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Characterization and Biological Activity of Ethanol Extract of *Chrysobalanus icacao* seed

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Abstract : *Bioactive compounds, antioxidant and antimicrobial activities of the ethanol seed extract of Chrysobalanus icacao were investigated using Gas Chromatography-Mass Spectrometry (GC-MS) 1,1-diphenyl-2-pircrylhydrazyl assay (DPPH) free radical scavenging and ferric reducing ability of plasma model and well in agar diffusion method respectively. The results obtained from GC-MS analysis indicated that 37 compounds are present in the seed extract. The most abundant compounds identified with respect to their % peak areas were n-Hexadecanoic acid (2.07 %), Octadecanoic acid (1.24%), Isoborneol (0.90%), 2,4-Dimethyl-1-heptene (3.64%), Methane (4.13%), Phenol(1.88%), Methyl-2-[(2-chloroethyl) Thio] (1.60%), Diisooctyl phthalate(1.04%),Methyl ester(2.07%),and 2,3,5,7-tetrathioocatane 3, 3-dioxide (1.63%). The extract showed significant activity in all antioxidant assays compared with the reference antioxidant, ascorbic acid, in a dose-dependent manner, indicating that C. icacao possessed significant antioxidant activity, which may be attributed to its high natural abundance of oxygenated and polyphenolic compounds. In the DPPH scavenging assay, the IC_{50} value of the extract was found to be 4.45µg/ml, while the IC_{50} for FRAP was 3334 µg/ml. The susceptibility of these isolates towards the seed extract was compared with gentamycin and nystatin, which were used as a positive control for bacteria and fungi, respectively. Results obtained showed that the extract was able to inhibit the growth of the isolates at various concentrations. On comparing the zones of inhibition of the extracts with that of the standard (Gentamycin and nystatin) for bacteria and fungi, respectively, the results showed that the zone of inhibition of the standard for all the tested isolates was greater than that of the extracts. The results obtained in this study showed that C. icacao is a reservoir of bioactive compounds, which may be responsible for its significant broad-spectrum antimicrobial and antioxidant activities.*

Keywords: *Chrysobalanus icacao, Gas Chromatography-Mass Spectrometry, Isoborneol, Octadecanoic acid, Gentamycin*

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1.0 Introduction

Spices and herbs constitute a large group of natural plant-derived materials obtained from leaves, roots, bark, berries, rhizomes, buds, seeds, or floral parts and are widely used for culinary, medicinal, and preservative purposes. They are primarily used to enhance food flavour and aroma while also contributing functional and health-promoting properties.(Tapsell *et al*., 2006). Spices are an essential part of traditional African cuisines, with many cultivated or growing wild in

Nigeria (Okoh-Esene *et al*., 2011). Beyond culinary applications, increasing scientific attention has focused on spices as sources of bioactive compounds with antioxidant and antimicrobial properties. Spices have been used for centuries in traditional medicine, food and as dietary supplements. In addition to making food taste good, culinary spices have been used as food preservatives and for their health-enhancing properties for centuries (Kaefer and Milner,2011; Kunnumakkara *et al.,* 2009). The abundance of phytochemicals in spices is responsible for their preservative nature in foods. Antioxidants are known to protect food quality by arresting or repressing free radical oxidation of fats and oils (Buck 1991). Spices are important sources of natural antioxidants such as phenolic compounds, phospholipids, carotenoids, vitamin C, and protein-based constituents. (Choe and Min, 2009). They also contain appreciable amounts of carbohydrates, proteins, essential oils, fats, and mineral nutrients including, zinc, magnesium, phosphorus, and calcium. They are equally rich in both micro and macro nutrients, such as zinc, magnesium, phosphorus and calcium.

Herbs and spices contain some secondary metabolites that form part of the plant's chemical defense (Newman and Cragg, 2012). Some spices and plant materials not only provide flavor and aroma, they also possess antimicrobial properties that help in thereby inhibiting microbial growth in foods and enhancing shelf stability (Politeo *et al.,* 2006; Javanmardi *et al*., 2003; Rice-evans *et al*., 1996).

Several studies have demonstrated that herbs and spices possess therapeutic potentials, including antibacterial, anti-inflammatory, anti-ulcer, cardioprotective, and anticancer activities (Jager *et al*., 2013; Rajakannu *et al*., 2015; Farzaei *et al*., 2015; Volak *et al*., 2012; Kris-Etherton *et al*., 2002).

