DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

IDENTIFICATION OF VARIOUS CLASSES OF SECONDARY METABOLITES IN SYZYGIUM AROMATICUM (CLOVE)

BY

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CERTIFICATION

This is to certify that this project work presented by OGUNBONA RANTI OMOTOYOSI with Matriculation Number HND/23/SLT/FT/0082 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 GOD ALMIGHTY for His unending grace, and to my parents, MR and MRS OGUNBONA , whose sacrifices and unwavering support shaped this journey — especially my father, whose strength, love, and belief in me carried me through every challenge.

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Abstract

13.44g of pulverized sample of clove was extracted with 300ml of methanol. The crude extract was subjected to qualitative phytochemical analysis. The extract yield was 5.25g (about 39%). From the phytochemical analysis results, it shows the presence of alkaloids, steroids, flavonoids, tannins, lactones, diterpenes, triterpenes and glycosides were confirmed while saponin was absent.

CHAPTER ONE

1.1 Introduction

The role of plants in human life has been increasing day by day due to advancement in the nutritional and medicinal disciplines. Spices are dried root, seed, bark fruit or flowers of plants which served several functions including flavoring agents, food additives, coloring agents, preservatives and medicines. During prehistoric times the discoveries of spices have been a period of joy as they are used as flavoring agents (Osuntogun et al. 2004). For aeons now, spices are irreplaceable part of cuisions all over the world. Beginning from the Ayurveda, these spices are used to cure several aliments due to their medicinal properties. Several phytochemicals have been isolated from spices responsible for their medicinal properties (Parthasarathy et al. 2008). They also possessed several pharmaceutical and phytochemical properties and hence, helpful in preparation of many medicines (Osuntogun et al. 2004).

1.2 Distribution

Syzygium aromaticum (Clove) belongs to family Myrtaceace, a taxon of dicotyledon plants is one of most valuable and second most important spice in the world trade. Various synonymes used for the clove are Caryophyllus aromaticus, Caryophyllus silvestris, Eugenia caryophyllus, Jambosa caryophyllus and Myrtus caryophyllus (Soh and Parnell, 2015).

Clove is commonly used in cultivation and indigenous to North Maluku Islands in Indonasia. Major cultivator countries of clove are Pemba, Zanzibar, Indonasia, Madagascar and some of wild clove varieties are found in Bacan, Ternate, Motir, Tidore, Makian and Western parts of Irian Jaya. In India cultivation of clove is restricted to three states Karnataka, Tamil Nadu and Kerala. India becomes second largest consumer of clove after the Indonesia (Board 2010). Cloves are available throughout the year due to different harvest seasons in different countries. The different varieties of clove tree vary in canopy shape from pyramidal to cylindrical. The clove tree

can live upto 100 years and above. The tree prefers to grow in well-drained soil with sufficient soil moisture. Clove tree requires heavy sunlight with high atmospheric temperature (25 to 35°C), well-distributed rainfall above 150 cm and high humidity above 70% (Danthu et al. 2014). The crop cannot withstand water logged conditions. In India clove grows well in deep black loamy soil of humid tropics and successfully grows in the red soils of midlands of Kerala and in the hilly terrain of Western Ghats in Karnataka and Tamil Nadu (Byng 2016).

1.3. Morphology and taxonomy

Clove is an aromatic spice tree. The term clove is taken from French word ‘clove’ and ‘clou’ which means ‘nail’. Clove is conical myrtle, medium sized tree with straight trunk which grows up to 10 to 12 m in height. The branches are semi-erect, grayish in color and dense. Leaves are large oblong to elliptic, simple obovate opposite, glabrous and possess plenty of oil glands on the lower surface. Tree

begins flowering in about 7 years and continues flowering for 80 years or more.

Flowers are small, crimson in color and are hermaphrodite(bisexual) borne at the terminal ends of small branches. Each peduncle carries 3 to 4 stalked flowers and inflorescence length remains between 4 to 5 cm. Initially flower buds are pale yellow in color with glossy appearance and turn green to bright red at maturity. These are 1-2 cm long with cylindrical thick ovary consisting of four fleshy sepals. Buds are divided into elongated stem and a globose bulbous head which stimulates into nail. Commercially cloves used are air-dried unopened flower buds, 2.5 cm in length and 1.25 cm wide.

