ACTIVE CONSTITUENTS OF *LITSEA CUBEBA*

by

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Original scientific paper
https://doi.org/10.2298/TSCI190526047Z

Litsea cubeba is a plant of Lauraceae and Litsea. It is a valuable plant and has a wide range of uses, including in traditional Chinese medicine. Herein, Litsea cubeba wood was harvested from Henan Province, The active ingredients were extracted from Litsea cubeba wood by modern techniques such as gas chromatography-mass spectrometry (GC-MS), thermal gravimetric analysis, and thermal desorption gas chromatography-mass spectrometry. The analysis results show that the wood of Litsea cubeba contains a large amount of valuable active substances that can be utilized in medicine, bio-energy, and spices and flavorings, and large-scale cultivation of this plant could be beneficial.

Key words: *Litsea cubeba, active ingredient, energy crop, gas chromatography*

Introduction

Litsea cubeba is a deciduous shrub or small tree with aromatic branches and leaves. During July to August when it matures, it turns from cyan to deep red or black [1-3]. *Litsea cubeba* is mainly distributed in most parts of China and other countries in Southeast Asia. It possesses excellent traits such as fast growth, resistance to disease and pests, robust germination, easy reproduction, and high yield.

The traditional Chinese medicine (TCM) name for *Litsea cubeba* is *May chang* or mountain pepper, and the plant contains volatile oils, β-caryophyllene, eugenol, methyl-anthranilate, and other compounds [4-6]. The entire plant can be used as medicine, and it dispels wind, cold, and dampness and also relieves pain and inflammation. It can treat colds or prevent colds. Its wood is medium in size and resistant to moisture [7], but it is easy to split and can be used for ordinary furniture and architecture. The leaves are primarily used as the main raw material for the extraction of citral [3, 8, 9], which is a monoterpene aldehyde used in pharmaceutical products and the preparation of flavors. The use of its mature fruit to produce biodiesel is beneficial because it does little damage to the natural ecological environment, and can also be used for many years. Therefore, it is economically beneficial to use it as an energy crops, and for chemical and pharmaceutical raw materials.

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Material and methods

Experimental materials

The test materials were obtained from the mountainous area of Luanchuan County, Luoyang City, Henan Province, China. Wood samples were ground in a laboratory using a plant shredder (model: FW-400A, Beijing Zhongxing Weiye Instrument Co., Ltd.) and pulverized to 60-100 mesh before use. For extraction, 10 g of pulverized wood was placed in an Erlenmeyer flask, and this was repeated two more times for a total of 3 flasks; 70 g of the sample was set aside for further analysis. Each of the 3 flasks then received a 300 ml aliquot of one solvent: ethanol, methanol, or diethyl ether. The extractions were performed with the above solvents and then were filtered under high pressure and subjected to rotary distillation to carry out subsequent detection and analysis.

Experimental methods

The GC-MS

The wood of the *Litsea cubeba* was decomposed by GC-MS. GC: A quartz capillary column with a column length of 30 mm × 0.25 mm × 0.25 μm and a carrier gas of high purity helium was used. The initial temperature was set at 50 °C, 8 °C/min was raised to 250 °C, and 5 °C/min to 300 °C. MS: The scanning mass range of the program is 30-600 amu [10, 11], the voltage is 70 ev, the ion source and four stage temperature are set at 230 °C and 150 °C [12, 13].

The Py-GC-MS

The wood of the *Litsea cubeba* was analyzed by Py-GC-MS. The carrier gas was high purity helium. The capillary size is 30 mm × 0.25 mm × 0.25 μm [14, 15]. The initial temperature is set at 40 °C, 5 C/min to 120 °C and 10 °C/min to 200 °C. The program scanning quality range is 30-600 amu [16].

The TD-GC-MS

The TDS: Set its initial temperature to 30 °C, and keep it for 1 minute, at a rate of 10 °C/min, rise to 100 °C, and then rise to 200 °C after 5 minutes, without keeping it. The temperature of the transmission line is 230 °C.

