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Antimicrobial Activity of Essential Oil and Extracts of *Gongronema latifolium* Decne on Bacterial Isolates from Blood Stream of HIV Infected Patients

I.A. Adeleye, M.E. Omadime and F.V. Daniels

ABSTRACT

The essential oil as well as aqueous and ethanolic extracts of *Gongronema latifolium* leaves was evaluated for antimicrobial activity against bacteria isolated from blood streams of HIV patients in Lagos. Using agar diffusion method, the essential oil and the extracts showed moderate inhibitory activity against all the *Stphylococcus* sp., *Escherichia coli*, *Shigella* sp., *Salmonella* sp., *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Onchrobactrum anthropi* and *Candida albicans*. The zones of inhibition values recorded were comparable to control antibiotic ampicillin but less than that of Ciprofloxacin and Chloramphenicol. The MIC for essential oil ranged between 5-40 µg mL^{-1}, while MBC also ranged between 5-40 µg mL^{-1}, the MIC and MBC for ethanol extract ranged between 3.125-12.5 mg mL^{-1} and 3.125-25.0 mg mL^{-1}, while aqueous extract MIC range between 6.25-25.0 mg mL^{-1} and MBC also ranged between 6.25-25.0 mg mL^{-1}, respectively. Extracts of *Gongronema latifolium* may be useful in ethnomedicine and in the treatment of blood stream infections in HIV patients. Essential oil from *Gongronema latifolium* leaves (Endl.) Decne was obtained by hydrodistillation and analyzed using Gas Chromatography/Mass Spectrophotometry (GC-MS). The oil was dominated by linear aliphatic compounds (27.06%),unsaturated fatty acids which was characterized by high percentage of Phthalic acid (18.61%), oleic acids (5.2%), arachidic acid (2.34%) and fumaric acid (2.22%). Monoterpenes including camphor, β-Cymene and phytol.

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INTRODUCTION

Medicinal plants constitute an effective source of both orthodox and traditional medicine, herbal medicine has been shown to have genuine utility with about 80% of rural dwellers depending solely on it for primary health care (Akinyemi *et al.*, 2005).

In Nigeria, over 300 plants are used for treating various diseases including HIV/AIDS opportunistic infections such as pneumonia, diarrhea, typhoid fever, candidiasis, tuberculosis and other ailments (Sofowora, 1986; Enwereji, 2008).

Medicinal plants are known to owe their curative potentials to a certain biological active substances which are refered to as active principles or phytochemical substances and these include terpenes, flavonoids, saponins, anthraquinones, glycosides etc (Iwu *et al.*,1993).

Essential oils are important constituents of some higher plants comprising monoterpenes, sesquiterpene, arylpropanoids and fatty acid derivatives. They have been recognized long ago to possess antimicrobial activities (Del-Vechio *et al.*, 2009).

Gongronema latifolium (Endl.) Decne commonly called Utazi and Arokeke or Madumaro in the South eastern and south western part of Nigeria respectively belongs to the family Asclepiadaceae. It is a tropical rain forest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2003; Ugochukwu *et al.*, 2003). Few studies have been carried out on the phytochemical composition, antimicrobial properties and essential oil constituents of the plant. Aqueous and Ethanolic extracts of the plant have been reported to have inhibitory effect on pathogenic microoganisms (Eleyinmi, 2007). It's hypoglycemic, hypolipidemic and antioxidative properties had also been reported (Ugochukwu and Babasdy, 2003). Due to wide spread use of this plant by traditional healers detail scientific investigation is needed.

This present study was therefore embarked upon to achieve the following objectives; (1) To identify the essential oil constituents (2) determine the antibacterial and antifungal effects of the essential oil and other extracts of the plant on bacteria and Yeast causing blood stream infection in HIV patients in Lagos with a view to determine whether this plant can serve as an alternative medical therapy for managing HIV blood stream infections in this resource limited environment.

MATERIALS AND METHODS

Sources and collection of plants material: Fresh plant samples of *Gongronema latifolium* were collected from Ikorodu market and Mile 12 Market in Kosofe Local Government Area, Lagos State. The fresh leaves samples were authenticated at the Department of Botany and Microbiology, Faculty of science, University of Lagos. This study commenced in November 2008 and was concluded in September 2009.

