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CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF VOLATILE COMPONENT OF THE ETHANOL LEAF EXTRACT OF *MOMORDICA CHARANTIA*

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ABSTRACT: *GC-MS characterization of the leaf extract of Momordica charantia was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) The initial phytochemical screening of the sample revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids glycosides and tannins .the interpretation of the GC/MS spectrum revealed 13 absorption peaks Peak 1 was identified as Benzene propanoic acid,with molecular weight of 150g and a molecular formula of $C_9H_{10}O_2$. Similarly, peak 2-13 were also identified as 1- Octadecyne, 1-Tridecyne, Pentadecanoic acid methyl ester, Hexadecanoic acid ethyl ester, n-Hexadecanoic, 11-Octadecanoic acid methyl ester, Phytol ,Oleic acid, 1-fluoro decane, E-9-Tetradecenal, 9-Octadecenal, 1-Pentanol-4-methyl-2-propyl with the following molecular weights; 250g,180g,270g,284g,256g,296g,296g,282g,160g, 210g, 266g,144g and molecular formula of $C_{18}H_{34}$., $C_{13}H_{24}$, $C_{17}H_{34}O_2$, $C_{18}H_{36}O_2$. $C_{16}H_{32}O_2$, , $C_{19}H_{36}O_2$, $C_{20}H_{40}O$. $C_{18}H_{34}O_2$. $C_{10}H_{21}F$. $C_{14}H_{26}O$. $C_{18}H_{34}O$. $C_9H_{20}O$ respectively. **T**he leaf extract showed marked inhibition of some of the selected pathogens,. At concentrations of 200mg/cm^3 , the extract inhibited Staphylococcus aureus, 4mm, Trichophyton 2mm, Candida albican 4mm and streptococcus auerus 10mm The minimum inhibition concentrations are 200mg/cm^3 for trichophyton spp, 20mg/cm^3 for Candida albicans, 100mg/cm^3 for staphylococcus aureus and 50mg/cm^3 for streptococcus aureus .*

KEYWORDS: Antimicrobial Properties, Volatile Component, Ethanol Leaf, Momordica Charantia

INTRODUCTION

Momordica charantia also known as bitter melon, is a member of the cucumber family, Cucurbitaceae. The plant is grown in the tropical and subtropical regions of the world. (Taylor 2002). Traditionally, fruit juice of *Momordica charantia* has been used for treatment of diabetes for centuries Perumal et al 2015. Charantin, a natural steroidal glycoside present in the fruits of this medicinal plant, has been reported to possess potential hypoglycemic activity. Lotlikar and Rajarama, 2010. Charantin is steroidal glycoside and exist as equal mixture of stigmasterol glucoside and β- sitosterol glucoside It has blood sugar lowering property equivalent to insulin .Sonal and Pratima 2015. Bitter melon is composed of various chemicals that reduces the amount of sugar in the blood, Virdi et al 2003, stimulates appetite, helps in the entire digestion process. Hence it is used in treatment of digestive problems Sampath and Debjit 2010. Bitter melon has emetic, purgative, anthelmentic and anti-flatulent properties. It is useful for the dissolution of fats from the body. It is known to