The samples were analyzed by TD-GC-MS. In the absence of shunt, the carrier gas is high purity helium, and the gas flow rate is 1 ml/min. The initial temperature was set to 30 °C, 10 °C/min to 100 °C, 10 °C/min to 200 °C [17, 18].

The thermalgravimetric

Thermalgravimetric analysis was carried out on the samples of *Litsea cubeba wood*. The equilibrium gas was nitrogen, the gas release rate is 20 ml/min.The initial temperature was 30 °C and increased to 850 °C at different rates (10 °C/min, 20 °C/min, 40 °C/min) [19, 20].

Results and discussion

The GC-MS analysis results

According to the GC-MS analysis results, fig. 1, the main components of ethanol extraction from *Litsea cubeba* wood are 4H-1-benzopyran-4-one,5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy- (24.616%); dl-.alpha.-tocopherol (17.256%); sakuranin (12.999%); .gamma.-sitosterol (10.319%); coumarin (6.676%).

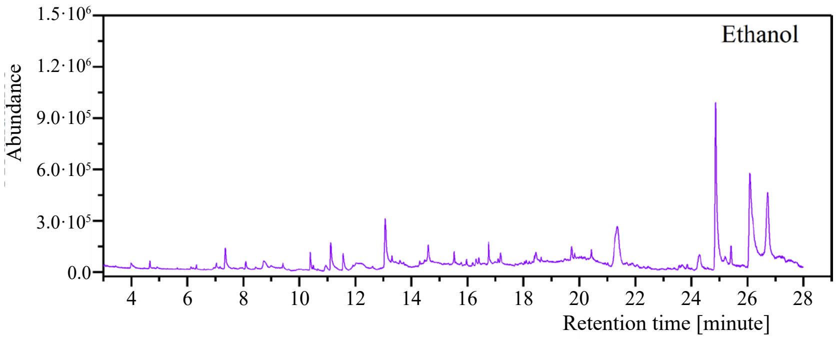

Figure 1. Total Ion Chromatography Analysis of Ethanol Extracts from *Litsea cubeba* wood

According to the GC-MS analysis results, fig. 2, the main components of the methanol extraction from *Litsea cubeba* wood are sakuranin (18.780%); vitamin (11.187%); gamma.-sitosterol (10.846%); melezitose (6.143%);coumarin (3.544%).

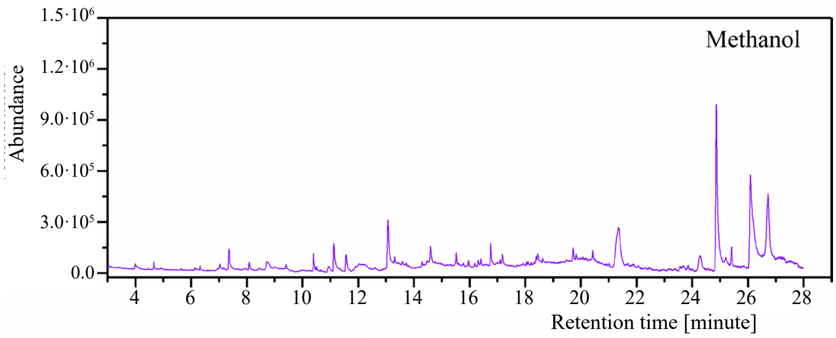

Figure 2. Total ion chromatogram of methanol extract from *Litsea cubeba (Lour.)* wood

According to the GC-MS analysis results, fig. 3, the main components of the methanol extraction from *Litsea cubeba* wood are butyl 9-tetradecenoate (18.697%); .gamma.-sitosterol (18.408%); dl-.alpha.-tocopherol (17.669%); trimethylsilylmethanol (10.412%); (2R,3R,4aR,5S,8aS)-2-hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphtha-

