DECLARATION

I, **Showket Hamid**, with this declare that the matter embodied in the project **"EVALUATION OF THE ANTI-MICROBIAL ACTIVITY OF ESSENTIAL OILS FROM THE FRUITS OF *ILLICIUM VERUM*"** submitted to Global Group of Institutes, Amritsar (Department of Pharmacy) is the result of my direct investigations and project has been composed under the supervision and guidance of **Dr. Manbir Kaur**, Principal (Department of Pharmacy), Global Group of Institutes, Amritsar, Supervisor **Ms. Pooja Kohar**, Global Group of Institutes, Amritsar. I also declare that nothing in part or full has been submitted for the award of any degree, diploma, or fellowship to any other Institute or University. All the ideas and references have been duly acknowledged. If anything wrong is detected in the future, I shall be held responsible.

Date:

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Ms. Pooja Kohar

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Writing, illustrating, and executing this thesis is never a one-man effort, but it is often the result of the invaluable contributions of several individuals directly or indirectly. This suitably applies to this title. Without help, encouragement, and blessings from several people, I could never have been able to finish my work.

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SHOWKET HAMID

CONTENT

LIST OF FIGURES

LIST OF ABBREVIATIONS

WHO: World Health Organisation

TCM: Traditional Chinese Medicine

TSD: Traditional Steam Distillation

OSE: Organic Solvent Extraction

HD: Hydro-Distillation

SCFE: Super-Critical Fluid Extraction

SAO: Star Anise Oil

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

SAWRE: Star Anise Water Reduce Extract

DPPH: 1,1-diphenyl-2-picrylhydrazyl

BOD: Biological Oxygen Demand

DIZ: Diameter of Zone of Inhibition

CHAPTER - 1

INTRODUCTION.

1. INTRODUCTION

Throughout history, traditional medical systems have employed plants as natural solutions for various ailments, with a proven track record of healing and averting illnesses. In both established and emerging nations nowadays, a sizable population depends on medicinal plants for economic, cultural, and healthcare growth as well as skincare and skincare-related issues. An abundance of data is accessible about the use of medicinal plants by native societies to support medical research, advance drug development, and preserve cultural traditions. India was a pioneer in the discovery of herbal medicine and one of the world's leading producers of medicinal plants. Presently, the World Health Organisation (WHO) is concentrating on developing nations like Hungary, Malaysia, Brazil etc.

Illicium verum (also known as Chinese Star Anise, Star Anise), is a woody species commonly known as *ba jiao hui xiang* in China, used as a therapeutic agent in Traditional Chinese Medicine [TMC]. The plant is also widely used in the food industry as a spice (1). In India, it is called by the name of "*chakra phool*". It is an important medicinal plant and is also commonly used as a spice. The main two raw materials obtained from the plant *Illicium verum* (fruit—*Anisi stellati fructus* and essential oil—*Anisi stellati aetheroleum)* were added in European Pharmacopoeia in 2002 [4th edition], both of these raw materials were good at exhibiting two distinct effects such as Expectorant and Spasmolytic respectively (2). It is characterized by a unique sweet and licorice-like odor, the flavor of oil present in the plant is very much similar to anise seed or fennel oil but compared to them it is very strong (3).

In the present scenario, *I. verum* is a medicinal plant that is important globally. Recent scientific studies have proved that the fruit and essential oil present in *I. verum* possess numerous pharmacological properties including antibacterial, antifungal, anti-inflammatory antiseptic, aromatic, and antioxidant properties, etc.

The main component of *I. verum* essential oil is ***trans-anethole.*** It is extensively used in the food, perfume, and pharmaceutical industries due to its sweet flavor and aromatic scent, and it is also commonly used as a spice. Moreover, recent studies have shown that trans-anethole has antioxidant, anti-inflammatory, and anti-obesity properties, which are also significant in cosmetology and medicine. Furthermore, some other common classes of

compounds were previously identified in the plant including Phenylpropanoids, sesquilignans, shikimic acid, flavonoids, phenolic acids, lignans, etc.

Illicium verum has been observed to have excellent ***anti-microbial*** properties. This effect of star anise is one of the important focuses of modern pharmacological research. Trans-anethole, the main component present in the plant exhibits anti-parasitic, antiviral, antibacterial, and antifungal properties **(4)**.

