

PROXIMATE COMPOSITION AND BIOACTIVE COMPOUNDS PRESENT IN DICHLOROMETHANE EXTRACTS OF *COCUSNUCIFERA L* (FRUITS)

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ABSTRACT

This study determined the proximate composition and bioactive compounds present in dichloromethane fruit extract of Cocusnucifera(L) to unknot details behind local wild usages in disease treatment and in diets. The dichloromethane fruit extract of dried C. nucifera(L) was analyzed for its bioactive compounds using gas chromatographic techniques (GC-MS) following standard laboratory procedures. The results revealed presence of 11 bioactive compounds in C. nucifera which include Coumarin (1.40%), Methyl (Methyl 4-0-methyl-alpha-d-mannopyranoside) uronate (6.79%), 3 methyl, decanoic acid (4.41%), 2, 2, 4 – trimethyl – 3 – pentanol (2.45%), Hexadecanoic acid, methyl ester (33.23%), n-hexadecanoic acid (1.89%), cis-vaccenic acid (4.72%), Neophytadiene (11.55%), Methyl stearate (22.33%), Tetracosylheptafluorobutyrate (1.34%), Bis (2 ethylhexyl) phthalic acid (9.80%). The most abundant was shown to be Hexadecanoic acid, methyl ester (33.23%). These bioactive compounds are known for their anti-helminthic, anti-inflammatory, antioxidant, antifungal, antimicrobial, and antitumor potentials, hence giving it its ethnomedicinal and therapeutic relevance and usages. The proximate profile gave Moisture content as 38.70%, Protein 5.37%, Ash 2.32%, Fibre 11.47%, Fat 31.00% and Carbohydrate 11.14%. The assayed proximate profile is suggestive that C. nucifera could be a good source of dietary fats, fibre and carbohydrates.

Keywords: *Cocusnucifera(L)*, Proximate Profile, Bioactive Compounds, Dichloromethane

INTRODUCTION

Trado-medicinal practitioners have used several plants to manage and treat a variety of disease conditions (Onyegeme-Okerenta and Essien, 2021). Overtime, these plants have been shown to contain lots of naturally occurring substances with potential antioxidant activity (Sunday *et al.*, 2016).

These compounds are referred to as bioactive compounds (Obia *et al.*, 2023).

Bioactive food components are substances found in foods or dietary supplements that, in addition to meeting fundamental nutritional requirements, have the potentials to alter the health of both humans and animals that consume them (Bernhoft, 2010). Nutrients are typically not considered to be "plant bioactive

compounds". These bioactive substances in plants are seen as secondary metabolites that are not essential for the plant's everyday operations (like growth), but they are crucial for competition, defence, attraction, and signalling (Bernhoft, 2010; Sunday *et al.*, 2016). The therapeutic potentials of medicinal plants as sources of antioxidants in preserving biochemical processes in the cells of the organisms containing them are thus being given consideration (Obia *et al.*, 2023). These plants which contain these bioactive compounds essential for metabolic processes in our body systems also are sources of vital nutrients hence providing nutritional values, and health benefits, however some provide us with no nutrients and could even be toxic to human health (Obia *et al.*, 2023).

Cocos nucifera (L.) is one of such plants widely used by locals for therapeutic purposes. *Cocos nucifera* (L.) is a member of Arecaceae family (palm family) known as coconut. The plant is an arborescent monocotyledonous tree. They are used industrially and for cooking at home (Sachs *et al.*, 2002). There is wide usage of this plant for treatment of several pathological conditions. Reports show that extract from husk fibre of *C. nucifera* is also used to treat diarrhoea (Yartey *et al.*, 1993). Coconut oil has been widely used in preventing hair loss, while coconut water is shown to be useful in treating renal disease (Singh, 1986). Coconut has been shown to be widely used in disease treatments such as an antipyretic, (hence reducing renal inflammation, as ointment for burns, injuries, abscesses and dermatitis and also used for oral treatment of amenorrhoea and asthma (Preetha *et al.*, 2013); for oral treatment of menstrual cycle disorders (Mitchell and Ahmad, 2006); as a wound ointment, as an oral contraceptive, they are used for treatment of fever, diarrhoea, diabetes, gonorrhoea (Mitchell and Ahmad, 2006), dysmenorrhoea and in treating disorders associated with urogenital tract infections (Hoste *et al.*, 2009). The phytochemistry of the coconut fibre (mesocarp) showed presence of phenols, tannins, leucoanthocyanidins,

