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Isolation and characterization of 4-allyl-2methoxyphenol (eugenol) from clove buds marketed in Tehran city of Iran

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Abstract: Clove oil was extracted from the buds of clove plant, Syzygium aromaticum. Hydrodistillation of clove buds purchased in the maket of Tehran, Iran, resultled in the formation of a mixture which contained the desired eugenol and eugenol acetate. The progress of isolation of the crude oil was monitored by FT-IR and HPLC. Separation of eugenol from eugenol acetate was achieved through chemical methods, by dissolving the mixture in ether and extracting it with aqueous NaOH (6M) solution. The aqueous layer was separated and to it was added concentrated HCl until its pH became about 7-8. Finally, ether was added to this solution until the free eugenol was migrated and dissolved completely in the ethereal layer. The purity of eugenol was determined by HPLC. The chemical structure of isolated eugenol was confirmed by its ¹HNMR, MS, FT-IR, UV-VISIBLE spectra. **Keywords:** Clove oil, eugenol, 4-allyl-2-methoxyphenol.

Introduction

Clove oil is an essential oil from the clove plant, **Syzygium aromaticum** (Eastern Hemisphere) or Eugenia caryophyllata and Eugenia aromaticum (Western Hemisphere). There are only small differences between these species and many consider them to be essentially the same. It is a natural analgesic and antiseptic used primarily in dentistry for its main ingredient eugenol (I). It can also be purchased in pharmacies over the counter, as a home remedy for dental pain relief, mainly toothache. The oil produced by cloves can be used in many things from flavouring medicine to remedies for bronchitis, the common cold, a cough, fever, sore throat and tending to infections. The therapeutic properties of clove oil are analgesic, antiseptic, antispasmodic, antineuralgic, carminative, anti-infectious, disinfectant, insecticide, stimulant, stomachic, uterine and tonic. The main clove oil-producing countries are Madagascar and Indonesia.¹







The scientific name of clove is **Syzygium aromaticum.** It belongs to the genus Syzygium, tribe Syzygieae, and subfamily Myrtoideae of the family Myrtaceae. It is classified in the order of Myrtales, which belong to super order Rosids, under Eudicots of Dicotyledonae. Clove is an angiospermic plant and belongs to division of Magnoliophyta in the kingdom Plantae.²

Clove oil is a naturally occurring food flavour and is extensively used in fragrance and flavor formulations for its spicy aroma. Clove oil and its primary ingredient eugenol (I) have been in widespread use as flavoring and fragrance agents in the United States since before 1900. The soap and detergent industry is a major user of both materials, and eugenol is typically used in such products at concentrations in the range of 0.05-0.1% (v/v). The Environmental Working Group's cosmetics database lists 278 cosmetic and personal care products available over-the counter that contain eugenol in low concentrations.

There are three types of clove oil:¹

- (i) Bud oil is derived from the flower-buds of S. aromaticum. It consists of 60–90% eugenol, eugenyl acetate (4-Allyl-2-methoxyphenyl acetate) (II), caryophyllene [4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene;
 β-Caryophyllene; trans-(1R,9S)-8-Methylene-4,11,11-trimethylbicyclo[7.2.0]undec-4-ene] (III) and other minor constituents.
- (ii) Leaf oil is derived from the leaves of S. aromaticum. It consists of 82–88% eugenol with little or no eugenyl acetate, and minor constituents.

(iii) Stem oil is derived from the twigs of S. aromaticum. It consists of 90–95% eugenol, with other minor constituents.

Clove essential oil is extracted from Eugenia caryophyllata (also known as Syzygium aromaticum, Eugenia aromatica, E. carophyllus) of the Myrtaceae family.

A native of Indonesia and the Malacca Islands, it is an evergreen tree that grows to about 10 meters (30 feet) tall and has bright green leaves and nail-shaped rosepeach flower buds which turn, upon drying, a deep red brown. These are beaten from the tree and dried.

