ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Effect of Storage Conditions on the Chemical Composition of Vietnamese Clove (*Syzygium aromaticum*) Essential Oil

Thi Kim Ngan Tran[1,2,3] Thien Hien Tran[1,2,3], Xuan Tien Le[4], Tran Quoc Toan[5,6], Thuy Trang Le Ngoc[7], Hoang Le Tuan Anh[8], Mai Huynh Cang[9]*

1. *Faculty of Environmental and Food Engineering, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.*
2. *Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.*
3. *NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.*
4. *Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam.*
5. *Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam.*
6. *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Viet Nam.*
7. *Institute of Applied Materials Science, Vietnam Academy of Science and Technology, 01 TL29, District 12, Ho Chi Minh City, Vietnam.*
8. *Mientrung Institute for Scientific Research, Vietnam Academy of Science and Technology, Vietnam.*
9. *Department of Chemical Technology, Nong Lam University, Ho Chi Minh City, Viet Nam.*

***Corresponding Author: Mai Huynh Cang**

Abstract

In this study, changes in the composition of the oil from clove buds (*Syzygium aromaticum*) and their blends were investigated by mass gas chromatographic analysis during different storage under light and temperature conditions for a period of four months. Samples that were taken periodically were used to assess the chemical composition. Under these conditions, clove essential oil retains its main ingredients, including Eugenol, ß-Caryophyllene and Acetyleugenol for four months. However, the oxidation rate of the essential oil under direct light and 45 ° C is rapid, contrasted by that essential oils stored in the dark and 4 ° C. The total Eugenol content in essential oils increased further at the end of the storage period under storage conditions. Changes in these compounds during storage can serve as an indicator of quality for clove essential oil.

Keywords: *Syzygium aromaticum, Essential oïl, Storage, Temperature, Light, Oxidation.*

Introduction

In recent years, people have tended to prefer food products, pharmaceuticals, and cosmetics that are natural, non-toxic, and can minimize the introduction of chemicals into the body and protect consumers' health. Therefore, valorization of the plants that produce precious essential oils, which are highly applicable in many fields has been the interest of by scientists [1, 10]. Applicable fields of essential oils include cosmetics, food and pharmaceutical industries [11, 19].

Most essential oils are extracted from the leaves, stems, flowers or bark of plants, so they are very pure and have good effects on health. Clove (*Syzygium aromaticum*) is a woody plant, perennial, with a height of up to 20m, belonging to the family of Myrtaceae. *Syzygium aromaticum* grows in warm climates and has oval leaves with flowers growing in clusters. Clove flower buds split into 4 distinct lobes. Flowers bloom with 4 petals, above the 4 lobes, to help protect the inner stamens. Buds about 10-17.5 mm long, about 2mm thick, dark red eye-catching. Cloves are very fragrant and intense, exerting numb taste on the tongue and can help reduce pain and spiciness. Cloves contain up to 14-21% volatile essential oils and have very specific aroma that is not mixed with other compound.

The stem contains 5% essential oil content while the oil in leaves only amounts to around 2%. In clove buds, essential oil and vitamin are predominant components. Clove essential oil contains valuable compounds in large quantities such as eugenol (80-90%), eugenol acetate (15%) and beta-caryophyllene (5-12%) [20]. Among them Eugenol (4-allyl-2-methoxyphenol) figures as a neuroprotective agent [21].

Eugenol exhibits an excellent bactericidal activity against many organisms such as *Escherichia coli*, *Staphylococcus* aureus and is also known for its antioxidant capacity [22, 23]. Many studies indicated that secondary compounds such as essential oils could be affected by genetic factors, climate, soil and farming techniques [24]. However, there have not been many reports on changes in chemical information about the composition and properties of essential oils during the storage of clove essential oil [25, 28].

Essential oils can degrade via oxidation processes in the presence of air and light, and temperature during storage. These changes often have adverse effects on the quality of essential oil products. In this study, four different storage conditions (light, dark, 4°C, 45 ° C) were established and lasted for four months to evaluate compositional changes and preservability of clove essential oil. The results are presented by the GC-MS analysis method to identify the modified components. Newly extracted essential oil was used as the control sample [29, 32]. Study results are expected to contribute in further development in preservation of sensitive compounds.

