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## Phytochemical Screening and Antimicrobial Sensitivity of Clove Flower (*Syzygium aromaticum*, L. Merrill and Perry) Bud on Dental Pathogens

			
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### ABSTRACT

Phytochemical screening and antimicrobial sensitivity of clove flower (*Syzygium aromaticum*) bud on dental pathogens was studied. Extract of Cloves flower (*S. aromaticum*) showed very strong activity against all the tested microbial isolates at various concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. The tested microbial isolates were *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Candida albicans*, *Rhizopus oryzae*. All the isolates were sensitive to the aqueous extract of Cloves flower (*S. aromaticum*). The highest sensitivity of 21.33 mm was observed in *S. epidermidis* at 100mg/ml while *A. flavus* showed the lowest sensitivity of 7.33 mm at 6.25mg/ml. Minimum Inhibitory Concentration (MIC) of 6.25 mg/ml was observed for all isolates while Minimum Bactericidal Concentration (MBC) of 12.5 mg/ml was observed for *S. epidermidis* and *Escherichia coli*, while MBC of 25 and 50 mg/ml were observed for *Proteus mirabilis* and *K. pneumoniae*. The Minimum Fungicidal Concentration (MFC) of 12.25 mg/ml was observed for all fungi isolates used. Various phytochemicals tested were present in *S. aromaticum* these include saponins, tannins, phenols, cardiac glycoside, flavonoids, alkaloids and anthracene. The bacteria isolates were resistant to few of the conventional antibiotics used including augmentin (30mcg), ceporex (10mcg) and ampicillin (30mcg). *K. pneumoniae* showed total resistance to Augmentin, Ceporex and Ampicillin. The results of this study showed that the extract of *S. aromaticum* has antimicrobial effect on Gram positive, Gram negative bacteria and fungi. The presence of phytochemical in the extracts may have been responsible for the antimicrobial activity exhibited by the plant extract.

## INTRODUCTION

**Aim:** Possible development and assessment of aqueous extract from the plant for various beneficial health as the functional effects in animal and human species are increasing in popularity.

**Review of literature:** Dental caries is a chronic disease of multifactor etiology and pathogens. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by *Streptococcus mutans*, a cariogenic bacterium [1]. Dental caries are one of the public health concerns for several reasons. Teeth affected with dental caries are sources of infection, which can cause an inflammation of dental pulp, periodontium and gums. If left untreated, this disease gradually leads to teeth loss, which causes chewing difficulties and aesthetic problems [2]. It remains one of the most widespread diseases of the mankind. In developing countries, dental caries is often at epidemic proportions, especially among the poor. Since the 19<sup>th</sup> century, when sucrose became a daily used sweetener by many people worldwide, the increasing prevalence of dental caries had also been noticed [3]. Despite a low mortality rate associated with dental diseases, they have a considerable effect on the self esteem, eating ability, nutrition and health both in childhood and older age. In the modern society the most important role of teeth is to enhance facial appearance. Teeth also play an important role in speech and communication. Dental decay also leads to tooth loss which reduces the ability to eat a varied diet [4], [5]. Tooth loss has also been associated with loss of enjoyment of food and confidence to socialize [5]. It is therefore clear that dental diseases have detrimental effect on quality of life both in childhood and older age [6].

Cloves (*S. aromaticum*) are dried aromatic unopened floral buds of an evergreen tree 10-20 m in height, belonging to the family Myrtaceae, indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon [7]. They are esteemed as a flavouring agent and also used as a spice for scenting, chewing tobacco and an ingredient of betel chew. Cloves have many therapeutic uses, they control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves [9, 10, 11, 12, 13]. In addition, the cloves are highly antiseptic [14], antimutagenic [15], anti-inflammatory [16], antioxidant [8], antiulcerogenic [17, 18], antithrombotic [19], antifungal [20, 21], antiviral [22] and antiparasitic [23]. Spices have been traditionally used since ancient times,

for the preservation of food products as they have been reported to have antiseptic and disinfectant properties [24]. *S. aromaticum* has been shown to be a potent chemo preventive agent, used by the traditional Ayurvedic healers of India since ancient times to treat respiratory and digestive ailments [25, 26]. Eugenol is the main volatile compound extracted from clove bud (*S. aromaticum*) and used in traditional medicine, as a bactericide, fungicides and anesthetic [27].

