Syzygium aromaticum Essential Oils Obtained by Different Methods to be Used in Dentistry

ANGELA CODRUTA PODARIU[1], DURMUS ALPASLAN KAYA[2]*, ALEXANDRA SABINA POTOROACA[1], RAMONA AMINA POPOVICI[1], LAURA CRISTINA RUSU[1]

[1] Victor BabesUniversity of Medicine and Pharmacy Timisoara, First Department of Faculty of Dentistry, 2 Eftimie Murgu Sq., 300041, Timiusoara, Romania

[2]Department of Field Crops, Faculty of Agriculture, Mustafa Kemal University, 31034, Antakya-Hatay, Turkey

The Syzygium aromaticum known as cloves has been used over one century because of its pharmaceutical properties given by main component – eugenol. The aim of this study was to obtain clove essential oils by two methods, hydrodistillation and microwave-assisted extraction, and to characterize by GC-MS to in order to establish composition in eugenol. The results showed that by classical hydrodistillation the yield was 15% and the main components were Eugenol (80.12%), Caryophyllene (11.80%) and Acetyleugenol (5.57%) and by microwave-assisted extraction the yield was 21% and there were same predominant components with different percentages: Eugenol (78.00%), Caryophyllene (12.45%), Acetyleugenol (5.12%). The eugenol in studied essential oils was over 78% from buds, a high amount which proved that the clove essential oils can be used successful in dentistry.

Keywords: Syzygium aromaticum, clove, essential oil, Microwave-assisted, Hydrodistillation

Well known as clove, *Syzygium aromaticum* (syn: *Eugenia cariophylata*) (Myrtaceae family) has been used for centuries as food preservative and in traditional medicine [1], especially in the preparations for gum and teeth [2]. Clove, though native to Indo-Malaysia, are mostly produced for export by Brazil, Madagascar and Tanzania [3]. It has been traditionally used as a carminative, antiemetic, toothache remedy, and counterirritant. Clove oil is believed to be a carminative, occasionally used in the treatment of ûatulent colic, and is commonly used topically for symptomatic relief of toothache [4]. Oil of cloves has been used to provide pain relief for teething infants, presumably via the interaction of eugenol and the vanilloid receptor [5]. The essential oil of clove shown special properties and pharmacological activities which recommend it to be used in medical field, such as anti-inflammatory, antioxidant, antimicrobial, antibacterial, antifungal, antiviral, antinociceptive, antimutagenic, and anticarcinogenic [6-8]. Other therapeutic effects, including anti-phlogistic, anti-vomiting, analgesic, antispasmodic, anti-carminative, kidney reinforcement, antiseptic and HCMV extracorporeal restraining effect were reported by Liu et al.[9].The main component of clove oil is eugenol, reported in dentistry over a century. According with The National Institute of Health [10] clove is used topically for toothache and for pain control during dental work. Also due to antiseptic and analgesic effects it has been using for the treatment of local infections in tooth cavities and killing the tooth nerves [11]. Depending on the part of where the oil is extracted (bud, leaf, or stem) the eugenol amount varying from 60 to 90%. Different studies showed that clove buds usually eugenol, β-caryophyllene, α-humulene, caryophyllene oxide and acetyl eugenol. The published results reveals a great variability in chemical compositions of the clove bud essential oils. The main components of Turkish clove bud (cultivated in Antalya-Turkey) are eugenol (87%), eugenyl acetate (8.01%) and β-caryophyllene (3.56%), [12].The main components of Bangladesh (cultivated in Chittagong-Bangladesh) are eugenol (47.70%), β-caryophyllene (18.90%) and benzdene-1-ethyl-3-nitro (11.10%) [13]. The main components of Indian clove bud (cultivated in Pencap-India) are eugenol (77.81%), eugenyl acetate (21.30%) and â-caryophyllene (8.44%) [14].

In this study, the components of essential oils clove buds obtained by hydrodistillation and microwave-assisted extraction methods were compared.

Experimental part

Materials and Methods

In the study, cloves buds obtained from spice markets in Turkey were used as material.