Phytochemical screening: Preliminary phytochemical tests as described by Harbone (1984), Sofowora (1986) were carried out on the aqueous extract of *Gongronema latifolium*.

These tests involve the addition of appropriate chemical agents to the aqueous extracts of the plant in a test tube.

Alkaloids, Saponins, Tannins, flavonoids and other compounds were tested for using these methods.

Preparation of extracts: The leaves sample were rinsed and air-dried. They were further dried in vacuum oven at 50°C for 10-15 h. The leaves were milled completely into coarse powder by grinding. The powdery form of the leaves was further treated to extract active ingredient.

Aqueous extract: Aqueous extract was carried out as described by Eleyinmi (2007) and Adeleye *et al.* (2008a). One hundred and eighty gram of dried milled leaves powder was soaked in 300 mL of sterile distilled water for 5 days at 4°C. The solution (i.e., powdered leaves and water) was centrifuged at 10,000 rev min^{-1} for 5 min and was filtered with Whatmann No. 1 filter paper.

The filtrate was poured into a 250 mL beaker and labeled appropriately. The filtrate was dried at 50°C for 2 weeks until a constant dry weight of the extract was obtained in a Vacuum oven.

Ethanol extract: Method of ethanol extraction was similar to the aqueous extraction as described by Eleyinmi (2007) and Adeleye (2008a). One hundred and eighty gram powder leaves material was soaked in 300 mL of 70% ethanol for 5 days at room temperature.

The solution was also centrifuged at 10,000 rev min^{-1} for 5 min and this was filtered with whatman No. 1 filter paper into 250 mL conical bottle flask. The ethanol filtrate was placed in a vacuum oven at 50°C and dried for 2 weeks to evaporate the alcohol. This was also labeled accordingly.

Extraction of essential oil: Extraction of essential oil from vegetable material was carried out by hydro-distillation process as described by Nenad *et al.* (2007).

Anti-microbial assay

Sources of microorganisms: The test organisms employed for screening plant for antimicrobial activity of essential oil and *Gongronema latifolium* extracts were isolates from the blood stream infections of HIV infected patients obtained from the Lagos University Teaching Hospital Complex (LUTH) and Nigeria Institute of Medical Research (NIMR) Yaba, Lagos (Adeleye, 2008b).

The organisms were *Shigella dysentriae*, *S. flexneri*, *Staphylococcus aureus*, *S. chromogenes*, *S.c. cohnii, S.c. urealyticum*, *S. warnei*, *S. sciuri*, *S. epidermdis*, *Escherichia coli*, *Salmonella typhi*, *S. typhimunium*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *P. flourescence*, *Onchrobactrum anthropi* and *Candida albicans*.

They were all sub-cultured unto fresh nutrient agar plates and incubated 24 h before use.

Preparation of inoculum: Active cultures for screening were prepared by transferring a loopful of cells from the stock cultures to test tube of Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi and were incubated without agitation for 24 h at 37°C and 72 h at 25°C, respectively. The cultures were serially diluted with fresh Mueller-Hinton broth and Sabouraud dextrose broth to achieve a McFarland standard of 0.5 corresponding to a cell density of $1.5x10^8$ cfu mL^{-1} for bacteria and $1.5x10^5$ spore mL^{-1} for fungal.

These were use to inoculate the Mueller-Hinton plates by using 0.1% inoculum suspension to swab uniformly using sterile cotton wool.

Antimicrobial screening: The agar diffusion method of NCCLS in 2003 was employed for the screening of antimicrobial activities of extracts. Sterile cork borer of 6.0 mm diameter were used to bore holes into the organisms seeded plates and three drops of the reconstituted water and ethanol extract were dropped, into the holes.

Three drops of essential oil was also dropped in other holes. Sterile distilled water was used as positive control, while Ampicillin, Ciprofloxacin and Chloramphenicol were used as negative control. These were done in triplicates under aseptic condition.