The antibacterial activity of *I. verum* essential oil, as well as anisic and shikimic acid which are present in fruits of *I. verum* against several bacteria, both Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus) pyogenes* and Gram-negative bacteria: (*Escherichia coli* and *Salmonella typhi*) were evaluated and a positive outcome was obtained **(5)**.

CHAPTER- 2

REVIEW OF LITERATURE

2.2. Classification

Illicium verum is commonly called *star anise*, while scientifically it is placed in Kingdom: Plantae; Class: Magnoliopsida-Dicotyledons; Order: Austrobaileyales; Family: Schisandraceae; Genus: Illicium; Species: *Verum, Anisatum;* Botanical name: ***Illicium verum* Hook. f.**

2.3. Geographical Distribution

Star anise is a plant that grows best in deep, well-drained, fertile, moist, and acidic sandy loam or loamy soil. It does not grow well in dry, barren, or low-lying, waterlogged areas. This plant is mainly found in Southeast Asia and North America, with 80% of its source coming from Asia. In Southeast Asia, China is the main producer of star anise, followed by Vietnam, Cambodia, and Myanmar. In China, star anise is mainly produced in Guangxi, specifically in Baise, Nanning, Qinzhou, Wuzhou, and Yulin. The plant is typically grown at 200-700 meters **(10)**.

2.4. Chemical Characteristics

The medicinal properties of a plant are attributed to its active phytoconstituents, which can be volatile or non-volatile. ***Illicium verum*** is a plant with diverse activities due to numerous different phyto-constituents. Studies on the chemical composition of star anise have been conducted for a long time. Recent research has shown that different parts of the plant, such as its roots, leaves, seeds, and fruits, contain various chemical components. The major chemical components present in ***I.verum*** are phenylpropanoids, flavonoids, neolignans, monoterpenoids, and sesquiterpenoids.

- Click on the link below for more details about the chemical composition of ***I.verum*** **:**

Table 2.4. Chemical composition of *I.verum*

The essential oil isolated from I. verum fruit is mainly composed of trans-anethole, a phenylpropanoid compound. The trans-anethole content in the oil usually ranges from 72% to 92% **(11).**

Star anise is a well-known source of carbohydrates, proteins, vitamin A, and ascorbic acid. It contains 2-4g of protein, 65-75g of carbohydrates, and 4-6g of fats, dietary fibers, and sugars. Star anise is also rich in minerals such as sodium, calcium, zinc, magnesium, potassium,

iron, and copper. Almost 359Kcal energy can be obtained per 100g of star anise. The aromatic odor of ***Illicium verum*** is due to the presence of essential oil, which is 2.5–3.5% in fresh fruit and 8–9% in dried material. GCMS is generally used to determine the chemical profile of essential oils **(12).** This fragrant essential oil is mainly composed of trans-anethole and shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) (**Fig.2.4**). The cancer-preventing and antiviral actions of star anise oil are because of the high concentration of trans-anethole. It is also used as a substrate for making different pharmaceutical products, for example, chloral and anticonvulsive agents **(13).**

