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Characterization and antimicrobial studies of *Ficus capensis* methanolic leaf extract

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ABSTRACT: The study was carried out to access phyto-chemical properties using Gas Chromatography- Mass Spectrometry and antimicrobial potency in *Ficus capensis* against some microbial pathogens at different concentrations. The chromatogram revealed seven peaks indicating the presence of seven phyto-compounds in the extract of *Ficus capensis* leaf. The most abundant compounds were Benzene 1, 2, 3- triol (peak area: 38.70%) and Sorbic acid (peak area: 38.36%). The extract was tested at varied concentrations of 250mg/ml, 200mg/ml, 100mg/ml, 50mg/ml and 10mg/ml on four organisms, *Staphylococcus aureus*, *Proteus vulgaris* , *Escherichia coli* and *Candida albicans*. The Mean Inhibition Zone Diameter (IZD) of *Staphylococcus aureus* was 22.5 ± 1.29mm, 16.5 ± 1.29mm, 14.25 ± 0.89mm, 11.00 ± 0.81mm and 8.75 ± 0.50mm respectively compared to the reference drug (chloramphenicol) 10.00 ± 0.81mm. That of *Proteus vulgaris* was 18.0 ± 0.82mm, 14.5 ± 0.57mm, 9.25 ± 0.96mm, 8.0 ± 1.15mm, 6.0 ± 0.81 compared to the positive control (chloramphenicol) 7.75±0.5mm. *Escherichia coli* Inhibition Zone Diameter (IZD) was 22.25 ± 1.71mm, 20.0 ± 0.81mm, 17.5 ± 0.58mm, 7.75 ± 0.5mm, 6.25 ± 0.5mm respectively compared to the reference drug (chloramphenicol) 17.50±0.58mm. The Mean Inhibition Zone Diameter (IZD) of *Candida albicans* showed 19.75 ± 0.5mm, 18.0 ± 0.82mm, 14.0 ± 0.82mm, 12.25 ± 0.5mm, 9.0 ± 0.82mm but the reference drug had no effect on *Candida albicans* 0.00 ± .00. This shows that both phyto-compounds in *Ficus capensis* are effective against bacteria and fungi therefore could be adopted for pharmacological use.

KEYWORDS: Antimicrobial, Benzene-1, 2, 3-triol, *Ficus capensis*, GC-MS, Sorbic acid.

I.INTRODUCTION

Ficus is a genus of plants collectively known as figs or fig trees. It has about 850 species of woody trees, shrubs, vines, epiphytes and hemiepiphytes in the family of Moreceae. They are seen throughout the tropics and few in the semi-warm temperate zone. They are mostly evergreen while some are endemic to areas outside of the tropics [1].Figs are relatively easy to identify and 70 or more figs can co exist in places like Asia [2].Fig plant can exist as a hermaphrodite or a hermaphrodite and a female[3]. *Ficus capensis* (family,Moraceae) with synonyms as *Ficus sur*, cape fig and Sycomorus capensis is a fig tree with medicinal properties found In the terrestrial Zones mostly found along the river banks. They are mostly found in tropical Africa and Cape Islands. It is an evergreen tree with spreading branches and roots. The leaves are broad and green and the tree produces fruit all through the year in a single or branched raceme along the branches. The leaves are used as vegetable with a reported blood boosting effect [4] and an antisickling effect of red blood cells [5]. The reported medicinal properties of its extract include being used in the treatment of diarrhoea and dysentery, treatment of sexually transmitted diseases, chest ailments, tuberculosis and anaemia. [6]. It is also used in circumcision, wound dressing, leprosy and epilepsy treatment [7, 8]

II MATERIAL AND METHODS

A. Plant Materials

Fresh leaves of was harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified by Prof M C Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

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B. Preparation of Plant Extract

The plant material of *Ficus capensis* was collected from the wild, shade dried for 10 days and pulverized to powder using mechanical grinder. The plant extract was prepared using Soxhlet method described by [9]. Thirty five grams (35 g) of powdered sample was introduced into the extraction chamber of the Soxhlet extractor using methanol as solvent. Temperature was maintained at 70^{o} C throughout the extraction period of 48 hrs. At the end of the extraction period, the extract was concentrated using oven at 35^{o} C to obtain dried extract which was sent for GCMS analysis.

C. GCMS analysis of *Ficus capensis.*

The characterization of the Phytochemicals in *Ficus capensis* ,was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5).The initial column temperature was 80^{o}C for 1min, and then increased linearly at 70^{o}C min^{-1} to 220^{o}C, held for 3 min followed by linear increased temperature 10^{o}C min^{-1} to 290^{o}C for 10 min. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

D. Identification of Phytocomponents in *Ficus capensis,*

GC-MS Chromatogram of *Ficus capensis,* revealed seven peaks showing that seven different compounds were present. Identity of the active components in the extract was done by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored in the database of National Institute of Standards and Technology (NIST) and also with published literature, NIST08.LIB [10], WILEY8.LIB [11], PESTEI-3.LIB and FA-ME.LIB library sources were used for matching the identified components from the plant material. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

E. Isolation of *Staphylococcus aureus*.

Nasal swabs were collected from goat and sample was cultured in mannitol salt media. Golden yellow colonies were observed on the media which is diagnostic for staphylococcus aureus. Gram staining and catalase test was carried out to confirm organism as staphylococcus.