All the plates containing the test organisms and extracts (Ethanol, Water and Essential oil) were incubated at 37°C for 24 h for bacteria and at 25°C for 48 h for yeast, respectively. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* NCTC 10148 were used as standard test organisms. Zones of inhibition were measured in millimeters (mm).

Minimal Inhibitory Concentration (MIC): The Minimum inhibitory concentration of the extract (aqueous and ethanol extracts were determined for each of the isolate using dilution susceptibility test. Two fold serial dilutions of each extract i.e., 100 mg mL^{-1} was carried out in seven tubes containing sterile 2 mL Mueller Hinton broth to give the following extract concentration of 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg mL^{-1}.

A loopful of the standardized test organisms was inoculated aseptically into the tubes containing the serially diluted extracts and incubated at 37°C for 24 h.

Minimum Bactericidal Concentration (MBC): This is the lowest extract concentration at which the organisms did not recover and grow when transferred into a fresh medium. This was determined by subculturing from the tubes not showing visible growth after 24 h on MacConkey agar plates for bacteria and incubated at 37°C for 24 h and Sabouraud dextrose agar plate for yeast at 25°C after 48 h.

Essential oil-MIC and MBC Determination: Serial dilutions of the essential oil was done using 10% Tween 80 in sterile nutrient broth and Sabouraud dextrose broth for bacteria and yeast (*Candida albicans*), respectively.

This was to facilitate essential oil dispersion. The 0.5 mL of the essential oil was added to 2 mL of the media and double fold serial dilution was carried out to give a concentration of 1, 2, 5, 10 and 40 µg mL^{-1}. Each strain of test organism was tested with essential oil by inoculating with 50 mL physiological saline containing $5x10^6$ cells for bacteria and $5x10^5$ for Yeast, respectively. They were incubated at 35°C for 18-24 h (bacteria) and at 22°C for 48-72 h (yeast) (Panizzi *et al.*, 1993; Del-Vechio *et al.*, 2009). MIC and MBC were determined as described above by sub-culturing from the tubes not showing visible growth after 24 h on MaConkey agar and sabouraud dextroxe agar and incubated at 37°C for 24 h (Bacteria) and 25°C for 48 h (yeasts). Results were recorded in millimeter after measuring the diameter of zone of inhibition.

GC/MS analysis of essential oils components: This was carried out using Gas Chromatography/Mass Spectrophotometry (GC/MS) method to analyze and identify the essential oil constituents.

The Gas chromatographic analyses/Mass spectrophotometry was performed with Agilent system consisting of a model 7890A, with the following parameters: Column 30x0.25 mm, idx0.25 µm HP-5 m sec fused silical capillary with a (5%phenyl)-methylpolysiloxane stationary phase (Agilentpart No. 19091S-433). Oven Temperature programme: 50 to 250°C gradient of 10^oC/min, up to 200°C; injector and detector temperature 250°C; Carrier gas Helium (3.325 mL min^{-1}), sample size: 0.5 µL.

The compounds of the oil were identified using their retention time indices(determined with reference to a homologous series of normal alkanes) and by comparison of their mass spectral fragmentation patterns(NIST data base (G 1 036 A,revision D.O.I.00/chem. Station data system (G170ICA, version CO.OO.1.08))13 and with data available in common literatures.

RESULTS

Phytochemical screening: Phytochemical screening revealed the presence of compounds such as Saponins, Alkaloids, Pyhlobatinnins, Glycosides and Flavonoids and the absence of Tannin (Table 1).

The results antimicrobial activity of aqueous, ethanolic and essential oil extracts of *G. latifolium* are shown in Table 2.

Staphylococcus sp., *Shigella* sp., *Salmonella* sp., *Klebsiella Pneumonia*, *Pseudomonas* sp., *Escherichia coli* and *Onchrobactrum anthropi* were all inhibited at 100 mg mL^{-1}. The essential oil showed the highest antimicrobial and fungicidal effects against all the test organisms including *Candida albicans* compared to aqueous and ethanolic extracts as zones of inhibition ranged between 7.5 mm for *Pseudomonas aeruginosa* to 11.25 mm for *Shigella flexneri*, respectively.

