Escherichia. coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853*, Klebsiella , A. baumannii* ATCC 1656-2*, P. mirabilis* ATCC 700603*pneumoniae* (ESBL) ATCC ATCC) 12453, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (MRSA *, and Enterococcus faecalis* (VRE) ATCC 51299. The tested fungi were 33591 *Aspergillus flavus*, *Aspergillus niger, Epidermophyton floccosum., Trycophyton mentagrophytes* and *Microsporum canis.* Two strains of yeasts were investigated in this study; *Candida albicans* and *Cryptococcus neoformans.*

Preparation of plant extracts

Dried powder of *Salvia officinalis, Thymus vulgaris, Eugenia caryophyllata* and *Artemisia absinthium* (100 g) was placed in soxhlet apparatus (Electromantle ME) with 450 ml of methanol for 12 h at 90˚C. The extract was dried over sodium carbonate anhydro (BDH, England) and filtered through Whatman filter paper No.1. The organic solvents were evaporated under reduced pressure in a rotary evaporator. The produced oil was dissolved in dimethyl sulfoxide (DMSO) and kept in sealed dark glass bottles at low temperature (4°C.).

Antimicrobial Activity

This test was carried out using agar well diffusion method according to Joshi *et al*. (2009). Bacteria or yeast were taken and shaken in the sterile distilled water corresponding to 10^8 CFU/ml for bacteria and 10^6 CFU/ml for yeast (Mihajilov-Krstev *et al*., 2010). Fungal inocula were prepared by flooding Petri dish with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was $1.5x10^4$ spore/ml (Adigüzel *et al*., 2005). Minimal inhibitory concentration was determined by the method recommended by Ter-Laak *et al*. (1991). Each antimicrobial agent was serially diluted by transferring 100 µl of the antimicrobial agent through sterilized microtitre plate containing 100 µl media (nutrient broth for bacteria, Sabouraud dextrose broth for fungi and yeast). Freshly prepared standard number of cells ($1.5x10^8$ CFU / ml for bacteria or yeast and $4x10^4$ spore/ml for fungal isolates) was added to the media that contained some drops of phenol red. Glucose metabolisms were measured by a change of the color of phenol red indicator from red to yellow. MIC was determined at the concentration with no color change and DMSO were used as a control.

Toxicity and antitumor activity of the plant extracts

Cytotoxicity is performed by Brine Shrimp Test (BST). Different dilutions of each plant extract made in DMSO were prepared (100, 200, 300 and 400 µg/ml) and 0.5 ml of each dilution was added to 4.5 ml of brine solution and maintained at room temperature for 24 h under the light (Krishnaraju *et al*., 2005). The surviving larvae were counted by light microscope and the number of dead shrimp in each vial was recorded. The concentration at which 50% of the larvae were killed (LD_{50}) was determined as the toxic concentration (Lachumy *et al*., 2010). The antitumor activity of the tested plants was determined against Ehrlich carcinoma and Lymphoma cell line. Cells were grown in RPMI 1640 medium (Sigma, USA) with 10% fetal calf serum (FCS) (Gibco, USA) at 37˚C under a humidified atmosphere consisting of 95% air and 5% CO2 for 48 h. The percentage of cell viability was assessed to determine the 50 % lethal dose by which 50% of cells are killed (LD50).

Chemical analysis of the crude extracts by GC-MS

The compounds and structures of major components were analyzed by GC-MS (Perkin Elmer).

RESULTS AND DISCUSSION

Antimicrobial activity of the essential oils of *S. officinalis, T. vulgaris, E. caryophyllata* and *A. absinthium* was investigated against some Gram positive bacteria, Gram negative bacteria, yeast and filamentous fungi. Table (1) and (2) are showing the effect of plant essential oils on pathogenic bacteria.