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have anti-lipolytic properties. the plant possesses all the essential vitamins in good amounts, such as vitamin A, thiamine, riboflavin, vitamin C and also minerals like iron. Kandangath et al 2015 it is used in the treatment of anorexia. It also has at least one substance that is like the insulin secreted by the human pancreatic glands. Bitter melon is extremely effective in the treatment of diabetes mellitus. The juice of the bitter melon is excellent remedy for hangovers. It is also very beneficial in preventing the liver damage that occurs due to excessive alcoholism.. Several medicinal properties of the bitter gourd have been studied by various researchers, such as anti-diabetic, anti-ulcerogenic ,Gurbuzi et al 2000, anti-mutagenic, antioxidant Scartezzini and Speroni 2000, anti-tumour, anti-lipolytic, analgesic, abortifacient, anti-viral, hypoglycemic and immunomodulatory, Spreafico et al 1983 *Anti-obesity* Khan and Flier , *Umesh et al 2005* . *Recent* studies reveals that the bitter gourd proteins (α-and β-monorcharin) has inhibitory effect against HIV virus. Ng et al. 1992, Raman and Lau1996, Basch et al.2003 , Bourinbaiar and Lee-Huang 1995, *Nerurka et al 2005. The* leaf extracts have broad-spectrum anti-microbial activity . Traditionally it has also been used in treating peptic ulcers, interestingly in a recent studies , the leaf extract has been shown to exhibit the growth of *Helicobacter pylori*. studies have shown its efficacy in treatment of various cancers (lymphoid leukemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumor, prostatic cancer, squamous carcinoma of tongue and larynx, human bladder carcinomas and Hodgkin's disease). *Cunnick et al 1990* . The Root extract is astringent. Abortifacient, anthelmintic, aphrodisiac and could treat burn, catarrh, constipation, dermatosis, diabetes, diarrhea, dyspepsia, eczema, fever, fhemorrhoids, hepatitis, hypoglycemia, inflammation (liver), leprosy, leucorrhoea, leukemia, malaria, menstrual colic, pain, pruritus, rheumatism, scabies, tumor, wound, vaginitis, cancer (breast), glucosuria, halitosis, hematuria, polyuria, bite (snake), anemia, colitis, kidney (stone), dysentery, gonorrhea, rhinitis, contraceptive, dysmenorrhea, fat loss, galactagogue, gout, hydrophobia, piles, pneumonia, psoriasis, sore, asthma, headache, scald, sprue, stomachache, cold, cough, hypertension, tonic , measle, rheumatoid arthritis and lupus. *Lee haung et al 1995, Scartezzini and Speroni 2000.Gurbuzi et al 2000, Guevara et al 1990* Jaspreet et al 2003, Grover and Yadav 2004

The fruit extract has demonstrated activity against the stomach ulcer-causing bacteria Helicobacter pylori. Sankaranarayanan and Jolly 1993 , Raman and Lau 1996 Shuo et al 2017. Despite the many uses of the different parts of this plant, it content has not been fully documented.

MATERIALS AND METHODS

Sample Collection

The leaves of *momordica charantia were* obtained in Eziobodo community in Owerri West L.G.A of Imo state, Nigeria. He were Identified and authenticated by Dr Ibeabuchi of crop science department federal university of technology owerri. They were then room dried for a period of one month before been milled into powder using a milling machine. The milled sample was then stored in airtight container till required for analysis, Iwu and Onu, (2018).

Frothing test for Saponins

This test is based on the ability of the saponins to produce froth in aqueous solution. 5g of the leaf sample was weighed into a test tube and 50cm^3 of water was added and extracted after

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4hours. The water extract was shaken vigorously in a conical flask. The production of a persistent froth when allowed to stand for 5 minutes indicates the presence of saponins in the sample. Iwu et al,(2016b)

Test for Flavonoids

5g of the sample was soaked with 50cm^3 of water and then filtered. To the filtrate were added drops of ammonia and 3cm^3 of concentrated H_2SO_4 was added. A yellow precipitate which disappears on storage indicates the presence of flavonoids. Okwu and Okwu, (2004)

Test for Alkaloids

5g of the sample was extracted using 20% acetic acid in ethanol .5cm^3 of the extract was treated with 3drops of Wagner's reagent (iodine crystals and KI). A yellowish brown precipitate indicates the presence of alkaloids. Iwu et al, (2018b).

Test for Tannins

5g of the leaf sample was weighed into a beaker and 50cm^3 of water was added and allowed to soak properly for 4 hours and extracted.10cm^3 of the leaf extract was treated with 3 drops of ferric chloride. A blue-black precipitate indicates the presence of tannins Iwu et al (2018c)

Test for Steroids

5cm^3 of the water extract was treated with concentrated H_2SO_4 in acetic anhydride. The formation of a blue-green color indicates the presence of steroids. Iwu and Onu,(2018)

Test for Phenols

20cm^3 of the water extract was treated with 5cm^3 of concentrated sulphuric acid and drops of sodium nitrate ($NaNO^3$). 2cm^3 of sodium hydroxide was added to the mixture. A blue precipitate indicated the presence of phenols. Iwu et al, (2018a)

Test for Glycosides

20cm^3 of the water extract was treated with Fehling solutions of A and B in equal amount and boiled. A brownish red precipitate indicates the presence of glycoside. Iwu et al, (2018b)

Preparation of Samples for GC-MS Analysis

Two hundred grams of the leaf sample was soaked in ethanol for 48 hours and then extracted. The extract was re-extracted using chloroform to obtain chloroform soluble extract. This was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GCMS analysis.

