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Antimicrobial Activity of Clove Plant Flower Bud Extract *(Syzygium aromaticum)* on *Salmonella typhi*

Elisha E[1], Ajobiewe HF[1*], Ibrahim AE[1], Alau KK[2], Umeji LC[3], Salami AO[3], Udefuna PA[3], Yashim AN[4], Ajobiewe JO[5]

[1]Biological Sciences Department Bingham University Karu Nasarawa State Nigeria
[2]Society for Family Health Gimbiya Street Garki Area 11 Abuja Nigeria
[3]United State Department of Defense, Walter Reed Program Nigeria, and US Embassy Abuja Nigeria
[4]Department of Basic Medical Sciences Baze University Abuja Nigeria
[5]National Open University Dutse Study Center Abuja Nigeria

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***Corresponding author:** Ajobiewe HF
Biological Sciences Department Bingham University Karu Nasarawa State Nigeria

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 *Salmonella typhi* 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 *Salmonella typhi*. 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) concentrations 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, Saponins, Phenols, Tannins, Flavonoids, Steroids, Soluble Carbohydrate, Glycosides, and Terpenes.

Keywords: Salmonella typhi, Alkaloids, Saponins, Phenols, Hot water, Cold water, and Ethanol *Syzygium aromaticu*.

STUDY BACKGROUND

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 [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 *Salmonella typhi* [2]. These were 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 bio resource 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, ant mutagenic, and anti-ulcerogenic properties [4].

This research is concerned with investigating the antimicrobial properties of the plant *Syzygium aromaticum* against *Salmonella typhi* and 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].

Salmonella typhi

The bacterium *Salmonella typhi* causes typhoid fever [7]. The bacterium is a gram-negative, motile, non-sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits and vegetables or via convalescent or chronic carriers [7]. It has also been linked with zoonotic transmission via reptiles and common domestic pets. Enteric fever (typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate [9]. In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics and a rapid rise in multidrug resistance. Resistance to the first line drugs chloramphenicol, ciprofloxacin and amoxicillin has been reported [10]. With the increase in resistance to ant typhoid drugs, medicinal plants have gained popularity among both urban and rural dwellers in the treatment of the ailment. Moreover, 80% of the world population are rural dwellers and rely on medicinal plants for their daily medications, and plants have been reported to have minimal or no side effects compared to antibiotics [6]. Clove plant is a plant specially introduced from Asia (exotic) and commonly cultivated in fuel plantations and elsewhere in and around towns and villages. In Burkina Faso, a decoction of the leaves with lemon juice is used for the treatment of fevers (Fern, 2019). In northern Nigeria, the tree is very popular for its local usage in the treatment of typhoid fever. The study was therefore carried out to investigate the antimicrobial property of the leaf extracts with the view to provide scientific evidence for its application as a medicinal plant.

Scientific Classification

Domain: Bacteria
Phylum: Proteobacteria
Class: Gamma proteobacteria
Order: Enterobacter ales
Family: Enterobacteriaceae
Genus: *Salmonella. Spp.*

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 colour 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 centre [11]. 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 [12].

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 [13].

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 [13].

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 [13]. 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 [14] 100% extraction occurs at 80 minutes in pressurized water of 125°C [24]. Ultrasound-assisted and microwave-assisted extraction methods provide more rapid extraction rates with lower energy costs [15]. Other important essential oil constituents of clove oil include acetyl eugenol, beta- caryophyllene and vanillin, crategolic acid, tannins such as bicornin [14], 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 [16].

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 Triterpenes, oleanolic acid. Narayanan and Natu (1974) isolated maslinic acid from clove buds. From clove, 2oc-hydroxyoleanolic acid was also isolated. Sterols isolated from clove include sitosterol, stigmasterol and campesterol [17].

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-xylopyranosy l-(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 [17].

Clove Bud Oil

Good-quality clove buds contain 15-20% essential oil n [16]. 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 [16].

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 [18]. 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 [4]. 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 (Pandey, and Singh 2009). 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.

