PHYTOCHEMICALS AND ORGANIC COMSTITUENTS OF EUGENIA CARYOPHYLLUS

BY

OLUWANISOLA ANJOLA

MATRIC NO: 16010102009

A DESSERTATION SUBMITTED TO THE BIOCHEMISTRY UNIT. DEPARTMENT OF BIOLOGICAL SCIENCE COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, IN PARTIAN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B Sc. HONS) IN BIOCHEMISTY

December 2020

CERTIFICATION

I certify that this project has been carefully supervised and approved as adequate for the partial fulfillment of the award of Bachelor of Science, B.Sc. (BIOCHEMISTRY) of Mountain Top University.

....

....

OLUWANISOLA ANJOLA DATE

STUDENT

....

PROF. A. I. AKINWANDE DATE

SUPERVISOR

....

DR. A. A. ADEIGA DATE

HOD

DEDICATION

I dedicate my project to God Almighty for His grace, strength, favour and mercies. He who was my support through this period of my life and to my amazing parents; Mr and Mrs Oluwanisola for their financial support throughout my days in school, I thank you for the love and trust you have in me.

ACKNOWLEDGEMENT

I would like to express my immense gratitude to Almighty God for his infinite mercy as I started and completed my project successfully.

I want to show gratitude to the Dean of College of Basic and Applied sciences (Prof. A. I. Akinwande) and to the Head of Department, Biological sciences (Dr. A. A. Adeiga) for their fatherly support through-out my stay in the university. I want to specially acknowledge my supervisor; Prof. A. I. Akinwade, who saw me through this crucial stage of my education to my heart and to all the lecturers and the technical staff of the Department of Biological sciences, College of basic and applied sciences. I am truly grateful for the help throughout my stay in the university. I also want to express my utmost gratitude to my parents, Mr and Mrs Oluwanisola and my siblings, friends for showing their undying support and encouragement.

Finally, my gratitude goes to my ever-supportive friends; especially Yacque of lagos, mudiyah, benzo, hil, jacuzzi, jerry and all the ever-supporting people I have around me, we gather dey.

Table of Contents

ABSTRACT

Eugenia caryophyllata (clove) was extracted by cold extract using deionised water and characterized to determine the phytochemicals and structures of the extract using qualitative study, GC-MS and NMR analysis. The results showed the presence of phytochemicals except Protein- Amino acids which is absent. The phytochemicals evaluated in the aqueous extract of Eugenia caryophyllata (clove) were in the order of total phenolics > flavonoids with the respective values of 6.6 > 2.6 mg/g of extract. Presence of high level of phenolic compounds revealed that Eugenia caryophyllata (clove) extract can have good antioxidant capacity. The resulting spectrum of ^1H NMR analysis found that the existence of distinct peaks signals at 2.36, intensity of 4134.6 and 4011.2 in the range of 2.47 – 2.25 with reference to the proton of acids H-C-OOH, carbonyl compounds HC–C=O, aromatic methyl group Ar–H (Ar–C–H, benzylic) and Methyl ketone group HC– (R)-C=O. The results demonstrate the characterisation of Eugenia caryophyllata (clove) revealing the phytochemical properties and isolated compounds in relation to its general use as a medicinal plant.

Keywords: Eugenia caryophyllata (clove); Erythrocyte Osmotic Fragility Test, GC-MS and NMR.

CHAPTER ONE

1.0 INTRODUCTION

<u>*Eugenia caryophyllus*</u> (cloves) locally known as "Kanafuru" are from the family *Myrtaceae, Syzygium aromaticium,* they are aromatic flower buds and they are widely used as spice. They possess anticancer, antidiabetic, antifungal, anti-inflammatory, antinociceptive, anti-bacterial, and antithrombotic properties including other biological constituents, functions and properties of this plant. They are available all year round due to their harvest seasons in different parts of the world. Their trees are evergreen and are found in various part of Africa and the middle east of the world, ranging from 8-12 meters high (26-39ft), they possess large leaves and crimson flowers grouped in terminal clusters. They are mostly harvested and used as spices throughout the world and smoked as cigarettes in some parts of Indonesia, and their bioactive ingredients can be used as an insect repellant too. They are also dried and burnt as incense in some parts of the world. The plant has a strong phenolic smell and sharp acrid taste.

A plant whose buds are commonly referred to as cloves, from the family *Myrtaceae, Syzygium aromaticium* which are native to Indonesia, India, Madagascar, Zanzibar, Pakistan, Sri Lanka, and Tanzania. They are aromatic flower buds and they are widely used as spice (Deladino etal.,2008). They possess anticancer, antidiabetic, antifungal, anti-inflammatory, antinociceptive, anti-bacterial, and antithrombotic properties including other biological constituents, functions and properties of this plant. They are available all year round due to their harvest seasons in different parts of the world. Their trees are evergreen and are found in various part of Africa and the middle east of the world, ranging from 8-12 meters high (26-39ft), they possess large leaves and crimson flowers grouped in terminal clusters. They are mostly harvested and used as spices throughout the world and smoked as cigarettes in some parts of Indonesia, and their bioactive ingredients can be used as an insect repellant too (Acosta,2009; Chen etal.,2006; Noubigh etal.,2013; Waterhouse,2001). They are also dried and burnt as incense in some parts of the world. The plant has a strong phenolic smell and sharp acrid taste, the plant is also used to control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension, and gastrointestinal spasm; relieve pain; cause uterine contractions; and stimulate the nerves (Shrivastava et al., 2014).

It is mostly harvested to be used for spices in culinary arts but more importantly for its popular Eugenia oil which is sold for a very high price because of its properties ranging from medical to cosmetics, it consists of 90-95% eugenol, and some other minor constituents. A major component of clove taste is imparted by the chemical eugenol. Eugenol is the component which is most responsible for clove aroma and comprises 72-90% of the essential oil extracted from cloves. Other important essential oil constituents of clove oil are acetyl eugenol, beta-caryophyllene and vanillin, crategolic acid, tannins such as bicornin, methyl salicylate (painkiller), gallotannic acid, the flavonoidseugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids such as oleanolic acid, campesterol and stigmasterol, and several sesquiterpenes (Uddin ma 2017). Therefore, products with great potential for application of clove essential oil are packaging for meats, chocolates, and dairy products due to the high lipid content contained in these products; in fruits, juices, jams, and sweets that have high water activity and are therefore, susceptible to develop microorganisms. In this sense, the use of eugenol as an antimicrobial agent adds quality to food products, by maintaining their sensory properties, increase their shelf life, and human health benefits (Cortés-Rojas DF 2014)

1.1 AIMS AND OBJECTIVES

1.1.2 AIM OF THE STUDY

The aim of this study is to characterise the phytochemical properties of *Eugenia caryophyllus* using qualitative study, GC-MS and NMR methods of analyses to evaluate the bioactive components of the plant in relation to previous studies

1.1.2 OBJECTIVES OF THE STUDY

This project includes;

- Characterisation of phytochemicals in E.caryophyllus.
- To evaluate the phytochemical properties of *E.caryophyllus* extract using qualitative studies
- To determine the prominent compounds present using GC-MC
- To determine the functional proton dominated compound using ^{1}H- proton NMR

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EUGENIA CARYOPHYLLUS

The genus Eugenia is one of 75 genera (3000 species) belonging to the family Myrtaceae which is native in the tropics, particularly in tropical America as well as Australia and plants of this family are known to be rich in volatile oils which are reported for their medicinal importance. Genus Eugenia has properties like anti-inflammatory, analgesic, antipyretic (Karla S, Carretero E and Villar, 2017). According to Rahhal (2017), antifungal and used in peptic ulcer treatment. Syzygium aromaticum (Linn.) Merr. and L.M. Perry (Syn. Eugenia aromatica) Kuntze, Eugenia caryophyllata Thunberg, belongs to family Myrtaceae, commonly known as clove, is an aromatic tree, native to Indonesia and used as a spice in cuisines in many parts of the world (Srivastava and Malhotra, 2017).

It is grown naturally in Moluku Islands of Indonesia and cultivated in many parts of the world like Tanzania, Madagascar, Sri Lanka, India, China, Indonesia, Malaysia, Brazil, Malagasy Republic, Jamaica and Guinea (Baytop, 2018). It is an ever-green plant of 10 to 20 m in height with spear-shaped leaves and racemiferous yellowish flowers, has a strong phenolic smell and sharp acrid taste, whereas, essential oil of clove is a colorless or light yellowish fluid extracted from dried flower buds. Flower buds collected twice a year, in the months of October and February when they change color from green to crimson, dried carefully and separated from their peduncles. Clove is broadly used in cooking (biryanis, salads, pickles and garam masala), pharmacy, perfumery and cosmetics.