All of plant essential oils showed antibacterial activity. The inhibition zones are and *S. officinalis* variable according to the type of the plant. *E. caryophyllata* recorded the same maximum inhibition against *K. pneumonia*. The former showed also the highest inhibition zone against *P. mirabilis*, while the latter showed the highest zone against Methicillin Resistant *S. aureus* 33591. For all other bacteria, *A.*

absinthium exhibited the largest inhibition zones than other plants essential oils ranging from 17 mm. to 28 mm. MIC values were 50-150 µg/ml. However, the moderate activity against *P. mirabilis* and the slight decreasing in the inhibition zones against *K. pneumonia* and MRSA than *S. officinalis* did not exclude *A. absinthium* essential oil as the strongest against most of bacterial strains in this study. In this respect, Sengul *et al*. (2011) obtained broad spectrum antimicrobial activity of *A. absinthium* methanolic extract against many Gram positive and Gram negative bacteria. Also, Erel *et al*. (2012) reported antimicrobial activity of *A. absinthium* essential oil against *E. coli, S. aureus, E. faecalis*. Zanousi *et al*. (2012) reported antimicrobial activity of *A. absinthium* against *E. coli, K. pneumonia, E. faecalis* and *S.aureus*. They found that the most effective essential oil of the plant has been extracted from fruits, but there was no antimicrobial activity against *P. aeruginosa* has been detected. *T. vulgaris* oil has the weakest antimicrobial activity against bacterial isolates. Inhibition zone diameter range was 12.7-18.6 mm.

The effect of plant essential oils on yeast and filamentous fungi is represented in Table (3) and (4). The effect of essential oils on the tested fungi has also revealed that the strongest activity was recorded for *A. absinthium* with considerable increasing in inhibition zones. Zone diameter range was 13-25 mm. and MIC was 50-100 µg/ml. Similarly, Taherkhani *et al*. (2013) found that the leaf essential oil of *A. absinthium* indicated significant activity against *C. albicans.* Also, Juteau *et al*. (2003) recorded the similar result. Only three strains are different in their susceptibility response. One of them is *T. mentagrophytes*, since it was more susceptible to the essential oil of *E. caryophyllata*. Others were *A. flavus* and *M. canis*. They were affected by the oil of *T. vulgaris* with the largest inhibition zone of 21 and 23 mm, respectively and MIC 100, 25 µg/ml, in that order.

Cytotoxicity and antitumor activity of the plants essential oils are shown in Table (5). Investigation of antitumor activity of plant essential oils showed that *A. absinthium*

was the third active essential oil against Lymphoma cell line and Erlich cell line. The highest active essential oil was *E. caryophyllata*, while *T. vulgaris* has no antitumor activity. In accordance, Jeong Seo (2003) reported that the artemisetin isolated from Afsantin (*Artemisia absinthium* Linn.) exhibited marked antitumor activity against melanoma. Taherkhani (2014) also confirmed that the essential oil displayed good cytotoxic action towards the human tumor cell line. Toxicity against *Artimia salina* has been studied, the highest cytotoxic effect was recorded for the essential oil of *A. absinthium* (LD_{50} 400). However this value means a moderate cytotoxic effect. Taherkhani (2014) reported that cytotoxicity of the oil towards human tumor cell line is much higher than that required for healthy human cells. These results indicate low adverse effects for the oil. Lust, (1979) reported that pure *A. absinthium* (wormwood) oil is very poisonous, but with proper dosage, it has little or no danger.

The essential oil of *A. absinthium* was investigated by GC-MS to identify active compounds. Two major compounds were identified, the first one was bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl; common name (fenchone) and the second compound is bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl; common name (camphor). Mass spectra for both compounds are shown in Figures (1) and (2). Fenchone is suggested to be involved in the antimicrobial activity of *A. absinthium.* Similarly, antibacterial activity of *Foeniculum vulgare* is attributed to fenchone present in its essential oil (Dinesh *et al*., 2014). In contrast, fenchone was not considered a potent antimicrobial agent as mentioned by Lis-Balchin and Roth (2000). The second compound may also be contributed to the antimicrobial activity of *A. absinthium* is bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl; common name (camphor). Nibret and Wink (2010) recorded that *A. absinthium* essential oil contains 3.7% camphor. Guvenalp *et al*. (1998), reported that camphor is one of the main components of the essential oils of some *Artemisia* species. According to Mukul (2013), camphor and davanone are the major components of *A. absinthium* essential