Chrysobalanus icaco L., a member of the family Chrysobalanaceae, is a tropical evergreen plant widely distributed in coastal and tropical regions (Watson & Dallwitz, 1992). It is locally called "ayim eto" in Akwa Ibom state, Southern Nigeria which translates to onion tree in English language (Bassey *et al*., 2011). *In Akwa Ibom State, Southern Nigeria, it is locally known as "ayim eto," meaning "onion tree," due to the characteristic aroma of its seeds (Bassey et al., 2011).*

It is small evergreen tree with thick and bushy foliage, its leaves are oblong elliptic, acuminate and cuneate at base, its flowers are hermaphroditic actinomorphic and arranged in cymes, while its fruits are pubescent when young. Its seeds are hard like nuts, spherical in shape, longitudinally rubbed round its entire body and have a harsh onion- like aroma and unique flavour (Bassey *et al*, 2011). The seeds are traditionally utilized as culinary spices and meat tenderizers, suggesting the presence of biologically active phytochemicals. The tree's height is up to 25m or more, but sometimes it starts flowering at 3m. Although Chrysobalanus *icacao* is one of the lesser-known spices, its dried seeds are usually used as a spice in foods, beverages and as a meat tenderizer (Bassey *et al.*2011). The seeds have a strong, peppery, unique and distinguishing flavour.

Previous studies have reported that *C. icaco* contains bioactive constituents such as flavonoids, alkaloids, and cardiac glycosides (Bassey *et al*., 2011). Pharmacological investigations have demonstrated hypoglycemic, anti-inflammatory, antiangiogenic, and antimicrobial activities associated with *C. icaco*, supporting its traditional medicinal applications (Oliveira *et al*., 2014; Ferreira-Machado *et al*., 2004; Feitosa *et al*., 2012; Fernandes *et al*., 2003). Despite these reported biological properties, limited information is available regarding the phytochemical characterization and biological activities of the ethanol extract of *Chrysobalanus icaco* seeds, particularly using comprehensive analytical techniques such as GC–MS combined with antioxidant and antimicrobial evaluation.

Therefore, this study aimed to characterize the bioactive constituents of ethanol seed extract of *Chrysobalanus icaco* using GC–MS analysis and to evaluate its antioxidant and antimicrobial activities. The findings from this study are expected to provide scientific validation for the traditional use of the plant and contribute to the development of natural antioxidant and antimicrobial agents for pharmaceutical and food preservation applications.

2.0 Materials and Methods

2.1 Sample Collection

The seeds of *Chrysobalanus icacao* were bought from Akpan andem market in Uyo Local Government area and Nung Udoe market in Ibesikpo Local Government area of Akwa Ibom state. The seeds were authenticated and identified by Dr. Imoh Johnny (pharm). The voucher herbarium specimens were deposited at the herbarium of the Medicinal Plant Research Centre in the faculty of pharmacy, University of Uyo (Voucher no: UUPH A (20)b).

2.2 Sample preparation

The seeds were thoroughly washed, sorted manually to remove bad ones and extraneous materials, peeled and air dried for two weeks. The dried seeds were pulverized and stored in a clean air-tight well-labelled container from which samples were collected for different analyses.

2.3 Extraction

The whole powdered sample (393.42g) was macerated with ethanol with intermittent shaking. . The extract was filtered off through a cotton plug and finally with a Whatman No. 1 filters paper. The liquid filtrate was concentrated and evaporated to dryness using a rotary evaporator (WG tv311-V, Wilmad-Lab Glass, USA) at 40 °C, and was transferred into well-labeled sterile glass vials and stored at 4 °C before use

2.4 Percentage Yield

The percentage yield of *chrysobalananus icacao* was calculated and given as

$$\%\text{Yeild} = \frac{weight\ of\ the\ extract}{weight\ of\ the\ sample} \text{ x } 100 \quad (1)$$

