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Mineral composition and chemical characterization of the oil constituents of *Hibiscus sabdariffa* calyxes (Zobo calyxes) by Gas chromatography mass spectrometry (GC-MS)

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Abstract

Medicinal plants are the first natural agents used traditionally to treat a wide range of illnesses, especially infectious diseases including malaria, diarrhea, fever, anaemia and colds especially in the rural areas. In this study, the calyxes of Hibiscus sabdariffa were investigated for their mineral composition, phyto constituents and oil components. Before extraction, twenty grammes (20g) of the powdered sample was analyzed for its mineral composition using X-ray fluorescence (XRF) spectrometry. The other part was extracted by cold maceration using methanol solvent. A portion of the concentrated extract obtained by rotary evaporator was investigated for phyto constituents using standard methods and procedures while the other portion was subjected to isolation by vacuum liquid chromatography (VLC) and characterization by gas chromatography mass spectrometry (GC-MS). Mineral elements were detected with varying concentrations of K (19.51 %), Ca (20.49 %), Na (1.18%) and Mg (6.02%). Glycosides, flavoinoids, alkaloids and saponins were present except tannins and eugenols. The oil constituents detected as major compounds were hexadecanoic acid (palmitic acid) methyl ester (Retention time (Rt): 21.475, 42.97%) and 9,12-Octadecadienoic methyl ester (Rt: 23.581:24.40%). The findings of this study prove that H. Sabdariffa calyxes is composed of essential minerals necessary for body functions, useful bioactive substances required for physiological

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activities and saturated (palmitic acid) and unsaturated (linoleic acid) fatty acids necessary for human nutrition.

Keywords: *Hibiscus sabdariffa,* mineral components, oil constituents, gas chromatography-mass spectrometry.

1. INTRODUCTION

Hibiscus sabdariffa, family- *Malvaceae,* locally called zobo plant in Nigeria is widely used as traditional drink mainly during the dry seasons to boost hydration of the human body with the supply of medicinal and nutritional chemical components. It is also used as herbal infusion, decoction and concoction for boosting blood (Obu, 2020). The calyx water decoction is consumed regularly by many people in Edo State and Nigeria in general. The herbal drink is mostly fortified with sugar to taste, garlic, ginger and cloves to enhance other physiological functions. Recent studies on the calyx reveals 49 calories, 84.5% water, 1.99 grams protein, 0.1 grams fat, 12.3 grams total carbohydrates and 2.3 grams fibre per 100 grams serving (Akabassi *et al.* 2016). The presence of some vitamins like E and ascorbic acid (vitamin C) has been reported by Evans and Helliwill (2001). Organic acids, phosphorus, copper, iron and calcium have also been reported by Okwuowu *et al.* (2008). Many medicinal plants have been analyzed for their oil constituents which possess physiological potentials in man. Among these are *Tetracarpidium conoforum* (Iyekowa *et al.* 2016a), *Phyllantus amarus* (Iyekowa *et al.* 2019), *Euphorbia hirta* (Iyekowa *et al.* 2016b), *Stigmaphyllum ovatum* (Iyekowa *et al.* 2017), *Dacryodes edulis* (Amoren *et al.* 2022) and *Abrus precatorius* (Iyekowa and Opara, 2022). *Hibiscus sabdariffa* have also been investigated for its diverse potential pharmacological potentials, including anti-anemic properties, anti-inflammatory, antifungal and antibacterial properties **(**Okwuowu *et al.* 2008; Prakash *et al.* 2015). Studies showed that zobo drink had no toxic effect in the internal organs (liver and kidney) of broiler birds according to (Ugwu *et al.* 2020). Many consumers have opined that the zobo drink showed evidence of lowering of high blood pressure (Mozaffarian *et al*., 2010). Studies have shown that *Hibiscus sabdariffa* flowers are rich in phytoconstituents like glycosides, phenolics, terpenoids, anthocyanins and saponins *(Prakash et al. 2015)*. Traditionally, it is generally considered safe for consumption in regular dietary amounts but this purported health benefits require further scientific investigation on the nature of the chemical constituents in the plant. Thus, this research is aimed at investigating the mineral and oil constituents of the *Hibiscus sabdariffa* calyx by XRF and GCMS analysis.