Traditional Medicinal Uses

Cloves are used in India Ayurvedic 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 anthelminitic [4] aid to warm the digestive tract. Applied to a cavity in a decayed tooth, it also relief toothache [11]. 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 [19].

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 [20].

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.

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 [21]. Many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat [22]. 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 [22]. 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 [1]. 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 [24]. 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.

Antibiotic Resistance of *Salmonella typhi*

Salmonella typhi, the causative agent of typhoid fever, is a gram-negative, motile, rod shaped, facultative anaerobe. It is solely a human pathogen and there is no animal reservoir. Antibiotic therapy is the mainstay for the treatment of typhoid fever and the complications associated with it. The drugs of choice are chloramphenicol, ampicillin, trimethoprim-sulphamethoxazole, quinolones and cephalosporins. Resistance of *S. typhi* against chloramphenicol was first reported in England in 1950. Several workers have reported occurrence of multidrug resistant *S. typhi* strains in recent years around the world and in different parts of Nigeria Mechanisms of antibiotic resistance in *S. typhi* include inactivation of drug, alteration of the target site, and active efflux. These mechanisms could either be chromosomal or plasmid mediated. Plasmids of incompatibility group HI1 and Care important vectors of antibiotic resistance in some strains of *S. typhi*. The chromosomal-mediated drug resistance phenomenon against fluoroquinolones has been reported recently and attributed to a single point mutation in the quinolone resistance determining region (QRDR) of the topoisomerase gene gyrA, which encodes DNA gyrase. It has been opined that the initial development of resistance by *S. typhi* and most other bacterial pathogens occurred as a result of human practices such as over prescription and indiscriminate use of antibiotics as well as inappropriate use in animals [25].

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 [23].

METHODS

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. The pure isolate of *Salmonella typhi* was gotten in Bingham University, Karu and sub cultured using nutrient agar for *Salmonella typhi*. The plates were labelled respectively and then incubated at 37^{o}C to obtain a viable culture of the susceptibilities of the test organism. In the preparation of plant extract 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 What man's no. 1 filter and stored in an air tight container in the refrigerator. In the 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. In the 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. For the 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). Samples were then processed for culture and sensitivity testing using clove as the antimicrobial agent 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.

RESULTS

Minimum Inhibitory Concentration

The *Syzygium aromaticum* showed antimicrobial activity against *Salmonella typhi* 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.

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.

Diameter of Inhibition Zones (DIZ) of antibacterial activity of clove pant extract against *Salmonella typhi* 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 *Salmonella typhi*.

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, Saponin, 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 *salmonella typhi*.

Isolates	Extracts	Diameter zone of inhibition (mm)					
		Plants concentrations (mg/ml)					
		500	250	125	62.50	31.25	15.63
Salmonella typhi	Hot water	13	10	9	6	5	0
	Cold water	21	19	15	10	8	5
	Ethanol	25	23	18	13	12	3
	Positive control	36	33	29	23	18	10

The results as shown in Table 2 above, gives the values of the Diameter of Inhibition Zones of the *Syzygium aromaticum* extracts on *S.typhi* 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 *Salmonella typhi* respectively.

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

Extracts	*Salmonella typhi*
Hot water	125
Cold water	62.5
Ethanol	15.63
Positive control (Ciprofloxacin)	15.63
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*. *S.typhi*, hot water extract was 125mg/ml, that of cold water was 62.5mg/ml and for ethanol extract was 15.63mg/ml. This shows that higher concentrations of the plant extracts are needed to show antimicrobial activity *S.typhi*.

RESULTS

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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.

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	Ethanol	25	23	18	13	12	3
	Positive control	36	33	29	23	18	10

The results as shown in Table 2 above, gives the values of the Diameter of Inhibition Zones of the *Syzygium aromaticum* extracts on *S.typhi* 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 *Salmonella typhi* respectively.

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

Extracts	*Salmonella typhi*
Hot water	125
Cold water	62.5
Ethanol	15.63
Positive control (Ciprofloxacin)	15.63
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*. *S.typhi*, hot water extract was 125mg/ml, that of cold water was 62.5mg/ml and for ethanol extract was 15.63mg/ml.