GC-MS Experimental Procedures

GC-MS analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the as follows: Temperature programming from 80 – 200^{o}C held at 80oC for 1 minute, the rate is 5^{o}C/min and at 200^{o}C for 20 minutes. FID Temperature of 300^{o}C, injection temperature of 250^{o}C, carrier gas is Nitrogen at a flow rate of 1 cm3/min and split ratio of 1:75. GC-MS Gas (chromatography, Mass spectrum) analysis was conducted using GC-MS QP 2010 Plus Shimadzu Japan with injector Temperature at 230oC and carrier gas pressure of 100kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50m/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5kv and sampling rate of 0.2 seconds. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank HERMCE Z 233 M-Z centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany (Iwu et al 2016a, b.) **Antimicrobial Analysis**

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The microorganisms
;Aspergillus niger,Staphylococcus aureus, Trichophyton ribrum Candida albican streptococcus aeurus *Proteus mirabilis and Pseudomonas aureginosa* were used for the analysis. They are clinical isolates of human pathogens obtained from the Federal Medical Centre Umuahia and were brought to the laboratory and resuscitated in buffered peptone broth (Secharian chemie) and thereafter into nutrient agar medium and incubated at 37ºC for 24 hrs Iwu et al (2016b).

Antibacterial Assay
The test solution of each extract was prepared by dissolving 0.1 g of the plant extract separately. 1.0cm^3 of dimethyl sulphoxide (DMSO) to get a concentration of 100mg/cm^3. The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (Whatman No 1.6 mm diameter) were placed in glass petridish and sterilized in hot air oven. Iwu et al 2018b, the media (10g nutrient Agar in 200cm^3 distilled water, autoclaved at115ºC for 30 minutes) was cooled to 50ºC. The sterile nutrient Agar media were poured into the sterile petridish and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2cm^3 of plant extract. Standard antibiotic Ciprofloxacin was used as a control on a disc with DMSO 100mg/cm^3. The discs were used after drying them in an incubator at 40ºC to remove any trace of solvent. Discs were introduced into the surface of the medium. The plates were microbated at 37ºC for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotics to minimize error and the average of these values were recorded.

Minimum Inhibitory Concentration (MIC)
The minimum inhibitory concentration of the extract was determined by incorporating constant volume of 0.2cm^3 of each diluents of the extract into the perforated disc on a seeded nutrient agar plate as described in the anti-microbial susceptibility test section. 0.1g of each extract was dissolved in 1cm^3 of DMSO to obtain 100mg/cm^3. This concentration of DMSO was then doubled to obtain 50mg/cm^3 then doubled again to obtain 12.5mg/cm^3 and again to obtain6.25mg/cm^3. Each concentration was then used in the method earlier described to obtain zone of inhibition. The least concentration that showed inhibitory zones was taken as the MIC. Ekundayo and Ezeogu, (2006)..

RESULTS AND DISCUSSION.
The initial phytochemical screening of the leaf extract of Momordica charantia are shown in table 1 below. The result reaveled the presence of alkaloids, saponins, flavonoids, phenols, steriods glycosides and tannins.

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TABLE 1: Phytochemical screening of the leaf of *momordica charantia*

Phytochemical	Inference
Alkaloids	++
Saponins	++
Flavonoids	++
Phenols	++
Steriods	++
Glycosides	++
Tannins	++

Key; ++ present

Alkaloids are vast and vary a lot in their activity when ingested by man and livestock. Some alkaloids are useful and important in medicine and constitute most of the valuable drugs currently used by humans. They are reported to have marked physiological effect on animals .Edeoga and Eriata 2001

Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules Tukappa and Londonkar 2013. In addition, tannin was found in the plant at a concentration range. Plant leaves with high tannin content have been used successfully as hops alternative in beer Hutchinson and Dalziel 1963.

