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Phytochemical Screening and Antimicrobial Evaluation of *Syzygium aromaticum* Extract and Essential oil

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A B S T R A C T

Extraction of *Syzygium aromaticum* powder using organic solvent (methanol) revealed the existence of alkaloids, flavonoids, terpenoids, tannins, aldehydes, ketones, alkaloids, glycoside, steroids, carbohydrates and phenolic compounds. A total of forty-six phenolic compounds were identified and quantified in *Syzygium aromaticum* extract using GCFID among which are gallic acid (847.36), syringic acid (259.04), protocatechuic acid (252.29), caffeic acid (151.01), eugenin (121.30), eugenitin (101.29), P-hydroxybenzoic acid (85.04), salicyclic acid (31.84), kaempferol (30.75), quercetin (27.68), rhamnetin (21.61), phenylacetic acid (18.09), myricetin (16.67) and isohamnetin (5.07) being of significant values. The percentage yield of *Syzygium aromaticum* oil extracted by hydrodistillation technique and isolated with Dichloromethane was 21.20%. The major phytoconstituents present are eugenol (75.10%), eugenyl acetate (13.57%), β-caryophyllene (5.27%), limonene (1.45%) and α-terpinolene (1.12%) respectively. The antimicrobial activity (minimum inhibitory concentration and minimum bactericidal concentration) of *Syzygium aromaticum* oil was investigated against six microorganisms namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus flavus* and *Penicillium* spp using agar well diffusion and nutrient broth dilution techniques. *Syzygium aromaticum* oil showed excellent antimicrobial activity compared to ciprofloxacin and ketoconazole used as control by inhibiting both bacteria and fungi with wide zones of inhibition.

Introduction

Spices and herbs have been used for thousands of centuries by many continents to enhance the flavor and aroma of foods; preserving foods and for their medicinal value. Spices are aromatic plant products which are frequently used to enhance food palatability. Most spices were originally indigenous to the tropics eg. cinnamon, pepper, clove and nutmeg (Viuda-Martos *et al.*, 2007). *Syzygium aromaticum* (Clove bud) is one of the most ancient and valuable spices of the Orient (Chaieb *et al.*, 2007) which are used as a carminative to increase hydrochloric acid in the stomach and to improve peristalsis.

Essential oil compounds are fat soluble thus possess the ability to permeate the membranes of the skin before being captured by the microcirculation and drained into the systemic circulation which reaches all targets organs (Adorjan and Buchbauer, 2010). Due to their

molecular structures (presence of olefenic double bonds and functional groups such as hydroxyl, aldehyde, ester); essential oils are readily oxidizable by light, heat and air. (Skold *et al.,* 2008).

The essential oil extracted from the dried flower buds of cloves is used for acne, warts, scars and parasites. Research has shown that clove oil is an effective mosquito repellent (Trongtokit *et al.,* 2005). Culinary spices and herbs contain a wide variety of active phytochemicals including flavonoids, terpenes, polyphenols, curcumins, coumarins (Fabio *et al.,* 2003). The aim of this research is to determine the phytochemical constituents and antimicrobial effect of *Syzygium aromaticum* oil and crude extract.

Materials and Methods

Chemicals

The phenolic acids standards and 1, 1-diphenyl-2-picrylhydrazyl (DPPH-90% purity) were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, WI) and Sigma-Aldrich Co., St. Louis, MO, USA respectively.

Growth condition of test organisms

Previously characterized isolates (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus flavus* and *Penicillium*) obtained from Medical Microbiology Laboratory of Fountain University were used. Bacterial isolates were subcultured on nutrient agar at 37°C for 24 h while fungal on Sabouraud Dextrose Agar at 28°C for 3 days respectively. All isolates were maintained at 4°C on Nutrient agar slope for further analysis.

Pretreatment and Processing of Sample

Syzygium aromaticum bud was procured from Oluode market in Osogbo, Osun State, Nigeria. Damaged and spoilt buds were removed; pulverized into powdery form, filtered to remove residues and stored in an air-tight container at room temperature for further analysis. The organoleptic characteristics of the smooth powder such as color, flavor, odor and intensity of odor were observed.