Materials and Methods

Clove buds (the main part of the plant) were collected when the buds begin to turn from green to reddish pink (petiole rate 17 - 25%). Collected buds were sun dried in the sun. Flower buds were collected around March-April 2019 of 2019 in India and were purchased by the Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam. Clove buds after being purchased were cleaned to remove impurities. Dried clove buds (1300g) were distilled by the steam distillation system. The mixture was continuously distilled for 4 hours. The resulting essential oil is anhydrous by sodium sulfate, with an efficiency of 6.85% based on dry material.

Storage Condition

To determine the effect of storage conditions on the composition of the essential oil during storage, the essential oil was analyzed immediately after extraction to act as a control sample. Other samples were divided equally and stored in glass jars. Each set of samples is stored at four different temperatures and lighting conditions: kept directly under daylight at room temperature (25 °C), in dark bottles at room and refrigerator temperatures (4°C) and oven (45°C) for 4 consecutive months. The GC-MS analysis method is used to assess the change of volatile compounds in essential oils, after the storage time of 1 month, 2 months and 4 months.

Gas Chromatography-mass Spectrometry Analysais

The chemical composition of the Clove essential oil was determined by GC-MS analysis using GC Agilent 6890 N instrument coupled with the HP5-MS column and MS 5973 inert. The pressure of the head column was 9.3 psi. 25µL of essential oil was added with 1.0 mL n-hexane and dehydrated with Na2SO4.

The flow rate was constant at 1 mL/min. Injector temperature is 250°C and the rate of division is 30. Thermal program for samples: 50°C kept for 2 minutes increased by 2°C/min to 80°C, continued to increase by 5°C/min to 150°C, continued to increase by 10°C/min to 200°C, increase 20°C/min to 300°C hold for 5 minutes. The compounds were determined by comparing retention indices with the Wiley library or with published mass spectra.

Result and Discussion

The impact of light, as well as time on the quality of clove essential oil during storage, is shown in Figures 1a, b and table 1. In general, the content of essential oil components fluctuates throughout storage conditions and periods. Visually, after 4 months, color change from light to darker yellow was observed in samples stored under normal conditions and in 45°C. The results also indicate that the concentration of the lower molecular weight components decreased with time of storage, especially at room temperature. Clove essential oils stored at room temperatures had ß-Caryophyllene, Acetyleugenol, and Eugenol as main components. Under the direct influence of light and in the dark, 3-Allyl-2-methoxyphenol was found in essential oil samples in the first two months of storage, but this ingredient was missing at the end of storage time in the same sample.

On the other hand, 3-Allyl-6-methoxyphenol content was only determined after four months of storage. In direct light conditions, Acetyleugenol content in almost all samples, compared with the control samples (18.111%), tended to decrease during storage after 4 months where the sharpest decrease was observed in February (to 16.877%) and modest increase was detected at the end of the storage period (to 17.533%). Another important ingredient showing an interesting change trend is Eugenol. Eugenol content is constantly changing during the storage period where a decrease after the first month (75.199%) of storage and a subsequent increase in the second month (76.985%) were observed. Overall, the Eugenol content at the end of the storage period (76.869%) was higher than the Eugenol content (76.542%) in the control sample. On the other hand, when storing essential oils in dark bottles, avoiding exposure to light, Eugenol content in the essential oil gradually increased over the storage period, achieving 76.95%, 76.668%, and 76.913% after one, two and four months, respectively.

Similar to Eugenol, Acetyl eugenol experienced gradual decline over the course of four months. To be specific, Eugenol amounted to 18.111% at the time of extraction, then decreased to 16.951%, 17.025% and 17.424% after one, two and four months respectively. Meanwhile, essential oils stored under light easily cause changes in the composition of essential oils because of the chemical changes of terpenoids. In the case of significant compositional changes occurring in the dark, the responsible factors may include the structure and the likelihood of their reaction [29].