F. Isolation of *E coli, Proteus vulgaris* and *Candida albicans*

The samples were re-isolated from pure stock in the Veterinary Microbiology laboratory in a MacConkey media. Gram staining was done to confirm a Gram negative organism

G. Preparation of single-antibiotic discs

100 discs were obtained from punching a No. 1 whatman paper disc and sterilized in a hot air oven at 160^{0} C and 1ml of the re-constituted chloramphenicol solution was pipette into bijou bottle containing the sterile disc and dried in a hot air oven at 60^{0} C.

H. Antibiotic Sensitivity Test

An agar well diffusion method was used for the antibiotic sensitivity. This was done by preparing a nutrient media and the organisms inoculated into the media and incubated for 2 hours thereafter, a template aseptically was made using a cork borer on one end and the different concentration of extract placed on it, then the chloramphenicol disc was placed on the other end of the dish. Then the plate was incubated over night and readings taken by observation and measurement of the diameter of zone of inhibition for each concentration.

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I. Data Analysis

Data collected were analyzed using SPSS version 20 software. All values were expressed as the mean value ± standard deviation by ANOVA for comparisons of the multiple means. It was followed by post hoc test to compare their differences. ($p<0.05$) was considered statistically significant difference between test and control groups for measured values.

III. RESULTS

GCMS chromatogram of the ethanolic extract of *Ficus capensis*, (Figure 1) showed seven peaks which indicated the presence of seven phytochemicals constituents.

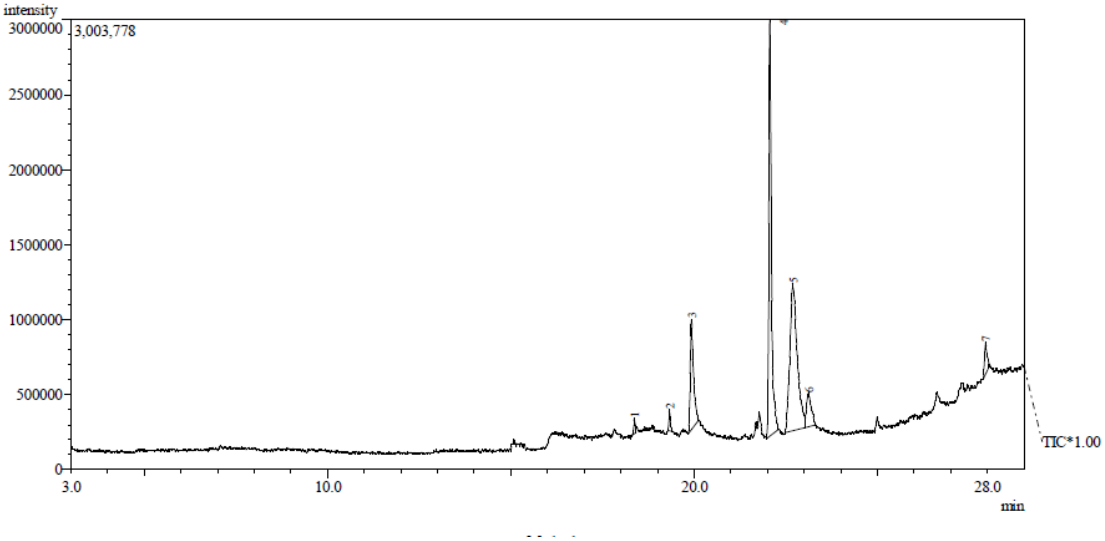
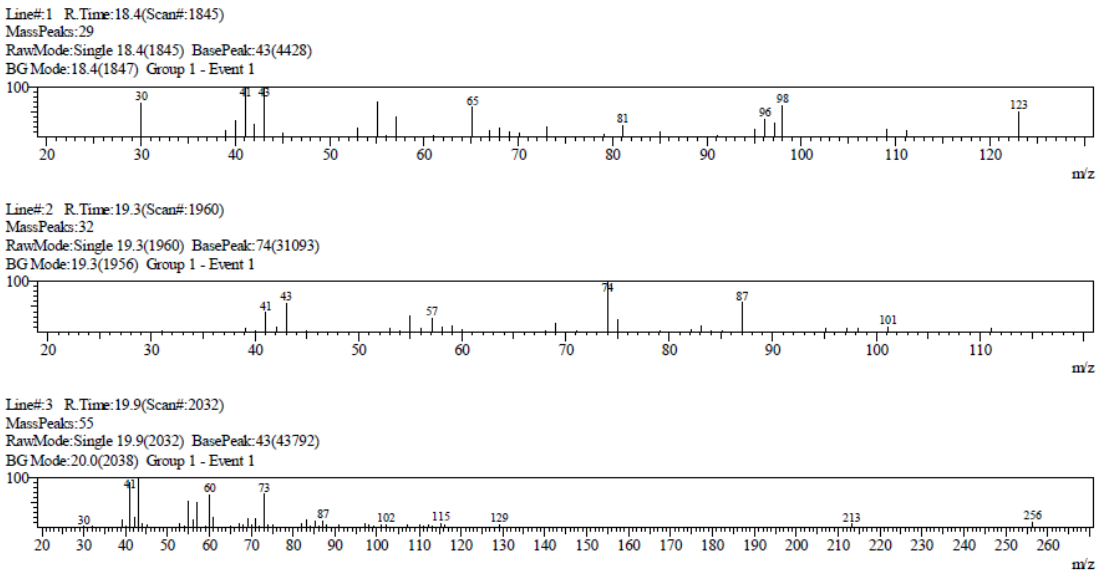


