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QUALITATIVE DETERMINATION OF ESSENTIAL OIL COMPONENTS OF STAR ANISE BY GAS CHROMATOGRAPHY(GC)

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ABSTRACT

Essential oils are used in various fields for different purposes. Essential oils are extracted from their specific raw materials by distillation process. The Star Anise is used as a Spice in Culinary (cooking) purposesand the essential oil of Star Anise is used in Medicinal purpose as in Aromatherapy to treat long term and short-term stress related issues. The star anise essential oil is extracted by distillation process, it is first subjected to preliminary tests such as Thin Layer Chromatography and aPhytochemical screening test for terpenoids. Further the extracted essential oil is Qualitatively Determined by using Gas Chromatography. The sample is injected through the inject port it is carried through carrier gas (mobile phase) to the column (stationary phase) and then to the detector (flame ionization detector).The result is observed in the software autochrome 300. The blank is run in which no peaks are observed ,sample is run showing many peaks it is compared with a the standard .Many peaks in the sample chromatogram indicates the presence of many components in it among them Anethol is one of the most important and abundant component of essential oil.

KEYWORDS: Star Anise oil, Gas Chromatography, flavoring, illiciumverum, aromatherapy, spicing agent.

INTRODUCTION

The attraction of medicinal and aromatic plants is continuously growing due to increasing consumers demand and interest in these plants for culinary, medicinal, and other applications by extracting Essential Oils. The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or dry distillation. The essential oil is then separated from the aqueous phase by physical means. Essential oils are soluble in alcohol, ether, and fixed oils, but insoluble in water. These volatile oils are generally liquid and colourless at room temperature. They have a characteristic odour, are usually liquid at room temperature. They are widely used in the cosmetics industry, perfumery, aromatherapy. The latter is intended as a therapeutic technique including massage, inhalations, or baths using these volatile oils.

BIO SYNTHESIS: Terpenoid and phenylpropanoid derivatives are the main components found in essential oils. In most plants, their essential oils contain terpenoids at around 80%. But the presence of phenylpropanoid derivatives affords the essential oils significant flavour, odour. These two groups of compounds are derived from two different pathways from different primary metabolites.

ESSENTIAL OIL EXTRACTION: Essential oil extraction is one of the critical points that can affect the

chemical profile of the essential oil. Many techniques have been developed to obtain essential oil such as microwave- assisted extraction, expression, enfleurage, and solvent extraction. Mostly the hydro distillation is used for the extraction of essential oil.

ESSENTIAL OIL USES: Essential oils are commonly used in aromatherapy to bring about mental and physical wellbeing. Aromatherapy is the practice of blending different therapeutic essential oils to stimulate a desired response. Essential oils can be applied or massaged into the skin, inhaled, or immersed in water. Examples: Eucalyptus oil: nasal decongestant, disinfectant. Clove oil: toothaches. Tea tree oil: antifungal, antibacterial, antiviral properties. Peppermint: digestive disorders. Lavender: anxiety, insomnia, and restlessness. (Jean Baptiste Hzounda Fokou etal., 2020).

INTRODUCTION ABOUT STAR ANISE: Star anise, dry fruits of the star anise tree (Illicium verum), used as a spice and source of pharmaceutical chemicals. The plant is indigenous to the south-eastern part of China and to Vietnam. The volatile, aromatic essential oil is commonly used for flavouring candies, liqueurs, and perfumes. The fruit takes its name from the star like arrangement of its carpels around a central axis. The dried fruit is about 0.25 to 0.5 cm in diameter; individual carpels are usually about 1 cm in length and contain a single seed. Dried carpels are hard, rough, and reddish brown; the seeds are smooth, and light brown. The dried fruits essential oil content is about 3 %, and its principal component is Anethole.

OTHER NAMES: Anis de Chine, Anis Estrellado, Anis Etoile, Anis Etoile Chinois, Aniseed Stars, Anise Stellate Fructus, Ba Jiao Hui, Badiane, Badiane, Badiane de Chine, Bajiro, Chinese Anise, Chinese Star Anise, Eight-Horned Anise, Eight Horns, and illicium verum.

