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Efficacy of the Essential Oils of Curcuma Longa and Syzygium Aromaticum against Bacteria Implicated in Diabetic Foot Ulcers

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Abstract: *Diabetes mellitus is a metabolic disease and one of the most feared complications is diabetic foot ulcers (DFUs) resulting in amputation and gangrene arising from the action of pathogenic bacteria. This study investigated the efficacy of essential oil extracts of Curcuma longa (turmeric) and Syzygium aromaticum (clove) on bacteria implicated in DFUs. Essential oils extracted from Curcuma longa and Syzygium aromaticum were used singly and in combinations to determine their antimicrobial activities on bacteria isolated from DFUs (Salmonella paratyphi, Salmonella arizona, Pseudomonas aeruginosa, P. fluorecens, Citrobacter sp, Enterococcus fecalis, Enterobacter sp, Escherichia coli, Enterobacter cloacae, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, Bacteroides fragilis, and Peptostreptococcus sp.). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined. The zone of inhibition was highest in Proteus mirabilis (36.0 mm ± 1.0 mm) and lowest in Salmonella arizona (10.0 mm ± 1.0 mm). Clove essential oil had the highest activity against the selected bacteria (82.76%). Turmeric was able to inhibit 2.97% of the pathogenic bacteria. Combinations of clove and turmeric inhibited 82.76% of the isolates. The MIC and MBC ranged between 0.90% and 7.5% respectively. This study showed that terpenoid extracts of the selected plants are active against pathogenic bacteria isolated from DFUs while the high activity of Syzygium aromaticum (clove) extract could serve as a pointer towards the development of antimicrobial compounds of natural origin to combat drug-resistant pathogenic bacteria associated with diabetic foot ulcers.*

I. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia caused either by an impaired response of body cells to insulin or because the body does not secrete enough insulin. One of the secondary implication of diabetes is diabetic foot ulcers. The rates at which amputation and gangrene of diabetic foot ulcers is increasing is alarming (Mathangi and Prabhakaran, 2013). Fifteen percent of people with diabetes will develop a foot ulcer at some time during their life and 85% of major leg amputations begin with a foot ulcer (Ramani *et al*., 1991).

Curcuma longa, known as turmeric, *ginger pupa* in Yoruba, is a medicinal plant that is botanically related to the ginger family, Zingiberaceae (Chattopadhyay *et al.*, 2004). Turmeric is widely used as a spice, food preservative and coloring material (McCaleb *et al*., 2000). There are several data indicating a great variety of pharmacological activities of turmeric, which exhibit antiinflammatory, anti-human immunodeficiency virus (HIV), antibacteria, antioxidant effects and nematocidal activities (Leon and Araujo, 2001) and its use for the treatment of tumors (Sharma *et al*,. 2005).

Turmeric contains phenolic compounds called curcuminoids that possess all the bio-protective properties of turmeric. Crude turmeric extracts have both antioxidant and antimicrobial capacities so that turmeric could be a potent alternative to common antibiotics. Turmeric extracts are found to show antibacterial activity against methicillin resistant *Staphylococcus aureus* (Kim, 2005, Smith-Palmer, 1998).

Clove spice belongs to the family of *Myrtaceae* in the genus *Syzygium* and scientifically named as *Syzygium aromaticum*, and known as "konafuru" in Yoruba language. Clove contains, among other compounds, gallotanins, triterpenes, flavonoids and phenolic acids. Oil derived from cloves contains additional compounds including b-caryophyllene and eugenol acetate. It contains an essential oil, eugenol which has been use for theraupetic purpose. Eugenol has been found to reduce blood sugar levels in diabetics. Clove oil is also used in aromatherapy. *Syzygium aromaticum* (clove) has been known to inhibit bacteria, certain fungi and parasites. Clove oil has been used for antiseptic for oral infections (Matan, 2006).

