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PHYSICOCHEMICAL AND PHYTOCHEMICAL SCREENING OF SIX PLANTS COMMONLY USED IN THE TREATMENT OF MALARIA IN NIGERIA.

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Abstract

Medicinal plants contain active compounds usually present as complex mixtures though at low concentrations, which accounts for the medicinal properties. Therefore it is important to identify and characterize these compounds. This study aims to quantify and characterize the physicochemical and phytochemical compositions of six plants commonly used in the treatment of malaria using High-Performance Liquid Chromatography (HPLC). These plants which are used individually or in combinations: Enantia chlorantha, Cymbopogon citratus, Curcuma longa, Carica papaya, Alstonia boonei and Mangifera indica, were extracted with hot water and the extracts characterized with HPLC using standard procedures. The results showed that *M. indica* stem bark had the highest yield with 81.48% and C. papaya had the lowest yield with 53.80%. Physicochemical properties of the extracts of E. chlorantha, C. citratus, C. longa, C. papaya, A. boonei and M. indica respectively are as follows: Melting point 90, 80, 95, 92, 96 and 96; pH 7.43, 8.02, 6.24, 6.81, 6.41, 6.85; moisture content 18.27, 22.77, 9.96, 9.62, 3.85 and 10.00; Total ash 1.45, 3.51, 0.34, 0.57, 9.10,10.21; refractive index 1.34, 1.34, 1.34, 1.34, 1.34, 1.34 and 1.34. Alkaloids, phenols, flavonoids, terpenoids, tannins, coumarin were some of the antimalarial active phytochemicals identified. Alkaloid (Atropine) was highest in C. longa (4382.2mg/g). M. indica (32982.8mg/g) had the highest Rutin Hydrate content. While Ouercetin was not detected in C. papaya, it was considerably present in A. boonei (491.1mg/g). All the analyzed six plants contain low phenol (gallic acid). The characterized physicochemical and phytochemical compositions of the examined plants suggests why the plants are effective in the treatment of malaria. The information reported herein describes the physicochemical and phytochemical contents of six commonly used antimalarial plants in Nigeria. It is expected that the information will be useful in understanding the pharmaceutical effects of how the plants work in the body and in the development of efficacious and safe antimalarial drugs.

Key Words: Antimalarial plants, Physicochemical properties, Phytochemical screening, HPLC quantification.

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1.0 Introduction

Plants are major sources of bioactive compounds with potential for developing new and original antimalarial drugs [1]. These simple medicinal preparations often mediate beneficial responses due to a variety of their active chemical constituents [2], which are responsible for their medicinal properties [3]. A wide variety of plants belonging to several families have been identified through ethnobotanical and ethnopharmacological studies as antimalarial medicinal plants [4], and are used for treating malaria by majority of the infected populations in most endemic countries [5]. However, very little scientific data exist to validate the antimalarial properties of most identified medicinal plants. Studies to establish the identity, purity and quality of natural products include macroscopic and microscopic evaluations, physicochemical and chemical characteristics of the crude plant extracts [6].

All herbal medicines are complex mixtures of more than one active ingredient. The non-nutrient plant chemicals or bioactive components are as referred to phytochemicals often or phytoconstituents and are responsible for the plants' therapeutic effects such as anti-oxidant, anti-microbial, anti-inflammatory, antimalarial activities [7-8]. The most important pure compounds which have exhibited antimalarial properties have been observed in plant derived phytochemicals include: alkaloids, terpenoids, flavonoids, coumarines, phenolics,

polyacetylenes, xanthones, guinones, steroids, and lignans. Knowledge about the active ingredients responsible for a given therapeutic effect frequently serve as a model for the synthetic preparation of new medicines, enabling the plants to be modified into drugs and made more effective. The active phytochemicals in medicinal plants are generally present in complex matrices at low levels. Purification methods are therefore required for their identification and characterization. Quantitative analysis is an important tool that provides information on the composition and concentration of the active components of the plant material [9]. Inadequate data on the concentrations of antimalarial active phytoconstituents responsible for their medicinal functions can be remediable through research. Some of the analytical methods currently available for the quantitative determination of plant extracts include High-Performance Liquid Chromatography (HPLC) [10]. High-Performance Thin-Layer Chromatography (HPTLC), Ultraviolet-Visible Spectrophotometry and Capillary Electrophoresis (CE) [11]. HPLC is known for its high efficiency and rapid separation.