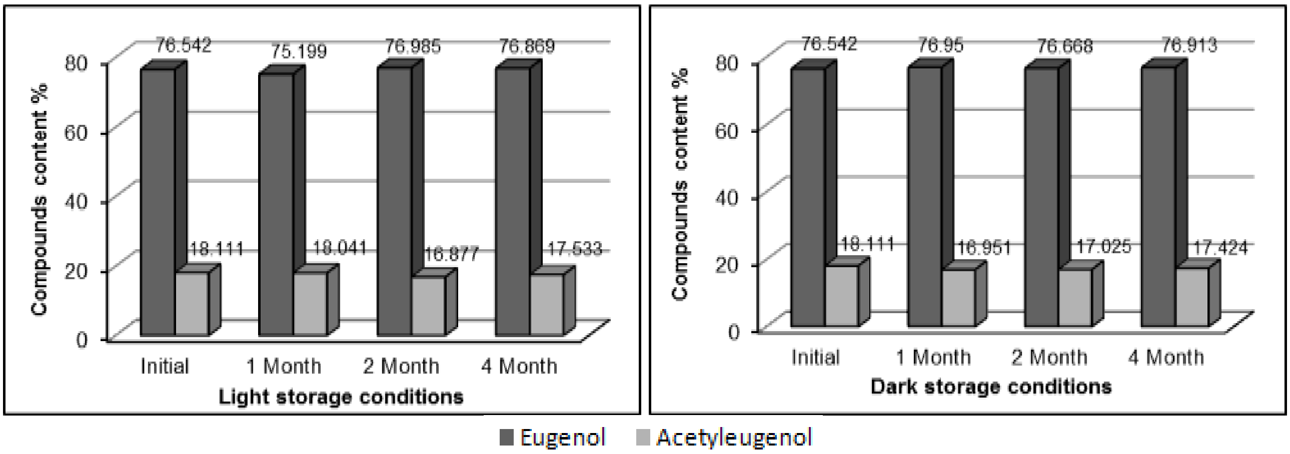

Figure 1: The influence of light on the storage of essential oils

Figure 2a,b and Table 1 indicate that compositional stability of the essential oil was undermined with prolonged storage and elevated temperature (from 4 to 45°C) [33]. Evidently, most compounds decreased in content markedly at 45 °C. At the temperature of 4 °C, essential oil composition tended to decrease in comparison to the newly extracted sample [25]. In this study, monoterpenoids account for most of detected compounds identified in essential oils. Furthermore, highest retentions of main compounds were observed in samples stored at low temperatures, especially at 4°C. For samples stored at 4°C, Acetyleugenol content was 18.111% after distillation, then gradually decreased to 15.56% after 2 months and finally increased to 17.417% at the end of the storage period. For Eugenol, the compound achieved the relative content of 76.258%, 78.294% and 76.983% after one, two and four months after storage respectively. We also found that an increase in storage temperatures has clearly led to an increase in unidentified oxidation products. Current data suggest that essential oils differ in their sensitivity to self-oxidation at different storage temperatures. It is obvious that with the longer storage time, at 45°C, the number of compounds decreased markedly (Table 1). The content of the Eugenol compound of the control sample was 76.542%. After two and four months, the Eugenol content decreased to 77.57% and 77.489%.

In contrast, the Acetyleugenol content (18.111% in the control sample) in essential oils stored at 45°C decreased over time, reaching 17.094% at the end of the storage period. This may be due to volatilization, oxidation and other undesirable changes in essential oil components during storage [34]. Turek et al (2012) reported the values of the physical and chemical parameters and quality changes of the select essential oils during different storage times.

It was shown that compositions vary according to the different storage regimes and rosemary essential oils exhibit marked stability at room temperature in the dark but are susceptible to oxidation under the influence of light [35]. However, in another study Misharina et al (2005), it was shown that Sweet fennel oil is unstable regardless of light condition and declined dramatically within 2 months under light, accompanied by formation of oxidation products, as well as some unidentified compounds. In the same study, it was also revealed that composition of laurel essential oil is independent of light condition [29].

Ambient temperature greatly affects the stability of essential oils in several respects mainly through acceleration of chemical reactions. Current results indicate that the number of components having small molecular weight declines extending storage time. Oxidation reactions are one of the main causes of essential oil damage and it is clear that access to oxygen acts as the critical factor in determining essential oil stability.

On the other hand, Blitzke 2009 has articulated that the lack of reliable and comprehensive studies involving assessing stability of essential oils stored in storage and specific specifications hindered development of proper storage conditions and determination of shelf life.