The antimicrobial activities of clove have been proved against several bacterial and fungal strains. Sofia *et al*. tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove (Sofia *et al.*, 2007). The only sample that showed complete bactericidal effect against all the food-borne pathogens tested *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* was the aqueous

extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action. The antibacterial activity of black pepper, geranium, nutmeg, oregano, thyme and clove have been tested against 25 strains of Gram positive and Gram negative bacteria (Dorman and Dean, 2000). The oils with the widest spectrum of activity were thyme, oregano and clove respectively. The antibacterial activity of clove, oregano, bay and thyme essential oil was tested against *E. coli* O157:H7 showing the different grades of inhibition of these essential oils (Burt and Reinders, 2003). Rana *et al*. (2011) determined the antifungal activity of clove oil in different strains and reported this scale of sensibility *Mucor sp*.>*Microsporum gypseum*>*Fusarium monoliforme* NCIM 1100>*Trichophytum rubrum*>*Aspergillus sp*.>*Fusariumoxysporum* MTCC 284. The chromatographic analyses showed that eugenol was the main compound responsible for the antifungal activity due to lysis of the spores. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was reported by Devi *et al* (2010).

II. Materials And Methods

Swab samples of Diabetic Foot Ulcer were collected from Diabetic Foot Ulcer patients at Endocrinology Department, Federal Medical Centre, Abeokuta, Ogun State, Nigeria. The swab samples were transported to the laboratory in a sterile bag containing ice packs.

The swab sticks were dipped into 9 ml normal saline for five minutes under sterile condition to allow the pus of the wound penetrate into the normal saline. Serial dilution was done from 10^{-1} to 10^{-6}. One ml of dilutions 10^{-2} and 10^{-4} was pipette into sterile petri dishes, sterile Nutrient Agar, MacConkey Agar, Manitol salt agar was poured into different sterile petri dishes. It was incubated (Gallenkamp incubator, Netherlands) for 24 hrs at 37^{o}c. For isolation of anaerobes, charcoal agar (transport medium) was prepared and the used swab stick was inserted in it. It was taken back to the laboratory and incubated at 37°c for 72 hrs.

Fresh rhizomes of *Curcuma longa* and flowers of *Sygizium aromaticum* were purchased at Lafenwa market in Abeokuta, Ogun state Nigeria.

Extraction method

Essential oil extract of *Curcuma longa* (turmeric) and *Sygizium aromaticum* (clove) were obtained by hydrodistillation (Kim *et al.,* 2008). The hydrodistillation was done using the Clavenger's apparatus. The Clavenger's apparatus comprises of the heating mantle, round bottom flask (3000 ml), condenser and the separating funnel. During hydrodistillation, the samples were exposed to temperature of 78^{o}C for turmeric, and 60^{0}c for Clove. The high temperatures lead to changes in thermolabile components. The essential oils were trapped in the condenser, collected and dried with anhydrous H_2SO_4 and kept in a brown bottle. They were stored in the refrigerator at a temperature of 4°C until use.

Sensitivity testing of the extract on the bacterial isolates

The sensitivity testing of the essential oils of clove and turmeric was determined using agar well diffusion method as described by Irobi *et al.*(1994), Russell and Furr (1997) with some modifications. The bacterial isolate were first cultured in nutrient broth for18 h before use. Exactly 0.2 ml of the standardized test isolates (0.5 McFarland standards) was then sub cultured into Muller-Hinton agar (Oxoid). The medium was allowed to set, wells were bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with the prepared solutions of the extract and care was taken not allow solution spill on the surface of the medium. The plates were allow to stand on the laboratory bench for 1 h to allow proper inflow of the solution into the medium before incubating the plates in an incubator (Gallenkamp, Netherlands) at 37^{0}C for 24 h. the plates were later observed for the zones of inhibition.

Phytochemical screening of the essential oil.

The essential oils were subjected to phytochemical screening using Trease and Evans (2002); Harborne (1998) methods to test for alkaloids, tannins, flavonoids, steroids, saponins and reducing sugars.

Determination of the extent and rate of kill of the test isolates by essential oils.

The extent and bactericidal rates were determined using the method described by Odenholt *et al.,* (2001) with some modifications. This was determined using each of the active fractions of *Enterococcus faecalis* representing the Gram-positive and *Pseudomonas aeruginosa* representing the Gram-negative isolates. Viable counts of the test organisms were initially determined. A 0.5 ml volume of known cell density (by viable count 10^6 cfu/ml) from each organism suspension was added to 4.5 ml of different concentrations of the essential oil. The suspension was thoroughly mixed and held at room temperature (28-30°C) and the killing rate was determined over a period of 2 h. Exactly 0.5 ml of each suspension was withdrawn at the appropriate time intervals and transferred to 4.5 ml nutrient broth (Oxoid Ltd) recovery medium containing 3% "Tween 80" to neutralize the effects of antimicrobial compounds carried over from the test suspensions. The suspensions were

shaken properly and serially diluted up to 10^{-5} in sterile physiological saline. Exactly 0.5 ml of the final dilution of the test organism was transferred into pre sterilized nutrient agar at 45^{o}C and plated out. The plates were allowed to set and incubated upside down at 37^{o}C for 72 h. Control experiment was set up without the inclusion of antimicrobial agent. Viable counts were made in three replicates for each sample. Depression in the viable count indicated killing by the antimicrobial agents.

