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Antimicrobial Activity of Clove Plant Flower Bud Extract *(Syzygium aromaticum)* on *Escherichia coli*

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Abstract **Original Research Article**

The continuous use of antibiotics for the treatment of bacterial infections has led to the increase in bacterial resistance to these antibiotics. Many bacteria, including *E. coli* are currently multi-drug resistant, which poses a great problem to the medical and pharmaceutical industry [1]. This research was conducted to investigate the antimicrobial properties of *Syzygium aromaticum* against the above-mentioned organisms. The plant extracts were in Hot water, Cold water, and Ethanol, and the susceptibility of the organisms was tested using agar well diffusion and disk diffusion methods. The plant extracts showed antimicrobial activity on all isolates used i.e *E.coli*. From the result, the minimum inhibitory concentration (MIC) was 0.24mg/ml, and the minimum bactericidal concentration (MBC) was 3.91mg/ml. The diameter zone of Inhibition in (mm) against the plant concentration in (mg/ml) conentrations were 500mg/ml, 250mg/ml, 125mg/ml, 62.50mg/ml, 31.25mg/ml, and 15.63mg/ml. The values of the diameter zone of inhibition against the plant concentrations ranged from 16mm to 0mm while control ranged from 38mm to 10mm. The Phytochemical components determined in Cloves were Alkaloids, Saponin, Phenols, Tannins, Flavonoids, Steroids, Soluble Carbohydrate, Glycosides, Terpenes. This research showed that all the clove extracts against *E. coli* had the lowest MBC values, which proved to be the most effective and all the clove extracts against *E. coli* had the lowest MIC value of 0.24mg/ml, which proved to be the most effective of the three.

INTRODUCTION AND LITERATURE REVIEW

Background of the Study

Antimicrobial activity of a plant or its substance can be defined as its ability to kill or inhibit the growth, metabolism, and replication of a microorganism, ultimately leading to its death. The discovery of the antimicrobial effects of certain plants, chemicals, and substances (synthesized or found in nature) has been of great impact and importance in microbiology and medicine. It has led to the development of antibiotics, and alternative herbal remedies for the treatment and control of contamination and infection caused by microorganisms. Antibiotic resistance is an increasing threat to global health. (Maliehe *et al*., 2015) [2]. The development of new antimicrobial drugs is a priority to combat the increasing spread of antibiotic-resistant bacteria. Since the discovery of antibiotics, majority of new antibiotics have been isolated from actinomycetes, especially *Streptomyces spp*. however, the number of newly discovered antibiotics continues to decrease. Therefore, in this study extracts of organic solvents of a medicinal plant *Syzygium aromaticum* was evaluated for their antibacterial activities against *Escherichia coli* (Orwa *et al*., 2009)[3]. These was carried out by taking the organic extracts of both the leaf and stem parts of the plants. From day one nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Due to limited choice of antibiotics medicinal plant extracts have gained interest because of their known antimicrobial nature. Medicinal plants are the richest bioresource of drugs for traditional systems of

medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. Many spices around the world have been used for several medicinal purposes and as food preservatives, and out of those *Syzygium aromaticum* is widely used as it has got anti-inflammatory, antimicrobial, antithrombotic, antioxidant, antimutagenic, and anti-ulcerogenic properties [4].

This research is concerned with investigating the antimicrobial properties of the plant *Syzygium aromaticum* against *Escherichia coli* reducing the problem of wound infections and microbial resistance to antibiotics by proffering a cheaper and more accessible alternative to antibiotics, which, is successful, was of tremendous advantage and impact to the medical and scientific field [5].

LITERATURE RIVIEW

Antimicrobial Activity

Antimicrobial activity of a plant or a substance can be defined as its ability to kill or inhibit the growth, metabolism, and replication of a microorganism, ultimately leading to its death. The discovery of the antimicrobial effects of certain plants, chemicals, and substances (synthesized or found in nature) has been of great impact and importance in microbiology and medicine. It has led to the development of antibiotics, and alternative herbal remedies for the treatment and control of contamination and infection caused by microorganisms [6].

Escherichia coli

Escherichia coli also known as *E. coli* (Wells, 2000) [7] is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms) [8]. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination 9]. The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K 2, [10] and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship [11]. *E. coli* is expelled into the environment within faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards [12]. *E. coli* and other facultative anaerobes constitute about 0.1% of gut microbiota [13], and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination [14]. A growing body of research, though, has examined environmentally persistent *E.coli* which can survive for extended periods outside a host [15]. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favourable conditions, it takes up to 20 minutes to reproduce [16].

