



## Phytochemical Screening and Identification of Bioactive Constituents of the Chloroform Extract of *Icacina Trichantha* Tuber Peel Oliv

\*AKOH, OU; MAC-KALUNTA, OM

Department of Chemistry, Michael Okpara University of Agriculture Umudike, P.M.B. 7267, Umuhia, Abia state, Nigeria  
\*Corresponding Author Email: [onyilac@gmail.com](mailto:onyilac@gmail.com)

**ABSTRACT:** This study was carried out to identify the bioactive constituents of the tuber peels of *Icacina trichanthalinna* of the *Icacinales* family. This plant is basically selected due to its widespread use in herbal treatment. Nine compounds were identified in the chloroform extract of the tuber peel. Undecane (43.254%), 2-hexanone (23.299%), Dodecanoic acid ethyl ester (6.244%) and 9-octadecynoic acid (5.915%) appeared to be the most prevailing compounds and they are known to possess antibacterial, antiviral, antioxidant, antimycobacterial, hypercholesterolemic activities. Also identified was triarachine which has been reported to have some biological activity.

DOI: <https://dx.doi.org/10.4314/jasem.v25i7.3>

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**Dates:** Received: 10 May 2021; Revised: 28 June 2021; Accepted: 01 July 2021

**Keywords:** Medicinal plants, *Icacinatrichantha*, chloroform extract, GC-MS, Analysis.

Medicinal based plants also called medicinal herbs are of great use today, particularly in the developing countries of the world. This is because they are very available, cheap, and safe. Medicinal plants have been used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insect, fungi, diseases, and herbivorous mammals. Folkloric stories and ideas have always played an important role in creating of novel bioactive components for therapeutic purposes (Awa et al., 2012). Drug research makes use of ethno botany to search for pharmacologically active substance in nature, and has in this way discovered hundreds of useful compounds. Medicinal plants have been known to have a very high efficacy and that is why most people (about 78%) of the people in the rural communities rely on them for effective method for their primary health care because of the presence diverse bioactive components present in them, which have some pharmacological activities and nutritional values in the body of living organisms (Burkhill, 1985). *Icacina hantha* belongs to the family of *Icacinales*. Other species of this plants includes *Icacinatric laessensii* De wild, *Icacinaguess feldtii* Asch, *Icacina mannii oliv*, *Icacina oliviformis* (poir), *Icacinatric hantha oliv*.

*Icacinatric hantha* Oliv. (*Icacinaceae*) is a perennial shrub up to 2m with scan dent growth above. It is commonly found in field crops, forest re-growth and

waste areas in most part of Nigeria (Burkhill, 1994; Della Loggia, 1999). *Icacinaceae* comprises over 8 species including *Icacinatrichantha* as one of the genera in the family. *Icacina trichantha oliv* is popularly referred to as Gbegbe in Yoruba western Nigeria (meaning carry away) while Igbos in the eastern part of Nigeria calls it Ibugo (Edorios and Ekpete, 2015). It thrives better in colonies. The plant is reported to become a weed of rice-padis in former Bendel State presently Edo and Delta States in Nigeria. The peel has been used extensively by herbalists and traditional doctors in the treatment of poisoning, constipation, to induce emesis and to cure malaria. It is used in folk medicine as anti-inflammatory, anti-convulsion, anti-diabetic and anti-malarial in different part of African (Holldobler, 1990). Some already isolated compounds from different parts of this plant has been reported to possess anti-inflammatory, anti-diabetic, anti-microbial, anti-oxidant, anti-gene toxic, anti-cytotoxic and hepatoprotective activity (Ezeigbo, 2010). Phytochemical examination of *Icacina trichantha* peels indicated the presence of alkaloids, phenols, tannins, flavonoids, saponins, steroids, terpenoids and glycosides (Shagal et al., 2014). The leaves and seeds, when crushed and macerated in local gin, can be used for the treatment of hypertension and asthma (Ajibensin et al., 2008). Tubers are used by traditional healers to treat various medical conditions including constipation, poisoning, malaria, rheumatism, toothache, as well as to induce emesis and abortion.