CLASSIFICATION: It belongs to the family of Illiciaceae, dicotyledonous angiosperm species. It is a tropical evergreen tree, tall between 5-10 m. Star anise has large glossy green foliage, its white flowers are beautiful and of great decorative value. Star anise fruit has eight carpels that together form the star-shaped fruit (hence called Star anise).Natural Resource: Star anise is a spice made from the fruit of the Chinese evergreen tree *Illicium verum.*

USES: Star Anise is widely used in Asian cuisines to flavour dishes especially meat and curries. It is also used in desserts and beverages. It's an addition to other popular Indian spices makes a special spicy ingredient, which is called Garam masala..(Article - Balcony GardenWeb).

Culinary uses of star anise: Star anise enhances the flavour of meat. It is used as a spice in preparation of biryani and masala chai all over the Indian subcontinent. It is widely used in Chinese, and in Indonesian cuisines. It is widely grown for commercial use in China, India, and most other countries in Asia.

Medicinal uses: The oil produced from star anise contains thymol, terpineol and anethole, which is used for treating cough and flu. Anise also helps improve digestion, alleviate cramps, and reduce nausea. Consuming star anise tea after meals helps treat digestive ailments such as bloating, gas, indigestion, and constipation.

SIDE EFFECTS: While growing star anise, don 't confuse it with Japanese star anise *(Illicium anisatum*) or Shichimi, which is a poisonous plant and native to Japan. Its seeds or fruits are somewhat like those of star anise and are only slightly smaller and looks like cardamom, having a more rounded shape and have a small hook.

MATERIALS AND METHOD

To Perform Gas Chromatographic method for Qualitative Determination ofEssential oil components of Star Anise seed by using GC Instrument with Capillary column (Stationary phase)HP-50+(30 m x0.32mm x0.25µm) wasused. Theinstrumentisalso equipped with Nitrogen as Carrier gas (Mobile phase), FlameIonizationDetector (Hydrogen and Oxygen are supplied for flame) used as the detection system and Autochrome 300 software is used to read it.Injectport is usedforinjectingthesamples.

Chemicals and Solvents

Essential oil extracted from the Star Anise seed is used. Volatile solvents like benzene, ethylacetate, acetone, hexane, methanol are used in preliminary tests.

Sample Injector

A sample port is necessary for introducing the sample at the head of the column. Modern injection techniques often employ the use of heated sample ports through which the sample can be injected and vaporized in a near simultaneous fashion. A calibrated micro syringe is used to deliver a sample volume in the range of a few microliters through a rubber septum and into the vaporization chamber. Most separations require only a small fraction of the initial sample volume, and a sample splitter is used to direct excess sample to waste. Commercial gas chromatographs often allow for both split and split less injections when alternating between packed columns and capillary columns. The vaporization chamber is typically heated 50 °C above the lowest boiling point of the sample and subsequently mixed with the carrier gas to transport the sample into the column.

Carrier Gas: The carrier gas plays an important role and varies in the GC used. Carrier gas must be dry, free of oxygen and chemically inert mobile phase employed in gas chromatography. Helium is most used because it is safer than, but comparable to hydrogen in efficiency, has a larger range of flow rates and is compatible with many

detectors. Nitrogen, argon, and hydrogen are also used depending upon the desired performance and the detector being used. Both hydrogen and helium, which are commonly used on most traditional detectors such as Flame Ionization (FID), thermal conductivity (TCD) and Electron capture (ECD), provide a shorter analysis time and lower elution temperatures of the sample due to higher flow rates and low molecular weight. For instance, hydrogen or helium as the carrier gas gives the highest sensitivity with TCD because the difference in thermal conductivity between the organic vapor and hydrogen/helium is greater than other carrier gas. Other detectors such as mass spectroscopy, uses nitrogen or argon which has a much better advantage than hydrogen or helium due to their higher molecular weights, in which improve vacuum pump efficiency. All carrier gasses are available in pressurized tanks and pressure regulators, gages and flow meters are used to meticulously control the flow rate of the gas. Most gas supplies used should fall between 99.995% - 99.9995% purity range and contain a low level (< 0.5 ppm) of oxygen and total hydrocarbons in the tank. The carrier gas system contains a molecular sieve to remove water and other impurities. Traps are another option to keep the system pure and optimum sensitive and removal traces of water and other contaminants. A two-stage pressure regulation is required to use to minimize the pressure surges and to monitor the flow rate of the gas. To monitor the flow rate of the gas a flow or pressure regulator was also require onto both tank and chromatograph gas inlet. This applies different gas type will use different type of regulator. The carrier gas is preheated and filtered with a molecular sieve to remove impurities and water prior to being introduced to the vaporization chamber. A carrier gas is typically required in GC system to flow through the injector and push the gaseous components of the sample onto the GC column, which leads to the detector.