Plate 1: Fleshy and dried calyces of *Hisbiscus sabdariffa*

2. MATERIALS AND METHOD

2.1 Sample collection, treatment and extraction

The dried calyces of *H. sabdariffa* used were purchased from Uselu market in Egor local government area of Edo state, Nigeria. Sand, stones and unwanted debris were hand-picked out from the calyces. The calyces were identified by a Taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The dry calyces were air dried in the laboratory for 14 days and later pulverized into fine powder using electric blender in preparation for extraction by maceration. Three hundred grammes (300 g) of the powdered sample were packed in a beaker and 1L of methanol was used for the extraction by maceration for 72 hours. The filtrate was recovered via separation by Whatman's number 4 filter paper and concentration done through rotary evaporator (model RE, 200) at 50^{o}C to obtain a reddish dark crude extract.

2.2 Determination of mineral element by X-ray fluorescence spectrometry

The energy Dispersed X-ray Fluorescence (EDXRF) spectrometer was used for the determination of the mineral element. Here, X-ray beam was used to excite each sample and spectra were recorded with high resolution detectors. Mineral elements including potassium (K), calcium (Ca), magnesium (Mg), Iron (Fe) and copper (Cu) among others were estimated and elemental concentrations were determined in percentage.

2.3 Phytochemical screening of Hibiscus sabdariffa extract

The chemical constituents in *H. sabdariffa* calyxes extract were qualitatively determined using standard methods and procedures described by Sofowora

(1993) and Trease and Evans (1987). The phyto constituents examined were steroids, glycosides, saponins, phenolics, eugenols, tannins, terpenoids, flavonoids and alkaloids.

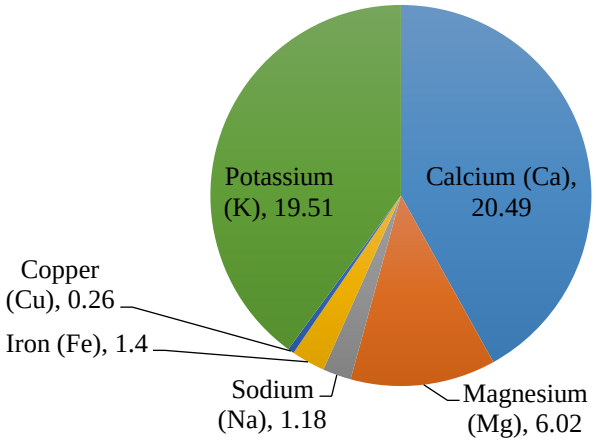
2.4 *Isolation of oil*

The methanol crude extract (67g) was mixed with silica gel (particle size 200- 425 mesh) as the solid phase and 100% N-hexane as the mobile phase. A light yellow phase obtained was concentrated to recover the pure isolate. The isolated oil was further analyzed using GC-MS and this was obtained on a Shimadzu, GC-MS–QP2010 spectrophotometer.

3. RESULT AND DISCUSSION

3.1 Mineral composition

The mineral composition of the powdered *H. sabdariffa* calyse is shown in the Pie chart (Figure 1) below.



The concentration of macro-elements in the powdered dried calyces of *Hibiscus sabdariffia* decreases in the following order: Ca> K> Mg> Fe, while Na and Cu had 1.18% and 0.26% concentrations respectively. The presence of calcium in high quantity in the calyx of the plant indicates the plant usefulness in bone tissue development and ossification (Iyekowa *et al.* 2016a). Calcium helps to rebuild the human bones. About 99% of the body's calcium is stored in the bones and it is responsible for muscle contraction, formation of bone and teeth, normal functioning of many enzymes, normal heart rhythm and blood clotting (Kanu *et al.* 2015).