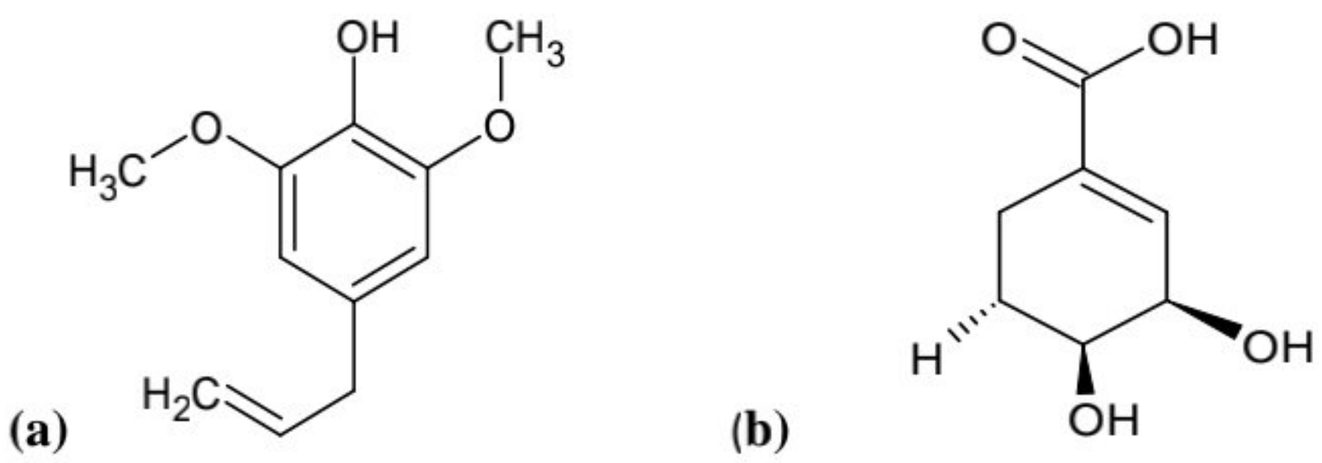


Fig 2.4. Essential oil components

a: Anethole b: Shikimic acid

2.4.1 Star Anise Oil

Star Anise Oil (SAO) is a highly aromatic and volatile oil that is extracted from the star anise fruits, seeds, branches, and leaves. It is classified as a clear liquid that is colorless or pale yellow, and it has a smell similar to that of star anise. When exposed to cold temperatures, it often becomes turbid or may precipitate as crystals, but it turns clear again after warming. SAO is readily soluble in 90% ethanol and has been studied for its chemical properties that make it the main component of star anise.

This oil is widely used in the food industry as a flavoring agent in alcoholic drinks, beverages, candy, baked goods, and chewing gum. It is also used as a masking agent to cover up unpleasant odors, which makes it a popular choice in fragrances for soap, mouth gargles, and toothpaste. Anisole, one of the ingredients in SAO, is used in the fragrance industry to synthesize anisaldehyde, anisole, anisic acid, and its esters. These monomer fragrances are widely used in toothpaste, foodstuffs, soap, and cosmetics. There are different methods used to extract SAO from star anise fruits. These methods include traditional steam distillation (TSD), organic solvent extraction (OSE), headspace solid-phase micro-extraction (HSMS)

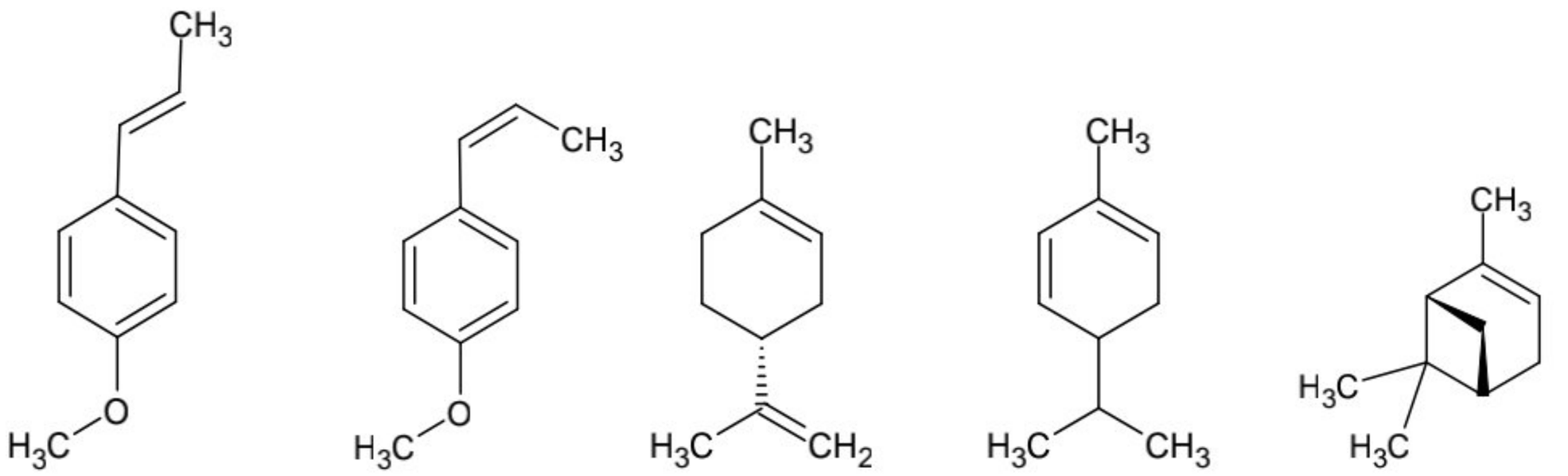
, microwave-assisted extraction (MAE), subcritical CO2 (SCOD), hydro-distillation (HD), and supercritical CO2 fluid extraction (SCFE). Among these methods, TSD is commonly used because it is easy to operate, low in cost, and relatively stable in oil yield. However, SCFE is a new technology that has been widely used in recent years. This technique operates at a lower temperature, which is close to ambient temperature, but with a higher separation efficiency. In this method, the extraction and separation process are combined into one, and CO2 is used as the solvent, so no solvent residue is produced. Some experiments have shown that the chemical composition of TSD-obtained extracts is significantly reduced compared to that of SCFE and OSE. Extracts obtained using SCFE have a richer chemical composition, including various saturated and unsaturated fatty acids.