Column Oven

The thermostatted oven serves to control the temperature of the column within a few tenths of a degree to conduct precise work. The oven can be operated in two manners: isothermal programming or temperature programming. In isothermal programming, the temperature of the column is held constant throughout the entire separation. The optimum column temperature for isothermal operation is about the middle point of the boiling range of the sample. However, isothermal programming works best only if the boiling point range of the sample is narrow. If a low isothermal column temperature is used with a wide boiling point range, the low boiling fractions are well resolved but the high boiling fractions are slow to elute with extensive band broadening. If the temperature is increased closer to the boiling points of the higher boiling components, the higher boiling components elute as sharp peaks but the lower boiling components elute so quickly there is no separation. In the temperature programmingmethod, the column temperature is either increased continuously or in steps as the separation progresses. This method is well suited to separating a mixture with a broad boiling point range. The analysis begins at a low temperature to resolve the low boiling components and increases during the separation to resolve the less volatile, high boiling components of the sample. Rates of 5-7 °C/minute are typical for temperature programming separations. Open Tubular Columns and Packed Column.

Detection System

The detector is the device located at the end of the column which provides a quantitative measurement of the components of the mixture as they elute in combination with the carrier gas. In theory, any property of the gaseous mixture that is different from the carrier gas can be used as a detection method. These detection properties fall into two categories: bulk properties and specific properties. Bulk properties, which are also known as general properties, are properties that both the carrier gas and analyte possess but to different degrees. Specific properties, such as detectors that measure nitrogen-phosphorous content, have limited applications but compensate for this by their increased sensitivity. Each detector has two main parts that when used together they serve as transducers to convert the detected property changes into an electrical signal that is recorded as a chromatogram. The first part of the detector is the sensor which is placed as close the the column exit as possible in order to optimize detection. The second is the electronic equipment used to digitize the analog signal so that a computer may analyse the acquired chromatogram. The sooner the analog signal is converted into a digital signal, the greater the signal-to-noise ratio becomes, as analog signal are easily susceptible to many types of interferences. An ideal GC detector is distinguished by several characteristics. The first requirement is adequate sensitivity to provide a high-resolution signal for all components in the mixture. This is clearly an idealized statement as such a sample would approach zero volume and the detector would need infinite sensitivity to detect it. In modern instruments, the sensitivities of the detectors are in the range of 10-8 to 10-15 g of solute per second. Furthermore, the quantity of sample must be reproducible, and many columns will distort peaks if enough sample is not injected. An ideal column will also be chemically inert and should not alter the sample in any way. Optimized columns will be able to withstand temperatures in the range of -200 °C to at least 400 °C. In addition, such a column would have a short linear response time that is independent of flow rate and extends for several orders of magnitude. Moreover, the detector should be reliable, predictable, and easy to operate.

Flame ionisation detectors

Flame ionization detectors (FID) are the most generally applicable and most widely used detectors. In FID, the sample is directed at an air-hydrogen flame after exiting the column. At the high temperature of the air-hydrogen flame, the sample undergoes pyrolysis, or chemical

decomposition through intense heating. Pyrolyzed hydrocarbons release ions and electrons that carry current. A high impedancepicometer measures this current to monitor the sample's elution. It is advantageous to use FID because the detector is unaffected by flow rate, non-combustiblegases, and water. These properties allow FID high sensitivity and low noise. The unit is both reliable and relatively easy to use. However, this technique does require flammable gas and destroys the sample.(chemistry of libre texts).

Instrumentation

S NO	NAME OF INSTRUMENT	MODEL
1	GC Instrument	Chemato GC Instrument
2	Column	Capillary column HP 50+
3	Detector	FID-510
4	Software	Autochrome – 300 Younglin
5	Syringe	Hamilton syringe
6	Sonicator	Ultrasonicbath sonicator (1.5L)

Sample collection

Star Anise Seeds were purchased in local area market.