DNA Extraction and Amplification of 16S rRNA gene

Bacteria genomic DNA was extracted from bacteria isolates using PureLink Genomic DNA kits (Invitrogen Life Technologies, CA, USA) followed by amplification of 16S rRNA gene in 25 µL reaction premix (17.8 µL of Nuclease Free H_2O, 2.5 µL of 10X Buffer, 1.5 µL of 25 mM $MgCl_2$, 1 µL of 5 mM dDNTP, 1 µL of forward primer, 1 µL of reverse primer and 0.2 µL of Taq DNA Polymerase enzyme) using 27F (5'-AGAGTTTGATCMTGGCTCAG–3') and 1492R (5'–ACCTTGTTACGACTT–3') primers (Lane, 1991). The conditions of PCR amplification were as follows: 15 min at 96 ^{o}C followed by 35 cycles of denaturing at 95 ^{o}C for 30 sec, annealing at 51 ^{o}C for 30 sec, and extension at 72 ^{o}C for 2 min, and a final extension at 72 ^{o}C for 7 min. PCR amplification was performed in a Thermocycler (Agilent Surecycler 8800).

The amplified 1500-bp fragments were resolved by electrophoresis on a 1% agarose gel prepared in 0.5X TBE, stained with Gelred™. The gel was run for 50 min at 100V and visualized under UV transilluminator. PCR product of the 16S rRNA amplified region was purified using QIAGEN PCR product purification kit [GmbH, D-40724 Hilden, Germany] and directly sequenced using 27F (forward primer) and 1492R (reverse primer) by Big dye Terminator method with ABI 3730 Genetic Analyzer at STAB VIDA Sequencing Technologies at Portugal.

Sequencing, editing and database matching

Bi-directional sequences obtained with forward and reverse primers were edited and aligned to generate a consensus sequence using BioEdit sequence Alignment Editor (version 7.1.9) (Hall, 1999). Consensus sequences were then aligned with sequences deposited in the National Centre for Biotechnological Information (NCBI) gene bank by using the Basic Local Alignment Search Tool (BLAST) to establish identities of the bacteria isolates.

III. Results

Morphology and biochemical identification of bacterial isolates.

The Gram positive bacteria isolated from DFUs include *Staphylococcus aureus* and *Enterococcus faecalis* while the Gram negative isolated are *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella paratyphi*, *Salmonella arizona, Enterobacter cloaca, Citrobacter specie* and *Enterobacter sp.*.

Essential oil extract obtained from *Curcuma longa* and *Syzygium aromaticum*.

The extract obtained from *Curcuma longa* was a light yellow liquid, smelling like a herb. The extract was stored in a brown bottle. The percentage yield of the essential oil extracted from *Curcuma longa* was 0.67% (W/V). The extract obtained from *Syzygium aromaticum* was a colourless, densed liquid, it was stored in a brown sterile bottle and kept in a refrigerator. The percentage yield of the essential oil obtained from *Syzygium aromaticum* was 5.23% (W/V).

Antimicrobial activities exhibited by the essential oils of *Curcuma longa* and *Syzygium aromaticum* against test isolates.

Table 1 shows the sensitivity pattern exhibited by essential oils of *Curcuma longa*, (Turmeric) and *Syzygium aromaticum* (Clove) against various strains of bacteria isolated in this study. For the combinations of Clove and Turmeric (CT), 86.21% of the isolates were susceptible to the activity of the essential oils of clove and turmeric with the zone of inhibition ranging from 11.0± 1.0 mm to 32.5 ± 2.5 mm. About 82.76% of the bacterial isolates were susceptible to the activity of essential oil of clove only. Zones of inhibition exhibited by the extract ranged between 9.5 ± 0.5 mm and 36.0 ± 1.0 mm. Turmeric only was able to inhibit 6.90% (that is 2 of the 29) of the bacterial isolates and zones of inhibition ranged between 7.0 ± 0.7 mm and 11.0 ± 1.0 mm. Therefore clove a lone (C) and a combination of clove and turmeric (CT) were selected for further studies, while turmeric alone (T) was discarded due to its relatively low antimicrobial activities.

