Isolation and Identification of cloves oil from eugenia caryophyllata using Ultrasonic extraction technique

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Abstract:

This study is designed to isolate and identify of essential oil eugenol from cloves, an important medical plant used in various pharmaceutical formulations .The Isolation process is carried out by Ultrasonic bath technique and simple distillation, with water and extraction with various organic solvents. Optimum organic extractant and optimum pH for both techniques extraction are determined. The oil was determined spectrometrically at 640nm with Folin- Ciocalteau reagent, the maximum extraction yield was estimated in ultrasonic extraction technique. The collected oil is identified via Thin Layer Chromatography (TLC) using a mixture of Ethylacetate: tolune (1:9) as chromatographic eluent. Spectroscopic studies Ultraviolet – Visible (UV-Visible) spectrometry and Infra-Red (IR) spectrometry are also conducted for identification eugenol oil from cloves.

Introduction

Cloves (Eugenia caryophyllata) is also called Eugenia Aromatica A small evergreen tree, pyramidal, trunk soon divides into large branches covered with a smooth greyish bark; leaves large, entire, oblong, lanceolate (always bright green colour), which stand in pairs on short foot-stalks, when bruised very fragrant [1]. The cloves contains volatile oil, gallotannic acid; two crystalline principles - Caryophyllin, which is odourless and appears to be a phylosterol, (Eugenol), gum, resin, fibre[2].

Medicinal Action of the most stimulating and carminative of all aromatics; given in powder or infusion for nausea emesis, flatulence, languid indigestion and dyspepsia, and used chiefly to assist the action of other medicines. The medicinal properties reside in the volatile oil. The Eugenol cool place. If distilled with water, salt must be added to raise the temperature of ebullition and the same Cloves must be distilled over and over again to get their full essence. The Eugenol oil is frequently adulterated with fixed oil and oil of Pimento and Copaiba. As a local irritant it stimulates peristalsis. It is a strong germicide, a powerful antiseptic, a feeble local anaesthetic applied to decayed teeth, and has been used with success as a stimulating expectorant in phthisis and bronchial troubles. Fresh infusion of Cloves oil astringent matter as well as the volatile oil. The infusion and Clove water are good vehicles for alkalis and aromatics [3].

Ultrasonics. branch of physics dealing with high-frequency sound waves, usually in the range above 20,000 hertz (Hz), that is, above the audible range. Modern ultrasonic

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generators can produce frequencies up to more than several gigahertz (1 GHz = 1 billion Hz) by transforming alternating electric currents into mechanical oscillations. The science of ultrasonics has many applications in various fields of physics, chemistry, technology, and medicine. The high-energy produced from ultrasonic waves can be used as a tool in destroying plant cell wall instead of heating which required along time and many tedious steps furthermore care must be taken from flame hazardous[4-5].

The aim of this study is to establish a new procedure Ultrasonic extraction technique for isolation and Characterization of Eugenol and to compare with simple distillation technique. The new method can be applied successfully to produce industrial quantities from eugenol for pharmaceutical preparations.

Materials and Methods

I-Chemicals and Apparatus

a)Chemicals:

Cloves powder, sodium sulfate (Fluka), Organic solvents: 1,2-dichloroethane, dichloromethane, ethylacetate, chloroform, diethyl ether, toluene carbon tetrachloride, and hexane all from (BDH),Ethanol absolute (AnalaR), KBr (BDH), Acetic acid (Fluka), Anisaldehyde (Rederde-Haine),Eugenol (BDH), TLC-silica plate (20x20 ,250 Whatmman), Folin-Ciocalteau reagent (BDH), NaOH (BDH), HCl 37%(BDH).

b)Apparatus:

1. UV-Visible Spectrophotometer (Shemadzu UV-120-20).
2. IR Spectrophotometer (Pye-Unicam ,sp3-300).
3. Digital pH-meter (Orion).
4. Ultrasonic bath (Decon FS300,England).
5. Digital balance (Sartorius,BL 210S).
6. Distillation apparatus.

