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Gas Chromatography-Mass Spectrometry Profiling of Extracts of *Markhamia platycalyx* (Baker) (Bignoniaceae)

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A B S T R A C T

Plants are important source of both traditional and modern medicine. Over the last 50 years, studies on plant secondary metabolites have been growing. The main objective of this study was to investigate the chemical constituents of *Markhamia platycalyx* using GC-MS and its anti-plasmodial potential. Specifically, the study measured the percentage yield of the extracts of *Markhamia platycalyx*, extracted the phytochemicals from leaves, root and stem barks of *Markhamia platycalyx* and analyzed the chemical constituents of *Markhamia platycalyx* that are responsible for anti-plasmodial properties. Phytochemicals from *Markhamia platycalyx* were extracted from, leaves, root and stem barks. The samples were analyzed for the presence of various phytoconstituents that are responsible for antimalarial properties through Gas chromatography-Mass Spectrometry (GC-MS). The samples for this study were collected from Bondo Kosiemo Village, Karungu-Sori area of Migori County. Extraction of phytochemicals was conducted at the Chemistry Laboratory at Masinde Muliro University of Science and Technology. GC-MS profiling was done at the Chemistry department at Jomo Kenyatta University of Agriculture and Technology. The extracts of *Markhamia platycalyx* were screened for the presence of phytochemical constituents by following the standard methods (Manga & Oyeleke, 2008). The phytochemicals analyzed included tennins, alkaloides. balsams, saponins, steroids, flavonoids, anthraquinones, cardiac glycosides and resins. GC-MS was performed to analyze the chemical constituents in the plant sample using Japan Kyoto's SHIMADZU GC-MS QP2010SE. Values for yield of the extract were expressed as percentages. Statistical difference between percentage yield among the extracts were determined through Analysis of Variance, One-way ANOVA. The study established that ethyl acetate produces the best yield of *Markhamia platycalyx* extracts, but the difference in the yield as per the solvents were not significant. Leaves of *Markhamia platycalyx* produces highest yield of extract followed by stem and root barks and significantly differed. Water, ethanol, ethyl acetate, hexane, dichloromethane and methanol extracts of the leaves of *Markhamia platycalyx* have balsams, tannins, saponins, flavonoids, steroids, saponins glycoside, terpenes and glycosides but lack cardiac glycoside and alkaloids. Leaves, root and stem barks extracts of *Markhamia platycalyx* were found to be majorly composed of fatty acids. We recommend phytochemical and GC-MS analysis of the flowers of *Markhamia platycalyx*, further identification and characterization of the specific fatty acids with properties against *Plasmodium falciparum*.

Keywords: *Markhamia platycalyx*, *Plasmodium falciparum*, anti-plasmodial potential, phytochemical analysis, Gas chromatography and Mass Spectrometry

Introduction

Plants are important source of both traditional and modern medicine. Over the last 50 years, studies on plant secondary metabolites have been growing. Furthermore, in the treatment of diverse diseases and disorders worldwide, pharmaceutical firms use certain plant formulations (Johnson *et al*., 2016).

The Bignoniaceae family has several genera that produce secondary metabolites of high economic and therapeutic values (Abdel-Wahab *et al*., 2015). It has a few species in the tropical and subtropical areas with a few in temperate climatic conditions (Abdel-Wahab *et al*., 2015). This family is composed of 104 genera and 860 species (Abdel-Wahab *et al*., 2015). *Markhamia platycalyx* tree is an important species that belongs to this family. In Kenya, the tree is said to produce fine timber and is identified by the native names: Siala (Luo) and Lusiola (Luhya) (Omara, 2020). The tree is also with medicinal value (Mahmoud et al., 2017). *Markhamia platycalyx* is mainly found in Western part of Kenya (Omara, 2020). By looking into literature, other scholars considered *M. platycalyx* in place *M. lutea* However, in a most recent study, which was done in Egypt on cultivated plants, Abdel-Hameed, (2014) classified both of them into two different lineages.