**Objectives:** To determine zone of inhibition of aqueous extracts of the test plants on selected oral microorganisms and to compare zones of inhibition of the extract with that of the commercial antibiotics on selected oral microorganisms.

## MATERIALS AND METHODS

### Collection and Preparation of Plant Material

*Syzygium aromaticum* flower were purchased from Lagos Street Market, Ring Road, Benin City. The *S. aromaticum* were washed to remove soil and it was then washed, sliced and dried. The dried materials were pounded to powdered and then subsequently sieved. 50g of the powdered *S. aromaticum* was weighed into bottle and 500ml of distilled water was added. This was to carryout in aqueous extract. The plant material was soaked in the solvent for 24hrs and then filtered. The filtrate was concentrated to get the crude extract from which different concentrations were prepared. All extract were stored at 4 °C when not in use.

### Phytochemical Screening of Extract

The phytochemical screening of *Z. aromaticum* flower bud extracts were carried out to determine the presence of the following compounds; Saponins, tannins, phenols, cardiac glycoside, Anthracene, flavonoids and alkaloids using the standard procedures described by [28] and [29].

### Collection of Bacteria Isolates

The microbial isolates of *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Candida albicans*, *Rhizopus oryzae* and *Aspergillus flavus* were obtained from Medical Microbiology Laboratory of the University of Benin Teaching Hospital (UBTH), Benin City.

### **Preparation of Culture Media**

The media used were nutrient agar and potato dextrose agar and were prepared according to manufacturer's instruction (T M MEDIA, ISO 13485:2003 CERTIFIED).

### **Antimicrobial Susceptibility Testing**

#### **Preparation of Different Concentrations**

Following the method of [30] concentration of 100mg/ml of the extract was prepared by dissolving 0.1g of the extract in 1ml of sterile water. Then concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were prepared from the stock concentration (100mg/ml) by double dilution procedure.

#### **Microbial inoculum preparation**

The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37<sup>0</sup>C. After incubation, one milliliter of the cultures was inoculated onto solidify nutrient agar at 45<sup>0</sup>C using a Pasteur pipette.

#### **Antimicrobial Assay**

Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms [31]. Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Nutrient agar and potato dextrose agar plates for bacteria and fungi, respectively.

Three (3) adequately spaced wells of diameter 4mm per plate were made on the culture agar surface respectively using sterile metal cup-borer. 0.2ml of each extract and control were put in each hole under aseptic condition with the aid of pipette pump, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Conventional antibiotics were used as positive controls for bacteria and fungi respectively; distilled water was used as the negative control. The plates were then incubated at 37<sup>0</sup>C for 24 hours for the bacteria strains and at 28<sup>0</sup>C for 72 hours for fungal isolates. The zones of inhibition were measured and recorded after incubation. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms.

The diameters of these zones were measured diagonally in millimeter with ruler and then mean value for each organism from the triplicate cultured plates was recorded. Using the agar-well diffusion technique, an already made gram positive and gram negative (Asodisks Atlas Diagnostic, Enugu, Nigeria) standard antibiotic sensitivity disc bought from a laboratory chemical equipment store in Benin City was used as positive control for bacterial while Metronidazole was used as positive control for fungi. Distilled water was used as negative control for all the test organisms.

#### **Determination of Minimum Inhibitory Concentration (MIC)**

The Nutrient agar was prepared and sterilized, then poured into sterile petri dishes and allowed to solidify. The surface of the medium was inoculated with the test isolates. The discs soaked in different concentrations of the extract were placed on the surface of the seeded nutrient agar. The plates were incubated at 37°C for 24 hours, after which they were examined for the presence of growth inhibition. The MIC was taken as the lowest concentration that prevented the growth of the test microorganisms.

#### **Minimum Bactericidal Concentrations (MBC) and Minimum Fungicidal Concentrations (MFC)**

A loopful of the content of each plate in the MIC determination above, which did not show any visible growth after the period of incubation was streaked unto freshly prepared Nutrient agar, to determine their MBC and then incubated at 37°C for 24 hours after which it was observed for visible growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration.

