



Phytochemical Characterization and Cytotoxicity of *Carissa edulis*, *Azadirachta indica*, *Cassia siamea* and *Harrisonia abyssinica* from Masumbi Village, Siaya County-Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author OPL designed the study, wrote the protocol, identified the species of plant and wrote the first draft of the manuscript. Authors SSR, AMH and MC supervised the study. Author NSN managed the literature searches and analyses of the study performed by EPA probit analysis version 1.5 and author RC managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the phytochemicals and cytotoxicity of methanolic crude extracts of *Carissa edulis*, *Azadirachta indica*, *Cassia siamea* and *Harrisonia abyssinica*, from Masumbi village, Siaya county-Kenya.

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Study Design: Qualitative analysis of the phytochemicals and brine shrimp cytotoxicity assay were done using standard procedures.

Place and Duration of Study: Plant samples were collected from Masumbi village, Siaya county-Kenya. Botanical identification of the plants' samples was done in the Department of Biological Sciences, Egerton University and their voucher samples deposited in the departmental herbarium. Extraction, concentration, spotting and phytochemical analysis were carried out at the Department of Chemistry, Egerton University-Kenya.

Methodology: Chemical tests were done to determine the class of phytochemicals present in the methanolic crude extracts while brine shrimp cytotoxicity assay was carried out to predict the potential toxicity of the methanolic plant extracts.

Results: Extracts of four different species of plants were analyzed for their phytochemical composition. Alkaloids, flavonoids, glycosides, saponins, steroids, tannins and terpenoids were detected in the extracts. The methanolic crude extracts had LC₅₀ values between 198.498 and 450.022 µg/ml, and were categorized as being cytotoxic.

Conclusion: In the current study the methanolic crude extracts demonstrated LC₅₀ values less than 1000 µg/ml indicating that these plants extracts were cytotoxic.

Keywords: *Phytochemicals; cytotoxicity; brine shrimps; Carissa edulis; Azadirachta indica; Cassia siamea; Harrisonia abyssinica.*

1. INTRODUCTION

Herbal remedies as cheap alternatives to conventional medicine have contributed significantly to rural livelihoods. Apart from the traditional healers practicing herbal medicine, many people are involved in collecting and trading in medicinal plants. The World Health Organization (WHO) estimates that 80% of the world's population depends on medicinal plants for their primary health care [1-3]. The use of traditional medicine has been explored globally and is in use as folklore medicine among people of developing countries in their health care system as an alternative where conventional medicine is less available [3,4].

Natural products are an important source of new anti-microbial agents which are in the form of secondary metabolites [5]. Drugs derived from unmodified natural products or semi-synthetic drugs obtained from natural sources accounted for 78% of the new drugs approved by the United States Food and Drug Administration (FDA) between 1983 and 1994 [6]. This evidence contributes to the support and quantification of the importance of screening natural products. The bioactivities of plants have been investigated by a number of researchers worldwide giving very positive and promising results [5,7]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [8,9]. Scientific interest in medicinal plants has burgeoned in recent times due to increased

efficiency of new plant derived drugs and rising concerns about the side effects of modern medicinal substances, hence the need to look for new molecular structures as lead compounds from the plant kingdom [9].

The bio-activity of natural products is mainly associated with secondary metabolites, often elaborated for the plant defense. Some of these phytochemicals accidentally protect humans against pathogens. Phytochemicals are known to have several properties important to cells including; prophylactic properties, therapeutic properties, provide nutrition for normal cell health and repairs, inhibit carcinogens and act as antioxidants [10]. The phytochemical screening of plant materials to determine the presence of bioactive chemical constituents is thus vital in the knowledge of their therapeutic properties [11]. Despite the beneficial effects of phytochemicals, studies have established that they can be toxic [10,12]. These chemicals are produced as part of the plant's defense against pests and herbivores or to gain an advantage over competing agents. Therefore, medicinal plants are not always safe [12]. It is therefore appropriate to evaluate the potential toxicity of the plant extracts said to indicate useful antineoplastic activity. This will be used to create awareness of the safety of the plant extract(s) as an alternative medication or it be harnessed for its potential as antineoplastic leads [6,12,13].