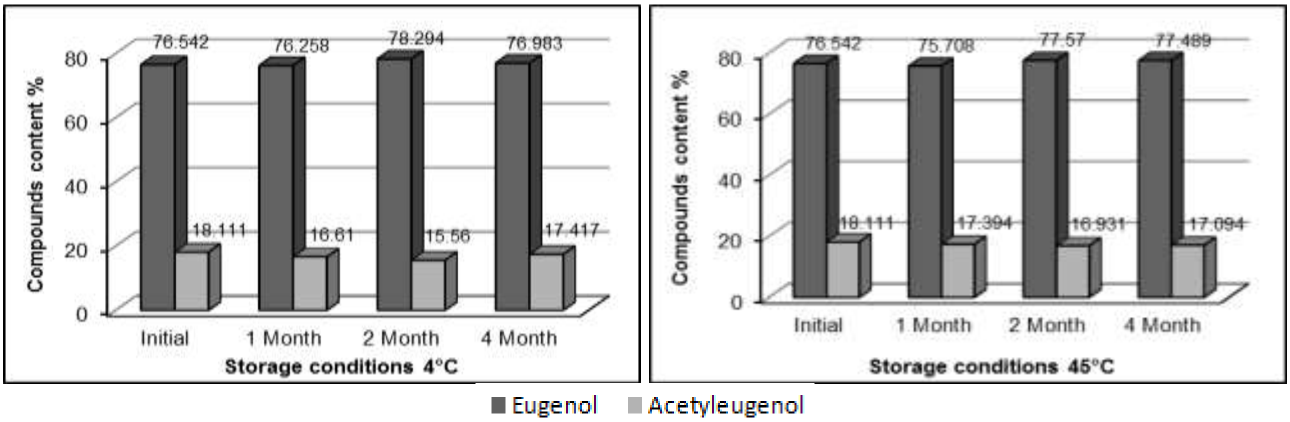

Figure 2: The influence of temperature on the storage of essential oils

Table 1: Ingredients of Clove essential oil after for 4 months stored under the conditions of light and temperature

Name	Initial	1 Month				2 Month				3 Month			
		Light	Dark	4°C	45°C	Light	Dark	4°C	45°C	Light	Dark	4°C	45°C
Eugenol	76.542	75.199	76.95	76.258	75.708	76.985	76.668	78.294	77.57	76.869	76.913	76.983	77.489
3-Allyl-2-methoxyphenol	-	1.639	1.057	1.769	1.998	1.019	1.309	1.038	0.776	-	-	-	-
Caryophyllene	4.319	4.018	4.137	4.442	3.689	4.109	3.949	4.622	3.418	-	-	-	-
α-Caryophyllene	0.456	0.426	0.441	0.468	0.401	0.433	0.417	0.486	0.374	-	-	-	-
Acetyleugenol	18.111	18.041	16.951	16.61	17.394	16.877	17.025	15.56	16.931	17.533	17.424	17.417	17.094
3-Allyl-6-methoxyphenol	-	-	-	-	-	-	-	-	-	0.18	0.186	-	-
Caryophyllene oxide	0.192	0.258	0.251	0.185	0.447	0.313	0.326	-	0.652	-	-	-	-

These variations indicate that the contents of the essential component in aromatic plants may be related to the expression of different genes in different seasons. In addition, it was found that an isomer of eugenol that was found in the MS spectrum of the product has the same form as the standard spectrum in the data banks. Eugenol has been shown to have antibacterial properties [37, 38].Therefore; the clove essential oil is suggested to have good antibacterial activity due to the presence of eugenol in high content. In addition, eugenol has also been demonstrated to exhibit strong antifungal activity against different fungal species [39]. The chemical composition of eugenol varies from region to region, possibly due to a number of environmental factors, the part of the tree being used, the age of the tree and the period of the growing season, or even genetic factors.

