International Journal of Tropical Medicine 10 (4-6): 21-29, 2015
ISSN: 1816-3319

Chemical Composition, Antioxidant and Antimicrobial Potentials of *Icacina Trichantha* Oliv. Leaf Extracts

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Abstract: The chemical composition of the leaf extracts of *Icacina trichantha* plant extracted successively with n-hexane, ethyl acetate and ethanol was analyzed by GC/GCMS. The preliminary phytochemical screening conducted on the crude leaf extracts revealed the presence of tannins, flavonoids, phenols and glycosides which are known to support the bioactive activities of the plant in folk medicine. The antibacterial activity of *Icacina trichantha* of the three extracts against Gram-negative bacteria namely; *Eschericha coli*, *Pseudomonas aeruginosa* and *Klebsillia oxytoca* was evaluated in the present research study. It was found that the hexane and ethyl acetate extracts are more effective than ethanol against the three bacteria strains. The free radical scavenging activity of the extracts by spectrophotometric assay on the reduction of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) was also examined. The n-hexane extract possessed the highest antioxidant activity with half maximal inhibitory concentration (IC_{50}) of 0.21 μg mL^{-1} followed by ethyl acetate extract; 3.917 μg mL^{-1} and ethanol ; 4.812 μg mL^{-1}. We hereby report for the first time the major compounds from the hexane, ethyl acetate and ethanol extracts of *Icacina trichantha* to be stearolic acid (30.74%), oleic acid (36.04%) and erucic acid (29.01%), respectively all of which contribute individually and or synergistically to the biological activities reported in this study.

Key words: 1,1-diphenyl-2-picrylhydrazyl, *Icacina trichantha,* phytochemical, antibacterial, antioxidant, GC/GCMS

INTRODUCTION

Man has been exploring the nature particularly plants in search of new drugs since antiquity. This has led to the use of large number of medicinal plants with therapeutic properties to treat several diseases (Verpoorte and Memelink, 2002). The World Health Organization (WHO) estimates that nearly 80% of the world's inhabitants (primarily those living in developing countries) relies on traditional medicine for their primary health care, most of which involves the use of plant extracts (Ajay *et al*., 2009). This may be related not only to the cost and difficulty in obtaining modern orthodox medical care but also the proven efficacy and tolerability of the herbal preparations a practice that has been with indigenous groups for ages (Okoli *et al*., 2007). Rural areas of many developing countries including Nigeria still rely on traditional medicine for their primary health care needs and have found place in day to day life. These medicines are safer and cheaper when compared with synthetic or modern medicine (Iwu *et al*., 1999; Idu *et al*., 2007; Mann *et al*., 2008).

The plants are a rich source of secondary metabolites with interesting biological activities. Therefore, these secondary metabolites have an important source with a variety of structural arrangements and properties (Vickers, 2002; El-Shemy *et al*., 2003, 2007). Several active compounds have been isolated from plants and used directly as patented drugs like taxol, artemisinin and maprouneacin (Goodman and Walsh, 2001; Klayman, 1993; Carney *et al*., 1999). Due to the multitargeting effects, cheap and safety of herbal drugs compared to synthetic ones, there is great need to search for potential drugs from plants.

Many of these plants contain some bioactive compounds which have not been fully discovered. Most research studies on biological systems are based on the use of plant extracts which do not seem to provide adequate and accurate analyses with respect to the phytochemical compounds responsible for the observed effects. As a consequence, the evaluation of bioactive principles needs to be examined in the light of the traditional use and preparation of the plant (Taylor *et al*., 2001; Holmstedt and Bruhn, 1995). This should include

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chemical and biological evaluation of original drug preparations in order to establish dose-effect relationships for the quantitative use of the remedy. (Taylor *et al*., 2001).

Icacina trichantha Oliv. (Icacinaceae) is a perennial shrub up to 2 m with scandent growth above. It is commonly found in field crops, forest regrowths and waste areas in most part of Nigeria. The leaves are simple, alternate and broadly-elliptic. The stem is straggling, semi-wood, round in cross-section has soft brown hairs and arises from an underground tuber that also has soft brown hairs.

