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Antiplasmodial and cytotoxic activities of selected medicinal plants in Western Kenya

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ABSTRACT

Malaria is a potentially lethal illness that is transmitted through the bite of mosquitoes and is caused by a parasitic organism. Individuals who are pregnant, small children, and the elderly are considered to be especially susceptible to the condition. The presently accessible antimalarial medications are associated with adverse effects and substantial expenses, particularly in regions with little financial resources. Medicinal plants present a viable option owing to their reduced incidence of adverse effects, decreased financial burden, and convenient availability. Nevertheless, the existing body of research pertaining to the utilization of medicinal plants for the treatment of malaria is somewhat restricted. The objective of this study was to investigate the cytotoxic and antiplasmodial characteristics of various medicinal plants found in Kakamega County, located in Western Kenya. *In vitro* studies were conducted using organic and aqueous extracts derived from the plants. The extracts were used to assess both chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Additionally, an evaluation was conducted to determine the safety and cytotoxicity of the plant extracts. The plant extracts obtained from dichloromethane exhibited the lowest yield, whereas the water extracts yielded the highest proportion. Plants belonging to the Leguminosae family, namely *Senna didmobotrya* and *Senna occidentalis*, exhibited the most substantial productivity when subjected to water and methanol solvents. Several plant extracts shown significant antiplasmodial action against both the chloroquine-sensitive and chloroquine-resistant strains of the malaria parasite. A number of extracts had a moderate level of antiplasmodial action, but a small subset exhibited poor or negligible activity. Of the three examined extract types (water, methanol, and dichloromethane), it was shown that the methanol extracts exhibited the greatest prevalence of plants with significant antiplasmodial activity against the chloroquine-sensitive strain. The majority of the methanol extracts exhibited moderate action against both strains, although a minority shown low or no activity. The extracts of dichloromethane also exhibited a variety of antiplasmodial properties. In general, the study unveiled the therapeutic potential of medicinal plants found in Kakamega County, located in Western Kenya, for the treatment of malaria. The efficacy of these plants in inhibiting the growth of both chloroquine-sensitive and chloroquine-resistant strains of the malaria parasite was demonstrated. The results of this study offer significant insights for stakeholders who are interested in investigating the potential of herbal remedies as an alternate strategy for the treatment of malaria.

Keywords: Medicinal plants, Antiplasmodial, Cytotoxic, Extracts.

INTRODUCTION

There are five distinct species of Plasmodium that have been discovered as the primary causative agents of malaria. These species are *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, Plasmodium knowlesi*, and *Plasmodium falciparum* [1, 2]. *P. falciparum* remains the most perilous among these species. Malaria is responsible for significant levels of both mortality and morbidity in regions such as sub-Saharan Africa, Asia, and Latin America [3].

According to available data, around 1.2 billion individuals are deemed to possess a heightened susceptibility to malaria, constituting a subset of the projected 3 billion individuals who are anticipated to encounter the disease year [4]. According to recent statistical data [4-6], malaria remains the predominant ailment in tropical regions, with a staggering number of over 219 million symptomatic cases and an estimated 619,000 fatalities. Pregnant women and children under the age of five are particularly vulnerable, with Africa exhibiting the greatest incidence rates and mortality rates in both categories [1].

Following the transmission of Plasmodium parasites by the bite of a female Anopheles mosquito, these parasites traverse the circulatory system, undergo growth, and then undergo multiplication inside the liver. It is within the liver that the manifestation of malaria-specific symptoms occurs, including but not

limited to headache, weakness, discomfort, stomach troubles, fever, and nausea [3]. Untreated cases of malaria for an extended period of time can result in several complications, including but not limited to severe anemia, damage to brain tissue, renal failure, pulmonary edema, and jaundice [7]. Despite significant advancements in the fight against malaria, there is growing concern regarding the emergence of drug-resistant strains of P. falciparum, specifically in relation to artemisinin combination treatments (ACTs) [4].