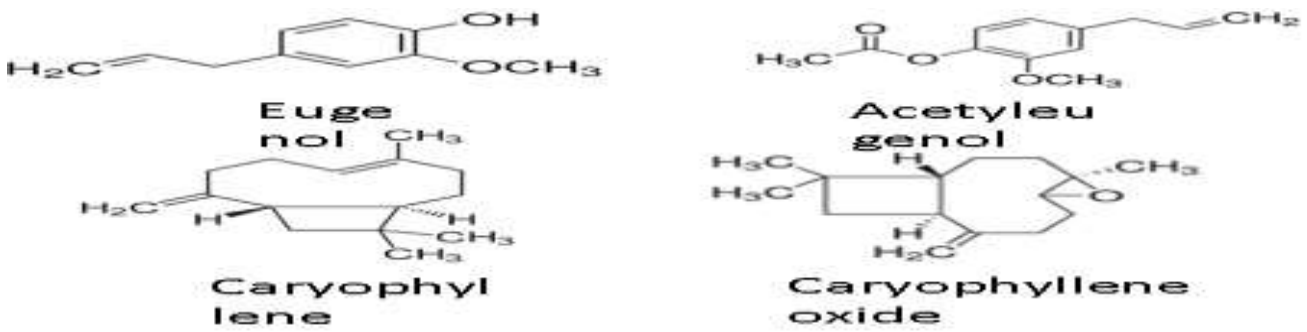

Figure 3: The major components of *Syzygium aromaticum* essential oil

Conclusion

This assessment provides a basis for understanding and identifying future research pathways to attain better preservability. The stability of essential oils isolated from clove essential oil was observed for four months under light and dark light condition and at temperature of 4°C and 45°C. The compounds of clove essential oil have undergone a number of changes depending on the temperature and light impact on storage conditions. It was concluded that *Syzygium aromaticum* essential oil stored in a refrigerator maintained its main ingredients better than samples stored at room temperature and those stored at 45°C. Storing *Syzygium aromaticum* essential oil at low temperatures prevents the reduction of the concentration of the oil components and helps maintain the essential quality of the essential oil with minimal changes. These findings can be extended to industrial applications in preservation of plant-derived essential oils.

Acknowledgements

This research is funded by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

References

1. TTK NGAN et al (2020) “Chemical Components of Agarwood (Aquilaria crassna) Essential Oils Grown in Various Regions of Asia,” Asian J. Chem., 32 (1): 36-40.
2. TT Kim Ngan et al (2019) “Physico-chemical characteristics of Rosmarinus officinalis L. essential oils grown in Lam Dong province, Vietnam,” Asian J. Chem., 31 (12): 2759-2762.
3. TTK Ngan et al (2019) “Physico-chemical profile of essential oil of Kaffir lime (citrus hystrix DC) Grown in An Giang Province, Vietnam,” Asian J. Chem., 31 (12): 2855-2858.
4. TAN PHAT DAO et al (2018) “Extraction of Essential Oils from Vietnam’s Orange (Citrus sinensis) Peels by Hydrodistillation: Modeling and Process Optimization,” Asian J. Chem., 30 (18): 2424-2430.
5. TT Hien, NPT Nhan, ND Trinh, HTT Van, B Giang (2018) “Optimizing the Pomelo Oils Extraction Process by Microwave-Assisted Hydro-Distillation Using Soft Computing Approaches,” Solid State Phenom., 279: 217-221.
6. TH Tran, PTN Nguyen, VTT Ho, THN Le, LG Bach, TD Nguyen (2019) “Using soft computing approaches for orange (Citrus

nobilis Lour. var. nobilis) oils extraction process," IOP Conf. Ser. Mater. Sci. Eng., 479: 012015.