The Yorubas in Nigeria call it Gbegbe (meaning carry away) while Igbos call it Ibugo (Burkhill, 1985). The plant is very extensively used in the rural areas and this is regarded as a major handy household medicine for emergency treatment; hence, virtually all household have the macerated tuber in ethanol which is stored in corked bottles (Dalziel, 1937). The plant is reported to become a weed of rice-padis in former Bendel State presently Edo and Delta States in Nigeria. The leaf is said to be used as a wrapper for processed oil bean seeds known as 'ugba' in Igbo. The leaves are also used by the Yorubas for coronating their chiefs called 'Obas' (Asuzu and Egwu, 1998). The tuber has been used extensively by herbalists and traditional doctors in the treatment of poisoning, constipation to induce emesis and to cure malaria. The species is reportedly used as medicines in rural communities in Nigeria (Timothy and Idu, 2011). The Igbos consider the plant to be aphrodisiac (Burkhill, 1985).

The ethanol and water extracts of *Icacina trichantha* leaves were screened for their phytochemicals and antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., *Candida albicans* and *Klebsiella pneumonia*. The result indicated that alkaloids, tannins, saponins and phenols were present in the plant part. Both the ethanol extract and the water extract were active against all the microorganisms. Moreover, Shagal *et al*. (2014) carried out the phytochemical screening of hexane and methanol extracts of *Icacina trichantha* tuber. Phytochemical screening of the extracts revealed the presence of alkaloids, steroids, reducing sugars and cardiac glycosides. This attests to the fact that *Icacina trichantha* tuber contains bioactive compounds of potentially therapeutic and prophylactic significance and thus could be a promising candidate for drug development. Timothy and Idu (2011) carried out the preliminary phytochemistry and *in vitro* antimicrobial properties of aqueous and methanol extracts of *Icacina trichatha*. The leaf of *Icacina trichantha* possessed a broad spectrum of antimicrobial property which is significantly dependent on the solvent of extraction.

Though, a large number of plants worldwide show strong antioxidant activities (Katalinic *et al*., 2006), there is no report to the best of our knowledge on the antioxidant properties of the leaf extracts of *Icacina trichantha* as well as the characterization of the constituents of the leaf extracts of this plant using GC/GCMS in any experimental protocol. In the light of this, we have investigated the *in vitro* antioxidant potential of the extracts by DPPH assay and their antibacterial effects against different bacterial strains. Phytochemicals responsible for these activities were also examined. The plant leaf was selected for this study because of the reported phytochemicals which include phenols, naphthaquinones, among others.

The present study provides basic data on the natural antioxidant and antimicrobial potentials of *Icacina trichantha* leaf for the food, pharmaceutical or cosmetic industries and also offers scientific reference for the large scale usage and exploitation of *Icacina trichantha* as a vital resource.

MATERIALS AND METHODS

Reagents and chemicals: The 1,1-Diphenyl-2-Picrylhydraxyl (DPPH) was obtained from Sigma-Aldrich (Germany). n-Hexane, ethyl acetate, Hydrochloric acid (HCl), tetraoxosulphate (VI) acid (H_2SO_4), chloroform ($CHCl_3$) and ethanol (C_2H_5OH) were obtained from the chemical store of the Chemistry Department of Kwara State University Malete, Nigeria. Solvents were redistilled before use while reagents were used without further purification. All other chemicals and reagent were of analytical reagent grade.

Microorganisms: Three Gram-negative bacteria namely; *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Escherichia coli* were used in this study. Nutrient agar was used as the growth media for the bacteria.

Instrument (GC-MS analysis): A Gas Chromatography mass Spectroscopy, GCMS System, GCMS QP 2010 PLUS (Shimadu Japan) interfaced with a firigan mat ion, trap detector ion source temperature was used and it was carried out at National Research Instituite for Chemical Technology, Zaria, Kaduna State, Nigeria. Identification of the volatile component was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS, their spectra data were compared with those of NIST library mass spectra.

Collection and identification of plant samples: Fresh leaf of *Icacina trichantha* was obtained from Lekki metropolis

in Lagos State Nigeria. The leaf was taxonomically authenticated and documented at the herbarium of Plant and Environmental Biology Department, Kwara State University Malete, Nigeria. The leaf material was air dried and pulverised.

Preparation of extracts: The scheme for the extraction is shown in Fig. 1. The pulverised plant material weighing 82.3 g was extracted exhaustively with n-hexane at room temperature for 5 days. The extract was decanted, filtered and concentrated under reduced pressure using rotary evaporator to afford 4.61 g of a yellow extract which was coded ITLH. The remaining plant material was subsequently extracted with ethyl acetate for 5 days. The ethyl acetate extract was decanted, filtered using a Whatman No. 1 filter paper and concentrated using rotary evaporator to yield 0.77 g of light green extract coded ITLEA. Finally, the remaining extracted plant material was extracted again for 5 days with ethanol. The ethanol extract was decanted, filtered and concentrated in a rotary evaporator to yield 0.77 g of a darkish green extract coded ITLE. The extracts were stored in a cool dark place until further analysis.