Scientific Classification of *Escherichia coli*

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: *E. coli*

Description of Cloves

Cloves are the aromatic dried buds of a tree (*Syzygium aromaticum*) used as a spice in virtually all the world's cuisine. The term 'Clove' is derived from the French word 'Clou' and the English word 'Clout both meaning 'nail'- from the likeliness of the flower bud of the Clove tree to a broad headed nail. The Clove tree is an evergreen tree, which grows to a height ranging from 8-12m, having large square leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds are at first of a pale color and gradually become green, after which they develop into a bright red, when they are ready for collecting. Cloves are harvested when 1.5-2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals, which form a small ball in the center [17]. The seeds should be collected from fully ripe fruits for raising seedlings. Fruits for seed collection known commonly as "mother of clove" are allowed to ripe on the tree and drop down naturally. Such fruits are collected and sown directly in the nursery or soaked in water overnight and the pericarp removed before sowing. The second method gives quicker and higher percentage of germination It is advisable to sow the seeds immediately after harvest. Heaping the fruits or keeping them tied up in air tight bags hastens the death of the seeds. Beds of 15-20 cm height, 1m width and conventional length are prepared for sowing seeds. The fertilizers must be applied in two equal split doses during the months of May-June and September-October in shallow drenches dug around the plant about 1 -11m away from the base Harvesting and processing [21]. The trees begin to flower in 6 years. Full bearing is achieved by about 20 years and the production continues for 80 years or more. Bearing between years shows much variation. Clove clusters are handpicked, when the buds reach full size and turn pink but before

they open. At this stage, they are less than 2 cm long. They are spread thinly on mats and stirred frequently for uniform drying. Well dried cloves will snap cleanly with a sharp click across the thumb nail and weigh about one third of the green weight. The opened flowers are not valued as a spice. Harvesting has to be done without damaging the branches, as it adversely affects the subsequent growth of the trees. On an average, a clove tree yields 3.5-7.0 kg/year, depending upon the age, size and condition of the tree [18].

Fig-1: Clove Plant

Scientific Classification of *Syzygium aromaticum*
Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: *S. aromaticum*

Climate and Soil

Clove trees grow well in rich loamy soils of the humid tropics and can be grown successfully in the red soils of the midlands of Kerala as well as in the hilly terrain of Western Ghats at higher elevations in Tamil Nadu and Karnataka. A cooler climate with well distributed rainfall is ideal for flowering; it thrives well in areas receiving an annual rainfall of 150-300 cm. The site selected for cultivation of clove needs good drainage, since crop cannot withstand water logging [19].

Nutrient Content of Cloves

The composition of the clove varies according to the agro climatic conditions under which it is grown, processed and stored. The dried clove bud contains carbohydrates, fixed oil, steam-volatile oil, resins, tannins, proteins, cellulose, pentosans and mineral elements. Carbohydrates comprise about two-thirds of the weight of the spice. The dried dark and flower buds also contain nutrients like proteins, minerals, vitamins, etc.21 [19].

1.6.7 Chemical Constituents

Volatile Constituents Clove yields different types of volatile oil extracted from i. leaves, ii. the stem, iii. The buds and iv. The fruit. These oils differ considerably in yield and quality. The yield and composition of the oil obtained are influenced by its origin, season, variety and quality of raw material, maturity at harvest, pre- and post-distillation treatments and method of distillation. The chief component of all the types of oil is eugenol [19]. The compound eugenol is responsible for most of the characteristic aroma of cloves Eugenol composes 72–90% of the essential oil extracted from cloves and is the compound most responsible for clove aroma [20]. 100% extraction occurs at 80 minutes in pressurized water of 125°C [23]. Ultrasound-assisted and microwave-assisted extraction methods provide more rapid extraction rates with lower energy costs [21]. Other important essential oil constituents of clove oil include acetyl eugenol, beta- caryophyllene and vanillin, crategolic acid, tannins such as bicornin [20], gallotannic acid, methyl salicylate (painkiller), the flavonoids eugenin , kaempferol, rhamnetin, and eugenitin, tri terpenoids such as oleanolic acid, and stigmasterol

Non-volatile Constituents

A few non-volatiles have been isolated from clove, which include tannins, sterols, triterpenes and flavonoids [22].

Tannins

Glycoside, galloyl and hexahydroxydiphenyl esters of 2, 4, 6- trihydroxy acetophenone- 3-ducopyranoside were isolated from clove Ieaves6. Further, two ellagitannins, namely, syzyginin A (1, 2, 3-tri-O-galloyl-4, 6-(S) - tergalloyl-p-D-glucoside) and syzyginin B, were also isolated from the leaves. Triterpenes cloves contain about 2% of the triterpene, oleanolic acid. Narayanan and Natu [23] isolated maslinic acid from clove buds. From clove, 2oc-hydroxyoleanolic acid was also isolated. Sterols isolated from clove include sitosterol, stigmasterol and campesterol [24].

