DOI: http://dx.doi.org/10.4314/star.v3i1.6
ISSN: 2226-7522(Print) and 2305-3327 (Online)
Science, Technology and Arts Research Journal
Sci. Technol. Arts Res. J., Jan-March 2014, 3(1): 31-38
Journal Homepage: http://www.starjournal.org/

Original Research

GC-MS Analysis of Essential Oil from Long Pepper Growing in Tepi, South-west Ethiopia

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Abstract

The objectives of the study were to extract and identify essential oils present in Long pepper by using gas chromatography-mass spectrometry (GC-MS). The essential oil of Long pepper growing in Tepi, Ethiopia, was obtained by hydro distillation and gas chromatography-mass spectrometry was used to identify the component. Five components were identified and eugenol was the major constituent (98.979%) while trans-caryophyllene, (0.643%), Preg-4-ene-3-one (0.149%), Phenol, 2-methoxy-4-(2-propenyl)-, acetate (0.145%), and 1, 3, 6-Octatriene, 3, 7-dimethyl (0.084%) were minor constituents. Eugenol comprises 98.979% of the essential oil extracted from Long pepper, and is the compound most responsible for the Long pepper s' aroma and therapeutic effect.

Article Information

Article History:
Received : 03-01-2014
Revised : 12-03-2014
Accepted : 19-03-2014

Keywords:
Long pepper
Hydro distillation
Extraction

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INTRODUCTION

Essential oils (also called volatile or ethereal oils); are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) (Guenther, 1948). The essence or aromas of plants are due to volatile or essential oils, many of which have been valued since antiquity for their characteristic odors. The essential oils have characteristic fragrances and tastes are mixtures of known and unknown compounds. They may contain hydrocarbons, terpene alcohols, aldehydes, ketones, phenols and esters (Denston, 1939). An estimated 3000 essential oils are known, of which about 300 commercially important are destined chiefly for the flavors and fragrances market (Van de Braak and Leijten, 1999). It has long been recognized that some essential oils have antimicrobial properties (Guenther, 1948; Boyle, 1955) and these have been reviewed in the past (Shelef, 1983; Nychas, 1995) as have the antimicrobial properties of spices (Shelef, 1983) but the relatively recent enhancement of interest in 'green' consumerism has lead to a renewal of scientific interest in these substances (Nychas, 1995; Tuley de Silva, 1996). Besides antibacterial properties (Deans and Ritchie, 1987; Carson *et al.,* 1995a; Mourey and Canillac, 2002), essential oils or their components have been shown to exhibit antiviral (Bishop, 1995), antimycotic (Azzouz and Bullerman, 1982; Akgul and Kivanc, 1988; Jayashree and Subramanyam, 1999; Mari *et al*., 2003), antitoxigenic (Akgul *et al*., 1991; Ultee and Smid, 2001; Juglal *et al*., 2002), antiparasitic (Pandey *et al*., 2000; Pessoa *et al*., 2002), and insecticidal (Konstantopoulou *et al*.,1992; Karpouhtsis *et al*., 1998) properties. These characteristics are possibly related to the function of these compounds in plants (Guenther, 1948; Mahmoud and Croteau, 2002).

Due to their antimicrobial, insecticidal, antifungal, and antibacterial activities, essential oils have been intensely screened and applied in the fields of pharmacology, medical and clinical micro-biology, phytopathology and food preservation (Daferera *et al*., 2000).

Techniques commonly employed for extracting essential oils include hydro distillation (ASTA, 1968), steam distillation (Chialva *et al*., 1982), solvent extraction (Burbott and Loomis, 1967), supercritical fluid extraction. Hydro distillation or steam distillation is the most widely utilized physical method for isolating essential oils from the botanical material (Whish, 1996 and Masango, 2004). Although steam distillation is much popular for the isolation of essential oils on commercial scale and 93% of the oils are produced by this process, but it is not a preferred method in research laboratories (Masango, 2004). This is probably due to unavailability of steam generators and suitable distillation vessels. Most studies which focus on the essential oil of herbs have made use of hydro distillation in Clevenger-type apparatus (Kulisic *et al*., 2004; Sokovic and Griensven, 2006; Hussain *et al*., 2008).

Several authors have compared the composition of essential oil obtained by hydro/steam distillation and the product obtained by super critical fluid extraction. They found that hydro/steam-distilled oil contained higher percentages of terpene hydrocarbons. In contrast, the super critical extracted oil contained a higher percentage of oxygened compounds (Reverchon, 1997; Donelian *et al*., 2009). Khajeh *et al*., (2004) reported variation in the chemical composition of *Carum copticum* essential oil isolated by hydro distillation and supercritical fluid extraction methods. Silva *et al*., (2004) reported that essential oils from leaves of *Ocimum gratissimum*, *Ocimum micranthum* and *Ocimum selloi* obtained by steam distillation, microwave oven distillation and supercritical extraction with CO_2 showed different composition by GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS) is the most popular method for the determination of essential oil composition. Components existing in the essential oil can be identified by comparison of their relative retention time or indices and their mass spectra (MS). Identification of individual components of essential oils, however, is not always possible using MS data alone. Often different spectra are reported in a library for a single compound, with different common names, or systematic name, corresponding to an individual component sometimes apparent (Sheille *et al*., 2002). The spectral similarity of a great number of essential oil components causes difficulty in obtaining positive identification of individual components; mass spectra for sesquiterpenes are often identical or nearly identical (Konig *et a*l., 1999). Some authors have also evaluated different techniques for essential oil analysis, like the more comprehensive two-dimensional gas chromatography (Dimandja *et al*., 2000; Sheille *et al*., 2002). However, GC-MS analysis is still the most widely used method for routine analysis of essential oils, and care must be taken to optimize the chromatographic conditions in order to obtain the most accurate results. The present study aims to investigate the chemical composition of Long pepper essential oil by GC-MS analysis.