Phytochemical screening of the plant extracts: Each extract of the leaves of *I. trichantha* was subjected to preliminary phytochemical screening for the detection of different secondary metabolites such as phenols, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, etc. (Harborne, 1999; Trease and Evans, 1985; Sofowora, 2001).

Test for tannins: The 2 cm^3 of the extract was boiled and allowed to cool. To the cooled extract was added 3 drops of ferric chloride ($FeCl_3$) solution.

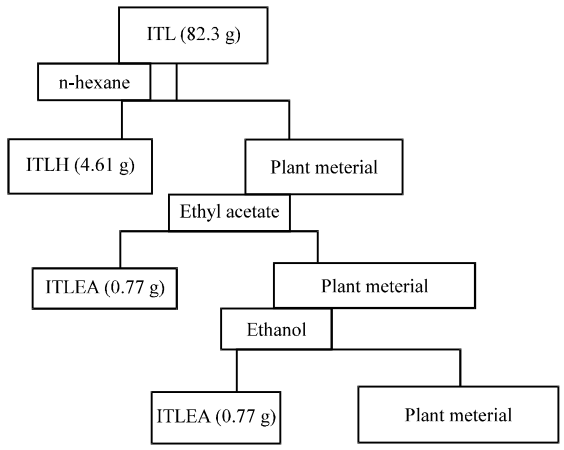

Fig. 1: Extraction schematics

Test for saponins: To 3 cm^3 of each extracts was added 2 cm^3 of distilled water and shaken vigorously for 2 min. Persistent frothing indicates the presence of saponin.

Test for phenols: The 1 cm^3 distilled water and 3 drops of 5% NaOH were added to 2 cm^3 of each extract, respectively.

Test for terpenoids: The extracts of the plant material was taken in a clean test tube, 2 mL of chloroform was added and it was vigorously shaken, then evaporated to dryness. To the 2 mL of concentrated sulphuric acid was added and heated for about 2 min.

Test for glycosides: The extract of the plant material was mixed with 2 mL of chloroform and 2 mL of concentrated sulphuric acid was carefully added and shaken gently, then the observation was made.

Test for steroids: The 2 mL of the extract was put in a test tube and 10 mL of chloroform was added and filtered, 2 mL of the filtrate was mixed with 2 mL of a mixture of acetic acid and concentrated sulphuric acid was added along the side of the test tube.

Test for flavonoids: A portion of the extracts was added in a test tube. To the 5 mL of dilute ammonia and 2 mL of concentrated sulphuric acid were added.

Estimation of antioxidant activity using DPPH assay: The antioxidant activity was measured using (DPPH) assay. The free radical-scavenging ability of the extracts against 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free radical was evaluated as described by Cervato. Briefly, appropriate dilution of the extracts (1 mL) was mixed with 3 mL of 60 μM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 min before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH˙ on addition of test samples in relation to the control was used to calculate the percentage inhibition (Inhibition %) following the equation:

$$\text{Inhibition (\%)} = \left[\frac{(A517_{control} - A517_{sample})}{A517_{control}}\right] \times 100$$

The IC_{50} which stands for the concentration of extract required for 50% scavenging activity was calculated from the dose-inhibition linear regression equation of each extract

Antibacterial activity

Preparation of Mueller Hinton Agar (MHA): MHA was prepared according to the manufacturer's instruction. The

38 g of MHA was dissolved in 1 L of sterile distilled water. It was allowed to homogenize before sterilizing inside the autoclave at 121°C for 15 min. The sterilize MHA was asceptically poured into sterile disposable petri dishes and allowed to set.

Preparation of bacteria innoculum: Innoculum of *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Escherichia coli* was prepared according to Kirby-Bauer Method. Little quantity of the bacteria culture was suspended in 9 mL sterile normal saline and serially diluted until the colour of the serially diluted bacteria and normal saline resembles the colour of 0.5 Mc Farland standard. The suspension was swabbed on the surface of the prepared MHA plates.

