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Study of Fluid Extraction from Clove Buds

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ABSTRACT: Various extraction methods have been practiced globally for the extraction of eugenol and other nutraceutics from plants. The most extensively employed approaches in this regard include solvent extraction, hydro-distillation, microwave-assisted extraction, supercritical carbon dioxide extraction and ultrasound-based extraction. Phytogenic components present in a variety of medicinal plants have been extensively used for the prevention and treatment of different lifestyle related risk factors. Traditionally, extracts of different parts of plants have been recommended to cure various complications including bronchitis, diarrhea, skin diseases, cancer, hyperlipidemia, liver ailments, hyperglycemia arthritis, cardiovascular diseases and inflammatory disturbances. The functionality of these plants is proposed due to the presence of a plethora of bioactive ingredients found in them. The most common plant based functional components include sterols, flavonoids, phenols, tocopherols and organic acids that possess tangible health benefits and have wide applications in the development of functional and nutraceutical foods. Hence in present study, we would like to extract fluid from clove buds to obtain its secondary metabolites for medicinal purposes

KEYWORDS: plants, phytogenic, hyperlipidimia, diseases, cancer, secondary metabolites, clove buds, medicinal

I.INTRODUCTION

Solvent extraction is one of the most common and extensively employed methods for the extraction of fluid from clove buds. Accordingly, eugenol has also been extracted using various solvents like methanol, ethanol, petroleum ether and *N*-hexane. The major hindrances of solvent extraction are inclusion of other soluble residues undesirable flavor changes in the food. However, still this method has wide applications for the extraction of eugenol and other fluids from various aromatic herbs.[1] In a typical solvent extraction process of fluid from clove, the clove buds are ground and wrapped in filter paper followed by subjecting the filter paper to the extraction thimble and inserting into the reflux flask having 500 mL capacity. Afterwards, extraction is carried out by using a suitable organic solvent in Soxhlet apparatus. The process ends by concentrating the obtained extracts at 50 °C using rotary vacuum evaporator.[2]

Several modifications have been made in the conventional solvent extraction process, which show higher efficiency as compared to the traditional method. As an instance, batch extraction process is an attractive alternative to the Soxhlet extraction. [3]This method employs the use of reactor equipped with agitator having four blades and motor having 1200 rpm speed. Recently, this method was studied by Garkal *et al.* who extracted eugenol from leaves of tulsi plant using methanol as solvent and reported satisfactory extraction efficiency. They further reported that extraction efficiency of eugenol was not affected by agitation speed.[4]

Hydro-distillation is also one of the mostly used methods for the extraction of fluid. During hydro distillation method, powdered sample (100 g dried and ground clove buds) is soaked into water. To carry out hydro-distillation, dried clove sample is taken into 500 mL volumetric flask and subjected to hydro-distillation for 4–6 hours. Subsequently, the volatile distillate is collected and saturated with sodium chloride following the addition of petroleum ether or other suitable organic solvent. Later, hydro and ether layers are separated and dehydrated by using anhydrous sodium sulphate. Eventually, the sample is heated in water bath at 60 °C for the recovery of ether and concentration of extract. The average yield of oil using hydro-distillation is about 11.5% whereas reported eugenol concentration is 50.5–53.5%. However, extraction yield can be increased by reducing the particle size of ground clove buds.[5]

II.DISCUSSION

Cloves are the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum* (/sɪˈzɪdʒiːəm ˌærəˈmætɪkəm/).[2][3] They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice, flavoring, or fragrance in consumer products, such as toothpaste, soaps, or cosmetics.[4][5] Cloves are available throughout the year owing to different harvest seasons across various countries.[6] The clove tree is an evergreen that grows up to 8–12 metres (26–39 ft) tall, with large leaves and crimson flowers

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grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green, then transition to a bright red when ready for harvest. Cloves are harvested at 1.5–2 centimetres (⅝–¾ in) long, and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball.[7]

Clove *stalks* are slender stems of the inflorescence axis that show opposite decussate branching. Externally, they are brownish, rough, and irregularly wrinkled longitudinally with short fracture and dry, woody texture. *Mother cloves (anthophylli)* are the ripe fruits of cloves that are ovoid, brown berries, unilocular and one-seeded. *Blown cloves* are expanded flowers from which both corollae and stamens have been detached.[8] *Exhausted cloves* have most or all the oil removed by distillation. They yield no oil and are darker in color

Cloves are used in the cuisine of Asian, African, Mediterranean, and the Near and Middle East countries, lending flavor to meats (such as baked ham), curries, and marinades, as well as fruit (such as apples, pears, and rhubarb). Cloves may be used to give aromatic and flavor qualities to hot beverages, often combined with other ingredients such as lemon and sugar. They are a common element in spice blends, including pumpkin pie spice and speculaas spices.[9]

In Mexican cuisine, cloves are best known as *clavos de olor*, and often accompany cumin[10] and cinnamon.[12] They are also used in Peruvian cuisine, in a wide variety of dishes such as *carapulcra* and *arroz con leche*.