\*Corresponding Author Email: [onyilac@gmail.com](mailto:onyilac@gmail.com)

The tuber juice can be used for treating mumps (Ubom, 2010). This study is geared at identifying the bioactive constituents in the chloroform extract of *icacina trichantha* tuber peel *oliv* as well as the phytochemical study.

## MATERIALS AND METHODS

**Sample Collection:** The Plant (sample) was harvested at Umunakukwu Chokoneze Mbaise, Imo State in August 2019 and identified in Forestry Department of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and given a herbarium number ICA DALZ. 1094.



Fig 1. *Icacinatri chantha* tuber

**Preparation and Extraction:** The tuber was well washed in running water to remove sands and unwanted debris, rinsed with sterile distilled water and then peeled. The tuber peel was air dried for four weeks, crushed into pieces with mortar and pestle and weighed. The weight was found to be 500g. The pulverized peel was kept in a clean tight waterproof till use. It was extracted using 1500ml of chloroform for 48 hours. The extracts were then filtered using Whatman filter paper. The filtrate was concentrated using rotary evaporator. 2 g of the chloroform extract was used for GC-MS because of the clearer separation from the TLC done.

**Phytochemical Screening:** The phytochemical screening of the plant extract was qualitatively analyzed in details using a standard protocol.

**Test for alkaloids:** 1g of the chloroform extract was dissolved in 5ml of 10% ammonia solution and the extracted with 15ml of chloroform. The portion was evaporated to dryness and the resultant residue dissolved in 15ml dilute sulphuric acid.

**Meyer reagent test:** drops of the reagent was added to a portion of the acidic solution in test tube and observed for a yellowish precipitate indicative of the presence of alkaloids.

**Test for steroid:** 2 ml of chloroform and concentrated  $H_2SO_4$  were added with 5ml of aqueous plant crude extract. In lower chloroform layer, red colour appeared indicating the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated  $H_2SO_4$  and acetic were poured into the mixture. The greenish coloration indicated the presence of steroids.

**Test for glycosides:** 2ml of glacial acetic acid was used to treat the extract, then 1 droplet of  $FeCl_3$  was added and concentrated  $H_2SO_4$  of 1 ml. the presence of brown colouration showed the presence of glycoside.

**Test for saponins:** 20 ml of distilled water was mixed with aqueous crude extract in a test tube and it was shaken by hand for 15 minutes. Foam later was obtained on the top of the test tube. The Frothing was mixed vigorously with few drops of olive and it persisted. This foam layer indicates the presence of saponins.

**Test for tannin:** 2g of the chloroform extract was boiled with 5ml of 45% ethanol. The mixture was allowed to cool, therefore it was filtered. The filtrate was further subjected to ferric chloride test: 1ml of the filtrate was diluted with distilled water and added 2 drops of ferric chloride. A greenish to black colour indicates the presence of tannins.

**Test for Flavonoid (Alkaline reagent test):** 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which become colourless when added 2 drops of dilute acid to mixture. This result showed the presence of flavonoid.

**Test for phenol:** 5 ml of 90% ethanol was added to 2 ml of the extract, then a drop of freshly prepared 10% ferric chloride. The formation of a darkish blue colour indicates a positive result.

**Test for terpenoid (salkowski tests):** To 5ml of the sample was added 2 ml of chloroform, then 3ml of sulphuric acid. A reddish brown ring at the interface indicated a positive test.

**GC-MS Analysis:** 2ul of the sample extract was injected into the GC column for analysis. The GC (Agilent 6890N) and MS (5975B MSD) is equipped with DB-5ms capillary column (30 m $\times$ 0.25 mm; film thickness 0.25  $\mu$ m). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/min. The temperature was again increased to 230°C at the rate of 5°C/min. The process continued till the temperature reached 280°C at the rate of

20°C/min which was held for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C then. Helium was used as the carrier gas with a flow rate of 1 mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively. To identify the unknown phytochemical components present in the extract, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014. Then the phytochemicals were identified after comparing the unknown peak value and chromatogram from GC-MS against the known chromatogram, peak value from the NIST Library database. Subsequently, the details about their molecular formula, molecular weight, retention time and percentage content were also obtained.