Antimicrobial susceptibility test: All the inoculated plates were bored at the centre with 6 mm cork borer and each extracts was introduced to the hole with the aid of capillary tubes. The plates were allowed to stand uprightly for about 20 min before they were transferred to the thermostatically stable incubator. All the plates were incubated at 35±2°C (normal body temperature).

Qualitative and quantitative analysis of the *Icacina trichantha* chemical constituents: The afore-mentioned GC/GCMS instrument was used for the qualitative and quantitative analysis of the constituents of *Icacina trichantha* leaf extracts. The compounds were identified on the basis of their retention times and mass-spectral fragmentation patterns compared with those of reference compounds stored in the spectrometer database and the NIST library quantification of the identified constituents was performed by injecting 1 μL of the samples (on column injector, hydrogen as carrier gas) and calculations from the electronic integration of the FID peak areas.

Statistical analysis: The group mean±SEM was calculated for each analyte and significant difference between means evaluated by Analysis of Variance (ANOVA). Post-hoc test analysis was done using the Duncan multiple comparison test values at $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening results: The result of the preliminary phytochemical screening of the crude extracts of *Icacina trichantha* is as shown in Table 1.

The phytochemical analysis conducted on the *I. trichantha* leaf extracts revealed the presence of tannins, phenols, glycosides, steroids and flavonoids. These phytochemicals are known to support bioactive activities in medicinal plants and may therefore be responsible for both the antioxidant and antimicrobial potentials of the leaf extracts of *I. trichantha*. This finding corroborates the research by Mohammed and Kubmarawa who also reported the presence of some of these phytochemicals in the ethanolic leaf extracts of *I. trichantha*.

Table 1: Phytochemical screening results of *Icacina trichantha* leaf extracts

Phytochemical compounds	HEX/IT	EA/IT	ET/IT
Tannins	+	+	+
Saponins	-	-	-
Phenols	++	++	+
Terpenoids	+	++	++
Glycosides	++	++	++
Steroids	++	++	+
Flavonoids	++	++	+

HEX/IT = n-Hexane Extract, EA/IT = Ethyl Acetate Extract, ET/IT = Ethanol extract; + = trace amount, ++ = strongly present, - = absent

Tannin was present in all the leaf extracts of the plant in trace amount while saponin was conspicuously absent in all the extracts. The n-hexane and ethyl acetate extracts exhibit the highest phenolic content while it was only present in trace amount in the ethanolic extract. Moreover, terpenoids, glycosides and steroids were strongly present in all the three extracts while flavonoids were strongly present in both the n-hexane and ethyl acetate extracts and only present in trace amount in ethanol extract. The difference observed in the phytochemical constituents in all the three leaf extracts may be due to the difference in the extraction solvents.

However, tannins are generally known to be useful in the treatment of inflamed or ulcerated tissues and have remarkable activity in cancer prevention (Ruch *et al*., 1989; Motar *et al*., 1985). Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li *et al*., 2003). Flavonoids also serves as health promoting compound as a result of its anion radicals (Havsteen, 1983) while phenolic compounds have been attributed with antioxidant and anticancer effects in cells and animal models (Fresco *et al*., 2006; Liu, 2004). Thus, the presence of these constituents in *I. trichantha* supports the antioxidant potentials of this plant as well as its use in the treatment of cancer.

Antimicrobial activity results: The results of antimicrobial activity of *Icacina trichantha* leaf extracts against three different Gram-negative bacteria strains are presented in Table 2 and Fig. 2.

The antibacterial results for different extracts (Fig. 2) indicates that hexane and ethyl acetate extracts were found to be more effective against the three different bacteria tested for when compared with the ethanol

Table 2: Antimicrobial efficacy of leaf extracts of Icacina trichantha

Extracts	Zone of inhibition (mm)		
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella oxytoca*
Hexane (HEX/IT)	22.50	9.75	12.95
Ethyl acetate (ET/IT)	27.00	10.25	10.75
Ethanol (ET/IT)	18.75	0.00	9.50

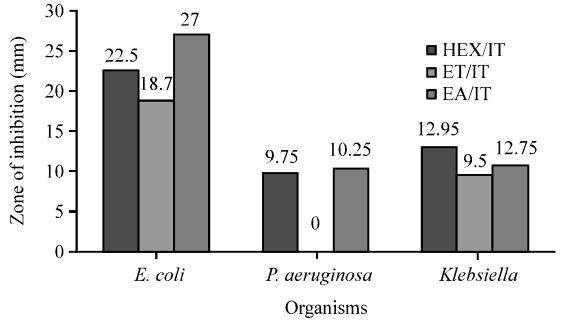

Fig. 2: Graphical representation of antimicrobial efficacy of leaf extracts

extract. This variation in the activity of the extracts may be due to the different phytochemicals contained in them as shown in Table 1 in which the phenolic, flavonoids and steroids contents were higher in both n-hexane and ethyl acetate extracts than the ethanolic extract.