flavonoids, triterpenes, steroids, and alkaloids, triterpenes, saponins, and condensed tannins. Condensed tannins are reported to possess anti-helminthic activity (Yook *et al.*, 2010). The ethyl acetate extracts, from *C. nucifera* fibre are shown to be rich in polyphenols, such as; catechins, epicatechins and flavonoids. *C. nucifera* is shown to be rich in vitamins such as vitamin B, nicotinic acid (B3), pantothenic acid (B5), biotin, riboflavin (B2), folic acid it also contains trace levels of vitamins B1, B6, C, pyridoxine, thiamine (Akinyele *et al.*, 2011).

Aqueous crude extracts of husk fibre of *C. nucifera* are used to treat arthritis and other inflammatory ills. They also have anti-bacterial, antifungal, and anti-viral activities (Akinyele *et al.*, 2011). Evidence suggests that diets rich in phenolic compounds can significantly enhance human health because of the effects of phenolic antioxidants (Adebayo *et al.*, 2013; Naczk and Shahidi, 2004). *C. nucifera* plants have proven to contain phenolic compounds and flavonoids that support antioxidant activity (Adebayo *et al.*, 2013). They were also shown to be cardioprotective (Adebayo *et al.*, 2013) hence possessing several important pharmacological effects with low toxicity. It has been observed that various extracts contain different constituents hence the need to examine the proximate composition and bioactive constituents of dichloromethane extracts of *Cocosnucifera* L.

MATERIALS AND METHODS

Sampling and Sample Preparation

Fresh *C. nucifera* were obtained from the popular fruit garden market in Port Harcourt Rivers State. They were identified at the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt and deposited at the herbarium with the voucher no: UPH/P/357

The fresh, mature white fruit of *C. nucifera* were cut into smaller pieces, dried and grounded into powdered form. Ten grams (10

g) of the powdered samples were weighed to a constant weight at room temperature ($29\pm 1^\circ\text{C}$) for 4 weeks, packaged in sterilized containers and labelled appropriately before taking them to the laboratory for extraction using Soxhlet extraction method with dichloromethane as solvent. After extraction the solvent was removed from the thimble, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was discarded.

Proximate Analysis: The proximate analysis of the samples for moisture, ash and carbohydrate contents were determined as described by AOAC (2005). Crude protein, fibre and fat contents were determined by the methods of Pearson (1976).

Sample Preparation and Analysis of Bioactive Compounds

Bioactive compounds present in *C. nucifera* fruit were analyzed using gas chromatographic (GC-MS) methods as described by (Ukwubile et al., 2019; Onyegeme-Okerenta and Essien, 2021). Ten grams (10 g) of the powdered sample were weighed into a well-stoppered bottle and 20 ml of dichloromethane was added. The mixtures were vigorously agitated and were left to stand for 5 days. The extract was collected by filtering into a quartz beaker and the process was repeatedly carried out for two more consecutive times. The combined aliquots of the extract collected were concentrated on a steam bath to about 5 ml. This was purified by passing through a Pasteur's pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air-dried to about 2 ml for gas chromatographic analysis.

GC-MS Analysis of *C. nucifera* Fruit Extract

The dichloromethane concentrate of the various extracts was diluted with 98% hexane and 1 μl of each diluted sample was automatically injected into the Gas chromatographic Model: 7890A (GC)

interfaced with Mass Selective Detector model: 5975C (MSD) for Gas Chromatography/Mass Spectrometry analysis and characterization of the various bioactive compounds present (Ukwubile et al., 2019).

Identification of chemical constituents present in *C. nucifera* fruit extract

Bioactive compounds present in the extract were identified based on GC retention time on HP- 5 column and matching of the spectra with computer software using the Chem-software attached to the MS library. Detection of compounds present in the sample was confirmed by comparing the spectrum of the identified compounds to the database library of the National Institute of Standards and Technology (NIST) which houses more than 62,000 patterns. The name, molecular weight, structure of the components in the test material were then ascertained (Ukwubile et al., 2019).