II. Extraction

a) Ultrasonic bath technique:

Approximately 25g of finally powdered cloves (Eugenia caryophyllata) and 150mL of water was placed and mixed in a 250mL beaker, and then transferred to five 25mL screw vials placed in the Ultrasonic bath and sonicated for 15min.The extracted solution in the five vial were companied and filtered to remove any insoluble materials .About 120mL of the crude extract were prepared to the purification step.

b) Simple Distillation:

Approximately 25g of finally divided cloves (Eugenia caryophyllata) and 100mL of water were mixed and placed in a round bottom flask, distillation apparatus then setup for distillate the cloves oil. The flask heated strongly until boiling started, and then the flame reduced just enough to prevent foam from being carried over into the receiver. About 60ml of the distillate was collected, and after removing the flame 60ml of water was added to the flask. The distillation process resumed and additional 60ml of distillate was collected[1].

III. Purification of Eugenol

The collected 120ml of distillate from the two techniques placed in a 250ml separatory funnel and extracted with three 15ml portion of organic solvent. The organic solvent extracts were combined and 2g of sodium sulfate then added. The flask swirled for 2min and then filtrated. The organic solvent then evaporated on a steam bath in a hood.

To separate Eugenol from acetyleugenol, the remaining four-fifths of the organic solvent solution (about 30ml) was extracted with 5% aqueous NaOH solution. This extraction was carried out three times, using 10ml portion of NaOH each time. The organic layer dried over anhydrous sodium sulfate, the solvent

then filtered and evaporated. The pH=1 was adjusted by conc. HCl and the Eugenol then extracted with three 8ml portion of organic solvent and evaporate the solvent[1].

IV. Quantitative determination of total eugenol

The quantitative determination of total eugenol was conducted spectrometrecally by using Folin-Ciocalteau reagents (Phosphomolybdotungstic reagent) to determine phenol and phenolic derivatives at 640 nm .The calibration curve was constructed as shown in figure(2).

V. Determination of Optimum extraction pH

A set of 16 aqueous test solution of cloves were treated with 0.1N. HCl or 0.1N NaOH to adjust pH values from 0.1 to 10, and then percent of extraction determined spectrometrically from calibration curve.

VI. Identification of eugenol

a) TLC examination

Examined by thin-layer chromatography using a TLC silica plate

Test solution. A 50 µl of the substance to be examined was dissolved in ethanol 70% and dilute to 25 ml with the same solvent

Reference solution (1): A 50 µl of eugenol was dissolved in ethanol 70% and dilute to 25 ml with the same solvent.

Reference solution (2): A 50 µl of anisaldehyde was dissolved in ethanol 70% and dilute to 25 ml with the same solvent.

A 5 µl of each solution was applied to the plate. Develop in a bath of 15 cm using a mixture of 10 volumes of ethyl acetate and 90 volumes of toluene (eluent). The plate was dried in a current of cold air and examined in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with anisaldehyde solution. Heat at 100°C to 105°C for 10 min. The reference solution principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

b) IR-spectrum examination

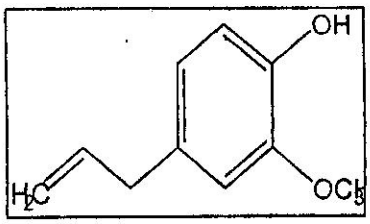
A1 to 2 mg substance was triturated with 0.3 to 0.4 g of dried, finely powdered potassium bromide. These quantities are required for a disc 13 mm in diameter. The mixture was carefully grind and spread uniformly in a suitable die and compressed at a pressure of about 800 MPa. then the disc transferred to Infra-Red spectrophotometer and the IR-spectrum was recorded.

c) UV spectrum examination

A 1% (v/v) eugenol solution in dichloroethane was prepared, then 3ml of the prepared solution was transferred to 1cm cell in a UV-Visible spectrophotometer and the spectrum from 200nm to 370nm was scanned.

