DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

HYDRODISTILLATION, PHYTOCHEMICAL PROFILING AND ELUCIDATION OF THE CHEMICAL COMPONENTS IN THYMUS VULGARIS (THYME) LEAVES

By

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CERTIFICATION

This is to certify that this project work presented by JOHN GLORIA OLUWADUNSIN with Matriculation Number HND/23/SLT/FT/1140 has been read, approved and submitted to the Department of Science Laboratory Technology (Biochemistry Unit), Institute of Applied Sciences, Kwara State Polytechnic, Ilorin.

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DEDICATION

This project is dedicated to Almighty God, the God of mercy and the creatures of the universe. I give him glory for sustaining me up to this moment including my beloved Father, Mother (Mr. &Mrs. John)

Also, I give thanks to God for making this journey successful and fruitful.

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My profound gratitude to God Almighty, the All-knowing one. First and foremost, I am deeply grateful to my supervisor, Mr. Adeyemo Elijah, for his guidance, insightful feedback, and unwavering support. Thank you for your expertise encouragement they were invaluable.

This journey wouldn't have been possible without my sweet caring parents. Mr and Mrs. John. Thank you so much for everything mum and dad.

I want to give a special gratitude to my big daddy (Mr. Tope) My sponsor, thank you for all you do sir, Words can't express how grateful I am. Your support and words of encouragement keeps me moving. Thank you for believing in me sir.

Last but not the least, I want to thank me for believing in me, i want to thank me for doing all this hard work, I want to thank me for having no days off, I want to thank me for not quitting. I want to thank me for being a giver and trying to give more than I receive. I want thank me for doing more right than wrong, I want to thank me for just being me at all times.

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Abstract

Thymus vulgaris was steam-distilled i.e. hydrodistilled to obtain the volatile components while the non-volatile constituents were extracted with ethanol as solvent. The hydrodistillate was analyzed using GC-MS to elucidate the structures of the components while the crude ethanolic extract was screened for the presence of phytochemicals. The results showed that there are 36 volatile components in the hydrodistillate ranging from organic acids to hydrocarbons. The phytochemicals present include steroids, triterpenes, flavonoids and fixed oil. These components identified in both hydrodistillate and crude extract are believed to be responsible for the bioactivities of the plants and facilitated its culinary and medicinal usage.

Keywords: Thymus vulgaris, hydrodistillation, extraction, phytochemicals,

CHAPTER ONE

1.0 INTRODUCTION

(Thyme) is an aromatic small perennial woody plant belonging to the lamiaceae family used for medicinal and spice purpose globally. Thyme grows well under dry and sunny climatic conditions in unshaded areas[2].

Thymus vulgaris is also called a common thyme this standard cultivar in extensively cultivated in numerous nations, with a particular emphasis on Mediterranean region such as Greece, Italy, and spain[3]. The UK and other European countries are particularly fond of this variety. Which is distinguished by its silver-edged leaves. 'Narrow-leaved French' the name of this variety implies that it is frequently associated with France. It is frequently employed in French cuisine and is characterized by its narrower leaves and strong and fragant fragrance[4].

1.1 PROMBLEM STATEMENT

Thyme is a popular herb used in cooking, but it can also be used for medicinal purposes. However, there are some potential problems associated with using thyme. For example, thyme can interact with certain medications, and it may not be safe for pregnant women or people with certain medical conditions.

Additionally, some may be allergic to thyme. It is important to talk to a doctor before using thyme to any medical purpose.

1.2 AIM AND OBJECTIVE OF THE STUDY

Thyme has been used for centuries for medicinal purposes, and some studies suggest that it may have several benefits including: antioxidant properties (thyme contains antioxidants that may help protect cells from damage), anti- inflammatory properties (may help reduce inflammation, which can be beneficial for conditions such as arthritis), anti-microbial properties (have anti-microbial properties that can help fight infections). Others are respiratory benefits (help in relieving symptoms of respiratory conditions such as coughs and colds). The potential risks include allergic reactions (some people may be allergic to thyme), interactions with medications (thyme may interact with certain medications, so it’s important to talk to your doctor before using it). All of these are due to the activities of the various compounds present in thyme.

The study is aimed at extracting these natural compounds in thyme both as volatile and non-volatile components, identifying each class of compounds and elucidating them by means of an instrumental method namely GC-MS analysis.

The specific objectives are:

(i) To collect samples of thyme leaves by procuring commercially available products

(ii) To extract the volatile components by hydro-distillation methods using Dean-Stark apparatus from a portion of the sample

(iii) To extract another portion of the thyme sample with ethanol for non-volatile components soluble in the solvent

(iv) To detect and identify the presence of phytochemicals in the crude ethanolic extract using conventional phytochemical screening methods

(v) To carry out GC-MS analysis (total scan) of the hydro-distillate of *thymus vulgaris* sample

(vi) To identify by structural elucidation of the constituents of the hydro-distillate of the thyme sample.

1.3 JUSTIFICATION OF THE STUDY

Thyme has been used for medicinal purpose for centuries, there is limited scientific evidence to support its effectiveness. This study will fill this knowledge gap by investigating the chemical components of thyme so as to account for its potential benefits and risk of using thyme of medicinal purposes. The findings of the study will help inform healthcare professionals and consumers about the safe and effective use of thyme. And possible restrictions on its use due to allergic reactions which may result from its consumption, resulting from the presence of a particular chemical components. The composition may also be indicative of its medicinal properties.

1.4 THE SCOPE OF THE STUDY

This study will focus on the qualitative profiling of the phyto-constituents of *thymus vulgaris* and the subsequent elucidation of the compounds in the extract to establish it function as a medicinal alongside its use for culinary purpose.