2,5 DPPH Radical Scavenging Assay

The free radical scavenging capacity of the extracts were estimated in vitro by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) method according to Choi *et al.,*(2002) with slight modifications. . A solution of the radical was prepared by dissolving 0.02g of DPPH in 100ml of methanol to obtain a concentration of 0.3mM. 200µL solution of DPPH was added to sample solutions at different concentrations (150–950µg/ml). A control (Abs Control) containing methanol and DPPH solution was also prepared. All solutions obtained were then incubated for 1 hour at room temperature. Absorbance was measured at 517 nm. Vitamin C was used as a standard, and the same concentrations of it were prepared as the test solutions. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical inhibition using equation 2

$$\%\ inhibition = \frac{A_b - A_s}{A_b} \times \frac{100}{1} \quad (2)$$

where A_b and A_s are the absorbance of the blank and the sample, respectively. Based on the results obtained from the application of equation 1 for various samples, a plot of percentage inhibition versus concentration curve was developed and applied for the determination of the sample concentration required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

2.6 Ferric Ion Reducing Anti-Oxidant Power Assay (FRAP)

The reducing property was determined by assessing the ability of the ethanol extract of *Chrysobalanus icacao* to reduce $FeCl_3$ as described by (Benzie and Strain, 1996) with modification. 300mM acetate buffer pH 3.6, 10mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$_ $6H_2O$ solution were mixed for

preparation of stock. FRAP reagent was prepared right away before analysis by mixing 25ml acetate buffer, 2.5ml TPTZ solution, and 2.5ml $FeCl_3$_ $6H_2O$ solution. Plant extract (1000 µg/ml) was prepared by different solvent. 200 µg/ml of the
extracts was mixed with 1.8 ml of the FRAP reagent and was incubated at 37 ºC for 30 min in the dark condition before using. Then readings of the colored products (ferrous tripyridyltriazine complex) were determined at 595 nm against a distilled water blank. FeSO4-7H2O (0.1 -1,5 mM) was used for calibration. Ascorbic acid was used as a positive control. Results are expressed as mM Fe2+/ mg sample

2.7 Gas chromatography-mass spectrometry (GC-MS)

The ethanol extract of the seed of *Chrysobalanus icacao* was subjected *to* the Gas Chromatography and Mass Spectrometry (GC–MS) analysis to reveal their bioactive components. One microliters (1 uL) of the sample extracts was injected into the GC column for analysis. 7890A Agilent Technologies with 5975C mass spectroscopy detector (GC-MS) was (crosslinked 5% phenyl-methyl siloxane) column (30m x 0.25mm ID with DF =0.25 film thickness) was used. Column flow was set to 1.0mL/min. Using Helium as the carrier gas. The temperature program started with a temperature of 140ºC held for 1 minute, a ramp of 2ºC per minute to 218ºC , followed by a ramp of 10ºC per minute to 280ºC . Final hold was two minutes. Injection temperature was 280ºC with a splitless injection of 1µL. The transfer line was held at 280ºC and the mass spectrometer had a delay of five minutes. The split ratio and ionization voltage were 110:1 and 70 eV, respectively. To identify the unknown chemical components present in the sample, the individual mass spectral peak value was compared with the database of the National Institute of Science and Technology, 2014. Thereafter, the bioactive compounds were identified by comparing the unknown peak value and chromatogram from GC–MS against the known chromatogram peak value from the National Institute of Standards and Technology (NIST). Subsequently, the details about their molecular formula, molecular weight, retention time and percentage content were also obtained.

2.8 Antimicrobial Assay of Chrysobalanus icaco

2.81 Collection of microbial test organisms

Test organisms used for this work. Were three (3) G+ve, five(5) G-ve, one (1) yeast and one (1) mould. (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella sp, Escherichia coli, Pseudomonas aeruginosa, Klebsiella sp, Shigella sp, Candida albicans, and Aspergillus niger*.). These organisms were obtained from Microbiology stock culture unit, University of Uyo. Isolates were sub-cultured and preserved as pure cultures on Nutrient agar and Sabouraud Dextrose agar slants and stored at low temperatures until required.

2.82 Preparation of test organisms

G+ve and fungi test organisms were serially diluted using 10-fold dilution to factor three and G-ve to factor five and thereafter the last dilutions compared with McFarland standard.