Statistical Analysis

All statistical analysis were done using the Statistical Package for Social Sciences (SPSS) version 21.0. The obtained data were presented with bar charts depicting means of triplicate values from analyzed samples.

RESULTS AND DISCUSSION

Plants have been shown to contain several medicinal values which is inherent in some chemical substances, giving it a definite physiologic action on human body (Himal et al., 2008, Nwaichi&Olua, 2015). The most important of these bioactive compounds of plants include flavonoids, alkaloids and phenolic compounds (Nwaichi&Olua, 2015; Adele et al., 2024). *C. nucifera L* is amongst several plants known for its usage in treatment of diseases and as source of basic nutrients.

The Proximate composition and bioactive compounds present in the dichloromethane extract of *Cocos nucifera L* fruit was evaluated in this study. The obtained results from the proximate profile gave moisture content as 38.70%, protein 5.37%, ash 2.32%, fibre 11.47%, fat 31.00% and carbohydrate 11.14%. The assayed proximate profile is suggestive

that *Cocos nucifera* (L) could be a good source of dietary fats, and moisture content, but not rich in minerals as shown from the ash content. Results from this study corroborates that of Chuku and Mathias, (2021), who also reported that fats has highest composition in sampled coconut followed by fibre composition then carbohydrates. Chuku and Mathias, (2021) also reported low ash content which shows that coconut is not rich in minerals. This finding however contradicts the reports by Chinedu *et al.*, (2018) who reported higher carbohydrates and crude fibre levels in coconut than lipids.

However, the results from this study showed that coconut is also a viable source of carbohydrates and proteins. The significant

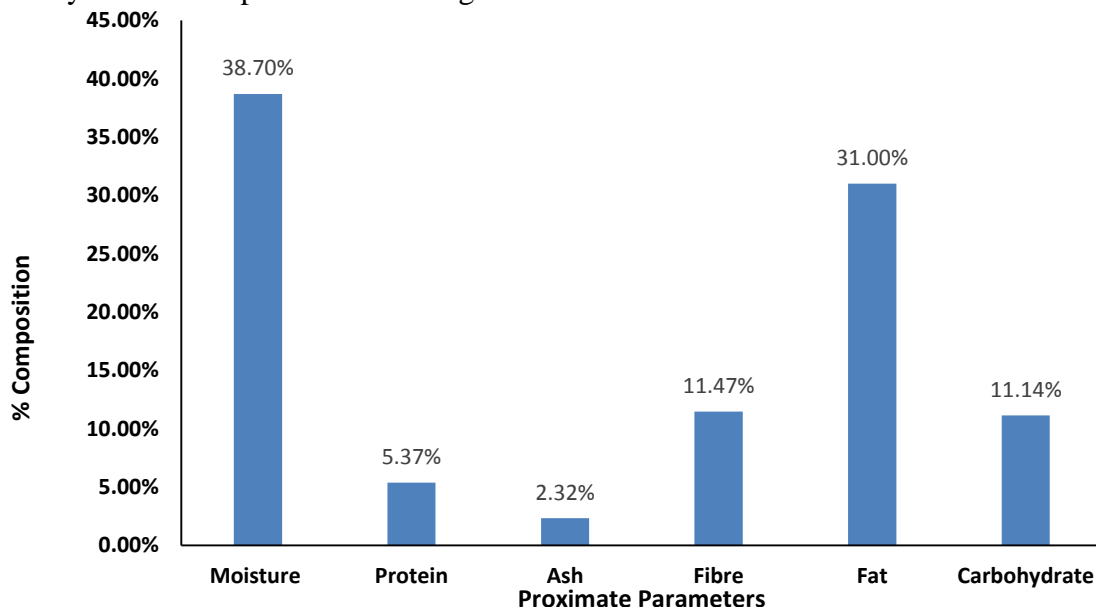


Figure 1. Proximate composition of *C. nucifera*

Coconut's fibre content suggests its usefulness in the reduction of constipation and intestinal motility control. The moisture content indicates that coconut will deteriorate over time when kept in its fresh form. The ash content depicts the corresponding amount of mineral elements in coconut.