1.5 RELEVANCE OF THE STUDY

The study is relevant because it will provide valuable information about the potential benefits and risks of using thyme for medicinal purposes and further enhance its use in culinary. This information could help to inform nutritionist, dieticians and ‘alternative’ healthcare professionals and consumers about the safe and effective use of thyme. This will boost its consumption and contribution to medicinal applications.

CHAPTER TWO

2.0 LITERATURE REVIEW

Thyme is a medicinal plant use as flavoring substance in food, its products such as powder, extracts and oil, have antioxidant, anti-diabetic, antilipidemic, antitumor and antimicrobial acting attributed to thyme's active components such as canverciol and thymol in combination with other biological components[5].

2.1 THYME VULGARIS DSCRIPTION

Thyme (Thyme vulgaris L.) is a main medicinal plant which belongs to the lamiaceae family[5]. Carvarol (5-methy1-1-2-isopropy1 phenol) are the main phenolic components in thymus vulgaris, which form about 2055% of oil extract[5] many studies demonstrating that thyme volatile oil is among the main essential oils used in cosmetics as antioxidants and preservatives and in food manufacturing[6]. Essential oils of thymus vulgaris is a combination of monoterpenese, the main substances of this oil are phenol isomer carvacrol and its nature terpenoid thymol, which have antimicrobial, ant oxidative, antibacterial, antitussive, antispasmodic and expectorant actions[7]. Phenolic acid, terpenoids and flavonoids glycosides also present in thymus vulgaris L[8]. In addition, other active biochemical compounds of Thyme species are flavonoids (e.g Thymonin, cirsilineol and 8-methoxy-airsilineol), caffeic acid, triterpenoids, aliphatic aldehydes, long-chain saturated hydrocarbons and "Labiate tannin (rosmarinic acid)[9]

2.2 COMPOSITION OF THYME

Several studies have reported that thyme is a source rich in bioactive compounds. Among the phenolic components of thyme oil are thymol and cavacrol, the latter of which is an isomer of the former. Thymol provides thymol oil with its olfactory peculiarities. Depending on the place of origin and species of thyme, this oil offers percentage of phenolic content that range from 40 to 80 percent of thymol and up to 55 percent of carvacrol[10]. Thyme contains monoterpene phenols, including carvacrol (iso-propyl-ortho-cresol; 0.4-20.6%), thymol (2-iso-propulmeta-cresol; CLOHIHOJLO-64%) and p-cymene (9.1-22.2%) and other monoterpenes, such as ∝- pinene (0.9-6.6%), 1, 8-cinede (0.2-14.2%), Camphor (0-7.3%), linalool (2.2-4.8%) and borneol (0.6-7.5%)[11].

Table 2.1: Components in thyme leaves

Compounds	Quantity (%)	
	Thymus zygis, subsp. *gracilis*	*Thymus vulgaris* L.
α-Pinene	1.10–3.50	1.9
Camphene	0.40–1.50	1.2
Sabinene	0.10–0.20	-
Myrecene	1.10–2.90	1.1
α-Terpineol	0.50–1.30	0.3
1.8-Cineole	1.70–3.10	2.1
γ-Terpinene	2.90–9.70	5.2
p-Cymene	24.70–40.90	29.1
Linalool	3.50–4.20	3.7
Terpinen-4-ol	1.10–3.50	1.3
α-Terpineol	1.00–3.10	0.3
Caryophyllene Oxide	0.40–1.00	0.5
Thymol	22.30–43.30	38.1
Carvacrol	1.50–2.70	2.3
β-Caryophyllene	-	3.1

It is important to consider the main factor is that influence the effectiveness of natural extract and essential oils; for examples, the biotic characteristics of the plantis, such as the season and geographical source, have an influence on the concentration and composition of the bioactive compounds[12].

2.3 PHYTOCHEMICAL CONSTITUENTS OF THYMUS VULGARIS

Numerous dives chemical substances, including as the saponins the tanins, flavonoids, which alkanoids substances, terpenoids that and steroid hormones are found in the vulgaris shrub L. one of the main herbs that yield monoterpene phenolic chemicals was thyme. Carvacrol, which p-cymeme, -pinene, borneal, thymol, and 1, 7-cineole represent a few of their main component.[11,] . Heidari et al.[13] Employing Gas Chromatography-Mass spectrometry (GC-MS) to identity the creosol 1-methoxy-3-methyl-phenol, thiophenol (benzene thiol), loliolide, 8-methoxy -7-methylphenol, and quinic acid, as demonstrated within[13].