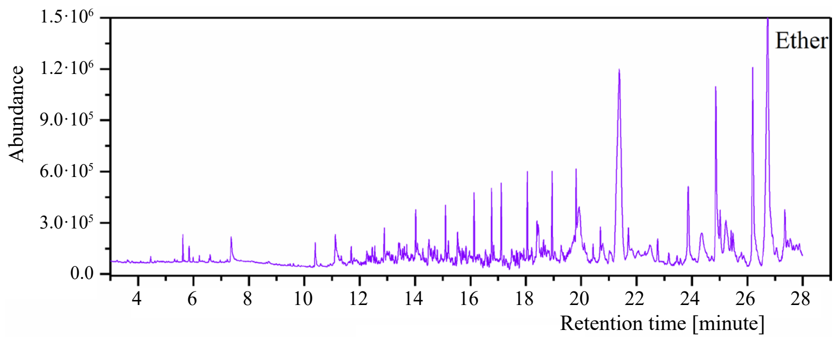

Figure 3. Total ion chromatogram of the ether extract from *Litsea cubeba* wood

len-1(2H)-one (10.222%); 1-decanol,2-hexyl-(8.056%); 2-dodecen-1-yl(-)succinicanhydride (5.979%); and undec-10-ynoic acid, tetradecyl ester (5.145%).

By analyzing the composition and content of each extraction constituent, materials with high utilization value contained in *Litsea cubeba* wood were identified, and are described:
– Linoleic acid, which is used as a hypolipidemic agent for the treatment and prevention of atherosclerosis, is also broken down into metabolites that are important nutrients for the brain [21, 22] and have a good preventive effect on cardiovascular and cerebrovascular diseases.
– Vitamin E is a highly effective antioxidant that protects cell membranes from free radicals and protects the blood vessels, heart, breasts, eyes, skin, and glands. At present, vitamin E is widely used for its anti-aging capabilities because it can eliminate the deposition of lipofuscin in cells [23, 24], improve the normal function of cells, and slow the aging process of tissue cells.
– Benzoic acid can be used as a disinfectant, preservative, and anti-bacterial agent.
– Coumarin is present in many plants in the form of glycosides, and is used in the manufacture of perfumes, as a fixative, a fluorescent indicator, and also in the electroplating industry [25].
– Hydrogenated cinnamic acid lactone is used in food and tobacco flavors, and soap and cosmetic scents.

The Py-GC-MS analysis results

According to the PY-GC/MS results, fig. 4, the main components in the saplings are phenol, 3-methyl-(4.968%);1,3,5-cycloheptatriene(4.881%);4-penten-1-ol(4.841%);benzene(4.253%);1,3-cyclopentadiene (4.050%); and 1-pentene, 3,4-dimethyl- (2.895%).

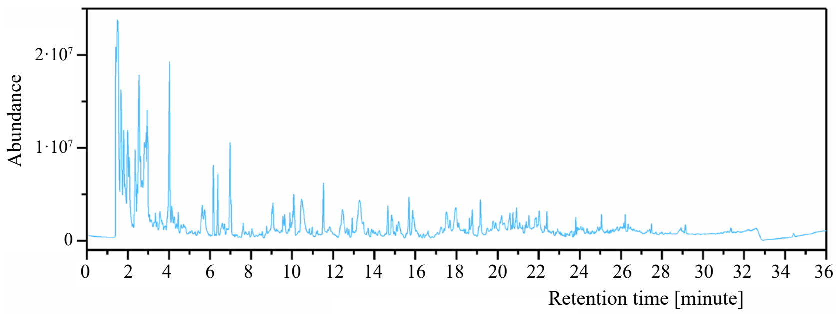

Figure 4. Pyrolysis ion map of *Litsea cubeba* wood

Data analysis reveals that the following important substances are contained in *Litsea cubeba* wood:
– Falcarinol, which is a polyacetylene, is widely distributed in the plant kingdom [26, 27] and possesses anti-cancer, anti-bacterial, anti-fungal, and neuro-cell protection functions, and has a calming and analgesic effect.
– The 3-Hydroxybutanal is commonly used as an intermediate in organic synthesis, and it can also be used to prepare butenol, antioxidant AH, antioxidant AP, perfume, mineral flotation agents, and pharmaceutical sedatives and sleeping pills.
– The 2, 6-Dimethylphenol can be used in the production of photographic chemicals, pesticides, polyesters, and polyether resins, and is used to manufacture an arrhythmia drug for slow heart rhythm [28]. It can also be used in organic synthesis, and in antiseptics, pharmaceuticals, solvents, and antioxidants.