METHODS AND MATERIALS

Plant Material

The plant material (Long pepper) was collected from local market, Tepi, in February 2013. Plant material was identified by Ethiopian Institute of Agricultural Research Tepi national Spice Research Center and transported to chemistry laboratory for essential oil extraction.

Hydro Distillation (Extraction of essential oil)

300gm of cleaned and dried plant material was powdered using metal mortar and pestle and placed in a round bottom flask fitted with condenser hydro distilled for 3hrs at atmospheric pressure and constant temperature. The strongly aromatic oil was separated from the water layer using diethyl ether and the solvent was removed by boiling.

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectrum of the essential oil was obtained using prinks Elmer spectrum65 FT-IR spectrometer in Addis Ababa University and functional groups were determined with the help of correlation charts. The IR spectra were reported in % transmittance**.** The wave number region for the analysis was 4000-400cm^{-1} (in the mid-infrared range).

GC-MS Analysis

The component identification was achieved by the GC-MS analysis using HP 5890 series GC equipped with mass selective detector (MSD), HP 5972 series (German) in Addis Ababa University. Helium was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1µl was employed, injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 50°C (isothermal for 4min.), with an increase of 3°C/min, to 280°C and held for 10min. isothermal at 280°C. Total GC running time was 90.67 min.

Identification of Components

The components of essential oil was identified on the basis of comparison of their retention time and mass spectra with published data (Massda, 1976; Adams, 2001) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling the GC-MS system, in Addis Ababa University, Ethiopia. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The retention times and chemical composition of phytocomponents present in Long pepper essential oil are presented in Table 1.

Determination of the Functional Groups using FT-IR

The functional groups present in the essential oil were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer with those of an IR correlation chart. In the FT-IR spectrum of Long pepper essential oil the absorption band or frequency from 3500 cm^{-1} - 3200cm^{-1} (strong and broad), showed the presence of O-H stretch, H-bonded for alcohol and phenol and 3000cm^{-1}-2850cm^{-1} (medium) indicate C-H stretch for alkane. The absorbance band from 1600cm^{-1}-1580 cm^{-1} (medium) revealed the presence of C-C stretch (in-ring) for aromatics. A strong absorption band between 1550cm^{-1}–1475 cm^{-1} indicated the presence of N-O asymmetric stretch for nitro compounds. A medium-weak band between 1680-1600cm^{-1} showed the presence of alkenes C=C stretch. The presence of strong band between 1320cm^{-1} -1000cm^{-1} indicates the presence of C-O stretch for alcohols functional group (Figure 1).

GC-MS Chromatogram

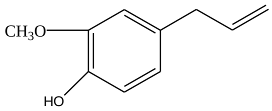
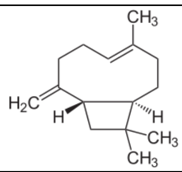
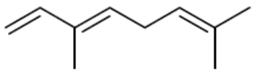
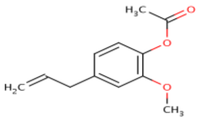
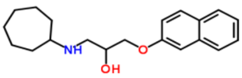
A total of five components, with different retention times, were eluted from the GC column as indicated by the chromatogram (Figure 2) and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in figure 3 to 7. The relative amount of individual components was calculated based on GC peak areas.

Comparison of the GC-MS spectrograph obtained with the instruments data bank together with computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling the GC-MS system revealed that the essential oil of Long pepper contained different organic compounds that eluted at different retention times depending on the boiling point of the eluted component. The instruments data bank was also able to identify the presence of eugenol (98.979%), trans-caryophyllene (0.643%), 1, 3, 6-Octatriene, 3, 7-dimethyl (0.084%), Phenol, 2-methoxy-4-(2-propenyl), acetate (0.145%), Preg-4-ene-3-one (0.149%), with retention times of 34.39, 35.313, 36.598, 39.591 41.933 minutes respectively.

Reported Biological Actions Eugenol

Eugenol, an essential oil and the chief component of clove oil, is commonly used in the food industry, in aromatherapy and as a therapeutic agent in dentistry (Leal-Cardoso *et al.*, 1994). Otherwise, despite its commercial use, eugenol may be found in large amounts among the essential oils of many plants most of them widely used in folk medicine (Leal-Cardoso *et al.*, 1994). Although the action of eugenol is thought to be analgesic (Feng and Lipton, 1987; Thompson and Eling, 1989), authors reported that eugenol appears to have other actions (Sticht and Smith, 1971; Brodin and Roed, 1984). This compound reduces arterial blood pressure in dogs after intravenous injections, and increases blood flow after both intra-arterial and intravenous injections. Sticht and Smith, (1971), suggesting that the action of eugenol on the cardiovascular system might be on blood vessels. It was also reported that methyleugenol, an analogue of the phenolic compound eugenol, relaxes the isolated ileum and inhibits contractions induced by stimulation of voltage-dependent and receptor-operated channels (Lima *et al.*, 2000).

Table 1: Phytocomponents identified in Long pepper essential oils.