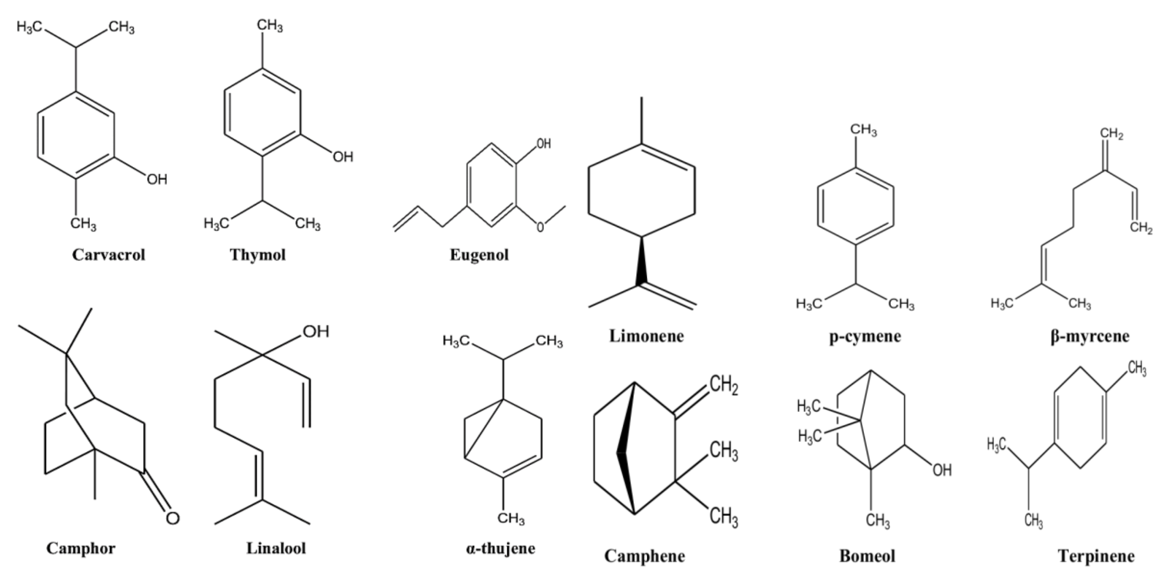


Figure 2.1: Some terpenoidal compounds in thyme

Thyme has a comparable abundance of flavonoids.

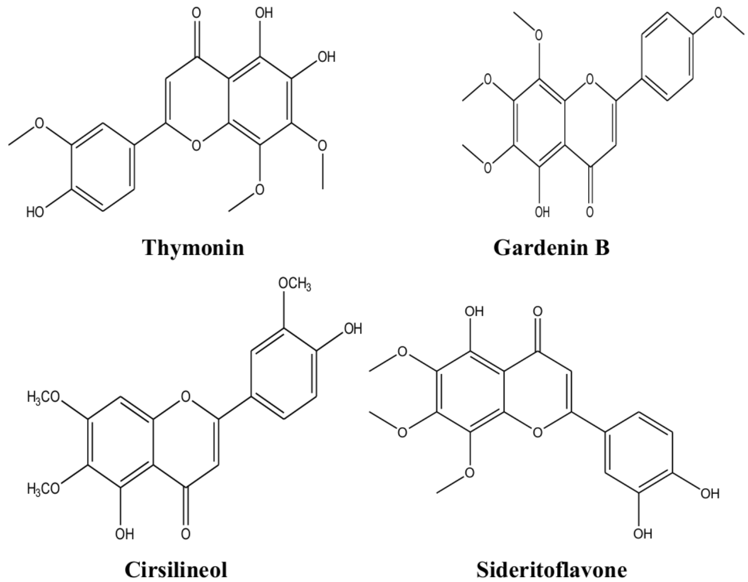


Figure 2.2: Some essential oils in thyme

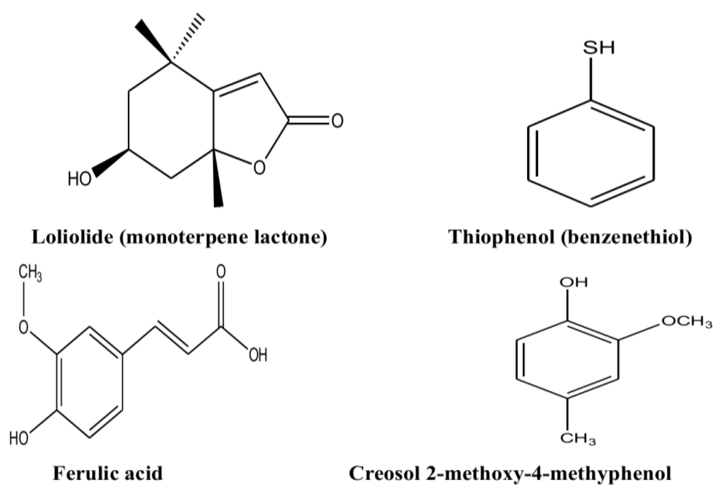


Figure 2.3: Some flavonoids in thyme

Leadenin, 6-hydroxyluteolin, which and methyl flavone including 6-demethylnobiletin, 8-methoxy-crisilineol, cirsimaritin, and 8-methoxy luteolin are the main flavone, Gardenin B, which is the thymonin, sideroto flavone, and xanthomicrol are some more important flavones[14]

2.4. PHARMACOLOGICAL ACTIVITIES OF THYMUS VULGARIS

Thymus vulgaris, commonly known as common thyme, is an herb with a long history of medical use. Its pharmacological properties are attributed to the presence of various bioactive compounds.

2.4.1 Antioxidant activity

According to research on liver cells, experimental mice given extracts of thyme or sage showed increased resistance to oxidative stress[19]. The result shows that consuming extracts from Thymus Vulgaris and S. officinalis increases the rat liver cells' ability to withstand oxidative damage and may have liver protective effects[15]. The study looked at the quantity and composition of plants extracts, their capacity to stop hydrogen peroxide and 2,3-jimethoxy-1,4 naphlhoquinone-induced damage to DNA, and the concentration of antioxidants that are enzymatic and non-enzymatic in human HepG2 cells, such as peroxidase from glutathione, glutathione, and superoxide dioxide dismutase. According to the findings, pretreating the cells with the investigated plant extracts significantly reduced the amount of oxidant-induced damage to the DNA in the cells. The measured DNA-protective effect may be explained by the antioxidant properties of SO and TV as well as the increased GPX activity present in cell that were pretreated with them[16]. The sample classified as C-T and T exhibited the least degree for oxidation of lipids and the highest levels of tocopherol, and n-3 fatty acids in their tissues. The two types furthermore showed a substantial drop in drip loss[17].