– Hexyl sterol is used in cosmetics, detergent industry dispersants, and fiber emollients, printing ink auxiliaries, advanced lubricant additives, biopharmaceuticals and other fields [29-31].
– Cyclopropanol is an organic synthesis intermediate and pharmaceutical intermediate that can be used in laboratory research and development and chemical and pharmaceutical synthesis processes.

The TD-GC-MS analysis results

According to the TD-GC-MS results, fig. 5, the main components in the saplings are 2-naphthalenemethanol,decahydro-.alpha.,4a-trimethyl-8-methylene-,[2R-(2.alpha.,4a.alpha., 8a.beta.)]-(13.634%);1,6,10-dodecatrien-3-ol,3,7,11-trimethyl-,(E)-(9.054%);4-terpinenylacetate(8.141%);bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-,(1.alpha.,2.alpha.,5.alpha.)-(4.176%);n-hexadecanoic acid (4.151%).

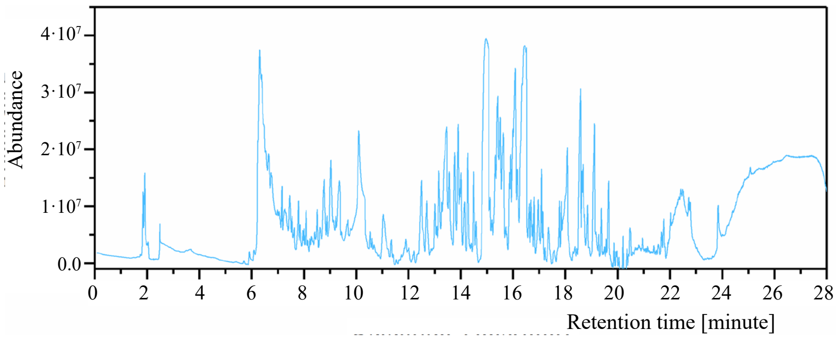

Figure 5. Thermal desorption ion diagram of *Litsea cubeba* wood

Thorough analysis of the data revealed that the following important substances are contained in the *Litsea cubeba* wood:
– Linalool is a well-known compound used in perfumes. It can also be used in the preparation of cosmetics, soaps, detergents, and foods. It is used to prepare artificial oils such as that of orange leaves, bergamot, and lavender [32-34].
– Stearic acid is widely used in cosmetics, plastic plasticizers [35, 36], mold release agents, stabilizers, surfactants, and other organic chemicals. It is used in medicine to prepare ointments and suppositories.
– Myristic acid is a raw material for spices and medicines, and can also be used as a defoaming agent and flavoring agent [37].
– Isobornyl acetate is used in the perfume industry and is a raw material for the synthesis of camphor [38, 39]. It can also be added to products used on a daily basis, such as soap, talcum powder, toilet water, air spray, and other scent and flavoring agents.

The TGA-DTG analysis results

Figure 6 shows the TGA and DTG curves of *Litsea cubeba* wood. There are three stages during the thermogravimetric analysis of *Litsea cubeba* wood. A weight loss phenomenon occurs, the expression of which is basically the same in the three heating modes shown. The first stage is the dehydration stage, when the temperature range is 100-200 °C. At this stage, the combined loss of water and free water is approximately 11%. During the second stage, the temperature varies from 200-500 °C during this stage, and the decomposition of cel-

