EXTRACTION OF COMPOUNDS FROM THYME LEAVES AND THIER ANTIMICROBIAL ACTIVITY

Nazar Muhammad Samein[1] **Fouad Hussein Kamel[2,3]**

[1,2]Asist. Lec., Khabat Technical Instit. & Prof., Erbil Medical Instit., respectively, Polytechnic Univ., Erbil, Iraq.

[3]Corresponding author: fouadhk@epu.edu.iq

ABSTRACT

Thyme plant leaves were collected from locally Khanaqin marked-Diyala, Iraq. Volatile oil successfully was extracted from leaves of thyme using aqueous extract method. The aqueous extracted oil yield was 4 %. Thin layer chromatography (TLC) analysis appeared five zones; four of these zones were identified. The composition of the oil extract present were carvacrol (23.7%), p-Cymene (10.4%), Camphor (0.9) and thymol (19.7%) besides othor unknown compounds. The volatile oil possessed antimicrobial activity against some human pathogenic bacteria and more over fungi, while, the activity was mainly attributed to thymol and carvacrol. The study can confirm possible use of volatile oil as food preservative to prevent growth of food born bacteria.

Key words: Thyme, volatile oil, chromatography, antimicrobial activity.

INRODUCTION

The life force of a plant was called the volatile oil or essential oil. Volatile oils are highly concentrated materials extracted from various parts of plants. These plants and oils have been used for many years dating back to ancient civilizations that used them as sources of force heal (Svoboda and Deans, 1995).

The Treatment with medicinal plants continued till the 19th century when chemistry science started its progress and herbalist time began, then chemists started to extract active compounds from different plants and trees, like atropine from *Atropa belladonna* and ephedrine from *Ephedra alata* (Azaizeh *et al.*, 2006).

Crudes of drugs are used medically because of their volatile oil contents; in several cases, the volatile oils separated from the plants are also used as drugs themselves. Similarly, various crude drugs are powdered and employed as spices and condiments (anise, clove and sage) (Liu *et al.*, 2017). Oil of thyme is derived from thyme, also known as thymus velar's (Sahib, 2013). The perennial herb, a member of the main family is used in aromatherapy, cooking, potpourri, mouth washes and elixirs, as well as, added to ointments (Abu-Rghif *et al.*, 2016). Thyme has also a number of medical properties, which is due to the herb's essential oil (Chinou *et al.*, 1996). The medical properties of thyme oils

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(which are extracted through steam distillation of fresh flowers and leaves) are due to their component Acne, Anticancer, Antispasmodic, Ant rheumatic, Antiseptic, Bactericidal, tonic, cordial, carminative , insecticide stimulant, yeast killer and others (Al-Balany, 2003).

The aim of this research was to study the antimicrobial activities of the extracted oil on some common pathogenic species of bacteria and possibility using the volatile oil as food preservative to prevent the growth of food born bacteria.

MATERIALS AND METHODS

Thyme leaves were obtained from the Khanaqin local market. Dried thyme was powdered by using laboratory mill before extraction procedure. Water extract was prepared by instrument extraction Soxhlet adding 15 gram of thyme with 100 ml of sterile distilled water in a 250 ml flask. Later, exposed the mixture to higher temperature of 100°C using electro mental for 2.5hr. Then the volatile oil was separated from the aqueous phase adding 25 ml of Di Ethyl Ether and dried with 5 gram Na_2SO_4 anhydrous. Filtrate; evaporate in water bath at 40 ^oC for 30 min with stirring then obtained oils were stored in dark bottle (Al-Balany 2003).

Thin layer chromatography: used solvents was mixed in ethyl acetate and toluene (5%and 95%, respectively) as mobile phase, while suitable plastic paper coated with silica gel was chosen as a stationary phase. The chromatogram showed five zones, each zone have been scratched isolate and dissolve in ether, and then filtration removal of the solvent gave the desired compounds (Omidbaigi *et al.*, 2010).

The identification process depends on:
UV-VIS. spectra: UV-VIS. spectra were recorded using Schimadzo UV- VIS spectrophotometer 715 over the range 200-800 nm, by using DCM as solvent, to analyses the compounds that have been isolated in this study from the preparative TLC (Kamel, 2017).

IR spectra: IR spectra were recorded in University of Al-Montasyria-Baghdad using Schimadzo spectrophotometer over the range 600-400 cm^{-1} to analyses the compounds that have been isolated (Al-Sharifi *et al.*, 2011; Fayad *et al.*, 2013).

Antimicrobial activity: The activity of oil extracted against some pathogenic microorganisms performed depending diffusion method (Kamel *et al.*, 2017b). Test microorganisms (*Staphylococcus aureus*, *E. coli* and *Pseudomonus* aerogenus and *Candida albicans* were obtained from local isolates in the clinical microbiology laboratories.

RESULTS AND DISSCUSSION

Fig. 1 appears the TLC test of thyme oil, from this chromatogram, observing five zone, four zones were characterized when compared with standard material, the results of these comparisons were explained in table 1.