Furthermore, the SCFE method led to the highest concentration of other chemical compounds and the finest quality of the essential oil. In a separate study, it was revealed that the hydro distillation (HD) method produced a smaller amount of trans-anethole (47.16%) compared to the SCFE method employed by Wang et al **(14).**

Many researchers have used GC-MS to analyze the chemical constituents of SAO due to its many complex chemical compounds. A list of various chemical components identified in SAO can be found in **Table 2.4.1.** The main component, trans-anethole, was found to be present in over 80% of SAO. SAO has been shown to have broad-spectrum antibacterial properties. Trans-anethole has also been found in other plant essential oils and has been found to have insecticidal, larvicidal, and antimicrobial properties. When the antifungal properties of trans-anethole were compared to those of SAO, it was found that trans-anethole had similar inhibitory activity towards the test fungi, with IC50 values similar to the oil and an average difference of 0.018. This suggests that trans-anethole plays a significant role in the antifungal properties of SAO **(15).**

The essential oil of I. verum fruit contains various compounds, including estragole (p-allyl anisole, methyl chavicol) in a concentration of approximately 2%, limonene at 2%, and cis-anethole at 0.5% **(16).** Other compounds present in the oil consist of monoterpenoids like α-pinene, p-cymene, eugenol, linalool, camphene, β-myrcene, trans-ocimene, terpinen-4-ol, α-terpineol, γ-terpineol, terpinolene, and γ-terpinene, as well as sesquiterpenoids such as trans-α-bergamotene, α-copaene, cubebene, cyperene, (+)-9-epigene, β-elemene, α-phellandrene, foeniculin, α-caryophyllene, β-caryophyllene, and α-muurolene**.** Additionally, p-anisaldehyde, 2-(1-cyclopentenyl)-furan, isobornyl thiocyanoacetate, and trans-chalcone have also been

detected **(17). Figure 2.4.1** displays the chemical structures of some selected I. verum essential oil compounds.



Trans-anethole **Cis-anethole** **Limonene** **α- phellandrene** **α-pinene**

Figure 2.4.1. Chemical structures of selected compounds of *I. verum* essential oil.

- Click on the link below for more details about the chemical components of Star anise essential oil.**:**

Table 2.4.1. chemical components of star anise essential oil

2.5. Pharmacological activities/properties

2.5.1. Antimicrobial activity

The antimicrobial properties of star anise have been a key focus of modern pharmacological research. Star anise oil (SAO) has a broad inhibitory spectrum against plant pathogenic fungi **(21).** The SAO's minimum inhibitory concentration (MIC) of 0.5 µL/mL had a stronger antimicrobial effect on Bacillus subtilis than common preservatives like paraben **(22).** There was also a strong inhibition of *Magnaporthe oryzae* spore germination when using an inhibition assay, and the IC_{50} value of the oil was determined to be 0.32 mg/mL. At all concentrations in the medium, *trans*-anethole displayed a very similar inhibitory rate to that of SAO against the test fungi, which suggested again that this was the main active component among the volatiles in the oil **(23).** Ibrahim et al. conducted a study to evaluate the antibacterial

activity of star anise waste residue extract (SAWRE) against several multi-drug-resistant strains of bacteria including Streptococcus pneumoniae, S. aureus, Klebsiella pneumoniae, A. baumannii, Escherichia coli, and P. aeruginosa. The researchers used agar disc diffusion methods, agar plate dilution techniques, and MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) determinations to assess the antibacterial properties of SAWRE. The results of the study showed that SAWRE was highly effective in inhibiting the growth of all tested bacteria. The MIC values ranged from 16 to 128 µL/mL. The bacteriostatic properties of star anise are attributed to various components present in the extracts that act synergistically to damage the bacterial cell walls, cytoplasmic membranes, and membrane proteins. This leads to a loss of glucose, proteins, and DNA from the cell, causing anabolic disorders, and eventually resulting in the death of the bacteria and fungi **(24).**