The Latin word 'Clavus' means nail shaped, referring to the bud. It was often used by the Greeks, Roman and the Chinese, to ease toothache and as a breath sweetener, especially when talking to the Emperor. It has antiseptic properties and was used in the prevention of contagious diseases, such as the Plaque. It was an important commodity in the spice trade and is still used in perfumes, mulled wines and liqueurs, love potions, dental products and, stuck in an orange as pomade, an insect repellent.

Clove oil can be used for acne, bruises, burns and cuts, keeping infection at bay and as a pain reliever. It helps with toothache, mouth sores, rheumatism and arthritis. It is beneficial to the digestive system, effective against vomiting, diarrhea, flatulence, spasms and parasites, as well as bad breath. Clove oil is valuable for relieving respiratory problems, like bronchitis, asthma and tuberculosis. The disinfecting property is useful in cases of infectious diseases. Placing a few drops of clove oil on a cotton ball and then placing the cotton ball in a linen cupboard will not only fragrance the cupboard, but will help to keep fish moths at bay. It is also of use for skin problems - especially for skin sores and leg ulcers and as an insect repellent.

Although clove oil is a very potent oil that should be used with great care in aromatherapy, it does have wonderful properties - from stimulating the mind and lifting depression, to aiding digestion, relieving pain in arthritis and rheumatism, easing respiratory problems and assisting leg ulcers. If it is used in oil, lotion or cream applied to the skin, the concentration should be well below 1%. It may cause irritation to the skin of some individuals and can easily irritate the mucus membranes. It should be avoided during pregnancy. Clove oil has a warm, strong, spicy smell and the oil is colorless to pale yellow with a medium to watery viscosity. In summary³:

Clove oil is useful for its disinfecting properties, relieving of pain, especially toothache, arthritis and rheumatism. It is effective when used for complaints of the digestion system.

(i). Burners and vaporizers: In vapor therapy, clove oil can be useful for bronchitis and dizziness and to help lift depression, while strengthening memory and fighting weakness and lethargy.

(ii). Massage oil: Clove oil can be used in a blended massage oil to assist with diarrhea, bronchitis, chills, colds, muscular numbness, spasms, rheumatism and arthritis. For toothache the outer jaw can be massaged with this oil. Use a low dilution of less than 1%.

(iii). In cream or lotion: When used in a cream or lotion, the positive effects of clove oil are the same as those of a massage oil and can furthermore help to sort out leg ulcers and skin sores. Use in low dilution of less than 1%.

(iv). Mouth wash: Clove oil can be included at a low rate as part of a mouthwash for toothache.

The main chemical components of clove oil are eugenol (I), eugenol acetate (II), iso-eugenol (III) and caryophyllene (IV), (figure 1).

Experimental

Reagents and solvents (reagent grade) were purchased from Sigma-Aldrich and Merck and used without further purification. Evaporation and concentration in vacuo were performed at water aspirator pressure. ¹HNMR spectra were recorded in CDCl₃ 500 MHz Bruker instruments at 20 °C. Chemical shifts are reported in ppm relative to the signal of Me₄Si. Mass spectra were recorded on Mass spectrometer (Vannian Mat, 311-A, Germany) instrument. Reversed Phase HPLC was carried out on a Kenauer, Germany instrument. FT-IR spectrum was measured on a Bruker (Nicolet 550) instrument. UV-Visible spectrum was measured on an Optizen (South Korea) instrument.