Magnesium has been found in most researches to regulate and facilitate many essential bodily functions. It is involved in enzyme activities of the human body including regulation of heart, nerve and muscle function of the body. This function also embraces the regulation of blood pressure, cholesterol production and neurotransmitters of the brain and nervous system.

Sodium is crucial for fluid balance, active transport mechanism and proper regulation of the acid-base balance of the body. However, its low concentration of 1.18% suggests the plant can be used in controlling high blood pressure as high sodium content triggers high blood pressure (Akabassi *et al.* 2016). Although, high sodium value of 23.1% was reported for the seed of *Hibiscus sabdariffa* by Akabassi *et al.* (2016) and this suggest caution for high dosage consumption of the seeds of *Hibiscus sabdariffa.* The high value of potassium (19.51%) over sodium suggests that the zobo extract possess the potential of lowering high blood pressure. Potassium helps in maintaining fluid levels in the body and supports the functioning of vital organs like heart, muscles, kidneys and sensory neurones. Sodium and potassium help in balancing nerve stimulation and muscle contraction. The abnormal high serum sodium levels, in extreme cases, can also affect action of the muscles, especially of the heart; this can result to coma or lethal consequences (*Hibiscus* Tea Evaluation Network, 2012).

3.2 *Phytochemical constituents*

The phytochemicals detected in the methanol extract of *H. sabdariffa* is shown in Table 1.

Table 1: *Phytochemical constituents in methanol extract of H. sabdariffa*

S/N	Phytochemical constituents	methanol extract of *H. sabdariffa*
1	Glycoside	+
2	Saponin	+
3	Flavonoid	+
4	Phenolics	+
5	Tannin	-
6	Eugenol	-
7	Phytosterols	+
8	Terpenoids	+
9	Alkaloids	+

In Table 1, apart from tannins and eugenols, the methanol extract of *H. sabdariffa* showed the presence of alkaloids, saponins, glycosides, flavonoids, terpenoids and alkaloids. However, phenolics and steroids were absent. These constituents are responsible for most physiological and pharmacological activities of medicinal plants (Sofowora, 1993). Several claims of wellness, antimicrobial potency, antioxidant activities and anti-inflammatory properties are attributed to these phytochemicals (Doughari, 2012). Meanwhile, according to Adegunloye *et al.* (1996), the leaves extract of *H. sabdariffa* contained glycosides, alkaloids, flavonoids and phenolics but in this study, the methanol extract of the calyces contained more

components including glycosides, saponins, flavonoids, phenolics, phytosterol, terpenoids and alkaloids. The absence of tannins in this work was also reflected in the research of Okereke *et al.* (2015) who also adopted the same methanol extract of *H. sabdariffa* calyces. The presence of phenolics, flavonoids and anthocyannins in the calyces of Zobo plant has been identified as strong antioxidants with potential of protecting the heart for optimal function(Guyton and Kensler 1993). The report of Ugwu *et al.* (2020), was slightly different with the presence of tannins in hot aqueous extract of Zobo plant when compared to our findings with the absence of tannins in the cold methanol extract. This result could be attributed to temperature and polarity of extracting solvents with water being more polar than methanol.

3.3 *GC-MS Analysis*

The GC-MS chromatogram of the isolated light yellow oil given in Figure 1 showed twelve (12) peaks indicating from the search list of the chemical abstract service twelve compounds. The chemical compounds identified (according to NIST Library) in the oil fraction are presented in Table 2 below.