2.5.2. Antioxidant property

The fruits of star anise are often used as spices and are an excellent source of antioxidants that have been used for a long time. Fine powder and extracts of star anise are prepared using water and ethanol under normal or supercritical CO2 conditions, as well as SAO, all of which have antioxidant properties. The antioxidant activity of star anise and its extracts have been verified by conducting different studies using linoleic acid peroxidation, the β-carotene-linoleic acid system, and DPPH (1,1-diphenyl-2-picryhydrazyl) radical-scavenging methods **(25).** Adding fine powder of star anise to lemongrass oil can improve its oxidative stability. This combination creates a natural antioxidant. The scavenging ability of the star anise antioxidant was tested using the DPPH assay at concentrations of 500, 1000, and 1500 ppm. The results showed that the scavenging ability of the antioxidant was 80.67%, 81.38%, and 81.73%, respectively **(26).** The study evaluated the ability of star anise to act as an antioxidant against H2O2-induced DNA damage and lymphocyte death in humans. The assessment was done by measuring the inhibitory effect on lipid peroxides, hydroxyl-radical-scavenging, DPPH, and superoxide free-radical-scavenging activities. The findings showed that the water extracts of star anise demonstrated the most effective antioxidant activity. The results also indicated that the aqueous extract of star anise acted as an antioxidant at a dose of 25 µg, which protected DNA against peroxides **(27).**

2.5.3. Other Activities

I. verum also shows Anti-inflammatory, Antihelminthic, Insecticidal activities, etc.

2.5.4. TOXICOLOGY

Star anise is a traditional Chinese herb that has been used for a long time. Initially, it was used as a cooking ingredient, but people later discovered its medicinal properties. Previous literature suggests that most cases of anise poisoning occur due to accidental ingestion of anise analogs. However, cases of dietary overdose were reported in the 19th century, although the exact mechanism of poisoning is still not clear. In 1992, the first case of poisoning caused by over-consumption of anise was reported. The patient had used star anise plants crushed to make cakes and experienced paroxysmal vomiting, weakness, and chills in the extremities after consumption **(30).**

LIST OF TABLES

Table 2.4. Chemical composition of *I.verum*

Group of Compounds	Raw Material	Compound name	References
Phenolic Compounds	Essential oil	*Trans*-anethole, *cis*-anethol Estragole	**(16)**
	Fruit	Shikimic acid	**(6)**
	Root	Illiverin A, 4-allyl-2-(3-methyl but-2-enyl)-1,6-methylenedioxybenzene-3-ol, illicinole, 3-hydroxy-4,5-methylenedioxyallyl-benzene, (−)-illicinone-A, 4-allyl-4-(3-methylbut-2-enyl)-1,2-methylenedioxycyclohexa-2,6-dien-5-one, 3,4-seco-(24Z)-cycloart-4(28),24-diene-3,26-dioic acid, 26-methyl ester	**(18)**
Monoterpenoids	Essential oil	α-Pinene, p-cymene, limonene, linalool, terpinen-4-ol, α-terpineol, eugenol, γ -terpineol, σ-3-carene, camphene, β-myrcene, *trans*-ocymene, terpinolene, γ -terpinene	**(6,15)**

Sesquiterpenoids	Essential oil	α-Phellandrene, α-muurolene, β-caryophyllene, α-copaene, *trans*-α-bergamotene, foeniculin, β-elemene, cyperene, α-caryophyllene, (+)-9-epiledene, cubebene	**(6,15)**
	Root	Tashironin, tashironin A, 11-*O*-debenzoyl-11α-*O*-2-methylcyclopent-1-enecarboxyltashironin, veranisatins A–C	**(1,19)**
Flavonoids	Essential oil	*Trans*-chalcone	**(17)**
	Fruit	Kaempferol and glucosides, quercetin and glucosides	**(1)**
Fatty acids	Fruit	Linoleic acid, stearic acid, myristic acid	**(6)**
Alkyl glucosides	Fruit	R-sec-butyl-D-glucopyranoside	**(6)**
Biphenyl-type neolignans	Leaf	Verimol G and verimol H, 4,4′-dihydroxy-3,3′-dimethoxy-9,9′-epoxylignan	**(19)**
Aldehydes	Essential oil	*p*-Anisaldehyde	**(6,17)**
Other	Essential oil	Anisoxide, 2-(1-cyclopentenyl)-furan, isobornyl thiocyanoacetate	**(20 ,15)**