This shows that higher concentrations of the plant extracts are needed to show antimicrobial activity *S.typhi*.

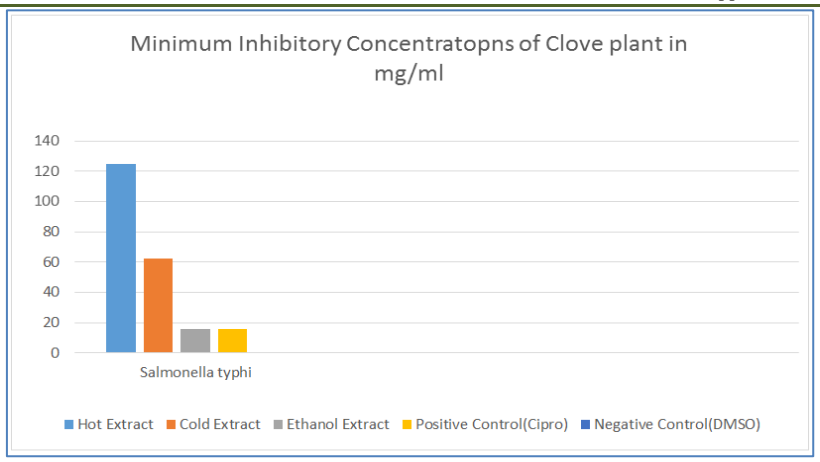

Chart Showing Minimum Inhibitory Concentrations of Clove plant (*Syzygium aromaticum*) against *Salmonella typhi*.

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

Extracts	***Salmonella typhi***
Hot water	-
Cold water	500
Ethanol	250
Positive control (Ciprofloxacin)	7.71
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*. The MBC value of the extracts only showed on cold water, ethanol and Positive control against *S.typhi*.

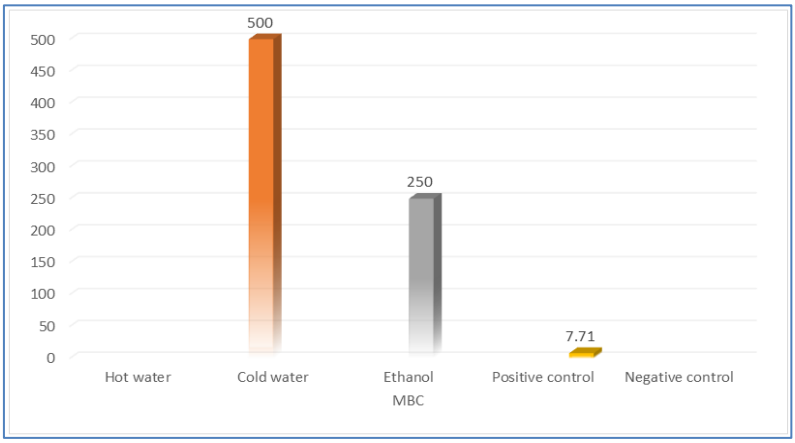

Chart Showing Minimum Bactericidal Concentrations of Clove plant (*Syzygium aromaticum*) against *Salmonella typhi*.

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, Saponins, 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. The ethanol extract of the plant proved to be the most effective of the three plant extracts.

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. *S. typhi*, hot water extract was 125mg/ml, that of cold water was 62.5mg/ml and for ethanol extract was 15.63mg/ml. This shows that higher concentrations of the plant extracts are needed to show antimicrobial activity for *S. typhi*.

Syzygium aromaticum has been by this research, seen to have low antimicrobial activity on and no activity on *S. typhi*, which are known as multi-drug resistant organisms. 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. The MBC value of the extracts only showed on cold, ethanol and positive control of the plant against *S.typhi*.

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, Saponins 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, antidiuretic, and analgesic activities and for central nervous system activity. From the aim of this research, Clove is expected to be active against *Salmonella typhi* and also resistant to the antibiotic. Phytochemical components determined are Alkaloids, Terpenes, Steroids, Soluble Carbohydrate, Flavonoids, Saponins, 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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