Markhamia lutea has been investigated for its several therapeutic activities including antiviral (Kernan *et al*., 1998), antileishmanial and antimalarial (Lacroix *et al*., 2009). Nevertheless, the bark extract of *M. tomentosa* produced a good in-vitro antimalarial activity as reported by (Tantangmo *et al*., 2010). In Egypt, Ali et al., (2015) reviewed the phytochemicals of *Markhamia* species and their therapeutic values. They established that Phenyl propanoids, triterpenic acids and anthraquinones are the major phytochemicals reported in this genus and were used in curing anaemia and bloody

diarrhoea and had other ethnopharmacological uses in Africa. From their review, Ali et al., (2015) reported that the activity of the extracts of various species of *Markhamia* is a potential anti-fungal, anti-inflammatory, anthelmintic, antiparasitic, analgesic, antimicrobial and anti-viral agents.

Specifically, Mahmoud et al., (2017) investigated the *in-vitro* antimalarial and antileishmanial of *Markhamia platycalyx* leaves. They established the highest percentage of inhibition (87%) in petroleum ether fraction against *P. falciparum* D_6 strain relative to chloroquine. They also established a good antimalarial activity (47%) inhibition in dichloromethane fraction, followed by 24% obtained from ethyl acetate fraction, while weak activity (14%) was established in the aqueous fraction but was higher than that of the total ethanol extract (8%).

In Kenya, Omara, (2020) did a review on antimalarial plants used by Kenyan communities and identified 286 species of plants which belongs to 75 botanical families. Among these, *M. platycalyx* was reported to be used by the Luo community in Nyanza region for the treatment of malaria but parts of the plant used were not specified (Omara, 2020). In addition, Omara, (2020) did not establish the part of *M. platycalyx* used for the treatment of malaria. However, Mahmoud et al., (2017) identified the leaves of *M. platycalyx* for their investigation. They used standard phytochemical procedures by Harborne, (1998) to screen the phytochemicals of *M. platycalyx.* From their screening, total ethanol extract showed the presence of anthraquinones, carbohydrates, flavonoids, glycosides, triterpenes and unsaturated sterols. Alkaloids, saponins, cardenolides tannins, coumarins and crystalline sublimate substances were absent.

On the other hand, Uzor (2020) established that alkaloids are the major antimalarial phytoconstituent among several classes such as terpenes, steroids, and flavonoids. With the absence of alkaloids, Mahmoud et al., (2017) predicted antimalarial activity of *M. platycalyx* as a result of sterols and triterpenes. The high percentage of inhibition of *Markhamia platycalyx* leave extract in the absence of alkaloids prompted this study to investigate the anti-plasmodial potential and gas chromatography-mass spectrometry (GC-MS) analysis of *Markhamia platycalyx* extracts.

Malaria is one of the top major challenges for global health that killed an estimated 655,000 people in 2010. From this figure, 91% of the deaths were in Africa and 86% of these were children under 5 years of age (WHO, 2011). WHO (2017), reported that the more susceptible continent to malaria is Africa and estimated that there are 2 million deaths annually. About 2 billion people in more than 100 countries globally are exposed to malaria, and this situation is severe in Africa where it is attributed to limited access to healthcare services and poverty (WHO, 2017). In the East African region and Kenya in particular, malaria is an endemic disease in two regions, the Coast and the Lake Victoria basin. These regions record the highest rate of malaria infection in the country at a rate of 27% (6 million cases) recorded in 2015 from 38% recorded in 2010 (Machini *et al*., 2016). In the East African region, *Anopheles gambiae* and *A. funestus* are the main vectors of malaria (Sinka *et al*., 2016), while *Plasmodium vivax* and *Plasmodium falciparum* are the deadliest parasites causing malaria in sub-Saharan Africa (Omara, 2020).

The global emergence of parasites that are resistant to chloroquine has been attributed to the misuse of chloroquine during the management of malaria (Mekonnen *et al*., 2014). In Kenya, chloroquine was discontinued as first line malaria treatment because of the prevalence of resistant *P. falciparum* resistance strains (Mwai *et al*., 2009). White (2007) reported that quinolones not only had cardiotoxicity as a side effect, but also malaria parasites had developed resistance against them. Therefore, currently the only option for treating malaria is artemisinin-based combination therapy (ACT) (Omara, 2020). Unfortunately, *P. falciparum* has also developed resistance to artemisinin (Rahmatullah *et al*., 2012; Ashley *et al*., 2014; Tilley *et al*., 2016; Lu *et al*., 2017).