Zones of inhibition of Aqueous extract at 100 mg mL^{-1} ranged from 7.2 mm (*Klebsiella pneumonia*) to 10.00 mm for *Staphylococcus urealyticus*, respectively. Ethanolic extract also ranged from 7.5 mm for *Klebsiella pneumonia* to 11.0 mm for *Escherichia coli* at 100 mg mL^{-1}, respectively.

Table 1: Bioactive compounds *in Gongronema latifolium*

	Saponins	Alkaloids	Flavonoids	Glycosides	Tannins	*Phylobatinnins*
Gongronema latifolium	+	+	+	+	_	+

Table 2: MIC and MBC of aqueous, ethanolic and essential oil extracts

	Aqueous extract			Ethanolic extract			Essential oil		
Test organisms	MIC (mg mL^{-1})	MBC (mg mL^{-1})	Zone of inhibition (mm)	MIC (mg mL^{-1})	MBC (mg mL^{-1})	Zone of inhibition (mm)	MIC (µg mL^{-1})	MBC (µg mL^{-1})	Zone of inhibition (mm)
Staphylococcus. urealyticus	6.25	6.25	10.0	6.25	12.50	10.0	5.0	5.0	10.00
Staphylococcus C. aureus	6.25	6.25	9.0	6.25	12.50	9.3	10.0	10.0	10.50
Staphylococcus chromogene	6.25	6.25	9.3	6.25	12.50	9.0	5.0	10.0	10.00
Staphylococcus cohnii cohnii	12.50	25.00	8.8	12.50	25.00	9.8	5.0	10.0	10.00
Staphylococcus sciuri	12.50	12.50	9.5	12.50	25.00	10.0	5.0	10.0	10.70
Staphylococcus warneri	6.25	12.50	9.5	12.50	25.00	9.5	5.0	5.0	9.75
Staphylococcus epidermidis	6.25	6.25	9.5	12.50	25.00	9.8	5.0	5.0	10.00
Pseudomonas fluorescence	6.25	6.25	9.3	3.125	3.125	9.5	5.0	10.0	9.75
Pseudomonas aeruginosa	6.25	6.25	9.0	3.125	3.125	7.8	10.0	10.0	8.50
Shigella flexneri	6.25	12.50	9.7	6.25	12.50	10.3	10.0	10.0	11.25
Shigella dysentriae	12.50	12.50	10.0	6.25	12.50	10.3	5.0	20.0	11.00
Salmonella typhi	12.50	25.00	8.2	3.125	6.25	9.5	20.0	20.0	10.00
Salmonella typhimurium	12.50	25.00	7.5	3.125	6.25	8.3	10.0	20.0	7.50
Klebsiella Pneumonia	25.00	25.00	7.0	3.125	6.25	7.3	10.0	40.0	6.30
Onchrobactrum anthropi	12.50	12.50	6.2	6.25	12.50	6.5	40.0	10.0	6.30
Escherichia coli	6.25	6.25	6.5	6.25	12.50	7.0	10.0	10.0	7.00
Candida albicans	-	-		-	-		5.0	10.0	11.20
Escherichia coli NCTC 10148	6.25	6.25	7.0	6.25	12.50	7.8	10.0	10.0	7.00
Staphylococcus aureus ATCC 25923	6.25	12.50	8.6	12.50	12.50	9.0	10.0	10.0	8.40