Rt (min.)	Compound	Structure	Molecular Formula	MW	Peak area
34.39	Eugenol [Phenol, 2-methoxy-3-(2-propenyl)-		$C_{10}H_{12}O_2$	164	26554529674
35.313	Trans-caryophyllene,		$C_{15}H_{24}$	204	172593749
36.598	1,3,6-Octatriene, 3,7-dimethyl,		$C_{10}H_{16}$	136	22514527
39.591	Phenol, 2-methoxy-4-(2-propenyl)-, acetate		$C_{12}H_{14}O_3$	206	39014030
41.933	Preg-4-ene-3-one [1-(Cycloheptylamino)-3-(2 naphthyloxy) propan-2-ol]		$C_{20}H_{27}NO_2$	313	39894375

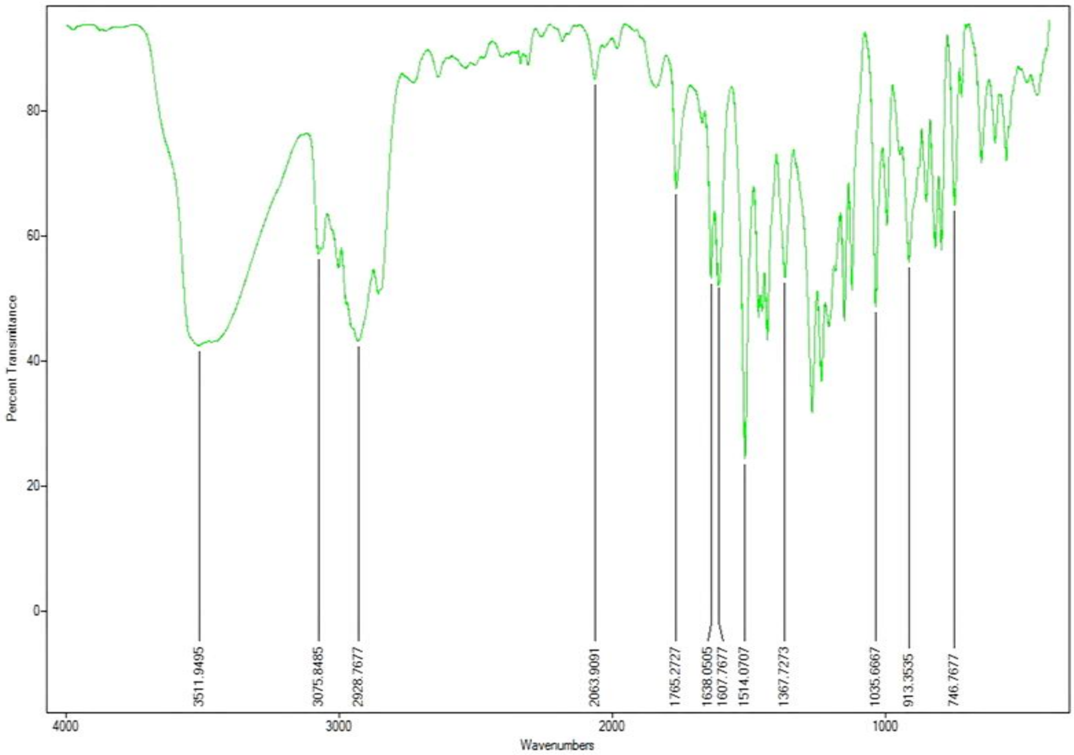


Figure1: FT-IR spectra of Long pepper essential oil.