Carissa edulis, is locally known in Luo community as "Ochuoga"; *Azadirachta indica*, locally known as "mwarubanne"; *Cassia siamea*,

locally known as “ndege”, and *Harrisonia abyssinica*, locally known as “pedo”, are widely used for their perceived medicinal value in treating various ailments (e.g. malaria, diabetes, eczema, leprosy, fever, dysentery, gastrointestinal, chest pain, haemorrhoids and snakebite). The phytochemical screening of the four target study plant materials to determine their bioactive chemical constituents is thus vital in unearthing their therapeutic properties [11]. The most important of these phytochemicals found in the medicinal plants are the alkaloids, tannins, saponins, steroids, terpenoids and flavonoids [14-16]. There has been significant increase in the use of medicinal plants due to their minimal side effects, availability and acceptability to the majority of the people. Therefore, the aim of this study was to investigate the phytochemical composition and cytotoxicity analysis of *Carissa edulis*, *Azadirachta indica*, *Cassia siamea*, and *Harrisonia abyssinica*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

The plants' roots, stems, and leaves for the study were collected from Masumbi village, Luo community, Siaya county-Kenya from January – February, 2015. The plants were identified through ethnobotanical approach by consulting the herbalist from the village. Roots of *Carissa edulis* were dug and young tap roots collected from several plants of the same species. This was repeated with *Harrisonia abyssinica* roots. Collection of other parts (leaves, and stem bark) for *Azadirachta indica* and *Cassia siamea* were collected by physically pruning the young leaves and slashing of stem bark using a knife respectively. For every plant that was sampled, its leaves, branches and / or fruits were carried for taxonomic identification to the Department of Biological Sciences, Egerton University, Kenya.

2.1.1 Identification of plant samples

The plants samples were taxonomically identified by a botanist at the Department of Biological Sciences, Egerton University – Njoro campus. The voucher numbers; V/SO/014/01EU, V/SO/014/02EU, V/SO/014/03EU and V/SO/014/04EU were prepared and assigned to the plant samples. The voucher samples were banked in the Department of Biological Sciences herbarium.

2.1.2 Preparation of plants' root bark, stem bark and leaves

The plants' root bark, stem bark and leave, were prepared by separating them from undesirable materials. They were cut into small pieces using a knife and air dried in the dark for four week to avoid decomposition of light sensitivity bioactive compound. This was done at room temperature to achieve a constant weight before being ground into coarse powder by the help of a mechanical mill (Thomas - Wiley Laboratory mill, Model 4). The dry samples of *Carissa edulis*, *Cassia siamea*, and *Azadirachta indica* and *Harrisonia abyssinica* were weighed using a balance. The dry weight before grinding and powdered weight of each sample were recorded. Sample A (*Carissa edulis* – root bark) weighed 555.173 g and 341.898 g after grinding. Sample B (*Azadirachta indica* – leaves) weighed 264.479 g and 159.019 g after grinding. Sample C (*Cassia siamea* - stem bark) weighed 737.315 g and 198.632 g after grinding and sample D (*Harrisonia abyssinica* – root bark) weighed 382.314 g and 158.084 g after grounded into powdered form.

2.2 Preparation of Plant Extracts

A batch of 100 g of *Carissa edulis* root bark powder (coarse granule), *Cassia siamea* stem bark powder (coarse granule), *Azadirachta indica* leaves powder (coarse granule), and *Harrisonia abyssinica* root bark powder (coarse granule) was macerated with intermittent shaking in 300 ml of methanol solvent for three days using 500 ml conical flasks. The preparation was left to stand at room temperature for 72 hours with intermittent shaking after every 12 hours. The four different extracts were decanted into different conical flasks. Fresh solvent (100 ml of methanol) was subsequently added into each soaked samples and agitated for ten minutes, and further decanted and mixed with previous extract of each sample. Filtration was carried out by (Whatmann filter paper No.1) by gravity into 1000 ml volumetric flasks.