The Eugenia caryophyllata tree is an evergreen tree, which grows to a height ranging from 10-20 m. Flower buds are first of a pale color and gradually become green after which they develop into dark brown or dusty red. Flower buds are generally 10-17.5 mm in length and consist of a sub-cylindrical, slightly flattened, four sided hypanthium. Upper portion of hypanthium consists of two celled inferior ovary with numerous ovules attached to an axile placenta, surmounted by four thick, divergent sepals and covered by unopened corolla consisting of four membranous imbricate petals, frequently detached, enclosing numerous incurved stamens, odor, strongly aromatic; taste, pungent, aromatic followed by slight tingling of the tongue (The Ayurvedic Pharmacopoeia of India, ND).

In British Columbia, Flower of Clove is widely used in the treatment of roundworms and tapeworms in pets and pigs whereas, flower bud of E. caryophyllata has been used as traditional medicine for the treatment of asthma and various allergic disorders; as vermifuge, antibacterial agent and in toothache in China, Japan and Korea. The flower bud is a well-known food flavor for exotic food preparations and a popular remedy for headache, soar throat, dental and respiratory disorders, digestive system ailments, in traditional medicines of Australia and Asian countries (Pawar and Thaker, 2019). In addition, the clove is widely used as traditional medicine for treatment of dyspepsia, gastritis and diarrhea; as antipyretic, aphrodisiac, appetizer, expectorant, antiemetic, anxiolytic, myorelaxant, analgesic, decongestant, anti-inflammatory and hypnotic (Harborne and Baxter, 2019). Clove is widely used in Indian Ayurvedic medicines, Chinese medicines, Unani medicines of Asian countries and Western herbal medicines and considered as warm, aromatic, to fortify the kidney yang (hypochlorhydria), carminative, in toothache, as aphrodisiac and in the treatment of male sexual disorders (Sharma, 2019).

Clove oil has been used to improve peristalsis, as anodyne, in anaesthesia and is said to be a natural anthelmintic (Lopez, Sanchez, Batlle, and Nerin, 2020). Due to its frequent use in teeth problems, it is widely used as an ingredient in popular toothpastes and mouth fresheners in India. Clove is broadly used in cooking, food processing, pharmacy, perfumery and cosmetics (Lopez et al., 2020). The essential oil of this plant has been proved efficacious repellency on the mosquitoes Ades aegypti, Culex quinquefasciatus and Anopheles dirus, insecticidal activity on Pediculus capitis as well as acaricidal activity against Dermatophagoides farinae and D. pteronyssinus. It is also effective against Psoroptes cuniculi, a mange mite and possesses anti-inflammatory, cytotoxic, anesthetic and antimicrobial properties. It is commonly associated with dental caries and periodontal disease (Mishra and Singh, 2018). Sesquiterpenes, found in clove, were investigated as potent anticarcinogenic agents (Li, Xu, Zhang, Liu, and Tan, 2019).

Eugenol, the major aromatic constituent of clove oil, has been reported to have a variety of different applications, e.g. as an antioxidant (Li et al., 2019), antimycotic (Li et al., 2019), antibacterial antifungal (Dan, Steven, Erich, and Andrew, 2019), participates in photochemical reactions (Li et al., 2019), as insecticidal , in photocytotoxicity (Lee and Shibamoto, 2015) and also as an additive used in certain cigarettes (Li et al., 2019). It has been reported that eugenol at low concentration acts as an antioxidant and anti-inflammatory agent, whereas its higher concentrations act as a pro-oxidant resulting from the enhanced generation of tissue-damaging free radicals (Isaacs, 2018) and it induced apoptosis of human cancer cells (Isaacs, 2018). It also prevents the transmission of HSV-2 in a mouse model of intra vaginal HSV-2 challenge and was found to give protection in guinea pig model of HSV (Soto and Burhanuddin, 2019). It has been reported that the essential oil of clove has anticonvulsive effect in tonic seizures but not in colonic seizures in mice and produces a sustained increase in the mounting frequency of normal male rats and mice (Voie, Adams, Reinhardt, Rivenson and Hoffman, 2017).

On the other hand, Eugenol, a chief constituent of clove oil, causes desquamation of the inner secretory columnar cell layer and exerts adverse effects on secretory activity of seminal vesicle (Chogo, and Crank, 2017), spermicidal activity on ejaculated human spermatozoa and possesses significant anti-inflammatory activity at 0.025 mL/kg . It has been reported that eugenol at various concentrations (Taylor and Roberts, 2018) and 100 µM exhibited a remarkable DPPH free radical scavenging potential and showed the cytotoxic effect. The IC50 values of eugenol were reported to be 700 µM in HepG-2 cells; 1000 µM in Caco-2 cells and 700 µM in VH10 cells. The terpenes, beta-caryophyllene, beta-caryophyllene oxide, alpha-humulene, alphahumulene epoxide I and eugenol isolated from essential oil of Eugenia caryophyllata induce glutathione S-transferase enzyme which plays a vital role in detoxification in liver and intestines (Lane, Ellenhorn, Hulbert, and McCarron, 2017).

It is stated that induction of glutathione Stransferase inhibits chemical carcinogens, hence these terpenes are promising anticarcinogens (Guidotti, 2018). However, clove oil is toxic to human cells26. If ingested or injected in large quantity, it has been shown to cause life threatening complications, including Acute Respiratory Distress Syndrome, Fulminant Hepatic Failure and Central Nervous System disorder. The lethal oral dose of clove has been reported as 3.752 g/Kg body weight Kirsch (2017) and the median lethal dose of Eugenia caryophyllata and its etheric oil were reported as 0.613 mL/kg and 0.863 mL/kg in mice respectively (Kirsch, 2017). Many Eugenia species were reported as a good source of polyphenols, gallic and ellagic acid derivatives (Mihara, and Shibamoto, 2020), tannins (Tajuddin, Latif, and Qasmi, 2020) and flavonol glycosides. Eugenia caryophyllata is a rich source of essential oil. It contains 15-20%

essential oil, 13% tannins, 10% fixed oil and 6-12% non-essential ether extract. Essential oil of clove is a colorless or light yellowish fluid, a distillate of dried flowers, stalks and leaves (Park, Gwak, Yang, Choi, Jo, and Chang, 2017). Several constituents of clove oil have been identified but eugenol (C10H12O2), eugenyl acetate and β-caryophyllene represent the major components of the oil. Eugenol (4-allyl-2- methoxyphenol), makes up 70 to 90% by weight90,91, eugenol acetate (> 17%) and cariofilen (> 12%), β-caryophyllene (9%), 1,8-Cineole (0.1%), Linalool (0.2%), α-Copaene (1.2%), αHumulene (3.5%), β-Cadinene (0.5%), Epizonarene (0.1%), α - Muurolene (0.1%), Eugenyl acetate (4.2%), δ-Cadinene (3.6%), αcopaen (1.0%), methoxy benzaldehyde, benzyl alcohol, benzaldehyde, carvacrol, 2-heptanone, methyl salicylate, isoeugenol, methyl eugenol, phenyl propanoides, dehydrodieugenol, transconfireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid, oleanolic acid, thymol, cinnamaldehyde, acetyl salicylate, vanillin, and crategolic acid. It has been reported that clove also contains tannins (gallotannic acid), flavonoids (eugenin, rhamnetin, and eugenitin), triterpenoids (oleanolic acid, stigmasterol and campesterol). The characteristic smell and taste of clove oil is due to the presence of a wide range terpene compounds (Park, et al., 2017).

2.2 TAXONIMIC TREE.

Domian: Eukaryota

Kingdom: Plantae

Phylum: Tracheophyta

Subphylum: Spermatophytina

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceac

Genus: Syzygium aromaticium

Spices: Eugenia caryophyllus

2.3 Macroscopical features

The volatile oil is situated in the schizolysigenous oil glands or ducts which are present in all 1 parts of the flower buds (hypodermis).

Size: Length varies from 12 to 17 mm.

Type: Actinomorphic, bisexual, epigynous. The flower bud has a spherical head and a sub-cylindrical hypanthium tapering at the lower end.

Calyx: Polysepalous, 4 hard and thick sepals with oil glands

Corolla: Polypaptalous, 4 petals imbricate, enclose the stamens and forms the head of the bud Androecium- numerous stamens, free and introrsely;

Gymnasium: Binocular, inferior with ovules stamens, free Placentation axial.

Style: Single and erect.

Colour: Dark brown;

Odour: Aromatic, spicy, Strong.