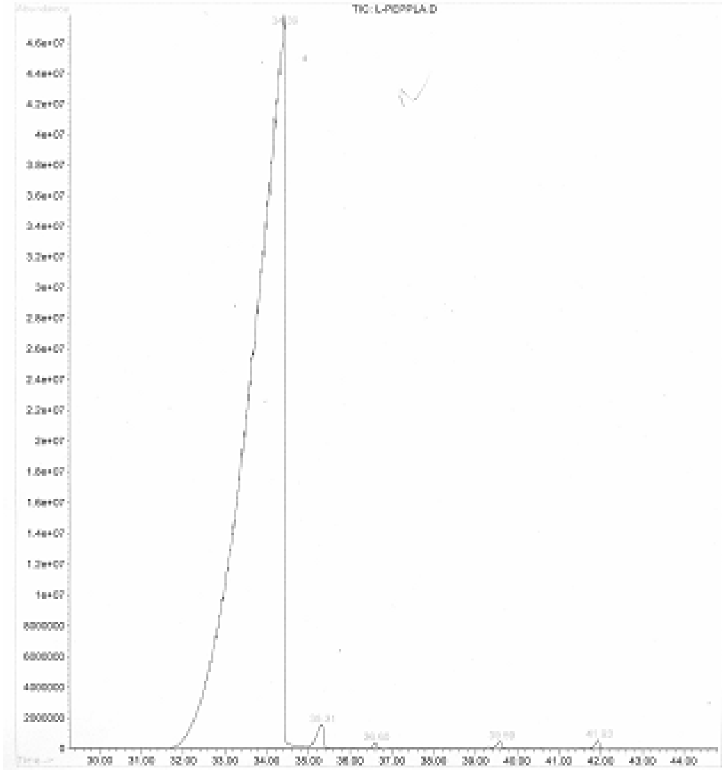


Figure 2: GC-MS profile of Long pepper essential oil

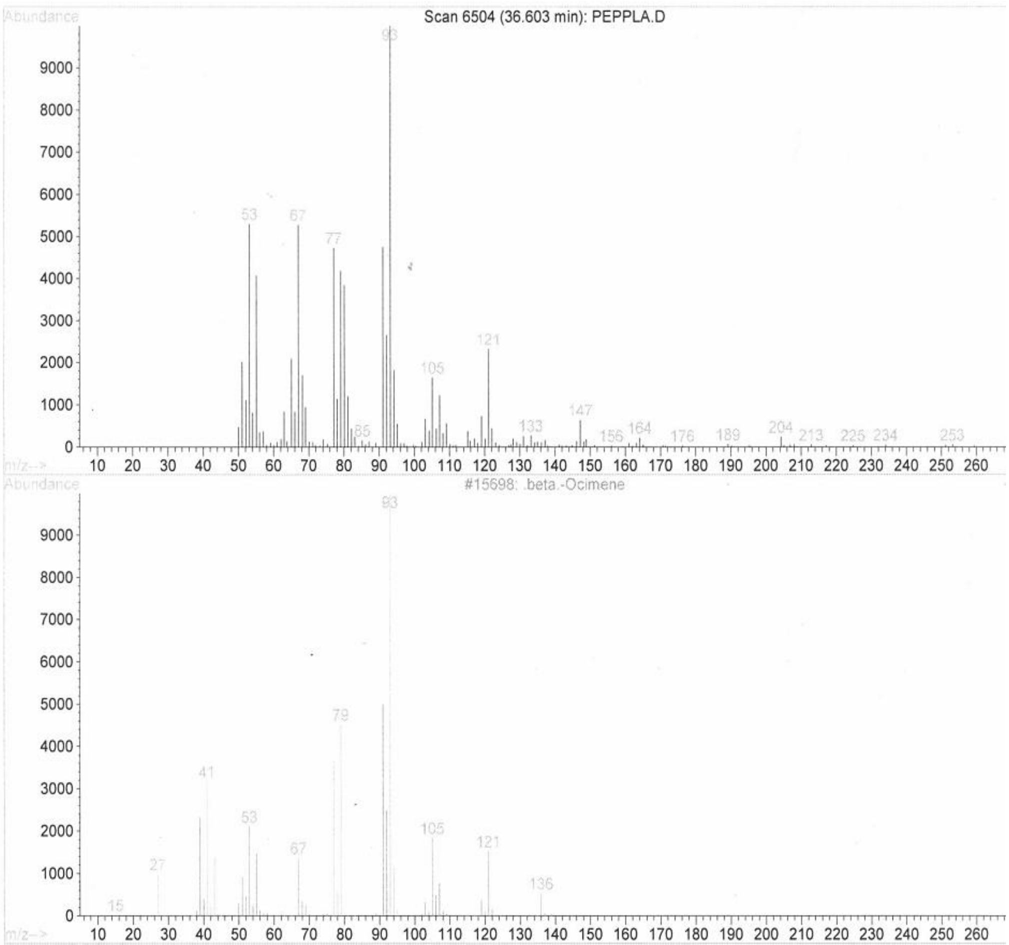


Figure 3: Mass profile of peak at Rt 35.313min; A GC- MS of peak eluted at Rt 35.313min; Trans-caryophyllene,

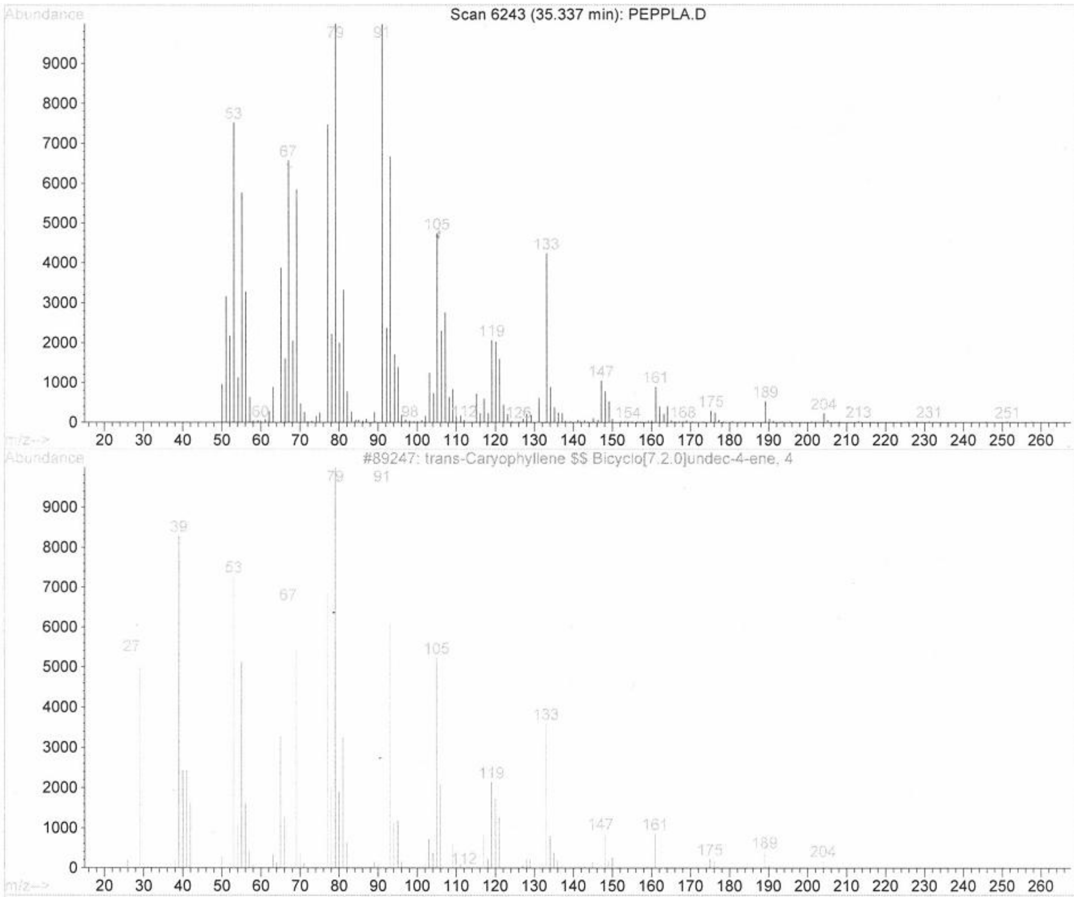


Figure 4: Mass profile of Peak at Rt 34.39min; A GC- MS of peak eluted at Rt 34.39min; Eugenol.