Enantia chlorantha (African yellow wood), *Cymbopogon citratus* (Lemon grass), *Carica papaya* (Pawpaw), *Mangifera indica* (mango), *Curcuma longa* (Tumeric), *Alstonia boonei* (Pattern wood) are some of the predominantly used antimalarial medicinal plants in most of the endemic countries including

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Nigeria. The knowledge of some antimalarial active constituents of each plant species was studied in order to gain insight into their antimalarial potentials.

2.0 Materials and Methods

2.1 Collection and identification of plant materials

E. chlorantha, C. citratus, C. longa, C. papaya, E. chlorantha, A. boonei and M. indica plant parts were collected from medicinal plants growers at Oje, Ibadan. Identification and authentication of the plants were done by a plant taxonomist, in the Department of Botany and Microbiology, University of Lagos, Lagos Nigeria. Voucher specimens were kept for future reference at the herbarium unit of the Department.

2.2 Processing and extraction of plant materials

Preparation and extraction of plant materials was done at the Ethno-Survey Unit, Research and Training Department, Nigeria Natural Medicine Development Agency, Federal Ministry of Science and Technology, Lagos, Nigeria. Plant parts were dried at 38^oC in a solar sun dryer machine [12] locally designed and produced in 2013 by the Natural Medicine Development Agency, Lagos. The dried plant parts were separately ground to powder using an industrial grinding machine, weighed (Shimadzu electronic balance ATX224), labeled and stored in airtight bottles at room temperature. Hot water extraction of 80, 35, 35, 50, 50, and 50 g of powdered *E*. chlorantha, C. citratus, C. papaya, M. indica, C. longa and A. boonei respectively was done in a solute-solvent proportion of 1g: 15 mL. The plant material was homogenized and left to macerate at room temperature with intermittent shaking for 72 h to allow for complete extraction and obtain concentrated extracts. Each of the six separate mixtures were then filtered using a muslin cloth. The filtrate collected in six separate, labeled flasks were placed in an oven at 44°C to dry. The percentage yield for dried extract obtained was determined using the formula: (Weight of extract recovered / Initial weight of powdered sample) X 100. The extracts were then labeled and stored in airtight containers at room temperature of about 27°C till further analysis.

2.3 Physicochemical properties of the plant extracts

The extracts from the six plants were analyzed for some physicochemical parameters according to the methods described by the World Health Organization [13].

Description: This included evaluation of each extract by colour, shape, and special features including odour, texture and melting point.

Determination of pH range: The pH of different formulations in 1% w/v (1g: 100 ml) and 10 % w/v (10g: 100ml) of water soluble portions of each powdered extract was determined using standard glass electrode pH meter HI2209 manufactured in England.

Determination of moisture content: This was done by placing 1.0 g of each powdered extract in

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an accurately weighed moisture disc, allowed to dry at 105°C for 3 h in an oven, cooled in a desiccator for 30 min and weighed. The loss of weight was calculated as the content in percent of air-dried material.

Determination of total ash: Two grams of each powdered extract was placed separately in a previously ignited (350°C for 1 h) and tarred crucible accurately weighed. They were spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550°C for 5 h in a maffle furnace until it was white, indicating the absence of carbon. They were then cooled in a desiccator and weighed. Total ash content for each sample was calculated.

Determination of refractive index: Refractive index for each powdered extract was measured using Abbe Refractometer model number 10450 produced in England.

Solubility: Solubility of the extracts were checked by dissolving a sample of each powdered extract in water, chloroform, ethanol and diethyl ether.