Table 2.4.1. chemical components of star anise essential oil

S No	Chemical constituents	Molecular formula	References
1	2-actonylcycloheanone	$C_9H_{14}O_2$	(28)
2	Limonene	$C_{10}H_{16}$	(28)
3	γ-terpinen	$C_{10}H_{16}$	(28)
4	Linalool	$C_{10}H_{18}O$	(28)
5	spiro[4.5] dec-1-ene	$C_{10}H_{16}$	(28)
6	1-terpenen-4-ol	$C_{10}H_{18}O$	(28)
7	3-undecyne	$C_{11}H_{20}$	(28)
8	*p*-llylanisole	$C_{10}H_{12}O$	(28)
9	*p*-cumic aldehyde	$C_{10}H_{12}O$	(28)
10	propanal,2-methyl-3-phenyl	$C_{10}H_{12}O$	(28)
11	trans-anethole	$C_{10}H_{12}O$	(28)
12	benzaldehyde,3-methoxy	$C_8H_8O_2$	(28)
13	*p*-anisaldehyde	$C_8H_8O_2$	(28)
14	Anethole	$C_{10}H_{12}O$	(28)
15	anisole methyl ester	$C_9H_{10}O_3$	(28)
16	anisyl acetone	$C_{10}H_{12}O_2$	(28)
17	acetic acid, geraniol ester	$C_{12}H_{20}O_2$	(28)
18	Copaene	$C_{15}H_{24}$	(28)
19	iso-caryophyllene	$C_{15}H_{24}$	(28)
20	Caryophyllene	$C_{15}H_{24}$	(28)
21	2-norpinene,2-6-d	$C_{15}H_{24}$	(28)
22	β-farnesene	$C_{11}H_{14}O_2$	(28)
23	Benzene-1,2-dimethoxy-4-(1-propenyl)	$C_{11}H_{17}NO_2$	(28)
24	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl	$C_{11}H_{18}O_2$	(28)

25	Bicyclo[3,1,1]hept-2-ene, 2-ethanol,6,6-dimethyl	$C_{15}H_{24}$	**(28)**
26	Bicyclo[3,1,1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)	$C_{15}H_{24}$	**(28)**
27	γ-lermene	$C_{15}H_{24}$	**(28)**
28	α-amesene	$C_{15}H_{24}$	**(28)**
29	cyclohexene, 1-methyl-4-5(5-methoxy-1-methylene-4-hexenyl)	$C_{15}H_{24}$	**(28)**
30	germacrene D	$C_8H_{11}NO$	**(28)**
31	Phenylethanolamine	$C_2H_6N_2O$	**(28)**
32	Acethydrazide	$C_{12}H_{22}O_2$	**(28)**
33	surfynol 102	$C_{16}H_{16}O_4$	**(28)**
34	*p*-anisoin	$C_{15}H_{26}O$	**(28)**
35	*trans*-nerodilol	$C_{14}H_{18}O$	**(28)**
36	1-(3-methyl-2-butenoxy)-4-(1-propenyl) benzene	$C_{12}H_{16}O_4$	**(28)**
37	Bicyclo[2,2,1]heptane-2,3-dione, 6-(acetyloxy)-1,5,5-trimethyl, endo	$C_3H_8N_2O_2$	**(28)**
38	Hydrazinecarboxylic acid, ethyl ester	$C_{14}H_{24}O_2$	**(28)**
39	Propanoic acid, 2-methyl, 3,7-dimethyl-2, 6-octadiethyl ester(*E)*	$C_9H_{10}O$	**(28)**
40	*p*-allylphen	$C_{24}H_{46}O_2$	**(28)**
41	hexyl oleate	$C_{10}H_{16}$	**(29)**
42	α-pinene	$C_8H_{24}O_4Si_4$	**(29)**
43	Cyclotetrasiloxane, octamethyl-	$C_{10}H_{10}$	**(29)**