A major component of clove taste is imparted by the chemical eugenol,[13] and the quantity of the spice required is typically small. It pairs well with cinnamon, allspice, vanilla, red wine, basil, onion, citrus peel, star anise, and peppercorns.[10,11]

The spice is used in a type of cigarette called *kretek* in Indonesia.[1] Clove cigarettes were smoked throughout Europe, Asia, and the United States. Clove cigarettes are currently classified in the United States as cigars,[14] the result of a ban of flavored cigarettes in September 2009.[15]

Clove essential oil may be used to inhibit mold growth on various types of foods.[16] In addition to these non-culinary uses of clove, it can be used to protect wood in a system for cultural heritage conservation, and showed the efficacy of clove essential oil to be higher than a boron-based wood preservative.[17] Cloves can be used to make a fragrant pomander when combined with an orange. When given as a gift in Victorian England, such a pomander indicated warmth of feeling.[18]

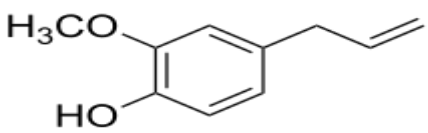
Use of clove for any medicinal purpose has not been approved by the US Food and Drug Administration, and its use may cause adverse effects if taken orally by people with liver disease, blood clotting and immune system disorders, or food allergies.[5]

Cloves are used in traditional medicine as the essential oil, which is used as an anodyne (analgesic) mainly for dental emergencies and other disorders.[18] There is evidence that clove oil containing eugenol is effective for toothache pain and other types of pain,[5][19][20] and one review reported efficacy of eugenol combined with zinc oxide as an analgesic for alveolar osteitis.[21] Clove essential oil may prevent the growth of *Enterococcus faecalis* bacteria which is often present in a root canal treatment failure.[22]

Studies to determine its effectiveness for fever reduction, as a mosquito repellent, and to prevent premature ejaculation have been inconclusive.[5][19] It remains unproven whether blood sugar levels are reduced by cloves or clove oil.[19] The essential oil may be used in aromatherapy.[5]

Eugenol comprises 72–90% of the essential oil extracted from cloves, and is the compound most responsible for clove aroma.[13][37] Complete extraction occurs at 80 minutes in pressurized water at 125 °C (257 °F).[38] Ultrasound-assisted and microwave-assisted extraction methods provide more rapid extraction rates with lower energy costs.[39]

Other phytochemicals of clove oil include acetyl eugenol, beta-caryophyllene, vanillin, crategolic acid, tannins, such as bicornin,[13][40] gallotannic acid, methyl salicylate, the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin, triterpenoids such as oleanolic acid, stigmasterol, and campesterol and several sesquiterpenes.[5] Although eugenol has not been classified for its potential toxicity,[37] it was shown to be toxic to test organisms in concentrations of 50, 75, and 100 mg per liter.[41]



Eugenol

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III.RESULTS

A clear supernatant liquid was obtained by filtering the extract through a tightly packed cotton-plug in a Pasteur pipet. Alternatively, a clear supernatant liquid can be obtained by decanting the extract into a disposable syringe and filtering through a 0.45-micron syringe filter or via centrifugation of the extract followed by decantation of the clear liquid. The same procedure was followed when extracting with chloroform.[23] Alcoholic solutions of eugenol and acetyl eugenol standards were prepared for color testing, TLC, and GCMS. A concentration of 100 mg/mL was used for color testing and TLC analysis, whereas the concentration was reduced to 1 to 10 mg/mL for analysis by GC-MS. [24]The standards are more convenient to work with in diluted form for several reasons. Color testing responses of the pure liquids are too intense and not representative of the lower concentrations of analytes found in most plant extracts.[25] Spotting of the analytes on TLC plates using standard solutions gives a more appropriate analyte loading than the pure liquids, which would surely overload the capacity of the TLC plate. Likewise, analysis by GC-MS requires analytes in a highly diluted form to avoid overloading of the GC column. Eugenol and acetyl eugenol standards were purchased from TCI Chemicals. Flexible, plastic-backed TLC plates were purchased from EMD Millipore (20 x 20 cm, silica gel, 200 mcm thickness,[26] 60-angstrom pore size, F254). Spots were visualized under a 254 nm UV light. Plates are also available with a thin aluminum backing. These flexible plates are convenient for teaching and research purposes due to their ability to be cut to any size with a sharp scissors, or preferably a paper trimmer, and stored in a laboratory notebook. The analyte solutions were spotted with micropipettes that were prepared by heating and stretching glass capillary tubes on a Bunsen burner and cutting each to a useful length.[27] The smaller diameter of micropipettes allows for a controlled loading of solution onto the TLC plate, which produces small, concentrated spots at the origin. This is important since longitudinal diffusion causes spots to enlarge as they travel up the TLC plate via capillary action in the mobile phase.[28]