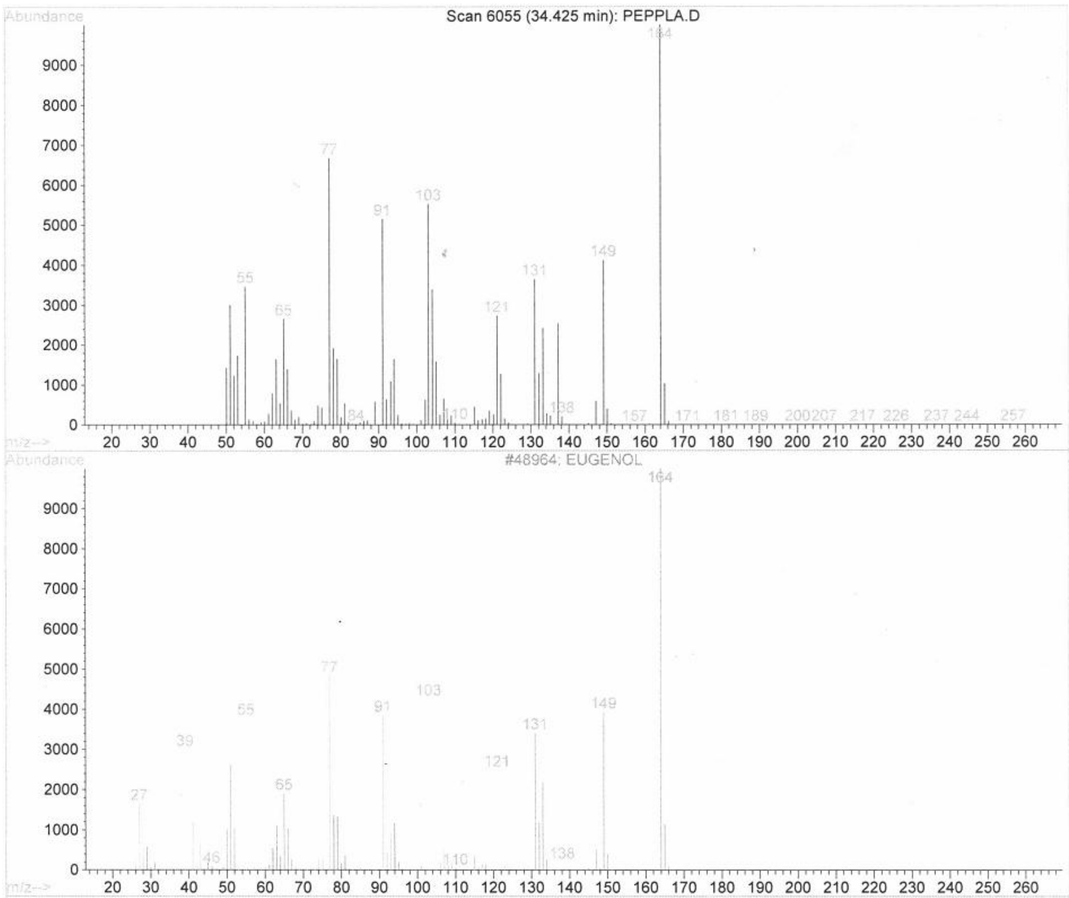


Figure 5: Mass profile of peak at Rt 36.598 min; A GC- MS of peak eluted at Rt 36.598min; 1, 3, 6-Octatriene, 3, 7-dimethyl.

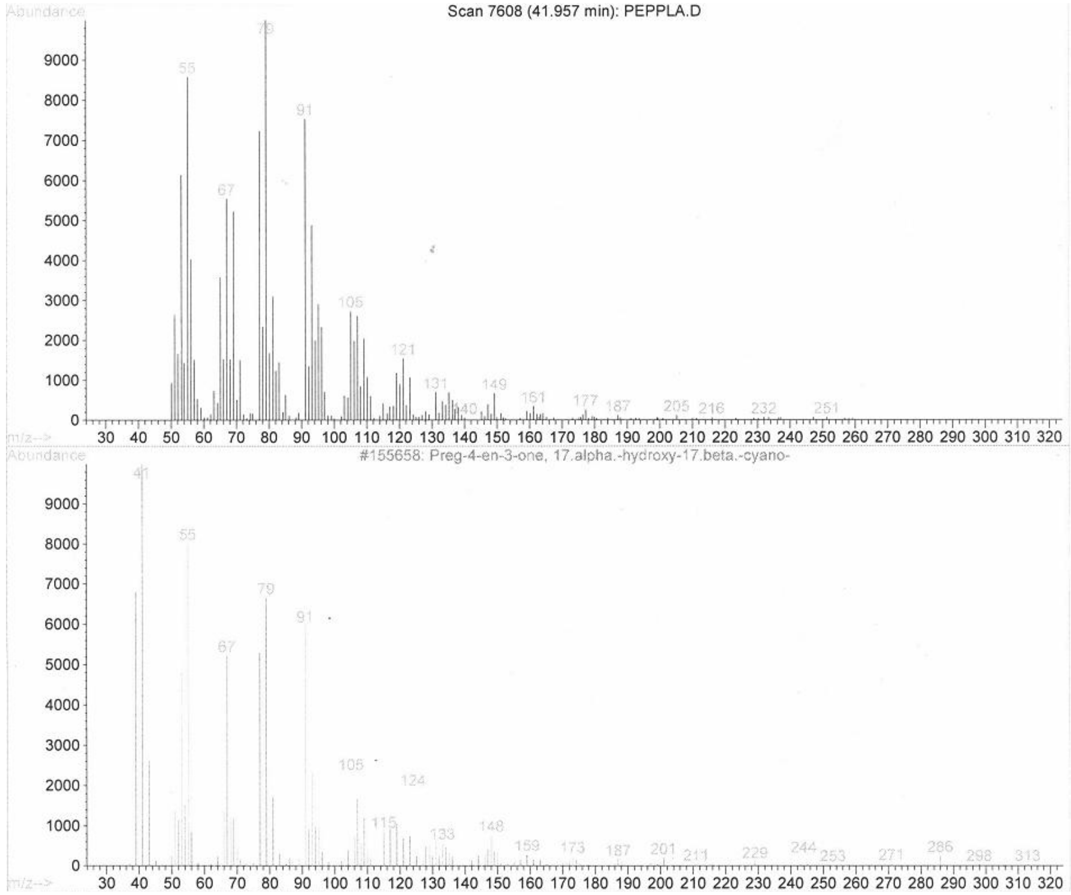


Figure 6: Mass profile of peak at Rt 39.591min; A GC- MS of peak eluted at Rt 39.591min; Phenol, 2-methoxy-4-(2-propenyl)-, acetate.