Extraction Star Anise oil from Star Anise seeds

Anise oil was extracted from Star Anise Seeds using distillation apparatus. 100 grams of Anise Seeds were slightly grinded and taken in the round bottom flask and was extracted continuously using distillation technique. The extracted Anise Seed oil was separated using a separating funnel. The obtained Anise Seed Oil was used for gas chromatography analysis.

Preliminary tests

TLC Analysis

Thin Layer Chromatography Analysis is performed for the extracted Anise Seed Oil by taking Four different combination of solvents to see the separation of compounds present in the extracted Anise Seed Oil.

Mobile Phase 1: Ethyl Alcohol: Hexane Ratio: 8.8: 1.2

Mobile Phase 2: Hexane: Acetone
Ratio: 9:1

Mobile Phase 3: Benzene: Ethyl Acetate Ratio: 2.5:1.5

Mobile Phase 4: Ethyl Acetate: Methanol Ratio: 9.5:0.5.

Phytochemical Screening

In Phytochemical Screening a test for Terpenoids is performed. In this test 5ml of the extracted Star Anise Oil is taken to this 2ml of Chloroform is added. Now 1ml of concentrated HSO_4 is added slowly from the walls then a reddish-brown colouration is formed at the interface of two layers.

Standard testing procedure- for the analysis of Star Anise Oil by gas chromatography

Temperature settings

Inject port : 260˚C

Oven : 40–250˚C with a gradual increment of 3˚C/min.

Detector : 280˚C

Gaseous flow settings

Carrier gas : 1.1kg/cm^2

Hydrogen : 1.5kg/cm^2

Oxygen : 2kg/cm^2

Stationary Phase : Capillary column

Detector : Flame ionization detector

Sample volume : 0.1 to 0.2 µL

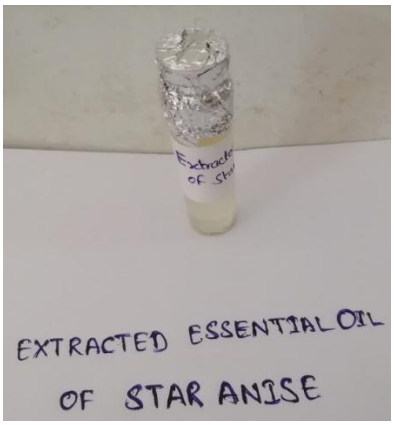
Run time : 80 minutes.

RESULT AND DISCUSSION

RESULTS

Extraction results

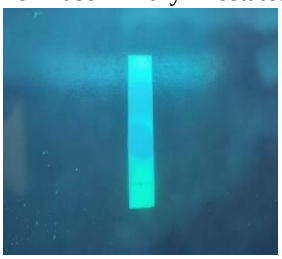
Star Anise Oil was isolated from Star Anise Seed. The extracted Star Anise Oil was analysed using Thin Layer Chromatography and Terpenoids test. Star Anise Oil was isolated from the Star Anise Seed. The extracted Star Anise Oil was analysed using Gas Chromatography method.



PRELIMINARY TEST RESULTS

Thin Layer Chromatography Result

Mobile Phase 1- Ethyl Acetate: Hexane Ratio- 8.8: 1.2



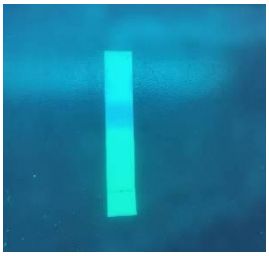
$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_{f\,1} = \frac{6.3}{6.9} = 0.913$

$R_{f\,2} = \frac{5.0}{6.9} = 0.7246$

$R_{f\,3} = \frac{3.5}{6.9} = 0.5072$

Mobile Phase 2 – Hexane : Acetone Ratio- 9:1



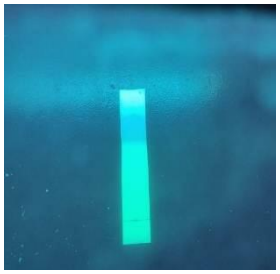
$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_{f\,1} = \frac{5.3}{7.6} = 0.6973$