The results for the GC-MS analysis of the bioactive compounds present in *C. nucifera* fruit revealed is presented in Figure 2 while the percentage composition of the 11 identified bioactive compounds and their individual spectra are presented in Figures 3 and 4 (a-k).

level of lipids and carbohydrates is suggestive of the reason for the notable usage of coconut as a dietary nutrient source globally as it will no doubt serve as a potential ATP source for biological processes. Studies has shown that carbohydrates sremain vital in providing energy in living things (Edeogaet *al.* 2005).

The high lipid and carbohydrates contents is thought to be one of the reasons coconut and its products easily ameliorates hunger. The high lipid profile is also indicative of probable role as a boost to hormone synthesis. It could also be helpful in the functioning of fat-soluble vitamins. Proteins supports growth regulation and catalytic fronts of enzymes.

The compounds are coumarin (1.40%), Methyl (Methyl 4-0-methyl-alpha-d-mannopyranoside) uronate (6.79%), 3 methyl, decanoic acid (4.41%), 2, 2, 4 – trimethyl – 3 – pentanol (2.45%), Hexadecanoic acid, methyl ester (33.23%), n-hexadecanoic acid (1.89%), cis-vaccenic acid (4.72%), neophytadiene (11.55%), methyl stearate (22.33%), Tetracosylheptafluorobutyrate (1.34%), Bis (2 ethylhexyl) phthalic acid (9.80%). Themost abundant was shown to be Hexadecanoic acid, methyl ester (33.23%). These bioactive compounds are known for their anti-helminthic, anti-inflammatory,

antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor potentials, hence giving it its ethno-medicinal and therapeutic relevance and usages.

3 methyl, decanoic acid is used as a detergent, and medically as a local anaesthetic, and as a sclerosing agent for the treatment of oesophageal and gastric varices and varicose

veins. They also serve as chemical agents injected into blood vessels and lymphatic sinuses to shrink or cause localized thrombosis; fibrosis, and obliteration of the vessels. This treatment is applied in a number of conditions such as varicose veins; haemorrhoids; gastric varices; oesophageal varices; peptic ulcer haemorrhage.