In Kenya, this resistance has resulted in frequent change of drugs for the first line treatment for malaria (Omara, 2020). In addition to the resistance, conventional treatment is costly, rural dwellers in Kenyans and world over. Due to this reason, many rural dwellers often use plants for the treatment and prevention of malaria (Cock *et al*., 2019). Based on this background, the current study investigated the chemical constituents of *Markhamia platycalyx* using GC-MS and its anti-plasmodial potential.

Therefore, the main objective of this study was to investigate the chemical constituents of *Markhamia platycalyx* using GC-MS and its anti-plasmodial potential. The study investigated the following specific objectives: To measure the percentage yield of the extracts of *Markhamia platycalyx*, to extract the phytochemicals from leaves, root and stem barks of *Markhamia platycalyx* and to analyze the chemical constituents of *Markhamia platycalyx* that are responsible for anti-plasmodial properties.

In addition, the following hypothesis were tested:

H_0**:** There is no significant difference between the yields of *Markhamia platycalyx* in terms of:

Type of solvent (distilled water, ethyl acetate, hexane, dichloromethane, ethanol and methanol)

Part of the plant (leaves, stem and root barks).

Materials and Methods

Phytochemicals from *Markhamia platycalyx* were extracted from, leaves, root and stem barks. Samples were analyzed for the presence of various phytoconstituents that are responsible for antimalarial properties through Gas chromatography-mass spectrometry (GC-MS).

Preparation of Plant Material

Fresh samples of different plant parts (leaves, stem and root barks) from *Markhamia platycalyx* were collected from Karungu and were botanically identified based on characterization by Kokwaro, (2009). Prior to being brought to the research laboratory, the plant was identified by the local herbalist

who use it traditionally in the area. The plant was also verified online to confirm its identity. All samples that were collected were washed twice using distilled water for the removal of adhering dusts and other associated organisms. The plant materials were chopped into small pieces and air dried under room temperature (20 ± 3^0C) for 14 days. A sample of the plant specimen was deposited in the herbarium facility which is maintained by the Department of Biological Sciences of UEAB. The dried materials were powdered using a laboratory mill at Kenya Industrial Research & Development Institute (KIRDI) Kisumu and were kept in an air tight container until use.

Extraction of Phytochemicals

The plant's active ingredients were obtained by extraction method described by (Ijaiya, Arzika & Abdulkadir, 2006). This procedure was used for distilled water, ethanol, ethyl acetate, hexane, dichloromethane and methanol. Distilled water was used to replicate the practice by herbalists and serve as polar solvent including methanol and ethanol. The non-polar solvents used were ethyl acetate, hexane and dichloromethane. This was obtained by soaking 20g of the grounded (sieved) samples in 175ml of cold distilled water in a sterile conical flask and left to stand for 24 hours was then filtered with sterile filter paper after which the filtrate was then evaporated in a rotary evaporator at 100^0C to evaporate excess water. The extract obtained was then stored in refrigerator for further investigation. The same procedure was adopted for ethanol, ethyl acetate, hexane, dichloromethane and methanol extracts were evaporated at 78^0C, 77.1^0C, 68^0C, 40^0C and 64^0C respectively.

The following formula was used to determine the percent weight-volume of extracts. Thereafter, the extracts were filtered, evaporated using rotary evaporator and final volume measured as the yield of the extract.

Percent weight-volume (%(w/v)) = 100 x (weight of extract / ml of solvent).

Qualitative Analysis of the Phytochemicals

The extracts of *Markhamia platycalyx* were screened for the presence of phytochemical constituents by following the standard methods (Manga & Oyeleke, 2008). The phytochemicals analyzed included tennins, alkaloides. balsams, saponins, steroids, flavonoids, anthraquinones, cardiac glycosides and resins.