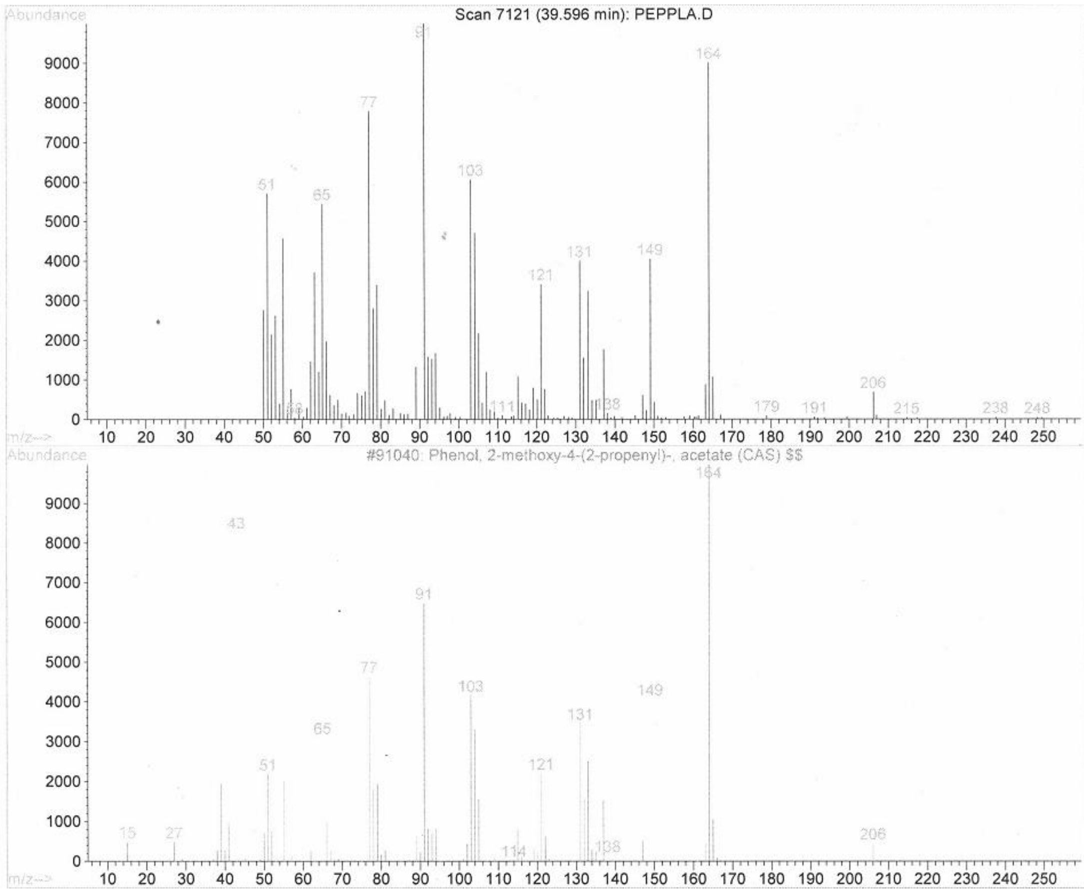


Figure 7: Mass profile of peak at Rt 41.933min; A GC- MS of peak eluted at Rt 41.933min; Preg-4-ene-3-one

Trans-Caryophyllene

The terpenes, found in essential oils of different plants, are micro-constituents most commonly used as flavor additives in food, toiletries, and perfumes (Craveiro *et al*., 1981). Recent studies from different groups around the world have shown that terpenes and terpenoids exert a plethora of pharmacological effects (Leal-Cardoso and Fonteles, 1999). *Trans-Caryophyllene* is an important constituent of the essential oil of several species of plants. It is the major chemical constituent (20.6%) of the essential oil of *Pterodon polygalaeflorus* (EOPp). EOPp blocks the electromechanical excitation-contraction coupling without affecting the pharmacomechanical coupling (Evangelista *et al*., 2007). The blocking effect caused by EOPp was suggested to result from inhibition of dihidropyridine sensitive Ca^{2+} channels, but there were no evidence to support that suggestion (Evangelista *et al*., 2007). *Trans-Caryophyllene* has been reported to possess many pharmacological effects. For example, it displays antimicrobial (Astani *et al*., 2011) and analgesic activity (Chavan *et al*., 2010). It activates the endocannabinoid system (Gertsch, 2008). *Trans-Caryophyllene* also has a well-documented anti-inflammatory activity (Fernandes *et al*., 2007; Medeiros *et al*., 2007). Additionally, *trans*-caryophyllene is effective on intestinal smooth muscle, blocking the electromechanical and pharmacomechanical excitation-contraction coupling (Leonhardt *et al*., 2010). Those activities allow it to be considered as a potential anti-spasmodic agent in tracheal smooth muscle.

CONCLUSIONS

GC-MS analysis revealed that five different chemical components were identified in the essential oil of Long pepper, including eugenol (98.979%), trans-caryophyllene (0.643%), 1, 3, 6-Octatriene, 3,7-dimethyl (0.084%), Preg-4-ene-3-one (0.149%), and Phenol, 2-methoxy-4-(2-propenyl)-, acetate (0.145%). Eugenol comprises 98.979% of the essential oil extracted from Long pepper, and is the compound most responsible for the Long pepper s' aroma/ fragrant odor. The ingredients obtained from this study indicate that the oil can be fully utilized for the manufacture of perfumery products, antimicrobial and antiseptic agents.