Flavonoids

A chromone C-glycoside, isobliflorin (5,7-dihydroxy-2- methoxychromone-8-C-p-D-glucopyranoside) and biflorin were isolated from the ethanolic extract of cloves9. From the ethanol extract of the seeds, apigenin 6-C-[p-D-xylopyranosyl-(l-2)-(3-D galactopyranoside]-7-O-p-D-glucopyranoside and apigenin-6-C-[3- D-xylopyranosyl- (1-2)-[3-Dgalactopyranoside]-7-O-p-D-(6-0-pcoumarylglucopyranoside) were isolated [24].

1.7 CLOVE BUD OIL

Good-quality clove buds contain 15-20% essential oil [25]. The oil is dominated by Eugenol (70-85%), eugenyl acetate (15%) and p-caryophyllene (5-12%), which together make up 99% of the oil. The

constituents of the oil also include methylamylketone, methyl salicylate, a-and p-humulene, benzaldehyde, p-ylangene and chavicol. The minor connstituents like methylamylketone, methyl salicylate etc., are responsible for the characteristic pleasant odour of cloves. The clove bud and stem oils from Madagascar were also dominated by eugenol, eugenyl acetate and p-caryophyllene. The stem oil contained a higher level of eugenol, whereas the eugenyl acetate content was higher in the bud oil. The oil from clove bud contained 73.5-79.7% eugenol and 4.5- 10.7% eugenyl acetate, while the stem oil contained 76.4-84.8% eugenol and 1.5-8.0% eugenyl acetate. Both contained 7.3-12.4% p-caryophyllene and 1.0-1.4% a-humulene. Pino identified 36 compounds from the volatile oil of clove buds. Clove buds from India contained 12.9-18.5% oil, of which 44-55% was eugenol, whereas the pedicels contained 3.0-7.7% oil with 60.0-72.4% eugenol [22]. Leaf Oil Clove leaves yield 3.0-4.8% essential oil. The essential oil content during the different stages of leaf growth revealed that the eugenol content in the leaves increased from 38.3 to 95.2% with maturity, while the contents of eugenyl acetate (51.2 to 1.5%) and caryophyllene (6.3 to 0.2%) decreased 4. Clove bud and leaf oil contain various classes of compounds, e.g. monoterpenes, sesquiterpenes, aldehydes and ketones. Clove Stem Oil clove stem yields 6% volatile oil. The oil is a pale to light yellow liquid containing 80.2% eugenol and 6.6% P-caryophyllene, besides several minor components. Fruit Oil Ripe fruits yield 2% of oil, which is comprised of 50-55% eugenol [22].

Medical Uses

Clove is known to possess antibacterial properties and is used in various dental creams, tooth pastes, mouth washes, and throat sprays to cleanse bacteria. It is also used to relive pain from sorghums and improves overall dental health. In dentistry, eugenol in combination with zinc oxide is used for temporary filling of cavities. Clove is an anodyne (an agent that soothes or relives pain) for dental emergencies. Cloves are aphrodisiac (an agent for arousing or increasing sexual desire or potency). Clove is used as an anti-inflammatory agent, due to its high content of flavonoids. Aroma therapists use pure clove oil to cure the symptoms of rheumatism and arthritis [32]. Clove is used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. Apply the paste of clove powder in honey to treat acne. Paste of clove powder in water promotes faster healing of cuts and bites. Cloves can effectively cure many digestive problems. It is having medicinal qualities to cure flatulence, loose motions, indigestion and nausea [33]. Cloves are useful in relieving the symptoms of diarrhoea, gastric irritability and vomiting. Clove and promote the discharge of mucous and secretions in the respiratory passage. The aromatic clove oil, when inhaled can help soothe certain respiratory conditions like cold, cough, asthma, bronchitis, and sinusitis. It also helps in clearing the nasal tract. Cloves can effectively prevent the lung cancer as well as the skin cancer. Eugenol helps in minimizing the harmful effects of environmental wastes that can cause cancer of digestive system. Clove oil stimulates blood flow and circulation making it useful for the people having cold extremities.

Cloves benefit the diabetic patients by controlling the blood glucose levels. Eugenol is powerful enough for preventing blood clots. Sucking of a clove bud reduces desire for alcohol. Muscular cramps are often relieved, when the oil of clove is applied as a poultice near the affected area. Cloves also help prevent the breakdown in retina of the eye, which slows down macular degeneration and aids vision in the old age. The underlying mechanism is through the prevention of the breakdown of docosahexaenoic acid, which preserves vision in elderly people [34]. Researchers found that sniffing the spicy aroma of cloves reduces drowsiness, irritability and headaches. One drop of clove oil applied to the roof of the mouth can instantly relieve many headaches. Clove enhances memory retention. It is recommended for relieving brain fog, lethargy and depressive state of mind. Research has shown that clove oil is an effective mosquito repellent. Clove may be looked upon as the champion of all the antioxidants known till date. The Oxygen Radical Absorption Capacity test (ORAC) is a scale developed by U.S. Department of Agriculture for comparing antioxidant activity. The ORAC score, of clove is over 10 million. A drop of clove oil is 400 times more powerful as an antioxidant than wolf berries or blueberries.