Fig. 1 shows the chromatogram of *Ficus capensis*, with seven peaks indicating seven phytocompounds



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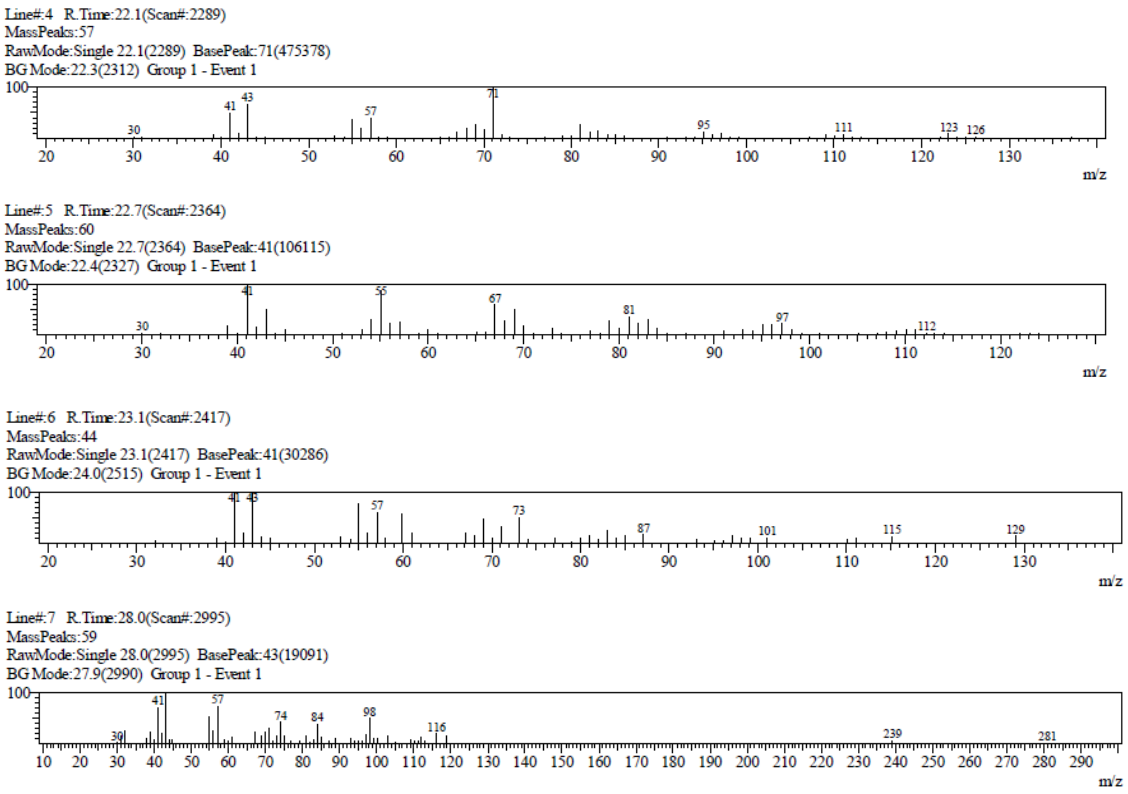
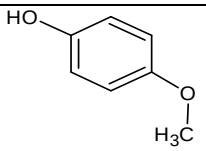
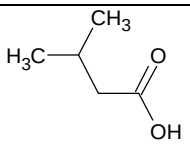
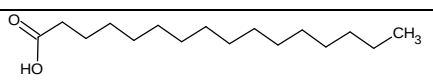


Figure 2: Shows the mass spectra of the seven phytocompounds in *Ficus capensis* identified by GCMS analysis

Table 1 shows the phytochemical compounds of *Ficus capensis*, by GC-MS analysis

S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	Mequinol	18.369	0.82	124.13	$C_7H_8O_2$		antineoplastic agent and antioxidant
2	**3-Methylbutanoic acid**, or more commonly **isovaleric acid**	19.325	1.08	102.13	$C_5H_{10}O_2$		anticonvulsant agent in valerian [12]
3	n-Hexadecanoic acid or Palmitic acid	19.929	12.63	256.42	$C_{16}H_{32}O_2$		Mild antioxidant and anti-atherosclerotic activity [13]

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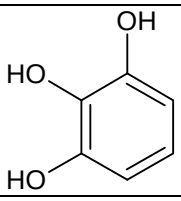
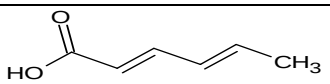
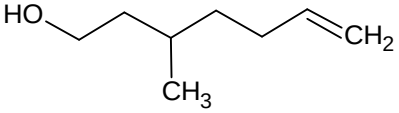
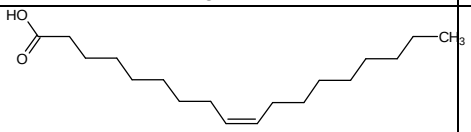
4	Benzene-1,2,3-triol	22.063	38.70	126.11	$C_6H_6O_3$		Antiseptic, antioxidant, antidermatic, fungicidal and insecticide
5	Sorbic acid	22.686	38.36	112.12	$C_6H_8O_2$		antibacterial drug fungicide
6.	3-Methyl-6-hepten-1-ol	23.122	5.74	128.21	$C_8H_{16}O$		Glucocorticoid receptor
7	cis-.Octadecenoic acid or cis-Oleic Acid	7.948	2.65	282.46	$C_{18}H_{34}O_2$		Protective against metabolic syndrome and cardiovascular disease risk factors [14].