2.4 Phytochemical screening

The six crude extracts were each qualitatively analyzed for the presence of important phytochemical constituents including steroids, saponins, tannins, terpenoids, alkaloids, flavonoids, phenols, carboxylic acids and quinones [14-16]. High-performance liquid chromatography quantification of phenols (gallic acid), alkaloids (atropine), flavonoid (rutin hydrate and quercetin) contents of the plant extracts was carried out [17-19].

The phenolic content of the extract was determined from 1.0 g of each plant powder dissolved in methanol. The analysis was performed on an HPLC system equipped with a C-18 column. Samples were each filtered prior to injection in the sample loop. Gallic acid, one of the major phenolic acids was used as standard [20-22], with methanol/acetonitrile (40:60) as the mobile phase. A calibration curve of the gallic acid standard was plotted from the HPLC and used to determine the amount of gallic acid (phenol) present in each sample. The separated gallic acid constituents were detected by a UV detector as they migrate down the column. The resulting detector signals were plotted against time as the chromatograms.

For the Alkaloids and Flavonoids determination, 1.25 mL each of the standard solutions were pipetted and mixed together in a sample bottle to form mixed standards of Atropine sulphate (alkaloid), Rutin hydrate and Quercetin (flavonoids) of 50 µg/mL concentration and made up to 5 mL with 1.25 mL of methanol. The solution was filtered using a milipore filter of 0.45 µm and 20 µL of the filtrate injected for HPLC analysis and the chromatograms obtained with aid of Chemstation software. One gram each of the pulverized samples were weighed and 20 mL of methanol added and sonicated for 5 min after which they were made up to 50 mL with methanol. These solutions were allowed to stand for 3h to enhance exhaustive extraction. The

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solutions were then filtered using whatman filter paper of 1100 mm. The filtrates were allowed to stand for 30 min and later filtered again using 0.45 micromolar (μ m) filter. Twenty microliter (μ L) of 1:10 dilution of the solutions were injected for HPLC analysis. The amount of each constituent present was detected using the internal standards in the machine. Calculation of concentrations/amount detected in each sample was done using the formula:

Peak Area (in units of mAU) of Sample X Concentration of Standards X Volume of Diluent Used / Peak Area (in units of mAU) of Standard X Weight of Sample

Where: mAU is the milli-Absorbance Units.

3.0 Results

3.1 Percentage yields of the extracted plant materials

The hot water extract used was able to extract components of the 6 plants studied in varied quantities (Table 1). *M. indica* stem bark had the highest yield with an extractive value of 81.48%, while the *Unripe fruits of C. papaya* had the lowest yield of 53.8%.

3.2 Physicochemical properties of extracts

Physicochemical properties of *C. papaya*, *C. longa*, *M. indica*, *E. chlorantha*, *A. boonei* and *C. citratus* extracts investigated are shown in Table 2. Total ash contents was as high as 10.21% in *C. citratus*, but 0.34% in *M. indica*. Compared to choloroform, ethanol and diethyl ether, water was a more universal solvent for the extracts with all

except *C. papaya* extract completely dissolving in it within minutes.

3.3 Phytochemical composition of extracts

Results obtained from the qualitative determination of phytochemicals of the hot water extracts of *A. boonei, C. papaya, C. citratus, C. longa, M. indica and E. chlorantha* showed saponin, alkaloid, flavonoid, phenol and lactones were present in all the extracts, while some other constituents including tannins, terpenoid, steroids and quinones quassinoids were found to be present in some of the extracts, but absent in others as shown in Table 3.

HPLC quantitative analysis showed the concentration of phytochemicals in the plant species varied considerably (Table 4). Of the extracts quantified, M. indica and A. boonei had the highest Rutin Hydrate content. While Quercetin was not detected in C. papaya, it was considerably present in A. boonei. The analysis also showed Alkaloid (Atropin sulphate) was higher in C. lounga and C. papaya. Low total gallic acid (phenol) was detected in all six extracts evaluated. Extracts had the same total peaks for flavonoids and alkaloid but this varied for gallic acid. Figure 1 shows the calibration curve of gallic acid constituent of the samples, Figure 2 shows HPLC chromatograms of the mixed standards (atropine sulphate, rutin and quercetin), while Figures 3 (a-f) shows the HPLC chromatograms of alkaloids (atropine sulphate) and flavonoid (rutin hydrate and quercetin) contents of hot water extract of C. papava, C.