Fruit matures nine months after flowering and the red ovary gradually turns to reddish purple. The fruit nearly contains one or two seeds known as ‘mother of clove’. The cultivated trees are rarely allowed to reach fruit stage. These are harvested when they develop dark red ellipsoid berry (Kamatou et al. 2012, Ortes-Rojas et al. 2014).

Taxonomically classification of Syzygium aromaticum (L.) from kingdom Plantae down to Species

Kingdom – Plantae

Sub kingdom – Tracheobionta

Super division – Spermatophyta

Division – Magnoliphyta

Class – Magnoliopsida

Subclass – Rosidae

Order – Myrtales

Family – Myrtaceae

Genus – Syzygium

Species – aromaticum (L.)

1.4 Chemical constituents of clove essential oil

From clove species three essential oils are available: clove stem oil, clove bud oil and clove leaf oil. Each clove essential oil differs in the chemical composition, flavour and color. In clove essential oil amount of secondary metabolites are affected by the nature of soil, climate, cultivation techniques and genetic factors (Vezar-Petri et al. 1985, Arslan et al. 2004).

1.4.1 The clove bud essential oil

The clove bud essential oil is yellow in color and denser than water. Alma et al. (2007) reported the presence of 18 components in clove bud essential oil. The main components characterized were eugenol (I, 87%), chavibetal (II, 19.7%), β-caryophyllene (III, 13%), eugenol acetate (IV, 8.01%), trisiloxane1, 1,1,5,5,5-hex-methyl-3,3bis[(trimethylsilyl)oxy] (V, 1.7%) etc. Further studies by (Khan et al. 2009, Matta 2010, Marya et al. 2012 and Kasai et al. 2016) reported eugenol (I, 74.32%) followed by the β-caryophyllene (III, 15.94%) and eugenol acetate (IV, 5.8%) as major compounds of clove bud essential oil (Kasai et al 2016).

1.4.2 The cloves leaf essential oil

Cloves leaf essential oil has characteristic pleasant odor and faint yellow color. Jirovetz et al. (2006) reported the presence of 23 compounds with eugenol (I, 76.8%), β-caryophyllene (III, 17.4%), eugenol acetate (IV, 1.2%), α-humulene (XII, 2.1%) as major compounds (Matta FB, 2010).

1.4.3 The cloves stem oil

The cloves stem oil is not commercially used as the cloves bud oil as the constituents responsible for fruity odor of clove oil are present in lesser amount and results in the flatter odor of clove stem oil but free eugenol (I) was present in much higher quantity in stem oil than the bud oil (Matta FB, 2010).

1.4.4 The clove root oil

The clove root oil was obtained by steam distillation with yield of about 6%. Freshly distilled root oil was bright yellow in color and having 85-95% of eugenol (I) (Pruthi 2001).

1.4.5 Proximate and Phytochemical composition of clove

Various researches have reported the nutritional value of clove through proximate and phytochemical analysis as Sulieman et al. (2007) determined the presence of (%) moisture (10±0.006), ash (5.2±0.01), crude fat (12.1±0.45), crude fibre (20±0.1), carbohydrates (51.5±0.02) and crude protein (1.2±0.02) content in clove bud powder. Bello and Jimoh (2012) also revealed the presence of (%) moisture (23.35±0.02), carbohydrates (30.95±0.17), crude fat (18.90±0.04), crude fibre (10.65±0.03), ash (9.10±0.05) and crude protein (7.00±0.01) content in clove bud and mineral composition in mg/kg as magnesium (1259.86±10.65), calcium (782.54±0.62), iron (710±12.45), potassium (2.69±0.02) and sodium (2.56±0.01) in clove seed powder. Ereifej et al. (2015) also showed the presence of (%) dry matter (83.6), ash (7.8), crude fat (4.3), crude protein (9.3), crude fibre (31.2) and carbohydrate (31) content and presence (mg/100g) of 9 minerals namely magnesium (196.8), calcium (117.5), potassium (111.6), sodium (61.6), manganese (20.9), iron (8.3), phosphorus (1.6), zinc (1.4) and copper (0.4) in cloves. Kumar et al. (2010) analysed the

phtyochemical composition of dichloromethane extract of clove bud oil which showed the presence of carbohydrates, terpenoids, glycosides, steroids, sterols, tannins and phenolic compounds.