Table 2 Table 2 shows mean inhibition zone diameter of different organisms at varied concentrations

Test organisms	chloramphenicol	extract concentration(mg/ml)				
		250	200	100	50	10
Staphylococcus aureus	10.00±0.81^{ad}	22.50±1.29^{e}	16.5±1.291^{d}	14.25±25^{c}	11.00±0.81^{b}	8.75±0.50^{a}
Proteus vulgaris	7.75±0.50^{b}	18.00±0.82^{e}	14.50±0.57^{d}	9.25±0.96^{c}	8.00±1.155^{b}	6.0±0.817^{a}
Escherichia coli	17.50±0.58^{e}	22.25±1.71^{d}	20.00±0.81^{c}	17.50±0.58^{c}	7.75±0.5^{b}	6.25±0.50^{a}
Candida albicans	0.00±0.00^{f}	19.75±0.50^{e}	18.0±0.82^{d}	14.0±0.82^{c}	12.25±0.5^{b}	9.00±0.82^{a}

a - f on the same row with different superscripts are significantly different (P<0.05).

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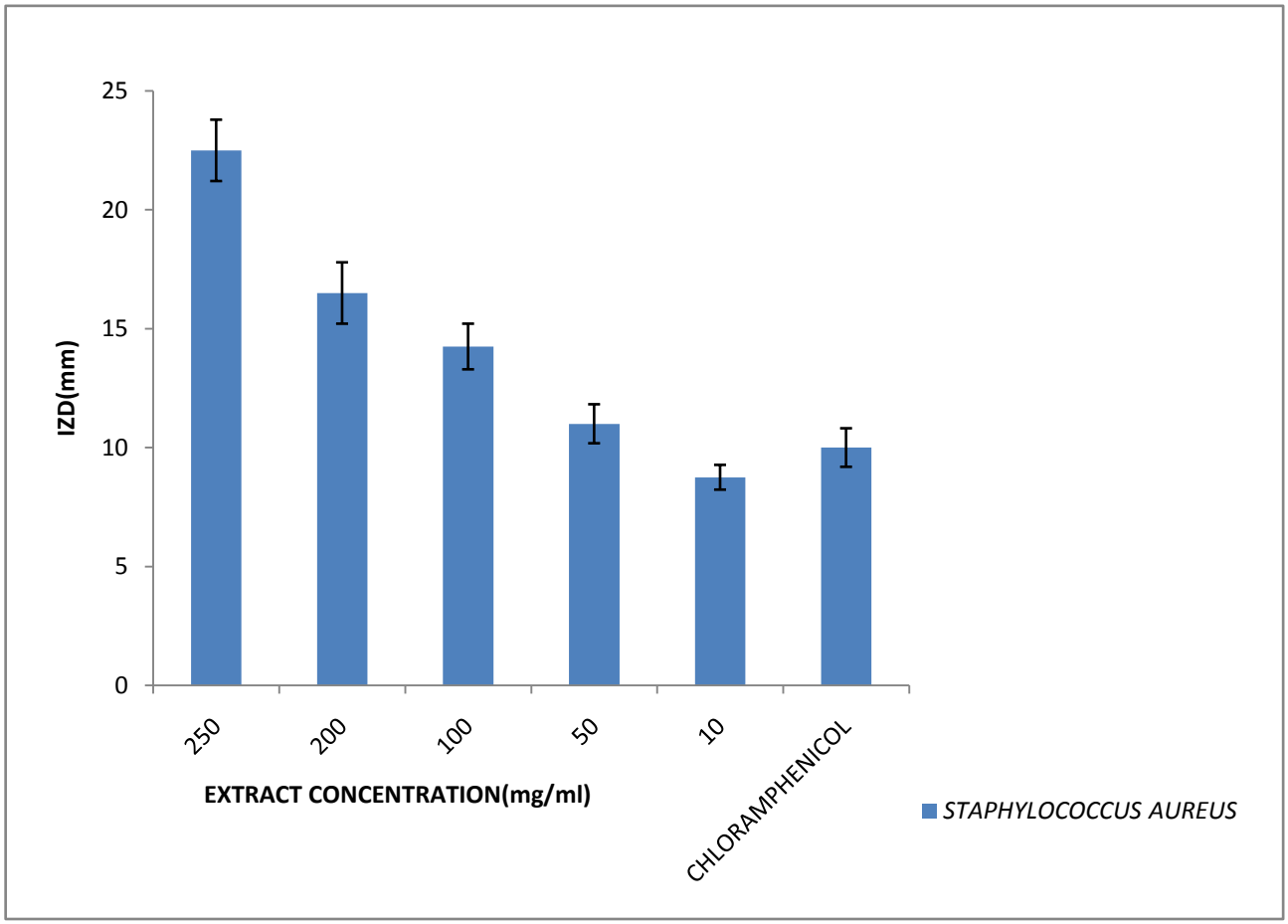

Fig. 3 Graph showing mean inhibition zone diameter of *Staphylococcus aureus*

From the graph Fig 3 it is shown that there was a concentration dependent increase in inhibition of the organisms by the extract 8.75±0.50 11.00±0.81, 14.25±0.895 ,16.5±1.29, 22.50±1.29, when compared to the reference drug 10.00±0.81 at $p<0.05$. The extract inhibited the growth of *Staphylococcus aureus* significantly.