44	Myrcene	$C_{10}H_{10}$	**(29)**
45	α-phellandrene	$C_{10}H_{10}$	**(29)**
46	δ-3-carene	$C_{10}H_{10}$	**(29)**
47	Sabinene	$C_{10}H_{18}O$	**(29)**
48	1,8-cineole	$C_{10}H_{18}O$	**(29)**
49	Linalool	$C_{10}H_{18}O$	**(29)**

CHAPTER- 3

RATIONALE AND OBJECTIVES OF CURRENT INVESTIGATION

3. RATIONALE AND OBJECTIVES OF CURRENT INVESTIGATION

The use of herbal products is significant worldwide due to their fewer side effects, easy availability, and affordability in comparison to conventional medicines. ***Illicium verum***, commonly known as Star Anise, is a popular ingredient in traditional Chinese and indigenous systems of folk medicine. It is commonly used to make tea, which is beneficial in treating respiratory infections, nausea, constipation, and other digestive problems.

3.1. AIM

Evaluation of the Antimicrobial activity of essential oils from the seeds of ***Illicium verum,*** In-vitro investigation using bacterial strains (***Escherichia coli***).

3.2. OBJECTIVES

- To induce Antimicrobial action from ***Illicium verum*** in an *in-vitro* bacterial culture medium (MacConkey agar)

- Evaluation of Zone of Inhibition obtained from volatile oil of ***Illicium verum.***

3.3. Plan of work

A. Exhaustive literature review and survey on the antimicrobial activity.

B. Selection and examination of plant material.

C. Authentication of plant

D. Preparation of protocol for ***in vitro*** investigation.

E. Prepare plant material for the Clevenger apparatus.

F. Phytochemical screening of plant material.

G. Isolation of *Illicium verum* essential oil.

I. Determination of Antimicrobial activity on prepared culture media and evaluate the zone of inhibition.

J. Prepare research report and final thesis

CHAPTER- 4

MATERIALS AND METHODS

MATERIALS AND METHODS

4.1. PURCHASE OF PLANT

The plant was purchased from the local area of Amritsar, Punjab.

4.2. MATERIAL FOR ISOLATION

Dried fruit powder of *Illicium verum*, in Distilled Water

4.3. CULTURE MEDIA USED

MacConkey Agar

TABLE 4.3.1: Composition of MacConkey Agar

INGREDIENTS	AMOUNT
Peptone(Pancreatic digest of gelatin)	17gm
Proteose peptone (meat and casein)	3gm
Lactose monohydrate	10gm
Bile salts	1.5gm
Sodium chloride	5gm
Neutral red	0.03gm
Crystal red	0.001gm
Agar	13.5gm
Distilled water	Add to make 1 litre

4.4. BACTERIAL STRAIN USED

Escherichia coli was used, which was collected from **Tuli Diagnostic Centre**, Amritsar.

CHAPTER-5

RESULT

CHAPTER-6

CONCLUSION

6. CONCLUSION

Illicium verum, also known as star anise, is a significant plant species that has been widely used in Traditional Chinese Medicine (TCM) for many years. Both the fruit and essential oil are used as raw materials in pharmacopeia and are effective in treating conditions such as rheumatism, insomnia, and digestive disorders. The unique chemical composition of ***I. verum*** fruits is responsible for their valuable properties, which are attributed to the presence of abundant amounts of flavonoids phenylpropanoids, mono- and sesquiterpenoids. etc.

Numerous studies have shown that the essential oil of *I. verum* exhibits strong biological activities such as antibacterial, antifungal, anti-inflammatory, and antioxidant activity, etc. This makes *I. verum* useful in the cosmetic industry. The essential oil extracted from *I. verum* fruit contains a high concentration of trans-anethole, ranging from 72% to 92%. Trans-anethole is an isomer of anethole and is responsible for the characteristic aroma of *I. verum*. Due to this unique scent, *I. verum* is commonly used in the perfume and cosmetic industries.

In the present study, we confirmed the antimicrobial activity of star anise essential oil demonstrated that the EO of ***I. verum*** was effective against *Escherichia coli* strains.

The obtained results confirm the alternative use of this plant to treat human diseases because of its effectiveness and safety. *I. verum* essential oil is a considerable natural antibacterial agent and might be used as a natural preservative in the food industry.

CHAPTER-7

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