1.6. Pharmacological Activities of Clove

1.6.1 Antibacterial activity

Antibacterial activity of clove essential oil has been reported against Staphylococcus aureus (Mishra and Sharma 2014) and Listeria monocytogenes in pasteurized milk (Cava et al. 2007). Matan (2012) reported that clove oil showed strong antimicrobial resistance against Penicillium sp., Aspergillus flavus and Staphylococcus aureus found on dried fish (Decapterus maruadsi). Zengin and Baysal (2014).

1.6.2. Antioxidant activity

High antioxidant activity shown by clove oil was due the presence of phenolic compounds like eugenol, thymol and eugenol acetate (Yadav and Bhatnagar 2007, Dai et al. 2013 and Nam and Kim 2013). Eugenol present in clove oil possessed high antioxidant activity

which was comparable with the activities of synthetic antioxidants pyrogallol and BHA (Dorman et al. 2000).

1.6.3. Antifungal activity

Several workers have reported antifungal activity of clove oil and eugenol against filamentous fungi, yeast as human pathogenic fungi (Gayoso et al. 2005) and food born fungal species (Hammer et al.1999 and Eugenia et al. 2009). Pinavaz et al. (2004) found that clove oil killed Candida albicans (C. albicans) by producing lesions in the plasma membrane due to presence of carvacrol.

1.6.4. Anti-inflammatory activity

Al-Ameedi et al. (2017) evaluated anti-inflammatory action of alcoholic Syzygium aromaticum extract (SAE) by using formalin test with twentyfour (24) mice divided into four groups. T1 and T2 groups were fed with 100 and 200 mg/kg (SAE) respectively whereas T3 group was fed with 0.3 mg/kg of meloxicam and T4 fed with distilled water. The results showed significant increase in analgesia time ($p<0.05$) and decrease ($p<0.05$) in licking number in animals exposed to various concentration of SAE.

1.6.5. Anticancer activity

Kumar et al. (2014) investigated the anticancer potential of various concentrations of water, ethanol extract and essential oil of clove in vitro through MTT and brine shrimp lethality test (BSLT) assay towards MCF-7 human breast cancer cells. In both MTT and BSLT essential oil showed cytotoxic effect with LD50 value of 37 µg/ml in BSLT after 24 hours. For MTT assay IC50 values after 24 and 48 hours were 36.43 and 17.6 µg/ml respectively.

1.7 Aims and objectives

1.7.1 Aim

The aim of this study is to identify the various classes of secondary metabolites present in Syzygium aromaticum (Clove) through phytochemical analysis (Qualitative analysis).

1.7.2 Objectives

- To extract the secondary metabolites from Syzygium aromaticum (Clove) using methanol as solvent by infusion method at room temperature.
- To calculate the percentage yield of the crude methanolic extract from Syzygium aromaticum (Clove).
- To carry out preliminary qualitative phytochemical screening to detect the presence of major classes secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, sterols, diterpene and triterpene.

CHAPTER TWO

2.0 MATERIALS AND METHODOLOGY

2.1 PLANT MATERIAL

The syzyglum aromaticum were freshly purchased at Oja Oba market Ilorin South Kwara State, Syzyglum aromaticum bud purchased from the market were of good quality, and the vendor was knowledgeable about the plant material.

2.2 APPARATUS AND GLASSWARES

Beakers, weighing balance, Burette, Measuring Cylinder, around bottom flask, Waterbath, , Conical flask, Spatula, Soxhlet extractors, heating Mantle, magnetic Stirrer, foils, Multifunctional Kitchen Blender, Test-tube, Test-tube racks, Separator Funnel, Test-tube holder, Cellulose thimble, UV/Vis Spectrophotometer, glassrood, distillation apparatus.