2.4.2 Antibacterial activity

Thyme essential oils have anti-bacterial properties[23]. Evaluated the bacterial activity and resistance of several antibiotics, including parahaemolyticus and fluvialis that were recovered from shrimps. As shown by the findings, the ethanol extract exhibited 20 antibacterial activities in the test and a 23 m zone of inhibition against. Parahaemolyticus and fluvialis. Thymus vulgaris intervened and totally blocked the microbial load in 150 and 60 minutes[18].

2.4.3 Antifungal activity

In chronic experimental toxoplamosis, the potential effectiveness of Thymus Vulgaris ethanolic extract against. Toxoplamosis gondii infection was evaluated. If was revealed that aberrant lesions in the brain and retina were greatly improved. The findings suggest Thymus Vulgaris potential efficiency as a new natural therapeutic and preventative agent for the treatment of chronic toxoplamosis[19].

2.4.4 Anti-plasmodic activity

Investigation by Begrow et al[19] of two different thyme extract arrangements: one with very little concentration of the carvacrol and other with a normal amount of the thymol. We additionally looked at both carvacrol and thymol individually. These comprised functional tests on the ileum including the trachea, two distinct organs, and exvitro clearance of mucociliary tissue (velocity of ciliary transport). Thymol and cabavacrol are effective in smooth muscle tissue such as the riot the trachea despite the kind of activation (Bastt, Kt, or acetylcholine). Utilizing endothelin or Batt as stimulant medications, thyme extract with the thymol concentration less than 40% decreased contractions, suggesting that the thymol is not the primary constituent of interest[20]. Vulgaris exhibited soothing effects in Kt-depolarized gastrointestinal muscle tissues in the intestinal tract and stomach[21].

2.4.5 Cytotoxicity activity

Milaidi et al[21]. The essential oils of Thymus Vulgaris and Rosmarinuus officinalis were tested for their in vitro cytotoxicity effects on the A549 human respiratory epithelial cell line. Using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphynyltetrazolium Bromide (MTT) colorimetric test, cytotoxicity was assessed, the A549 cell line we not cytotoxicity to these extracts at any of the tested doses, according to dose dependent tests that showed IC50 valves of 8.50 ± 0.01 Ng\ml after 72 hour[22]. The antibacterial and cytotoxicity properties of petroleum either, chloroform, and hydroalcoholic extracts of thymus leaves that were harvested in oman were investigated by Al-Balushi et al[22] the cytotoxicity activity was estimated using a brine shrimp assay. Almost all of the shrimp larvae have been destroyed by petroleum either and chloroform extracts at greater does of 1000 Ng\ml. The two extracts respective lethal concentrations (LC50) were determined to be 85, 2 and 95.8 Ng\ml extremely low cytotoxicity activities were shown by polar components such as hydro alcoholic extract[23].

2.4.6 Cardiovascular activity

Ramchoun et al[23] examined the impact of water-based extract of Thymus Vulgaris on the amount of antioxidants, hypotriglycerides, and high cholesterol level. The FRAP test, the radical-scavenging technique, and the reduction of 2, 2-azobis (2-amidinopropane) hydrochlorideinduced reactive RBC hemolysis were implemented to evaluate the antioxidant potential of polyphenol rich solutions. To induce high cholesterol among the rats, an intraperitioneal medication of 200mg per pound of body weight drug Triton WR-1339 was injected. The plasma concentration of overall cholesterol and triglycerides were not significantly altered after a 24hour therapy with a thymus vulgaris extract that contained polyphenols[24].

2.4.7 Antimicrobial activity

The study looked as the antibacterial properties essential oils extract from Thyme, sage and oregano seeds grown under various condition. The antibacterial effect of the essential oils extracted from aromatic herbs grown under water compared to those produced without showed significant differnce[25].

2.4.9 Anti-inflammatory activity

Vigo et al[26] reported that thyme oil decreased independent on dose way, the production of nitrogen oxide in a murine macrophage cell line via endoxin and interferon-gamma. They proposed both their nitrieoxide metabolism and their inhibitory impact on the replication of the gene that encodes an inducible synthase of nitric oxide might be the reason for the reduction it next nitric oxide generation. The key ingredient that has anti-inflammatory effect is thymol[27].

2.4.10 Anti-viral activity

Rezatofighi et al.[27] evaluated thymus vulgaris extract in OVO adjacent to the Newcastle. Illness virus. The maximal non-toxic concentration was obtained by performing an egg toxicity experiment on embryonated eggs. The extract capacity to lessen the viral potency by more than 56 times was shown by the inhabitation percentage of 10, which was found to be 1.75[27].

The effects of water-soluble extract of plants in the Lamiaceae genus that suppress the simplex systems virus (HSV). The thymus vulgaris extract had an inhibitory impact on HSV-1, which is types 2 (HSV-2), or an acyclovir-resistant form of HSV-1, was evaluated by invitro testing on the RG-37 cells utilizing the mechanism of plaque removal test[28].

The antiviral effects of hydrosols of Thymus Vulgaris and Nepeta cataria versus a pathogenic virus which harms pork's reproductive organs and lungs. They discovered a substantial decrease in the park Reproduction and pulmonary Disease Virus (PRRSV) burden via their thymus vulgaris hydrosol. Both pre-entry and post entry phases showed anti-PRRSV action[29].

2.4.11 Thyme as functional food

The use of herbs and species dates back to 5000 B.C. therefore, they might be considered one of the first functional foods. Experimental evidence supports the health benefits attributes to spices and herbs for example, cardio, protective and anti-atherogenic potential, digestive stimulant action, antidiabetic effects, antilithogenic properties, cancer-preventive potential and anti-inflammatory properties[30-32].