2.2.1 Concentration of plants' extracts

Concentration of each sample extract was carried out using a rotary vacuum evaporator (BüCHI ROTAVAPOR R-205 V805, Flawil Switzerland). The temperature of the rotary vacuum evaporator was set to 60°C. The rotation was set at 50 revolutions per minute (rpm) and the pressure at 15 Hg (mercury). Each filtrate

obtained as a result of filtration was transferred into a 250 ml round bottom flask. The flask was connected to the rotary vacuum evaporator and the suction pump set to allow the process to start. The four volumes of filtrates were concentrated in a rotary vacuum evaporator (BüCHI ROTAVAPOR R-205 V805, Flawil Switzerland) in the Department of Chemistry, Egerton University. The concentrated preparations were transferred into 250 ml flat bottom beakers and covered with perforated aluminium foil. The four were left to air dry in at room temperature in a flow laminar hood till when dry. The dry weight of each concentrated sample was recorded, kept at 4°C for later use.

2.3 Phytochemical Analysis

The plant extracts were analysed to test the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. They were identified by characteristic colour changes. The results were reported as (-) for absence of detectable colouration, (+) for presence of slight colouration, (++) for presence of deep colouration, and (+++) for presence of very deep colouration. Each methanolic crude extract was prepared and transferred to test tubes labeled (A) for methanolic crude extract for *Carissa edulis*, (B) for methanolic crude extract *Azadirachta indica*, (C) for methanolic crude extract for *Cassia siamea* and (D) for methanolic crude extract for *Harrisonia abyssinica*.

2.3.1 Alkaloids

0.5 g of each methanolic crude extract was boiled for 15 minutes in 1M HCl (25.0 ml, 1%). Equal volumes of the resulting suspension were filtered into four test tubes (A, B, C and D). To each test tube, 5 drops of freshly prepared dragendorff's reagent was added. Formation of a precipitate was noticed in tubes B and C which indicated the presence of alkaloids while not noticed in tubes A and D.

2.3.2 Cardiac glycosides

Solutions of methanolic crude extracts were prepared by weighing 2 g of each extract and emulsified in 5 ml of water. About 5 ml of each methanolic crude extract was measured using a cylinder and transferred into different glass test tubes (A, B, C and D). 2 ml of glacial acetic acid containing one drop of ferric chloride solution was measured using a measuring cylinder and added to each methanol crude extract. About 1

ml of concentrated sulphuric acid (H_2SO_4) was underlaid slowly to each methanol crude extract. A brown ring at the interface or violet ring appearing below the ring or a greenish ring formed gradually throughout the thin layer was considered to be positive results for cardiac glycosides.

2.3.3 Flavonoids

5 ml of dilute aqueous ammonial solution was added to each portion of the aqueous filtrate of the methanolic crude extracts in test tubes (A, B, C and D). This was followed by addition of concentrated sulphuric acid (H_2SO_4) to every test tube. The disappearance of yellow colourations on standing was noticed in all the test tubes and was considered positive for flavonoids.

2.3.4 Saponins

2 g of each methanolic crude extracts was transferred into glass test-tubes (A, B, C and D). In each test tube, 20 ml of distilled water was added. Each preparation was boiled in a water bath and then filtered. 10 ml of each filtrate was mixed with 5 ml of distilled water and shaken vigorously. 3 drops of olive oil was added to each preparation and then shaken vigorously. Formations of emulsion were noticed in all test tubes and were considered positive for saponins.

2.3.5 Steroids

2 ml of acetic acid and about 2 ml of Sulphuric acid (H_2SO_4) was added to 0.5 g to each methanolic crude extract in separate glass test tubes (A, B, C and D). Changes in coloration from violet to green were noticed in all the test tubes and were considered positive for steroids.

2.3.6 Tannins

0.5 g of each methanolic crude extract was boiled in 20 ml of distilled water in different glass test-tubes (A, B, C and D). The preparations were filtered and a few drops of 0.1% ferric chloride added to each test tube using a dropper. A brown green colouration was noticed in tubes A, B, C and D. They were considered positive for tannins.

2.3.7 Terpenoids

About 5 ml of each methanolic crude extract was mixed with 2 ml of chloroform ($CHCl_3$) and 3 ml of concentrated sulphuric to form a layer in

different glass test tubes (A, B, C and D). Red colourations were noticed in tubes A, B and D and were considered positive for terpenoids. This was not noticed in tube C.

2.4 Brine Shrimp Lethality Toxicity Bio-assay

The brine shrimp (*Artemia salina*) are used in the laboratory bio-assay for toxicity through estimation of medium lethal concentration. Brine shrimp toxicity (BST) tests have been used as bench top simple bio-assay for the discovery and purification in research of natural products. It is used to measure the concentration required to kill half of a group of shrimp (LC₅₀).