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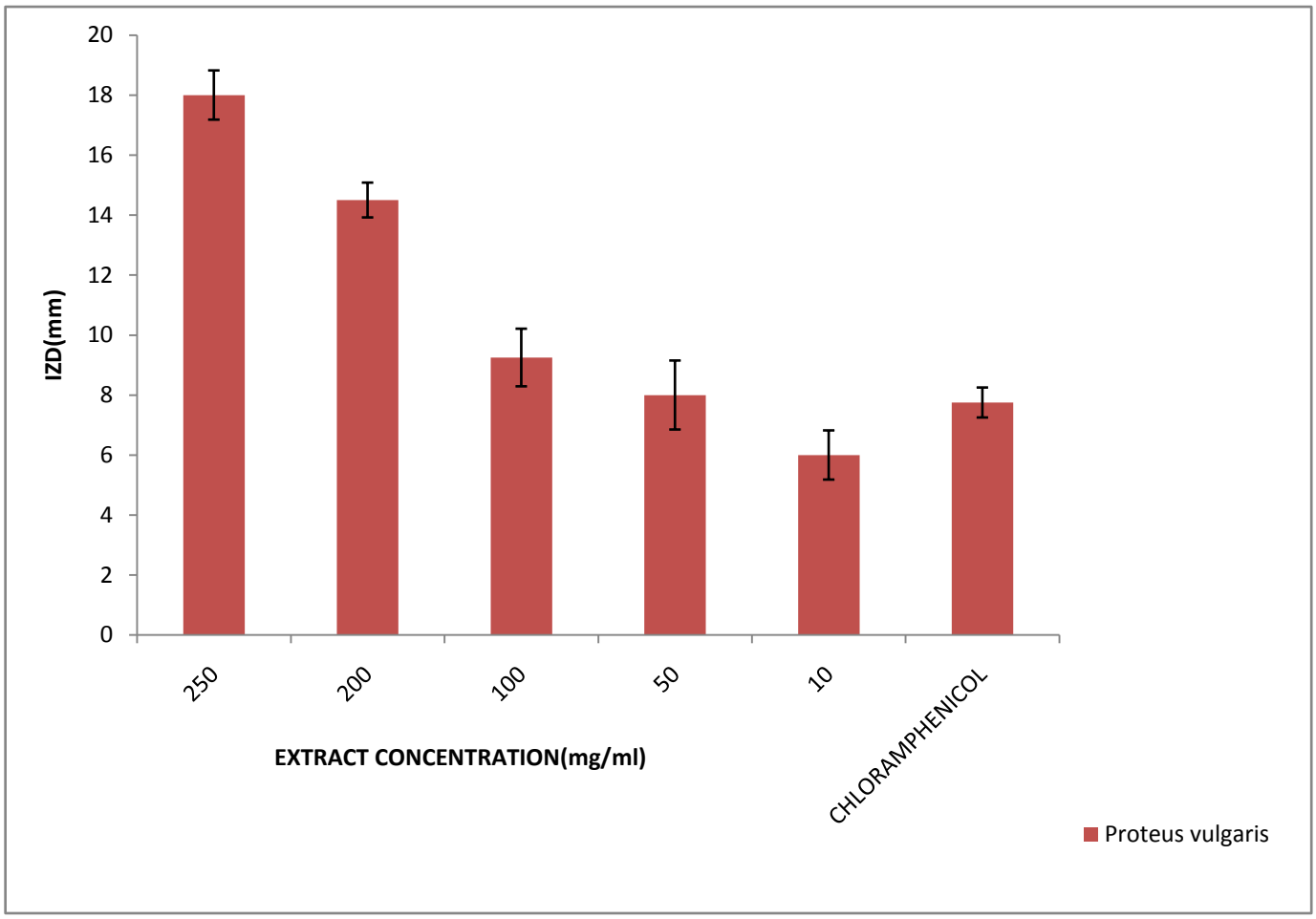

Fig. 4 Graph showing mean inhibition zone diameter of *Proteus vulgaris*

The graph Fig. 4 showed a concentration dependent increase in inhibition of *Proteus vulgaris* by the extract 6.0±0.817, 8.00±1.155, 9.25±0.96, 14.50±0.57, 18.00±0.82, when compared to the reference drug 7.75±0.50 at $p<0.05$. The extract inhibited the growth of the *Proteus vulgaris* significantly.