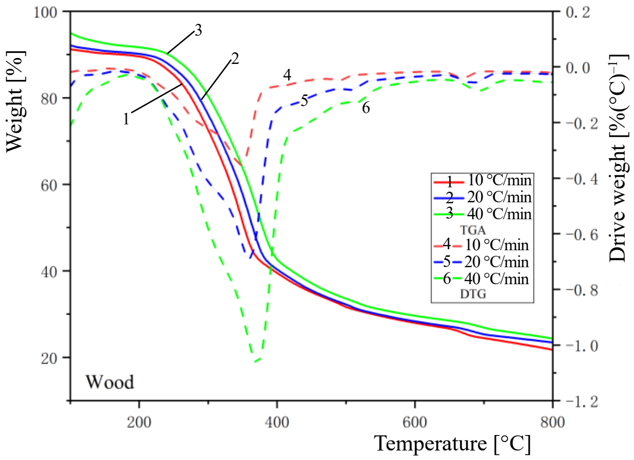

Figure 6. The TGA-DTG analysis of the *Litsea cubeba* wood

lulose, hemicellulose, and lignin mainly occurs. This is the main stage of pyrolysis, with weight loss of approximately 68%. The temperature range of carbonization in the third stage is 500-700 °C, and the weight loss is about 10%. After the above three stages, there is little change to the quality of the sample [40].

At three different heating rates, the sample's weight loss rate reached two peaks. When the sample is no longer losing weight, it contains 24% of its original mass. The maximum weight loss rate of the sample is different at different heating rates. The maximum weightlessness rates of the samples were 7 %/min, 15.6 %/min and 12.9 %/min, when the heating rates were 10 degree °C/min, 20 degree °C/min and 40 degree °C/min.

Conclusions

Our results show that a large number of compounds are contained in the wood of *Litsea cubeba* saplings and have high utilization value. The main components detected by GC-MS are falcarinol, vitamin E, and benzoic acid. They play an important role in disease prevention, utilization of biological resources, and preparation of drugs. The main components detected by Py-GC-MS are falcarinol, 3-hydroxybutanal, and 2, 6-dimethylphenol. These are anti-fungal compounds that protect cells, and can also be employed as fragrances and solvents for development and utilization. The main components detected by TD-GC-MS are linalool, stearic acid, and myristic acid. These ingredients can be used in perfume industry, organic chemical production and energy production.

The *Litsea cubeba* wood component is versatile and valuable, and can be used to treat arrhythmia, and possesses anti-tumor, bactericidal, anti-inflammatory, and analgesic properties; its essential oil also has wide uses as a flavoring and scent. This study is conducive to further research and promotion of *Litsea cubeba*, as well as expanding its development prospects.

References

[1] Wei, P., *et al*. Docking Study and Antiosteoporosis Effects of a Dibenzylbutane Lignan Isolated from Litsea Cubeba Targeting Cathepsin K and MEK1. *Medicinal Chemistry Research*, *27* (2018), July, pp. 2062-2070
[2] Nguyen, H. V., *et al*., Antibacterial Activity of Litsea Cubeba (Lauraceae, May Chang) and Its Effects on the Biological Response of Common Carp Cyprinus Carpio Challenged with Aeromonas Hydrophila, *Journal of Applied Microbiology*, *121* (2016), 2, pp. 341-351
[3] Lin, B., *et al*., Anti-Inflammatory Constituents from the Root of Litsea Cubeba in LPS-Induced RAW 264.7 Macrophages, *Pharmaceutical Biology*, *54* (2016), 9, pp. 1741-1747
[4] Si, L. L., *et al*., Chemical Composition of Essential Oils of Litsea cubeba Harvested from Its Distribution Areas in China, *Molecules*, *17* (2012), 6, pp. 7057-7066