GC-MS Analysis of the Chemical Constituents of *Markhamia platycalyx* that are Responsible for Anti-plasmodial Properties

Gas chromatography and mass spectrometry were performed to analyze the chemical constituents in the plant sample. The plant sample extracts were analyzed using Japan Kyoto's SHIMADZU GC-MS QP2010SE equipped with CTC Analytics autosampler and a BPX5 column (length 30M, thickness 0.25um Internal diameter 0.25mm) and the samples were injected in split mode (10:1) at an injection temp of 200 Degree Celsius; column oven temperature was set at 70°C and a temperature program used to bring about separation of compounds starting from 70oC then ramped at rate of 10°C/min to 205°C and then increased to285°C at rate of 5dc/min and held for 9 minutes. The interface temperature was set at 250°C, ion source temperature set t 200°C and the solvent cut time as 4.5. The mass spectrometer was run in SCAN mode for masses starting from 35Hz to 500Hz.

The chemical constituents were identified based on their retention time, MS fragment ions were generated and the percentage of the constituents were evaluated from the total peak area. Identification of the chemical constituents was done based on the MS spectrum patterns compared to the standard mass spectra available at the National Institute of Standards and Technology (NIST) Mass Spectra Database.

Research Instruments and Materials

The following instruments and materials were used in this study: *Markhamia platycalyx* samples. For plant preparation, double distilled water and laboratory mill were used. For the preparation of the extract, double distilled water, ethanol, ethyl acetate, hexane, dichloromethane, methanol, Millipore sterile filters and rotary evaporator. For the GC-MS analysis, Japan Kyoto's SHIMADZU GC-MS QP2010SE instrument was used.

Data Analysis

Descriptive statistics was employed to analyze data of this study. Values for yield of the extract were expressed as percentages. Statistical difference between percentage yield among the extracts were determined through Analysis of Variance, One-way ANOVA.

Results and Discussion

Yield of the Extracts of *Markhamia platycalyx*

Table 1 – Yield of the Extracts.

Plant Part	Solvent	Percent weight-volume (%(w/v)	Yield of Extract (ml)
Leaf	Distilled water	11.43	87
	Ethyl acetate	11.43	114
	Hexane	11.43	105
	Dichloromethane	11.43	98
	Ethanol	11.43	113
	Methanol	11.43	109
Root Bark	Distilled water	11.43	44
	Ethyl acetate	11.43	103
	Hexane	11.43	89
	Dichloromethane	11.43	83
	Ethanol	11.43	85
	Methanol	11.43	85
Stem Bark	Distilled water	11.43	69
	Ethyl acetate	11.43	93
	Hexane	11.43	86
	Dichloromethane	11.43	78
	Ethanol	11.43	94
	Methanol	11.43	93

Table 1 indicates the different percentage yield of extracts of *M. platycalyx*. However, the plant samples had equal weight volume percentage. This was to ensure that there was a uniform concentration of the plant samples.

Yield Per Solvent

Table 2 – Yield per solvent.

Solvent	Mean	N	Std. Deviation	Minimum	Maximum
Distilled water	66.67	3	21.595	44	87
Ethyl acetate	103.33	3	10.504	93	114
Hexane	93.33	3	10.214	86	105
Dicholoromethane	86.33	3	10.408	78	98
Ethanol	97.33	3	14.295	85	113
Methanol	95.67	3	12.220	85	109
Total	**90.44**	**18**	**16.769**	**44**	**114**

From table 2 above, the yield of the extracts was determined based on the solvents. With the same concentration of the samples, the minimum yield was exhibited by dichloromethane and maximum by ethyl acetate. Specifically, ethyl acetate had the best yield of the extract (103.33ml) followed by 97.33ml, 95.67ml, 93.33ml, 86.33mland 66.67 ml for ethanol, methanol, hexane, dichloromethane and distilled water respectively. This variation can be explained by the fact that different solvents have varying solubility capabilities for different phytochemical components. The findings of this study are in line with the findings of Muthaura et al., (2015) who established varied yields from water, hexane, methanol and ethyl acetate extracts from medicinal plants from Kwale County, Kenya.

Yield Per Solvent

Table 3 – Yield per plant part.