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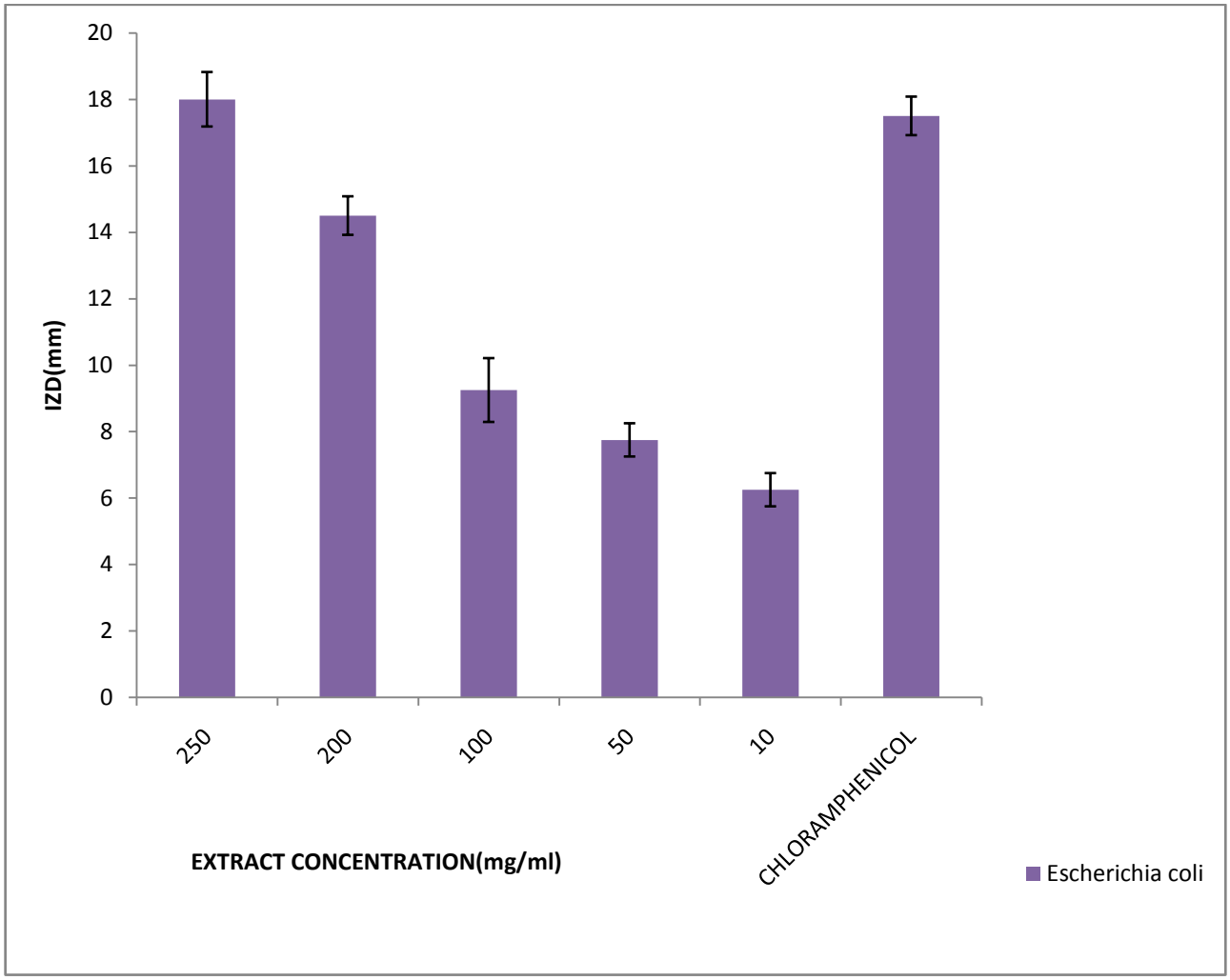

Fig. 5 Graph showing mean inhibition zone diameter of *Escherichia coli*

From the graph Fig. 5 it is shown that there was a concentration dependent increase in inhibition of *Escherichia coli* by the extract 6.25±0.50 7.75±0.5, 17.50±0.58, 20.00±0.81, 22.25±1.71, when compared to the reference drug 17.50±0.58 at $p<0.05$. The extract also inhibited the growth of *Escherichia coli* significantly.