Taste: Pungent, aromatic. (Kaur et al 2017)

2.4 THE BIOCHEMICAL EFFECTS OF THE APPLICATION OF *E. CRAYOPHYLLUS*

Numerous medicinal plants have traditionally been employed in the treatment and management of different ailments and diseases. The ameliorative efficacy of these medicinal plants, which play an important role in therapeutics, could be attributed to the synergistic impact of all the biologically active constituents of these plants, which enhances the antioxidant defense systems and attenuates lipid peroxidation (Wang, Mehendale, and Yuan, 2017). *Eugenia caryophyllus* (clove), a member of the Myrtaceae family, is an important medicinal plant and a common household spice often used for culinary purposes (Singh, Dhamanigi, and Asad, 2019). Cloves may be drunk as a tea or smoked in cigars (Malson, Lee, Murty, Moolchan, Pickworth, 2019). *Eugenia caryophyllus* has also been used for the topical treatment of toothache (Alqareer, Alyahya, and Andersson, 2018). Alqareer et al., (2018) states that in West Africa, the Yoruba use a hot water infusion of cloves to treat stomach upset, vomiting, and diarrhea. Growing evidence in recent years suggests that *Eugenia caryophyllus* possesses antioxidant, antiherpetic, antipyretic, anticandidal, anticarcinogenic, antiplatelet inhibitory, local anesthetic, and aphrodisiac properties.

The primary chemical constituents of *Eugenia caryophyllus* include eugenol, caryophyllene, and tannins. Clove is made up of 14% - 20 % volatile oils, which include eugenol, acetyl-eugenol, sesquiterpenes (α-and β-caryophyllenes), and small quantities of esters, ketones, and alcohols. Clove also contains tannins, sitosterol, and stigmasterol. Eugenol is the compound primarily responsible for the cloves' aroma; interestingly, 72% - 90% of the essential oil extracted from clove consists of eugenol. Cloves are the aromatic flower buds of a tree in the family

Myrtaceae, *Eugenia caryophyllus*. They are native to the Maluku Islands in Indonesia, and it is mainly used as a spice. Cloves are commercially harvested primarily in India, Pakistan, Indonesia, Madagascar, Zanzibar, Sri Lanka and Tanzania. But, Indonesia and Madagascar are the main clove buds oil producer.

According to Hatano, Nozaki, Takahashi, Okamoto, Ito, Bao (2018), there are three types of clove oil; bud oil, leaf oil and stem oil. Bud oil is derived from the flower-buds of *Eugenia caryophyllus*. It contains mainly of 60-90% eugenol, eugenyl acetate, caryophyllene and other minor constituents. Leaf oil is derived from the leaves of *Eugenia caryophyllus*. It consists of 82-88% eugenol, little amount of eugenyl acetate, and other minor constituents. Stem oils are evolved from the twigs of *Eugenia caryophyllus*. It consists of 90-95% eugenol, and some other minor constituents. A major component of clove taste is imparted by the chemical eugenol. Eugenol is the component which is most responsible for clove aroma and comprises 72-90% of the essential oil extracted from cloves. Other important essential oil constituents of clove oil are acetyl eugenol, beta-caryophyllene and vanillin, crategolic acid, tannins such as bicornin, methyl salicylate (painkiller), gallotannic acid, the flavonoidseugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids such as oleanolic acid, campesterol and stigmasterol, and several sesquiterpenes.

Hatano et al., (2018) states that it is now believed to the FDA that there are not enough evidence indicates clove oil or eugenol is effective for toothache pain or other types of pain [9]. Studies to determine its effectiveness for fever reduction, as a mosquito repellent, and to prevent premature ejaculation have been inconclusive. Clove may reduce blood sugar levels but it is not proven yet and studies going on to determine this. Furthermore, clove oil is used in preparation of some Clovacaine solution, and toothpastes which are the local anesthetic used in oral ulceration and inflammation. Eugenoland zinc oxide are mixed together to form a temporary tooth cavity filling. Clove oil can be used to anesthetize of fish. It also can be considered as a humane means of euthanasia with higher doses and the recommended dose is 400 mg/l. Eugenol is a colorless or pale yellow oily liquid which are extracted from different essential oils especially from clove oil, nutmeg, basil, cinnamon and bay leaf. In clove bud oil it is present in concentrations of about 80-90% where clover leaf contain 82-88% of eugenol. Eugenol is used as the perfumeries, flavorings, essential oils and also in the medicine as a local antiseptic and anaesthetic.

Eugenol can be produce zinc oxide eugenol by mixing with zinc oxide which is used in dentistry as the restorative and prosthodontics application. For root canal sealing, zinc oxide eugenol is used. It is also used in some mousetraps and kills particular human colon cancer cell lines *in vivo* and *in vitro*. Eugenol may have potential therapeutic effects against diseases characterized by excessive osteoclast activity. Other important essential oil constituents of clove seed include acetyl eugenol, β-caryophyllene, and vanillin; crategolic acid; tannins; gallotannic acid; methyl salicylate (an anesthetic); the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin; triterpenoids such as oleanolic acid, stigmasterol, and campesterol; and several sesquiterpenes. Hypercholesterolemia has been identified as a key risk factor for the development of cardiovascular diseases. Continuous ingestion of high amounts of fat seems to be directly related to abnormal lipid levels in humans. Hyperlipidemia in laboratory animals has been studied in order to better understand the relationship between disorders in cholesterol metabolism and atherogenesis and to test possible treatments to reduce circulating cholesterol levels.

2.4.1 The phytochemical properties of *E.caryophyllus* Extract

Clove (Eugenia caryophyllata Thunb.) is widely cultivated in Madagascar, Sri Lanka, Indonesia and the south of China. Clove bud oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavouring agent and antimicrobial material in food Clove oil was effective against L. monocytogenes and S. Enteritidis in tryptone soya broth (TSB) and cheese (Smith-Palmer, Stewart, and Fyfe, 2018). The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability (Huang, Ho, Lee, and Yap, 2019). Essential oils are well known inhibitors of microorganisms. Clove oils are natural preservative and flavouring substances that are not harmful when consumed in food products. There have been a number of reports of substances in each of clove oils that inhibit the growth of molds, yeasts and bacteria. Clove oil added at 2% in potato dextrose agar (PDA) completely inhibited the growth of seven mycotoxigenic molds (A. flavus, A. parasiticus, A. ochraceus, Penicillium sp. M46, P. roqueforti, P. patulum, and P. citrinum) for various times up to 21 days (Azzouz, and Bullerman, 2018) . Similarly reported that cinnamon oil and clove oil could separately inhibit many other microbes including Lactobacillussp, Bacillus thermoacidurans, Salmonella sp., Corynebacteriummichiganense, Pseudomonas striafaciens, Clostridium botulinum, Alternaria sp., Aspergillus sp., Canninghamella sp., Fusarium sp., Mucor sp., and Penicillium sp. The essential oil, isolated from the fruits of E. cardamomum showed antimicrobial. The objective of the research was to study the inhibitory effects of cardamom and clove oils, added singly and in various combinations on growth of bacteria. Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. Natural products have been an integral part of the ancient traditional medicine systems like Ayurveda, Chinese and Egyptian. It is estimated that 40% of the world population depends directly on plant based medicine for their health care. India has rich medicinal plant flora of some 25,000 species, out of which 150 species are commercially used for extracting medicines or drug formulation. Over the last few years, researchers have aimed at identifying and validating plants derived substances for the treatment of various diseases. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. In this context, it is worth mentioning that Indian plants are considered as vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments. The focus of this review is to provide information on the phytochemicals, ethno medicinal uses and pharmacological activities of Syzygium aromaticum commonly known as clove. Clove (Syzygium aromaticum (L.) Merril. and Perry, syn. Eugenia aromaticum or E. caryophyllata) is one of the most ancient and valuable spices of the Orient. It is a member of the family Myrtaceae. The clove of commerce is its dried unopened flower buds. Whole and ground cloves are used to enhance the flavor of meat and rice dishes and used widely in curry powders and masalas. They are highly valued in medicine as a carminative and stimulant and are said to be a natural anthelmintic. It is used throughout Europe and Asia and is smoked in a type of cigarette, known locally as kretek in Indonesia and in occasional coffee bars in the West, mixed with marijuana to create marijuana spliffs. In the last several years, it has been recognized as an effective anesthetic for sedating fish for a number of invasive and noninvasive fisheries management and research procedures (Smith-Palmer et al., 2018).

According to Smith-Palmer et al., (2018), oil of clove is used extensively for flavoring all kinds of food products, such as meats, sausages, baked goods, confectionery, candies, table sauces, pickles, etc. It is used in medicine for its antibacterial, antiseptic and antibiotic properties. It has also been successfully used for asthma and various allergic disorders by oral administration. Sesquiterpenes, found in clove were also investigated as potential anti-carcinogenic agents. The oil has many industrial applications and is used extensively in perfumes, soaps and as a clearing agent in histological work. In addition, the cloves are anti-mutagenic, anti-inflammatory, antioxidant, antiviral, anti-thrombotic and anti-parasitic.