Phytochemical screening of the essential oils of clove and turmeric.

The phytochemical screening of the essential oils of clove, turmeric and garlic showed the presence of terpenoids, the absence tannins, saponins, steroids and alkaloids as shown in Table 2.

Minimum inhibitory concentrations (MIC) and the Minimum bactericidal concentration (MBC) exhibited by the essential oils of clove and turmeric against the test isolates.

The minimum inhibitory concentration and the minimum bactericidal concentration exhibited by the test isolates are shown in table 3. The MIC exhibited by CT ranged between 0.90% and 3.75%. C also exhibited the same range of MIC which is between 0.9% and 3.75%. The MBC exhibited CT ranged between 0.9% and 3.75%. The MBC exhibited by C also ranged between 0.9% and 3.75%. There is no significant difference in the antimicrobial actions of the essential oils of CT (clove and turmeric) and C (clove only). Considering the antimicrobial activities of T (turmeric only) in table 1, it is apparent that C (clove only) does the whole job. Therefore clove only was selected for further studies.

The extent and rate of kill of *Enterococcus faecalis* exhibited by clove essential oil

The extent and the rate of kill of *Enterococcus faecalis* exhibited by the clove extract at 1 X MIC, 2 X MIC and 3 X MIC are shown in figure 1. The percentage of the cells killed by the essential oil at 1 X MIC in 5 minutes was 83.09% while the percentage of the cell killed at 10 minutes was 96% and at time 15 minutes, all the cells were already killed. When the MIC was doubled, the percentage of cells killed by clove essential oil within 5 minutes was 67%. At time10 minutes, all the cells were already killed. At MIC X 3, 100% kill was achieved within the first 5 minutes.

The extent and rate of kill of *Pseudomonas aeruginosa* exhibited by clove essential oil.

The extent and the rate of kill of *Pseudomonas aeruginosa* exhibited by clove extract at 1 X MIC, 2 X MIC and 3 X MIC are shown in figure 2. The percentage of the cells killed by the essential oil at MIC X 1 at 5 minutes was 83%. The percentage at 10 minutes was 94% and after 15 minutes 100% kill was achieved. At MIC X 2, the percentage killed at 5 minutes was 70% and at 10 minutes, a 100% was achieved. At MIC X 3, 100% kill was achieved within the first 5 minutes.

Table 1: The sensitivity patterns exhibited by the essential oils of clove and turmeric against the test isolates.

ISOLATES	**CT**	**C**	**T**
*Salmonella paratyphi_*	20.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
E. coli	23.0 ± 2.0	22.5 ± 2.5	0.0 ± 0.0
Salmonella arizona	0.0 ± 0.0	18.0 ± 2.0	0.0 ± 0.0
Proteus mirabilis	32.5 ± 2.5	31.0 ± 1.0	11.0 ± 1.0
*Salmonella paratyphi_*	19.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
*Salmonella paratyphi_*	23.0 ± 2.0	0.0 ± 0.0	0.0 ± 0.0
Salmonella arizona	16.0 ± 2.0	12.0 ± 2.0	0.0 ± 0.0
P. aeruginosa	14.5 ± 2.5	16.0 ± 1.0	0.0 ± 0.0
P. fluorescens	6.0 ± 6.0	13.5 ± 1.5	0.0 ± 0.0
P. fluorescens	15.0 ± 1.0	22.0 ± 2.0	0.0 ± 0.0
E. coli	18.0 ± 2.0	20.0 ± 2.0	0.0 ± 0.0
P. aeruginosa	20.0± 0.0	24.5 ± 0.0	7.0 ± 0.7
Salmonella arizona	15.5 ± 1.5	22.0 ± 2.0	0.0 ± 0.0
Proteus mirabilis	26.5 ± 5.5	36.0 ± 1.0	0.0 ± 0.0
P. aeruginosa	16.5 ± 2.5	32.5 ± 2.5	0.0 ± 0.0
E. coli	18.5 ± 1.5	22.5 ± 2.5	0.0 ± 0.0
Proteus vulgaris	15.5 ± 3.5	18.5 ± 1.5	0.0 ± 0.0
Staphylococcus aureus	0.0± 0.0	19.5 ± 0.5	0.0 ± 0.0
E. coli	12 ± 0.0	17.6 ± 0.1	0.0 ± 0.0
*Salmonella paratyphi_*	30 ± 0.0	35.0 ± 0.0	0.0 ± 0.0
Enterobacter cloacae	0.0± 0.0	0.0± 0.0	0.0 ± 0.0
P. aeruginosa	16.0 ± 3.0	15.5 ± 1.5	0.0 ± 0.0
P.fluorescens	0.0± 0.0	0.0± 0.0	0.0 ± 0.0
P. aeruginosa	0.0± 0.0	9.5 ± 0.5	0.0 ± 0.0
Enterococcus faecalis	14.5 ± 2.5	20. ± 0.5	0.0 ± 0.0
Salmonella arizona	11.0± 1.0	16.0 ± 4.0	0.0 ± 0.0
Proteus mirabilis	24.5 ± 2.5	20.0 ± 0.0	0.0 ± 0.0
Enterobacter sp	13.5 ± 1.5	16.0 ± 4.0	0.0 ± 0.0
Citrobacter sp	11.0 ± 1.0	16.5 ± 2.5	0.0 ± 0.0