Furthermore, it is evident from Fig. 2 that the ethyl acetate extract has the highest activity against *Escherichia coli* followed by n-hexane and then ethanol extract. The same trend is also observed for *Pseudomona aeruginosa* except for the ethanol extract which shows no activity. And in *Klebsiella oxytoca*, the n-hexane extract has the highest activity followed by ethyl acetate extract and then ethanol. These findings partly support the research by Mohammed and Kubmarawa where the ethanolic extract and aqueous extracts of *I. trichantha* leaf screened for *Escherichia coli*, *Staphylococcus aureus*, *streptococcus* sp., *Candida albicans* and *Klebsiella pneumonia* were active against all the tested microorganisms.

DPPH antioxidant activity of the plant extracts: The antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of samples (Sakanaka *et al*., 2005). The antioxidant activities of the n-hexane, ethyl acetate and ethanolic extracts of *I. trichantha* leaves are given in Table 3 and Fig. 3.

The result of the *in vitro* antioxidant activity of the extracts of *I. trichantha* is as shown in the table and bar chart above. It is evident from the chart that the n-hexane extract has the lowest minimum inhibitory concentration

Table 3: Half maximal inhibitory concentrations of the extracts of *I. trichantha*

Samples	IC_{50} (μg mL^{-1})
HEX/IT	0.210
EA/IT	3.917
ET/IT	4.812

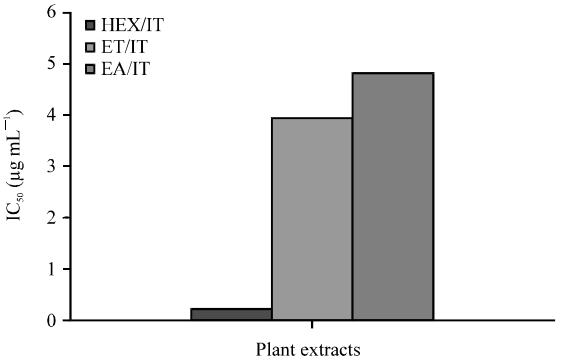

Fig. 3: Graphical representation of *in vitro* antioxidant activity of *I. trichantha* plant extracts

(IC_{50}) value and hence the highest antioxidant activity. Also, the antioxidant activity of ethyl acetate extract is higher than that of the ethanolic extract which has the highest IC_{50} value. This variation is due to the higher phenolic contents exhibited by the n-hexane and ethyl acetate extracts as shown in the phytochemical screening result when compared with the ethanolic extract.

The screening and characterization of antioxidants derived from natural sources has gained much attention and efforts have been put into identifying compounds as suitable antioxidants to replace synthetic ones. The search for phytochemicals with potent antioxidant continues to be of great importance in the search for remedies against free radical-mediated diseases, prevention of oxidative reactions in foods, protection against DNA damage and carcinogenesis and possible substances with wide range of pharmacological activities such as anti-inflammatory, antibacterial and antifungal properties (Lin *et al*., 2013).

***Icacina trichantha* leaf extracts composition**

GC-MS analysis results of n-hexane extract of icacina trichantha leaf: The GC-MS analysis results of the various constituents of the n-hexane extract of *I. trichantha* is presented in Table 4.

In the GC-MS analysis, 9 significant bioactive compounds were identified in the n-hexane extract of *I. trichantha* as shown in Table 4. Stearolic acid ($C_{18}H_{36}O_2$), Fig. 4 with RT 20.708 and percentage composition 30.74% was the major compound identified in the n-hexane extract of the plant. Other notable

Table 4: *Icacina trichantha* leaf n-hexane extract profile obtained from the GC/GC-MS