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longa, A. boonei, M. indica, E. chlorantha and C. citratus.

Table 1: Percentage yield of plants extracted

Plant samples	Powder (g)	Water (mL)	Yield (g)	Yield (%)
Enantia chlorantha (Stem bark)	80	1200	57.45	71.81
Cymbopogon citratus (leaves)	35	525	21.28	60.80
Carica papaya (unripe fruits)	35	525	18.83	53.80
Mangifera indica (Stem bark)	50	750	40.74	81.48
Curcuma longa (roots)	50	750	30.65	61.30
Alstonia boonei (Stem bark)	50	750	36.13	72.26

Table 2: Physicochemical properties of plant extracts

S/No	Description	СР	CL	MI	EC	AB	CC
1	Colour	Clay-ash	Orange-	Creamy-	Bright	Pale yellow	Olive
			yellow	yellow	yellow		green
2	Odour	Sweet	Repulsive	Pleasant	Repulsive	Characteristic	Sweet
		fragrance		smell			fragrance
3	Shape	Lumpy	Shapeless	Shapeless	Shapeless	Shapeless	Shapeless
4	Texture	Rough	Fine	Fine	Fine	Fine	Fine
5	Melting point	Burn at	Burn at	Burn at	Burn at	Burn at 96°C	Burn at
		90°C	80°C	95°C	92°C		101°C
6	pН	7.43	8.02	6.24	6.81	6.41	6.85
7	Moisture (%)	18.27	22.77	9.96	9.62	3.85	10.00
8	Ash (%)	1.45	3.51	0.34	0.57	9.10	10.21
9	Refractive	1.34	1.34	1.34	1.34	1.34	1.34
	Index						
10	Solubility Tests	5					
a.	Water	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
b.	Choloroform	Insoluble	Partially	Partially	Insoluble	Insoluble	Partially
c.	Ethanol	Insoluble	Partially	Insoluble	Partially	Insoluble	Partially
d.	Diethyl ether	Insoluble	Soluble	Insoluble	Partially	Partially	Insoluble
CP: Carica papaya, CL: Curcuma longa, MI: Mangifera indica, EC: Enantia chlorantha, AB: Alstonia							
boonei, CC: Cymbopogon citratus							

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TESTS	СР	CL	MI	EC	AB	CC
Tannins	-	-	+	-	+	-
Saponin	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Terpenoid	-	+	+	+	+	-
Phenol	+	+	+	+	+	+
Steriods	-	+	-	-	+	-
Quinones	-	+	+	+	+	+
Quassinoids	-	+	-	+	+	+
Lactones	+	+	+	+	+	+

Table 3: Results from the qualitative determination of phytochemicals present in the extracts.

CP: *Carica papaya*, **CL:** *Curcuma longa*, **MI:** *Mangifera indica*, **EC:** *Enantia chlorantha*, **AB:** *Alstonia boonei*, **CC:** *Cymbopogon citratus*

Phyto-constituents		Extracts	Total	Component	Qty (mg/g)
			peak	peak no	
Flavonoids	Rutin Hydrate	СР	06	01	508.53
		CL	11	01	552.40
		MI	14	03	32982.80
		EC	13	01	541.40
		AB	21	02	2597.80
		CC	04	01	955.30
	Quercetin	CP	06	05	0.00
		CL	11	04	99.60
		MI	14	08	31.68
		EC	13	03	0.44
		AB	21	04	491.10
		CC	04	02	0.007
Alkaloid	Atropin	CP	06	06	1122.90
		CL	11	06	4382.20
		MI	14	09	0.13
		EC	13	04	96.52
		AB	21	05	0.001
		CC	04	03	44.29
		СР	71	59	0.002