oil. Nibret and Wink (2009) also reported that *A. absinthium* oil containing 38.37% camphor. Camphor is hypothesized to contribute to the antimicrobial activity of *A. absinthium* oil. This is agreed with Mahboubi and Kazempour (2009), they investigated the antimicrobial activity of camphor against Gram positive bacteria, Gram negative bacteria, yeast and filamentous fungi. Camphor is known to be toxic when ingested in large amounts, and may lead to seizures, confusion, irritability and neuromuscular hyperactivity (Liebelt and Shannon, 1993 and Ryno, 2009). Thus the cytotoxic effect of *A. absinthium* essential oil may be attributed to its camphor content.

CONCLUSION

Our results pointed out the potential value of *A. absinthium* essential oil against some bacterial and fungal isolates, as it exhibits the strongest antimicrobial activity among all of the tested plant essential oils with moderate cytotoxic effect and antitumor activity. Fenchone and/or Camphor are the major compounds may contribute to the biological activities of the plant essential oil.

REFERENCES

Adigüzel, A.; Güllüce, M.; Şenqül, M.; Öğütcü, H.; Şahian, F. and Karaman, I. (2005) Antimicrobial effects of *Ocimum basilicum* (Labiatae) extract, Turkish J. Biotechnol., 29: 155-160.

Arina, B. and Iqbal A. (2002). *In vitro* fungitoxicity of the essential oil of *Syzygium aromaticum*. World J. Mocrobiol. Biotech., 18(4): 317-319.

Azaz AD; Huseyin A; Irtem HA; Lu MK and Baser KHC. (2004). Composition and the *in vitro* Antimicrobial activities of the essential oils of some *Thymus* species. *Z. Naturforsch.* **59, 7**5.

Bae EA; Han MJ; Kim NJ and Kim DH. (1998). Anti-Helicobacter pylori activity of herbal medicines. Biol Pharm Bull., 21(9):990-2.

Baytop T. (1984) Türkiye'de Bitkiler ile Tedavi. I.U. Yayinlari No: 3255, Eczacilik Fak. No: 40 Ýstanbul 444.pp

Betoni, J.E.; Mantovani R.P; Barbosa L.N.; De-Stasi L.C. and F.A. Junior. (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus* diseases. Mem. Inst.Oswaldo Cruz., 101(4): 387-390.

Bozin, B.; Mlmica-Dukic, N.; Samojlik, I. and Jovin, E.; 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. J. Agric. Food. Chem., 55: 7879-7885.

Burt SA and Reinders RD. (2003). Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett Appl Microbiol., 36(3):162-7

Cai, L. and Wu, C.D. 1996. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. J. Nat. Prod., 59(10): 987-990.

Chaieb, K.; Hajlaoui H.; Zmantar T.; Nakbi K.A.B.; Rouabhia M.; Mahdouani K. and Bakhrouf A.. 2007a. The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. Phytother Res., 21(6): 501-506.

Cowan, MM (1999). Plant Products as Antimicrobial Agents, Clin. Microbiol. (12): 564-582

Davis PH. (1982). Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Scotland 311.pp

Delamare, A.P.L.; Moschen-Pistorello, I.T.; Artico, L.; Atti-Serafini, L. and Echeverrigaray, S., 2007. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem., 100: 603-608.

Dinesh S. B.; Menon K.R.K. and Manish Kumar Singhal (2014). Comparative antimicrobial activity of essential oils of *Cuminum cyminum* L. and *Foeniculum vulgare* Mill. seeds against

Salmonella typhimurium and *Escherichia coli*. Journal of Essential Oil Bearing Plants, 17:4, 617-622, DOI: 10.1080/0972060X.2014.956675.

Erel Ş.; Reznicek G.; Şenol S. G.; KARABAY YAVAŞOĞULU N. U.; KONYALIOĞLU S. and ZEYBEK A. U. (2012). Antimicrobial and antioxidant properties of *Artemisia L*. species from western Anatolia. Turk J Biol., 36 75-84

Ernst E. (2001) The Desktop Guide to Complementary and Alternative Medicine. China. Mosby.