## RESULTS AND DISCUSSIONS

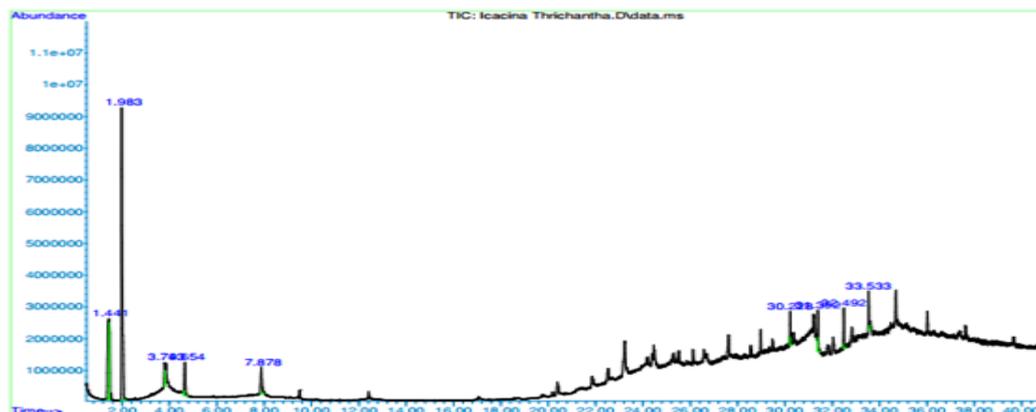
The physicochemical characteristics of the chloroform extract of *Icacinatri chantha* tuber is presented in table 1. While the GC-MS Chromatogram of the chloroform

extract of the tuber peel of *Icacinatric hantha oliv* is shown in figure 2 and the signal peaks of the identified compounds are presented in table 2. *Identification of the peaks:* Nine peaks for bioactive compounds were identified in the chloroform extract of the tuber peels of *Icacinatricanthaoliv* by GC-MS. The peak 1 is presented in figure 1. The compound identified in peak 1 is 3,3-dimethyl hexan-2-one with a formula  $C_8H_{16}O$  and molecular weight 128. The R-time is 1.441 and total abundance of 23.299%. The chemical structure is shown in figure 1.

**Table 1:** Phytochemical screening results from the Chloroform crude extract

Compound	Observation
Alkaloids	+
Steroids	+
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Phenol	+
Terpenoids	+

Key: + = present



**Fig 2:** GC-MS Chromatogram of the chloroform extract of the tuber peel of *Icacinatric hanthaoliv*

**Table 2:** Signal peaks of the identified compound

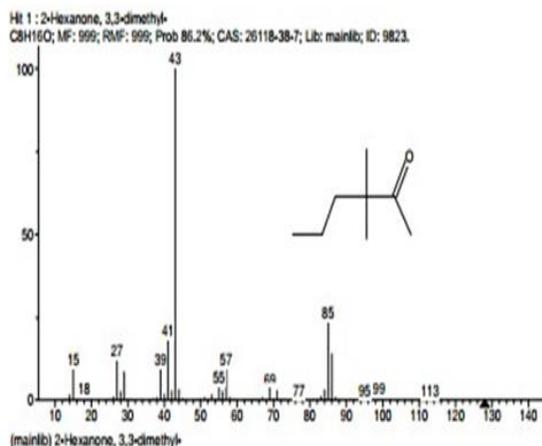
Peak #	R.T. min	First scan	Max scan	Last scan	PK TY	Peak height	Corr. area	Corr.% max	% of total
1	1.441	147	164	167	rBV4	2556602	12207325	53.87%	23.299%
2	1.983	248	257	281	rBV	9216694	22662592	100.00%	43.254%
3	3.793	562	568	573	rBV4	751879	1798518	7.94%	3.433%
4	4.654	708	716	733	rVB3	1051659	3271549	14.44%	6.244%
5	7.878	1261	1270	1289	rBV2	829643	3099188	13.68%	5.915%
6	30.228	5106	5110	5118	rVB2	1063790	1764013	7.68%	3.367%
7	31.392	5307	5310	5322	rVB4	1310170	2579709	11.38%	4.924%
8	32.492	5494	5499	5511	rVB2	1259369	2631987	11.61%	5.023%
9	33.533	5674	5678	5686	rBV5	1218883	2379027	10.50%	4.541%