Peaks	Compound	Yield (%)	RT	MF	Base peak	Mass spectra data
1	3,3-dimethyl-2-hexanone	4.22	3.113	$C_8H_{16}O$	43	27, 41, 47, 57, 85
2	Undecane	3.22	5.525	$C_{11}H_{24}$	57	27, 41, 43, 57, 71, 156
3	Palmitic acid	27.74	18.052	$C_{16}H_{34}O_2$	43	27, 41, 43, 60, 73, 129, 284
4	5-octadecenoic acid methyl ester	0.68	19.860	$C_{19}H_{36}O_2$	43	27, 43, 67, 74, 264
5	Stearolic acid	30.74	20.708	$C_{18}H_{36}O_2$	41	27, 41, 55, 67, 87, 196
6	Stearic acid	11.31	21.143	$C_{18}H_{36}O_2$	43	27, 41, 43, 60, 73, 85, 198
7	9,12-octadecadienoic acid	6.08	21.296	$C_{18}H_{32}O_2$	41	41, 53, 67, 81, 95, 110
8	9,17-octadecadienal	3.56	21.712	$C_{18}H_{40}O$	67	41, 55, 67, 81, 264
9	Eicosanoic acid	1.39	23.121	$C_{20}H_{40}O_2$	43	41, 43, 57, 73

Table 5: *Icacina trichantha* leaf ethyl acetate extract profile obtained from the GC/GC-MS

Peaks	Compound	Yield (%)	RT	MF	Base peak	Mass spectra data
1	1,2-ethanediol monoacetate	0.85	5.381	$C_8H_8O_3$	43	31, 43, 74, 86, 103
2	1,2,3-propanetriol monoacetate	0.42	7.384	$C_5H_{10}O_4$	43	43, 61, 74, 103
3	4-tetradecene	0.56	9.500	$C_{14}H_{28}$	41	41, 55, 57, 97
4	1-tetradecane	0.45	11.988	$C_{14}H_{28}$	41	41, 57, 83
5	Palmitic acid	11.33	17.104	$C_{16}H_{32}O_2$	43	41, 43, 60, 73
6	Lauric acid ethyl ester	6.57	17.577	$C_{14}H_{28}O_2$	88	73, 88, 101, 228
7	Palmitic acid ethyl ester	3.14	18.099	$C_{18}H_{36}O_2$	88	73, 88, 101, 281
8	9,12-octadecadienyl chloride	32.48	20.416	$C_{18}H_{31}C_1O$	55	41, 55, 83, 151
9	Oleic acid	36.04	20.640	$C_{18}H_{34}O_2$	55	41, 55, 83, 151
10	Stearic acid	5.08	20.863	$C_{18}H_{36}O_2$	43	41, 43, 60, 73
11	9,12-decadienoic acid methyl ester	3.08	21.558	$C_{19}H_{34}O_2$	67	41, 55, 67, 81, 95

RT = Retention Index and MF = Molecular Formula

Fig. 4: Stearolic acid

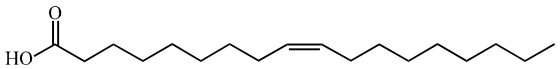

Fig. 5: Oleic acid

compounds present in significant amount include palmitic acid (27.74%, RT 18.052), stearic acid (11.31%, RT 21.143) and 9,12-octadecadienoic acid (6.08%, RT 21.296) all constituting 75.87% of the total compounds.

Stearolic acid (9-octadecynoic acid) is the acetylenic analogue of the ubiquitous oleic acid which has been implicated for the blood pressure reducing (hypotensive) effects of olive oil (Teres *et al*., 2008).

GC-MS analysis results of ethyl acetate extract of icacina trichantha leaf: The GC-MS analysis results of the chemical composition of the ethyl acetate extract of *I. trichantha* is presented in Table 5.

The GC-MS spectrum showed 11 peaks indicating 11 different bioactive compounds with oleic acid ($C_{18}H_{34}O_2$), RT 20.640, 36.04%, being the most abundant compound. Other compounds present in large quantities include 9,12-octadecadienyl chloride (32.48%, RT 20.416), palmitic acid (11.33%, RT 17.104), lauric acid ethyl ester (6.57%, RT 17.577), all constituting 50.38% of the total compounds.

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is odourless, colourless oil, although commercial sample may be yellowish.in chemical terms, oleic acid is classified as a monosaturated omega-9 fatty acid (Thomas, 2000). Oleic acid is a common monosaturated fat in human diet.