Preparation of *Syzygium aromaticum* Extract

Syzygium aromaticum aqueous extraction was done using methanol and distilled water. Twenty five grams of *Syzygium aromaticum* powder was soaked in distilled water and methanol (250 ml each) separately at room temperature for 7 days. The mixtures were stirred intermittently every 18 hours by gently swirling of the flask thus slurry obtained was filtered after 7 days and crude residue was used for the analyses. The filtrate was concentrated under reduced pressure in a rotary vacuum evaporator (NYC R- 205D) at 40°C (Bag *et al.,* 2009) thus; semisolid substance obtained was dried in hot air oven at 45°C to obtain crude extract (Jonathan and Fasidi, 2003). The crude extracts obtained were reconstituted in Dimethyl Sulfoxide (DMSO) and subjected to preliminary phytochemical screening, scavenging property and antimicrobial analysis. Confirmatory analysis was done using Gas chromatography flame ionization detector in order to identify the chemical constituents.

Preliminary Phytochemical Screening of *Syzygium aromaticum* Extract

Phytochemical constituents {such as tannins, saponins, phlobatanins, phenolics, reducing sugar, trepenoid, steroid, glycosides, alkanoids (Hager's and Wanger's tests) and flavonoids (ferric chloride, sodium chloride and lead acetate tests)} of the crude extracts

were analyzed. *Syzygium aromaticum* extract (2 ml each) was utilized separately for each analysis thus formation of precipitate, color change or frothing indicates presences of the phytochemical mentioned above (Sofowora, 1993).

DPPH radical scavenging assay

The antioxidant activity of *Syzygium aromaticum* extracts was assayed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Burits *et al.,* 2000). Fifty microlitres of varying concentrations (10-50%) of the extracts were added to 5 ml of 0.004% methanol solution of DPPH and incubated for 30 minutes at room temperature. Absorbance was measured against quercetin at 517 nm using UV–Visible spectrophotometer. Thus, inhibition rate (I %) on free radical DPPH was calculated as stated below;

Inhibition % (I%) = $\{(A_{blank} - A_{sample}) \div A_{blank}\} \times 100$

A_{blank} = absorbance of the control reaction
A_{sample} = absorbance of the test compound
Quercetin = Standard reference (blank)

Confirmatory Analysis

Quantification of Phenolic Compounds Present in *Syzygium aromaticum* Extract

Phenolic compounds were extracted using two- stage extraction procedures according to Kelley *et al.,* (1994) and Provan *et al.,* (1994). At the initial stage, 50mg of *Syzygium aromaticum* powder was extracted with 5 ml of 1M NaOH for 16 hours on a shaker at ambient temperatures. After extraction, the sample was centrifuged at 5000 rpm, rinsed with water repeatedly and supernatant obtained was heated at 90ºC for 2 hours to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4M HCI to pH <2.0, diluted to 10 ml, with deionised water and centrifuged to remove the precipitate. During the final stage, the residue (precipitate) was extracted with 5 ml of 4M NaOH, heated to 160ºC in Teflon and allowed to cool before filtering. The supernatant obtained was adjusted to pH<2.0 with 4M HCI and further subjected to purification.

Purification of Phenolic Compounds Present in the Extract

An aliquot (5-15 ml) of the supernatant was passed through a conditioned Varian Bond Elut PPL (3 ml size with 200 mg packing) solid-phase extraction tube at 5 ml/min attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were placed under a vacuum (-60 kPa) until the resin was thoroughly dried and phenolic acids were eluted with 1 ml of ethyl acetate into gas chromatography autosampler vials. The PPL tubes were conditioned by passing 2 ml of ethyl acetate followed by 2 ml water (pH <2.0).

Derivatisation (Silylation) of Extract

After extraction, 2 ml of concentrated extract present in gas chromatography vial was derivatized by adding 20 µl of bis(trimethylsilyl) trifluoroacetamide (derivatising agent) with a magnetic stirrer at 45ºC for10 minutes. This was done using gas chromatography (HP 6890) powered with HP Chemstation Rev.A09.01 [1206] Software, Split injection at split ratio 20:1 using Nitrogen as carrier gas, inlet temperature of 250°C and flame ionization detector. The oven was programmed at initial temperature of 60°C for 5 minutes, first ramping at 15ºC/min for 15 minutes and second ramping at 10ºC/min for 4 minutes respectively.