Sum of corrected areas: 52393908

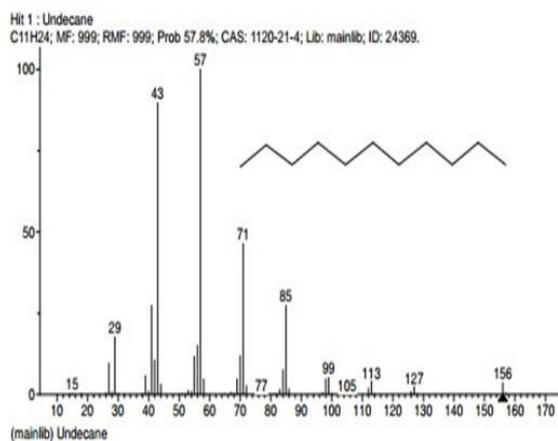
The peak 2 is presented in figure 2. The compound identified in peak 2 is Undecane with a formula  $C_{11}H_{24}$  and molecular weight 156. The R-time is 1.983 and total abundance of 43.254%. The chemical structure is shown in figure 2. The peak 3 is presented in figure 3. The compound identified in peak is Hexadecanoic

acid, 1, 1-dimethyl-1-2-ethanedyl ester with a formula  $C_{36}H_{70}O_4$  and molecular weight 566. The R-time is 3.793 and total abundance of 3.793%. The chemical structure is shown in figure 3. The peak 4 is presented in figure 4. The compound identified in peak is Dodecanoic acid, ethyl ester with a formula  $C_{14}H_{28}O_2$

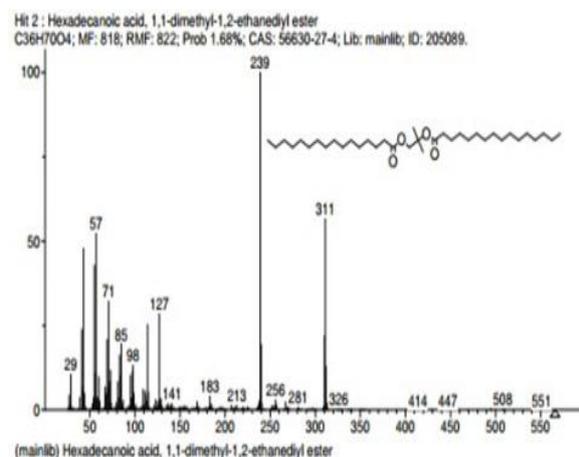
and molecular weight 228. The R-time is 4.654 and total abundance of 6.244 %. The chemical structure is shown in figure 4.



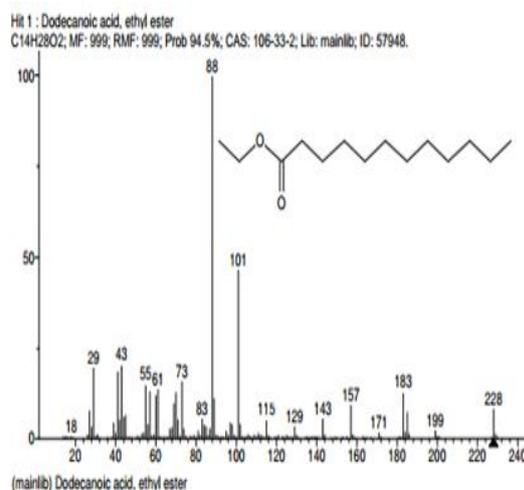
**Fig.1:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinaric hanthaoliv* by GC-MS in peak 1