Table 2: *GC-MS Analysis of Hexane fraction (Yellow oil) of Sabdariffa*

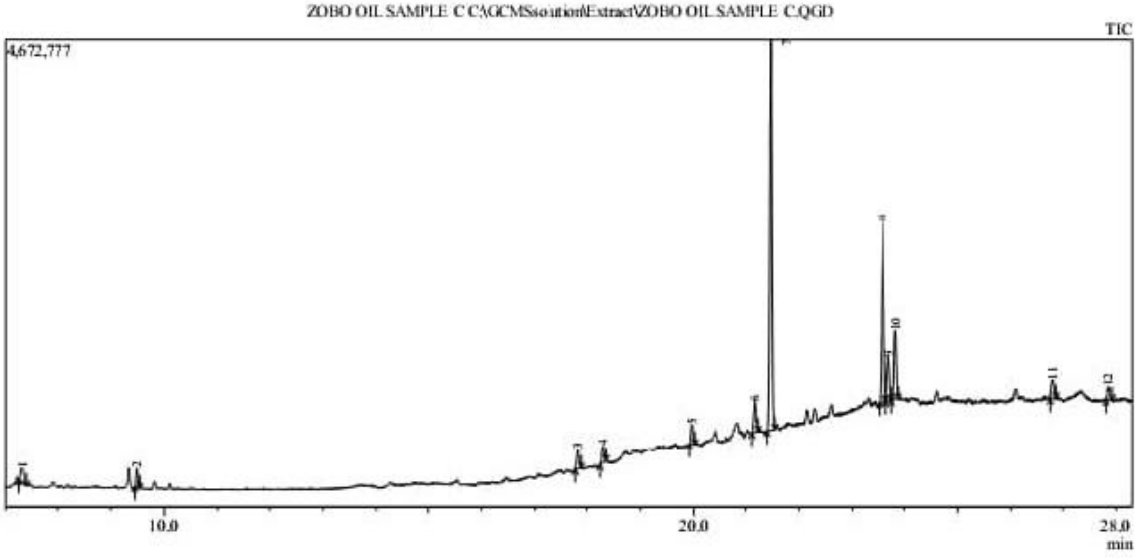
Peak	Retention time	Organic compound	Area (%)
1	7.299	2-Hexanone, 4-methyl-	2.49
2	9.491	Hydroperoxide, 1-methylpentyl	1.81
3	17.834	Lyxitol, 1-O-decyl-	2.28
4	18.299	Diethyl Phthalate	2.16
5	19.965	Tetradecane	2.29
6	21.177	1,2-Benzenedicarboxylic acid	3.62
7	21.475	Hexadecanoic acid, methyl ester	42.97
8	23.581	9,12-Octadecadienoic acid, methyl ester	24.40
9	23.697	9,12,15-Octadecatrienoic acid, methyl ester	5.55
10	23.830	Methyl stearate	8.13
11	26.802	Eicosanoic acid, methyl ester	2.49
12	27.856	Pentadecane, 8-hexyl-	1.80
TOTAL			100

LEEDEX LABORATORIES

Sample Information

Analyzed by	: Ronald Ibia
Analyzed	: 3/1/2024 4:56:48 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: ZOBO OIL SAMPLE C
Sample ID	: ZOBO OIL SAMPLE C
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 0.20
Data File	: C:\GCMSsolution\Extract\ZOBO OIL SAMPLE C.QGD
Org Data File	: C:\GCMSsolution\Extract\ZOBO OIL SAMPLE C.QGD
Method File	: C:\GCMSsolution\Extract\Extract md.qgm
Org Method File	: C:\GCMSsolution\Extract\Extract md.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\EXTRACT TUNNING 01-03-2024.qgt
[Comment]	
ZOBO OIL SAMPLE C	
Modifier by	: Admin
Modified	: 3/1/2024 5:43:32 PM