$R_{f\,2} = \frac{4.8}{7.6} = 0.6315$

$R_{f\,3} = \frac{4.2}{7.6} = 0.55264$

Mobile Phase 3 - Benzene : Ethyl Acetate Ratio- 2.5 : 1.5



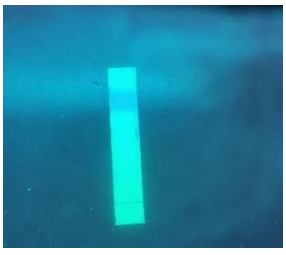
$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_{f\,1} = \frac{6.6}{7.2} = 0.9166$

$R_{f\,2} = \frac{5.4}{7.2} = 0.750$

$R_{f\,3} = \frac{5.1}{7.2} = 0.7083$

Mobile Phase 4 – Ethyl Acetate : Methanol Ratio – 9.5 : 0.5



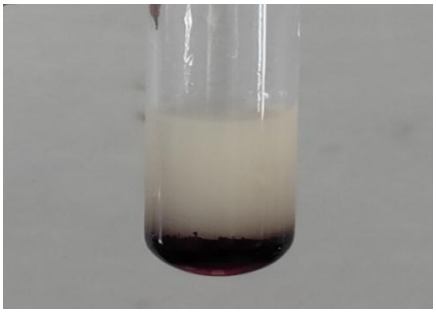
$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_{f\,1} = \frac{7.0}{7.3} = 0.9589$

$R_{f\,2} = \frac{6.1}{7.3} = 0.8356$

Phytochemical Screening Result

The preliminary test, Phytochemical screening for Terpenoids is “POSITIVE” for the StarAnise Oil(Essential Oil).

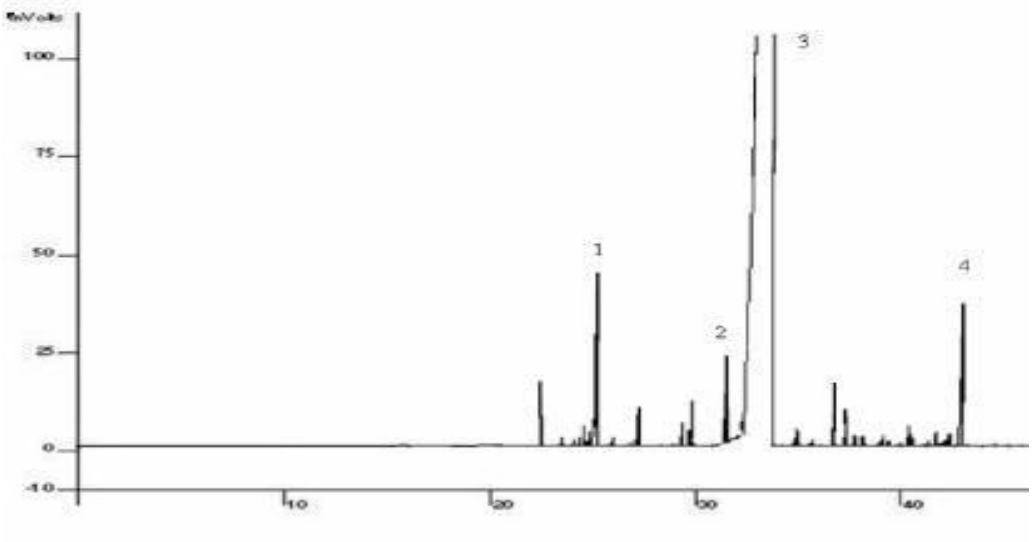


GC RESULT

1	**Blank**	**No Peak is Observed**	**……**
2	Oil Extracted from Anise Seed	More number of peaks observed	29.4667 41.9833

Results confirmed that the oil extracted from Anise seed oil shows the peaks as shown in the chromatogram, hence confirmed that the extracted oil found to having components.