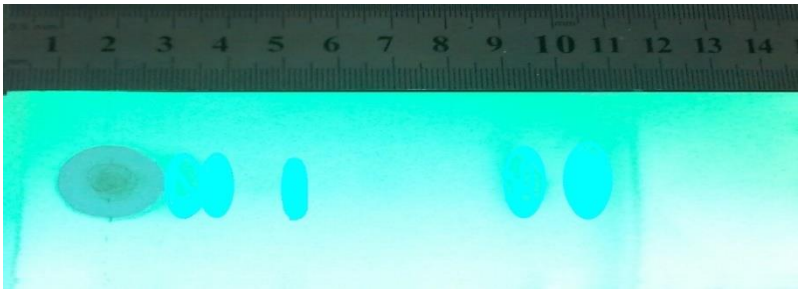

Fig 1. TLC chromatogram of thyme oil

Table 1. Retention factor (R_f) of identification compound for volatile oil extract

Compound	R_f
P-Cymene	0.96
Thymol	0.88
Carvacrol	0.68
Unknown	0.28
Camphor	0.25

Ultraviolet and Visible Spectroscopy (UV-Visible): To identify each component of this chromatogram by spectroscopic methods. Each zone was crushed carefully isolated and dissolved in diethyl ether, which on filtration and removal of solvent gave the desired compounds.

The electronic spectra of the four compounds that obtained from preparative (TLC) were recorded using 95% ethanol in the range of (200-400) nm. Tables 2 and 3 showed the spectra and the absorption of all different compounds, the available result has been found to be superimposed with authentic sample. P-cymene showed λ_{max} at 254 nm and this is due to the π-π* transitions. In thymol and carvacrol compounds showed λ_{max} at 233 nm and 266 nm for thymol, and λ_{max} at 228 nm and 274 nm for carvacrol; this is due to n-π* and π-π* transitions. Saturated ketone camphor shows λ_{max} at 285 nm due to *n*-π* transition.

Table 2. Results of UV-Visible spectra of volatile oil extract

	compound	Absorption maxima λmax (nm)
1	Thymol	256,290,374
2	Carvacrol	256,298,365
3	p-Cymene	257,265,274
4	Camphor	290

Table 3. Quantitative and qualitative composition (w/w%) of the *Thymol* essential oils studied

Components	Retention time (min.)	w/w%
Thymol	3.91	0.38
P-Cymene	8.98	5.75
Camphor	22.25	0.46
Carvacrol	46.08	29.5

IR spectrum of isolated compounds (1-4) were shown in table (4), from table can observed that compounds 1 and 2 exhibits a broad band appearing at 3382-3300 assigned to the stretching vibration of (OH) group. While compound (3) exhibits the band 1750 was related to C=0 stretching.

Compound (4) exhibits the following bands, 3050 was due to aromatic C-H stretching band, 2965-2865 was stretching due to C-H methyl group, 1514 was due to C=C stretching of ring besides other characteristic bands.

Table 4. Some characteristic bands of compounds 1-4

com poun d	Name	Assignment cm^{-1}				
		OH	CH Str.	C=C Str.	OH bending	Isopropy l group
1	Thymol	3300 broad	2962-2870	1570,1480,1430	12,87-1345	1285
2	Carvacrol	3382	2960-8860	1585,1460,1458	1458-1241	1395
3	Camphor	-	2950	-	-	1390-1375
4	p-Cymene	CH aromatic str. 3050	2965-2865	1514		

C-O str. In phenol	CH aromatic bending	C
1246 1251 -	800 802 750	

The difference in the results from specification can be ascribed to the type and percentage of chemical components of medial Iraq thyme compared with other countries, which is probably due to the season of collection and the nature of the plant. In addition to that, there are other factors such as genotype, chemo type, and geographical origin. Environmental and agricultural conditions can all influence the composition of the final natural product of the plant.

Antimicrobial Activity

The sizes of inhibition of bacterial and fungal growth culture are summarized in table 5 due to volatile oils in concentration of 50% (v/v) thyme used. It has been

reported that the concentration of essential oils of thyme at 10% (v/v) and 20% (v/v) has no antimicrobial activity.

Four pathogenic microorganism (Brito-Arias, 2016; Sarker *et al.*, 2005; Svoboda and Deans, 1995) Gram-negative *(E.coli)* gave inhibition zone 29 mm, while the Gram-positive bacteria *(Staph. aureus)* gave inhibition zone 36 mm, Gram-negative *(pseudomonas aeruginosa)* gave inhibition zone 30 mm, and fungi *(Candida)* gave inhibition zone 56 mm from the extracted oils of Thymol.

Table 5. Effects of extracted volatile oil on the microbial growth

Microorganism	Inhibition zone (mm)
E.coli	29
Staph.aureus	36
Pseudomonas aeruginosa	30
Candida albicans	56

The most important findings in this investigation are that the Gram-positive bacteria are more susceptible to the essential oils than Gram-negative bacteria but less susceptible than fungi. Whatever, the activity is mainly attributed to thymol and carvacrol. From the results of the antibacterial activity, the study can confirm the possibility of using the volatile oil as food preservative to prevent the growth of food born bacteria.