Isolation of clove oil from clove buds: clove buds (30 g) was placed into a 1 liter three necked round bottomed flask equipped with a separatory funnel. The apparatus was set up for a direct steam distillation. 600 mL distilled water was added to the flask and the flask was heated with a Bunsen burner. During the steam distillation water was added through a separatory funnel at a moderate rate just to maintain the original level of the liquid in the distilling flask. Distillation was continued until the distillate became almost colourless. Then, the distillate was extracted with methylene chloride (3×50 mL). The aqueous layer was discarded. The methylene chloride layer was dried over anhydrous sodium sulphate and filtered it over a filter paper. Removal of the solvent on a rotary evaporator, resulted in the formation of a brown colour oil(5 g). Its FT-IR showed a mixture of eugenol and a carbonyl compound. The crude oil (5 g) was column chromatographed several times on silica gel and the main fraction was collected and anyalysed (4g, 16.6%), its n_D was 1.5369. Its FT-IR was exactly identical with that of pure eugenol and showed v^- (cm⁻¹): 3514.3 (br,s, OH str.), 3074.7 and 3003.0 (=C-H aromatic and olefinic, m-w), 2972.9, 2937.9, 2841.1 (m,C-H str., Aliphatic), 1637.6 (w, sharp, C=C olefinic), 1608, 1513.8, 1459.5 (m-s, sharp, C=C aromatic), 1432.0 (m, sharp, CH₂, aliphatic), 1367.9 (w, sharp, CH₃), 1268.8, 1234.2 (s, C-O str.); its UV-Viisble spectrum showed (λ_{max} 230 nm, A=3; λ_{max} 275 nm, A=1.41); its HNMR (CDCl₃, 500 MHz) showed (δ): 3.335 (d, CH₂, benzylic), 3.892 (s, CH₃O), 5.06 – 5.11 (m,=CH₂, allylic group),5.496 (s, OH phenolic), 5.94 - 5.99 (m, -CH=, olefinic), 6.69 - 6.71 (m, H-3 + H-5), 6.852 - 6.869 (d, H-6, aromatic ring); its mass spectrum (E.I.) showed m/z: 164 $[M^+, 100\%]$, 149 $[(M^+, 100\%)]$, 140 $[(M^+, 100\%)]$ $- CH_3)^+$, 32%], 137[(M $- CH_2 = CH_2)^+$, 21%], 133[(M - $CH_2CH=CH_2)^+, 15\%$, 131 (22%), 121[(149– CO)⁺, 17%], 103 (25%), 91(20%). Based upon these results and comparison of the spectra with the corresponding standard spectra of eugenol, this fraction was identified as 4-allyl-2-methoxyphenol.4,5

Discussion

Clove oil can be extracted from the leaves, stem and buds of clove plant, **Syzygium aromaticum**. The main chemical components of clove oil are eugenol, eugenol acetate, iso-eugenol and caryophyllene. Eugenol belongs to the group of phenylpropanes such as anethol, estragole and cinnamaldehyde, which are formed via the shikimate pathway and are frequently found in essential oils. Eugenol is slightly soluble in water, a prerequisite for all other uses. Eugenol is a clear, pale yellow oil with a pleasant scent and (in dilute aqueous solution) a spicy taste. Together with other spice constituents, eugenol is under detailed investigation for its biological effects in the human body. Eugenol as a phenol is OH-acidic (pK_a 10.3) and can be deprotonated by NaOH solution. The ionic phenolate form is soluble in water; this property was used for separation from other organic constituents of the clove oil. In this work, we isolate the main component of the essential oil derived from clove buds marketed in the city of Tehran, Iran, by steam distillation.

Steam distillation of the clove buds was carried out until the distillate became almost colourless. The distillate was extracted with methylene chloride (3×50)

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2. "Syzygium aromaticumn (L). Merr and Perry L.M.",Germplasm Resources Information Network (GRIN) online database. http://www.ars-grin.gov/cgibin/npgs/html/taxon.pl?50069 (retrieved on June 9, 2011). mL) and the aqueous layer was discarded. The methylene chloride layer was dried over anhydrous sodium sulphate and filtered it over a filter paper. Removal of the solvent on a rotary evaporator, resulted in the formation of a brown colour oil (5g, 17.7%). Its FT-IR showed a mixture of eugenol and another carbonyl compound. Therefore, the crude oil (5 g) was column chromatographed several times on silica gel and the main fraction was collected and analysed (4g,13.3 %, based on the weight of clove buds used), its n_D was determined 1.5369 which was very close to authentic sample. Its FT-IR, ¹HNMR, mass spectrum and UV-Visible spectra were taken and analyzed carefully with those of the authentic sample. Based upon these results and comparison of the spectra with the corresponding standard spectra of eugenol, this fraction was identified as 4-allyl-2-methoxyphenol.

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