Table 4: HPLC quantified chemical constituents present in plant extracts

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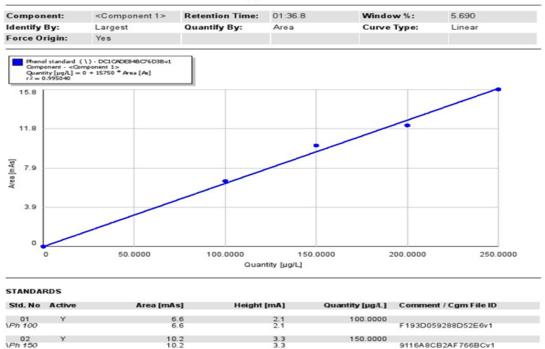
Phyto-constituents		Extracts	Total	Component	Qty (mg/g)
			peak	peak no	
Phenol	Gallic acid	CL	82	67	0.12
		MI	96	75	0.001
		EC	108	94	0.002
		AB	72	59	0.001
		CC	104	85	0.001

CP: Carica papaya, CL: Curcuma longa, MI: Mangifera indica, EC: Enantia chlorantha, AB: Alstonia

boonei, CC: Cymbopogon citratus

03 \Ph 200

04 \Ph 250



3.8

4.7

200.0000

250.0000

Component Calibration Report Phenol standard (\) - DC1CADE84BC76D3Bv1

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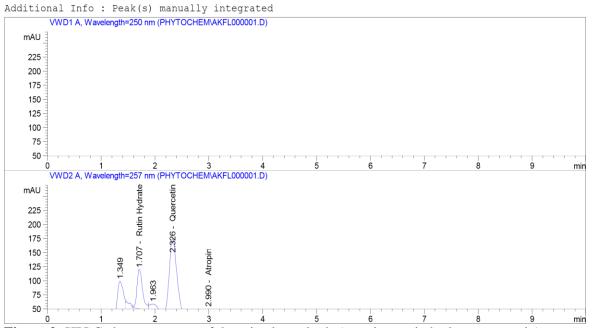
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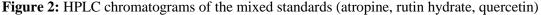
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Figure 1: HPLC standard calibration curve for phenol (gallic acid)



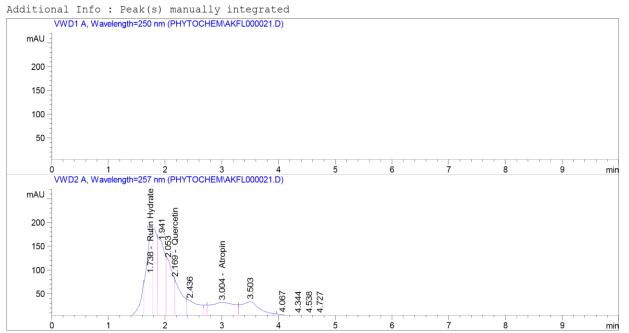


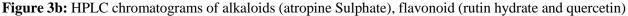
Additional Info : Peak(s) manually integrated VWD1 A, Wavelength=250 nm (PHYTOCHEM\AKFL000010.D) mAU min VWD2 A, Wavelength=257 nm (PHYTOCHEM\AKFL000010.D) - Rutin Hydrate mAU 2.949 - Atropin Λ min

Figure 3a: HPLC chromatograms of alkaloids (atropine Sulphate flavonoid (rutin hydrate and quercetin) content of water extract of *Carica papaya*

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content of water extract of Curcuma longa

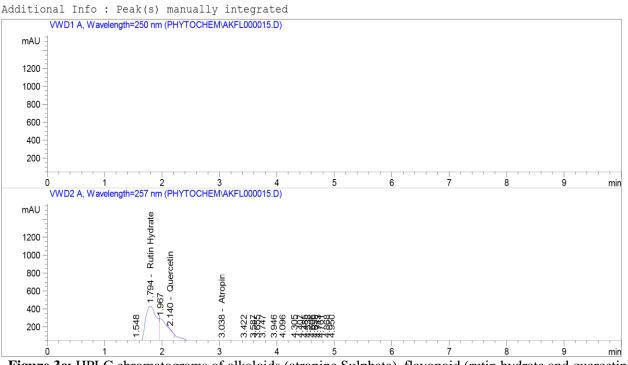


Figure 3c: HPLC chromatograms of alkaloids (atropine Sulphate), flavonoid (rutin hydrate and quercetin) content of water extract of *Alstonia boonei*