Extraction of *Syzygium aromaticum* Oil

Syzygium aromaticum oil was extracted from

10% w/v *Syzygium aromaticum* powder using hydro-distillation technique at 100oC and continuous agitation with magnetic stirrer (Schnaubelt, 2005). The essential oil was separated from the distillate by mixing with chloroform in a seperatory funnel; the lower layer (organic layer) contains *Syzygium aromaticum* oil and chloroform while the upper layer is the aqueous layer. Furthermore, organic layer was dried by mixing with 2 g of anhydrous Sodium sulfate and allowed to stand overnight. The residue was removed by decanting while the volatile chloroform was separated from the essential oil by exposure to air thus percentage yield of essential oil obtained was determined.

Quantification of *Syzygium aromaticum* Oil

Essential oil obtained was analyzed by Gas Chromatography-Flame Ionization Detector HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] Software in order to determine the constituent and corresponding concentration. The chromatography was done in HP 5MS column (0.25 µm interior diameter x 30 m long) with a particle size of 0.25 µm, at a flow rate of 1.0 ml/min using Flame Ionization Detector (FID) signal and hydrogen as the mobile phase at injection temperature of 150°C and 300°C detector temperature respectively.

Antimicrobial Effectiveness of *Syzygium aromaticum* oil

Antibacterial and antifungal activity of *Syzygium aromaticum* oil was determined using agar well diffusion method. Suspension [10 µL of cell suspension containing $1.1x10^4$ CFU/mL of each test organism (bacterium and fungus)] was inoculated separately on sterile Muller-Hinton agar using spread plate method. The plates were allowed to dry and a sterile cork borer of diameter 6 mm was used to bore wells in the agar plates (Okeke *et al.,* 2001). Subsequently, 50 µL of the essential oil was transferred into triplicate wells; sterile DMSO (50 µL) was the negative control for the assay while ciprofloxacin (50 µL) and ketoconazole (50 µL) served as positive controls for antibacterial and antifungal assays respectively. The plates were incubated after 1 hour at 37°C for 24 hours for bacteria growth (Khokra *et al.,* 2008) and 28°C for fungi (Fiori *et al.,* 2000). Zones of inhibition were recorded to the nearest diameter in millimeter according to Moreira *et al.,* (2007).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Syzygium aromaticum* Oil

The MIC of *Syzygium aromaticum* oil for bacterial strains was determined by broth microdilution assay (Hammer *et al.,* 1996). Overnight cultures were prepared by inoculating Mueller hinton broth (2 ml) and incubating at 37°C for 24 hours. After incubation, culture broths were adjusted to McFarland standard number 1 by diluting with sterile distilled water to obtain a concentration of $1.1x10^4$ CFU/mL. Serial dilution of the culture broth with varying concentrations (1, 3, 5, 7 and 9 µl/ml) of clove oil was prepared separately while sterile DMSO and ciprofloxacin served as negative and positive controls. MIC for fungal strains was determined using agar dilution method. Saboraud dextrose agar plates were prepared separately with increasing concentration of *Syzygium aromaticum* oil from 1 µl/ml to 9 µl/ml (v/v) in intervals and dissolved with sterile DMSO (20%) in the medium; inoculated with 10 µl of fungal suspension ($1.1x10^4$ CFU/mL) and incubated at 28°C for 3 days. Sterile DMSO and ketoconazole served as negative and positive controls respectively. Zones of inhibition were measured, thus agar plate with lowest concentration of *Syzygium aromaticum* oil

inhibiting visible growth of bacterial and fungal after incubation period was regarded as the MIC (Lee *et al.,* 2007). Ten microliters of the invisible culture broth were inoculated onto Mueller Hinton agar and incubated at 37°C for 24 hours. MBCs were determined from the lowest concentration of clove oil that inhibited growth on Mueller Hinton agar.

Results and Discussion

Organoleptic Characteristics of *Syzygium aromaticum*

The organoleptic characteristics of *Syzygium aromaticum* powder such as color, flavor, odor, and intensity of odor observed are reddish- brown, burning and spicy, strong aroma and pungent while the characteristics of the essential oil include color (colorless to light yellow, becoming brownish when aging), appearance (slightly murky), odor (spices cloves like), intensity of color (strong, pungent) and flavor (warm, almost burning spicy flavor) respectively.