2.4.2 Phytochemical Constituents of Clove

Various studies have been carried out to find various constituents of S. aromaticum. Clove buds contain 15–20% essential oil, which is dominated by eugenol (70–85%), eugenyl acetate (15%) and β-caryophyllene (5–12%). Other essential oil ingredients of clove oil are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin and triterpenoids like oleanolic acid. The constituents of the oil also include methyl amyl ketone, methyl salicylate, α and β-humulene, benzaldehyde, β-ylangene and chavicol. The minor constituents like methyl amyl ketone, methyl salicylate etc., are responsible for the characteristic pleasant odour of cloves. Gopalakrishnan et al. (1984) characterized six sesquiterpenes, namely: α-cubebene (1.3%), α-copaene (0.4%), βhumulene (9.1%), β-caryophyllene (64.5%), γ-cadinene (2.6%) and δcadinene (2.6%) in the hydrocarbon fraction of the freshly distilled Indian clove bud oil (Khandelwal, 2018).

2.4.3 Pharmacological Activity of Clove

Antibacterial activity several studies have demonstrated potent antibacterial effects of clove. The inhibitory activity of clove is due to the presence of several constituents, mainly eugenol, eugenyl acetate, βcaryophyllene, 2-heptanone, (Matan, Rimkeeree, Mawson, Chompreeda, Haruthaithanasan, Parker (2018) acetyl-eugenol, α-humulene, methyl salicylate, iso-eugenol, methyl-eugenol, phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnetin, myricetin, gallic acid, ellagic acid and oleanolic acid. These compounds can denature proteins and react with cell membrane phospholipids, changing their permeability. Jamal, Javed, Aslama, and Jafri (2017), found clove oil effective against non-toxigenic strains of E. coli O157:H7. Similarly in another study clove oil was found to be active against food borne gram positive bacteria (S. aureus, B. cereus, E. faecalis, L. monocytogenes) and gram negative bacteria (E. coli, Y. enterocolitica, S. choleraesuis, P. aerugenosa). Azzouz and Bullerman (2018) showed that aqueous and ethanolic extracts of clove buds inhibit growth of methicillin resistant clinical isolates at 1000 and 500mg/ml concentration (Taylor and Roberts, 2019). The isolates were multi drug resistant, mostly against beta-lactams, aminoglycosides, tetracyclines, floroquinolones and macrolide antibiotics. In another study eugenol at 2µg/mL inhibited growth of 31 strains of Helicobacter pylori, after 9 hours of incubation, which is being more potent than amoxicillin and doesn’t develop resistance (Jamal et al., 2017).

2.4.4 Antifungal activity

Many studies have reported antifungal activity for clove oil and eugenol against yeasts and filamentous fungi, such as several foodborne fungal species and human pathogenic fungi (Matsumura, Matsuda, Sato, Minami, Ramawat, Mérillon, 2019). Clove oil and eugenol have also been tested as antifungal agents in animal models (Srivastava, 2020). The phenolic components of clove, carvacrol and eugenol, are known to possess fungicidal characteristics (Srivastava, 2020), including activity against fungi isolated from onychomycosis. Rana et al. determined antifungal activity of clove oil in different strains and reported following scale of sensibility- Mucor sp.> Microsporum gypseum> Fusarium monoliforme NCIM 1100> Trichophytum rubrum> Aspergillus sp.> Fusarium oxysporum (Matsumura et al., 2019). In chromatographic analysis eugenol was found to be the main compound responsible for the antifungal activity, due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was also reported by Devi et al (Matsumura et al., 2019). The large spectrum of fungicidal activity of clove oil and eugenol was reported on Candida, Aspergillus and dermatophytes and the mechanism of action was attributed to the lesions of the cytoplasmic membrane (Matsumura et al., 2019). Burt proposed that different modes of action can be involved in the antifungal activity of essential oils. The activity may in part be due to their hydrophobicity, which is responsible for their partition into the lipid bilayer of the cell membrane, leading to an alteration of permeability and a consequent leakage of cell contents (Jamal et al., 2017).

2.4.5 Antioxidant/Free radical scavenging activity

Clove essential oil has the highest antioxidant capability and perhaps one of the best known oil for food or supplement. For this reason, it has been included in some longevity formulae. Clove and eugenol possess strong antioxidant activity, which is comparable to the activities of the synthetic antioxidants, BHA and pyrogallol (Huang, Ho, Lee, and Yap, 2019). Clove oil inhibited 97.3% lipid peroxidation of linoleic acid emulsion at 15 µg/mL concentration. The essential oil demonstrated scavenging activity against the -diphenyl-1-picryl hydrazyl (DPPH) radical at concentrations lower than the concentrations of eugenol, butylated hydroxytoluene (BHT), and butylated hydroxyl anisole (BHA). Abojid et al observed enhanced liver functions, kidney functions, and antioxidant status in clove treated rats and showed that its protective role against H2O2.

2.4.6 Anticarcinogenic activity

Induced cell damages might be due to the effect of active compounds found in essential oil and plant extract (Huang et al., 2019). A recent study by Calleja et al reported that βcaryophyllene isolated from clove essential oil protects rat liver from carbon tetrachloride induced fibrosis by inhibiting hepatic stellate cell activation (Huang et al., 2019). Clove essential oil has also been reported to show anticarcinogenic (Deepak, Kasonga, Kruger, Coetzee, 2018) and antimutagenic potential because of its strong free radical scavenging activity. Several Preliminary studies suggested chemo preventive role of clove oil, particularly in cases of lung, skin and digestive cancers. Ethyl acetate extract of clove inhibits tumor growth and promotes cell cycle arrest and apoptosis. Oleanolic acid one of the components of ethyl acetate extract of clove was found to be responsible for its antitumor activity. Its mechanism was attributed to the promotion of Go/G1 cell cycle arrest and induction of apoptosis in a dose-dependent manner (Deepak et al., 2018). Eugenol acts as a potential molecule that can interfere with several cellsignaling pathways, specifically the NF-κB. Eugenol was found to suppress growth of malignant melanoma WM1205Lu of both anchorage-dependent and anchorage independent growth, decreased size of tumors and inhibited melanoma invasion and metastasis by the inhibition of two transition factors of the E2F family. Hussain et al. studied the effect of eugenol combined with gemcitabine on cervical carcinoma and found that the combination of eugenol and gemcitabine can inhibit cancer cell growth, even in low concentrations (Deepak et al., 2018). Studies on related gene also found that eugenol can reduce the possibility of apoptosis of B-cell lymphoma-2 (Bcl-2), Cyclooxygenase-2 (COX-2), and interleukin-1β (IL-1β), reduce inflammation, and increase the treatment efficacy of gemcitabine. Moreover, Eugenol showed better curative effects in skin cancer and melanoma (Deepak et al., 2018).

2.4.7 Analgesic activity

Eugenol is a routine analgesic agent widely used in dental clinics due to its ability to alleviate tooth pain. Its anesthetic effects in dental pain as well as analgesic and anti-inflammatory effects in animal models have been well documented (Mittal, Gupta, Parashar, Mehra, Khatri, 2020). The effects have been attributed to its capability to suppress prostaglandins and other inflammatory mediators such as leukotriene. It is also believed to depress the sensory receptors involved in pain perception, inhibits the conduction of action potential in sciatic nerves and N-methyl-D-aspartate (NMDA) receptors but potentiates ionotropic γ-aminobutyric acid (GABAA Anti-inflammatory activity) receptors, which are both involved in pain sensitivity (Mittal et al., 2020). Clove oil clear respiratory passages, acting as an expectorant for treating many upper-respiratory conditions including colds, eye sties, bronchitis, sinus conditions, cough and asthma. One of the studies showed that the essential oil possess significant antiinflammatory effect at doses of 0.05 ml/kg (90.15% inhibition) and 0.200 ml/kg (82.78% inhibition) (Mittal et al., 2020).

2.4.8 Anti-inflammatory Activity

Clove has been used in traditional public medicine to relieve nasal obstruction and musculoskeletal pain which implies its anti-inflammatory activity and the activity is due to COX-2 inhibition. The aromatic oil, when inhaled, can help relieve certain respiratory conditions like coughs, colds, asthma, bronchitis and sinusitis. Clove also contains a variety of flavonoids including kaempferol, rhamnetin and βcaryophyllene which also contributed to its anti-inflammatory and antioxidant properties.

2.4.9 Anti-diabetic activity

Several reports have documented the potential role of clove as an antidiabetic agent. It was found that clove and insulin regulate the expression of diabetes-related genes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene, in a similar manner (Prasad et al, 2005)

Plants extracts have also been shown to reduce blood glucose levels in animal models via its ability to increase muscle glycolysis and mitochondria function by activating AMP-activated protein kinase andsirtuin (Tu et al., 2014).