Using analytical balance, 30 mg of each methanolic crude extract was weighed and transferred into four different universal bottles each containing 3ml of 1% aqueous dimethyl sulfoxide (DMSO). The concentration of 10 mg/ml for each extract was kept in the refrigerator at 4°C to be used for brine shrimp toxicity bio-assay. Artificial sea water was made by dissolving sea salt (18.0 g) in half litre of distilled water in a beaker. The brine shrimp eggs were then added to one side of the divided beaker with aluminium foil, and covered. The preparation was incubated at room temperatures, 25°C for 48 hours to allow the eggs to hatch. Concentrations (1000, 500, 250, 125, 62.5, and 31.25 µg/ml) of the methanol crude extracts of *Carissa edulis*, *Azadirachta indica*, *Cassia siamea* and *Harrisonia abyssinica* were prepared by a serial dilution in 1% aqueous dimethyl sulfoxide. Each concentration was tested in triplicate. Dimethyl sulfoxide 1% aqueous solution was used as a negative control. By the help of a sterile Pasteur pipette, brine shrimp larvae (life *nauplii*, 10) were picked and added to each microtitre plate wells containing

concentration of 10 mg/ml of each crude extract. The microtitre plate was covered with perforated aluminium foil and incubated at room temperature for 24 hours. The cultured microtitre plate was placed on the inverted microscope stage after incubation period and the *nauplii* viewed out. The numbers of dead and survived brine shrimp larvae were confirmed by the help of an inverted microscope, and then recorded. The viewing and the counting of the dead *nauplii* was repeated after 48 hours. The brine shrimp lethality was expressed in percentage by;

$$\frac{\text{Number of shrimps} - \text{life shrimp}}{\text{Number of shrimps}} \times 100$$

2.5 Statistical Analysis

The determination of the LC₅₀ at 95% confidence interval was determined from the count by Probit Analysis using EPA Computer Probit Analysis Program (Version 1.5) [7]. The criterion for toxicity for fractions was established as value > 1000 µg/ml (non toxic), ≥500≤1000 µg/ml (weak toxicity) and <500 µg/ml (toxic) [10,12,17].

3. RESULTS

3.1 Phytochemical Screening

The crude extracts were subjected to chemical group tests to identify various types of important chemical constituents present in the plant extracts (Table 1). Cardiac glycosides were absent in methanolic crude extracts of *Carissa edulis*, *Cassia siamea*, and *Harrisonia abyssinica*. Terpenoids were observed to be absent in methanolic crude extracts of *Cassia siamea*, while alkaloid was absent in extracts of *Carissa edulis* and *Harrisonia abyssinica* respectively. Steroids, saponins, tannins and flavonoids were present in all methanolic crude extracts.

Table 1. Results of phytochemical screening

Methanolic extracts	Phytochemicals						
	Steroids	Saponins	Glycosides	Tannins	Flavonoids	Terpenoids	Alkaloids
<i>C. edulis</i> root bark	+	+	-	+++	+	++	-
<i>A. indica</i> leaves	++	+	+	+++	+++	+	++
<i>C. siamea</i> stem bark	+	+	-	++	+	-	+
<i>H. abyssinica</i> root bark	++	+	-	+	+	+	-

Presence of slight colouration (+), presence of deep colouration (++) , presence of very deep colouration (+++) and absence of colouration (-)

3.2 Brine Shrimp Cytotoxicity Bioassay

Cassia siamea stem bark methanolic extract demonstrate cytotoxicity activity at LC₅₀ 450.022. *Carissa edulis* root bark methanolic extracts, *Azadirachta indica* leaves methanolic extract and *Harrisonia abyssinica* root bark methanolic were equally cytotoxic at LC₅₀ 255.606, 233.061 and 198.498 respectively (Table 2).