KEY: C = Clove, CT= Clove and Turmeric,

Table 2: Phytochemical screening of the essential oils of clove, turmeric and garlic

Chemical test	Clove	Turmeric	Garlic
Terpenoids	Positive	Positive	Positive
Tannins	Negative	Negative	Negative
Saponins	Negative	Negative	Negative
Steroids	Negative	Negative	Negative
Alkaloids	Negative	Negative	Negative

Table 3: The minimum inhibitory concentrations and minimum bactericidal concentrations exhibited by the essential oils of clove and turmeric against the test isolates

S/N	Isolates	CT		C	
		MIC (%)	MBC (%)	MIC (%)	MBC (%)
1	*Salmonella paratyphi*	3.75	3.75	3.75	3.75
2	*E. coli*	3.75	3.75	3.75	3.75
3	*Salmonella arizona*	1.80	0.90	1.80	1.80
4	*Proteus mirabilis*	0.90	0.90	0.90	0.90
5	*Salmonella paratyphi*	3.75	3.75	0.00	0.00
6	*Salmonella paratyphi*	3.75	3.75	3.75	3.75
7	*Salmonella arizona*	1.80	1.80	1.80	1.80
8	*P. aeruginosa*	1.80	1.80	1.80	1.80
9	*P. fluorescens*	0.90	0.90	0.90	0.90
10	*P. fluorescens*	0.90	0.90	0.90	0.90
11	*E. coli*	0.90	0.90	1.80	1.80
12	*P. aeruginosa*	0.90	0.90	0.90	0.90
13	*Salmonella arizona*	0.90	0.90	0.90	0.90
14	*Proteus mirabilis*	1.80	1.80	0.90	1.80
15	*P. aeruginosa*	0.90	0.90	0.90	0.90
16	*E. coli*	0.90	0.90	0.90	0.90
17	*Proteus vulgaris*	0.90	0.90	0.90	0.90
18	*Staphylococcus aureus*	0.90	0.90	0.90	0.90
19	*E. coli*	0.90	0.90	0.90	0.90
20	*Salmonella paratyphi*	0.90	0.90	0.90	0.90
21	*Enterobacter cloaca*	0.90	0.90	0.90	0.90
22	*P.aeruginosa*	0.90	0.90	0.90	0.90
23	*P.fluorescens*	0.90	0.90	0.90	0.90
24	*P.aeruginosa*	0.90	0.90	0.90	0.90
25	*Enterococcus faecalis*	3.75	3.75	3.75	3.75
26	*Salmonella arizona*	3.75	3.75	3.75	3.75
27	*Proteus mirabilis*	1.80	0.90	1.80	1.80
28	*Enterobacter sp*	0.90	0.90	0.90	0.90
29	*Citrobacter sp*	3.75	3.75	0.00	0.00