2.4.10 Anti-Thrombotic activity

Clove oil was reported as an inhibitor of platelet aggregation and thromboxane synthesis and may act as an antithrombotic agent. In fact, clove oil inhibited human platelet aggregation induced by arachidonic acid, a compound found in the plant extract (Grespan et al., 2012)

2.4.11 Organ-protective effects

Clove extract was shown to reduce the activity of liver enzymes, such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, and to elevate levels of antioxidants indices, such as glutathione, ascorbic acid, superoxide dismutase, and catalase, highlighting its hepatoprotective effects (Adefegha et al., 2014)

2.4.12 Odontalgic properties

Periodontal disease is caused by bacteria is dental plaque. Plaque is the sticky substance that forms on teeth soon after have brushed. In an effort to get rid of the bacteria, the cells of immune system release substances that inflame and damage the gums, periodontal ligament or alveolar bone. This leads to swollen, bleeding gums, a sign of gingivitis (the earliest stage of periodontal disease). This plant is the only natural remedy to this teeth disease.

2.5 Toxicity

2.5.1 Plant Toxicity

Eugenol slows blood clotting and can lead to an increased risk of bleeding. It should be avoided by people with bleeding disorders, those scheduled for surgery, and people on anticoagulants medications. Cloves have been shown to lead to lowered blood sugar levels, and should be avoided by people who are hypoglycemic (have low blood sugar). Most of its bioactive ingredients are toxic in higher dosages. The eugenol in cloves can cause allergies. The compound reacts directly with the body proteins and causes contact dermatitis. It can also cause localized irritation

Cloves may also cause respiratory allergies in a few individuals. This was especially true in the case of workers involved in the spice (clove) processing factories, who inhaled the spice dust. Irritation of the upper and lower respiratory tracts and impaired lung functioning were two of the major symptoms. The clove essential oil is generally recognized as safe substance when consumed in concentrations lower as 1 500mg/kg.

CHAPTER THREE

3.0 Materials and Methods:

3.1 Plant Material

3.1.1 Sample Collection and preparation

The plant Eugenia caryophyllata (clove) used for this study was collected in October, 2020 from an individual her seller at, Magboro, Ogun state, Nigeria through Prof. Amos Akinwande, my project supervisor and also the Dean of the College of Basic and Applies Sciences, Mountain Top University, Ibafo, Ogun state

The buds of the cloves were oven-dried for three days at 60 because of their dried nature, the drying was to remove all moisture present in the sample, and I used a mortar and pestle to grind the sample into powder, it was then weighed and stored in an air-tight jar. Twenty-five grams

(25g) of the powder was weighed into a jar and was macerated in 100ml of water with occasional shaking at room temperature for 96 hours twice. Filtration was done using a muslin cloth, the filtrate was concentrated in a vacuum at 60c to about one-tenth the original volume. The concentrates were then kept in the refrigerator at -4c.

3.2 Methodology

3.2.0 Phytochemicals- Qualitative study

3.2.1 Test for flavonoids

Alkaline reagent test: about 2 ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow coloration with disappeared on the addition of dilute HCL

3.2.2 Gum and mucilage test

The plant extract was dissolved in 20ml of distilled water and to this 50ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage.

3.2.3 Test for tannins

The extract of the sample was treated with 15% ferric chloride test solution. The resultant colour was noted. A blue colour indicated the presence of hydrolysable tannin or into 5ml of freshly prepared potassium hydroxide (KOH) in a beaker; 1ml of the extract was added and shaken to dissolve. A dirty precipitate observed indicates the presence of tannin.

3.2.4 Test for alkaloids

Dragendroff's test: The plant extracts was dissolved in chloroform and the solution was extracted with dil H_2SO_4 and acid layer taken and tested for presence of alkaloids. To 5ml of acid layer of test solution, 5ml of Dragendroff's reagent (potassium bismuth iodide solution) and 5ml of dil HCl were added. An orange-red precipitate indicated presence of alkaloids

Cardiac glycosides

Keller-kilian's test: 0.5ml of glacial acetic acid was dissolved in 50ml of test solution containing one drop of ferric chloride solution. This was then under layer with 0.5ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of cardiac glycosides

3.2.4 Test for terpenoids

Salkowski's test: 1ml of each the extract was added to 4ml of chloroform. 6ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids

3.2.5 Test for phenols

Ferric chloride test: to 5ml of alcoholic solution of extract, 1ml of distilled water followed by few drops of 10% ferric chloride ($FeCl_3$) solution was added. Formation of blue colour indicates the presence of phenols

3.2.6 Test for steroids

Liebermann burchard test: to 2ml to extract, 2ml of glacial acid and 2ml of acetic anhydride and 3-4 drops of concentrated sulphuric acid were added. The solution becomes red, then blue and finally bluish green, indicates the presence of steroids.

3.2.7 Fatty acids test:

10ml of test solution was mixed with 10ml of ether. This extract was allowed to evaporate on filter paper and dried the filter paper. The transparency on filter paper indicates the presence of fatty acids.

3.2.8 Proteins and amino acid:

Ninhydrin test: 2-3 drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) was added to the small quantity of extract solution and heat it. Development of blue color reveals the presence of proteins, peptides or amino acids

3.2.9 Test for carbohydrates

Molisch's test: to 4ml of the test solution, 2ml of a-naphthol solution was added, concentrated sulphuric acid (H2SO4) was poured through the sides of the test tube. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates

3.3 GCMS METHOD FOR PHYTOCHEMICAL ANALYSIS

This analysis was performed using 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) and electron impact source (Agilent Technologies).

The stationary phase of separation of the compounds was carried out on HP- 5 capillary column coated with 5% of Phenyl Methyl Siloxane (30 m length × 0.32 mm diameter × 0.25 μm film thickness) (Agilent Technologies).

The carrier gas was helium used at a constant flow rate of 1.573 ml/min, an initial nominal pressure of 1.9514 psi and at an average velocity of 46 cm/s. One microliter of the samples were injected in splitless mode at an injection temperature of 260°C. Purge flow was 21.5 ml/min at 0.50 min with a total gas flow rate of 23.355ml/min; gas saver mode was switched on.

The oven was initially programmed at 60°C (1 min), then ramped at 4°C/min to 110°C (3 min), followed by temperature program rates of 8°C/min to 260°C (5 min) and 10°C/min to 300°C (12 min). Run time was 56.25 min with a 3 min solvent delay.

The mass spectrometer was operated in electron- impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. Scanning of possible compounds was from m/z 30 to 550 amu at 2.62s/scan scan rate and were identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library.

3.4 NMR method of analysis

1H NMR spectra for was performed with a thermoscientific FOURIER 300 spectrometer, operating at 3,000,000 MHz. solutions were prepared using deionized water.

Chapter 4

4.0 Results and Discussion

4.1 Result

Table 4.1 Phytochemical analysis of the aqueous extract of Eugenia caryophyllata (clove)

	Phytochemicals	**Test**	**Water extract**
1	Carbohydrates	Molisch's test Braford's test Benedicts test	+ + +
2	Tannins test	Using FeCl3	+
3	Alkaloids	Dragendroff's Mayer's test	+ +

		Wagner's test	+
4	Cardiac Glycosides	Keller- Killani test	+
5	Fatty acids test	Paper test	+
6	Flavonoids	Shinoda test Alkaline reagent test Lead acetate test	+ + +
7	Gum and Mucilage		+
8	ProteinAmino Acid	Ninhydrin test	-
9	Phenols	Ferric chloride test	+
10	Steroids	Liebermann burchad	+
11	Terpenoids	Salkowski's	+

+ = Present, - = Absent

Table 4.2 Phytocomponents identified in the aqueous extract of Eugenia caryophyllata (clove) by GC-MS

No	Retention time	Name of compound	Molecular formula	Peak Area (%)	Molecular weight
1	5.039	Oxirane, 2-ethyl-2-methyl	$C_5H_{10}O$	0.04	86.13 g/mol
2	10.502	Pyrrolidine,1-(1-cyclohexen-1-yl), Phosphinic acid	$C_{10}H_{17}N$	0.71	151.25 g/mol
3	14.516	Neophytadiene, Phytol, acetate	$C_{20}H_{38}$	3.77	278.5 g/mol
4	15.694	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	5.51	256.42 g/mol
5	16.774	Phytol	$C_{20}H_{40}O$	3.84	296.5 g/mol
6	17.184	Ethyl 9,12,15-octadecatrienoate 9,12,15-Octadecatrienoic acid, ethyl ester	C20H34O	3.65	306.4828g/mol
7	19.599	Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O	0.59	358.5558g/mol
8	23.341	Vitamin E dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	2.91	430.7 g/mol
9	24.750	.gamma.-Sitosterol	$C_{29}H_{50}O$	4.18	414.7 g/mol
10	26.829	Phytyl palmitate	C36H70O2	1.92	534.9398g/mol
11	29.376	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	0.96	280.4 g/mol

		(Z,Z)- 1,3-Dioxolane, 4-ethyl-5-octyl-2,2 -bis(trifluoromethyl)			
12	29.607	Ethanone, 2-(2-benzothiazolylthio) -1-(3,5-dimethylpyrazolyl)- 1,14-Dibromotetradecane	$C_4H_6O_2$	0.82	86.09 g/mol

Table 4.3 Peak Values of the 1H NMR

^{1}H NMR (82 MHz,) δ 2.36, 2.36.