Plant part	Mean	N	Std. Deviation	Minimum	Maximum
Leaves	104.33	6	10.309	87	114
Root bark	81.50	6	19.756	44	103
Stem bark	85.50	6	10.134	69	94
Total	**90.44**	**18**	**16.769**	**44**	**114**

From table 3 above, the yield of the extracts was determined based on the part of the plant. This was determined with the uniform concentration of the extracts. As indicated, the minimum yield was recorded by root barks while maximum yield was recorded by leaves. Specifically, leaves exhibited the highest yield (104.33ml) followed by stem barks 85.5% and root barks 81.5%.

The most important sources of phytochemicals that can be screened from plant components are thought to be plant extracts. These phytochemicals can be extracted from medicinal plants using a variety of solvents and extraction techniques. Therefore, distilled water, ethyl acetate, hexane, dichloromethane, ethanol and methanol were selected to enable the extraction and separation of a wide range of components that are present in the samples. The study's choice of the extraction solvents and parts of the plant was an effort to obtain the best possible separation of components in each extract. The findings on the yield of the crude extracts above was based on the weight/volume of the samples.

Phytochemical Analysis of *Markhamia platycalyx* Extracts

Table 4 – Phytochemical Analysis of *Markhamia platycalyx* Extracts

Plant Part	Phytochemical	Distilled water	Ethyl acetate	Hexane	Dichloromethane	Ethanol	Methanol
LEAF							
	Alkaloids	Absent	Absent	Absent	Absent	Absent	Absent
	Balsams	Present	Absent	Absent	Absent	Present	Present
	Tannins	Present	Absent	Absent	Present	Present	Present
	Saponins	Absent	Present	Absent	Present	Present	Present
	Flavonoids	Present	Absent	Absent	Absent	Absent	Absent
	Steroids	Present	Present	Present	Absent	Present	Present
	Saponins glycoside	Present	Present	Absent	Absent	Present	Present
	Terpenes	Present	Present	Present	Present	Present	Present
	Glycosides	Absent	Present	Present	Absent	Absent	Absent
	Cardiac glycoside	Absent	Absent	Absent	Absent	Absent	Absent
	Resins	Present	Absent	Absent	Absent	Absent	Absent
	Carbohydrates	Present	Present	Absent	Present	Present	Present
	Anthraquinones	Absent	Absent	Absent	Absent	Absent	Absent
STEM							
	Alkaloids	Absent	Absent	Absent	Absent	Absent	Absent
	Balsams	Present	Absent	Absent	Absent	Present	Present
	Tannins	Present	Absent	Absent	Absent	Present	Present
	Saponins	Absent	Present	Absent	Absent	Present	Present
	Flavonoids	Absent	Present	Absent	Absent	Present	Present

Steroids	Present	Present	Absent	Present	Absent	Present
Saponins glycoside	Absent	Present	Absent	Absent	Present	Present
Terpenes	Absent	Absent	Absent	Present	Present	Present
Glycosides	Absent	Absent	Present	Present	Absent	Absent
Cardiac glycoside	Absent	Absent	Absent	Absent	Absent	Absent
Resins	Present	Absent	Absent	Present	Present	Present
Carbohydrates	Present	Absent	Absent	Present	Present	Present
Anthraquinones	Absent	Absent	Absent	Absent	Absent	Absent
ROOT						
Alkaloids	Absent	Absent	Absent	Absent	Absent	Absent
Balsams	Present	Absent	Absent	Absent	Present	Present
Tannins	Present	Absent	Absent	Absent	Present	Present
Saponins	Absent	Present	Absent	Absent	Present	Present
Flavonoids	Absent	Present	Absent	Absent	Present	Present
Steroids	Present	Absent	Absent	Absent	Absent	Present
Saponins glycoside	Absent	Present	Absent	Present	Present	Absent
Terpenes	Absent	Absent	Absent	Present	Absent	Present
Glycosides	Absent	Absent	Present	Present	Absent	Absent
Cardiac glycoside	Absent	Absent	Absent	Absent	Absent	Absent
Resins	Present	Absent	Absent	Absent	Present	Present
Carbohydrates	Absent	Present	Absent	Absent	Present	Present
Anthraquinones	Present	Present	Present	Present	Present	Present

Table 4 above indicated that water, ethanol, ethyl acetate, hexane, dichloromethane and methanol extracts of the leaves of *Markhamia platycalyx* were positives for balsams, tannins, saponins, flavonoids, steroids, saponins glycoside, terpenes and glycosides but negative for Cardiac glycoside. The leaf, stem and root barks extracts were negative for alkaloids.