Essawi T, Srour M. (2000) Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 70: 343-349,

Faleiro, M.L.; Miguel, M.G.; Ladeiro, F.; Venancio, F.; Tavares, R.; Brito, J.C.; Figueiredo, A.C.; Barroso, J.G. and Pedro, L.G. (2003). Antimicrobial activity of essential oils isolated from Portuguese endemic species of Thymus, Lett Appl Microbiol., 36(1); 35-40.

Gangoue-pieboji J.; Pegnyemb D. E. and Niyitegeka D. (2006). "The *in-Vitro* antimicrobial activities of some medicinal plants from Cameroon," Annals of Tropical Medicine and Parasitology, 100(3); 273- 243. doi:10.1179/136485906X86365

Giordani, R.; Regli P.; Kaloustian J.; Mikail C.; Abou L. and Portugal H.. 2004. Antifungal effects of various oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris.* Phytother. Res., 18(12): 990-995.

Guvenalp, Z.; Cakir, A.; Harmandar, M. and Gleispach, H. (1998). The essential oil of *Artemisia austriaca* Jacq. and *Artemisia spicigera* C. Koch from Turkey. Flavour Fragrance J. 13: 26-28.

Jeong, Seo (2003). Antitumour activity of flavonoids isolated from Artemisia. Plant Medicine, 69(3):218-222.

Joshi, B.; Lekhak, S. and Sharma, A. (2009): *Antibacterial Property of Different Medicinal Plants*: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana.* Journal of Science, Engineering And Technolgy.,5 (I): 143- 150.

Juteau F; Jerkovic I; Masotti V; Milos M; Mastelic J; Bessiere JM and Viano, J. (2003). Composition and antimicrobial activity of the essential oil of *Artemisia absinthium* from Croatia and France. Planta Med., 69: 158-161.

Juven, B. J.; Kanner J.; Schved F.; and Weisslowicz H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacteriol. 76:626-631.

Kalemba D; Kusewicz D and Swiader K. (2002). Antimicrobial properties of the essential oil of *Artemisia asiatica* Nakai. Phytother. Res. 16: 288-91.

Karaman S; Digrak M and Ravid V. (2001). Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* from Turkey. J. Ethanopharamacol., **72;** 183.

Krishnaraju, A.V.; Rao, T.V.N.; Sundararaju, D.; Vanisree, M.; Tsay, H. and Subbaraju, G. V. (2005): Assessment of bioactivity of indian medicinal plants using brine shrimp (*Artemia salina*) Lethality Assay. International Journal of Applied Science and Engineering., 3(2): 125-134.

Lachumy, S. J.; Zuraini Z. and Sasidharan, S. (2010): Antimicrobial activity and toxicity of methanol extract of *Cassia fistula* seeds. Research Journal of Pharmaceutical, Biological 398. and Chemical Sciences., 1(4): 391-

Li, Y.; Xu C.; Zhang Q.; Liu J.Y. and R.X. Tan. 2005. *In vitro* anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. J. Ethnopharmacol., 98(6): 329-333.

Liebelt, E.L. and Shannon, M.W. (1993). Small doses, big problems: A selected review of highly toxic common medications. Pediatr. Emerg. Care, 19; 292–297.

Lis-Balchin M. and Roth G., (2000). Composition of the essential oils of *Pelargonium odoratissimum, P. exstipulatum*, and *P. x fragrans* (Geraniaceae) and their bioactivity. Flav. Fragr. J., 15; 391-394

Lopez, P.; Sanchez C;. Batlle, R. and Nerin C.. 2005. Solid- and Vapor-phase antimicrobial activities of six essential oils: susceptibility of selected food borne bacterial and fungal strains. J. Agric. Food Chem., 53(17): 6939-6946.

Lust John ND. (1979). The Herb Book. Bantam Books.

Mahboubi M and Kazempour N. (2009). The antimicrobial activity of essential oil from Perovskia abrotanoides karel and its main components.Indian J Pharm Sci.,71:343-347.

Marino M; Bersani C and Comi G. (1999). Antimicrobial activity of the essential oils of *Thymus vulgaris* L measured using a biopediometric method. J. Food Protection., **62;** 10-17.