2.83 Determination of extract concentration

The extracts were dissolved using sterile water to constitute different concentration of 100mg/ml, 200mg/ml 300mg/ml, 400mg/ml and 500mg/ml

2.84 Determination of Antimicrobial Assay of Chrysobalanus icacao

Antimicrobial activity of the extracts was evaluated using the well in agar diffusion technique (Okeke *et al.*, 2001). Test organisms were diluted using Nutrient broth and Malt Extract broth for bacterial and fungal isolates, respectively. They were further sub-cultured into Peptone water and cells adjusted to the McFarland Turbidity standard. 0.1ml of each diluted test organism was aseptically

transferred and spread on the surface of the Muller Hinton agar (MHA). Sterile swab sticks were used to spread the inoculum on the surface of the medium and allowed to dry on the bench. A sterile cork borer of 5mm was used to drill holes in the surface of the medium that was seeded with the test organisms. In each of the wells previously seeded with the test organisms, 0.2ml of the extract dilution of different concentrations was introduced into the wells. Control experiments were set up alongside the extracts using commercial antibiotics and antifungal drugs (Gentamycin and Nystatin) at 10 mg/ml and 40mg/ml for bacterial and fungal, respectively.

All plates were left on the bench for 1 hour before incubating at optimum temperature for 24hrs for bacterial and fungal isolates respectively. After incubation, antimicrobial activities were determined by measuring the Inhibition Zone Diameter (IZD) in all the activities of the extracts.

3.0 Results and discussion

3.1 Percentage yield

The percentage yield of *Chrysobalanus icaco* seed extract was determined gravimetrically. The mass of the extract was obtained from the difference between the weight of the beaker containing the extract (78.83 g) and the empty beaker (51.65 g), giving an extract weight of 27.18 g. The initial mass of the seed sample used for extraction was 393.42 g.

The percentage yield was calculated according to equation 1, resulting in a yield of 6.9%. The relatively low yield obtained may be attributed to the extraction solvent employed and its limited efficiency in extracting the bioactive constituents of *Chrysobalanus icaco* seeds.

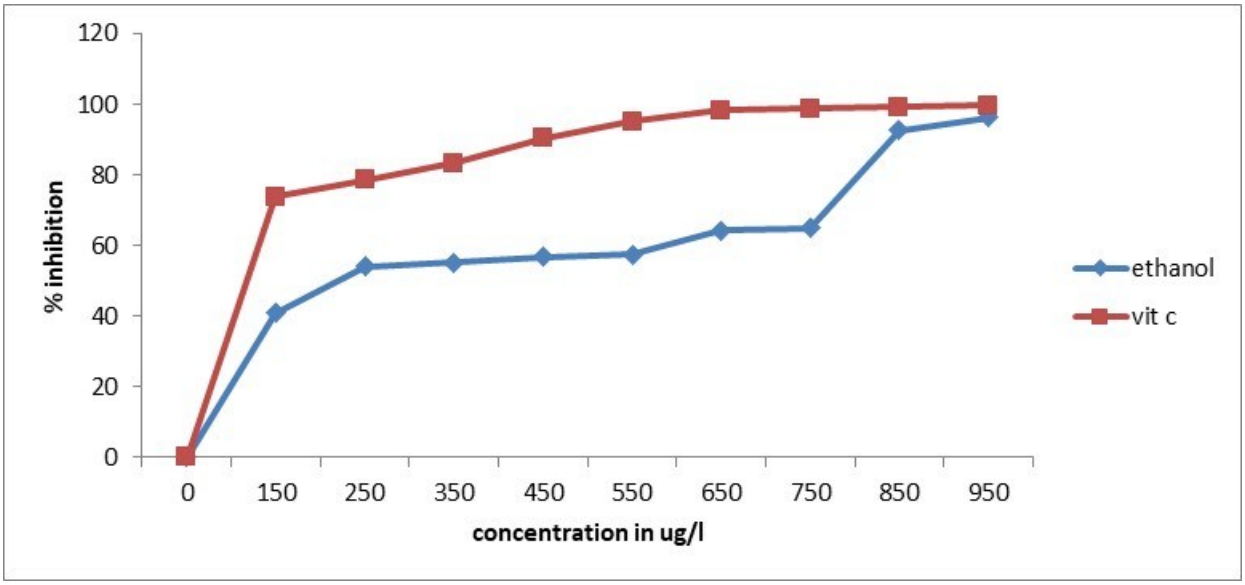

Fig 1. DPPH Free Radical Scavenging Activity of Seed Extract of *Chrysobalanus icacao*