[5] Nazeer, W. A., *et al*., In-Situ Species, Temperature and Velocity Measurements in a Pulverized Coal Flame, *Combustion Sciences and Technology, 143* (1999), 2, pp. 63-77
[6] Feng, J. S., *et al*., Identification of Essential Oils with Strong Activity against Stationary Phase Borrelia burgdorferi. *Antibiotics*, *7* (2018), 4, E89
[7] She, Q. H., *et al*., Chemical Composition, Antimicrobial Activity and Antioxidant Activity of Litsea Cubeba Essential Oils in Different Months. *Natural Product Research*, On-line first, https://doi.org/10.1080/14786419.2019.1557177, 2019
[8] Feng, W., *et al*., Hydrothermal Liquefaction of Litsea Cubeba Seed to Produce Bio-Oils. *Bioresource Technology*, *149* (2013), Dec., pp. 509-515
[9] Bing, L., *et al.,* Inhibitory Effects of the Root Extract of Litsea cubeba (lour.) pers. on Adjuvant Arthritis in Rats, *Journal of Ethnopharmacology*, *147* (2013), 2, pp. 327-334
[10] Chen, Y. C., *et al.,* Biology and Chemistry of Litsea Cubeba, a Promising Industrial Tree in China. *Journal of Essential Oil Research*, *25* (2013), 2, pp. 103-111
[11] Xiao, C. Z. *et al*., The Effect of Alternative Solvents to N-hexane on the Green Extraction of Litsea Cubeba Kernel Oils as New Oil Sources, *Industrial Crops and Products*, *126* (2018), Dec., pp. 340-346
[12] Hu, W., *et al*., Antibacterial Activity and Mechanism of Litsea Cubeba Essential Oil Against Methicillin-Resistant Staphylococcus Aureus (MRSA), *Industrial Crops and Products*, *130* (2019), Apr., pp. 34-41
[13] Peng, W. X., *et al*., Characteristics of Antibacterial Molecular Activities in Poplar Wood Extractives, *Saudi Journal of Biological Sciences*, *24* (2017), 2, pp. 399-404
[14] Hadian, Z., *et al*., Pesticide Residues Analysis in Iranian Fruits and Vegetables by Gas Chromatography-Mass Spectrometry, *Iranian Journal of Pharmaceutical Research: IJPR*, *18* (2019), 1, pp. 275-285
[15] Li, H. H., *et al*., Characterization of Key Aroma Compounds in Chinese Guojing Sesame-Flavor Baijiu by Means of Molecular Sensory Science, *Food Chemistry*, *284* (2019), June, pp. 100-107
[16] Peng, W. X., *et al*., Characteristics of Antibacterial Molecular Activities in Poplar Wood Extractives, *Saudi Journal of Biological Sciences*, *24* (2017), 2, pp. 399-404
[17] Ge, S. B., *et al*., Properties of Nonvolatile and Antibacterial Bioboard Produced from Bamboo Macromolecules by Hot Pressing, *Saudi Journal of Biological Sciences*, *25* (2018), 3, pp. 474-478
[18] Jiang, S. C., *et al*., Preparation and Properties of Novel Flame-Retardant PBS Wood-Plastic Composites, *Arabian Journal of Chemistry*, *11* (2018), Sept., pp. 844-857
[19] Zhao, J. C., *et al*., Studies on the Thermal Behaviors of Hexogen, Ammonium Perchlorate and Their Mixtures by TG-DSC, *DEStech Transactions on Environment, Energy and Earth Sciences*, 3rd Int. Conf. on New Energy and Renewable Resources, Guangzhou, China, 2018
[20] Frank, D., *et al*., Two Decades of Stir Bar Sorptive Extraction: A Retrospective and Future Outlook, *Trends in Analytical Chemistry*, *112* (2019), Mar., pp. 102-111
[21] Scherer, N., *et al*., New Studies on Leachables in Commercial Scale Protein Drug Filling Lines Using Stir Bar Sorptive Extraction Coupled with TD-GC-MS and UPLC/QTOF-MS/MS Analytics, *International Journal of Pharmaceutics*, *555* (2019), Jan., pp. 404-419
[22] Vergroesen, A. J., Dietary Fat and Cardiovascular Disease: Possible Modes of Action of Linoleic Acid, *Proceedings of the Nutrition Society*, *31* (1972), 3, pp. 323-329
[23] Qin, L. B., *et al*., Thermal Degradation of Medical Plastic Waste by In-Situ FTIR, TG-MS and TG-GC/MS Coupled Analyses, *Journal of Analytical and Applied Pyrolysis*, *136* (2018), Nov., pp. 132-145
[24] Knekt, P., Role of Vitamin E in the Prophylaxis of Cancer, *Annals of Medicine*, *23* (1991), 1, pp. 3-12
[25] Lee, A. Y. Y., *et al*., Low-Molecular-Weight Heparin *vs.* A Coumarin for the Prevention of Recurrent Venous Thromboembolism in Patients with Cancer, *Acc Current Journal Review*, *12* (2003), 5, pp. 19-20
[26] He, Q. Q., *et al*., TG-GC-MS Study of Volatile Products from Shengli Lignite Pyrolysis, *Fuel*, *156* (2015), Sept., pp. 121-128
[27] Christensen, L. P., *et al*., Inhibitory Effects of Feeding with Carrots or (?)-Falcarinol on Development of Azoxymethane-Induced Preneoplastic Lesions in the Rat Colon, *Journal of Agricultural and Food Chemistry*, *53* (2005), 5, pp. 1823-1827
[28] Awawdeh, M. S., *et al*., Multiple Injections of Vitamin E and Selenium Improved the Reproductive Performance of Estrus-Synchronized Awassi Ewes, *Tropical Animal Health and Production*, *51* (2019), 6, pp. 1421-1426
[29] Mezine, I., *et al*., Analysis of Plant Sterol and Stanol Esters in Cholesterol-Lowering Spreads and Beverages Using High-Performance Liquid Chromatography?Atmospheric Pressure Chemical Ionization?Mass Spectroscopy, *Journal of Agricultural and Food Chemistry*, *51* (2003), 19, pp. 5639-5646