7. TP Dao, TH Tran, TCQ Ngo, HTK Linh, LNY Trung, VT Danh, TTL Ngoc, NDY Pham, PM Quan, TQ Toan (2019) Extraction of Essential Oils from Vietnam's Orange (Citrus sinensis) Peels by Hydrodistillation: Modeling and Process Optimization, Asian J. Chem., 31: 2827-2833.
8. TH Tran, LK Ha, DC Nguyen, TP Dao, LTH Nhan, DH Nguyen, TD Nguyen, DVN Vo, QT Tran, LG Bach (2019) The Study on Extraction Process and Analysis of Components in Essential Oils of Black Pepper (Piper nigrum L.) Seeds Harvested in Gia Lai Province, Vietnam, Processes, 7: 56; doi:10.3390/pr7020056
9. TP Dao, DC Nguyen, DT Nguyen, TH Tran, PTN Nguyen, NTH Le, XT Le, DH Nguyen, DVN Vo, LG Bach (2019) Extraction Process of Essential Oil from Plectranthus amboinicus Using Microwave-Assisted Hydrodistillation and Evaluation of It's Antibacterial Activity, Asian Journal of Chemistry; 31: 977-981.
10. VM Thanh, LM Bui, LG Bach, NT Nguyen, HL Thi, TTH Thi (2019) Origanum majorana L. Essential Oil-Associated Polymeric Nano Dendrimer for Antifungal Activity against Phytophthora infestans, Materials, 12: 14-46.
11. HC Mai, TTT Le, TT Diep, THN Le, DT Nguyen, LG Bach (2018) "Development of solid lipid nanoparticles of Gac (momordica cocochinensis spreng) oil by nano-emulsion technique," Asian J. Chem., 30 (2): 293-297.
12. NPT Nhan et al (2018) "Application of Response Surface Methodology to Optimize the Process of Saponification Reaction from Coconut Oil in Ben Tre - Vietnam," Solid State Phenom., 279: 235-239.
13. H Mai, T Nguyen, T Le, D Nguyen, L Bach (2019) "Evaluation of Conditions Affecting Properties of Gac (Momordica Cocochinensis Spreng) Oil-Loaded Solid Lipid Nanoparticles (SLNs) Synthesized Using High-Speed Homogenization Process," Processes, 7 (2): 90.
14. Toan Duy Nguyen Chinh Thuy Nguyen, Van Thanh Thi Tran, Giang Vu Nguyen, Hai Viet Le, Lam Dai Tran, Giang Long Bach, Hoang Thai (2018) Enhancement of the Thermomechanical Properties of a Fly Ash- and Carbon Black-Filled Polyvinyl Chloride Composite by Using Epoxidized Soybean Oil as a Secondary Bioplasticizer, International Journal of Polymer Science, Article ID 8428736, 8.
15. TH Tran, PTN Nguyen, TN Pham, DC Nguyen, TP Dao, DT Nguyen, ND Hai, DVN Vo, XT Le, NTH Le, LG Bach (2019) Green technology to optimize the extraction process of turmeric (Curcuma longa L.) oils, IOP Conference Series: Materials Science and Engineering, 479: 012002.
16. TP Dao, DC Nguyen, Thien Hien Tran, Phan Van Thinh, Vu Quang Hieu, Dai Viet Vo Nguyen, Trinh Duy Nguyen, Long Giang Bach (2019) Modeling and optimization of the orange leaves oil extraction process by Microwave-assisted Hydro-distillation: The response surface method based on the central composite approach (RSM-CCD Model), Rasayan J. Chem., 12(2): 666-676.
17. HC Mai, ND Dao, TD Lam, BV Nguyen, DC Nguyen, LG Bach (2019) Purification Process, Physicochemical Properties, and Fatty Acid Composition of Black Soldier Fly (Hermetia illucens Linnaeus) Larvae Oil, Journal of the American Oil Chemists' Scoiety, 96: 1303-1311
18. LP Doan, TT Nguyen, MQ Pham, QT Tran, QL Pham, DQ Tran, VT Than, LG Bach (2019) Extraction Process, Identification of Fatty Acids, Tocopherols, Sterols and Phenolic Constituents, and Antioxidant Evaluation of Seed Oils from Five Fabaceae Species, Processes, 7: 456; doi:10.3390/pr7070456
19. DTT Thuy, TT Tuyen, TTT Thuy, PTH Minh, QT Tran, PQ Long, DC Nguyen, LG Bach, NQ Chien (2019) Isolation Process and Compound Identification of Agarwood Essential Oils from Aquilaria crassna Cultivated at Three Different Locations in Vietnam, Processes, 7: 432. doi:10.3390/pr7070432.
20. MH Alma, M Ertaş, S Nitz, H Kollmannsberger (2007) "Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum L.)," BioResources, 2 (2): 265-269.