3.2 *DPPH scavenging activity of Chrysobalanus icacao extract*

The free radical scavenging activity of the seed extract of *Chrysobalanus icacao* was assessed by the DPPH free radical scavenging method and the results are presented in Fig 1. The result indicated that the seed extract of *Chrysobalanus icacao* was able to reduce the stable, purple-coloured radical DPPH to the yellow coloured DPPH-H form. The results showed a dose-dependent scavenging power, where activity increased as the concentration increased. On comparing the scavenging activity of the seed extract with ascorbic acid (control), it was observed that the % scavenging activity at 950µg/L was 96.40% and 99.76%, respectively, showing that the seed extract of *Chrysobalanus icacao*

possessed significant antioxidant activity. The antioxidant properties reported in this study may be attributed to phenolic compounds and organic acids present in the seed extract as obtained from the GC-MS analysis. Phenolic compounds are also electron- rich molecules, they donate electrons to free radicals which are highly reactive and unstable molecules, effectively stabilizing them and preventing further damage to the body (Kruk *et al*., 2022; Zeb, 2020) It had been reported that correlation was established between total phenolic content, phytoconstituents and observed antioxidant activities (Ademiluyi & Oboh 2011, Chen *et al.,* 2011).

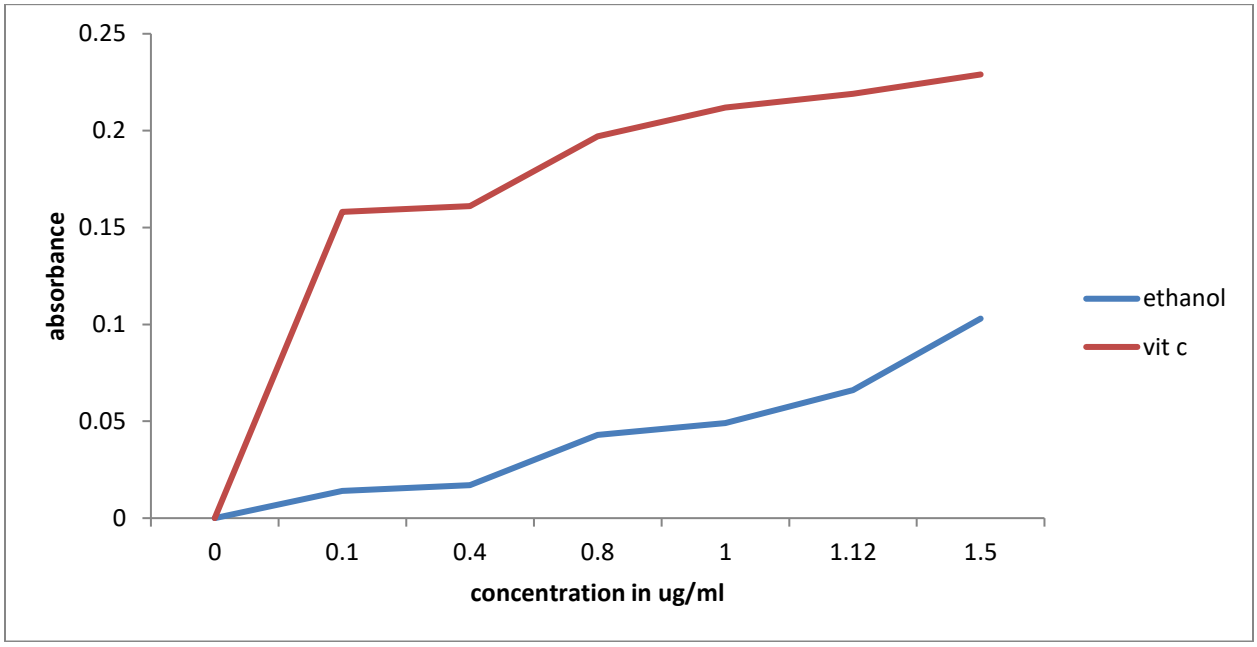

Fig 2: Result of Ferric Reducing Antioxidant Power (FRAP) of *Chrysobalanus icacao*

3.3 *Ferric reducing property of Chrysobalanus icacao extract*

Fig.2 shows the result of the ferric reducing activity of *Chrysobalanus icacao*

FRAP assay assesses the ability of seed extracts to reduce Fe^{3+} (ferric ions) to Fe^{2+} (ferrous) ions. The results obtained showed that the absorbance increased linearly with the concentration of *chrysobalanus icacao* seed extract. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron (Antia *et al.,* 2015). The result obtained in this study showed that *Chrysobalanus icacao* seed extract possesses antioxidant properties, hence its ability to reduce Fe^{3+} to Fe^{2+}.

The IC_{50} value was used as a significant indicator of antioxidant ability, and the result is presented in Table 1. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of the radical. According to the US NCI to consider a plant extract as a potential cytotoxic agent, IC_{50} values should be <20 µg/mL and for isolated compounds <4 ug/mL (Lin and Chang 2012). The results obtained indicated that *Chrysobalanus icaco possesses* a very strong scavenging activity (IC_{50}= 4.45 µg/mL).

Table 1: IC_{50} in ug/ml for antioxidant activity of *Chrysobalanus atacorensis.*

DPPH	4.45
FRAP	3334

Table 2: Results of antimicrobial activity of seed extract of *Chrysobalanus icacao*

Test Organisms	40 mg/mL	80 mg/mL	120 mg/mL	160 mg/mL	200 mg/mL	Gentamycin (10 mg/mL)	Nystatin (40 mg/mL)
Staphylococcus aureus	10	13	15	17	18	32	—
Bacillus subtilis	11	15	17	21	25	15	—
Staphylococcus epidermidis	8	10	14	16	19	37	—
***Salmonella* sp.**	10	12	13	16	20	18	—
***Shigella* sp.**	8	11	15	18	22	16	—
Escherichia coli	13	15	18	22	25	36	—
Pseudomonas aeruginosa	18	21	24	27	30	41	—
***Enterobacter* sp.**	14	16	18	23	25	33	—
Candida albicans	15	18	22	25	27	—	30
Aspergillus niger	12	15	17	21	14	—	24

3.4 *Antimicrobial assay of C. icacao extract*

The threat of antibiotic resistance from common pathogens has increased; hence, the search for medicinal plants as alternatives has intensified. Ethanol extract of *Chrysobalanus icacao* was evaluated for antimicrobial activity against microbial strains: Gram ve+ bacteria (*Staphylococcus aureus, Bacillus subtillis, staphylococcus epidermidis*), Gram ve- bacteria (*Salmonella sp, Shigellasp, Escherichea Coli,pseudomonas aeruginosa, Entrobactersp)* and two fungi *(Candida albicans and aspergillus niger)* and the result presented in Table 2. The Extent of sensitivity of the test organisms to the plant extracts was assessed by measuring the zone of inhibition after 24 hours of incubation. The result revealed varying degree of inhibition on the different test isolates, with more significant inhibition recorded with a higher extract concentration. Ethanol extract of *Chrysobalanus icacao* showed maximum inhibition at 200 mg/ml against *Pseudomonas aeruginosa* at 30mm followed by *Bacillus subtilis, Escherichia coli* and *Enterobactersp* whose zones of inhibition at 200mg/ml was 25mm. *Stahylococcus aureus* showed the least susceptibility (18mm at 200mg/ml). The antifungal activity of the various concentrations of ethanol extract of *Chrysobalanus icacao* against the various strains of fungi such as *Candida albicans* and *Aspergillus. niger* revealed that the extract inhibited the growth of the fungi at different concentrations. The zones of inhibition for *Candida albicans* and *Aspergillus. niger* at 200 mg/ml was 27 and 14mm, respectively. On comparing the antimicrobial results obtained in this study with the standards used (Gentamycin and nystatin) for bacteria and fungi, respectively, it was observed that the seed extract of *icacao* possess significant antimicrobial activity. According to Junior and Zanil, (2000), diameter of the inhibition zone: <9 mm is inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

It has been reported that the synergistic effect of the mixture of phytochemicals play important role in the use of plant extracts as antimicrobial agents (Amit Kumar *et al*, 2012). Generally, the antimicrobial activity of the The seed extract of *Chrysobalanus icacao* revealed that gram-positive and gram-negative bacteria as well as fungi were susceptible to the extract, indicating that it may be used as a broad-spectrum antimicrobial agent.

Table 3: Gas chromatography mass spectrometry (GC-MS) analysis of the seed extract of *Chrysobalanus icacao*

PK	RT	% composition	Name of the compound	Ref	CAS	Qual
1	2.707	3.64	2,4-Dimethyl-1-heptene	18551	042474-44-2	52
2	2.822	0.91	Methenamine	18651	000100-97-0	10
3	3.130	0.43	2-Butanone	4405	003393-64-4	38
4	3.216	0.42	Propanoic acid	22377	016883-50-4	28
5	3.347	0.36	p-Dioxane-2,3-diol Methane	10445	033577-16-1	37
6	3.416	4.13	Methane	19774	1000226-62-2	25
7	3.742	0.31	4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid	21932	1000191-37-7	38
8	3.908	0.90	Isoborneol	27470	1000191-37-7	43
9	4.125	0.25	2-Butenal	114884	021083-08-9	22
10	4.250	1.88	Phenol	28298	000091-10-1	93
11	4.325	0.30	Methane (chloromethylthio)-(methylthio)-Disulfide	19774	1000226-62-2	43
12	4.405	0.55	Chlorotrimethyl-Phosphorodithioc acid	5345	000075-77-4	9
13	4.776	0.36	Trifluoroacetamide	27069	000383-65-3	38
14	4.868	0.31	Bicyclo[3.2.1] octan-3-one	38013	074876-25-8	44
15	5.108	1.60	Methyl-2-[(2-chloroethyl)Thio]-	40103	125787-98-6 000483-76-1	38
16	5.216	0.62	Napthalene	68798	002298-36-4	95
17	5.216	0.50	Methyl ester acetic acid	37592	000143-07-7	16
18	5.536	0.49	n-Dodecanoic acid	64980	002564-36-4	92
19	7.400	2.07	n-Hexadecanoic acid	130822	000112-39-0	99
20	8.680	1.24	Octadecanoic acid	144272	000057-11-4	99
21	5.874	0.49	ThiodiglycolLinuron	9863	00011-48-8	43
22	5.959	0.29	2-Propenoic acid	59201	087087-42-1	46

23	6.108	0.24	2,9-Dithiadecane	46631	056348-40-4	25
24	6.457	0.21	Butyl angelate	29911	007785-64-0	20
25	6.599	0.49	2,4-methane-1H-ind	39508	0908640-25-6	25
26	6.645	0.26	4-fluorohistamine	13121	000143-28-2	46
27	6.765	0.72	1-propanol,2-methyl-2-(propylamino)-,benzoate	21932	022029-76-1	51
28	6.857	1.63	2,3,5,7-tetrathioocatane 3, 3-dioxide	81799	000112-39-0	98
29	7.074	0.29	9-octadecen-1-ol	128820	088496-84-8	37
30	7.137	0.30	3-Buten-2-ol	59820	000057-10-3	99
31	7.400	2.07	Methyl ester	59801	000112-62-9	97
32	8.240	0.40	9,12-octadecadienoic acid (z,z)-methyl ester	130820	000112-62-9	97
33	8.268	0.72	9-octadecenoic acid(z)-, oleic acid	81801	00060-33-3	96
34	8.377	0.29	9,12-octadecadienoic acid	117419	000112-80-1	95
35	8.777	0.36	8-methyl-6-nonenamide	155748	1000293-20-9	60
36	10.112	1.04	Diisooctylphthalate	155748	000131-20-4	55
37	12.943	0.31	Cyclohexene	140137	301643-32-3	53

where PK = Number of peaks, RT= Retention Time, CAS= Chemical Abstracts Service, Ref = References, Qual = Qualitative.