1.7.2 Traditional Medicinal Uses

Cloves are used in India Ayurveda medicine, Chinese medicine and western herbalism where the essential oil is used as an anodyne (pain killer) for dental emergencies. Cloves are used as carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. Clove are said to be natural anthelmintic [35] aid to warm the digestive tract. Applied to a cavity in a decayed tooth, it also relief toothache [36]. In Chinese medicine, cloves or ding xiang are considered acrid. In addition, clove oil is used in preparation of some toothpastes and clovacaine solution, which is a local anaesthetic used in oral ulceration and inflammation. Clove oil is mixed with zinc oxide to form a temporary tooth cavity filling [37].

EFFICACY AGAINST MICROORGANISMS

Bacteria and fungi

Initial screening of potential antibacterial and antifungal compounds from plants may be performed

with pure substances or crude extracts. The methods used for the two types of organisms are similar. The two most commonly used screens to determine antimicrobial susceptibility are the broth dilution assay and the disc or agar well diffusion assay. Clinical microbiologists are very familiar with these assays. Adaptations such as the agar overlay method may also be used. In some cases, the inoculated plates or tubes are exposed to UV light to screen for the presence of light-sensitizing photo chemicals. Other variations of these methods are also used. For instance, to test the effects of extracts on invasive *Shigella* species, noncytotoxic concentrations of the extracts can be added to Vero cell cultures exposed to a *Shigella* inoculum. The decrease in cytopathic effect in the presence of the plant extract is then measured [39].

In addition to these assays, antifungal phytochemicals can be analysed by a spore germination assay. Samples of plant extracts or pure compounds can be added to fungal spores collected from solid cultures, placed on glass slides, and incubated at an appropriate temperature (usually 25°C) for 24 h. Slides are then fixed in lacto phenol-cotton blue and observed microscopically for spore germination. After initial screening of phytochemicals, more detailed studies of their antibiotic effects should be conducted. At this stage, more specific media can be used and MICs can be effectively compared to those of a wider range of currently used antibiotics.

1.7.4 Bacterial Resistance to Antibiotics

Antibiotic resistance is defined as the ability of bacteria (and other microorganisms) to resist (not be affected by) the effects of an antibiotic to which they were once sensitive. The three fundamental mechanisms of antimicrobial resistance are:

1. Enzymatic degradation of antibacterial drugs,
2. Alteration of bacterial proteins that are antimicrobial targets, and
3. Changes in membrane permeability to antibiotics.

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives [40]. Many decades after the first patients were treated with antibiotics; bacterial infections have again become a threat [41]. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [42]. The Centre for Disease Control and Prevention (CDC) has classified a number of bacteria as presenting urgent, serious, and concerning threats, many of which are already responsible for placing a substantial clinical and financial burden on the health care system, patients, and their families. The management of microbial infections in ancient Egypt, Greece, and China is well-documented [43]. The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928. Since then, antibiotics have transformed modern medicine and saved millions of lives [44]. Antibiotics were first prescribed to treat serious infections in the 1940s. Penicillin was successful in controlling bacterial infections among World War II soldiers. However, shortly thereafter, penicillin resistance became a substantial clinical problem, so that, by the 1950s, many of the advances of the prior decade were threatened. In response, new beta-lactam antibiotics were discovered, developed, and deployed, restoring confidence. Unfortunately, resistance has eventually been seen to nearly all antibiotics that have been developed.

1.7.5 Antibiotic Resistance of *Escherichia coli*

E. coli is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer. The most problematic mechanisms in *E. coli* correspond to the acquisition of genes coding for extended-spectrum β-lactamases (conferring resistance to broad-spectrum cephalosporin's), carbapenemases (conferring resistance to carbapenems), rRNA methylases (conferring pan-resistance to aminoglycosides), plasmid-mediated quinolone resistance (PMQR) genes (conferring resistance to fluoroquinolones), and *mcr* genes (conferring resistance to polymyxins). Although the spread of carbapenemase genes has been mainly recognized in the human sector but poorly recognized in animals, colistin resistance in *E. coli* seems rather to be related to the use of colistin in veterinary medicine on a global scale. For the other resistance traits, their cross-transfer between the human and animal sectors still remains controversial even though genomic investigations indicate that extended-spectrum β-lactamase producers encountered in animals are distinct from those affecting humans [45].