**Fig.2:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinaric hanthaoliv* by GC-MS in peak 2

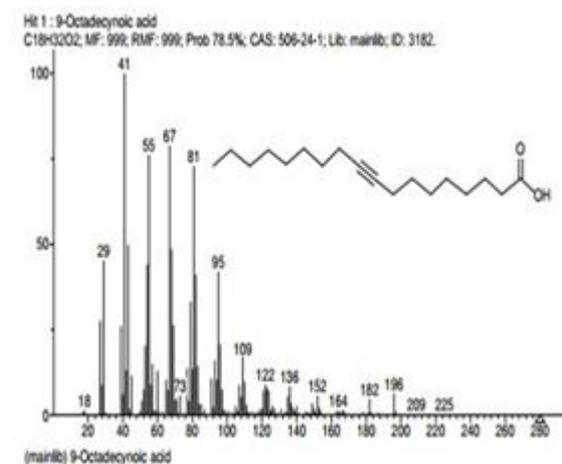


**Fig.3:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinaric hanthaoliv* by GC-MS in peak 3

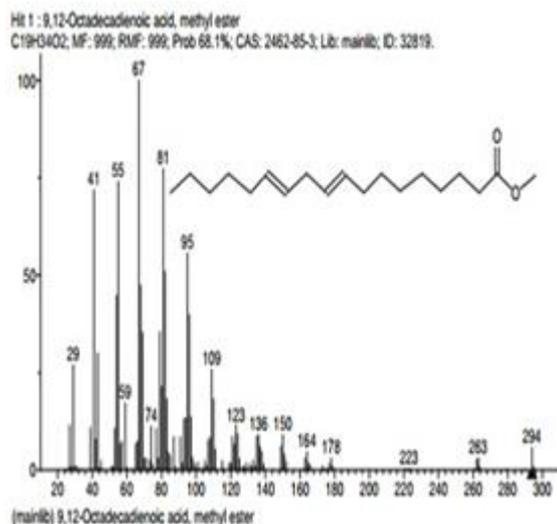


**Fig.4:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinaric hanthaoliv* by GC-MS in peak 4

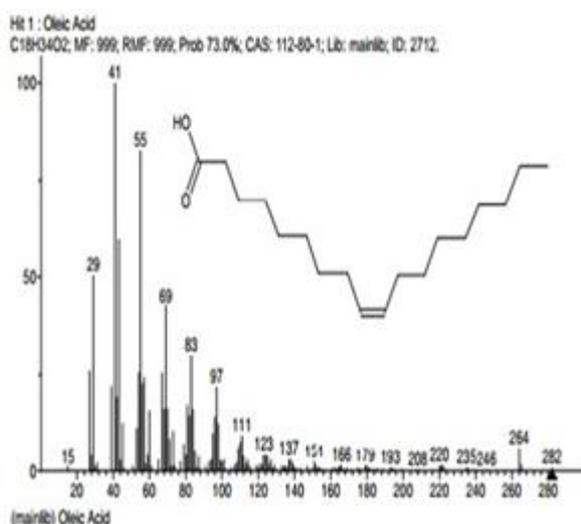
The peak 5 is presented in figure 5. The compound identified in peak is 9-Octadecynoic acid with a formula  $C_{18}H_{32}O_2$  and molecular weight 280. The R-time is 7.878 and total abundance of 5.915%. The chemical structure is shown in figure 5. The peak 6 is presented in figure 6. The compound identified in peak is 9,12-Octadecadienoic acid, methyl ester with a formula  $C_{19}H_{34}O_2$  and molecular weight 294. The R-time is 30.228 and total abundance of 3.367 %. The chemical structure is shown in figure 6. The peak 7 is presented in figure 7. The compound identified in peak is Oleic acid with a formula and molecular weight 282. The R-time is 31.392 and total abundance of 4.924%. The chemical structure is shown in figure 7.