2.3 REAGENTS

The reagents used were of high analytical grade and include Methanol, Distilled water, Concentrated Sulphuric acid (H_2SO_4), acetic anhydride, acetic acid, Chloroform Butylated hydroxianisole, Sodium nitroprusside (Sup) ferric oxide, Sodium hydroxide, Pyridone, Wagner's reagent, Hager's reagent, Mayer' regent, Dragendroff's regent.

2.4 EXTRACTION OF PLANT MATERIAL

The dried clove bud were Pulverized using a high-powered multifunctional kitchen blender SAMSUNG (Model No:2022L) with 5000vd and 32000RP, made in Japan. The powdered clove sample was kept on a plastic container and used for the Solvent extraction.

13.44g of dried pulverized clove sample was packed into a cellulose thimble and placed in a 1L beaker, 300mL of

methanol/solvent was measured and transferred in the beaker containing the thimble.

A magnetic bar was placed at the bottom of the beaker the beaker and it's content were placed on a magnetic-stirrer temperature regulated hot-plate. The extraction was done for about 2 hour, the coloured extract solution was removed and another 200mL of fresh methanol was added, and the extraction process repeated until the sample was exhaustively extracted. All the extraction were poured together and transferred into a 1L round bottom flask, the extraction solution was distilled to remove the methanol solvent.

The concentrated extract was subsequently transferred into a beaker and placed in a water bath, heating was done until all Solvent almost completely evaporated. (Silva Go et al 2017). The beaker and it's content were left to cool at ambient temperature and until it dried. The weight of the crude extract obtained was determined from the extract field was calculated. The crude methanol extract of clove obtained was labelled as (CMESA) and kept for further analysis.

The yield was Calculated as follows:

% Extract yield = x 100

2.5 PHYTOCHEMICAL TEST OF EXTRACT

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

2.5.1 Test for Steroids:

2.5.1.1 Salkowski Test: The extract mixed with 2ml of chloroform and concentrated Sulphuric acid and on standing yield red colour.

2.5.1.2Lieberman Burchardt Test: Chloroform solution of the extract with few of drop of acetic anhydride and one ml of concentrated sulphuric acid from the sides gives reddish ring at the junction of 2 layers (Singh V et al., 2017).

2.5.2 Test for Triterpenes

2.5.2.1 Lieberman Burchardt Test: Chloroform solution of the extract with few drops of acetic acid and one ml concentrated sulphuric acid gives deep red at the junction of 2layers.

2.5.3 Test for Alkaloids

The extract was dilute with ammonia and the extracted with chloroform solution. To this dilute hydrochloric acid was added. The acid layer was used for chemical test for alkaloids.

2.5.3.1 Mayer Test (Potassium Mercuric Iodide): The acid layer with few drops trip of Mayer's reagent gives a creamy white precipitate.

2.5.3.2 Wagner's Test (Sodium of Iodide in Potassium Iodide): The extract with a few drop of Wagner's reagent gives reddish brown colored precipitate.

2.5.3.3 Hager's Test (Solution of iodine in potassium iodide): The extract with Hayer's reagent gives yellow precipitate (Silva GO, et al 2017).

2.5.3.4 Dragendoff's Test (Solution of Potassium Bismuth Iodide): The extract with a few drops of dragendoff's reagent gives a reddish brown precipitate.

2.5.4 Test for Tannins

Ferric Chloride Test: the extract with 10% ferric chloride solution gives brownish green colour (Uma KS et al 2018).

2.5.5 Test for Lactones

Legal's Test: The extract with mixture of Sodium nitroprusside and Pyridine and treated with methanol alkali gives deep red colour. (Uma KS et al 2018).

2.5.6 Test for Flavonoid

Lead acetate Test: The extract was mixed with few drops of 10% lead acetate giver a yellow precipitate (Gul R, et al., 2017)

2.5.7 Test for Diterpenes

Copper acetate Test: The extract was mixed with copper acetate solution gives green colour.