Genetic Basis of Antimicrobial Resistance

Bacteria have a remarkable genetic plasticity that allows them to respond to a wide array of environmental threats, including the presence of antibiotic molecules that may jeopardize their existence. As mentioned, bacteria sharing the same ecological niche with antimicrobial-producing organisms have evolved ancient mechanisms to withstand the effect of the harmful antibiotic molecule and, consequently, their intrinsic resistance permits them to thrive in its presence [46].

METHODS

Sample collection of clove plant

The plant material cloves was collected from Maraba Central market Karu, Nasarawa state by observing its physical characters like shape, smell, odour and colour of the plant. Sample collection of isolates: the pure isolate of *Escherichia coli* was gotten in Bingham university, Karu Mueller Hilton agar for *E.coli* and inoculated using streaking method plate. The plates were incubated at 37^{o}C to obtain a viable culture of the susceptibilities of the test organism.

Preparation of Plant Extracts

Crushing; 20g of fresh leaves was weighed and put in a blender. 200ml of water was added and the mixture was blended to crush the leaves. The extract was filtered using Whatman's no. 1 filter and stored in an air tight container in the refrigerator. Cold Water Extract: 20g of dried, crushed leaves was weighed and soaked in 200ml of distilled water for about three hours and about thirty minutes with constant stirring and shaking. The extract was stored in an air tight container in the refrigerator until required for use. Hot Water Extract; the plant leaves were washed in distilled water and air dried for about five hours. 20g of the leaves were boiled in 200ml of distilled water for about thirty minutes with constant stirring and shaking. The therapy was allowed to cool to room temperature, and stored in a sterile, air tight container. Ethanolic Extract ; Dried flower buds of *Syzygium aromaticum* were extracted three times with 70% ethanol at room temperature overnight. The extracts were combined and concentrated using a rotary evaporator, and freeze-dried under a vacuum and dissolved in dimethyl sulfoxide (DMSO).

SAMPLE PROCESSING AND CULTURE

Culture Media Preparation

The media was weighed according to manufacturer's instruction, and mixed in distilled water. It was stirred and mixed on a Vortex Mixer. It was then autoclaved at 121^{o}Cfor 15 minutes. The media was allowed to cool to about 45-50^{o}C, and poured into petri dishes. The media was allowed to solidify, and stored face-down in the refrigerator until required for use. Preparation of nutrient agar; 28grams of nutrient agar powder was weighed using a weighing balance and transferred into a conical flask containing 1000mls of distilled water. This was then autoclaved at 121^{o}C for 15mins..Preparation of Mueller Hilton agar; this was carried out in accordance with the manufacturer's instruction. 37g of Mueller Hilton agar powder was dissolved in 1000mls of distilled water in a conical flask. It will now be autoclaved at temperature of 121^{o}C for 15mins. The flask was be removed and allowed to cool. 15mls was dispensed in to sterile Petri dishes and allow solidifying.

Preparation of McFarland Turbidity Standard; Barium sulphate suspension at 1.0% w/v was prepared as follows: One percent (1% w/v) solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 in 99mls of water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of barium chloride in 50mls of distilled water. Barium chloride solution (0.6mls) was added to 99.4mls of sulphuric acid solution to yield 1.0% w/v barium suspension. The turbid solution formed was transferred into a test tube as the standard for comparison. Standardisation of Bacterial Inoculum; For inoculum preparation, the density of the isolated bacterial cultures was adjusted equal to that of 0.5 McFarland standard (1.5 x 108 CFU/ml) by adding sterile distilled water. McFarland standard was used as a reference to adjust the turbidity of microbial suspension so that number of microorganisms was within a given range (Abdulsarsheed *et al.,* 2018).

2.5 Qualitative phytochemical analysis

The extract of the plant is to be screened for the presence of various secondary metabolites (phytochemical) such as Flavonoids, Alkaloids, tannins, saponins, glycosides, Phenols, Soluble Carbohydrate, Steroids and Terpenes.

2.5.1 Test for Alkaloids

Two mL of extract was taken in a test tube and then 0.2 mL dilute HCl was included, followed by 1 mL of Meyer's reagent. A yellowish coloration indicates alkaloid's presence (Rao *et al.,* 2014).

2.5.2 Test for Saponins

A 10mls filtrate is going to be mixed with 5mls distilled water and shacked vigorously for a stable persistent growth. Three (3) drops of olive oil is to be added and shaken vigorously, then observed (Cappuccino and Sherman 2014).

2.5.3 Test for phenols

A small amount of the ethanolic extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl3) was added. A blue, green, red or purple color is a positive test (Rao *et al.,* 2014).