4. DISCUSSION

4.1 Phytochemical Results

In this study, the following phytochemicals were detected: alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. They are normally produced by plants as an evolutionary adaptation to harsh environment or in response to attack by other organisms [11]. Tannins were detected in all the methanolic extracts. They have physiological role by acting as antioxidants through free radical scavenging activity, chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation. They also inhibit tumor growth by inducing apoptosis [18]. They exhibit anti-microbial activity by complexing proteins such as adhesins, substrates, cell wall and cell membrane proteins, hence inactivating microbial adhesion which is the first step in establishment of infections, and also causing cell wall/membrane disruption [19]. They also inactivate microbial enzymes and cell envelope transport proteins by processes that may involve reaction with sulfhydryl groups or through non-specific interaction with the proteins [20,21]. Tannins have endocrine role, and they function by interacting with estrogen receptors. They are also antiinflammatory, molluscicidal and hence important in the control of schistosomiasis [22]. They also have anti-diarrheal, anti-septic, anti-viral, anti-fungal, anti-parasitic, anti-irritant properties, used in curbing hemorrhage, in

wound healing, and improving vascular health by suppressing peptides that harden arteries [23].

Flavonoids were also detected and they exert their roles as chain breaking anti-oxidants, and by preventing oxidation of low-density lipoprotein by macrophages and metal ions like copper. This reduces the oxidative stress [11]. They also act as 'nature's biological modifiers', as anti-allergens, anti-inflammatory, and induces phase two enzymes that eliminate mutagens and carcinogens. They also act as anti-microbial by complexing extracellular and soluble proteins, and bacteria cell wall [11]. Saponins were also detected in all the methanolic extracts. They boost respiratory system as expectorant, and hence activity against cough. This could perhaps justify the already traditionally established function of the plant in the treatment and management of dry coughs. They also have anti-protozoa with cholesterol in the protozoal cell membranes causing cell lyses. Also, saponins functions as vaccine adjuvant, as anti-inflammatory, emetics, anti-viral, antifungal, insecticidal, molluscicidal, piscidal and as anti-bacterial by inhibiting colonization and boosting the immunity [24]. Saponins increase the blood flow of the coronary arteries, prevent platelet aggregation and decrease the consumption of oxygen by heart muscles [25]. They also have anti-edema, anti-tussive, purgative, antihypercholesterol, and hypotensive, cardiac depressant and immuno-regulatory properties [23]. Terpenoids were also detected in all fractions except hexane fraction. They exert their roles as antibacteria, anti-amoebic, anti-fungi, anti-viral, antiprotozoan, anti-allergens, as immune boosters and as antineoplasia. The mechanism of action is speculated to involve membrane disruption by these lipophilic compounds [11,20]. This may involve perturbation of the lipid fraction of bacterial plasma membranes, altering membrane permeability hence causing leakage of intracellular materials.

Table 2. Cytotoxicity results

Methanolic crude extracts	LC ₅₀ (µg/ml)	95% CI	
		Upper limit	Lower limit
<i>C. edulis</i> root bark	255.606	212.354	303.185
<i>A. indica</i> leaves	233.061	203.404	266.828
<i>C. siamea</i> stem bark	450.022	294.099	692.249
<i>H. abyssinica</i> root bark	198.498	161.312	244.669

LC₅₀- means the concentration that kills 50 % of the population (in µg/ml); 95% confidence interval gives the fiducial limit; 1% DMSO solution was used as negative control and caused no mortality to brine shrimps;

CI Represent confidence interval

4.1.1 Phytochemical results of *Carissa edulis*

The phytochemical results of *Carissa edulis*, methanolic crude extract of root bark was confirmed to be in consistent with the finding of [26] who confirmed the presence of steroids, terpenes, tannins, and flavonoids. This was also in tandem with [27] who established the presence of saponins in his extraction. This work was also found to be in line with [28] who used ethanol extracts of *Carissa edulis* Vahl root bark in rats and mice to establish its acute toxicity. Ngulde et al [28] established the presence of saponins, flavonoids and terpenoids while the steroids, cardiac glycosides, and tannins were not detected.

4.1.2 Phytochemical results of *Azadirachta indica* A. Juss

The phytochemical results of *Azadirachta indica* A. Juss methanolic crude extract of leaves was found to be consistent with several investigators. Phytochemical analysis and antimicrobial activity of *Azadirachta indica* A. Juss done by [29] discovered similar results of alkaloids, steroids, saponins and flavonoids in acetone extracts of *Azadirachta indica* A. Juss. The result of this research demonstrated the same similarity with both ethanol and methanolic extracts where all the bioactive compounds (alkaloids, steroids, saponins, tannins, cardiac glycosides, flavonoids and terpenoids [30] discovered on a study on comparative study of the phytochemical properties of *Jatropha curcas* and *Azadirachta indica* A. Juss extracts. Cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids were phytochemicals identified in the foliage of *Azadirachta indica* A. Juss. Similar observations were made by [31] where the presence of glycosides and triterpene were present in the methanolic extract of *Azadirachta indica* A. Juss leaf.