Since plants are powerful biochemists and have long been used in phytomedicine, man can obtain a wonderful range of industrial chemicals from them. Natural substances formed from plants can come from any part of the plant, including the stem, leaves, roots, fruits, and seeds, for example. Active components may be present in any region of the plant (Süntar, 2020).

Markhamia platycalyx has been found to have antiviral, antifungal, antiprotozoal, analgesic, anti-inflammatory, and cytotoxic properties (Mahmoud et al., 2019). Therefore, the synthetic and phytochemical profiling of this plant with purpose of discovering the chemical constituents is a routine activity in many laboratories.

Gebrehiwot et al., (2019) established that tannins, alkaloids, flavonoids and phenols have anti-plasmodial activity in other plants. The current study has established the absence of alkaloids and phenols. However, there is presence of tannins and flavonoids in the extracts of *Markhamia platycalyx.* Therefore, the anti-plasmodial activity of *Markhamia platycalyx* could be as a result of these phytochemicals acting singularly or in synergy with one another to exert the existing antimalarial activity. *Markhamia platycalyx* can therefore help in managing malaria. This therefore justify using *Markhamia platycalyx* for treating malaria in traditional herbal medicine.

In addition, Zareen et al., (2021) reported that alkaloids and flavonoids of different plants have proven anti-plasmodial activities. Flavonoids have profound efficacy against *Plasmodium falciparum*. The presence of flavonoids further validates the anti-plasmodial activity of *Markhamia platycalyx* in this study. However, further chemical analysis of the chemical constituents was highly considered in this study to better understand the underlying anti-plasmodial activity of *Markhamia platycalyx* and related.

GC-MS Analysis of the Chemical Constituents of *Markhamia platycalyx* that are Responsible for Anti-plasmodial Properties

Table 5 – Methanolic Leaf Extract

Peak	Compound	Retention time (min)	Relative area (%)
1	Dodecanoic acid, methyl ester	12.584	7.61
2	Dodecanoic acid	13.128	35.02
3	3-Buten-2-one, 4-(2,5,5-trimethyl-3,8-dioxatricycl	14.961	2.74
4	Methyl tetradecanoate	15.074	1.12
5	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydr	16.350	2.66
6	2-Pentadecanone, 6,10,14-trimethyl-	16.548	1.33
7	Hexadecanoic acid, methyl esterester	17.665	9.67
8	9-Octadecenoic acid, methyl ester	20.186	13.40
9	Phytol	20.321	9.81
10	Hexadecanoic acid, n.-octyl ester	26.089	16.64

From table 5 above, 6 chemical constituents were detected in methanolic root extract by GC–MS. All the detected chemical constituents were fatty acids. Therefore, the major chemical constituent (83.46%) of the relative area was fatty acids.

Table 6 – Hexanoic Leaf Extract

Peak	Compound	Retention time (min)	Relative area (%)
1	Dodecanoic acid, methyl ester	12.586	1.57
2	Dodecanoic acid	13.155	70.55
3	4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)butan-2	14.968	2.57
4	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-o	16.353	2.76
5	2-Pentadecanone, 6,10,14-trimethyl-	16.553	3.18
6	Hexadecanoic acid, methyl ester	17.665	3.65
7	8-Octadecenoic acid, methyl ester	20.190	4.33
8	Phytol	20.323	11.39

From table 6 above, 4 chemical constituents were detected in methanolic root extract by GC–MS. Most of the detected chemical constituents were fatty acids. Therefore, the major chemical constituent (80.01%) of the relative area was fatty acids.

Table 7 – Methanolic Leaf Extract

Peak	Compound	Retention time (min)	Relative area (%)
1	Dodecanoic acid, methyl ester	12.587	16.48
2	Dodecanoic acid	13.128	23.12
3	Methyl tetradecanoate	15.070	4.39
4	Hexadecanoic acid, methyl ester	17.668	22.66

5	9-Octadecenoic acid, methyl ester	20.194	29.56
6	Methyl stearate	20.534	3.80

From table 7 above, 6 chemical constituents were detected in methanolic root extract by GC–MS. All the detected chemical constituents were fatty acids. Therefore, the major chemical constituent (100%) of the relative area was fatty acids.