REFERENCES

Abu-Raghif, A., A. M. Al-Kazzaz, Q. J. Fadheel. 2016. A comparative study of the effect of thyme and calcium with vitamin D_3 in treatment of postmenopausal women with osteoporosis. *Intern. J. pharm. Tec. Res.* 9(5): 260-268.

Al-balany, M. R. 2003. The impact of extracts vegetable raw alkaloid vaccine to plant adhatodavasica in some germs and pathological. M.Sc. Thesis, University of Baghdad.

Al-Sharifi, H. R., J. H. Mazin, and N M. Al-Janabi. 2011. Antimicrobial activity of aqueous and alcoholic extracts for hoary-cress, London-Rocket, and Salad Rocket against some microorganism. *Anbar Journal of Agricultural Sciences*. 9(3): 304-314.

Azaizeh, H., B. Saad, K. Khalil and O. Said. 2006. The State of the Art of Traditional Arab Herbal Medicine in the Eastern Region of the Mediterranean: A Review. *Evidence-based Complementary and Alternative Medicine*. 3(2): 229-35.

Brito-Arias, M., C. Aguilar-Lemus, P. B. Hurtado-Ponce, G. Martinez-Barron, and M. Ibanez-Hernandez. 2014. Synthesis of phenylazonaphtol glycosides, β-D-O-glycosidase evaluation as substrates for beta-glycosidase activity and molecular studies. *Org. Med. Chem. Lett.* 4(2): 1-7.

Chinou, I. B., V. Roussis, D. Perdetzoglou, and A. Loukis. 1996. Chemical and biological studies on two Helichrysum species of Greek origin. *Planta medica*. 62(4): 377-379.

Fayad, N. K., O. M. Al-Obaidi, T. H. Al-Noor and M. O. Ezzat. 2013. Water and alcohol extraction of Thyme plant (*Thymus vulgaris*) and activity study against bacteria, tumors and used as anti-oxidant in margarine manufacture. *Innovative Systems Design and Engineering,* 4(1): 41-51.

Liu, Q. X. Meng, Y. Li, C. –N. Zhao, G.- Y. Tang, H. –B. Li. 2017. Antibacterial and antifungal activities of species. Int. J. Mol. Sci. 18(6): 1283.

Kamel, F. H., S. M. Hussain, and Q. F. Nafea. 2017a. Extraction and characterization of some active organic compounds from Breckland thyme plant. *Journal of university of Anbar for Pure science*, 11(3): 63-68.

Kamel, F. H., M. Y. Mohammed and S. S. Sabir. 2017b. Antibacterial activity of *Pistacia khinjul* fatty acids extract on some pathogenic bacteria. *Diyala Journal of Medicine*. 12(1): 58-62.

Omidbaigi. R., F. Fattahi, and G. Karimzadeh. 2010. Harvest time affect on the herb yield and essential oil content of lemon thyme (*Thymus*×*citriodorus* (Pers.) Schreb). *Iranian Journal of Medicinal and Aromatic Plants*. 26(3): 318- 325.

Sahib, Ahmed Salih. 2013. Treatment of irritable bowel syndrome using a selected herbal combination of Iraqi folk medicines. *J. of ethnopharmacology*, 148(3): 1008-1012.

Sarker, S. D., Z. Latif, and A. I. Gray. 2005 .Methods in Biotechnology. Natural Products Isolation. 2nd Ed. Humana Press, Totowa, NJ. 2005. 515pp.

Svoboda, K. p. and S. G. Deans. 1995. Biological activities of essential oils from selected aromatic plants. *Acta Hortic.* 390: 203-209.

استخلاص المركبات من أوراق الزعتر وفعاليتها المضادة للميكروبات

نزارمحمد سمين[1] فؤاد حسين كامل[2،3]

[2،1] مدرس مساعد في معهد تكنيكي- خبات، وأستاذ في المعهد الطبي- أربيل على التوالي، جامعة بوليتكنك، إربيل، العراق

[3] المسؤول عن النشر: fouadhk@epu.edu.iq

المستخلص

جمعت اوراق نبات الزعتر من الاسواق ا لمحلية في خانقين- ديالى، العراق. تم الاستخلاص المائي وبنجاح للزيوت الطيارة من اوراق الزعتر، وتبين إن كمية الزيت التي تم الحصول عليها من المذيب المائي بلغت 4%. اظهر التحليل الكروموتوغرافي خمسة مناطق، تم تحديد أربع منها. وكان الزيت المستخلص يحتوي على الكارفكرول، وبارا-سايمين، والكافور والثايمول، وبالنسب 23.7 % و 10.4% و0.9% و19.7% على التوالي، فضلاً عن مركبات غير معروفة. كان للزيوت الطيارة نشاطا مضادا للبكتريا الممرضة للانسان وبنسبة اكبر للفطريات، ويعزى هذا النشاط بالدرجة الرئيسة إلى الكارفاكرول. يمكننا ان نؤكد اماكنية استخدام الزيوت الطيارة في المواد الغذائية مادة حافظة لمنع نموالبكتيريا في الغذاء.

الكلمات المفتاحية: زعتر، زيوت طيارة، كروموتوغرافيا، نشاط مضادات الميكروبات.