[30] Malviya, P. S., *et al*., Acousto-Optic Modulation in Ion Implanted Semiconductor Plasmas Having SDDC, *Applied Mathematics & Nonlinear Sciences, 3* (2018), 1, pp. 303-310
[31] Sardar, M. S., *et al*., Computing Topological Indices of the Line Graphs of Banana Tree Graph and Firecracker Graph, *Applied Mathematics & Nonlinear Sciences, 2* (2017), 1, pp. 83-92
[32] Trela, A., Szymanska, R., Less Widespread Plant Oils as a Good Source of Vitamin E, *Food Chemistry*, *296* (2019), Oct., pp. 160-166
[33] Nobuhiro, K., *et al*., Efficacy of Vitamin E for Mechanical Damage and Oxidation of Polyethylene Rim by Stem Neck Impingement, *Clinical Biomechanics*, *68* (2019), Aug., pp. 8-15
[34] Xia, P. G., *et al*., Comparative Study on Volatile Oils of Four Panax Genus Species in Southeast Asia by Gas Chromatography-Mass Spectrometry, *Industrial Crops and Products*, *74* (2015), Nov., pp. 478-484
[35] Kobaek, L. M., *et al*. Effect of the Dietary Polyacetylenes Falcarinol and Falcarindiol on the Gut Microbiota Composition in a Rat Model of Colorectal Cancer, *BMC Research Notes*, *11* (2018), 1, 411
[36] Heydenreuter, W., *et al*, Alkynol Natural Products Target ALDH2 in Cancer Cells by Irreversible Binding to the Active Site, *Chemical Communications (Cambridge, England)*, *51* (2015), 87, pp. 15784-15787
[37] Li, C., *et al*., Preparation and Characterization of a Novel Environmentally Friendly Phenol-Formaldehyde Adhesive Modified with Tannin and Urea, *International Journal of Adhesion & Adhesives*, *66* (2016), Apr., pp. 26-32
[38] Tholstrup, T., *et al*., Acute Effect of High-fat Meals Rich in Either Stearic or Myristic Acid on Hemostatic Factors in Healthy Young Men, *American Journal of Clinical Nutrition*, *64* (1996), 2, pp. 168
[39] Wu, J. H., *et al*., A Waterborne Polyurethane Coating Functionalized by Isobornyl with Enhanced Antibacterial Adhesion and Hydrophobic Property, *European Polymer Journal*, *108* (2018), Nov., pp. 498-506
[40] Xie, Y. Z., *et al*., Study on Biomolecules in Extractives of Camellia Oleifera Fruit Shell by GC-MS, *Saudi Journal of Biological Sciences*, *25* (2018), 2, pp. 234-236

Paper submitted: May 26, 2019
Paper revised: August 23, 2019
Paper accepted: September 11, 2019