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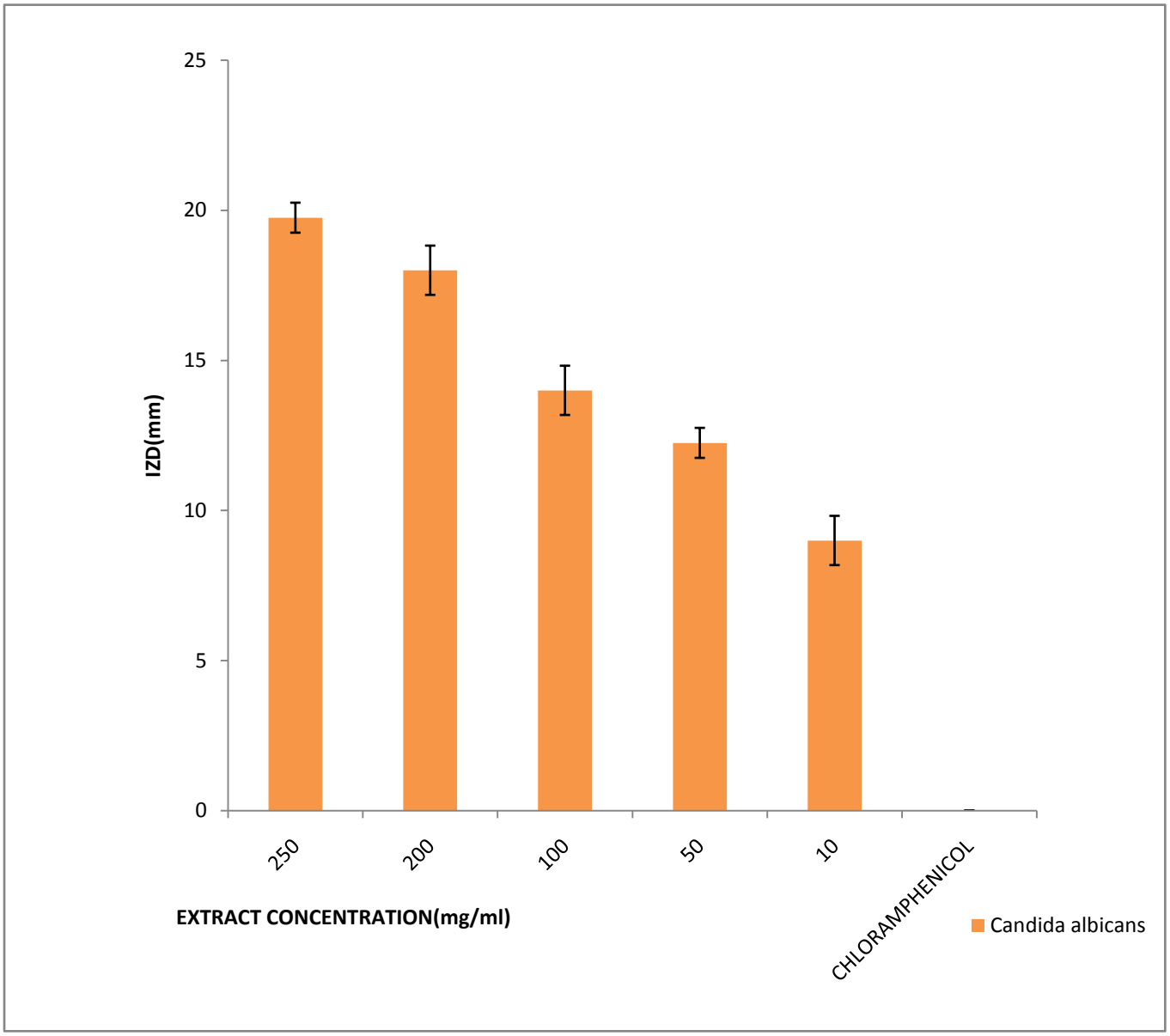

Fig. 6 Graph showing mean inhibition zone diameter of *Candida albicans*

The graph, Fig. 6 shows a concentration dependent increase in inhibition of *Candida albicans* by the extract 7.75±0.5, 9.00 ±0.82. 12.25 ±0.5, 14.0 ±0.82, 18.0 ±0.82. The reference drug had no effect on *Candida albicans*. There was high inhibition of the growth of *Candida albicans* by the *Ficus capensis* extract.

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IV. DISCUSSION

Identification and phytochemical screening of bioactive compounds in medicinal plants helps to understand their phytobiochemical potentials. The GC-MS analyzed the compounds that could inhibit microbial growth as shown in table1.The chromatogram in the GC-MS showed seven peaks shown in Fig. 1 which indicated the presence of seven phytocompounds in the extract. In table 1, the name, retention time, peak area percentage, molecular weight, structure and bioactivity are shown .The most abundant compounds were Benzene-I, 2, 3-triol and Sorbic acid which had the peak area percentage of 38.70% and 38.36% shown in the table 1. These phytocompounds identified by GCMS analysis exhibited anti-microbial activity indicating that methanolic extract of *Ficus capensis,* inhibits the growth of microbes .As the concentration of the extract increased, the inhibition zone increased showing that the extract is more effective at higher concentration. Valeric acid, or pentanoic acid, which is an alkylcarboxylic acid with the [RT:19.325,Peak area:1.08 % and Molecular formula $C_5H_{10}O_2$]was identified in the extract of *Ficus capensis,* by GCMS analysis. It is found naturally in the perennial flowering plant valerian (*Valeriana officinalis*), from which it gets its name. Its primary use is in the synthesis of its esters. Volatile esters of valeric acid tend to have pleasant odors and are used in perfumes and cosmetics. Ethyl valerate and pentylvalerate are used as food additives because of their fruity flavors. Valeric acid appears similar in structure to *gamma* –Hydroxybutyric acid (GHB) and the neurotransmitter, *gamma*-Aminobutyric acid (GABA) in that it is a short-chain carboxylic acid, although it lacks the alcohol and amine functional groups that contribute to the biological activities of GHB and GABA, respectively. It differs from valproic acid simply by lacking a 3-carbon side-chain. Mevalonic acid is derived from valeric acid by methylation [15]. Oleic acid [RT:7.948,Peak area:2.65% and Molecular formula $C_{18}H_{34}O_2$] protects against cardiovascular and cardiopulmonary risk factor [14]. It is also used to induce lung damage in certain types of animals, for the purpose of testing new drugs and other means to treat lung diseases,specifically in sheep, intravenous administration of oleic acid causes acute lung injury with corresponding pulmonary edema [16]. Sorbic acid, or 2,4-hexadienoic acid, a natural organic compound with [RT:22.686,Peak area:38.36% and Molecular formula $C_6H_8O_2$] is used as a food preservative. Sorbic acid and its salts, such as sodium sorbate, potassium sorbate, and calcium sorbate, are antimicrobial agents often used as preservatives in food and drinks to prevent the growth of mold, yeast, and fungi.[17] In the early 1980s, sorbic acid and its salts were used as inhibitors of *Clostridium botulinum* in meat products in order to replace the use of nitrites, which produce carcinogenic nitrosamines [18].