2.5.4 Test for Tannins

The aqueous extract was boiled in a water bath in the sterile test tube and 0.1 % of Ferric chloride was added and observed (Cappuccino and Sherman 2014).

2.5.5 Test for Flavonoids

5mls of diluted ammonia was added to a portion of the aqueous filtrate of each plant Extract by

the addition of concentrated H_2SO_4 was added and observed (Cappuccino and Sherman 2014).

2.5.6 Test for Steroids

About 100 mg of dried extract of sample was dissolved in 2 ml of CHCl3. H2SO4 was added carefully to form a lower layer. A reddish brown colour at the interface was an indicative of steroidal ring (Rao *et al.,* 2014).

2.5.7 Test for soluble carbohydrate

To about 2mls of extract few drops of Molish reagent was added. Then about 1ml of concentrated sulfuric acid was added along the side of the test tube. Appearance of reddish violet ring at the junction of the two layers indicates the present of carbohydrates (Harborne *et al.,* 1998).

2.5.8 Test for Glycosides

0.2gs of the extract was weighed and 2mls of sulphuric acid is to be added and boiled for 15mins after which 1ml of Fehling solution is added to aqueous 5% ferric chloride and observed (Cappuccino and Sherman 2014).

2.5.9 Test for Terpenes

5mls of solution of the extract was added in a test tube. Then 2mls chloroform and 3mls concentrated sulphuric acid solution was added, and observed for possible results (Clark *et al.,* 2009).

2.6 ANTIMICROBIAL DETERMINATION

2.6.1 Determination of Antibacterial Activity

Antibacterial activity was tested on Mueller Hinton Agar (MHA). The antibacterial activity of three extracts of *Syzygium aromaticum* on wound isolates were evaluated by using agar well diffusion method

2.6.2 Agar Well Diffusion Method

Agar plates were inoculated with standardized inoculum (1.5 x108 CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Five wells of 8 mm size were made with sterile borer into the agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. 0.04ml of each of the plant extracts were poured into a well of inoculated plates. Sterilized distilled water was used as a negative control which was introduced into a well instead of *Syzygium aromaticum* plant extract. Ciprofloxacin was used as a control. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar. After incubation for 24 hrs at 37^{o}C, the plates were observed. Antibacterial activity was present on the plates, indicated by an inhibition zone surrounding the well containing the plant extract. The Diameter of Inhibition Zone (DIZ) was measured and expressed in millimetres. The mean values of the diameter of inhibition zones were calculated (Jorgensen *et al.,* 2007).s

2.6.3 Determination of the Minimum Inhibitory Concentration (MIC).

Since the *Syzygium aromaticum* extracts exhibited antimicrobial activity against the pathogenic isolates, it was further assayed for its minimum inhibitory concentration (MIC). This was carried out by the two-fold serial dilution of the tested extracts in Mueller Hilton broth (2 ml volume), then inoculated with 100µl inoculum size with the test organisms. The aqueous extracts of the plant were prepared at concentrations of 500,250,125,62.5,31.25,15.63,7.71,3.91,1.95,0.98,0.49, and 0.24(mg/ml). The MIC was determined by the broth dilution method. Mueller Hilton broth samples (10 ml) were inoculated with different concentrations of the plant extracts and with active inoculum of bacterial isolates (1.5 x 108 CFU/ml) in tubes and incubated for 24 hrs at 37^{o}C. The MIC was determined as the lowest concentration of the extract which inhibited the organisms.

CHAPTER THREE

RESULTS

3.1 Minimum Inhibitory Concentration

The *Syzygium aromaticum* showed antimicrobial activity against *E.coli* hence the plant extract was assayed for its minimum inhibitory concentration by conducting a fourfold serial dilution of the extract and inoculating them with the test organisms. The minimum inhibitory concentration of the plant extracts were observed and recorded, as shown in table 3.

3 Minimum Bactericidal Concentration

Minimum bactericidal concentration (MBC) was specified as the least clove concentration resulting in removal of bacterial growth. Inocula were collected from the suppressive zones of MIC concentration and the two other sequential concentrations and plated onto Mueller-Hinton agar plates. The minimum Bactericidal concentration of the plant extracts were observed and recorded, as shown in table 4.

3.3 Diameter of Inhibition Zones (DIZ) of antibacterial activity of clove pant extract against *E.coli* using the Agar Well Diffusion Method.

The selected organisms were tested for susceptibility to the plant extracts, and the results were observed and recorded. The plant extracts exhibited strong antimicrobial activity against *E.coli* but showed weak activity against as shown in table two (2).