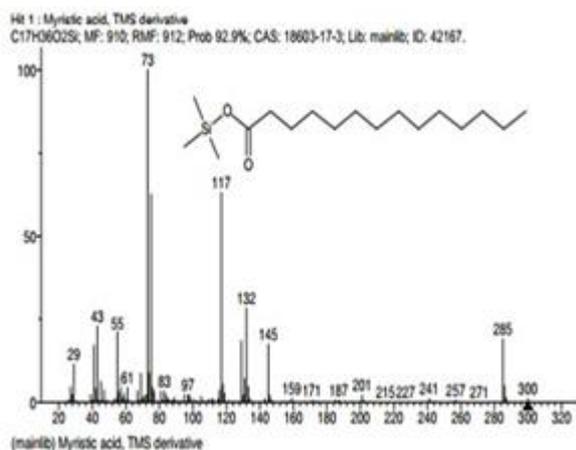
**Fig.5:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinarichanthaoliv* by GC-MS in peak 5



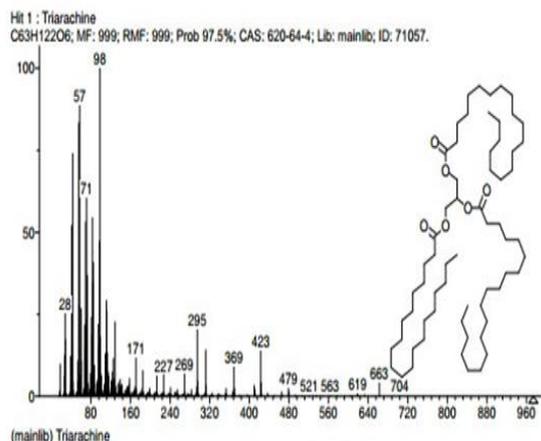
**Fig.6:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinartric hanthaoliv* by GC-MS in peak 6



**Fig.7:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacina trichanthaoliv* by GC-MS in peak 7



**Fig.8:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinartric hanthaoliv* by GC-MS in peak 8



**Fig.9:** Bioactive compound identified in the chloroform extract of the tuber peels of *Icacinartric hanthaoliv* by GC-MS in peak 9. Nine compounds were found in the chloroform extract of the peel of *icacinartric hantha* tuber peel

The peak 8 is presented in figure 8. The compound identified in peak is Myristic acid, TMS derivative with a formula  $C_{17}H_{36}O_2Si$  and molecular weight 300. The R-time is 32.492 and total abundance of 5.023%. The chemical structure is shown in figure 8. The peak 9 is presented in figure 9. The compound identified in peak is Triarachine with a formula  $C_{63}H_{122}O_6$  and molecular weight 974. The R-time is 33.533 and total abundance of 4.541 %. The chemical structure is shown in figure 9. Table 2 and Figure 2. The most prevailing compounds were Undecane (43.254%), 2-hexanone (23.299%), Dodecanoic acid ethyl ester (6.244%), 9-octadecynoic acid (5.915%), Myristic acid (5.023%), oleic acid (4.924%) and Triarachine (4.541). These compounds are known to possess antibacterial, antiviral, antioxidant proper ties (Fay, 1987). According to Wittko Francke (1999) Undecane is the sex pheromone of ant *Formica lugubris*, several other ant specie contain Undecane. Undecane has also been found to be the sex pheromone of the mite *Caloglyphus rodriguezii* and a component of aggregation pheromones of the ant *camponotus pennsylvanicus*. The presence of oleic acid and myristic acids indicates that this plant part has an antimycobacterial activity (Kalim *et al.*, 2010). The phytochemical results revealed the presence of all important phytochemicals which includes alkaloids, phenols, flavonoids, saponins, tannins, glycosides, steroids and terpenoids which comprises of six different compounds with more than 90% confirmation with the library data.

**Conclusion:** The use of *Icacina thricantha* tuber and its peel for the treatment of cough reflexes in the antibacterial, antimyco-bacterial and anti-oxidant properties. The facts that *Icacina thricantha* contains

undecane, as its most prevailing compound among other bioactive compounds will make it useful in agricultural industry as a pheromone and in cosmetics industry if properly explored.

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