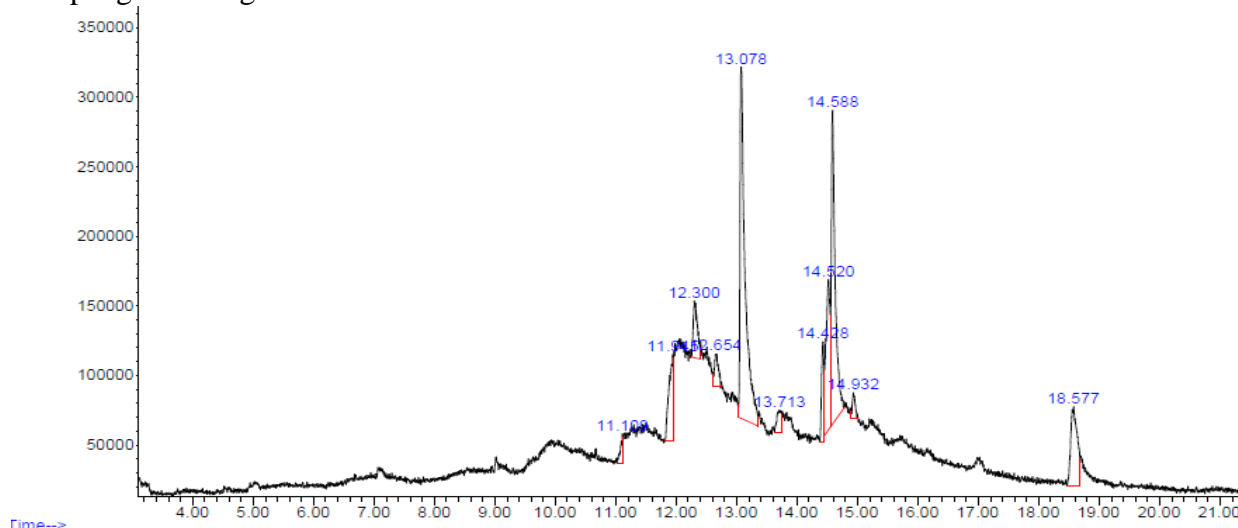


Figure 2: Chromatogram of dichloromethane extracts of *C. nucifera*

Bioactive compounds	Area under the peak (%)	Retention time (rt)	Molecular weight (g/mol)	Molecular formula
Coumarin	1.40	11.109	146.1439	C ₉ H ₆ O ₂
Methyl (Methyl 4-O-methyl-alpha-d-mannopyranoside)uronate	6.79	11.945	208.21	C ₈ H ₁₆ O ₆
3 methyl, decanoic acid	4.41	12.300	186.2912	C ₁₁ H ₂₂ O ₂
2, 2, 4 – trimethyl – 3 – pentanol	2.45	12.654	130.2279	C ₈ H ₁₈ O
Hexadecanoic acid, methyl ester	33.23	13.078	270.4507	C ₁₇ H ₃₄ O
n-hexadecanoic acid	1.89	13.713	256.4241	C ₁₆ H ₃₂ O
Cis-vaccenic acid	4.72	14.428	282.46	C ₁₈ H ₃₄ O ₂
Neophytadiene	11.55	14.520	278.5157	C ₂ OH
Methyl stearate	22.33	14.588	298.5038	C ₁₉ H ₃₈ O
Tetracosylheptafluorobutyrate	1.34	14.920	550.6763	C ₂₈ H ₄₉ F ₇ O ₂
Bis(2 ethylhexyl) phthalic acid	9.80	18.577	390.564	C ₂₄ H ₃₈ O ₄

Figure 3. Percentage composition of bioactive compounds present in dichloromethane extracts of *C. nucifera* fruit

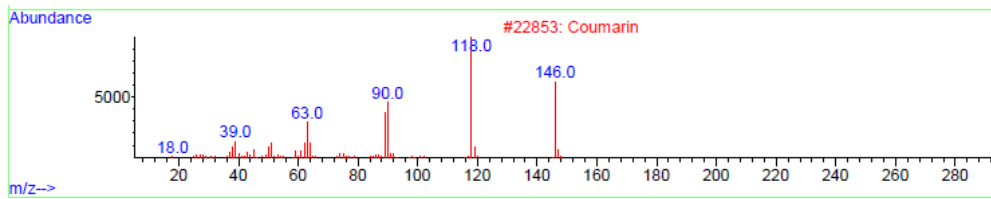


Figure 4a: GCMS Spectra of Coumarin

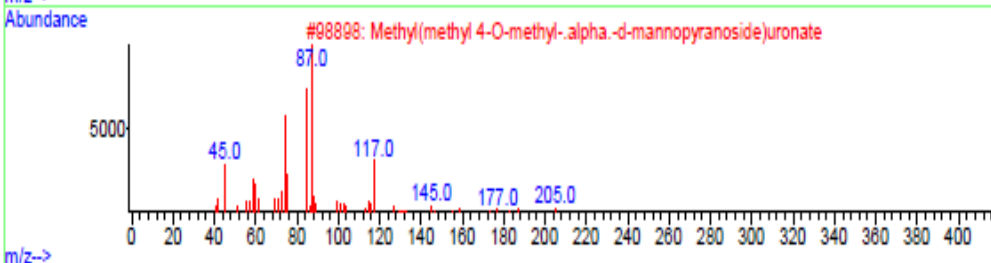
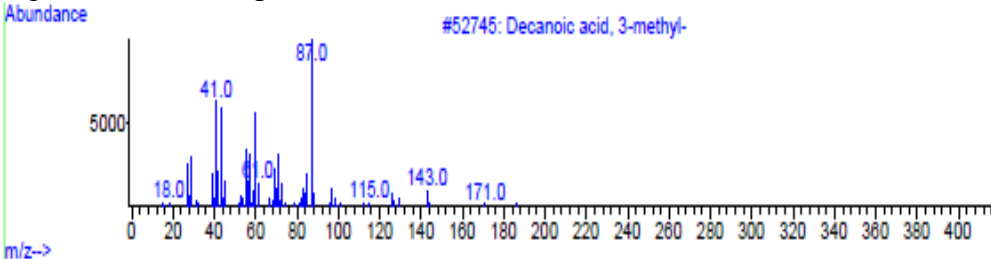