Preliminary Phytochemical Screening of *Syzygium aromaticum* Extract

Based on the preliminary screening, phlobatannins, reducing sugar and steroids were absent in methanolic extract of *Syzygium aromaticum* but present in distilled water extract. Color change such as dark green color indicates presence of tannins and phenolics, reddish brown interface (terpenoids and glycosides), greenish (steroids), yellow to colorless (flavonoids) respctively. Formation of precipitate such as red precipitate indicates presences of phlobatannins or reducing sugar, yellow or reddish brown (alkanoids) and yellow precipitate (flavonoids) while persistence of frothing indicates presences of saponins (Table I).

Quantification of Phenolic compounds Present in *Syzygium aromaticum* Extract

A total of forty-six phenolic compounds were identified and quantified in *Syzygium aromaticum* powder using GCFID (Table II). These include gallic acid (847.36), syringic acid (259.04), protocatechuic acid (252.29), caffeic acid (151.01), eugenin (121.30), eugenitin (101.29), P-hydroxybenzoic acid (85.04), salicyclic acid (31.84), kaempferol (30.75), quercetin (27.68), rhamnetin (21.61), phenylacetic acid (18.09), myricetin (16.67) and isohamnetin (5.07) being of significant values.

Quantification of *Syzygium aromaticum* oil using GC-FID

Percentage yield of *Syzygium aromaticum* oil obtained was 21.20%. A total of thirty nine compounds representing 100% of the total essential oil were analyzed and concentrations of the main components are eugenol (75.10%), acetyleugenol (13.57%), Beta caryophyllene (5.27%), limonene (1.45%) and alpha-terpinolene (1.12%) respectively (Table III).

Antioxidant Activity of *Syzygium aromaticum* extracts

Distilled water extract of *Syzygium aromaticum* has lower inhibition i.e scavenging effect on DPPH compared to the methanolic extract and reference antioxidant (quercetin) (Figure I).

Antibacterial and Antifungal Activity of *Syzygium aromaticum* Oil

Syzygium aromaticum oil has high inhibitory effect on bacterial isolates (*S. aureus*, *E. coli* and *P. aeruginosa*) with zones of inhibition of 30±0.02 mm, 28±0.11 mm and 21±0.05 mm and fungal isolates (*C. albicans*, *A. flavus* and *Penicillium* species) with 44±0.03 mm, 51±0.13 mm, 47±0.11 mm zones of inhibition respectively compared to ciprofloxacin (14±0.06 mm, 12±0.01 mm and 13±0.02 mm) and ketoconazole (14±0.13 mm, 10±0.18 mm and 11±0.08 mm).

Table.1 Preliminary Phytochemical Screening of *Syzygium aromaticum* Extracts

Parameters	Distilled Water Extract	Methanolic Extract
Tannins	Present	Present
Saponins	Present	Present
Phlobatannins	Present	Absent
Phenolic compounds	Present	Present
Reducing sugar	Present	Absent
Terpenoids	Present	Present
Steroids	Present	Absent
Glucosides	Present	Present
Alkaloids		
Hager's test	Present	Absent
Wagner's test	Present	Present
Flavonoids		
Sodium hydroxide test	Absent	Absent
Ferric chloride test	Present	Present
Lead acetate test	Absent	Absent