#### **Antibiotic Sensitivity Disc**

Two sensitivity discs for Gram-positive and Gram –negative organisms were obtained commercially from a pharmaceutical store in Benin City, Edo State. Different Gram positive sensitivity disc, Gram negative sensitivity disc and Metronidazole for fungal were used.

### **RESULTS**

The result of the antimicrobial activity of Cloves (*S. aromaticum*) extract at various concentrations such as 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml showed their activities against microbial isolates such as *Staphylococcus epidermidis*, *Escherichia coli*,

*Proteus mirabilis*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Candida albicans*, *Rhizopus* spp and *Aspergillus flavus* (Table 1).

Table 2 shows the minimum inhibitory concentrations (MIC) of 6.25 mg/ml for all microorganisms and minimum bactericidal concentrations (MBC) of 12.5, 25 and 50 mg/ml for *S. epidermidis*, *E. coli*, *P. mirabilis* and *K. pneumoniae* respectively; it also indicates minimum fungicidal concentration (MFC) of 12.5 mg/ml for all fungal isolates with *Syzygium aromaticum* extract. The result of the antifungal activity of *S. aromaticum* extract compared to antifungal drug (Metronidazole) is shown in Table 5

The result of phytochemical screening of the extract of *S. aromaticum* is presented in Table 3. The analysis revealed the presence of saponins, tannins, phenols, cardiac glycoside, anthracene, flavonoids and alkaloids in the extract.

Table 4 summarizes the inhibitory activities of test antibiotics against four bacteria as determined by the dist diffusion method. All antibiotics exhibited antibacterial activity against three gram-positive bacteria and one gram-negative bacterium with only *K. pneumoniae* resistance to three of the antibiotics; AU, CEP and PN. Table 5 likewise revealed the activity and compared the indices of the extract with Metronidazole against the four fungi used in this study, the extract showed better treatment against *A. flavus* and *C. albicans*.

**Table 1: Antimicrobial sensitivity testing of the aqueous extract of Cloves flower bud (*Syzygium aromaticum*) at various concentrations on dental microorganisms**

Organisms	Concentrations (mg/ml)					Sterile Distilled Water
	100	50	25	12.5	6.25	
<i>S. epidermidis</i>	21.33±1.15 <sup>a</sup>	18.33±0.58 <sup>a</sup>	18.0±2.00 <sup>b</sup>	15.33±3.51 <sup>b</sup>	9.67±0.58 <sup>a</sup>	-
<i>Escherichia coli</i>	16.33±2.31 <sup>a</sup>	12.33±1.53 <sup>b</sup>	11.33±1.16 <sup>b</sup>	10.67±1.16 <sup>b</sup>	9.67±3.22 <sup>b</sup>	-
<i>Proteus mirabilis</i>	16.33±2.31 <sup>a</sup>	14.67±1.53 <sup>a</sup>	11.00±1.00 <sup>c</sup>	12.67±1.53 <sup>b</sup>	10.67±1.53 <sup>b</sup>	-
<i>Klebsiella pneumoniae</i>	18.00±5.29 <sup>a</sup>	16.33±2.52 <sup>a</sup>	11.33±2.52 <sup>b</sup>	10.33±1.53 <sup>b</sup>	10.14±3.06 <sup>b</sup>	-
<i>Aspergillus niger</i>	15.00±3.00 <sup>a</sup>	13.33±1.53 <sup>b</sup>	11.00±0.00	9.00±1.00 <sup>c</sup>	9.33±3.06 <sup>c</sup>	-
<i>Candida albicans</i>	14.00±1.00 <sup>a</sup>	11.67±2.08 <sup>a</sup>	10.67±3.51 <sup>a</sup>	8.33±1.16 <sup>b</sup>	8.33±1.53 <sup>b</sup>	-
<i>Rhizopus oryzae</i>	16.0±1.00 <sup>a</sup>	10.67±4.51 <sup>b</sup>	9.00±1.00 <sup>b</sup>	8.33±1.53 <sup>b</sup>	8.33±2.08 <sup>b</sup>	-
<i>Aspergillus flavus</i>	16.00±3.61 <sup>a</sup>	14.33±2.89 <sup>a</sup>	9.00±1.00 <sup>b</sup>	7.67±2.08 <sup>b</sup>	7.33±2.31 <sup>b</sup>	-