Flavonoids are polyphenolic compounds containing a heterocyclic six-membered C-ring is sometimes replaced by a 5-membered ring . The oxidation state of the C-rings is used to classify flavonoids into different categories of which typical examples are Flavan-3-ols, flavanones, flavones, isoflavones and flavanols. Flavonoids are the major nutraceutical ingredients that are in plants. The best described property of almost every group of flavonoids is their capacity to act as anti-oxidants. The flavones seem to be the most powerful flavonoid for protecting the body against reactive oxygen species (ROS). Antibacterial activity has been displayed by a number of flavonoids, Quercetin has been reported to completely inhibit the growth of *Staphylococcus aureus* Havesteen1983. Flavonoids also posses anti-inflammatory and analgesic effect as well as anti-ulcerogenic activity Shahid et al 1998 .The extracts of Momordica charantia has shown proven activity against gonorrhea and jaundice Draughton 2004, mohammed et al 2013. This is probably due to antibacterial action of saponins. Saponins are foam forming in nature and have been implicated as a bioactive antibacterial agent of plant Mandal et al 2005. Saponins are also potential, sometimes for utilization in foods that need sustained foam volume such as ice-creams. Saponins are a class of chemical compounds, More specifically, they are amphipathic glycosides grouped, in terms of phenomenology by the soap-like foaming they produce when shaken in aqueous solution and in terms of structure by their composition of one or more hydrophilic glycosides combined with a lipophilic triterpene derivative. In plants saponins protect the plant against microbes and fungi. Some plant saponins may enhance nutrient absorption and aid in animal digestion. Saponins have been used as a pharmacological and/or immunological agent that modifies the effect of other agents in vaccines. Saponins from plants have been shown to significantly augment the cytotoxicity of immunotoxins and other target toxins directed against human cancer cells. Tannins are astringent, bitter plant polyphenol compounds that bind to and precipitate proteins and various other organic compounds including amino acids and

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alkaloids. The tannin compounds are widely distributed in many species of plants where they play a role in protection from predation and perhaps also as pesticides and in plant growth regulation. The astringency from tannin is what causes the dry puckery feeling in the mouth following the consumption of unripe fruits or red wine. Tannins are important ingredients used in process of making tannin leather. Medicinally, tannins are used as anti-diarrhea, haemostatic and anti-hemorrhoid compounds.

The presence of Phenolic compounds in the leaf of Momordica charantia indicates that this plant might be an anti-microbial agent. This is because phenols and phenolic compounds have been extensively used in disinfections and remains the standard with which other bactericides are compared Okwu and Okwu 2004. Phenolic compounds acts as electron donors and are readily oxidized to form phenolate ions. This gives rise to protonated phenol which is used as a cleaning agent. Extracts from leaves of *Momordica* charantia therefore have potent antiseptic or bactericidal properties. This finding supported the use of extracts of the leaves in treating wounds that not only heal fast but also prevent the formation of infection. Phenols have antioxidant properties The presence of phenol further indicates that Momordica charantia could act as anti-inflammatory, anti-clotting, immune enhancers and hormone modulators.

Glycosides are molecules in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plant store chemicals in form of inactive glycosides. Many such plant glycosides are used as medications. Some glycosides has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes but concluded that further study was required to determine proper dosage

GC/MS RESULT

The GC-MS spectrum of *Momordica charantia* is shown in fig 1 below with 13 absorption peaks, the interpreted data is contained in table 2 below. Peak 1 was identified as Benzene propanoic acid with percentage oil composition of 1.65, its molecular weight is 150g and a molecular formula of $C_9H_{10}O_2$. Fig 2. Similarly, peak 2 was also identified as 1- Octadecyne having a percentage oil composition of 15.13, with a molecular weight of 250g and molecular formula of $C_{18}H_{34}$. Peak 3 was identified as 1-Tridecyne with percentage oil composition of 2.51, molecular weight of 180g and a molecular formula of $C_{13}H_{24}$, the structure is shown in fig 2. Peak 4 was identified as Pentadecanoic acid methyl ester with percentage oil composition of 2.92, molecular weight of 270g and a molecular formula of $C_{17}H_{34}O_2$. Peak 5 was also identified as Hexadecanoic acid ethyl ester with percentage oil composition of 2.79, molecular weight of 284g and molecular formula of $C_{18}H_{36}O_2$. Similarly, peak 6 was identified as n-Hexadecanoic with percentage oil composition of 8.53, with a molecular weight of 256g and molecular formula of $C_{16}H_{32}O_2$, . Peak 7 was identified as 11-Octadecanoic acid methyl ester with percentage oil composition of 9.61, molecular weight of 296g and molecular formula of $C_{19}H_{36}O_2$