ZOBO OIL SAMPLE C C:\GCMSsolution\Extract\ZOBO OIL SAMPLE C.QGD



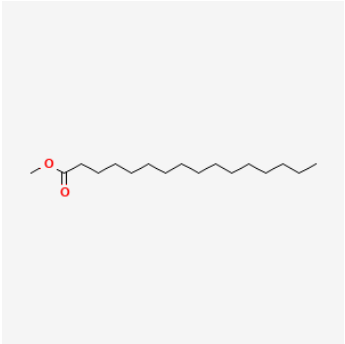
Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	7.299	638518	2.49	138679	1.66	4.60	2-Hexanone, 4-methyl-
2	9.491	464246	1.81	193384	2.31	2.40	Hydroperoxide, 1-methylpentyl
3	17.834	585101	2.28	200014	2.39	2.93	Lyxitol, 1-O-decyl-
4	18.299	554806	2.16	173758	2.08	3.19	Diethyl Phthalate
5	19.965	586532	2.29	200887	2.40	2.92	Tetradecane
6	21.177	929684	3.62	305612	3.65	3.04	1,2-Benzenedicarboxylic acid, bis(2-methylpr
7	21.475	11021223	42.97	3898911	46.59	2.83	Hexadecanoic acid, methyl ester
8	23.581	6258987	24.40	1810044	21.63	3.46	9,12-Octadecadienoic acid, methyl ester
9	23.697	1424638	5.55	438888	5.24	3.25	9,12,15-Octadecatrienoic acid, methyl ester, (Z
10	23.830	2086722	8.13	684205	8.18	3.05	Methyl stearate
11	26.802	638447	2.49	188863	2.26	3.38	Eicosanoic acid, methyl ester
12	27.856	462681	1.80	134612	1.61	3.44	Pentadecane, 8-hexyl-
		25651585	100.00	8367857	100.00		

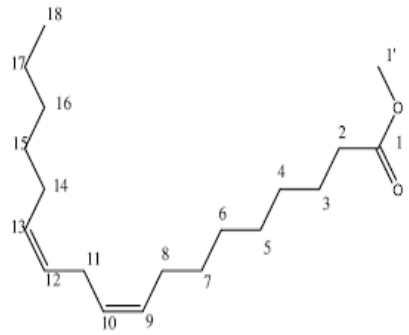
Library

The oil constituents of *H. sabdariffa* calyxes indicated the presence of 12 different compounds. Detected compounds includes hexadecanoic acid (palmitic acid) methyl ester (Retention time (Rt): 21.475, 42.97%) and 9,12-Octadecadienoic methyl ester (Rt: 23.581:24.40%) as the two major components. While methyl stearate (Rt: 23.830: 8.13%) was also implicated the oil fraction. These compounds over the years have gained relevance in the phytotherapy of several ailments and diseases. Methyl esters such as hexadecanoic acid and methyl stearate are reported to have antimicrobial activity (Iyekowa *et al.*2016a), antibacterial and antifungal

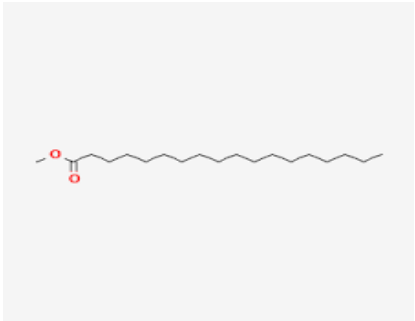
activity and lubricant potential (Mustapha *et al.* 2014). Palmitic acidals (hexadecanoic acid), a saturated fatty acid have been implicated as a useful fatty acid in controlling body inflammation (Momo *et al.* 2023). The most abundant fatty acid found in the oil sample was linoleic acid (9,12-octadecadienoic acid), an essential polyunsaturated fatty acid that offers a multitude of benefits for human health. The human body cannot synthesize large amount of this essential fatty acid but can be obtain from dietary sources, hence consumption of *H. sabdariffa* is worthwhile. Linoleic acid is an antioxidant and helps to build cell membranes, ensuring their proper fluidity and structure. It also influences the production of hormones including prostaglandins and male secondary sex hormone (testosterone). Some of the chemical compounds detected are shown in the structures below:



Palmitic acid ester



Linoleic acid methyl ester



Methyl stearate

4. CONCLUSION

The methanol extract of *H. sabdariffa* calyxes showed the presence of various secondary metabolites in the plant which is an indication of its usefulness in phytotherapy when consumed. Also, the oil constituents detected indicated both saturated and unsaturated fatty acids with reported physiological activities and these oils have been applied in medicinal products.

CONFLICT OF INTEREST

No conflict of interest was declared by the author.

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