Figure 4b and 4c: GCMS Spectra of Decanoic acid, 3-methyl, Methyl(methyl 4-O-methyl- α -d-mannopyranoside)uronate

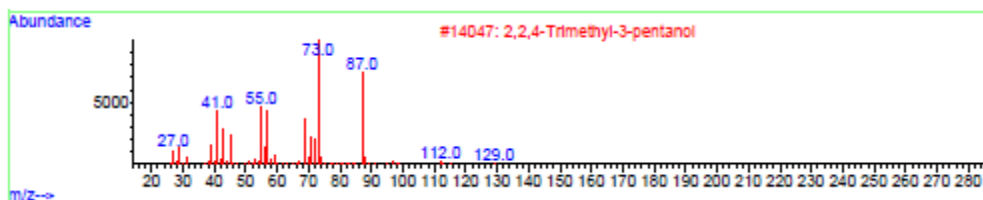


Figure 4d: GCMS Spectra of 2,2,4-Trimethyl-3-pentanol

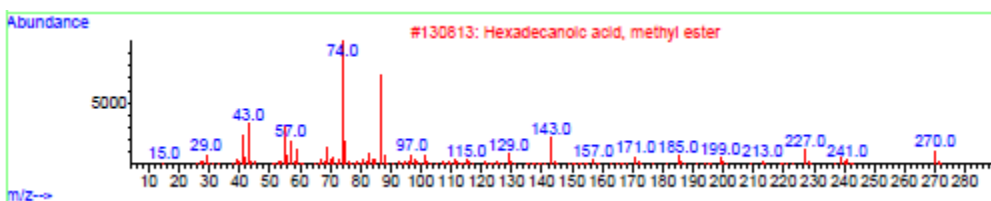


Figure 4e: GCMS Spectra of Hexadecanoic acid, methyl ester

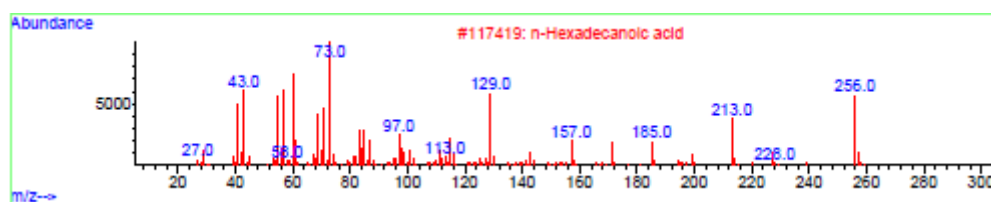


Figure 4f: GCMS Spectra of n-Hexadecanoic acid

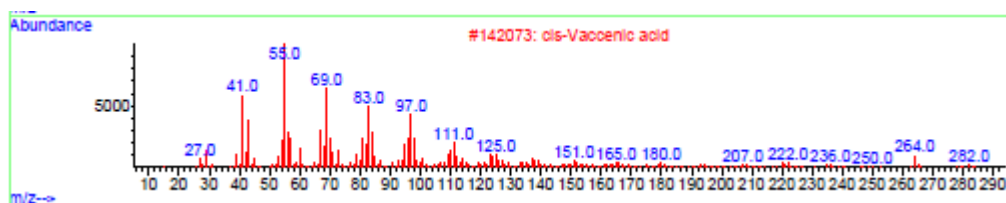


Figure 4g: GCMS Spectra of cis-Vaccenic acid

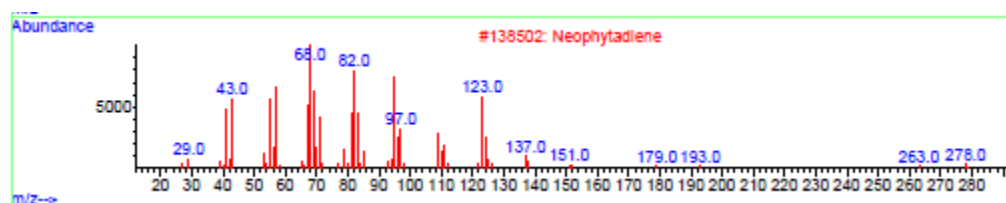


Figure 4h: GCMS Spectra of Neophytadiene

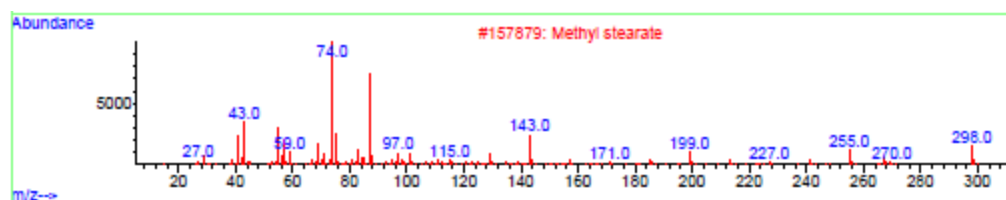


Figure 4i: GCMS Spectra of Methyl stearate

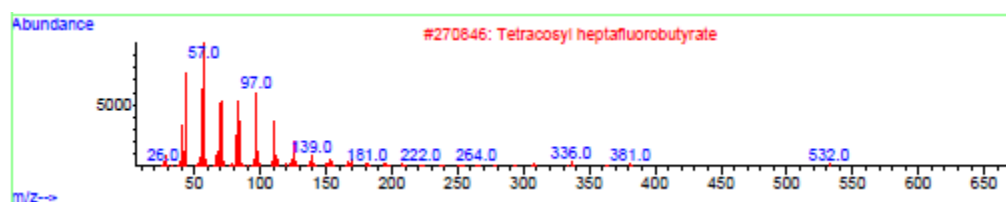


Figure 4j: GCMS Spectra of Tetracosylheptafluorobutyrate

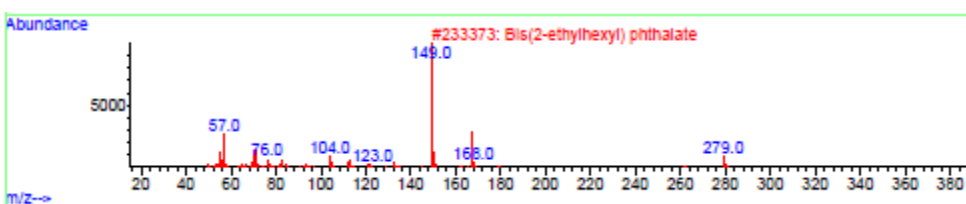


Figure 4k: GCMS Spectra of Bis(2-ethylhexyl)phthalate

Several authors have also reported presence of bioactive compounds in *C. nucifera*, compounds such as 2-Hexenal diethyl acetal, trans, Dodecanoic acid, ethyl ester, Dodecane and Hexadecanoic acid, ethyl ester which are known food additives (flavouring agents), Hexadecanoic acid, ethyl ester is known to induce expression of cyclooxygenase-2. Glutamic acid, N-isovaleryl-, Dimethyl ester is shown to play important physiological action as a neurotransmitter in nervous system (Meldrum, 2000). Mensink *et al.*, 2003 reported presence of Lauric acid in *C. nucifera*

which is known to elevate high-density lipoprotein (HDL). Similarly, Gold *et al.* (2015), also reported presence of Tetradecanoic acid, ethyl ester (used as flavourings agent) which elevated LDL-cholesterol with resultant effect being hypercholesterolemia. Most of these bioactive compounds were seen in ethanol and methanol extract hence entailing that dichloromethane extracts have selective extraction of the bioactive compounds which could be linked to the solubility and polarity of these bioactive compounds in dichloromethane solution.

These bioactive compounds could contribute to why coconut is widely used in traditional medicine.

CONCLUSION

The results from this study suggests that *Cocos nucifera* fruit is a viable source of lipids, carbohydrates, and proteins, as well as other vital bioactive compounds such as Myo-Inositol, 4-C-methyl, Heptaethylene glycol monododecyl Ether, 3-Undecanol, 3-ethyl, Hexadecanoic acid, methyl ester, Isopropyl palmitate, 9-Octadecenoic acid, (E)-, 12-Methyl-E,E-2,13-octadecadien-1-ol (11.55%), Heptadecanoic acid, 16-methyl-, methyl ester amongst others and hence could be a good nutrient source and a viable source for bioactive compounds.

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