Fig.1 DPPH scavenging effect of *Syzygium aromaticum* extracts

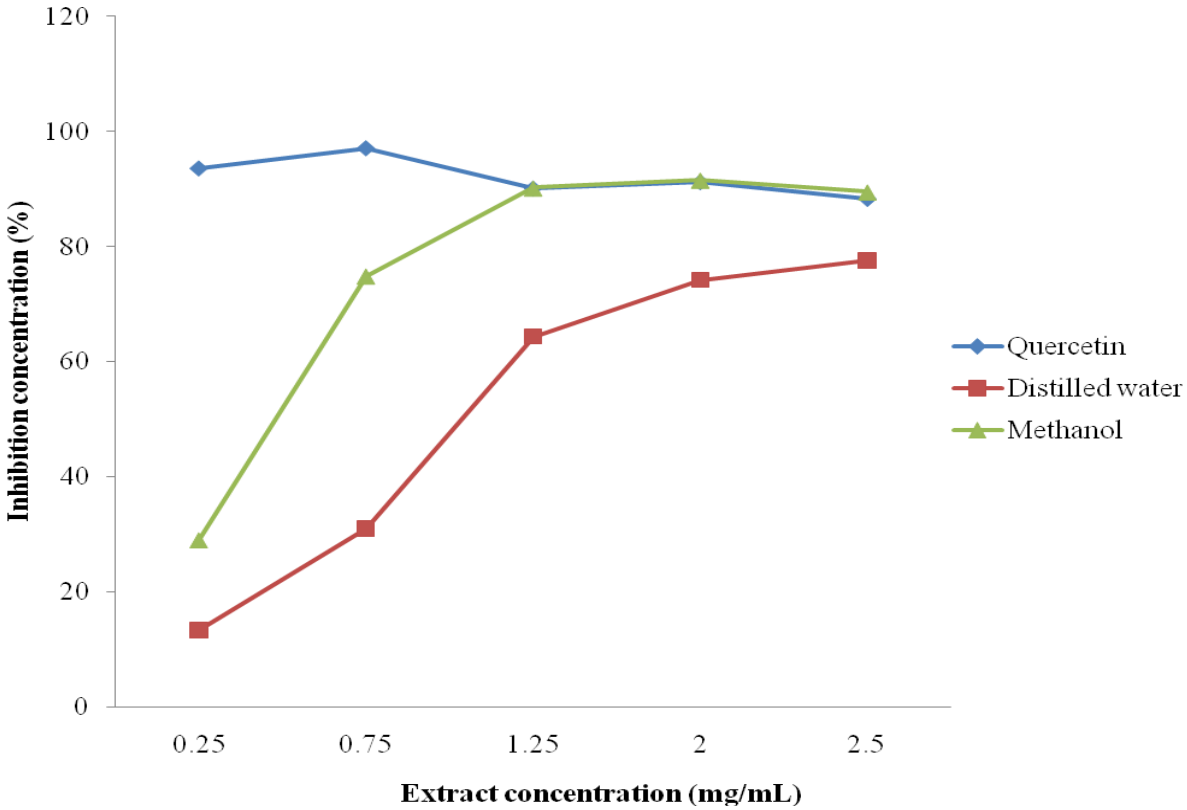

Table.2 Phenolic Compound Present in *Syzygium aromaticum* Extract

Ret Time [min]	Area [pA*s]	Amount/Area	Amount [mg/100g]	Compound
7.057	38.02240	7.96178e-6	3.02726e-4	Catechin
7.521	8.93904	7.96178e-6	7.11707e-5	Guaiacol
7.915	189.57533	7.96178e-6	1.50936e-3	Phloroglucinol
8.291	15.71195	1.15101	18.08466	Phenylacetic acid
8.513	33.36761	9.54198e-1	31.83932	Salicyclic acid
8.893	59.06726	7.96178e-6	4.70231e-4	Cinnamic acid
9.321	42.55415	7.96178e-6	3.38807e-4	Coumarin
9.731	96.59180	6.70241e-3	6.47398e-1	Carvacrol
10.174	28.37762	7.96178e-6	2.25936e-4	Gentisic acid
10.639	60.96240	4.13844	252.28940	Protocatechuic acid
11.054	34.66746	1.20773e-2	4.18689e-1	P-coumaric acid
11.349	78.53786	1.18596e-2	9.31426e-1	Vanillic acid
11.663	50.40628	7.96178e-6	4.01324e-4	o-coumaric acid
12.818	35.51277	2.39464	85.04016	P-hydroxybenzoic acid
13.416	62.43334	13.57220	847.35806	Gallic acid
13.736	96.05247	1.57213	151.00691	Caffeic acid
14.915	50.82818	3.61899e-3	1.83947e-1	Ferulic acid
15.307	39.70530	6.52401	259.03770	Syringic acid
15.469	65.69872	1.61463e-3	1.06079e-1	Piperic acid
16.034	180.49785	6.72010e-1	121.29645	Eugenin
16.243	83.44453	7.18370e-4	5.99440e-2	Sinapinic acid
16.668	136.01073	4.03226e-4	5.48430e-2	Daidzein
18.050	222.40218	4.55430e-1	101.28858	Eugenitin
18.350	230.75107	9.76562e-4	2.25343e-1	Genistein
18.838	204.81113	2.04750e-3	4.19351e-1	Apigenin
19.099	363.45380	7.96178e-6	2.89374e-3	Naringenin Chalcone
19.516	208.59067	1.47406e-1	30.74745	Kaempferol
19.952	114.59298	1.00000e-5	1.14593e-3	Naringenin
20.466	421.15018	822368e-4	3.46341e-1	Glycitein
21.819	162.15671	1.08600e-3	1.76971e-1	Luteolin
22.600	266.38889	8.11267e-2	21.61125	Rhamnetin
22.853	98.54902	7.96178e-6	7.84626e-4	Epicatechin
22.993	70.71553	7.96178e-6	5.63022e-4	Epigallocatechin
23.470	128.28236	9.68992e-4	1.34305e-1	Gingerol
23.621	85.01318	7.96178e-6	6.76856e-4	2-phenylethyl-beta-0-glucoside
23.965	117.02042	2.36563e-1	27.68273	Quercetin
24.185	138.33692	1.58028e-6	2.18610e-4	Delphinidin
24.609	170.39888	2.97435e-2	5.06826	Isohamnetin
24.789	77.48580	1.60051e-6	1.24017e-4	Malvidin
24.885	48.34124	3.44694e-1	16.66296	Myricetin
24.998	73.10680	7.961178e-6	5.82061e-4	Pentunidin
25.251	38.96031	8.16993e-8	3.18303e-6	3-0-caffeoylquinic
25.480	47.46246	1.60256e-6	7.60645e-5	Chlorogenic acid
26.291	57.78866	1.57233e-6	9.08627e-5	Rosmarinio acid
26.949	28.27051	8.09585e-7	2.28874e-5	Curcumin
28.192	18.42357	1.62127e-6	2.98696e-5	Rutin