Table 4.4: 1H NMR chemical shifts of Eugenia caryophyllata

	ppm	Intensity	Width	Area	Type	Flags	Impurity/Compound	Annotation
1	2.36	4134.6	6.53	738345.66	Compound	None		
2	2.36	4011.2	4.65	479595.33	Compound	None		

Table 4.5:

	Name	Shift	Range	H's	Integral	Class	J's	Method
1	A (m)	2.36	2.47.. 2.25	1	515987.85	m		Sum

Instrument: GC MSD

Using AcqMethod: PHYTO-SCAN.M

Sample Name: Eugenia caryophyllata (clove)

Misc. Info:

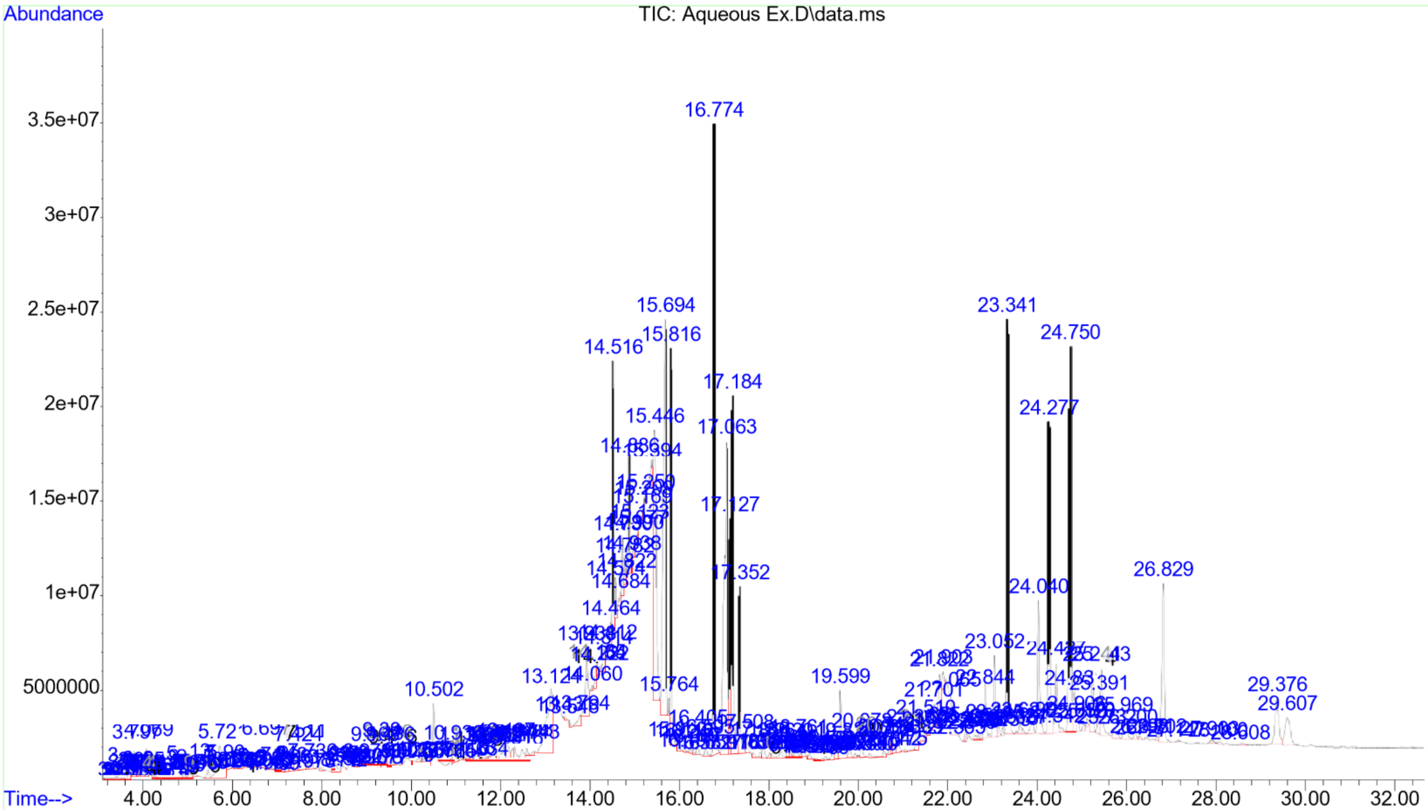
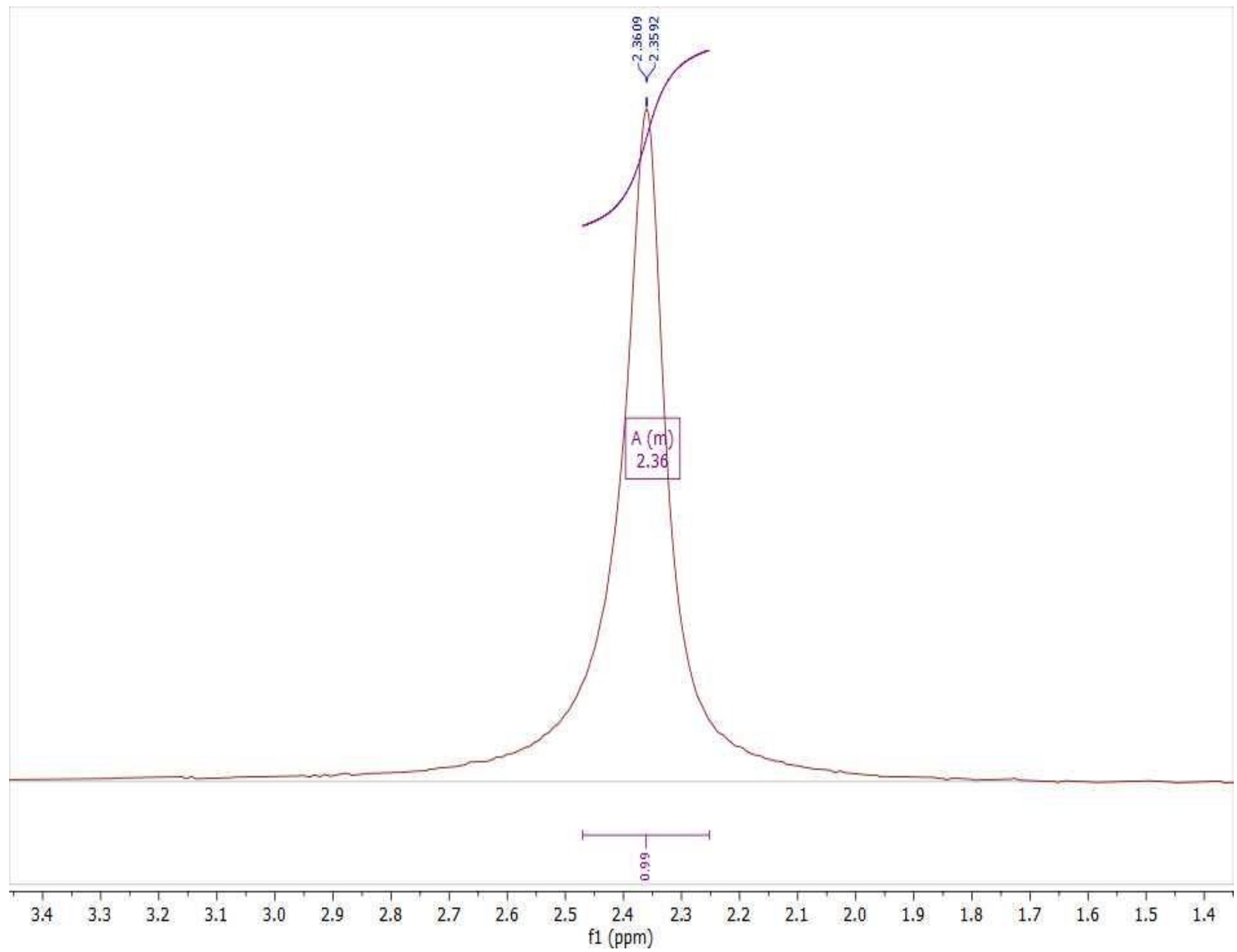