Results and Characterization

Eugenol is 2-methoxy-4- (prop-2-enyl) phenol. A colorless or pale yellow, clear liquid, darkening on exposure to air, with a strong odour of clove, practically insoluble in water, freely soluble in alcohol 70 % (V/V), practically insoluble in glycerol, miscible with acetic acid, alcohol, and ether. Eugenol structure shown in figure (1).



$C_{10}H_{12}O_2$

Figure (1): eugenol structure

Optimum organic solvent extractant and percent of yield were estimated from quantitative determination of Eugenol by spectrophotometric method using standard graph figure (2) and as shown in table (1).Various organic solvents 1,2-dichloroethane, dichloromethane, ethylacetate, chloroform, diethyl ether, carbon tetrachloride and hexane were utilized according to polarity. The results demonstrated that Ultrasonic extraction technique excellent in eugenol yield and more rapid from simple distillation technique.

From the table (1) we conclude that the maximum yield reached in Ultrasonic bath method with high polarity organic solvent. However 1,2-dichloroethane and dichloromethane is the best extractant for eugenol.

Table (1): Percent yield of eugenol for Ultrasonic and distillation techniques, and physical constants for the used organic solvent

Organic solvent	%yield Simple distillation	%yield Ultrasonic	Density g/cm^{3}[9]	Dielectric constant[9]
1,2-Dichloroethane	82	95	1.2572	10.4
Dichloromethane	81	90	1.3362	9.1
Ethylacetate	69	87	0.9012	6.0
Chloroform	34	25	1.4984	4.8
Ether(diethyl ether)	50	33	0.7192	4.3
Carbon Teteachloride	17	<10	1.5952	2.2
Hexane	<10	<10	0.6602	1.9

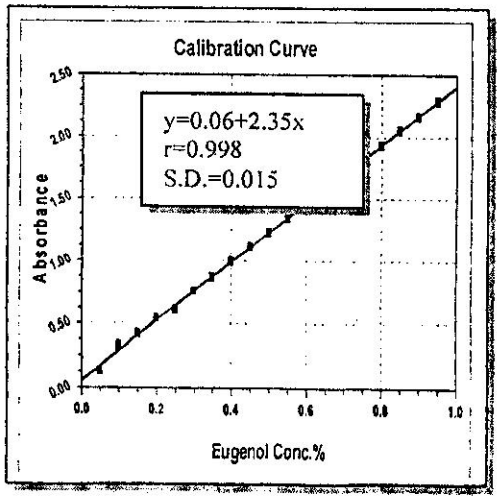


Figure (2): Calibration Curve for determination of eugenol at 640nm

ptimum pH is estimated from quantitative determination of eugenol from different pH solutions for the aqueous media by adding 0.1N HCl or 0.1N NaOH. Figure (3) show that the best pH for extraction eugenol from 0.5 to 2.

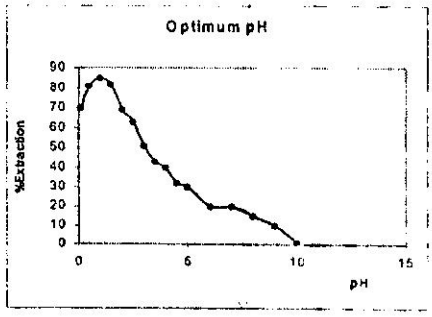


Figure (3): relationship between the pH for aqueous medium and percent of eugenol extraction

The TLC analysis demonstrates that the purified extracts contain one single spot similar in position and size to the spot in chromatogram obtained with reference solution.

IR spectrum examination shows many peaks related to eugenol chemical structure (see figure (1)) as shown in figure (4) and describe in table (2). The broad peak around 3000 cm^{-1} may be related to hydrogen bond usually occurred either from moisture or from intra-hydrogen bond.