Monosaturated fat consumption has been associated with decrease Low Density Lipoprotein (LDL) cholesterol and possibly increased High Density Lipoprotein (HDL) cholesterol. Oleic acid has been implicated to be responsible for the hypertensive (blood pressure reducing) effects of olive oil (Teres *et al*., 2008). Adverse effect also have been documented, however, since both oleic acid and monosaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer (Pala *et al*., 2001), although, the consumption of oleate in olive oil has been associated with a decreased risk of breast cancer (Martins *et al*., 1998).

The structure of the most abundant compound (oleic acid) from ethyl acetate extract of *I. trichantha* is as shown in Fig. 5.

GC-MS analysis results of ethanolic extract of icacina trichantha leaf: The GC-MS analysis results of the chemical composition of the ethanolic extract of *I. trichantha* is presented in Table 6.

The GC-MS spectrum showed 12 peaks indicating 12 different bioactive compounds with Erucic acid, ($C_{18}H_{34}O_2$) (Fig. 6a), RT 20.561, 29.01% as the major compound. Other notable compounds that are present in the ethanolic extract include stearic acid (24.65%, RT

Table 6: *Icacina trichantha* leaf ethanolic extract profile obtained from the GC/GC-MS

Peaks	Compounds	Yield (%)	RT	MF	Base peak	Mass spectra data
1	Acetoglyceride	2.54	5.396	$C_5H_{10}O_4$	43	40, 43, 61, 103
2	1,1,2-triacetoxyethane	0.35	7.378	C18H12O_6	43	29, 43, 103
3	3,5-diterbutyl phenol	0.70	11.047	$C_{14}H_{22}O$	191	41, 57, 91, 191, 206
4	Stearic acid	24.65	17.637	$C_{18}H_{36}O_2$	43	41, 43, 60, 73, 129, 241
5	Palmitic acid, ethyl ester	4.53	18.078	$C_{18}H_{36}O_2$	88	41, 57, 73, 88, 284
6	Phytol	5.21	20.052	$C_{20}H_{40}O$	71	41, 57, 71, 278
7	Erucic acid	29.01	20.561	$C_{18}H_{34}O_2$	41	41, 55, 69, 83, 264
8	Eicosanoic acid	14.81	20.790	$C_{20}H_{40}O_2$	43	41, 43, 57, 73, 312
9	7-tetradecyne	8.74	21.034	$C_{14}H_{26}$	67	27, 41, 54, 67, 81, 165
10	9,12-octadecadienoic acid methyl ester	6.31	21.530	$C_{19}H_{34}O_2$	41	41, 55, 67, 81, 109
11	Hexadecanoic acid	1.30	24.433	$C_{19}H_{38}O_4$	43	41, 57, 74, 84, 239
12	2,2-6,9-pentadecadien-1-ol	1.64	26.087	$C_{18}H_{28}O$	67	27, 41, 53, 67

RT = Retention Index, MF = Molecular Formula

Table 7: Classes of compounds present in the *I. trichantha* leaf extracts

	Composition (%)		
Class of compounds	HEX/IT	EA/IT	ET/IT
Fatty acid	77.26	52.65	68.47
Fatty acid ester	0.68	12.79	12.14
Alkane	3.22	0.45	0.35
Alkene	0.00	0.56	0.00

17.637), Eicosanoic acid (14.81%, RT 20.790), 7-tetradecyne (8.94%, RT 21.034), 9,12-octadecanoic acid methyl ester (6.31%, RT 21.530) all representing 54.71% of the total compounds.

The biological importance and toxicity of erucic acid, a monounsaturated fatty acid have remained controversial. Studies carried out on laboratory animals in the early 1970s showed that erucic acid appears to have toxic effects on the heart at high enough doses, although, an association between the consumption of rapeseed oil and increased myocardial lipidosis or heart disease has not been established for humans (FSANZ, 2003). While, there are no reports of toxicity from long-term use of Lorenzo's oil (which contains erucic acid and other ingredients), there are no reports of harm to people from dietary consumption of erucic acid. Meanwhile, phytol (3, 7, 11, 15-tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols (Fig. 6b) (Gloerich *et al*., 2007; McGinty *et al*., 2010). It is the product of chlorophyll metabolism in plants; hence, phytol is abundantly available in nature. Phytol is known to inhibit the growth of *Staphylococcus aureus* (Inoue *et al*., 2005) and to block the teratogenic effects of retinol (Arnhold *et al*., 2002).