Mehrgan H; Mojab F; Pakdaman S and Poursaeed M. (2008). Antibacterial activity of *Thymus pubescens* methanolic extract. Iranian J. Pharm. Res., **7(4):** 291-295

Mihajilov-Krstev, T.; Radnović, D. and Kitić, D. (2010) Antimicrobial activity of Satureja L. essential oils against phytopathogenic bacteria *Erwinia amylovora,* Biologica Nyssana.,1 (1-2): 95-98

Mukul Chauhan (2013). Chemical investigations of the essential oils of some *Artemisia* species of Ethiopia. IOSR Journal of Applied Chemistry (IOSR-JAC) e-ISSN: 2278-5736. 6, Issue 4, 01-07.

Nibret, E. and Wink, M. (2009). Volatile components of four Ethiopian *Artemisia* species extracts and their *in vitro* antitrypanosomal and cytotoxic activities. Phytomedicine, doi:10.1016/j.phymed.2009.07.016.

Nibret, E. and Wink, M. (2010). Volatile components of four Ethiopian *Artemisia* species extracts and their *in vitro* antitrypanosomal and cytotoxic activities. Phytomedicine, 17, 369–374.

Oliveira, F.Q.; Gobira, B.; Guimarães, C.; Batista, J.; Barreto, M. and Souza, M., 2007. Espécies vegetais indicadas na odontologia. Rev. Bras. Farmacogn. 17, 466-476.

Panizzi L; Flamini G; Lioni PL and Morelly I. (1993). Composition and antimicrobial properties of essential oils of four mediterranean lamiaceae. *J. Ethanopharmacol.*, **39,** 167.

Park, M.J.; Gwak K.S.; Yang I.; Choi W.S.; Jo H.J.; Chang W.J.; Jeung E.B. and Choi I.G.. 2007. Antifungal activities of the essential oils in *Syzygium aromaticum* (L.) Merr. Et Perry and *Leptospermum betersonni* Bailey and their constituents against various dermatiphytes. J. Microbiol., 45(5): 460-465.

Pawar, V.C. and Thaker V.S.. 2006. *In vitro* efficacy of oils against *Aspergillus niger. Mycosis,* 49(4): 316-323.

Ryno F. (2009). An Investigation into the antimicrobial and anticancer activities of *Geranium Incanum*, *Artemisia Afra* and *Artemisia absinthium.* Thesis for Master's Degree, Faculty of Health Science, Nelson Mandela Metropolitan University, p.27.

Sengul, M.; Ercisli S.; Hilal Y.; Neva G.; Arzu K. and Bülent Ç. (2011) Antioxidant, antimicrobial activity and total phenolic content within the aerial parts of *Artemisia absinthum, Artemisia santonicum* and *Saponaria officinalis*. Iranian Journal of Pharmaceutical Research, 10 (1): 49-56

Taherkhani, M.; Rustaiyan A.; Rasooli I. and Taherkhani T. (2013). Chemical composition, antimicrobial activity, antioxidant and total phenolic content within the leaves essential oil of *Artemisia absinthium L*. growing wild in Iran. African Journal of Pharmacy and Pharmacology., 7(2); 30-36

Taherkhani, M. (2014). *In vitro* cytotoxic activity of the essential oil extracted from *Artemisia absinthium*. Iranian Journal of Toxicology, 8(26): 1152-1156.

Ter-Laak, E.A.; Pijpers, A.; Noordergraaf, J.H.; Schoevers, E.C. and Verheijden, J.H. (1991) Comparison of methods for in vitro testing of susceptibility of porcine *Mycoplasma* species to antimicrobial agents. Antimicrob Agents Chemother **35**; 228–233.

Tomoo SAGA and Keizo Yamaguchi: History of antimicrobial agents and resistant bacteria; JMAJ, March/April 2009 . 52, (2), 103–108

Yang YC; Lee SH; Lee WJ; Choi DH; Ahn YJ. (2003). Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. J Agric Food Chem., 51(17):4884-8

Zanousi M. B.; Azar P. A. and Raeesi M .(2012). Chemical composition and antimicrobial activity of essential oils of different organs of three *Artemisia* species from Iran. Journal of Medicinal Plants Research., 6(42), 5489-5494.