Table 8 –Hexanoic Root Extract

Peak	Compound	Retention time (min)	Relative area (%)
1	Dodecanoic acid, methyl ester	12.583	6.19
2	Dodecanoic acid	13.121	3.16
3	Methyl tetradecanoate	15.064	1.61
4	Perhydro-3-(alpha-methoxy-4,5-methylenedio	16.399	2.25
5	Hexadecanoic acid, methyl ester	17.662	16.64
6	Dibutyl phthalate	18.369	2.15
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	20.118	15.09
8	9-Octadecenoic acid, methyl ester	20.186	30.07
9	Methyl stearate	20.529	4.94
10	6,6-Diethylhoctadecane	23.000	1.03
11	Eicosane	24.502	2.00
12	Tetracosane	26.005	4.63
13	Heptacosane	27.488	2.21
14	Tetratetracontane	28.944	3.57
15	Heptadecane, 2,6,10,14-tetramethyl-	30.364	0.94
16	Decanedioic acid, bis(2-ethylhexyl) ester	30.496	3.51

From table 8 above, a total of 16 chemical constituents were detected in hexanoic toot extract by GC–MS. Among the detected chemical constituents, 8 were fatty acids. Therefore, the major chemical constituent (67.21%) of the relative area was fatty acids.

Table 9 –Hexanoic Stem Extract

Peak	Compound	Retention time (min)	Relative area (%)
1	Isopropyl myristate	16.254	31.42
2	Dibutyl phthalate	18.369	4.05
3	Phenol, 2-(1,1-dimethylethyl)-4-(1-methyl-1-	19.493	4.84
4	Eicosane	26.006	4.31
5	Hexadecanoic acid, n.-octyl ester	26.085	20.70
6	2,4-Bis(dimethylbenzyl)-6-t-butylphenol	26.550	2.60
7	Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	26.745	18.07
8	Diisooctyl phthalate	26.830	2.51
9	Decanedioic acid, bis(2-ethylhexyl) ester	30.500	11.50

From table 9 above, a total of 9 chemical constituents were detected in hexanoic stem extract by GC–MS. Only 3 chemical constituents were fatty acids. Therefore, the major chemical constituent (34.71%) of the relative area was fatty acids.

Table 10 –Methanolic Stem Extract

Peak	Compound	Retention time (min)	Relative area (%)
1	Dodecanoic acid, methyl ester	12.584	12.01
2	Dodecanoic acid	13.131	2.78
3	Methyl tetradecanoate	15.074	2.76
4	Isopropyl myristate	16.261	0.79
5	Hexadecanoic acid, methyl ester	17.665	13.15
6	6,9-Octadecadienoic acid, methyl ester	20.126	2.30
7	9-Octadecenoic acid, methyl ester	20.189	22.35
8	7-Methyl-Z-tetradecen-1-ol acetate	20.260	3.64
9	Methyl stearate	20.534	3.54
10	Hexadecanoic acid, n.-octyl ester	26.090	36.67

From table 10 above, a total of 10 chemical constituents were detected in methanolic stem extract by GC–MS. Among the detected chemical constituents, 8 were fatty acids. Therefore, the major chemical constituent (95.56%) of the relative area was fatty acids.

The above findings of the GC-MS analysis have established that the leaves root and stem extracts of *Markhamia platycalyx* are majorly composed of fatty acids. By reviewing literatures, it showed that myristic acid, 5-octadecenoic acid, palmitoleic acid, palmitic acid, heptadecanoic acid, linoleic acid, stearic acid, linolenic acid has been reported in *Markhamia* genus. Mahmoud et al., (2015) also established fatty acids as the main chemical constituents of specifiable matter composition of *Markhamia platycalyx* leaves. Thu, the findings of the current study therefore is the same as the above literature by establishing fatty acids as the main chemical constituents of *Markhamia platycalyx*.