STANDARD CHROMATOGRAM



Sample Chrmaotogram

Compound	RT
α-Thujene	14.2000
α-Pinene	16.6833
Sabinene	19.9167
β-Pinene	24.2000
Herboxide	29.4667
β-Myrcene	33.0833
α-Phellandrene	35.8667
δ-3-Carene	39.2333
α-Terpinene	40.3833
Cymol	41.9833
1,8-Cineole	46.0333
Limonene	57.3500
γ-Terpinene	

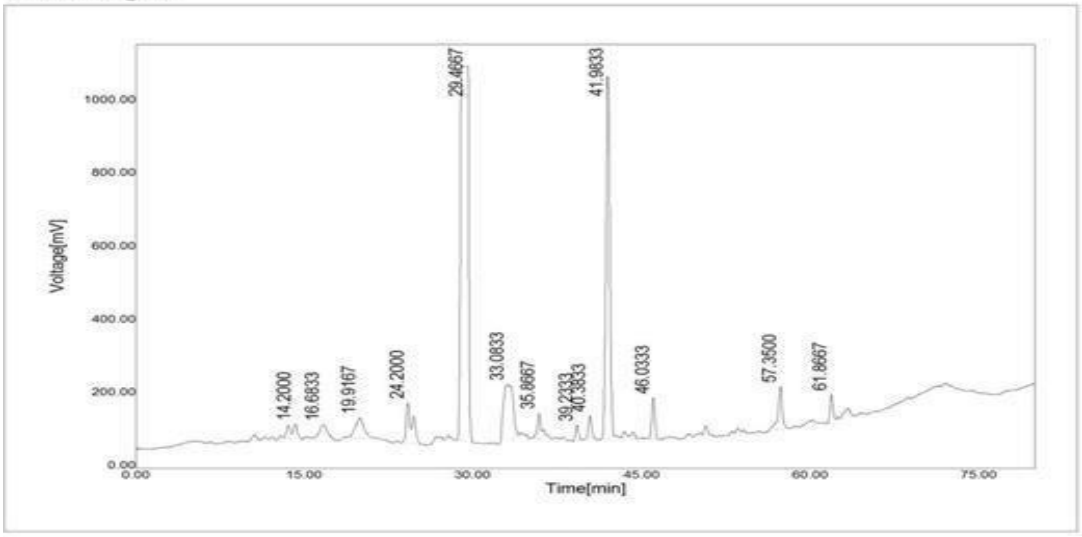
Analysis Report

Post: Prasanna	Name: Star anises

Analysis

Sample Name: STAR ANISE OIL Sample ID: File: 0059.RAW	Date: 2021-07-15 PM 01:52:08 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		14.2000	1780.5886	40.0732	0.0000
2		16.6833	744.9428	22.0715	0.0000
3		19.9167	3062.7070	55.0735	0.0000
4		24.2000	4382.6206	112.8135	0.0000
5		29.4667	49579.6719	1027.4041	0.0000
6		33.0833	9281.6611	153.4979	0.0000
7		35.8667	1924.6758	68.5552	0.0000
8		39.2333	787.8915	42.4532	0.0000
9		40.3833	1261.0146	61.6603	0.0000
10		41.9833	24317.7363	992.7823	0.0000
11		46.0333	2124.8101	112.9381	0.0000
12		57.3500	3091.8679	116.5523	0.0000
13		61.8667	1495.4945	74.6800	0.0000
Sum			103835.6875	2880.5552	0.0000

DISCUSSION

The essential oil from the star anise seed is extracted by distillation process and subjected to preliminary tests such as Thin layer chromatography and Phytochemical screening which gave positive and further the star anise oil is analysed by using Gas Chromatography.

CONCLUSION

Star Anise Oil was extracted by distillation from the Star Anise Seeds. The extracted Star Anise Oil was analyzed using gas chromatography method.

S No	Sample	Number of compounds identified
1	Blank	No peak Observed
2	Oil Extracted from Aniseseed	More number of peaks

Results confirmed that the oil extracted from Star Anise seed show 13 peaks in the chromatogram, confirmed that the extracted oil found to having the components.

Farther studies need to carry for the identification of chemical structure and medicinal activities of the extracted Star Anise Oil.

The Separation of the Essential Oil of Star Anise by Distillation process then the Preliminary test Thin Layer Chromatography and Phytochemical screening, then the final identification of the components of the star anise oil by Gas Chromatography is done successfully.

CONFLICT OF INTEREST

The authors have no conflict of interest regarding this investigation.

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