Although there is not only one definition of functional food, the following definition: A Natural or processed foods that contain known or unknown biologically-active compounds; which in defined, effective non-toxic amounts, provide a clinically proven and documented health benefits for the prevention, management or treatment of chronic disease.

2.5 FOOD APPLICATION

Since ancient times, herbs have been used to improve the look and taste of food; However, herbs and spices can also decrease the use of other unhealthy ingredient, such as synthetic additives (such as glulamate, synthetic antioxidants and antimicrobials) sugar, fat and salt[10].

The use of thyme in food is limited almost entirely the meat products, where it is used for technological purpose, mainly as an antioxidant and preservative[10].

The features of food (to which thyme is added) can influence the bioactivity of compounds and their preservative properties in foods; such factors include storage condition, composition and the types of microorganisms in food. All these aspects are related to their preservative properties.[33]

2.6 PUBLIC HEALTH AND DIETART IMPLICATION CONCERNING THE USE OF THYMES IN FOOD

The European Commission has accepted different EO components as flavoring in food, such as thymol, eugenol, carvacrol, citral, vanillin, limonene, linalool, carvone and cinnamaldehyde, because they do not present a risk to consumer health[10].

In general, recommendations for the intake of food for healthy eating do not yet include suggested amounts of spices and herbs. Therefore, the recommended intake of spices and herbs should be considered for incorporation into guides for healthy eating in different countries[10].

Before the application of thyme as a natural extract, several factors must be taken into account, such as nature of the extracts, fruiting stage, mode of extraction, the concentration of active extract component and the possible synergistic effect between thyme and other components[10].

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 REAGENTS AND EQUIPMENT

3.1.1 Reagents

The reagents used were of high analytical grade and include: Absolute ethanol, H_2SO_4, ferric chloride, Fehling's solution, acetic acid, hydrochloric acid, distilled water, methanol, pyridine, chloroform, acetic anhydride, sodium nitroprussicle (SNP), n-hexane, Lead acetate, Dragendoff's, Wagner's, Hagger's and Mayer's reagents, as well as sodium hydroxide, methanolic KOH, and cupric acetate.

3.1.2 Apparatus and Equipment

Beakers, weighing balance, measuring cylinder, round bottom flask, water bath, conical flask, spatula, Soxhlet extractor, heating mantle, magnetic stirrer, foils, Multifunctional kitchen blender, Separator funnel, mortar and pestle, test tube, test tube racks, test tube holder, thimble, Uv/Vis spectrophoto- meter, glass rod, Dean-stark apparatus, Dessicator, filter paper and distillation apparatus, GC-MS.

3.2 COLLECTION OF SAMPLES

The thymus vulgaris leaves were procured from the grocery section of a major shopping mall in Ilorin, Kwara State, Nigeria. The product was bought in sealed tamper-proof plastic container.

3.3 PREPARATION OF SAMPLES

The dried leaves were pulverized using a heavy-duty nutrition blender (Samsung Model: 2022L). The powdered leaves sample was kept in a plastic container, kept at room temperature away from direct sunlight in the Laboratory till the time of hydrodistillation and solvent extraction and used for the extraction.

3.4 HYDRODISTILLATION OF THYMUS VULGARIS

The hydrodistillation method was used to remove the volatile components in the leaf sample. The extraction was performed. Using a Dean-Stark apparatus operating at atmospheric pressure. After the extraction, it was partitioned with n-hexane in a separator funnel and the Crude essential oil kept in a clean dry amber glass bottle. The collected thymus vulgaris volatile oil was dried with anhydrous Sodium sulfate and stored at 40°C. The volatile component from the hydrodistillation of thymus vulgaris was labelled THD.

3.5 SOLVENT EXTRACTION OF PHYTOCHEMICALS IN THYMUS VULGARIS

50g of dried pulverized leaf sample of thymus vulgaris were weighed into a thimble and carefully placed in the extraction chamber of a 500ml capacity Soxhlet extractor 700ml of absolute ethanol was carefully measured with the 1L flask of the extractor. The sample was exhaustively extracted.

The extract solution was subsequently distilled to remove the solvent. The Crude extract was allowed to cool and the weight determined to calculate the yield. The Crude ethanolic extract of thymus vulgaris was eventually labelled as CAEE and kept for further analysis The yield was calculate as follows:

$$\% \text{ Extract yield} = \frac{\text{weight of Crude extract}}{weight\ of\ leaf\ sample} \text{ x } 100$$

3.6 PHYTOCHEMICAL PROFILING

3.6.1 Using Biuret test: 1 drop of 2% copper sulphate solution was added to thymus vulgaris extract, then 1mil ethanol is added, follows by potassium hydroxide pellet. Pink colour layers indicate the presence of protein.

3.6.2 Test for fixed oil and fat

A Small quantity of the extract is pressed between two filer papers. Oil Stain on the paper indicating the presence of fixed al.

3.6.3 Test for Tannins

Ferric chloride test**:** The extract with 10% ferrric Chloride solution gives brownish green colour

3.6.4 Test for Saponin

Coleus amboinicus extract was mixed with 2mL of distilled water in a test tube, the mixture was shaking vigorously and observed for the formation of persistent confirms the and observe foam that presence of saponin.

3.6.5 Test of Triterpenes

3.6.5.1 Saikowski Test Chloroform solution of the extract when shaken with concentration Sulphuric acid, lower layer turns to yellow on standing.

3.6.5.2 Lieberman test: Chloroform solution of the extract with few drop of acetic acid and 1ml concentrated sulphuric and gives deep red at the junction of the 2 layers.