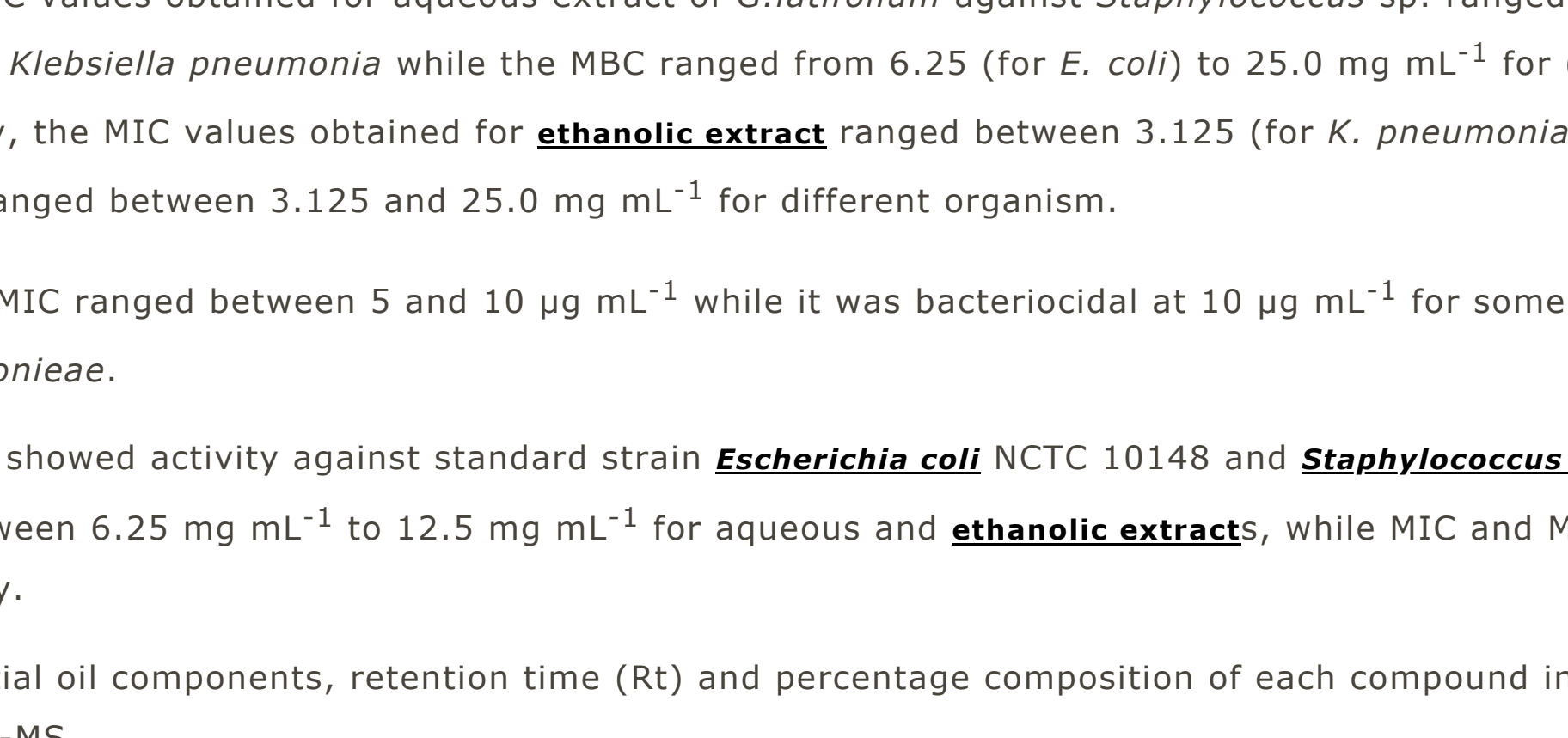

Fig. 1: (a, b)Bar chart representing zones of inhibition of extract @100 mg mL^{-1}

However, when compared to Ampicillin, Ciprofloxacin and chloramphenicol at 250 mg, the diameter recorded were less (Ampicilline 22.0 mm), (Ciprofloxacine 28.0 mm) and (Chloramphenicol 23.0 mm) (Fig. 1a, b).

Antimicrobial screening: The MIC and MBC values obtained for the crude extracts and essential oil varied from one organism to the other, for instance the MIC values obtained for aqueous extract of *G.latifolium* against *Staphylococcus* sp. ranged from 6.25 to 12.5 mg L^{-1} and 25.0 mg mL^{-1} for *Klebsiella pneumonia* while the MBC ranged from 6.25 (for *E. coli*) to 25.0 mg mL^{-1} for (*Staphylococcus cohnii cohnii*) (Table 2). Similarly, the MIC values obtained for ethanolic extract ranged between 3.125 (for *K. pneumonia*) and 12.5 mg mL^{-1} (for *S. scuri*) while the MBC ranged between 3.125 and 25.0 mg mL^{-1} for different organism.