In the light of the various reports on the toxicity of erucic acid, the therapeutic effect of the plant should be weighed alongside its toxicity when administered in folkloric medicine. Special attention may have to be given to the extraction solvent as erucic acid is conspicuously absent in n-hexane and ethyl acetate extracts of *I. trichantha* and only present in ethanol extract.

In summary, the percentage compositions of notable classes of compounds present in Hexane (HEX/IT), Ethyl

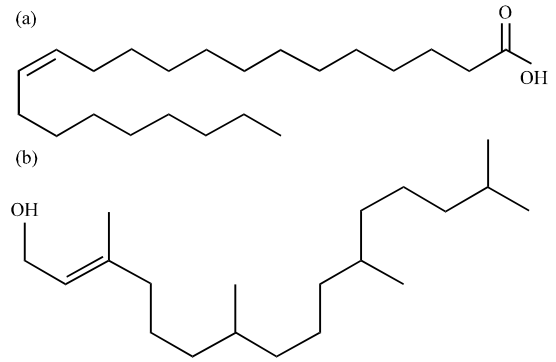

Fig. 6: Structures of erucic acid and phytol; a) erucic acid and b) phytol

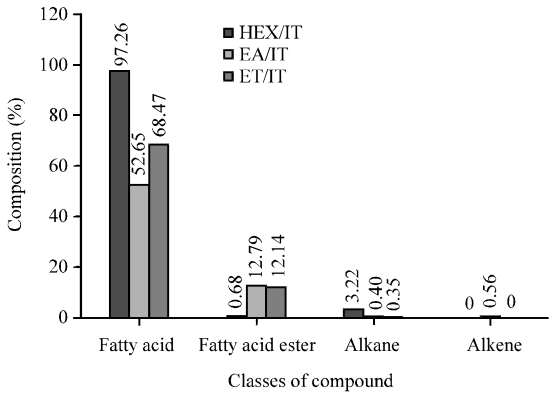

Fig. 7: Graphical representation of the classes of compounds of *I. trichantha* leaf extracts

acetate (EA/IT) and Ethanol (ET/IT) extracts of *Icacina trichantha* is presented in Table 7 and Fig. 7. The n-hexane extract possesses the highest percentage composition of the fatty acid and alkane while the ethyl acetate extract has the largest percentage composition of fatty acid ester and alkenes. The presence of these compounds is responsible for the antioxidant and antimicrobial efficacy of this plant.

CONCLUSION

Icacina trichantha leaf is rich in phytochemicals with proven antimicrobial and antioxidant activities. The phytochemical analysis conducted on *Icacina trichantha* extracts revealed the presence of tannins, flavonoids, phenolics, terpenoids and glycosides. This study confirmed antimicrobial properties of *Icacina trichantha* leaf that showed significant inhibition for *Eschericha coli*, *Klebsilla oxytoca* and *Psesudomonas aeruginosa* tested whose problem relates to the emergence of strains that possess multiple resistances to a range of antibiotics, thereby making them difficult to treat. The encouraging results indicate that *Icacina trichantha* leaf extracts especially the n-hexane and ethyl acetate extracts that possessed higher antimicrobial activity when compared with the ethanol extract might be exploited as natural antibiotic for the treatment of several infectious diseases and could also be useful in understanding the relationship between traditional cures and current medicines.

Similarly, the study indicates that the n-hexane extract of the leaf of the plant has higher antioxidant activity against DPPH than the hexane and methanol extract due to the presence of high content of phenolics which could be the most effective in protecting the body against different oxidative stressors.

Furthermore, the GC/GC-MS analysis results indicate that the major compound isolated from the n-hexane extract of *Icacina trichantha* is stearolic acid (30.74%), a monounsaturated fatty acid which is an acetylenic analogue of oleic acid that has been implicated for the hypotensive effect of olive oils. Other notable compounds present in the n-hexane extract include palmitic acid, stearic acid and 9,12-octadecadienoic acid. However, oleic acid (36.04%) is the most abundant compound obtained from the ethyl acetate extract of the plant while erucic acid (29.01%) is the major compound isolated from the ethanol extract. All these compounds contribute individually or synergistically for the various antioxidants and antimicrobial activities exhibited by *Icacina trichantha* leaves.

Though, the leaf extracts are highly regarded for their unique medicinal properties as established in the present study, the content of erucic acid present in ethanol extract calls for caution in the use of the plant in folkloric medicine. There is a need for *in vivo* studies and clinical to justify and further evaluate the antioxidant and antimicrobial potentials of the extracts.

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