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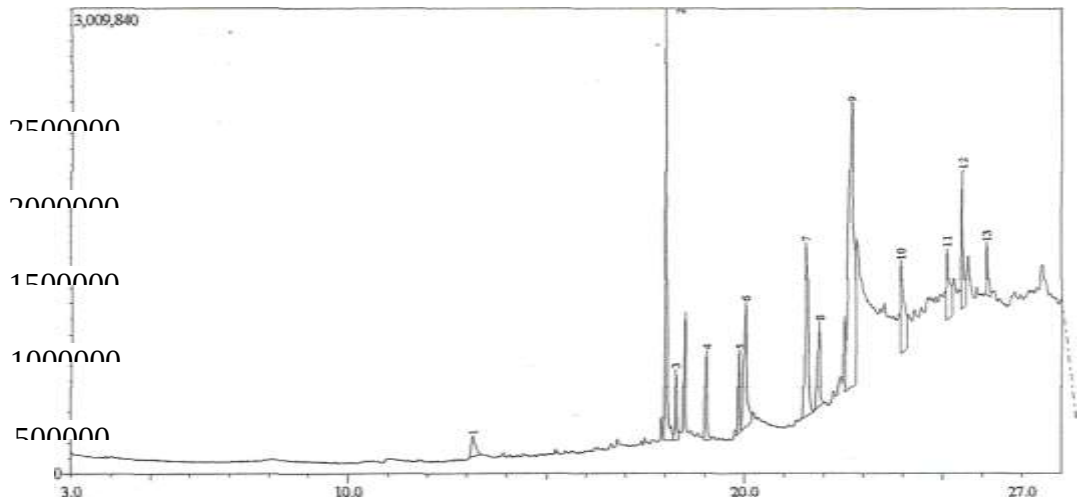

intensity3000000"
Fig 1 GC/MS spectrum of sample

Peak 8 was identified as Phytol, with percentage oil composition of 4.90 with a molecular weight of 296g and a molecular formula of $C_{20}H_{40}O$. Peak 9 was identified as also as Oleic acid, with percentage oil composition of 32.72 and molecular weight of 282g and molecular formula of $C_{18}H_{34}O_2$. Peak 10 was identified as 1-fluorodecane, with percentage oil composition of 6.79, molecular weight of 160g and molecular formula of $C_{10}H_{21}F$. Peak 11 was identified as E-9-Tetradecenal with percentage oil composition of 4.65, molecular weight of 210g and molecular formula of $C_{14}H_{26}O$. Peak 12 was identified as 9-Octadecenal with percentage oil composition of 5.73, molecular weight of 266g and molecular formula of $C_{18}H_{34}O$. Peak 13 was also identified as 1-Pentanol- 4-methyl 2-propyl ,having a percentage oil composition of 2.07, molecular weight of 144g and molecular formula $C_9H_{20}O$. The structures of the compounds are contained in fig 2 below

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TABLE 2. interpreted data of GC-MS spectrum of *Momordica* *charantia*

S/No	M.weight	M.formula	% oil	Name
1.	150	$C^9H_{10}O$	1.65	Benzene propanoic acid
2.	250	$C_{18}H_{34}$	15.13	1-Octadecyne
3.	180	$C_{13}H_{24}$	2.51	1-Tridecyne
4.	270	$C_{17}H_{34}O_2$	2.92	Hexadecanoic acid methyl ester
5.	284	$C_{18}H_{36}O_2$	2.79	Hexadecanoic acid ethyl ester
6.	257	$C_{16}H_{32}O_2$	8.53	n-Hexadecanoic acid
7.	296	$C_{19}H_{36}O_2$	9.61	11-Octadecanoic acid methyl ester
8	296	$C_{20}H_{40}O$	4.90	Phytol
9.	282	$C_{18}H_{34}O_2$	32.72	Oleic acid
10	160	$C_{10}H_{21}F$	6.79	1-Fluoro Decane
11.	210	$C_{14}H_{26}O$	4.65	E-9-Tetradecenal
12.	266	$C_{18}H_{34}O$	5.73	9-Octadecenal
13	144	$C_9H_{20}O$	2.07	1-Pentanol- 4-methyl 2-propyl

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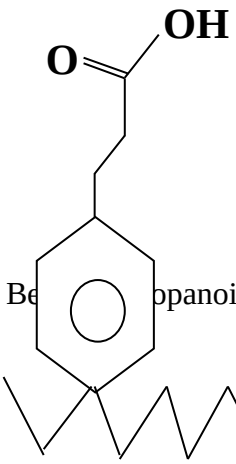
Fig 2: Structures of volatile compounds from the leaf of *Momodica charantia*