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Additional Info : Peak(s) manually integrated

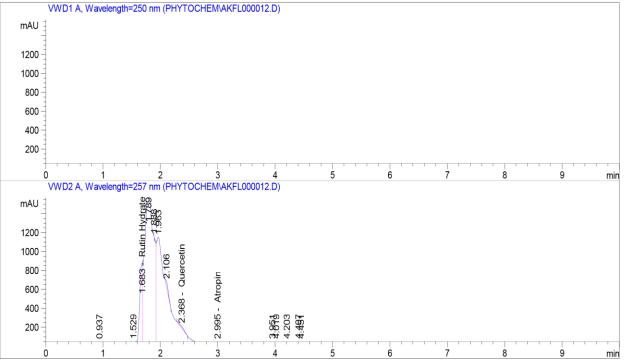


Figure 3d: HPLC chromatograms of alkaloids (atropine Sulphate), flavonoid (rutin hydrate and quercetin)

content of water extract of Mangifera indica

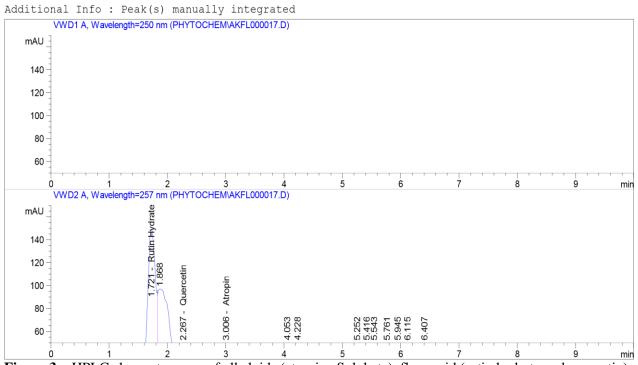


Figure 3e: HPLC chromatograms of alkaloids (atropine Sulphate), flavonoid (rutin hydrate and quercetin) content of water extract of *Enantia chlorantha*

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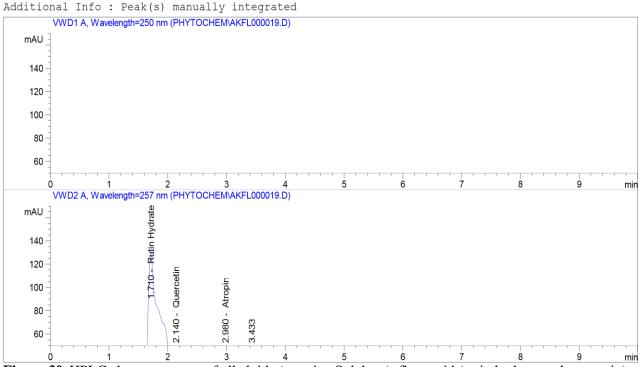


Figure 3f: HPLC chromatograms of alkaloids (atropine Sulphate), flavonoid (rutin hydrate and quercetin) content of water extract of *Cymbopogon citratus*

4.0 Discussion

The selected plants examined in this study are commonly used antimalarial options in Nigerian folk medicine [23-27]. Extraction is an important step in the processing of plant materials for the recovery of their bioactive constituents. In this work, just as in Seow *et al.*, [28], high yields of hot water extracts obtained is likely as a result of the thoroughly pulverized samples into powdered particle sizes pre-extraction. Extraction solvents have been reported to have an effect on the extraction yield and the content of bioactive compounds [29-31].

In accordance with Ibrahim *et al.* [32], extracts recovered from the samples varied in colour,

odour and texture, indicating difference in the composition of the extracts. Establishing identity and purity of medicinal plants is of utmost importance to establish their quality. Physicochemical criteria including moisture and ash content reported in this study indicated a safe level of 'cleanness' of the hot water extracts. Total ash values represents the total amount of material remaining after ignition. It gives information relative to the drugs adulteration with inorganic matter and also reflects the care taken in preservation, and the purity of crude [33].