Obtaining essential oils of Syzygium aromaticum buds

Essential oils of Clove bud were obtained by two different methods:

Classical hydrodistillation using Neo-Clevenger apparatus

The neo-Clevenger was used to obtain essential oil for hydrodistillation extraction. The buds were weighed and placed in a round bottom flask with a volume of distilled water (the solvent); mixture was refluxed about 3-4 h, during which the oil was collected in the side arm of the system (having a density more than water, oil separates out of the water). The installation was allowed to stand for about half an hour to prevent the oil to reach room temperature, the oil was dried over anhydrous sodium sulfate and then stored in dark color (amber) glass bottles and keep to refrigerator (about 4° C) until use for GC/MS analysis. The obtained volatile oil is a clearly liquid, slightly yellowish and with characteristic smell.

Microwave-assisted extraction

Using a microwave-assisted equipment, Minilabotron 2000 consisting of a 2.45 GHz microwave multimode resonant cavity equipped with a built-in CW (continuous wave) industrial generator (switch mode power supply and magnetron) with output power adjustable up to 2,000 W. A single extraction was performed by clove buds: It was weighed and placed in a round bottom flask with a volume of distilled water; the mixture was refluxed about 15 min, during which the oil is collected on the side arm of the system. During the experiment, the continuous control and monitoring of the time, forward power, reflected power

* email: dak1976@msn.com

and temperature are achieved via an integrated PLC/digital display: t = 15 min., P = 0.5 kW, T = 96°C. The steam produced in the reactor carrying the essential oil of clove is directed to a condenser with a Clevenger system. The separated oil was dried over anhydrous sodium sulfate and then stored in dark color glass bottles and keep in the refrigerator at 4 ° C until GC/MS analysis.

Analysis by GC/MS

Analysis of the essential oils carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 °C, MS Ionization source temperature was 220 °C, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1µL. Oven temperature was programmed in the range of 50 to 220 °C at 3 °C /min. The structure of each compound was identified by comparison with their mass spectrum (Wiley9 library). The data were handled using Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product no. 04070) and C_{21}-C_{40} (Fluka, product no. 04071).

Results and discussions

Yield of extraction clove essential oils

Essential oil rates obtained by different methods are given in table 1. The relative rate of clove essential oil was calculated as equation 1:

$$\textit{Essential oil rate}\ (\%) = \frac{g\ of\ extract}{g\ of\ dried\ material} \times 100 \quad (1)$$

Table 1

THE EXPERIMENTAL DATA OBTAINED FROM THE EXTRACTION OF OIL OF CLOVE BY DIFFERENT METHODS

Vegetable material	The method of obtaining	Practical weight (g)	Volume of distillated water (L)	Volume of essential oil (mL)	%
Clove buds	classical hydrodistillation	50.00	0.5	7.25	15.00
	microwave extraction	50.00	0.5	10.50	21.00

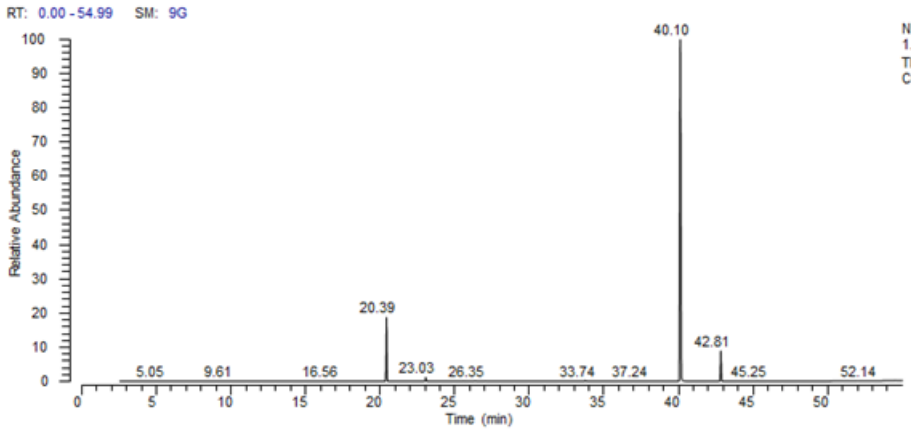

Fig. 1. GC-MS chromatogram of *Syzygium aromaticum* oil obtained by classical distillation