For the essential oil, the MIC ranged between 5 and 10 µg mL^{-1} while it was bacteriocidal at 10 µg mL^{-1} for some *Staphylococcus* sp. and 40 µg mL^{-1} for *K. pneumonieae*.

However, all the extracts showed activity against standard strain *Escherichia coli* NCTC 10148 and *Staphylococcus aureus* ATCC 25923 and MIC and MBC ranged between 6.25 mg mL^{-1} to 12.5 mg mL^{-1} for aqueous and ethanolic extracts, while MIC and MBC for the essential oil was at 10 µL, respectively.

Table 3 showed the essential oil components, retention time (Rt) and percentage composition of each compound in *Gongronema latifolium* leaves as analyzed by GC-MS.

Up to 56 peaks were detected in the Chromatogram of the oil and this represented 98.44% of the oil components. The leaf oil was characterized by the abundance of fatty acids. Total phthalic acids present (18.61%), fumaric acids (2.22%), oleic acids (5.2%), arachidic acids (2.34%).

The linear aliphatic compounds constitute (27.06%) tricontane (6.51%), dodecane (1.36%). Alphatic alcohols were present in fairly amount; methanol 0.84%, Pentadecanol 0.44%, Hexaconsanol 1.66%, policosanol 1.04%. Monoterpenes were also present in minute quantities; p-cymene 0.41%), camphor (1.20%) and Phytol (1.66%).

Table 3: Compounds identified from essential oil of *Gongronema latifolium*

IUPAC name	Common name	Retention time	Composition (%)
Dichloroxylenol	β-cymene	11.391	0.41
Hexadecane	Hexadecane	14.107	2.72
Dodecane	Dodecane	14.190	1.36
Octadecane	Octadecane	14.293	1.69
Nonadecane	Pelargonic	14.360	0.99
Heptadecane	Magaric acid	14.446	0.67
Eicosane	Arachidic acid	14.469	0.57
n-Octadecane	Octadecane	15.024	1.95
I-Bromodocosane	Camphor	15.144	1.20
Octadecane	Octadecane	15.144	1.20
2-bromododecane	Bromodocane	15.815	10.49
Hexadecane	Hexadecane	15.871	4.88
Triacontane	Tricontane	16.565	5.36
3_Chloro-IH-Pyridoquinoline	Quinoline	16.648	1.58
7,12-Dihydrobenzo(k) Fluoranthene	Fluoranthene	16.678	0.48
Ergoline-8-methanol	n-Methanol	16.721	0.84
2,4-dimethylpent-3-y-1undecyl ester	Fumaric acid	16.824	2.22
6H-indolo(2,3-b)quinoxaline	Quinoline	16.824	1.22
Octadecanoic acid	Oleic acid	16.878	1.36
Hesatriacotane	Hexatriancontane	17.163	5.46
1-nonadecene	Nonadecene	17.279	2.20
2-Pentadecanol	n-alcohol	17.711	0.44
4-trifluoroacetoxytradecane	Trifluoroacetate	17.944	1.87
1.2 Benzenedicarboxylic acid	Phthalic acid	18.083	6.06
Nonacosane	Nonacosane	18.302	1.18
Eicosyl acetate	Arachinoic acid	18.442	1.27
Octacosyl acetate	Linanyl acetate	18.545	0.51
1-H exacosanol	oleic acid	19.026	1.50
n-prophyl 11-octadecenoate		19.133	0.23
1,2-Benzenedicaboxylic acid	Phthalic acid	19.219	2.40
Trans-13-Octadecenoic acid	Oleic acid	19.302	2.26
3-Beta-acetoxy-6 nitroandrost	Phytol	20.970	1.66
1-hexacosanol	n- alcohol	21.282	1.66
Phthalic acid cycloheptyl isohesy ester	Phthalic acid	25.437	10.15
Eicosane	Arachidic acid	26.902	0.12
Eicosane	Arachidic acid	26.995	0.13
Octacosanol	Policosanol	27.420	1.04
Tetratriacontane,I-bromo		27.579	0.97
tricontane	Tricontane	27.835	1.15
Otacosane	Otacosane	27.885	0.60
1-hexacosene	Hexacosene	27.948	1.00
Eicosane	Arachidic acid	28.018	0.42
Octadecane	Octadecane	28.164	1.43
9-o.Methymaytansinol		28.253	1.11
Octacosane	Octacosane	28.363	1.25
Tricosane	Tricosane	28.393	0.27
Octacosane	Octacosane	28.459	0.77
Tetratriacontyl pentafluoro propion		28.669	2.86
Hexacosane	Hexacosane	28.695	0.54
Tricosane	Tricosane	28.722	0.47
Doctriacontyl heptafluro butyrate		28.758	1.03
Dotriacontyl trifluorocetrate	Trifluorocetrate	28.825	0.39
Cyclopntane	Cyclopentane	29.021	1.13
Eicosane	Arachidic acid	29.097	0.40
Cyclopentane	Cyclopentane	29.137	0.30
Octacosane	Octacosane	29.552	2.81