4.1.3 Phytochemical results of *Cassia siamea* Lam

The phytochemical results of *Cassia siamea* Lam crude extract of stem bark was in consistent with [32] that used different organic solvents in carrying out phytochemical analysis. The presence of alkaloids, tannins, flavonoids, terpenoids, steroids and cardiac glycosides in ethanol extract, methanol extract and ethyl acetate extract was in agreement with the discovery of this research. The presence of saponins in methanol extract and ethyl acetate

extract of *Cassia siamea* Lam has further supported the discovery of this research. In this research terpenoids were not phytochemically demonstrated in methanolic extract of *Cassia siamea* Lam. The presence of alkaloids, tannins, saponins and flavonoids from methanolic extraction of this research has been supported by [33].

4.1.4 Phytochemical results of *Harrisonia abyssinica* Oliv.

The phytochemical results of *Harrisonia abyssinica* Oliv methanolic crude extract of root bark of this research was in line with [34] who isolated steroids in their methanolic crude extract of *Harrisonia abyssinica* species of Tanzania. Other findings of chemical groups were not demonstrated in their work. A further comparison has been observed in [35] for the discovery of terpenes in his phytochemistry test. The same results have been observed with [36] in their methanolic crude extracts of *Harrisonia abyssinica* for traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan coast [37].

4.2 Brine Shrimp Cytotoxicity Bio-assay

Studies have showed that there is a positive correlation between the lethality to brine shrimp and corresponding oral lethal dose. Therefore the bioassay present a useful alternative model for predicting the oral acute toxicity of plant extract as well as a model for bioassay-guided fractionation of active cytotoxic and antimalarial agent [38]. According to Ayo et al. [17] LC₅₀ value less than 1000 µg/ml is considered toxic. All tested extract had a LC₅₀ value less than 1000 µg/ml, and hence were toxic.

The brine shrimp test was considered a rapid inexpensive and samples bioassay for testing plant extracts lethal concentration which correlated with the cytotoxicity properties of the plants. In this study each of the methanolic crude extracts showed different mortality rates at different concentrations. The percentage mortality increased with an increase in concentration of each methanolic crude extract. The variation in the results could have been due to different in the amount and kind of bioactive molecules present in the four methanolic crude extracts (e.g. steroids, saponins, glycosides, tannins, flavonoids, terpenoids and alkaloids). The present study was supported by [39] whose study was done on the phytochemical screening

& cytotoxicity potential of ethanolic extracts of *Senna siamea* leaves and established the presence of tannins, steroids and glycosides. The current study differed on organic solvent. This study was supported by [40] in her work on *In vitro* antiplasmodial activity and toxicity assessment of plant extracts used in traditional malaria therapy in the Lake Victoria region. Her assessment of cytotoxicity using shrimps on methanolic stem bark and root bark extracts gave inactive and moderately active results respectively. This study slightly differed with her work on *Carissa edulis* (Forssk) Vahl on stem bark analysis but gave similar outcome with root bark which showed presence of plant cytotoxic components of this part of the plant. The study was also supported by [41] in their work on *Azadirachta indica* acetone leaf extracts which gave high lethality of the brine shrimps. The organic solvent used for extraction was different from what was used in this study but the outcome supported the presence of potent cytotoxic components found in *Azadirachta indica* leaves.

5. CONCLUSIONS

The phytochemical testing demonstrated the presence of steroids, saponins, tannins, flavonoids, terpenoids, alkaloids and cardiac glycosides in the plant extracts. These bioactive compounds are normally associated with bioactivity of plant extracts. In the current study the methanolic crude extracts demonstrated LC₅₀ values less than 1000 µg/ml indicating that these plants may not make safe antimalarial drugs as their test concentration was below the acceptable toxicity limits. This calls for dose adjustment among the community using the crude extracts of the plants for the treatment of malaria and the chemical isolation of the bioactive compound responsible for the cytotoxicity activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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