1. Be opanoic acid

2 . 1-Octadecyne

3. 1-Tridecyne

4. Hexadecanoic acid methyl ester

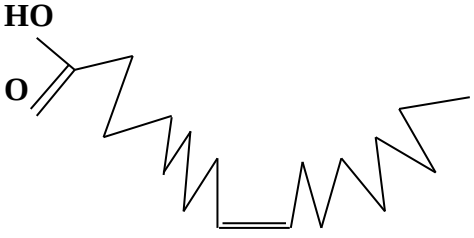
5. Hexadecanoic acid ethyl ester

6. n-Hexadecanoic acid

7. 11-Octadecanoic acid, methyl ester

HO

8. Phytol



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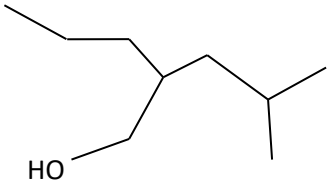
9. Oleic acid

10. 1-Fluoro Decane

11. E-9-Tetradecene

12. 9-Octadecenal



13. 1-pentanol, 4-methyl,2-propyl

ANTIMICROBIAL RESULT

The leaf extract showed marked inhibition of some of the selected pathogens,. At concentrations of 200mg/cm^3 the extract showed inhibition of *Staphylococcus aureus*, 4mm, Trichophyton 2mm, Candida albican 4mm streptococcus aeurus 10mm .the minimum inhibition concentrations are 200mg/cm^3 for trichophyton spp, 20mg/cm^3 for Candida albicans, 100mg/cm^3 for staphylococcus aureus and 50mg/cm^3 for streptococcus aureus table 3. These result are very close to those obtained when standard antibiotics Levoflaxcin and Kentoconazole

Table 3 ANTIMICROBIAL PROPERTIES *MOMORDICA CHARANTIA*

	Diameter of zone of inhibition (mm)						
Extract conc. Mg/ml	Aspergillus niger.	Candida albicans.	Trichophyton ribrum.	Pseudomonas aureginosa.	Staphylococcus aureus	Proteus mirabilis .	Streptacoccus spp.
200	-	4	2	-	4	-	10
100	-	-	-	-	2	-	6
50	-	-	-	-	-	-	2
25	-	-	-	-	-	-	-
Levo flaxacin							
200	X	X	X	14	10	8	14
Ketoconazole							
200	-	-	2	X	X	X	X

Key: x = not tested
- = no activity

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Staphylococcus aureus is a gram positive coccus that causes skin infection such as ; pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome , abscesses, pneumonia, toxic shock syndrome, osteomyeletis, endocarditis mastitis,,food poisoning characterized by vomiting and nausea, bacteremia and sepsis. Mwambete 2009.Diseases caused by this organisms could be treated with the leaf extract of *Momordica charantia* since it inhibited their growth, Similarly, Streptococcus causes sore throat, impetigo, scarlet fever, pneumonia sore blisters ,menegitis and bacteraemia .*Trichophyton ribrum* is a fungi that cause tinea, athelets foot, ring worm, jock itch , nail , beard and skin infection *Streptococcus*: This is a type of bacteria. There are two types of *Streptococcus*; group A and group B. Group A Causes strep sore throat, red throat, sometimes with white spots on the tonsils, scarlet fever, impetigo (a skin infection) toxic shock syndrome, cellulose. Group B Blood infection, pneumonia and meningitis in newborns. *Candida albicans*: it is fungi of the genus Candida. There are over 20 species that can cause infection in humans, the most common of which is C*andida albicans*. Candida yeast normally live in the skin and mucous membranes without causing infection; however, overgrowth of these organisms can cause symptoms to develop. They cause diseases based on the area of the body they affect; candidiasis that develop in the mouth or throat is called "thrush" , in the vagina is called "yeast infection". Invasive candidiasis occurs when Candida species enter the bloodstream and spread throughout the body. Cunnick et al 1990.

CONCLUSIONS

The leaf of *Momordica charantia* is full of phytochemicals ,these phytochemicals have marked medicinal application ,Ethanolic extract of the leaf of the plant contain volatile compounds some of which are fatty acids and esters and their molecular weights , formulas and structures have been properly elucidated. This research has therefore given credence to the tradomedicinal application of this plant in the treatment of various body ailments such as impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome , abscesses, pneumonia, toxic shock syndrome, osteomyeletis, endocarditis mastitis,,food poisoning characterized by vomiting and nausea, bacteremia , sepsis sore throat, impetigo, scarlet fever, pneumonia sore blisters ,menegitis and bacteraemia , tinea, athelets foot, ring worm, jock itch , nail , beard and skin infection sore and red throat, scarlet fever, pneumonia and meningitis in newborns *and* candidiasis

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