2.5.8 Test for Glycosides

Sodium hydroxide reagent: A small amount of alcoholic extract was dissolved to in one mL water and sodium hydroxide solution was added gives a yellow colour.

2.5.9 Test for Saponin

Close 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's preserves of saponin (Ezeonu, CS, et al 2016).

CHAPTER THREE

3.0 RESULT AND DISCUSSION

3.1 RESULTS

The results of the Syzygum aromaticum (clove buds) is present in the table below.

Table 3.1: Result of Phytochemical Test

Phytochemical	Result
Steroids	+
Triterpenes	+
Alkaloids	+
Tannins	+

Lactones	+
Flavonoids	+
Deterpenes	+
Glycosides	+
Saponins	+

Key: + = present - = Absent

3.1.1 Calculation of Percentage Extract Yield

The percentage crude extract yield of Syzygium aromaticum was calculated as follows:

% extract yield = x 100

Weight of crude extract = 5.25g

Weight of clove sample = 12.44g

% extract yield = x 100

% extract yield = 39%

The percentage extract yield is 39%.

3.2 DISCUSSION

The dark brown sticky paste crude extract gave an encouraging yield of 39%.

The result in (table 3.1) shows the phytochemical proving of Syzyygium aromaticum which include alkaloids, steroids, tannins, lactones, flavonoids, diterpene, glycosides, titerpene and saponin. That contribute to it's medicinal properties, (Silva GO et al., 2017).

Alkaloids are known to possess antimicrobial and analgesic properties which may support the use of Syzygium aromaticum in the treatment of infections and pain. Steroids and titerpenes suggest potential anti-inflammatory and anticancer properties, both classes of compound have been solely studied for their role in stabilizing cell membrane and interfering with tumor development. Flavonoids are

well known for their antioxidant and anti-inflammatory properties, they play a key role in protecting the body against oxidative stress and degenerative diseases such as cancer and cardiovascular, disorder. Tannis, which were detected in the extract are polyphenolic compounds with astringent properties, they have been reported to exhibit antimicrobial, antiparasitic and wound-healing activities, Glycosider, which were also detected, are important because they can act as prodrugs-biologically inactive compounds that are metabolized in the body to release active ingredients. Lactoner detected through the legal test are compounds, with known antibacterial and antifungal activities. Their present contributes to the broad-spectrum medicinal potential of the plant. Diterpenes have been associated with antimicrobial, antiviral properties. Their presence in Syzygium aromaticum indicates possible Pharmacological uses in managing infection and certain chronic diseases.

Several different plant sources for eugenol, a phenolic compound, including Cinnamon extract, clove oil and other plants. It has good health benefit making it a useful natural component. (Yang

et al., 2014). The pharmacological properties of eugenol includes antioxidant capacity, antibacterial, neuroprotective ability, hypolipidemic efficiency, anti-inflammatory action, anti-carcinogenic effects, and anti-diabetic effectiveness.

Eugenol is primarily found in aerial plants parts like flower, leaves and bark because these sections also contains many essential oils (Mohammed et al 2021).

However, this project was limited to qualitative analysis, further research involving quantitative phytochemical analysis and biological activity testing is essential to better understand and medicinal potential and safety of the plant.

CONCLUSION

The Phytochemical Analysis conducted on the various classes of Secondary metabolites of syzygium aromaticum (clove) bud, allow us to identify these bioactive compound (alkaloid, tannins, steroids, lactones, flavonoids, diterpenes, titerpenes, glycosides and saponin) providing

valuable insights into the potential health benefit of clove. Based on the information gathered, it is possible to infer that the intriguing plant known as clove is a rich sources of antioxidant and contain great medicinal value, and serve as good food preservative. They are also used as antibacterial to help increase the shelf life of food and protect it from food borne infection. Due to the antibacterial properties of clove it helps to alleviate footache.

Furthermore, clove has a distinct flavor and aroma, so it is used as spices in food and drink, chewing on clove can also assist in lowering blood pressure. Clove oil can be applied to burns, small open wounds and cuts to relieve pain, prevent infection and speed up healing.

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