Table.3 *Syzygium aromaticum* Oil Composition using GC-FID

Ret Time [min]	Area [pA*s]	Amount/Area	Norm %	Name
6.913	363.18304	2.42438e-5	1.445346	Limonene
8.050	4.45539	2.91183e-5	0.021296	Sabinene
10.049	14.64804	5.39835e-6	0.012980	Alpha Pinene
11.275	11.40357	3.75753e-6	0.007034	Beta Pinene
11.495	17.90532	1.39407e-5	0.040974	Benzyl Alcohol
12.844	21.89179	1.34116e-6	0.004820	Cis Ocimene
13.031	57.61564	1.56119e-6	0.014765	Myrcene
13.134	9.60553	1.07742e-5	0.016988	Allo Ocimene
13.792	8.80173	1.71522e-5	0.024782	Pinene-2-OL
14.191	40.71996	2.84261e-6	0.019001	Alpha Thujene
14.865	49.16263	5.50605e-6	0.044435	Gama Terpinene
15.354	66.18313	3.30316e-6	0.035886	Neral
15.414	20.02147	8.51826e-6	0.027996	Geranial
16.331	41.54738	2.48281e-6	0.016933	Isoartemisia
16.738	206.03482	1.25217e-6	0.042350	1,8-Cineole
17.928	80.16151	2.62150e-6	0.034495	Borneol
18.069	99.14284	6.85553e-5	1.115702	Alpha-Terpinolene
18.195	207.71317	4.37242e-7	0.014908	Linalool
18.693	90.20630	1.26570e-6	0.018742	Alpha Terpineol
19.035	113.34972	2.63841e-6	0.049092	Terpinen-4-OL
19.507	482.86920	9.47419e-4	75.096165	Eugenol
19.813	134.62978	1.01556e-6	0.022444	Thymyl Methl Ether
20.580	268.31439	1.19744e-4	5.274036	Beta Caryophyllene
20.933	176.67216	1.07038e-6	0.031042	Linalyl Acetate
21.250	95.38912	2.57061e-6	0.040251	Ethyl Cinnamate
21.396	232.67856	2.44694e-5	0.934599	Alpha Humulene
21.826	214.76151	1.43639e-6	0.050638	Borneol Acetate
22.026	130.76863	3.67621e-6	0.078913	Phenanthrene
22.468	109.80587	3.35833e-7	0.006053	Linaly Acetate
22.823	96.70632	2.87630e-6	0.045660	Beta Bisabolene
23.313	168.88454	3.57345e-7	0.009907	Beta Elemene
24.058	128.40918	1.84824e-6	0.038958	Germacrene D
24.651	240.91310	8.60279e-7	0.034021	Bicyclogermacrene
24.862	117.12291	4.51216e-5	0.867505	Alpha Copane
25.542	116.24159	1.58317e-6	0.030209	Alpha Bergamotene
26.264	156.40256	5.28725e-4	13.574370	Acetyleugenol
27.128	49.50246	1.04542e-6	0.008495	Elemicin
27.735	159.33490	1.04539e-6	0.027342	Benzyl Benzoate
27.995	111.86918	4.4700e-5	0.820867	Caryophyllene Oxide
Total (%)			100.000000	