This inhibitory effect was shown by measurement of the diameter of the zone of inhibition and its size was found to increase as the concentration increased. Therefore methanolic extract of *Ficus capensis,* at different concentrations showed significant inhibitory effect on some gram positive and gram negative organisms. The result also showed that although there is an inhibitory effect of *Candida albicans* by the extract, *Candida albicans* was not sensitive to the reference drug (chloramphenicol).

V. CONCLUSION

Ficus capensis, contain phyto-compounds that have anti-bacterial and anti-fungal effects. Among the seven compounds identified by GC-MS analysis, Benzene-1 2, 3 triol and sorbic acid were the most abundant compounds in the extract. Other phytochemicals were identified though at lower concentrations with bioactivity of anticonvulsant agent and preventive against cardiovascular risk factor. The extract was effective against bacteria and fungi and the activity of was concentration dependent and worked better at higher concentrations. This leaf extract has pharmacological potentials.

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REFERENCES

[1] Halevy, Abraham H. "Handbook of flowering " vol 6 of CRC; *CRC Press*. P .331. Retrieved 25-08-2009, 1989.
[2] Harrison, Rhett D. "Figs and the diversity of tropical rain forests". *Bioscience* 55(12):1053-1064, 2005.
[3] Armstrong, Wayne P. and Steven Disparti.." A key to subgroups of dioecious (gynodioecious) figs". *Waynesword. Palomar. Edu.* 1998-04-04. Retrieved 2012-01-05, 1998.
[4] Otitoju, G.T.O,Nwamarah, J.U, Otitoju,O; Odoh, E.C and Iyeghe, L.U. "Phytochemical composition of some underlisted green leafy vegetables in Nsukka urban L.GA. of Enugu state". *Journal of Biodiversity and Environmental Sciences*, 4(4),pp. 208-217 ,2014.
[5] Umeokoli, B.O., Onyegbule, F.A., Gugu, T.H and Igboeme, S.O. "Evaluation of the erythropoietic and the anti-sickling properties of Ficuscapensis leaf extract in the treatment of anaemia". *Planta Medica* 79, 2013.
[6] Sandabe U.K. and Kwari, H.D." Some aspects of ethno-Veterinary Medicine among Kanuri and Bura of Borno State". *Q. J. Borno Museum Soc.* Pp 44-45, 2000.
[7] Joshua K. "Conservation of Indigenous Medicinal Botanicals in Ekiti State, Nigeria", 2006.
[8] Wakeel, O.K., Aziba P.I., Ashorobi, R.B., Umukoro, S., Aderibigbe, A.O. and Awe E.O. "Neuropharmacological activities of *Ficus platyphylla* stem bark in mice". *Afr. J. Biomed Res*. 7(2): 75-78, 2004.
[9] Jensen W.B, "The origin of Soxhlex Extraction". *Journal Clinical Education. 84* (12),1913-1914, 2007
[10] Stein S. E., "National Institute of Standards and Technology (NIST) Mass Spectral Database and Software" Version 3.02,USA,1990.
[11] Mc Lafferty F. W., "Registry of mass spectral data." *Fourth electronic ed.Wiley New York* ,1986.
[12] Eadie, Mervyn J." Could Valerian Have Been the First Anticonvulsant?" *Epilepsia; Official Journal of the International League Against Epilepsy.* Volume 45, Issue 11, pages 1338–1343, 2004.
[13] Cho K.H "Monoacylglycerol oleic acid has stronger antioxidant, anti-atherosclerotic and protein glycation inhibitory activities than MAG-palmitic acid." *Journal of medicinal food,* 13(1): 99 – 107, 2015
[14] Gillingham, Leah G., Sydney Harris-Janz.,Peter and J. H. Jones "Dietary Monounsaturated Fatty Acids Are Protective Against Metabolic Syndrome and Cardiovascular Disease Risk Factors" *Springer International Publishing A.G.* Volume 46, Issue 3, pp 209-228, 2011.
[15] Watanabe M, Maemura K, Kanbara K, Tamayama T, and Hayasaki H. *"GABA and GABA receptors in the central nervous system and other organs"*In Jeon KW. Int. Rev. Cytol. International Review of Cytology **213**. pp. 1–47. *doi:10.1016/S0074-7696(02)13011-7*. *ISBN 978-0-12-364617-0*. *PMID 11837891*. 2002.
[16] Julien, M, Hoeffel, J.M., and Flick, M.R. "Oleic acid lung injury in sheep" *Journal of Applied Physiology* 60 (2): 433–40. PMID 3949648, 1986.
[17]A. S. Naidu " Natural food antimicrobial systems". p. 637,2000.
[18] Kinderlerer J.L,and Hatton P.V. "Fungal metabolites of sorbic acid". *Food AdditContam***7** (5): 657–69. doi:10.1080/02652039009373931. PMID 2253810,1990.