3.4 Results of Phytochemical Analysis of *Syzygium aromaticum* Extracts

Phytochemical analysis was conducted on the various aqueous *Syzygium aromaticum* extracts, and the following results were obtained and recorded. The plants extract showed the presence of flavonoids, tannins, alkaloids, and terpenoids. Glycosides were absent in all plant extracts, as shown in table one (1).

Table-1: Results of Phytochemical Analysis of *Syzygium aromaticum* (Clove Plant) extracts.

Phytochemical	Hot Extracts	Cold Extracts	Ethanol Extracts
Flavonoids	-	-	+
Tannins	-	-	-
Alkaloids	+	+	-
Glycosides	-	-	-
Terpenes	-	-	+
Saponins	+	+	-
Steroids	-	-	-
Soluble Carbohydrate	+	+	-
Phenols	+	+	+

Keys: + = Present
— = Absent

As shown in Table 1 above, the Hot water extracts of *Syzygium aromaticum* showed the absence of Flavonoids, Tannins, Steroids, Glycoside and Terpenes, but the Presence of Alkaloids, Saponins, Soluble Carbohydrate and Phenols. The Cold water extracts showed the presence of Saponins, Alkaloids, Terpens, Soluble Carbohydrate and Phenols, but Glycosides, Flavonoides, Tanins, Steroids were absent. The Ethanolic extracts contained Flavonoids, Phenols, and Terpenes, but showed the absence of Glycosides, Soluble Carbohydrate, Saponins, Tanins, Alkaloids, and Steroids

Table-2: Antibacterial activity of clove plant against *escherichia coli*

Isolates	Extracts	Diameter zone of inhibition (mm)					
		PLANTS CONCENTRATIONS (Mg/mL)					
		500	250	125	62.50	31.25	15.63
Escherichia coli	Hot water	11	9	7	5	3	0
	Cold water	27	22	20	17	13	9
	Ethanol	28	25	21	18	14	8
	Positive control	35	32	28	23	19	13

The results as shown in Table 2 above, gives the values of the Diameter of Inhibition Zones of the *Syzygium aromaticum* extracts on *E.coli* using the well diffusion method. The Ethanol, Hot and Cold-water extracts of S. *aromaticum* extracts showed positive controls DIZ values respectively. The control, Chloramphenicol, also showed antimicrobial activity against all isolates *E. coli*

Table-3: Minimum inhibitory concentration (mic) of clove plant IN mg/ml.

EXTRACTS	*Escherichia coli*
Hot water	0.24
Cold water	0.24
Ethanol	0.24
Positive control (Ciprofloxacin)	0.24
Negative control (DMSO)	-

The results observed in Table 3 above show the MIC values of the extracts which showed antimicrobial activity. The activities recorded were that of the *Syzygium aromaticum* extracts (hot, cold, and ethanol) against *all isolates*. The MIC value of the hot water extract of the plant against was 62.5 mg/ml, that of the cold water extract was 31.25mg/ml, and that of the ethanol plant extract was 15.63mg/ml. For *E.coli* all the given extracts showed the same values at 0.24mg/ml.

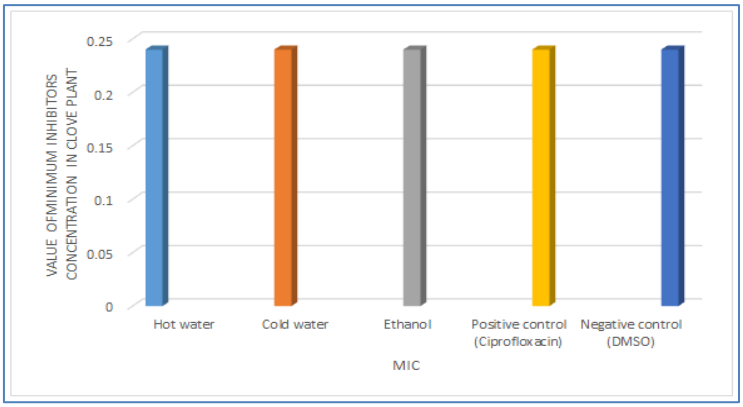

Chart Showing Minimum Inhibitory Concentrations of Clove plant (*Syzygium aromaticum*) against *Escherichia coli*.

Table-4: Minimum bactericidal concentration (mbc) of clove plant in mg/mL.

EXTRACTS	*Escherichia coli*
Hot water	250
Cold water	250
Ethanol	250
Positive control (Ciproflaxacin)	3.91
Negative control (DMSO)	-

The results observed in Table 4 above show the MBC values of the extracts which showed antimicrobial activity. The activities recorded were that of the *Syzygium aromaticum* extracts (hot, cold, and ethanol) against *all isolates*. For *E.coli* all the values were present. This shows that all the extracts of *E.coli* had the lowest MBC values, which proved to be the most effective.