KEY: C = Clove, CT= Clove and Turmeric,

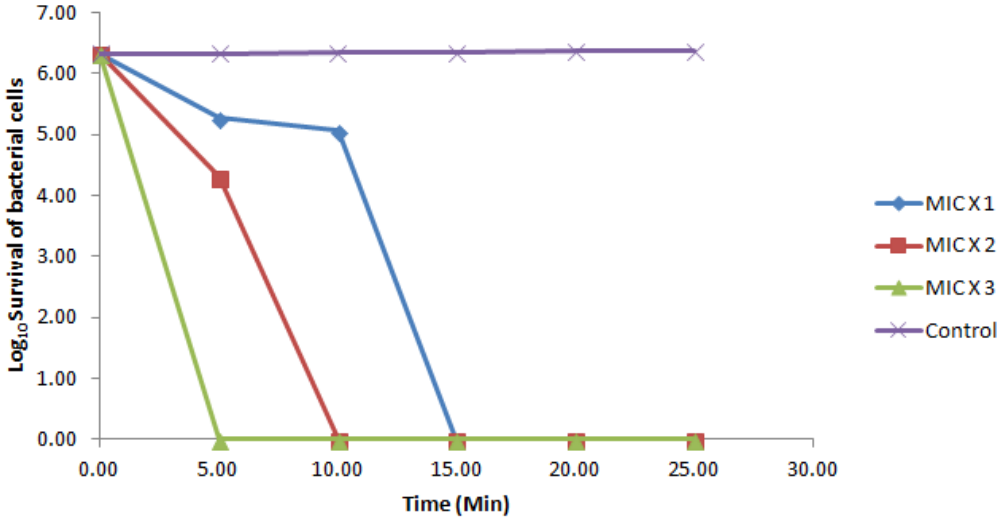

Figure 1: The extent and rate of kill of *Enterococcus faecalis* exhibited by clove essential oil at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the mean $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the oil.

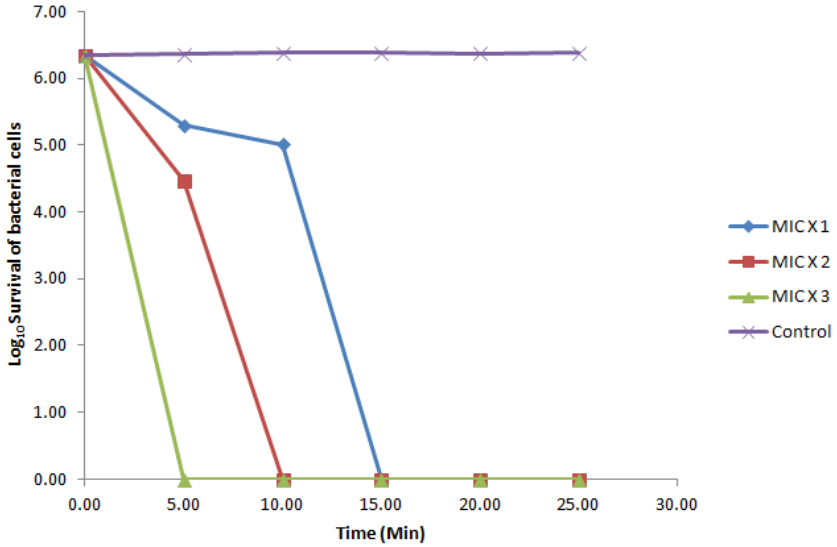

Figure 2: The extent and rate of kill of *Pseudomonas aeruginosa* exhibited by clove essential oil at 1 x MIC (), 2 x MIC (), 3 x MIC () and control (). Each point represents the mean $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the oil.

IV. Discussion

Essential oil of plant have been used for ages for antimicrobial activities (Sartoratto *et al.*, 2004). The essential oils of clove, turmeric and garlic have their active ingredients to be eugenol, curcumin and allicin respectively (Jirovetz, 2006). Curcumin have been found to have antimicrobial property so also is allicin and eugenol (Ankri and Mirelman, 1999). From this study, Turmeric only was able to inhibit 6.90% of the bacterial isolates. Turmeric essential oil was not active against *E. coli* isolated in this study. Rath *et al.* (2001) found that turmeric rhizome oil exhibit antibacterial activity against *Escherichia coli.* This result might be due to differences in strains of *E.coli*, variation in climate, altitude, soil composition, method of extraction and sample collection season (Ogunwande *et al*, 2005). Furthermore, antimicrobial properties can differ significantly within the same herb or spice species as the proportions of individual components in essential oils of plants are affected by genotype (Arrebola., *et al* 1994).