DISCUSSION

GC-MS analysis of *Gongronema lafifolium* obtained in Lagos for this study revealed high content of aliphatic linear compounds, unsaturated fatty acids and monoterpenes. Ogunwande *et al.* (2005) had also reported the presence of, camphor, phytol and phenol compounds and this is in line with our present findings.

Phytochemical screening of *Gongronema latifolium* plant extracts revealed the presence of Saponin, Alkaloids, Glycosides, Flavonoids. This is in line with the findings of Morebise and Fafunso, (1998). These compounds of *Gongronema latifolium* leaves extracts have been reported to have hypoglycemic, hypolipidemic ,antioxidiative and anti-inflammatory properties (Ugochukwu *et al.*, 2003; Morebise *et al.*, 2002).

The aqueous, ethanolic and essential oil extracts of *Gongronema latifolium* showed varying degrees of activities against organisms causing blood stream infections in HIV patients. Previous studies by Eleyinmi, (2007) has revealed antimicrobial activity of methanol extracts of *Gongronema latifolium* against *Salmonella enteritidis*, *Salmonella cholerasius ser typhimurium* and *Pseudomonas aeruginosa*.

Nwinyi *et al.* (2008) had also reported the inhibitory activities of aqueous and ethanolic extracts of *Gongronema latifolium* against *Staphylococcus aureus* and *Escherichia coli*, with MIC ranging between 2.5 and 10 mg mL^{-1} for ethanolic and aqueous extracts, respectively.

This is similar to our findings, as ethanolic extract of *Gongronema latifolium* inhibited all the organisms tested with MIC and MBC ranging from 3.125 mg-12.5 mg mL^{-1} and 3.125 mg-25.0 mg mL^{-1}, respectively. However, aqueous extract was less active and this may be due to the fact that the active ingredients are more soluble in ethanol than in water.

The essential oil evaluated in these studies was found to have shown the highest antimicrobial activity against tested organisms including *Candida albicans* as larger zones of inhibitions for MIC and MBC ranging between 5-40 and 5-40 µg mL^{-1}, respectively were recorded when compared to aqueous and ethanolic extracts. This findings agrees with Eleyinmi (2007) who reported the antimicrobial effect of *Gongronema lafifolium* fatty acids against *Staphylococcus aureus*, *Salmonella* sp and *Escherichia coli*. However, it is at variance with the study conducted by Ogunwande *et al.* (2005) who reported no inhibitory activity of the volatile oil of *Gongronema latifolium* against *Staphylococcus aureus* and *Escherichia coli*. However, previous studies conducted by Agoramoorthy *et al.* (2007) had confirmed that fatty acids extracts similar to the ones recorded in the present study, obtained from *Excoecaria allocha* plant, possessed antibacterial and antifungal activities, with MIC range similar to ours.

In the present study, the Gram-positive bacteria (*Staphylococcus* sp.) were more susceptible than the Gram-negative bacteria (*Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella typhimurium*). This is because Gram-negative bacteria are known to be more resistant to inactivation by medium and long chain fatty acids than Gram-positive bacteria because of their impermeability to hydrophobic compounds (Kabara, 1981).

CONCLUSION

Based on the results of this research, ethanolic and essential oil extracts of *Gongronema latifolium* may be useful in the practice of ethnomedicine and in the treatment of bacterial blood stream infection in HIV patients.

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