Similar letter indicate means that are not significantly different from each other

Note:

P<0.01 - Highly Significant, P<0.05 - Significant

**Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC) of Cloves flower (*Syzygium aromaticum*)**

Test isolates	MIC	MBC	MFC
<i>Staphylococcus epidermidis</i>	6.25mg/ml	12.25mg/ml	-
<i>Escherichia coli</i>	6.25mg/ml	12.5mg/ml	-
<i>Proteus mirabilis</i>	6.25mg/ml	25.0mg/ml	-
<i>Klebsiella pneumoniae</i>	6.25mg/ml	50mg/ml	-
<i>Aspergillus niger</i>	6.25mg/ml	-	12.5mg/ml
<i>Candida albicans</i>	6.25mg/ml	-	12.5mg/ml
<i>Rhizopus spp</i>	6.25mg/ml	-	12.5mg/ml
<i>Aspergillus flavus</i>	6.25mg/ml	-	12.5mg/ml

Key:

MIC – Minimum inhibitory concentration

MBC – Minimum bactericidal concentration

MFC – Minimum fungicidal concentration

**Table 3: Phytochemical screening of *Syzygium aromaticum***

Parameter	Aqueous extract
Saponins	+
Tannins	+
Phenols	+
Cardiac glycoside	+
Flavonoids	+
Alkaloids	+
Anthracene	+

Table 4: Antibiotic susceptibility test

Gram -ve	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
<i>Escherichia coli</i>	13(25.5)	13(25.5)	11(25.6)	10(33.3)	9 (24.3)	9 (23.7)	9 (33.3)	7 (20.6)	9 (25.7)	10(37.0)
<i>Klebsiella pneumoniae</i>	12 (23.5)	12(23.5)	10(23.3)	0 (0.0)	10(27.0)	10(26.3)	0(0.0)	8 (23.5)	6 (17.1)	0(0.0)
<i>Proteus mirabilis</i>	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)	9 (22.0)
Gram +ve	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV
<i>Staphylococcus epidermidis</i>	10 (24.4)	10(22.7)	10(26.3)	8 (22.9)	9 (26.5)	10(27.8)	9 (24.3)	6 (27.3)	8 (25.0)	9 (27.3)

P>0.05 = Not Significant

Table 5: Test of association between fungi (extract of clove flower) and treatment

Organisms	Treatment (%)		
	Metronidazole	Aqueous extract	Total (%)
<i>Aspergillus flavus</i>	Count	8	11
	% Within Fungi	42.1	57.9
<i>Candida albicans</i>	Count	10	11
	% Within Fungi	47.6	52.4
<i>Rhizopus oryzae</i>	Count	12	10
	% Within Fungi	54.5	45.5
<i>Aspergillus niger</i>	Count	12	12
	% Within Fungi	50	50
Total	Count	42	44
	% Within Fungi	48.8	51.2

## DISCUSSION

Extract of Cloves flower (*Syzygium aromaticum*) showed very strong activity against all the tested microbial isolates at concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml (Table 1). The antibacterial activity of Cloves flower (*S. aromaticum*) extract was read from the diameter zone of inhibition. It could be observed that Clove flower (*S. aromaticum*) extract had broad spectrum of activity on the test organisms. The tested microbial isolates include *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Candida albicans*, *Rhizopus oryzae* they were sensitive to extract of Clove flower (*S. aromaticum*). The highest sensitivity was observed in *S. epidermidis* as 21.33 mm at 100mg/ml, while *Aspergillus flavus* showed the lowest sensitivity of 7.33 mm at 6.25mg/ml. This is in agreement with the results of previous studies by [32] who reported that extracts from Clove flower (*S. aromaticum*) contained appreciable activity against Gram-positive and Gram-negative bacteria.