Table (1). Antimicrobial activity of essential oils using agar well diffusion method against bacterial pathogens

Plants / Bacteria	*Salvia officinalis*	*Artemisia absinthium*	*Eugenia caryophyllata*	*Thymus vulgaris*	Control
P. aerugenosa	16.7±1.5	28.00	18.6±8.1	18.6±8.1	ND
K. pneumoniae	26±1.1	24.00	26±0.0	16±0.0	ND
P. mirabilis	17.7±1.0	17.00	26.7±5.8	16.7±1.8	ND
A. baumannii	19±0.5	28.00	11±1.7	13±1.2	ND
E. faecalis	24±1.4	28.00	14±2.0	18±1.0	ND
S. aureus 33591	21.7±1.32	20.00	16.7±0.5	12.7±0.15	ND
S. aureus 25923	10±8.7	24.00	15	16	ND
E. coli	21±1.7	27.00	18.3±7.7	18.3±1.0	ND
Bacterial Index*	155.4	196	145.7	129.3	ND

*Bacterial Index: Total Activity (mm)

Table (2). The minimal inhibitory concentrations (µg/ml) of essential oils against bacterial pathogens

Plants / Bacteria	*Salvia officinalis*	*Artemisia absinthium*	*Eugenia caryophyllata*	*Thymus vulgaris*	Control
P. aerugenosa	50	50	25	150	>200
K. pneumoniae	100	50	50	50	>200
P. mirabilis	50	50	25	150	>200
A. baumannii	50	50	50	50	>200

Plants / Fungi	*Salvia officinalis*	*Artemisia absinthium*	*Eugenia caryophyllata*	*Thymus vulgaris*	Control
			100	100	>200
			25	100	>200
			50	150	>200
A.flavus	19±1.3	16±0.23	17±1.00	23±1.0	ND

Table (3). Antimicrobial activity of essential oils using agar well diffusion method against selected pathogenic yeasts and fungi

A. niger	19+0.18	21+.08	19.8±1.4	20+0.15	ND
E. floccosum	14±1.0	24±0.22	19±1. 0	21±0.18	ND
T.mentagrophytes	17±1.0	13±0.03	19±0.90	11±1.0	ND
M.canis	18±0.10	19±0.14	18±1.00	21±0.18	ND
C.albicans	18±0.11	26±0.16	18±0.10	10±0.15	ND
C.neoformans	19±0.18	25±0.01	19±0.17	17±2.16	ND
Fungal index*	140	165	147.8	140	ND

*Fungal Index: Total Activity (mm).

Table (4): The minimal inhibitory concentration (µg/ml) of essential oils against pathogenic fungi

Plants / Fungi	*Salvia officinalis*	*Artemisia absinthium*	*Eugenia caryophyllata*	*Thymus vulgaris*	Control
A.flavus	150	100	100	25	>200
A. niger	150	100	100	50	>200
E. floccosum	150	100	100	50	>200
T.mentagrophytes	150	100	50	50	>200
M. canis	150	100	100	100	>200
C.albicans	100	50	100	50	>200
C. neoformans	100	50	100	50	>200

Table (5). Toxicity against *Artimia salina* and antitumor activities of the different concentrations of selected plant essential oils (EOs)

Plants	Toxicity against *Artimia salina* LD_{50} (µg/ml)	Antitumor activity (LD_{50} µg/ml)	
		Lymphoma cell line	Erlich cell line
Salvia officinalis	600	400	400
Artemisia absinthium	400	600	400
Eugenia caryophyllata	600	200	400
Thymus vulgaris	≥ 600	≥ 600	600 ≥

Figure (1) Mass spectrum of bicyclo [2.2.1]heptan-2-one, 1,7,7-trimethyl-,(±)

Figure (2) Mass spectrum of bicyclo [2.2.1]heptan-2-one, 1,3,3-trimethyl-,(±)

(mainlib) Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl-