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Identification of Bioactive Compounds in *Chrysobalanus icaco* Seed Kernel Using Gas Chromatography-Mass Spectrometry

Oganezi N. C.[1*], Agbaeze T.[1], Kalu S. O.[1]

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ABSTRACT

Chrysobalanus icaco seed kernel (i.e Cocoplum) is good source of oil and is utilized as spice in soups or stews in some African cuisines. The work aimed to identify compounds in methanol and dichloromethane/methanol (1:1, v/v) flour extract from its seed kernels using gas chromatography-mass spectrometry. Eleven compounds were identified from the methanol extract while thirteen compounds were identified from the dichloromethane/methanol extract. The most abundant compounds from the methanol extract were cis-13-octadecenoic acid methyl ester, cis vaccenic acid and n-Hexadecanoic acid. They had relative abundance of 28.83, 17.14 and 15.40% respectively. 2,6-octadienal-3,7-dimethyl (E) (ie α-citral), citral, cis-3-hexenyl cis-3-hexenoate and trans-2,7-dimethyl-4,6-octadiene-2-ol were the most abundant in the dichloromethane/methanol extract. These had relative abundance of 12.86, 18.92, 10.56 and 20.59% respectively. Compounds which were common to both methanol and dichloromethane/methanol extracts of *C. icaco* seed kernel were 2,6-octadienal-3,7-dimethyl (E), hexadecanoic acid methyl ester, n-Hexadecanoic acid, heptadecanoic acid, 16 methyl, methyl ester, cis-vaccenic acid and oleic acid. Both solvents extracted varied concentrations of compounds which fall in different classes of either being an unsaturated long chain aldehyde, fatty acids, fatty acid methyl esters, indene derivatives, monoterpenoid alchohol. The compounds identified in both extracts of *Chrysobalamus icaco* seed kernels have various beneficial bioactivities and sensory attributessuch as being flavor compounds which confers *C.icaco* seed kernel a peculiar aroma. As such, *Chrysobalamus icaco* seed kernel is a functional food and can serve as a good raw material for edible oil from which bioactive compounds can be isolated and utilized in relevant food systems and in the preparation of neutraceuticals and pharmaceuticals. Therefore, its use in cuisines is greatly encouraged.

INTRODUCTION

A spice is dried seed, fruit, root, bark or flower of a plant or a herb used in small quantities for flavor, color or as a preservative (Kunnumakkara *et al.*, 2009). Herbs and spices have been utilized as additives globally not only to enhance the sensory attributes of food, but also to increase shelf life of foods by reducing or eliminating food borne pathogens (Lai and Roy, 2004). Spices can perform functions as nutrient sources, antioxidants, preservatives, insecticides and as medicinal plants for human use (Fasoyiro, 2015). Besides adding flavor and taste to dishes, they help prevent and alleviate various health problems due to the presence of various bioactive compounds which have different physiological and biochemical functions. Spices have shown many health benefits in preventing and curing a number of diseases such as cancer, aging, metabolic, neurological, cardiovascular and inflammatory diseases (Gottardi *et al.*, 2016). The active phytochemicals derived from these spices have provided the molecular basis for these actions (Kunnumakkara *et al.*, 2009).

Chrysobalanus icaco (Cocoplum) belongs to the family Chrysobalanaceae (Burkill, 1985). It is a low shrub or bushy tree which grows near sea beaches and inland throughout tropical Africa, tropical America, the Caribbean, southern Florida and the Bahamas (KWCSP,2023). It grows fruit for which its pulp and seed are consumed as food. The seed's kernel is utilized as soup spice either as pepper soup spice or 'ofeakwu' (ie palm fruit) soup spice in some parts of West Africa. The seed kernel is ground into a fine flour/powder and used as spice. *C. icaco*, commonly called 'gbafilo' seeds are economically and medicinally important as they are utilized for the preparation of special soup, control blood pressure, malaria fever and treatment of stomach disorder (Davies and Zibokere, 2011).In some climes, where it is common *C. icaco* plays a role in traditional medicine (Presta *et al.*, 2007). De Aguiar *et al.*(2017) reported that the seeds can produce an edible oil and they identified the types of fats using gas chromatography. Vargas *et al.* (2010) identified essential oil from the leaves of *C. icaco* after supercritical fluid extraction using gas chromatography.

Many bioactive compounds have been extracted from spices, providing a scientific basis for the use of such spices in our diet (Kunnumakkara *et al.*, 2009). The development of equipment and methods for identifying and evaluating natural products bioactive compounds to ensure their quality and discover new drugs is very necessary (Fu *et al.*, 2019). So many naturally synthesized compounds have bioactive functions and are being explored for different applications, particularly in the food and pharmaceutical industry (do' Nascimento *et al.*, 2021). Gas chromatography – mass spectrometry analysis is a convenient method to test the quantitative amount

[1] Department of Food Science and Technology, Abia State University, Uturu, Abia State, Nigeria
[*] Corresponding author's e-mail: chinonyeremwog@yahoo.com

of bioactive compounds in plant extracts (Gomathi *et al*., 2015). There is death of information on *C. icaco* seeds but a good number of research work has been done on leaf extracts and fatty acids of oil extract from the leaves and seeds. It is in view of this that gas chromatography mass spectrometry analysis was carried out on methanol and dichloromethane/methanol (1:1,v/v) extracts of whole seed flour of *Chroysobalanus icaco*.

MATERIALS AND METHODS

Sourcing of Materials and Extraction of Bioactive Compounds

Chrysobalanus icaco seeds were sourced from Afor Oru market, Oru Ahiara in Ahiazu Mbaise in Imo State, Nigeria. They were cracked to remove the husk and free the seed kernel. After which, the seed kernels were washed in clean water, drained off water, air dried before milling to get fine flour. The resultant *Chrysobalanus icaco* seed kernel flour was used for the extraction of bioactive compounds using methanol and dichloromethane/ methanol (1:1, v/v). To extract the bioactive compounds, twenty (20) grams of the pulverized flour was dispensed into two different labelled conical flasks. Two hundred (200) mL absolute methanol and dichloromethane/ methanol (1:1, v/v) were dispensed into the two different conical flasks respectively. The sample mixtures were shaked vigorously on a vortex mixer for 30mins and covered using aluminium foil. They were allowed to stand for 24h at room temperature and subsequently filtered through No1 Whatman filter paper respectively. Each sample extract was concentrated by heating over a boiling water bath to remove excess solvent. Hence, two extracts from whole seed flour of *Chrysobalanus icaco* were obtained. These extracts were subjected to gas chromatography/ mass spectrometry (GC-MS) analysis for the separation and identification of compounds.

Gas Chromatography Mass Spectrometry Analysis (GC–MS)

The GC–MS analysis of bioactive compounds from the two extracts were done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP- 5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Helium gas (99.995%) was used as carrier gas with flow rate of 1 mL/min. The initial column temperature was set at 50 –150 °C with increasing rate of 3 °C/min and hold time of 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter (1μl) of each extract was diluted with respective solvents and injected in a splitless mode into the gas chromatogram using hamilton syringe. The injector temperature was 250ºC while the mass spectrometer ion source temperature was 200ºC with an interface temperature of 280ºC but with electron impact ionization energy of 70 eV. Total run time of the gas chromatography-mass spectrometry analysis for the methanol extract was 20mins for both extracts. The relative abundance of the chemical compounds present in each extract was expressed as percentage based on peak area normalization produced in the chromatogram. Compounds were identified by mass spectroscopy. Bioactive compounds in the different extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards by comparing retention indices and mass fragmentation patterns of the compounds with those stored in the computer library software of the National Institute of Standard Technology (NIST/EPA/ NIH, Mass Spectral Library, Version 2.0). Quantitative determinations were made by relating respective peak areas to TIC areas of the GCMS.

RESULTS AND DISCUSSION

Table 1 shows results on methanol extract of *Chrysobarlanus icaco* seed kernel. Results indicated the presence of eleven compounds. The most abundant compounds were cis-13-octadecenoic acid methyl ester, cis- vaccenic acid, hexadecanoic acid methyl ester, and n-hexadecanoic acid. They had relative abundance of 28.83, 17.14, 16.40 and 15.40% respectively. These compounds have been reported to have various biological activities and nutritional values. Cis-13-octadecenoic acid methyl ester is a fatty acid methyl ester with therapeutic uses in medicine and surgery (Awonyemi *et al*., 2020). Cisvaccenic acid is a monounsaturated n -7 fatty acid and is reported to have antibacterial and hypolipidemic effects in rats (Hamazaki*et al*., 2016). Cis-vaccenic acid may have multiple modes of activity such as antioxidant activity which involves the neutralization of free radicals hence reducing oxidative stress, it may serve as an anti-inflammatory agent by decreasing the production of cytokines that promote inflammation; it may also act as a transcription factor regulating the expression of genes that are involved in metabolic processes (Aimola *et al*, 2016., Rontani *et al*, 2003). Hexadecanoic acid methyl ester was reported to have bactericidal effect against multi drug resistant bacteria (Shaaban *et al*., 2021). It has other biological activities such as anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nenaticid, insectifuge, antihistaminic, anticorona, and anti antiarthritic properties (Jegadeeswari*et al*., 2012). n-Hexadeanoic acid has been reported to posses antioxidant, antimicrobial and anti-inflammatory activities (Siswadi and Saragih, 2021).

Other bioactive compounds found in significant quantities in the methanol extract of *C. icaco* seed with well documented biological, physiological and pharmacological activities are oleic acid (6.02%) heptadecanoic acids, 16-methyl methylester (6.43%) and 2,6-octadienal-3,7-dimethy (E) (i.eα-citral). Oleic acid has been reported to induce apoptosis in carcinoma cells via the activation of different intracellular pathways involved in carcinoma cell development which could be the main mechanism of its antitumoreffects reported in clinical studies (Carrilo *et al*., 2012) Heptadecanoic acid, 16-methyl, methyl ester is a branched fatty acid methyl ester . It was reported to have the best activity against skin cancer protein and is considered for further in vitro studies towards development of anti skin cancer drug (Saravanakumar *et al.*, 2012). 2,6- octadienal – 3,7-dimethy (E) is reported to be a major component of lemon grass, volatile oil of *Cymbopogon, atratus* or of *C. flexuosus*. It has a role as a flavoring agent, a fragrance, an insecticide and aldehyde oxidase inhibitor (Aiemsaard *et al.*, 2011). It was reported to be suitable acaricidal and mite indicator ingredient and can be used for the control of dust mites (Park and Lee, 2018).

Table 1: Results on Gas Chromatography -Mass Spectrometry analysis of methanol extract of *Chrysobalamusicaco* (Cocoplum)seed kernel

Peak number	Retention time (mins)	Compound name	Relative abundance (%)	Molecular formular	Molecular weight
1	7.081	10-Undecen-4-one-2,2,6,6, tetramethyl	2.43	$C_{15}H_{28}O$	224.38
2	7.691	2,6-Octadienal 3.7-dimethyl ester (E)	4.35	$C_{10}H_{16}O_2$	152.23
3	16.993	Hexadecanoic acid methyl ester	16.40	$C_{17}H_{34}O_2$	270.45
4	17.641	n-Hexadecanoic acid	15.40	$C_{16}H_{32}O_2$	256.42
5	18.808	Cis-13-Octadecanoic acid, methyl ester	28.83	$C_{19}H_{36}O_2$	296.49
6	18.974	Furamic acid, pent-4-en-2-yl tridecyl ester	0.89	$C_{22}H_{38}O_4$	366.53
7	19.050	Heptadecanoic acid, 16-methyl, methyl ester	6.43	$C_{19}H_{38}O_2$	298.50
8	19.448	Cis-vaccenic acid	17.14	$C_{18}H_{34}O_2$	282.46
9	19.647	Oleic acid	6.02	$C_{18}H_{34}O_2$	282.47
10	19.697	12-methyl-E,E-2,13-Octadecadien-1-ol	0.44	$C_{19}H_{36}O$	280.41
11	19.799	1H-Indene,2-butyl-5-hexyloctahydro-	0.52	$C_{19}H_{36}$	264.49

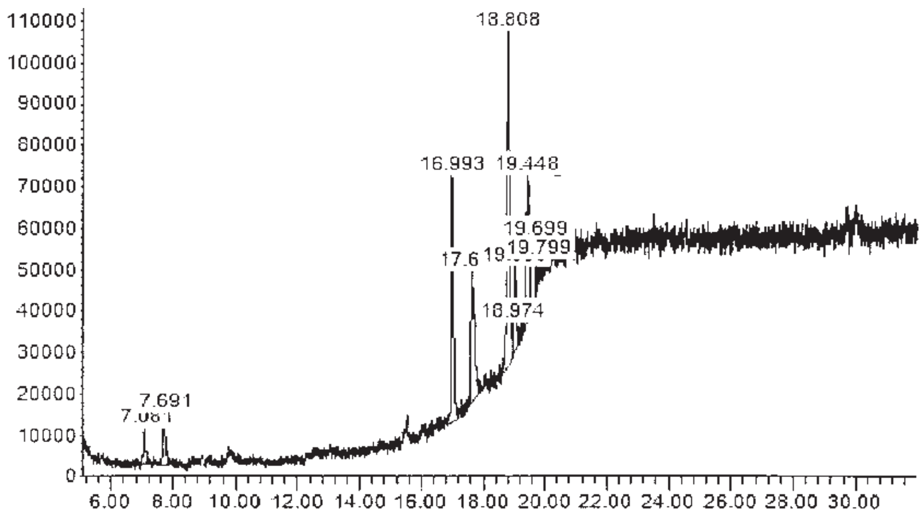

Figure 1: Chromatograph of Methanol extract of *Chrysobalanusicaco* (Cocoplum) seed kernel

Table 2 shows results on the compounds present in dichloromethane/methanol extract of *Chrysobalamusi caco* seed kernel. The most abundant compounds were 2,6-octadienal – 3,7-dimethyl (i.e α-citral),citral, Cis-3- hexehyl-cis-3-hexenoate and trans-2,7-dimethyl-4,6-octadiene-2-ol. They had relative abundance of 12.86, 18.92, 10.56 and 20.59% respectively. These compounds have been reported to have beneficial biological activities. In addition to already mentioned biological activities2, 6-octadienal-3,7-dimethyl and citral are similar compounds. Citral is anacyclic monoterpene aldehyde which consists of a racemic mixture of two isomers namely geranial and neral (Ganjewala *et al.*, 2012). It has biological activities such as antimicrobial, antioxidant, anticancer, antidiuretic and anti-inflammatory (Shama *et al.*, 2021). Cis-3-hexeny, cis -3-hexenoate is a flavor compound which has a fruity aroma and flavor and is widely used in the food industry as a flavor and fragrance ingredient (JECFA FAO/ WHO, 1997). Trans-2,7-dimethyl-4,6-octadiene-2-ol is a monoterpeniod alchohol. It has anti-inflammatory, antimicrobial, anticonvulsant, antiviral, antioxidant, anti-diabetic activity and also improves the functions of the endocrine system (Uju *et al*, 2022).9,7-octadecadienal, (Z) is an unsaturated long chain aldehyde which is reported to have antioxidant, anti-inflamatory activities (Geraci *et al.*, 2017, Adeoye-Isijola., *et al.*, 2018) as well as antimicrobial property(Karthika and Paulsamy, 2014).

Minor components were found in *Chysobalanus icaco* seed kernel for both extracts and they have important biological activities because of their synergistic effects based on several studies that have identified them from other sources. Compounds common to both extracts were 2,6-octadienal – 3,7-dimethyl(i.e α-citral), hexadecanoic acid methyl ester, n-Hexadecanoic acid, heptadecanoic acid-16- methyl, methyl ester, cis -vaccenic acid and oleic acid. Oleic acid is the most commonly occurring fatty acid in nature and its high content in olive oil is responsible for the hypotensive effect observed in olive oil (Ayed *et al*., 2018). This suggests that the presence oleic acid in *Chysobalanus icaco* seed kernel will proffer hypotensive activity in human biological system.

Table 2: Gas Chromatography and Mass Spectrometry analysis of bioactive compounds from Dichloromethane/methanol extract of *Chrysobalanus icaco* (Cocoplum) seed kernel

Peak number	Retention time (mins)	Compound name	Relative abundance (%)	Molecular formular	Molecular weight
1	7.067	2.6-Octadienal,3,7-Dimethyl	12.86	$C_{10}H_{16}O$	152.23
2	7.691	Citral	18.92	$C_{10}H_{16}O$	152.23
3	9.103	Cis-3-Hexenyl-cis-3-Hexenoate	10.56	$C_{12}H_{20}O_2$	196.29
4	9.767	Trans-2,7-Dimethyl 4,6-Octadien-2-ol	20.59	$C_{10}H_{18}O$	154.25
5	11.693	3.8.11-Trioxotetracyclo [4.4.1.0 (2,4). 0(7.9)] Undecane (1 α, 2 α, 4 α, 6 α, 7β, 9β)	3.08	$C_8H_{10}O_3$	154.16
6	12.544	3-Hexene, 2-methyl-(2)	3.48	C_7H_{14}	98.19
7	16.982	Hexadecanoic acid, methyl ester	3.75	$C_{17}H_{34}O_2$	270.45
8	17.631	n-Hexadecanoic acid	5.39	$C_{16}H_{32}O_2$	256.42
9	18.809	Cis-Vaccenic acid	4.93	$C_{18}H_{34}O_2$	282.46
10	18.972	6,11- Dimethyl-2,6,10-dodecatrien-1-ol	2.77	$C_{14}H_{24}O2$	208.34
11	19.041	Heptadecanoic acid,16-methyl-methyl ester	2.49	$C_{19}H_{38}O_2$	298.50
12	19.431	9,7 Octadecadienal	7.75	$C_{18}H_{32}O$	264.45
13	19.605	Oleic acid	2.33	$C_{18}H_{34}O_2$	282.47
14	20.087	Oleic acid	1.09	$C_{18}H_{34}O_2$	282.47

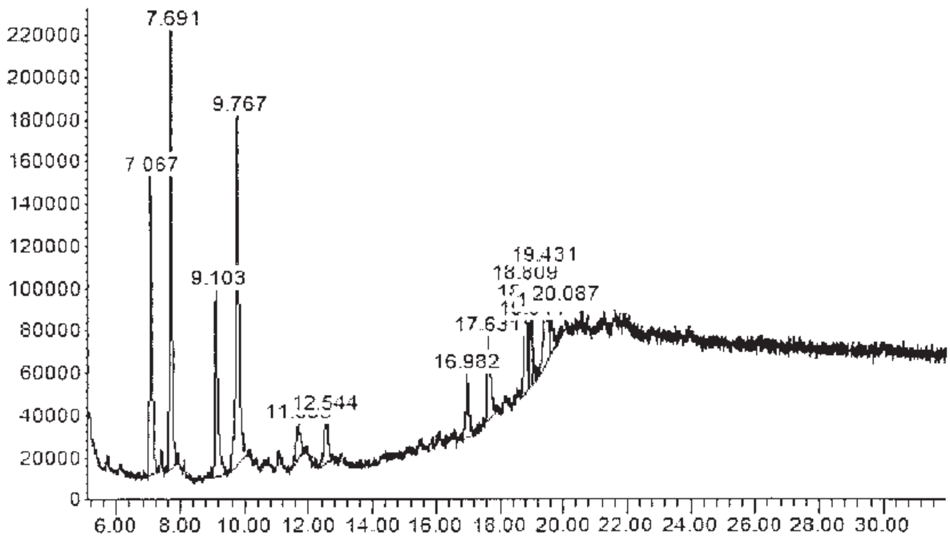

Figure 2: Chromatograph of Dichloromethane/methanol extract of *Chrysobalanus icaco* (Cocoplum) seed kernel

CONCLUSION

The seed kernel of *Chysobalanus icaco* fruit which is used as seed spice in some African cuisines contains beneficial bioactive phytochemicals with documented beneficial activities. These compounds have activities which serve as flavor compounds that proffer the seed spice its peculiar aroma, while some of the compounds found in substantial quantities are reported to promote good health via having antioxidant activities, anti-inflammatory, bactericidal effect, anticancer, antidiuretic effects to mention but a few. Hence, *Chrysobalanus icaco* (ie Cocoplum) seeds used as spice for culinary is a functional ingredient that can be beneficial in promoting good health by preventing degenerative diseases.

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