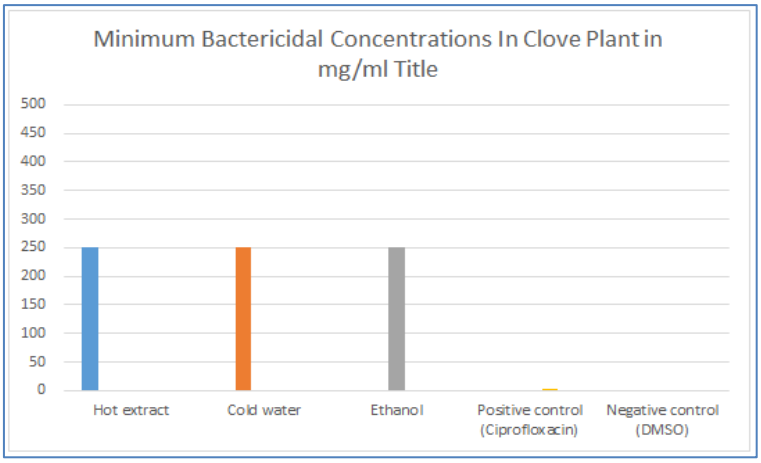

Chart Showing Minimum Bactericidal Concentrations of Clove plant (*Syzygium aromaticum*) against *Escherichia coli*.

DISCUSSION

The plant extracts were analysed to determine their phytochemical contents, and the results are shown in Table 1. The Hot extracts of *Syzygium aromaticum*

showed the presence of Alkaloids, Soluble Carbohydrate, Phenols and Saponins, but the absence of Glycosides, Terpenes, Steroids, Tannins and Flavonoids. The Cold extracts showed the presence of Alkaloids, Saponins, Phenols and Terpenes, but Glycosides, steroids, flavonoids, tannins, were absent. The Ethanol extracts contained Flavonoids, Phenols, and Terpenes, but showed the absence of soluble carbohydrate, steroids, Glycoside, Saponin, Tannins and Alkaloids. (Cappucino *et al.*, 2014).

The results in Table 2 show the values of the Diameter if Inhibition Zone (DIZ) which indicated antimicrobial activity of the *Syzygium aromaticum* extracts on the selected microorganisms, using the agar well diffusion method. It is shown that the activity of the plant extracts is concentration dependent, as the DIZs recorded are at high concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.50, 31.25 and 15.63mg/ml.

The results observed in table 3 show the MIC values of the extracts which showed antimicrobial activity. All activities of *Syzygium aromaticum* extracts (hot, cold and ethanol) were recorded against *all the isolates*, and the MIC values were determined as the lowest concentration of the plant extract that showed inhibition against the microorganisms. For *E. coli* all the given extracts showed the same values at 0.24mg/ml.

All the extracts of *E. coli* had the lowest MIC value of 0.24mg/ml, which proved to be the most effective of the three. (Clark *et al.*, 2009). *Syzygium aromaticum* has been by this research, seen to have low antimicrobial activity on *E. coli*. The results observed in table 3 show the MBC values of the extracts which showed antimicrobial activity. All activities of *Syzygium aromaticum* extracts (hot, cold and ethanol) were recorded against *all the isolates*, and the MBC values were determined as the Minimum Bactericidal concentration of the plant extract that showed inhibition against the microorganisms. For *E.coli* all the values were present. This shows that all the extracts of *E.coli* had the lowest MBC values, which proved to be the most effective.

CONCLUSION

Syzygium aromaticum has been used for a long time traditionally for the remedy of various illnesses and infections, and has been assumed to replace antibiotics in the treatment of ailments. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases for example alkaloid protect against chronic diseases, Saponin protect against hypercholesterolemia and other antibiotic properties. Phytochemical screening of clove extract used to study the presence of flavonoids, tannin, Saponin, glycoside, phenol, and terpenoids have various medicinal values such as anti-inflammatory, antidiabetic, and analgesic activities and for central nervous system activity. From the aim of this research, Clove is expected to be active against *Escherichia coli* also resistant to the antibiotic. Phytochemical components determined are Alkaloids, Terpenes, Steroids, Soluble Carbohydrate, Flavonoids, Saponin, Phenols, Tannins, Glycoside. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

RECOMMENDATION

1. We recommend that, cloves should be used at times instead of consumption of a lot of antibiotic drugs which may lead to resistance if taken excessively. The fact that the extract of these medicinal plants inhibited some medically important bacteria proves that the plant might have some potential as an alternative source of antibacterial substances.
2. The public should be enlightened on the use of herbs for the treatment of ailments and infections. The use of plants should not be indiscriminate, or based on rumours.

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