The finding of this study is corroborated by the report of [33] who equally reported *S. aromaticum* as active on *Staphylococcus epidermidis* at 100mg/ml. [32] reported *S. aromaticum* extracts as active on *E. coli*, *S. epidermidis*, *Proteus mirabilis*, *Aspergillus spp.* and *C. albicans* with zone of inhibition at various concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml respectively. [32] Have reported *S. epidermidis* to be most sensitive to extracts of *S. aromaticum* which is in disagreement with result of this study. Variations in the sensitivity of the bacterial species tested on the extracts might be as a result of differences in the strains employed for the research as [32] used clinical strains from hospital, while [33] used standard strains from the National Culture Type Collection (NCTC) which quite differ from the wild strains sourced from hospital samples used in this study. Wild strains of bacteria could possess genetic capabilities that could make them adapt well to the tough environments they dwell in as compared to stock cultures, which have been isolated and preserved. The antimicrobial activity of plant extracts has been linked by many researchers to be due to the presence of phytochemicals in them [34, 35]. The antimicrobial activity of the extracts tested *in vitro* could be higher than they are reported if active ingredients from the extracts are isolated and tested. [36] Reported that crude extracts of plant materials may contain inactive substances which may also antagonize the antimicrobial actions of one another.

The MIC of the extract is 6.25mg/ml for all organisms used including *E. coli* and *P. aeruginosa*, *S. aureus*. It was observed that the extract of Cloves flower (*S. aromaticum*) shows the broadest activity against bacterial isolates tested compared to fungal isolates. Perhaps its broad spectrum of activity on Gram negative and Gram positive bacteria coupled with its low MIC on most of the bacteria tested might give it an impetus as a potential antimicrobial agent.

Comparison of antifungal drug such as Metronidazole used in this study with the extract of Cloves flower (*S. aromaticum*) showed that Metronidazole was more effective in the treatment of *R. oryzae* infection with 54.5% as against 45.5% of the extract, but less effective with 47.6 and 42.1% as compared with the extract value of 47.6 and 57.9% against *C. albicans* and *A. flavus* respectively. Metronidazole and the extract of *S. aromaticum* showed equal efficacy of 50% in treatment of *A. niger* infection.

Not much has been reported on the antifungal activity of *S. aromaticum* extracts, except [37] who reported on the anticandida activity extract of *S. aromaticum*.

The results of the phytochemical analysis are presented in Table 3. saponins, tannins, phenols, cardiac glycoside, flavonoids, alkaloids and anthracene were detected in extract of Clove flower (*S. aromaticum*). The result of the phytochemical analysis corroborate the work of [32] who reported presence of Saponins, tannins, phenols, cardiac glycoside, flavonoids and alkaloids in extracts of *S. aromaticum*. Scrutiny of past works on *S. aromaticum* however, shows not much has been reported on the antimicrobial activity of the leaf extracts.

Literature related to antimicrobial and phytochemical constituents of *S. aromaticum* extracts is scanty. This might be attributed to the fact that *S. aromaticum* is a tree indigenous to West Africa and therefore research on the plant is scanty and claims by traditional herbalists on the usefulness of the plant as medicinal mostly centered on the use of the stem bark, root and leaf.

The antibacterial activities of extracts of *S. aromaticum* against gram negative and gram positive bacteria was comparable to those of conventional antibiotics. Comparison of antibacterial activity of extracts of *S. aromaticum* against tested bacteria isolates and commercial antibiotics showed that the bacteria were much more susceptible to the extract of *S. aromaticum* than to the conventional antibiotics. This may be due to the fact that the active ingredients in commercial

antibiotics are in refined and purified forms which may have reduced the medicinal properties in the antibiotics [38].

The bacteria test organisms were resistant to few of the conventional antibiotics used which included ciproflox (10mcg), norfloxacin (10mcg), gentamycin (10mcg), amoxil (20mcg), streptomycin (30mcg), rifampicin (20mcg), erythromycin (30mcg), chloramphenicol (30mcg), ampiclox (20mcg), levofloxacin (20mcg), tarivid (10mcg), reflacine (10mcg), ciproflox (10mcg), augmentin (30mcg), gentamycin (10mcg), streptomycin (30mcg), ceporex (10mcg), nalidixic acid (30mcg) and septrin (30mcg). *Klebsiella pneumoniae* showed total resistance to Augmentin, Ceporex and Ampicillin. The resistance of the bacterial isolates to some antibiotics could probably be due to frequent use of antibiotics and self medications.

## CONCLUSION

The results of this study showed that the extract of *S. aromaticum* has antimicrobial effect on Gram positive, Gram negative bacteria and fungi. The presence of phytochemicals in the extract may have been responsible for the activity possessed by the plant extracts.

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