21. H Kabuto, M Tada, M Kohno (2007) "Eugenol [2-methoxy-4-(2-propenyl)phenol] prevents 6-hydroxydopamine-induced dopamine depression and lipid peroxidation inductivity in mouse striatum," Biol. Pharm. Bull., 30 (3): 423-427.
22. Y-L Li, C-M Yeung, Y-ZC Lawrence, CM Chiu, VEC Ooi (2009) "Chemical Composition and Antioxidant Properties of Clove Leaf Essential Oil," Phyther. Res., 23: 140-142.
23. TE Masahiro OGATA, Midori HOSHI, Shiro URANO (2000) "Antioxidant Activity of Eugenol and Related Monomeric and Dimeric Compounds," Chem. Pharm. Bull., 10 (48): 1467-1469.
24. N Arslan, B Gürbüz, EO Sarihan, A Bayrak, A Gümüşçü (2004) "Variation in essential oil content and composition in Turkish anise (Pimpinella anisum L.) populations," Turkish J. Agric. For., 28 (3): 173-177.
25. V Rowshan, A Bahmanzadegan, MJ Saharkhiz (2013) "Influence of storage conditions on the essential oil composition of Thymus daenensis Celak," Ind. Crops Prod., 49: 97-101.
26. DTT Thuy, TT Tuyen, TTT Thuy, PTH Minh, QT Tran, PQ Long, DC Nguyen, LG Bach, NQ Chien (2019) Isolation Process and Compound Identification of Agarwood Essential Oils from Aquilaria crassna Cultivated at Three Different Locations in Vietnam, Processes, 7: 432. doi:10.3390/pr7070432
27. TH Tran, HHH Nguyen, DC Nguyen, TQ Nguyen, H Tan, LTH Nhan, DH Nguyen, LD Tran, ST Do, TD Nguyen (2018) Microwave-Assisted Extraction: Application to the Optimization of Essential Oil Extraction from Vietnamese Basil (Ocimum Basilicum) Using Response Surface Methodology, Processes, 6: 206. doi:10.3390/pr6110206.
28. TP Dao, DC Nguyen, DT Nguyen, TH Tran, PTN Nguyen, NTH Le, XT Le, DH Nguyen, DVN Vo, LG Bach, Extraction Process of Essential Oil from Plectranthus amboinicus Using Microwave-Assisted Hydrodistillation and Evaluation of It's Antibacterial Activity, Asian Journal of Chemistry
29. TA Misharina, AN Polshkov (2005) "Antioxidant properties of essential oils: Autoxidation of essential oils from laurel and fennel and of their mixtures with essential oil from coriander," Appl. Biochem. Microbiol., 41 (6): 610-618.
30. C Turek, FC Stintzing (2012) "Impact of different storage conditions on the quality of selected essential oils," Food Res. Int., 46 (1): 341-353.
31. TA Misharina (2001) "Influence of the duration and conditions of storage on the composition of the essential oil from coriander seeds," Appl. Biochem. Microbiol., 37 (6):9622-628.
32. M Usai et al (2011) "Influence of different stabilizing operations and storage time on the composition of essential oil of thyme (Thymus officinalis L.) and rosemary (Rosmarinus officinalis L.)," LWT - Food Sci. Technol., 44 (1): 244-249.
33. M Durante, MS Lenucci, B Laddomada, G Mita, S Caretto (2012) "Effects of Sodium Alginate Bead Encapsulation on the Storage Stability of Durum Wheat (Triticum durum Desf.) Bran Oil Extracted by Supercritical CO 2," J. Agric. Food Chem., 60 (42): 10689-10695.
34. D Mockutë, G Bernotienë, A Judþentienë (2005) "Storage-induced changes in essential oil composition of Leonurus cardiaca L. plants growing wild in Vilnius and of commercial herbs," Chemija, 2: October 29-32.
35. Turek C, Stintzing FC (2012) Impact of different storage conditions on the quality of selected essential oils. Food Res Int., 46: 341-53.
36. Blitzke T (2009) Atherische Ole und Aromaextrakte in der Kosmetik, der Aromatherapie und im Lebensmittelbereich. In: Hoppe B, Hoppe K, Junghanns W, Kabelitz L, Pank F, Schliephake E, editors. Handbuch des Arznei- und Gewurzpflanzenbaus. Band 1 Grundlagen des Arznei- und ¨Gewurzpflanzenbaus I. Bernburg, Germany: Verein fur Arznei- und Gewurzpflanzen Saluplanta., 383-94.
37. TT Adebolu, SA Oladimeji (2005) "Antimicrobial activity of leaf extracts of Ocimum gratissimum on selected diarrhoea causing bacteria in southwestern Nigeria," 4: July, 682-684.

38. S Cheng, J Liu, E Chang, S Chang (2008) "Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi," 99: 5145-5149.

39. E Varney, J Buckle (2012) "Effect of Inhaled Essential Oils on Mental Exhaustion and Moderate Burnout: A Small Pilot Study," J. Altern. Complement. Med., 19 (1): 69-71.