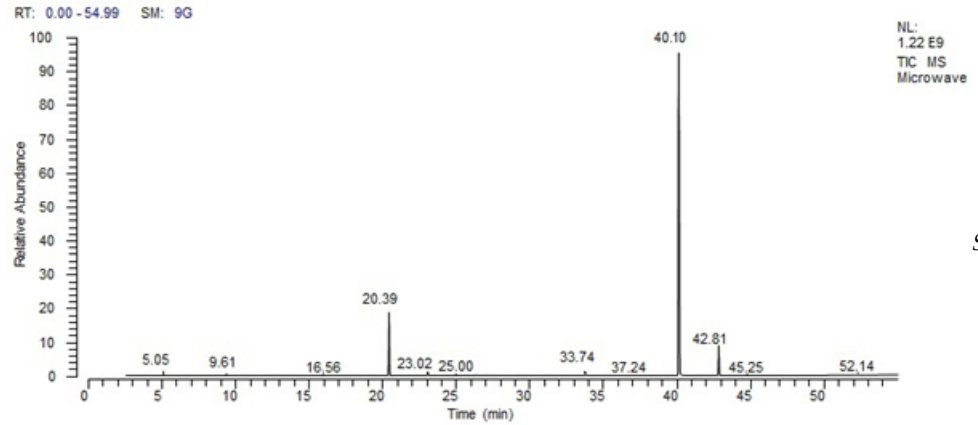

Fig. 2. GC-MS chromatogram of *Syzygium aromaticum* oil obtained by microwave extraction

Table 2

COMPARISON BETWEEN CHEMICAL COMPOSITIONS OF ***SYZYGIUM AROMATICUM*** OIL OBTAINED OBTAINED BY CLASSICAL HYDRODISTILLATION (CH) AND MICROWAVE-ASSISTED EXTRACTION (MA)

RT	*RI*	*Compound Name*	*SI*	*RSI*	*Cas #*	*CH*	*MA*
						%	%
7.08	742	Eucalyptol	759	894	470-82-6	0.02	0.35
8.86	814	1-Methylhexyl acetate	760	914	5921-82-4	0.02	0.23
9.61	838	Cyclohexanone	900	977	108-94-1	0.04	0.12
11.29	884	Oleic Acid	560	578	112-80-1	0.01	-
13.09	930	2-Nonanone	779	851	821-55-6	0.02	0.10
13.51	940	Cyclohexanol	886	963	108-93-0	0.04	0.09
16.56	1005	α-Copaene	924	952	3856-25-5	0.12	0.13
19.12	1061	Linalool	754	825	78-70-6	0.02	0.07
20.39	1086	Caryophyllene	977	977	87-44-5	11.80	12.45
21.64	1111	Methyl benzoate	617	741	93-58-3	0.01	-
22.78	1136	Cadina-1(10),4-diene	635	816	483-76-1	0.02	-
23.02	1141	Humulene	967	970	6753-98-6	0.81	0.65
23.27	1146	Ethyl benzenecarboxylate	681	800	93-89-0	0.02	0.23
23.83	1158	γ-Muurolene	855	907	30021-74-0	0.05	0.05
24.28	1167	3-Carene	694	766	13466-78-9	0.02	0.01
24.46	1171	α-Terpineol	574	805	98-55-5	0.01	0.02
24.80	1177	Eremophilene	735	804	10219-75-7	0.02	-
25.00	1181	Clovene	633	677	469-92-1	0.01	0.13
25.52	1191	Carvone	937	968	99-49-0	0.08	0.12
25.62	1193	α-Acetoxytoluene	816	967	140-11-4	0.03	0.01
27.08	1225	methyl salicylate	922	962	119-36-8	0.11	-
29.04	1267	Estragole	844	915	140-67-0	0.05	0.19
31.43	1319	Patchoulane	736	829	25491-20-7	0.03	0.01
33.74	1371	Caryophyllene oxide	966	973	1139-30-6	0.25	0.36
36.15	1426	Methoprene	635	703	40596-69-8	0.02	0.01
40.10	1521	Eugenol	985	987	97-53-0	80.12	78.00
42.81	1588	Acetyleugenol	963	966	93-28-7	5.57	5.12
45.25	1652	chavicol	883	945	501-92-8	0.06	0.09
52.14	1843	Farnesol	591	635	4602-84-0	0.04	0.02