3.6.5.3 Lieberman burchardt Test: Chloroform solution of the extracts were mixed with few drops of acetic anhydride, treated with the reagent Liebermann Burkhardt the appearance of a ring of blue green at the interphase, gives a positive position reaction.

3.6.6 Test for flavonoid

3.6.3.1 Ferric Chloride test: The extracts was mixed with few drops of natural ferric Chloride Solution gives a green colour.

3.6.3.2 Lead Acetate: The Extracts was mixed with few drops of 10% lead acetate give a yellow precipitate

3.6.4 Test for Carbohydrate

3.6.4.1. Benedict's Reagents. Coleus amboinicus extract was mixed with 2ml of Benedict reagent the mixture was heated on a boiling water bath for 2 minutes. A characteristics colour precipitate indicate the presence of sugar

3.6.4.2 Using Fehtling's Solution. The extract was mixed with equal amount of fehling A and B reagents and gently boiled. A brick red precipitate appears at the bottom of the test tube indicating the presence of reducing sugar.

3.6 PHYTOCHEMICAL SCREENING OF EXTRACT

The extract was tested for the presence bioactive compounds by using the following standard methods.

3.6.1 Test for alkaloid.

The extract was dilute with ammonia and then extracted with Chloroform solution to these dilute hydrochloric acids was added. The acid was used for Chemical test of alkaloids.

3.6.1.1 Mayer's test (potassium mercuric lodide) the acid layer with few drops of mayer's reagent gives a creamy white precipitate

3.6.1.2 Wagner's test (Sodium of lodide in potassium lodide). The extracts with a few drops of wagner's reagent gives reddish brown coloured precipitate.

3.6.1.3. Dragendoff's test (Solution of potassium Bismuth lodide) the extract with a few drops of dragendoff reagent gives a reddish-brown precipitate.

3.6.1.4 Hager's test (solution of lodine in potassium lodide) The acid layer with Hager's reagent gives yellow precipitate.

3.6.2 Test for Steroid (Salkowski Test): The extract was mixed with 2mil of chloroform and concentrated sulphuric acid was added along the sides of the test tube lower layer turn yellow on of Standing.

3.7 GC-MS ANALYSIS OF THE SAMPLE

The hydrodistillate obtained from the thymus vulgaris was analyzed by GC-MS to elucidate the structures of the components with the aid of GCMSD instrument using AcqMethod scan.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

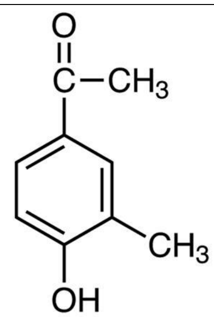
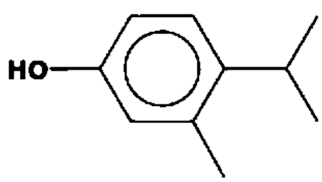
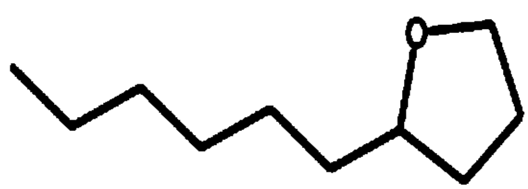
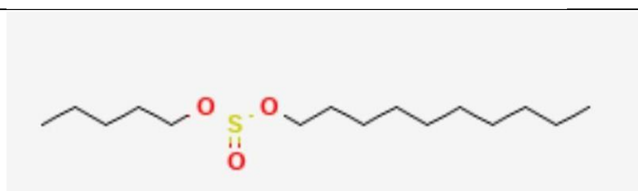
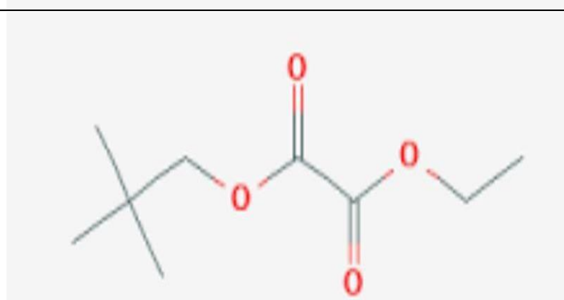
4.1 RESULTS

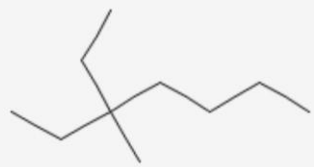
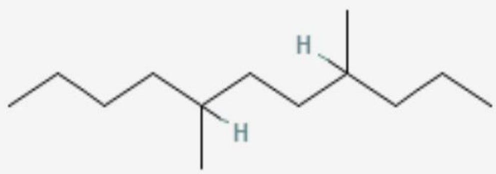
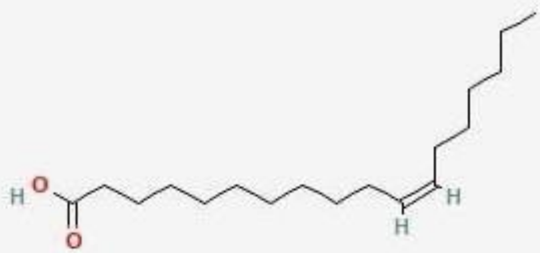
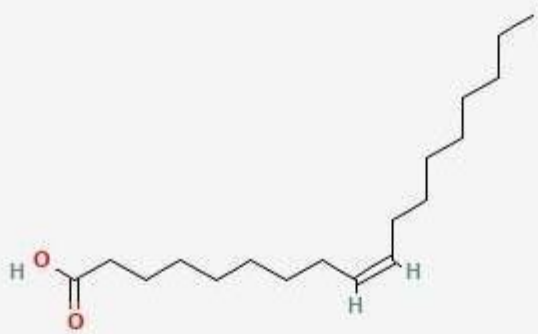
The results of the phytochemical screening of the ethanol extract of the thymus vulgaris sample is presented in the table 4.1 below.