Preliminary phytochemical analysis is an important process helpful in determining the chemical constituents of plant materials and

locating the source of pharmacologically active chemical compounds [28]. Phytochemicals revealed in this study corroborates other studies: *E. chlorantha* – alkaloids, saponins [34]; *C. citratus* – Alkaloids; *C. papaya* – alkaloids, phenol, flavonoids, saponins, steroids and tannins [35-36]; *M. indica* – phenols, terpenes, saponins, tannins, steroids [37]; Curcuma species – phenol [38-39]; *A. boonei* – saponin, alkaloids, tannins and steroids [40-42]. Work done so far have reported a large number of phytochemicals as important antimalarial-active substances that can play a role in the development of new antimalarial drugs [43].

According to Edeoga et al., [44], alkaloids, flavanoids, tannins and phenolic compounds are the most important of the bioactive compounds of plants. Phenols, alkaloids, and flavonoids have been previously reported to have antiplasmodial properties [45-50]. The present investigation quantified varying contents of phenol (gallic acid), alkaloid (atropine sulphate) and flavonoids (rutin hydrate and quercetin) of plant extracts studied. Fanta et al., [51] also reported HPLC analysis revealed the presence of quercetin, rutin, and gallic acid in different extracts of Carissa edulis Valh (Apocynaceae) Leaves. The phytoconstituents present in the plant extracts studied herein could also contribute to their antiplasmodial activity.

Total contents of some of the phytochemicals reported herein is in agreement with some previous studies. Chothani and Patel [52] reported low concentration of gallic acid quantified in fruit and leaves of *Careya arborea*. HPLC quantitative analyses of rutin in *M. indica* waterlily kernel extract was 2.5 mg/g dry weight [53]. These variations in the concentrations of phytochemicals reported by studies are due to a number of factors. Researchers studied different plant parts, carried out their evaluations with varying solvents and employed different techniques for quantification. Aqueous extraction of the plants was employed in this study based on how they are used locally in antimalarial therapies.

Alkaloids have been implicated in antimalarial activity of many plants [54]. A number of alkaloids target apicoplast, an organelle in the Plasmodium parasite, while others such as benzylisoquinoline alkaloids in *Cissampelos* mucronata, a plant belonging to the family Menispermaceae inhibits -protein synthesis in the parasite [55]. Phenols are strong antioxidants in human and plants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce atocopherol radicals and inhibit oxidases [56-57]. Gallic acid is a secondary metabolite present in most plants that are well known as powerful antioxidants, and the therapeutic applications for malaria treatment in vitro has been reported [58]. Flavonoids exhibit great antiplasmodial activity against different strains of the malaria parasite although the mechanism of antimalarial action is not clear. Some studies suggest that flavonoids impede the

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influx of myoinositol and L-glutamine in erythrocytes that are infected. Some flavonoids increase the level of oxidation of erythrocytes and inhibit protein synthesis in malaria parasites [55]. Furthermore, flavonoids are believed to inhibit fatty acid biosynthesis (FAS II) in Plasmodium [59]. Flavonoids demonstrated a marked and selective potentiating effect on the antiplasmodial activity of artemisinin [60].

5.0 Conclusion

This study identified and quantified the concentration of some antimalarial active phytoconstituents of C. papaya, C. longa, A. boonei, M. indica, E. chlorantha and C. citratus. Information presented in this study serves for the correct identification and characterization of each plant species studied. The findings indicate that these plants possess significant antimalarial active phytochemical constituents, therefore justifying their antimalarial use in ethnomedicine. Knowledge of these antimalarial active phytochemical constituents provided herein will guide their combinations for improved, more effective treatments against malaria. Further scientific attention is required to identify the specific classes and elucidate the chemical structures of the phytocontituents reported herein, to isolate and purify the antimalarial active compounds through bioactivity-guided assays, and to explore these important antimalarial-active substances for their wide applicability.

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