Clove only (C) and the combinations of clove and turmeric (CT) showed the same antimicrobial activity by inhibiting 82.76% of the isolates. This is a clear indication that only clove got the job done. The results of this study were also in agreement with the findings of Matan *et al.* (2006), who reported complete inhibition of *Staphylococcus aureus* by application of essential oil of clove.

The phytochemical analysis of the essential oils of clove, turmeric and garlic reveals the presence of terpenoids and the absence of Tannins, Reducing sugar, Flavonoid, Steroids, Saponins and Alkaloids. The absence of Tannins, Reducing sugar, Flavonoid, Steroids, Saponins and Alkaloids maybe due to the fact that the extract is an essential oil and essential oils contain only aromatic compounds that serve as the active ingredient (Shipra *et al*., 2012).

In vitro time-kill assays are expressed as the killing rate by a fixed concentration of an antimicrobial agent and are among the most reliable methods for determining tolerance (Nostro *et al*., 2001). The bactericidal rate of the essential oils of clove was determined using *Enterococcus faecalis* as the Gram positive representative and *Pseudomonas aeruginosa* as the representative of the Gram negative. From this study, it was observed that rate at which the essential oil eliminated the cells increased with increase in the concentration of the essential oil and contact time. For *Enterococcus faecalis* at MIC X 1, in 5 minutes 83% cells were killed by the essential oil, at 10 minutes, percentage killed was 96% and at 15 minutes, 100% bactericidal rate was achieved. It was also observed when MIC was doubled. For *Pseudomonas aeruginosa*, at MIC X 1, when the time was 5 minutes, 78% cells were killed. There was 94% bactericidal rate at 10 minutes and 100% killed rate was achieved at 15 minutes. Moreira *et al.* (2005) also reported that clove essential oil used at MIC level against *E. coli* exerted its maximum bactericidal effect after 15 min and its action was maintained during 20 h of incubation. The rate at which the essential oil eliminates these organisms at the shortest period of time is the generally accepted definition of the bactericidal activity in antibiotics as described by Pankey and Sabath, (2004). This ability of the plant extract to inhibit or kill these pathogens at minimum contact time and low concentration is an indication that clove essential oil could serve in preventing establishment of infections of diabetic foot ulcer caused by these pathogens.

V. Conclusion

The combination of turmeric and clove did show appreciable antimicrobial properties, however clove only was able to inhibit a larger percentage of the microorganisms. The essential oil of clove exhibited bactericidal activity on *Enterococcus faecalis* as well as on *Pseudomonas aeruginosa*, making it a broad spectrum essential oil. *Pseudomonas aeruginosa*, was susceptible to the bactericidal activity of clove essential oil at the smallest contact time and a low concentration. *Pseudomonas aeruginosa* is known to be inherently resistant to antibiotics but was susceptible to clove essential oil. This could serve as a pointer towards the development of antimicrobial compound of natural origin from clove to combat wound infection by pathogenic bacteria.

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APPENDIX

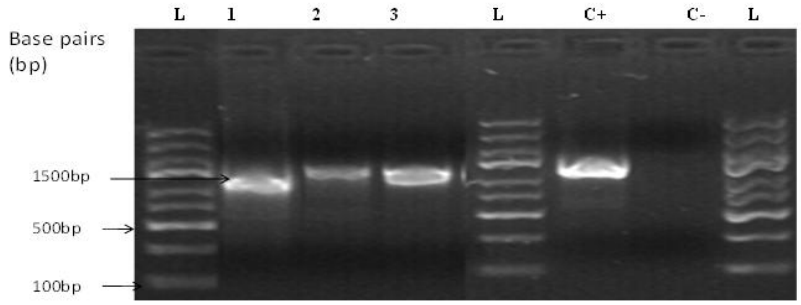

Plate 1: Electrophoresis in a 1.5% agarose gel of PCR-amplified 16S rRNA of the bacterial isolates from diabetic foot ulcers.

Keys: bp= base pair ladder, -: 1- *Salmonella enterica subsp enterica serovar Typhi,* 2- *Pseudomonas aeruginosa*, 3- *Enterococcus faecalis*. C+- Positive control , C—Negative control.