Color test reagents were prepared from sulfuric acid, formaldehyde, selenious acid, and ferric chloride, all purchased from Fisher Chemicals. Marquis reagent was prepared by mixing 10 mL of formaldehyde (40% by volume) in 90 mL of concentrated sulfuric acid . Mecke reagent was prepared by mixing 1 g of selenious acid in 100 mL of concentrated sulfuric acid .[29] Ferric Chloride reagent was prepared by mixing 10 g of anhydrous ferric chloride (which can be substituted with 16.5 g of the hexahydrate) in 100 mL of DI water [30].Alternatively, color test reagents can be purchased as kits. Color testing was performed by placing a few drops of the test reagent into a white porcelain test well, followed by addition of one drop of sample solution into the well. In this order of addition, placing the test reagent in the well first is important to ensure that an initial negative response is obtained prior to addition of the sample, which indicates an uncontaminated well. The practice serves as a negative control for the experiment. Chemical supplies can be sourced from various vendors; however, it should be noted that analytical grade items produce the best results.[31]

Infrared spectra were acquired on a Perkin-Elmer Spectrum 100 series Fourier Transform Infrared Spectrophotometer fitted with a Universal Attenuated Total Reflectance Sampling accessory containing a ZnSe crystal. Spectra were recorded in % transmittance and scanned from 4000 to 650 cm-1 for four scans per spectrum at a resolution of 2 cm-1[32] . Spectra of eugenol and acetyl eugenol standards were obtained neat, whereas the spectrum for the alcoholic cloves extract was obtained for the residue by placing several drops of the extract onto the ATR window and allowing the solvent to evaporate Total ion chromatograms and mass spectra were obtained on an Agilent 7890A/5975C fitted with a HP-5 column (30 m long x 0.320 mm diameter x 0.250 mcm film thickness),[33] a helium carrier gas flow of 1.3 mL/min (constant flow mode), inlet temperature of 275 deg C, MS transfer line temperature of 280 deg C, oven program of 60-320 deg C at a 30 deg/min ramp with a 2 minute hold and solvent delay at 60 deg C and a 3 minute hold at 320 deg C. The inlet split was 50:1. The quadrupole was set to scan 40-550 Daltons. The MS source temperature was 230 deg C and the MS quadrupole temperature was 150 deg C. Derivatization: 0.20 mg of ground cloves was extracted with 4.0 mL of chloroform and filtered as described earlier.[34] The derivation was conducted in two stages. First, 1-2 drops of acetic anhydride was added to each of two - 1 mL aliquots of the extract. Next, 1-2 drops of triethylamine was added to only one aliquot to facilitate quantitative conversion of eugenol into acetyl eugenol. Acetic anhydride and triethylamine were purchased from Aldrich Chemicals and chloroform was purchased from BDH. Dichloromethane can be substituted for chloroform[35]

IV.CONCLUSIONS

Marquis and Mecke are reagents in the “sulfuric acid series” of color tests. In the Marquis test, all three turned a red color. In the Mecke test, all three turned an initial and brief green that immediately turned to black. It is not surprising that the three samples respond similarly in the sulfuric acid tests.[36] The strongly acidic conditions unmask reactive groups to give a common and responsive product that contains a substituted catechol moiety. A similar effect is seen in the

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ecstasy family of drugs in which the unmasking of a 3,4- methylenedioxy group produces a catechol.[37] The Ferric Chloride test proved to be more discriminating. In the Ferric Chloride test, the cloves extract and eugenol standard turned light green, whereas acetyl eugenol standard showed no response. [38] The positive response for the cloves extract was due to the presence of eugenol. This ability of Ferric Chloride to distinguish a phenol from an aryl ether or ester is found in the opiate class of drugs, namely in the differentiation of morphine from heroin. [39] Morphine and heroin are indistinguishable in any of the sulfuric acid series of tests.[40] However, in the Ferric Chloride test, morphine responds with a color change (blue), whereas heroin has no response. In both the eugenol/acetyl eugenol and morphine/heroin examples, Ferric Chloride is a more discriminating test due to the milder chemical conditions.[41]

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