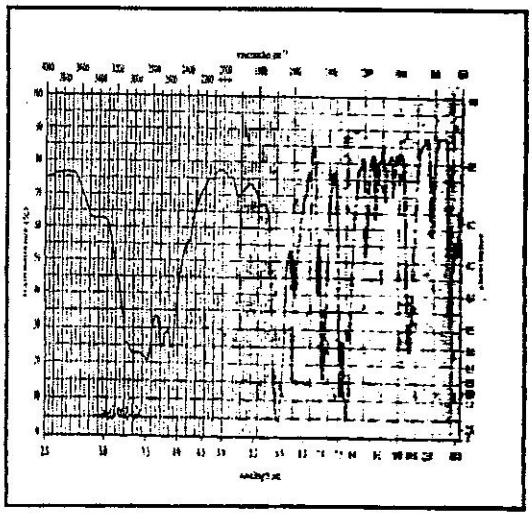


Figure (4): IR Spectrum for purified eugenol.

Table (2): IR spectral data, for eugenol from cloves

Group	Observe Wavenumber (cm^{-1})	Reference Wavenumber (cm^{-1})[7]
Aromatic ring	16,001,450	1600-1585 str*. 1500-1400 str.
Ar-O-R	1200, 1280-1300	1275-1200 str.
Phenolic O-H	3000-3200 1100-1310	3100-3500 str. 1100-1300 ben**.
Alkene C=C	1650-1700	1667-1640 str.
Aliphatic C-H	27,801,450	3000-2740 str. 1450-1375 ben.
Aromatic C-H	3000-3100 1100-1300	3100-3000 str. 1300-1000 ben.

*Stretching,**Bending.

The UV spectrum showed one sharp peak at 256nm as shown in figure (5).This observations identical with expected value for active aromatic electronic spectra due to ($\pi \longrightarrow \pi^*$) electronic transition that occurs in UV region at 255nm according to literatures[7].

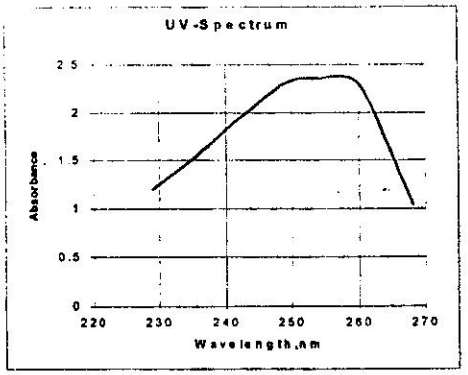


Figure (5): UV Spectrum for purified eugenol (dichloroethane reference)

method simple, rapid and more easy from simple distillation method. Furthermore the high percent yield makes the ultrasonic method suitable to the pharmaceutical industries.

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عزل وتشخيص زيت القرنفل من نبات Eugenia caryophyllata بأستخدام تقنية الآستخلاص بالموجات فوق الصوتية

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الخلاصة:

تم اعداد هذا البحث لعزل وتشخيص احد اهم الزيوت النباتية الطبية من نبات القرنفل وهو ما يعرف بزيت الايوجينول والمستخدم في العديد من المستحضرات الصيدلانية. تمت عملية العزل بأستخدام تقنية الآستخلاص بالموجات فوق الصوتية وتقنية التقطير البسيط ثم الاستخلاص بمذيبات عضوية مختلفة .تم تعيين كمية الزيت طيفيا عند 640 نانوميتر باستخدام كاشف فولن-سيوكالتيو ،ان اعلى قيمة لمنتوج الاستخلاص حددت باستخدام تقنية الاستخلاص بالموجات فوق الصوتية. كما تم خلال البحث تحديد المستخلص العضوي الامثل والرقم الهيدروجيني الامثل لكلا عمليتي الاستخلاص . شخص الزيت المستخلص من نبات القرنفل بطرائق تحليلية عدة ، الاولى تضمنت التشخيص بواسطة كروماتوغرافيا الطبقة الرقيقة حيث استخدم مزيج من الاثيل استيت : تولوين بنسبة 1:9 لمضهر في الكروماتوغرافيا . والثانية التحليل باطياف الاشعة فوق البنفسجية والمرئية ، والاخيرة التحليل باطياف الاشعة تحت الحمراء.