Each value represents mean of three independent replicate assays and zones of inhibition were recorded to the nearest diameter in mm and interpreted according to Moreira *et al.,* (2007). This implies that all test organisms are extremely sensitive to *Syzygium aromaticum* oil but slightly sensitive to antibacterial and antifungal drugs respectively.

S. aureus and *E. coli* had MIC of 5 µl/ml, *P. aeruginosa* had the broadest activity of 9 µl/ml while MIC for all fungal isolates was 1 µl/ml. Thus *Syzygium aromaticum* oil was bacteriostatic at concentrations 1 µl/ml, 5 µl/ml and 9 µl/ml respectively. MBC of *Syzygium aromaticum* oil against *S. aureus* and *E. coli* was 5 µl/ml.

Statistical Analysis

The experimental results are presented as mean of triplicate analysis.

Essential oils are natural products that plants produce for their own needs other than nutrition (i.e. protection or attraction). *Syzygium aromaticum* oil was subjected to chromatographic conditions in order to achieve a proper separation of the oil components required for qualitative analysis and quantification. Ability to assay for thirty-nine different compounds in *Syzygium aromaticum* oil (Table III) extracted through hydrodistillation method shows the effectiveness of the technique through separation of slightly volatile, water-immiscible substances by means of low temperature distillation because most of these compounds are susceptible to decomposition at high temperature (higher than 100ºC).

DPPH scavenging activity assay evaluates the hydrogen-donating ability of the chain-breaking antioxidants, the antioxidants that are capable to donate hydrogen to free radicals, leading to nontoxic species and inhibition of the propagation phase of lipid oxidation. The antioxidant activity of clove extract and oil in comparison with quercetin as a scavenger of the DPPH+ radical due to reduction in these radicals (Figure I) which shows free radical scavenging characteristics exhibited by *Syzygium aromaticum* oil and extract thus indicates its ability to interact and neutralize free radicals, thus preventing them from causing damage. This implies that *Syzygium aromaticum* can be used as dietary supplements for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation. (Dabelstein *et al.*, 2007).

The inhibitory activity exhibited by *Syzygium aromaticum* oil against the test organisms in this research is due to the presence of several constituents such as eugenol, beta-caryophyllene, limonene, alpha terpinolene (Chaieb *et al.,* 2007), acetyl eugenol, methyl salicylate, iso-eugenol, methyl-eugenol, phenylacetic acid, salicyclic acid, protocatechuic acid, p-hydroxybenzoic acid, eugenin, eugenitin (Yang *et al.,* 2003), phenolic compounds (kaempferol, rhamnetin, isorhamnetin, myricetin, quercetin, gallic acid, caffeic acid and syringic acid) (Table II) respectively (Cai and Wu, 1996). The broad spectrum antimicrobial activity exhibited by *Syzygium aromaticum* oil and crude extract agrees with the reports of Park *et al.,* (2007) and Fu *et al.,* (2007) that reported potent antifungal and antibacterial effects of *Syzygium aromaticum* on microorganisms due to its mechanism of action which include denaturation of proteins and reaction with cell membrane phospholipids thus changing the membrane permeability of the microorganism. The research confirms the effectiveness of *Syzygium aromaticum* oil and crude extract against test organisms at varying

inhibitory concentrations compared to antibacterial and antifungal drugs used as control. These findings justify the ethno-medicinal uses of the plant thus, represents an alternative source of natural antimicrobial substances for use in pharmaceutical industries and food system to prevent the growth of food-borne bacteria and extend the shelf-life of the processed food.

Syzygium aromaticum constituents (alkaloids, saponins, tannins and flavonoids) obtained in this research are known to have curative activity against several pathogens thus the essential oil and crude extract are hereby recommended for treatment of diseases caused by *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*s since some of these organisms are known to play a vital role in invasive skin diseases including superficial and deep follicular lesion (Usman and Osuji, 2007).

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