ACKNOWLEDGEMENT

The author wishes to acknowledge Mizan-Tepi University for financial support and granting permission to utilize the facilities of the chemistry laboratory for carrying out the research work.

REFERENCES

Adam, R.P. (2001). Identification of essential oils components by gas chromatography/ quadrupole mass spectroscopy. Allured Publishing Corp, Carol Stream, IL.

Akgu l,A., Kivanc¸, M., Sert, S. (1991). Effect of carvacrol on growth and toxin production by Aspergillus flavus and Aspergillus parasiticus. *Sciences des Aliments* 11: 361-370.

Akgul, A., Kivanc¸, M. (1988). Inhibitory effects of selected Turkish spices and oregano components on some foodborne fungi. *International Journal of Food Microbiology* 6: 263-268.

ASTA. (1968). Official analytical methods of the American Spice Trade Association. ASTA, Englewood Cliffs, 8-11.

Astani, A., Reichling, J., Schnitzler, P. (2011). Screening for Antiviral Activities of Isolated Compound from Essential Oils. *Evidence-Based Complementary and Alternative Medicine* Article ID 253643.

Azzouz, M.A., Bullerman, L.B. (1982). Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. *Journal of Food Protection* 45(14): 1298-1301.

Bishop, C.D. (1995). Antiviral activity of the essential oil of Melaleuca alternifolia (Maiden and Betche) Cheel (tea tree) against tobacco mosaic virus. *Journal of Essential Oil Research* 7: 641-644.

Boyle, W. (1955). Spices and essential oils as preservatives. *American Perfumer and Essential Oil Review* 66: 25-28.

Brodin, P., Roed, A. (1984). Effects of eugenol on rat phrenic nerve and phrenic nerve diaphragm preparations. *Archives of Oral Biology* 29: 611-615.

Burbott, A.J., and W.D. Loomis, (1967). Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiology* 42: 20-28.

Carson, C.F., Cookson, B.D., Farrelly, H.D., Riley, T.V. (1995). Susceptibility of methicillin resistant *Staphylococcus aureus* to essential oil of *Melaleuca alternifolia*. *Journal of Antimicrobial Chemotherapy* 35: 421-424.

Chavan, M.J., Wakte, P.S., Shinde, D.B. (2010). Analgesic and anti-inflammatory activity of Caryophyllene oxide from *Annona squamosa* L. bark. *Phytomedicine*, 17: 149-151.

Chialva, F., Gabri, G., Liddle, P.A.P. and Ulian, F. (1982). Qualitative evaluation of aromatic herbs by direct head space (GC) analysis. Applications of the method and comparison with the traditional analysis of essential oils, p. 183-195. In: Margaris, N. Koedam, A. and Vokou, D. (eds.). Aromatic plants–basic and applied aspect. Martinus Nijhoff, Netherlands

Craveiro, A.A., Fernandes, A.G., Andrade, C.H.S., Matos, F.J.A., Alencar, J.W., Machado, M.I.L. (1981). *Óleos Essenciais de Plantas do Nordeste*, UFC, Brazil.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G. (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. *Journal of Agricultural and Food Chemistry* 48: 2576-81.

Deans, S.G., Ritchie, G. (1987). Antibacterial properties of plant essential oils. International *Journal of Food Microbiology* 5: 165-180.

Denston, T.C. (1939). The volatile oils, A Textbook of Pharmacognosy. 3rd Edn.

Dimandja J.M., Stanfill S., Grainger J., Patterson D. Jr. (2000). Application of comprehensive two-dimensional gas chro-matography (GC/GC) to the qualitative analysis of essential oils. *Journal of High Resolution Chromatography* 23: 208-214.

Donelian, A., Carlson, L.H.C., Lopes, T.J. and Machado, R.A.F. (2009). Comparison of extraction of patchouli (*Pogostemon cablin*) essential oil with supercritical CO2 and by steam distillation. *The Journal of Supercritical Fluids* 48: 15-20.

Evangelista, G.L., Coelho-de-Souza, A.N., Santos, C.F., Leal-Cardoso, J.H., Lopes, E.A., dos Santos, M.V., Lahlou, S., Magalhães, P.J. (2007). Essential oil of *Pterodon polygalaeflorus* inhibits electromechanical coupling on rat isolated trachea. *Journal of Ethnopharmacology* 109: 515-522.

Feng, J., Lipton, J.M. (1987). Eugenol: antipyretic activity in rabbits. *Neuropharmacology* 26: 1775-1778.

Fernandes, E.S., Passos, G.F., Medeiros, R., da Cunha, F.M., Ferreira, J., Campos, M.M., Pianowski, L.F., Calixto, J.B. (2007). Anti-inflammatory effects of compounds alpha-humulene and (−)-*trans*-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *European Journal of Pharmacology* 569: 228-236.

Gertsch, J. (2008). Anti-inflammatory cannabinoids in diet: Towards a better understanding of CB (2) receptor action? *Community Integrated Biology* 1: 26-28.

Guenther, E. (1948). The Essential Oils. D. Van Nostrand, New York.

Hussain, A.I., Anwar, F., Sherazi, S.T.H. and Przybylski, R. (2008). Chemical composition. Antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry* 108: 986-995.

Jayashree, T., Subramanyam, C. (1999). Antiaflatoxigenic activity of eugenol is due to inhibition of lipid peroxidation. *Letters in Applied Microbiology* 28: 179-183.

Juglal, S., Govinden, R., Odhav, B. (2002). Spice oils for the control of co-occurring mycotoxin-producing fungi. *Journal of Food Protection* 65(4): 683-687.