In a review by Carballeira, (2008), fatty acids were reported to have properties against malaria, mycobacteria, and fungi. It was reported in this review that fatty acids' potential to disrupt the fatty acid biosynthesis machinery of *Plasmodium falciparum* had been considered as a potential method of parasite control, despite the fact that their antimalarial effects had long been known. In this review, scleropyric, docosahexaenoic and oleic acids were reported to be the best in killing *Plasmodium falciparum.* In addition, methyl esters of the fatty acids were reported to be as potent as the free acids in killing the parasite.

Extracts of *Markhamia platycalyx* has been established in this study to be rich in fatty acids. Owing to the fact that fatty acids have been established to have anti-plasmodial properties by Carballeira, (2008), it is therefore a signal that fatty acids are the chemical constituents responsible for the anti-plasmodial activities of the extracts of *Markhamia platycalyx.* Therefore, this finding confirms the claim by traditional practitioners that for the use of *Markhamia platycalyx* in treating malaria.

Significant Difference between Yield Per Solvent

Table 11 –ANOVA for Yield per Solvent

ANOVA Table

		Sum of Squares	Df	Mean Square	F	Sig.
Yield * Solvent	Between Groups (Combined)	2494.444	5	498.889	2.619	.080
	Within Groups	2286.000	12	190.500		
	Total	4780.444	17			

In this study. one-way ANOVA was used to determine whether there were any statistically significant differences between the mean yield of the six solvents. Significant difference was determined under 0.05 significance level. There were no statistically significant differences between the group means p =0.080.

The current study established that the difference in the yield as per the solvents were not significant. Therefore, it is worth noting that choice of solvent was not significant in obtaining extraction yield of *Markhamia platycalyx* for this study. This implies that any solvent used in this study was capable of yielding extracts for phytochemical analysis. However, the finding of this study deviates from the findings of Truong et al., (2019) who in their study established a significant difference in the extraction yield using different solvents.

Table 12 –ANOVA for Yield per Plant Part

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.
Yield * Plant part	Between Groups (Combined)	1784.111	2	892.056	4.466	.030
	Within Groups	2996.333	15	199.756		
	Total	4780.444	17			

The current study used one-way ANOVA to determine whether there were any statistically significant differences between the mean yield of the three plant parts. Significant difference was determined under 0.05 significance level. There were a statistically significant differences between the group means p =0.030.

The current study established that the difference in the yield as per plant parts were significant. Therefore, it is worth noting that choice of plant part was significant in obtaining extraction yield of *Markhamia platycalyx* for this study. This implies that any plant part used in this study differed significantly in yielding extracts for phytochemical analysis. Since leaves produced the highest yield, using leaves extract was significant in this study. The findings of this study are in line with the findings of a study done by Sembiring et al., (2018) who also established that extraction yield from leaves was higher than that from the other plant parts.

4. Summary

The types of phytochemicals existing in medicinal plants vary based on the solvents and part of the plant used. *Markhamia platycalyx* contains some essential phytochemical elements that have medicinal use. Thus, the study offers more proof of the plant's historical application in the treatment of diseases.

From the findings of this study, the following conclusions were drawn: Ethyl acetate produces the best yield of *Markhamia platycalyx* extracts, followed by ethanol, methanol, hexane, dichloromethane and distilled water. The difference in the yield as per the solvents were not significant. Leaves of *Markhamia platycalyx* produces highest yield of extract followed by stem and root barks. The difference in the yield as per plant parts were significant. Water, ethanol, ethyl acetate, hexane, dichloromethane and methanol extracts of the leaves of *Markhamia platycalyx* have balsams, tannins, saponins, flavonoids, steroids, saponins glycoside, terpenes and glycosides but lack cardiac glycoside. The leaf stem and root barks extract lack alkaloids. Leaves, root and stem barks extracts of *Markhamia platycalyx* are majorly composed of fatty acids.

Since Carballeira, (2008), reported fatty acids to have properties against malaria, the establishment of fatty acids in *Markhamia platycalyx* points to its anti-plasmodial activity. The finding of the current study is therefore a signal that fatty acids are the chemical constituents responsible for the anti-plasmodial activities of the extracts of *Markhamia platycalyx.* Therefore, the current study confirms the claim by traditional practitioners for the use of *Markhamia platycalyx* in treating malaria.

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