Fig. 1: GC-MS spectra of the aqueous isolated Eugenia caryophyllata (clove)


2.3609
2.3592
A (m)
2.36
0.99
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
f1 (ppm)

4.2 DISCUSSION

4.2.1 Phytochemical analysis of the aqueous extract of Eugenia caryophyllata (clove)

In this study, the aqueous solvent used for extraction of phytochemicals and their extractive values were determined. Extractions with distilled water yield 3.7 g. Aqueous extraction showed better extractive efficiency than other solvents and this result was found to be similar with the existing literature (Nazrul et al. 2010; Edziri et al. 2011). Eugenia caryophyllata (clove) extract was subjected to phytochemical analysis by both qualitative and quantitative methods. In qualitative analysis, various tests for phytochemicals were carried out and the results confirmed the presence of alkaloid, phenols, flavonoids, tannins and saponins in the crude extract. The phytoconstituents detected in the extract could be responsible for the antioxidant and antimicrobial activity (Peter and Wong 2006). In quantitative analysis, the phytochemicals evaluated in the aqueous extract of Eugenia caryophyllata (clove) were in the order of total phenolics > flavonoids with the respective values of 596.6 > 62.6 mg/g of extract. Presence of high level of phenolic compounds revealed that Eugenia caryophyllata (clove) extract can have good antioxidant capacity because of their ability to scavenge free radicals and active oxygen species (Miliauskas et al. 2004).

4.2.2 Phytocomponents identified in the aqueous extract of Eugenia caryophyllata (clove) by GC-MS

The identification of phytochemical compounds is based on their retention time (RT), molecular formula, molecular weight (MW), chemical structure and concentration (peak area %). GC-MS chromatogram of leaves of Eugenia caryophyllata (clove) analysis showed the presence of twelve different compounds namely Oxirane, 2-ethyl-2-methyl, Pyrrolidine,1-(1-cyclohexen-1-yl), Phosphinic acid, Neophytadiene, Phytol, acetate, n-Hexadecanoic acid, Phytol, Ethyl 9,12,15-octadecatrienoate, 9,12,15-Octadecatrienoic acid, ethyl ester, Octadecanoic acid, 2,39,12-Octadecadienoic acid, (Z,Z)- 1,3-Dioxolane, 4-ethyl-5-octyl-2,2 -bis(trifluoromethyl)-dihydroxypropyl ester, Vitamin E,di-.alpha.-Tocopherol, .gamma.-Sitosterol, Phytyl palmitate, as shown in Table 4.3. The individual fragmentations of the components are illustrated in Figures 1. The GC and MS running time for the aqueous extract of *Eugenia caryophyllata (clove)* were 32 min and spectrum is as shown Interpretation of mass spectrum (GC-MS) was done by using data base of msdchem library. Besides the presence of other phytochemicals such as eugenyl acetate, caryophyllene and 1, 2,3 benzenetriol, eugenol plays a vital role for antioxidant and antibacterial activities. Eugenol is the major components in clove extract and had good agreement with the results reported by Jasna et al. (2013). The high concentration of eugenol in clove extract makes it potential for antibacterial, and antioxidant properties. Studies on antibacterial action of eugenol represented that it disrupts the cell membrane and enhances its non-specific permeability (Gill and Holly 2006). Also, lipophilicity of eugenol enables it to disrupt the cell structure by incorporating with the lipopolysaccharide layer of bacterial cell membrane and results in the intracellular components leakage which leads to death (Burt 2004; Pandima Devi et al. 2010). Conjugation of carbon chain with aromatic ring of eugenol involved in the phenoxyl radical stabilization process by resonance (Diego et al. 2014). Eugenol has the capability to reduce two or more DPPH radicals by forming dimers. Pyrogallol or 1,2,3 benezenetriol also shown antibacterial activities.

GCMS analysis of the aqueous extract of leaves of *Eugenia caryophyllata* shows the presence of medicinally valued bioactive components like saponins, tannins, alkaloids, steroids, terpenoid and flavonoids. As the medicinal value of similar components in other plant extracts are already proved, no wonder if these components in *Eugenia caryophyllata* may also have equally effective.

Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as an emollient.20 Small amounts of oleic acid are used as excipient in pharmaceuticals, and it is used as an emulsifying or solubilizing agent in aerosol products.20

The NMR methods used is very important to determine the molecular structure of a chemical as a whole and provides information on the number of each type of hydrogen is sufficient to determine more about an unknown structure (Pavia et al., 2001).

The resulting spectrum of ^1H NMR analysis that gives important guidance in determining the structure with the value of Chemical shift. The results of the analysis found that the existence of distinct peaks signals at 2.36, intensity of 4134.6 and 4011.2 in the range of 2.47 – 2.25 with reference to the proton of acids H-C-OOH, carbonyl compounds HC–C=O, aromatic methyl group Ar–H (Ar–C–H, benzylic) and Methyl ketone group HC– (R)-C=O. Based on information from the reference in Pavia et al. 2009 and Chemdraw software, the value is respectively 2.47 ppm. Thus, the existence of the signal is then established that the ester product is methyl substituted compound in TMP. Besides, proton signals at 2.47-2.25 ppm appeared which is refer to the proton of C=C-H proton of olefin that the values are also present in the analysis Chemdraw and the reference in Pavia et al. 2009.

References

Aicardi, J. and F. Goutieres, 2019. Neonatal myoclonic encephalopathy (author's transl). Rev. Electroencephal. Neurophysiol. Clin., 8: 99-101.

Alqareer A, Alyahya A, Andersson L. (2018). The effect of clove and benzocaine versus placebo as topical anesthetics. *J Dent*;34(10):747-50

Aylwad, R.L., 2018. Epilepsy: A review of reports, guidelines, recommendations and models for the provision of care for patients with epilepsy. Clin. Med., 8: 433-438. DOI: 10.7861/clinmedicine.8-4-433

Azzouz MA, Bullerman LB (2018). Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. Journal of Food Protection 1982; 45: 1298– 1301

Azzouz MA, Bullerman LB (2018). Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. Journal of Food Protection 1982; 45: 1298– 1301.

Baytop T. (2018). Therapy with Medicinal Plants in Turkey. 2nd ed. Nobel Medical Bookstore: İstanbul-Turkey

Berg, A.T., S.F. Berkovic, M.J. Brodie, J. Buchhalter and J.H. Cross et al., 2010. Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and terminology. 2005-2009. Epilepsia, 51: 676- 685. DOI: 10.1111/j.1528-1167.2010.02522.x

Chogo JB and Crank G. (2017). Chemical composition and biological activity of the Tanzanian Plant Ocimum suave. J Nat Prod. 42:308-11

Chuetz F, Kumar S, Poronnik P, Adams DJ. (2019). Regulation of the voltage-gated K(+) channels KCNQ2/3 and KCNQ3/5 by serum- and glucocorticoid-regulated kinase-1. American journal of physiology Cell physiology. ;295(1):C73–80. Epub 2008/05/09. pmid:18463232

Dan B, Steven C, Erich S and Andrew G. (2019). Chinese Herbal Medicine: Materia Medica. 3rd. Ed. 2004.

Daroff, R.B., G.M. Fenichel, J. Jankovic and J.C. Mazziotta, 2017. Neurology in Clinical Practice. 6th Edn., Elsevier Health Sciences, Philadelphia, ISBN-10: 1455728071, pp: 2544.

Deepak V, Kasonga A, Kruger MC, Coetzee M. (2018). Inhibitory effects of eugenol on RANKL-induced osteoclast formation via attenuation of NF-κ B and MAPK pathways. Connective tissue research. 56(3):195-203.

Diego Francisco Cortes-Rojas, Claudia Regina Fernandes de Souza and Wanderley Pereira Olivera

Asian Pac J Trop Biomed 2014 :25182278

Ekberg JA, Boase NA, Rychkov G, Manning J, Poronnik P, Kumar S. (2017). Nedd4-2 (NEDD4L) controls intracellular Na(+)-mediated activity of voltage-gated sodium channels in primary cortical neurons. The Biochemical journal. 2014;457(1):27–31. Epub 2013/10/25. pmid:24152020

Fisher RS, Acevedo C, (2017). Arzimanoglou A, Bogacz A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. ;55:475–82.

Gallo, M., (2019). Lactic dehydrogenase and cancer: an overview. Front Biosci (Landmark Ed) 20: p. 1234- 49

Grant SG. (2019). Synaptopathies: diseases of the synaptome. Current opinion in neurobiology. 2012;22(3):522–9. Epub 2012/03/14. pmid:22409856

Guerrini, R., 2006. Epilepsy in children. Lancet, 367: 499-524.