Karpouhtsis, I., Pardali, E., Feggou, E., Kokkini, S., Scouras, Z.G., Mavragani Tsipidou, P. (1998). Insecticidal and genotoxic activities of oregano essential oils. *Journal of Agricultural and Food Chemistry* 46: 1111-1115.

Khajeh, M.Y., Yamini, F. Sefidkon and Bahramifar, N. (2004). Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. *Food Chemistry* 86: 587-591.

Konstantopoulou, I., Vassilopoulou, L., Mavragani-Tsipidou, P., Scouras, Z.G. (1992). Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. *Experientia* 48(6): 616-619.

Kulisic, T., Radonic, A., Katalinic, V. and Milos, M. (2004). Use of different methods for the testing activity of oregano essential oil. *Food Chemistry* 85: 633-640.

Leal-Cardoso, J.H.; Fonteles, M.C. (1999). Pharmacological effects of essential oils of plants of the northeast of Brazil. *The Anais da Academia Brasileira de Ciências* 71: 207-213.

Leal-Cardoso, J.H., Coelho de Souza, A.N., Souza, I.T., Figueiredo, I.M. (1994). Effects of eugenol on excitation–contraction coupling in skeletal muscle. *Archives internationales de pharmacodynamie et de thérapie* 327: 113-124.

Leonhardt, V., Leal-Cardoso, J.H., Lahlou, S., Albuquerque, A.A., Porto, R.S., Celedônio, N.R., Oliveira, A.C., Pereira, R.F., Silva, L.P. and Garcia-Teófilo, T.M. (2010). Antispasmodic effects of essential oil of *Pterodon polygalaeflorus* and its main constituent beta-caryophyllene on rat isolated ileum. *Fundam. Clin. Pharm.*, *24*, 749–758.

Lima, C.C., Criddle, D.N., Coelho-de-Souza, N.A., Monte, F.J., Jaffar, M., Leal-Cardoso, J.H. (2000). Relaxant and antispasmodic actions of methyleugenol on guinea-pig isolated ileum. *Planta Medica* 66: 408-411.

Mahmoud, S.S., Croteau, R.B. (2002). Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends in Plant Science* 7(8): 366-373.

Mari, M., Bertolini, P., Pratella, G.C. (2003). Non-conventional methods for the control of post-harvest pear diseases. *Journal of Applied Microbiology* 94: 761-766.

Masango, P. (2004). Cleaner production of essential oil by steam distillation. *Journal of Cleaner Production* 13: 833-839.

Massda, Y., (1976). Analysis of essential oils by Gas Chromatography and Mass Spectrometry. Halsted/Wiley, New York.

Medeiros, R., Passos, G.F., Vitor, C.E., Koepp, J., Mazzuco, T.L., Pianowski, L.F., Campos, M.M., Calixto, J.B. (2007). Effect of two active compounds obtained from the essential oil of *Cordia verbenacea* on the acute inflammatory responses elicited by LPS in the rat paw. *British Journal of Pharmacology* 151: 618-627.

Mourey, A., Canillac, N. (2002). Anti-Listeria monocytogenes activity of essential oils components of conifers. *Food Control* 13: 289-292.

Nychas, G.J.E. (1995). Natural antimicrobials from plants. In: Gould, G.W. (Ed.), New Methods of Food Preservation. Blackie Academic and Professional, London, pp. 58-89.

Pandey, R., Kalra, A., Tandon, S., Mehrotra, N., Singh, H.N., Kumar, S. (2000). Essential oil compounds as potent source of nematicidal compounds. *Journal of Phytopathology* 148(7-8): 501-502.

Pessoa, L.M., Morais, S.M., Bevilaqua, C.M.L., Luciano, J.H.S. (2002). Anthelmintic activity of essential oil of Ocimum gratissimum L. & eugenol against Haemonchus contortus. *Veterinary Parasitology* 109(1-2): 59-63.

Reverchon, E. (1997). Supercritical fluid extraction and fractionation of essential oils and related products. *Journal of Supercritical Fluids* 10: 1-37.

Shelef, L.A. (1983). Antimicrobial effects of spices. *Journal of Food Safety* 6: 29-44.

Sheille R., Mondello L., Marriott P., Dugo G. (2002). Characterisation of lavender essen-tial oils by using gas chromatography–mass spectrometry with correlation of linear reten-tion indices and comparison with compre-hensive two-dimensional gas chromatography. *Journal of Chromatography A* 970: 225-234.

Silva, M.G.V., Matos, F.J.A., Lopes, P.R.O., Silva, F.O. and Holanda, M.T. (2004). Composition of essential oils from three *Ocimum* species obtained by steam and microwave distillation and supercritical CO_2 extraction. ARKIVOC. 6: 66-71.

Sokovic, M. and Van Griensven, L.J.L.D. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. *European Journal Plant Pathology* 116: 211-224.

Sticht, F., Smith, R.M. (1971). Eugenol: some pharmacologic observations. *Journal of Dental Research* 50: 1531-1535.

Thompson, D., Eling, T. (1989). Mechanism of inhibition of prostaglandin H synthase by eugenol and other phenolic peroxidase substrates. *Molecular Pharmacol*ogy 36: 809-817.

Tuley de Silva, K. (Ed.) (1996). A Manual on the Essential Oil Industry. United Nations Industrial Development Organization, Vienna.

Ultee, A., Smid, E.J. (2001). Influence of carvacrol on growth and toxin production by Bacillus cereus. *International Journal of Food Microbiology* 64: 373-378.

Van de Braak, S.A.A.J., Leijten, G.C.J.J. (1999). Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, Pp. 116.

Whish, J.P.M. and Williams, R.R. (1996). Effects of post harvest drying on the yield of tea tree oil (*Melaleuca alternifolia*). *Journal of Essential Oil Research* 8: 47-51.