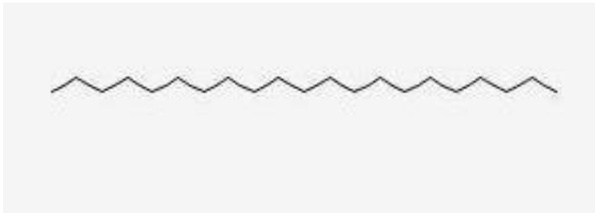
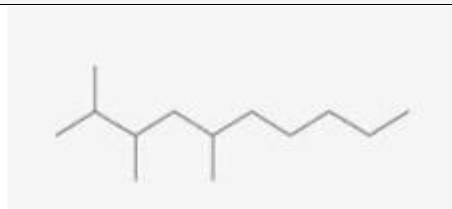
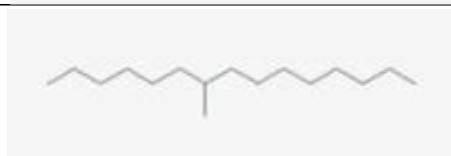
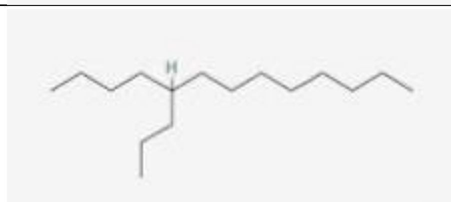
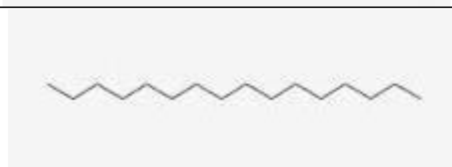
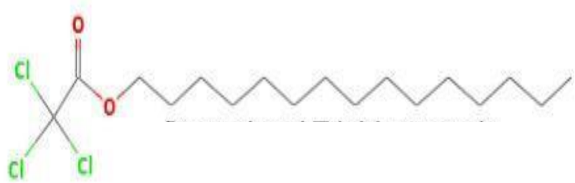
TABLE 4.1: RESULT OF PHYTOCHEMICAL SCREENING OF THYMUS VULGARIS ETHANOL EXTRACT

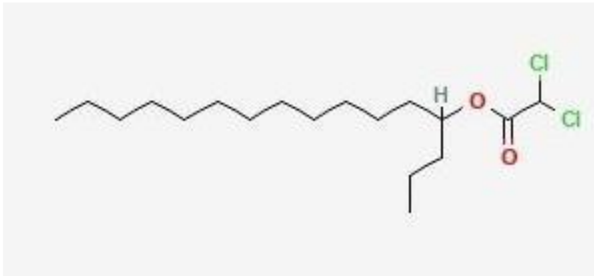
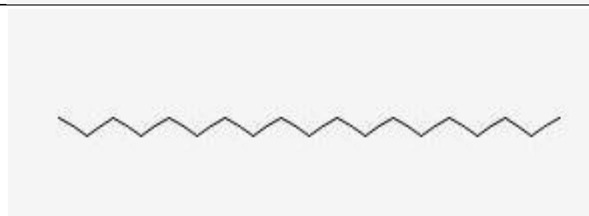
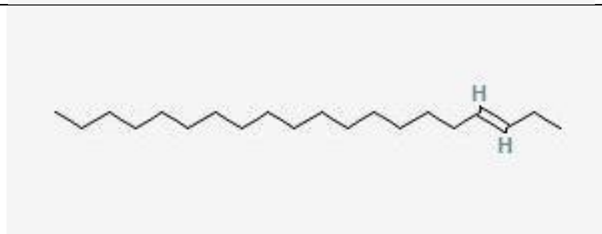
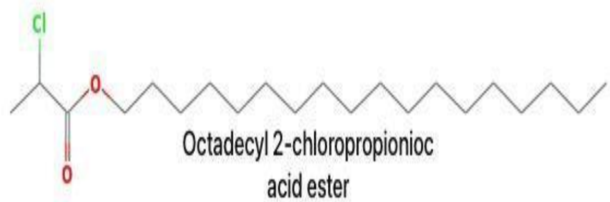
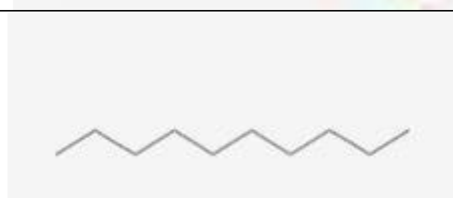
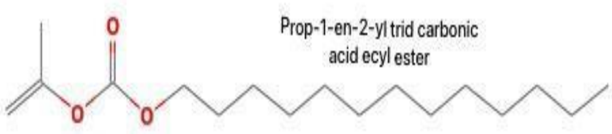
Phytochemical	Result
Alkaloids	-
Steroids	+
Tannins	-
Flavonoids	+
Lactones	-
Diterpenes	+
Glycosides	-
Saponin	-
Triterpenes	+
Protein	-
Carbohydrate	-
Fixed oil	+