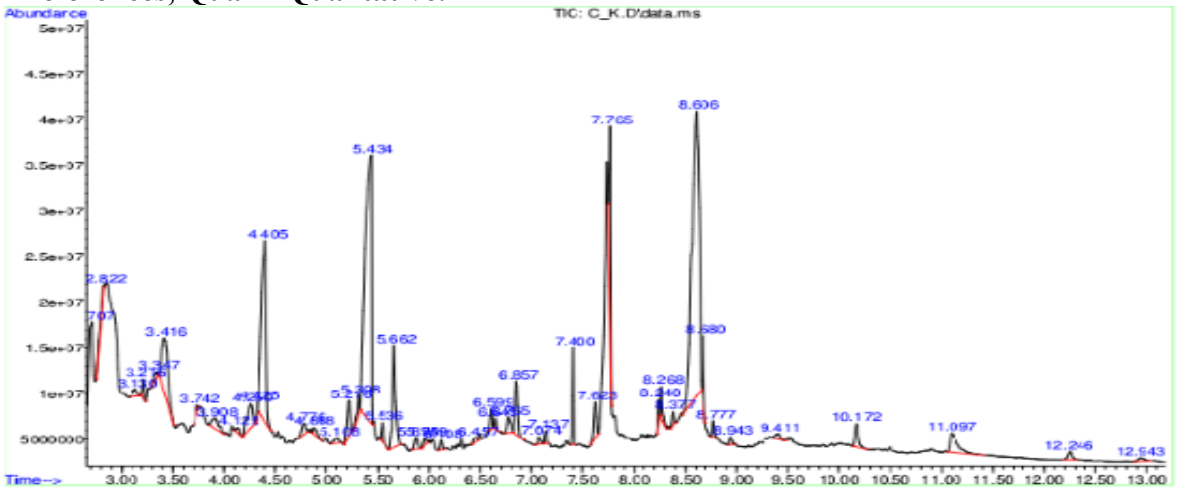

Fig. 3 Total ion chromatogram of ethanol extract of *Chrysobalanus icacao* seed

3.5 *GC-MS spectroscopy of the extract of C. icacao*

The results of the chemical composition of the seed extract of *Chrysobalanus icacao* were obtained by gas chromatography mass spectrometry (GC-MS) is presented in Table 3 and *Fig.* 3. A total of 37 compounds were identified. The major compounds in the seed extracts were n-Hexadecanoic acid (2.07 %), Octadecanoic acid (1.24%),Isoborneol (0.90%), 2,4-Dimethyl-1-heptene (3.64%), Methane (4.13%), Phenol(1.88%),Methyl-2-[(2-chloroethyl)Thio] (1.60%), Diisooctyl phthalate(1.04%), Methyl ester(2.07%), and 2,3,5,7-tetrathioocatane 3, 3-dioxide (1.63%) . The results also indicated the presence of alkanes, alkenes, ketones, aldehydes, carboxylic acids and alcohols. Octadecanoic acid, also known as stearic acid, is a saturated fatty acid with an 18-carbon chain and is known to have anti-inflammatory, antimicrobial, and antioxidant properties. (Singh and Chaturvedi ,2019; Lin,., *et al.*, 2018).

n-Hexadecanoic acid commonly known as Palmitic acid is a saturated fatty acid known to have antioxidant, antiinflammatory, hypo-cholesterolemic anti-inflammatory, anticancer, hepatoprotective, anti-arthritic, and anti-coronary properties (Adnan *et al;* 2019; Sera *et al.,* 2021; Siswadi & Saragih, 2021).

Phenolic compounds with the general formula C_6H_5OH are a diverse group of plant-derived bioactive secondary metabolites (flavonoids, phenolic acids) containing at least one aromatic ring with one or more hydroxyl groups. They are reported to possess potent antioxidant, antimicrobial, anti-inflammatory and anti-cancer properties (Kahkonen *et al.,* 1999). The phenols contain hydroxyls that are responsible for the radical scavenging effect, mainly due to redox properties (Tsai *et al;* 2008; Rice-Evans *et al.,* 1997). In addition, polyphenols have the ability to associate with other metabolites, e.g. protein, lipid, and carbohydrates, to form stable complexes and thus inhibit mutagenesis and carcinogenesis (Mukhopadhyay, 2001).

4.0 Conclusion

The ethanol extract of the seed of *Chrysabalanus icacao* obtained in this study was assessed for its bioactive compounds, antioxidant and antimicrobial activities. The results revealed that about 37 bioactive compounds were identified. The major bioactive compounds identified with respect to their %peak were Hexadecanoic acid, Octadecanoic acid, Isoborneol, 2,4-Dimethyl-1-heptene, Methane, Phenol, Methyl-2-[(2-chloroethyl)Thio], ,Diisooctyl phthalate, Methyl ester,and 2,3,5,7-tetrathioocatane 3, 3-dioxide. The result also revealed that the seed extract of *Chrysabalanus icacao* exhibited strong reducing and free radical scavenging properties when compared with standard compounds, which may be due to the presence of polyphenols and other bioactive compounds. The antimicrobial activity of the extract against tested bacteria and fungi strains showed that it has the potential to be used as a broad-spectrum antibiotic. The presence of significant bioactive compounds in the ethanol extract of *Chrysabalanus icacao* seed showed that the seed can be used as a potential source for the development of antimicrobial and antioxidant agents.

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