Guidotti T. (2018). Critigue of available studies of the toxicology of kretek smoke and its constituents by routes of entry involving the respiratory tract. Archives of Toxicology. 63:7-12

Harborne JB and Baxter H. (2019). Phytochemical Dictionary. Taylor and Francis: Washington DC.

Hatano T, Nozaki A, Takahashi E, Okamoto K, Ito H, Bao L. (2018). Hydrolysable Tannins Isolated from Syzygium aromaticum: Structure of a New C-Glucosidic Ellagitannin and Spectral Features of Tannins with a Tergalloyl Group. *Heterocycles.*2012;85(2):365

Hattersley, A.T. and F.M. Ashcroft, 2016. Activating mutations in Kir6.2 and neonatal diabetes: New clinical syndromes, new scientific insights and new therapy. Diabetes, 54: 2503-2513. DOI: 10.2337/diabetes.54.9.2503

Hema R, Kumaravel S, Sivasubramanian C, Panda S, Mohanty GC, Smal RN, Study On The Potentials Of Syzygium aromaticum. *Evaluation.* 2010;1:4.

Huang Y, Ho SH, Lee HC, Yap YL (2019). Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of Sitophilus zeamais Motsch. (Coleoptera: Curculionidae) and Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae).Journal of Stored Products Research 2002; 38:403–412.

Isaacs G. (2018). Permanent local anaesthesia and anhydrosis after clove oil spillage. Lancet;1:882-3

Jamal A, Javed K, Aslama M, Jafri MA (2017). Gastroprotective effect of cardamom, Elettaria cardamomum Maton. Fruits in rats. Journal of Ethnopharmacology 2006; 103:149–153

Karla S, Carretero E and Villar A. (2017). Antiinflammatory activity of leaf extracts of Eugenia jambos in rats. J Ethn Pharmacology. ;43:9-11.

Khandelwal KR (2018). :Book of practical Pharmacognosy. Nirali Prakashan Pune 2004:149-153

Kirsch CM (2017). Non-cardiogenic pulmonary edema due to the intravenous administration of clove oil. Thorax.;45(3):235-6

Lane BW, Ellenhorn MH, Hulbert TV and McCarron M. (2017). Clove oil ingestion in an infant. Human Exp Toxicol. 10(4):291-4.

Lee KG and Shibamoto T.(2015). Antioxidant property of aroma extract isolated from clove buds [Syzygium aromaticum (L.) Merr. Et Perry]. Food Chemistry. 2002;74:443-8

Li Y, Xu C, Zhang Q, Liu JY and Tan RX (2019). In vitro anti-Helicobacter pylori action of 30 Chinese herbal medicines used to treat ulcer diseases. J Ethnopharmacol, 98(6): 329-33

Lopez P, Sanchez C, Batlle R and Nerin C. (2020). Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected food borne bacterial and fungal strains. Journal of Agricultural and Food Chemistry. 53:6939-46.

Malson JL, Lee EM, Murty R, Moolchan ET, Pickworth WB. (2019). Clove cigarette smoking: biochemical, physiological, and subjective effects. *Pharmacol Biochem Behaviour* ;74(3):739-45

Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M: (2018). Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. International Journal of Food Microbiology 2007; 107: 180 – 185

Matsumura E, Matsuda M, Sato F, Minami H, Ramawat KG, Mérillon J-M (2019). Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes.

Mellish LC, Dunkley C, Ferrie CD, Paul DK. Antiepileptic drug treatment of Rolandic epilepsy and Panayiotopoulos syndrome: Clinical practice survey and clinical tial feasibility. Arch Dis Child. 2015;100(1):62–7.

Mellish LC, Dunkley C, Ferrie CD, Paul DK.(2018). Antiepileptic drug treatment of Rolandic epilepsy and Panayiotopoulos syndrome: Clinical practice survey and clinical tial feasibility. Arch Dis Child. 00(1):62–7.

Mihara S, Shibamoto T. (2020). Photochemical reactions of eugenol and related compounds: Synthesis of new flavor chemicals. J Agric Food Chemetry;30:1215-18

Mishra RK and Singh SK. (2018). Safety assessment of Syzygium aromaticum flower bud (clove) extract with respect to testicular function in mice. Food and Chemical Toxicology. 46:3333-38.

Mittal M, Gupta N, Parashar P, Mehra V, Khatri M. (2020) Phytochemical evaluation and pharmacological activity of Syzygium aromaticum: a comprehensive review. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(8):67-72.

Momose, A.,(2017). A case of fibrillary glomerulonephritis associated with thrombotic microangiopathy and antiglomerular basement membrane antibody. Nephron Extra, 2015. 5(1): p. 30-8.

Ottman, R., S. Hirose, S. Jain, H. Lerche and I. LopesCendes et al., 2010. Genetic testing in the epilepsies-report of the ILAE Genetics Commission. Epilepsia, 51: 655-70

Park MJ, Gwak KS, Yang I, Choi WS, Jo HJ and Chang WJ. (2017). Antifungal activities of the essential oils in Syzygium aromaticum (L.) Merr. Et Perry and Leptospermum betersonni Bailey and their constituents against various dermatiphytes. J Microbiol. ;45(5):460-5

Pawar VC and Thaker VS. (2019). In vitro efficacy of oils against Aspergillus niger. Mycosis 2006;49(4):316-23.

Rahhal M. (2017). M. Antifungal activity of some plant oils. Alexandria Sci Exch.;18: 225-30.

Rho, J.M. (2018). Inhibition of Lactate Dehydrogenase to Treat Epilepsy. N Engl J Med, 373(2): p. 187-9

Sada, N., (2017). Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. Science, 347(6228): p. 1362-7.

Sharma PV. (2019). Dravyaguna Vijnana. Chaukhambha Bharti Academy: Varanasi; 2001; 246–50

Sharma, S., N. Sankhyan, K. Ramesh and S. Gulati, 2017. Child neurology: Epilepsy of infancy with migrating focal seizures. Neurology, 77: 21-24

Shimomura, K., F. Hrster, H. De Wet, S.E. Flanagan and S. Ellard et al., 2017. A novel mutation causing DEND syndrome: A treatable channelopathy of pancreas and brain. Neurology, 69: 1342-1349.

Singh AK, Dhamanigi SS, Asad M. (2019) Anti-stress activity of hydro-alcoholic extract of Eugenia caryophyllus buds (clove). *Indian J Pharmacol.*;41(1):28-31

Smith-Palmer A, Stewart J, Fyfe L: (2018). Antimicrobial properties of plant essential oils and essences against five important food borne pathogens. Letters in Applied Microbiology; 26:118–122

Soto CG and Burhanuddin S. (2019). Clove oil as a fishanaesthetic for measuring length and weight of rabbitfish (Siganus lineatus). Aquaculture, 136:149-52.

Srivastava KC and Malhotra N. (2017). Acetyl euginol, a component of oil of cloves (Syzygium aromaticum L.) inhibits aggregation and alters arachidonic acid metabolism in human blood platelets. Prostaglandins Leukot Essent Fatty Acids. 1991;42(1):73-81

Srivastava KC. (2020). Antiplatelet principles from a food spice clove (Syzygium aromaticum L). J Prostaglandins Leukot Essent Fatty Acids 48:363-72.

Tajuddin AS, Latif A and Qasmi IA. (2020). Effect of 50 % ethanolic extract of Syzygium aromaticum (L) Merrand Perry. (clove) on sexual behaviour of normal male rats. BMC Complement Alter Med;4:17.

Tamber, M.S. and J.M. Mountz, 2017. Advances in the diagnosis and treatment of epilepsy. Seminars Nuclear Med., 42: 371-386.

Taylor PW and Roberts S. (2018). Clove oil: An alternative anaesthetic for aquaculture. North American Journal of Aquaculture. 61:150-5.

Taylor PW, Roberts SD. (2019). Clove oil:An alternative anesthetic for aquaculture. N Am J Aquacult;61:150-5.

The Ayurvedic Pharmacopoeia of India. Part-1.1;139-41.

Voie EJ, Adams JD, Reinhardt J, Rivenson A and Hoffman D. (2017). Toxicity studies on clove cigarette smoke and constituents of clove: determination of the LD50 of eugenol by instillation in rats and hamsters. Archives of Toxicology. ; 59:78-81

Wang CZ, Mehendale SR, Yuan CS (2017). Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness. *Am J Chin Med.*;35(4):543-58.

Yamatogi, Y. and S. Ohtahara, 2017. Severe epilepsy with multiple independent spike foci. J. Clin. Neurophysiol., 20: 442-448.