RT: Retantion time, RI: Retantion index, CH: Classical Hydrodistillation, MA: Microwave-Assisted Distillation

Composition of clove essential oils

Clove oil chromatograms obtained by two different methods are shown in figures 1 and 2, and the chemical compositions of essential oils are shown in table 2.

Table 2 shows that the main components of clove oil extracted by classical hydrodistillation were: Eugenol (80.12%), Caryophyllene (11.80%), Acetyleugenol (5.57%). It has been determined that the results obtained are in full agreement with the literature. The same main components were found also in microwave-assisted extraction: Eugenol (78.00%), Caryophyllene (12.45%), Acetyleugenol (5.12%).

Conclusions

In classical hydrodistillation 28 components with 99.38% yields, in microwave-assisted extraction 23 components with 99.56% yields have been identified. The eugenol in studied essential oils was over 78% from buds, a high

amount which proved that the clove essential oils can be used successful in dentistry.

Acknowledgement Bridge Grant PN-III-P2-2.1-BG-2016-0455/ 122BG

References

1. CORTES ROJAS, D.F,* DE SOUZA C.R.F, OLIVEIRA W.P., Asian Pac J Trop Biomed. 2014 Feb; **4**(2): 90–96
2. BHUIYAN, N.I, BEGUM, J., NANDI, N.C., AKTER, F., African Journal of Plant Science Vol. **4**(11), pp. 451-454, November 2010
3. DUKE, J. A., CRC Handbook of Medicinal Spices, CRC Press, Boca Raton. 2003, p. 25.
4. EBADI, M. Pharmacodynamic Basis of Herbal Medicine, 2nd Edition, CRC Press, Taylor & Francis Group, Boca Raton. 2007, p. 90.
5. CLIFFORD, M.N. J. Sci. Food Agric., **80**, 2000. p. 1033–1043.
6. RYU, B., KIM, H., WOO J.H., CHOI, J.H., JANG, D., Fitoterapia **115**, 2016. p. 46–51
7. SCOPEL, R, FALCAO, M.A., LUCAS, A.M., ALMEIDA, R.N., GANDOLFI, P.H.K, CASSEL, E., VARGAS, R.M.F.**,** Journal of Supercritical Fluids, **92**, 2014. p. 223-230.
8. MANAILA, E., BERECHET, M.D., STELESCU, M.D., CRACIUN, G., MIHAIESCU, D.A., PURCAREANU, B., CALINESCU, I., FUDULU, A., RADU, M., Rev. Chim.(Bucharest), **67**, no.1, 2016, p. 106
9.LIU H, MAO P, HONG S (1997).Med.J.Chin.People'sLiberationArmy,**1**, 1997. p.73-75]
10.*** http://www.nlm.nih.gov/medlineplus/druginfo/natural/patient-clove.html
11.SANDBERG,F.,CORRIGAN,D.,NaturalRemediesTheirOriginsandUses, Taylor&Francis, NewYork,2001,p.46.
12. ALMA, H.K, ERTAS, M., NITZ, S., KOLLMANNSBERGER, H., BioResources, 2007, **2**(2), 265-269.
13. BHUIYAN, M.N.I. Afr. J. Pharm. Pharmaco.,**6**, no. 16 (2012): 1260 - 1263.
14. GUPTA M, SINGH D, GULARIA P AND GUPTA S, J. Chem. Pharm. Res., **7** (1), 2015,568-572.

Manuscript received: 8.03.2016