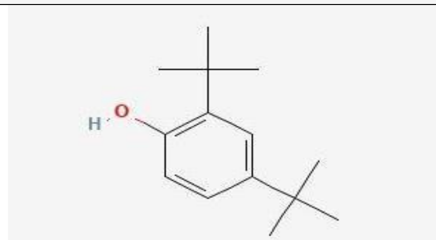
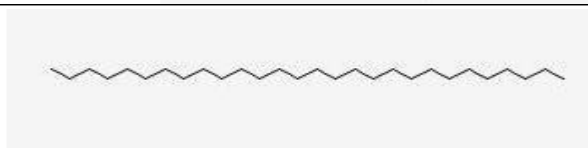
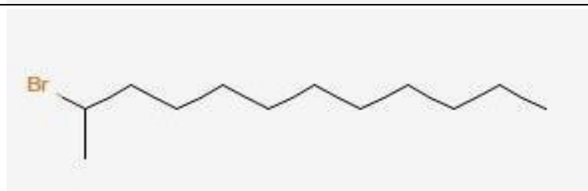
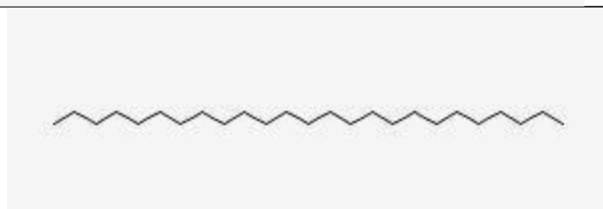
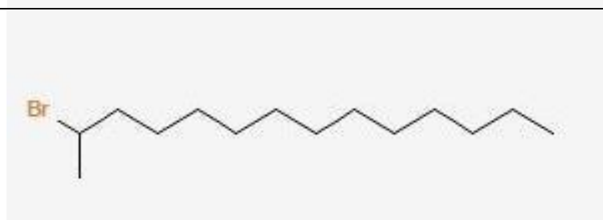
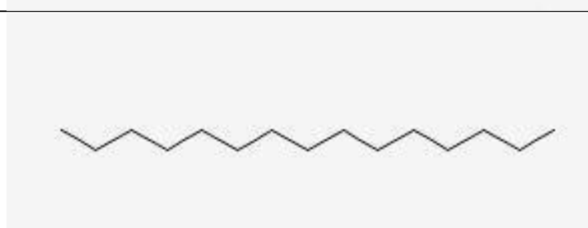
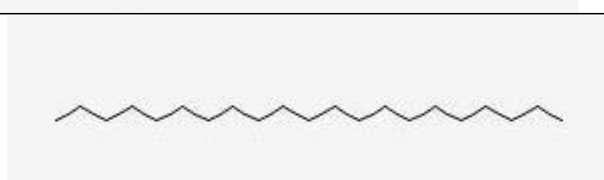
The results of the GC-MS analysis of the hydrodistillate of thymus vulgaris sample are presented in the following table 4.2.

S/N	STRUCTURES	NAMES
1		2,6-Dimethyl undecane
2		4-Hydroxy-3-Methylacetopheno
3		3-Methyl-4-Isopropyl phenol
4		Tetrahydrofuran, 2-hexyl
5		Sulfurous acid, decyl pentylester
6		Oxalic acid, ethyl neopentylester

7		3-Ethyl-3-methlheptane
8		9-octadecenoic acid
9		4,7-Dimethylundecane
10		Cis-vaccenic acid
11		Oleic acid
12		Pentadecane

13		Heneicosane
14		2,3,5-Trimethyl Decane
15		7-methyl Pentadecane
16		5-propyl Tridecane
17		Hexadecane
18		Pentadecyl Trichloroacetic acid ester
19		Hexadecyl Trichloroacetic acid ester

20		Heptadecyl Dichloroacetic acid ester
21		1-Nonadecene
22		3-Eicosene
23		Octadecyl 2-chloropropionioc acid ester
24		Allyl octadecyl oxalic acid ester
25		Decane
26		Prop-1-en-2-yl trid carbonic acid ecyl ester

27		Heptacosane
28		2,4-Di-Tert-butylphenol
29		Octacosane
30		2-Bromododecane
31		Pentacosane
32		2-Bromotetradecane
33		Pentadecane
34		Heneicosane

35		Hexadecane
36		1-Iodo-Dodene

4.2 DISCUSSION

Table 4.1 above present the classes of phytochemicals present in the crude ethanolic extract of thymus vulgaris. The presence of five (5) phytochemicals were established which include steroids, flavonoids, diterpenes, triterpenes and fixed oil, while alkaloids, tannins, lactones, glycosides, saponins, proteins, and carbohydrates. The phytochemicals present are responsible for the observed medicinal properties of thymus vulgaris.

From the table of the results of GC-MS analysis (Table 4.2) above, there are about thirty-six (36) chemical components in the hydro-distillate of *thymus vulgaris*. Eleven (11) of these components are acids e.g. oleic acid, Cis-vaccenic acid, 9-octadecenoic acid etc. which are possibly the fatty acids present in the thyme fixed oil. Twelve (12) of these components are hydrocarbons e.g. pentadecane, Hexadecane, 2-Bromotetradecane etc. Six (6) of these components are esters e.g. ethyl neopentyl ester, Allyl octadecyl oxalate etc. Two (2) of these components are aromatic phenols e.g 3-methyl-4-isopropylphenol, 2,4-Di-tert-butylphenol which may impart some form of antiseptic or antimicrobial activity. This explains why thymus vulgaris possesses antimicrobial activity. The remaining five (5) of these components are alkane e.g. 3-Eicosane, Octacosane, Pentacosane etc., and these are generally volatile acting as natural solvent for many of the organic compounds present in thyme.

CONCLUSION

Thyme is a rich source of chemical compounds, some of which are volatile while others are nonvolatile. These compounds spread across various classes of organic and phytochemicals. These definitely impart the medicinal capabilities and aroma to the plant.

More work should be done to also establish the possible health effects of some of these components, so as to account for its likely toxicity.

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