Chemotherapy remains the primary method of controlling malaria [8], however there are solo antimalarial medicines as well as combinations with other therapies that are also available [9, 10]. Non-artemisinin antimalarial drugs exhibit certain limitations, including reduced effectiveness [11], elevated expenses, and the possibility of inducing toxicity, leading to restricted adherence among patients. The primary challenge confronting antimalarial treatments is the emergence of resistance, a widespread phenomenon attributed to the chemical similarity among many of these drugs [12]. The phenomenon of cross resistance between 4-aminoquinolines, specifically chloroquine (CQ) and amodiaquine (AQ), serves as an illustrative instance of this occurrence [13]. Chloroquine (CQ) was widely utilized as an antimalarial drug because to its efficacy and cost-effectiveness prior to the emergence of resistance [14,15]. The utilization of artemisinin and its semi-synthetic derivatives, known as artemisinin-based combination therapy (ACTs), as the primary treatment for malaria caused by P. falciparum infection [17,18], was based on their superior efficacy compared to quinine [16]. The primary recommendation in most tropical countries is to utilize artemisinin-based combination therapies (ACTs) as the initial approach for treating malaria. However, the presence of artemisinin-resistant Plasmodium parasites remains a significant issue in numerous regions worldwide, necessitating more investigation into the development of alternative treatments that are both more secure and effective.

The utilization of botanical remedies has been well endorsed as a means to mitigate the development of resistance in Plasmodium parasites towards currently accessible antimalarial drugs [19, 20]. Herbal medicine has been a prominent therapeutic approach for the management of malaria for numerous centuries. The initial antimalarial drug, known as quinine, was identified within the bark of the Cinchona tree, which belongs to the botanical family Rubiaceae. During the early period of 1632, the treatment of human malaria involved the administration of a Cinchona bark infusion [2]. Artemisia annua, an ancient medicinal herb, was discovered in China, leading to the extraction of artemisinin from this plant [21]. The investigation of new antimalarial drugs derived from natural sources, which continues to be a viable approach in the management of malaria, has raised scientific concerns over the potential of herbal medicine. Herbal medications are seeing increasing popularity in developed and developing nations, mostly due to their accessibility, acceptance, affordability, and availability [12].

Between the years 2001 and 2017, a comprehensive examination revealed that a collective sum of 2000 chemicals originating from plants had efficacy in combating malaria caused by the Plasmodium falciparum parasite [9]. Asteraceae species are frequently employed in Kenya for the treatment of malaria, constituting around 15% of the total plant species exploited [22]. Within the realm of plant taxonomy, a comprehensive analysis reveals that among the 60 distinct plant families, a notable assemblage of more than 150 plant species derived from these families have been employed for the purpose of malaria treatment across various populations situated within the geographical boundaries of Kenya. The antimalarial properties exhibited by herbal plants can be attributed to the presence of many bioactive chemicals inside them [23]. The substances encompassed in this category consist of alkaloids, quinones, coumarins, flavonoids, terpenes, limonoids, sesquiterpenes, chalcones, and triterpenoids [19]. The concentration of these compounds is influenced by the geographical location of the herbal plants. It is imperative to ascertain the antiplasmodial and cytotoxic characteristics of diverse medicinal plants based on their geographical distribution. Consequently, our research conducted an identification of diverse medicinal plants throughout Kakamega County that exhibited antiplasmodial and cytotoxic characteristics.

METHOD AND MATERIALS

Study area and design. This study employed an experimental laboratory study design. A total of 16 medicinal herbs were meticulously collected, subjected to identification, and subsequently classified according to their taxonomic categorization. Subsequently, the foliage of the plant was extracted, followed by an assessment of its cytotoxic and antiplasmodial properties. This investigation was conducted in the region of Kakamega. This county exhibits high temperatures and prolonged wet seasons, both of which create optimal conditions for the proliferation of mosquitoes. Kakamega County is encompassed by a tropical rainforest. The county consists of a total of 12 sub-counties, among which Shinyalu and Malava sub-counties are notable for harboring the largest forested areas. Consequently, these sub-counties are home to a significant number of traditional medicine practitioners and those engaged in the utilization of medicinal plants. The incidence rate of malaria in Kakamega County is reported to be 33%. Evidence suggests that residents of Kakamega County employed herbal treatments as a means of treating malaria [24].

Collection of plants. The medicinal plants were obtained from Kakamega County with the assistance of local herbalists. To ensure their accuracy, a taxonomist at the Kenyan botanical museum in Nairobi, where the specimens were held, verified the legitimacy of the plants by considering their ethnomedical usage. The plant parts were subjected to air-drying in a chamber with adequate ventilation for a duration of one week at ambient temperature. Following this, the dried plant parts were further pulverized into a coarse powder. Subsequently, the granules that were matched were carefully placed into transparent plastic containers and strategically arranged on arid laboratory shelves to facilitate the extraction process.

Extraction methods. The plant material, which had been dried and transformed into powder form, underwent a maceration process for a duration of three days at ambient temperature. This process was carried out using 10 liters of each solvent, both individually and concurrently. The solvents employed were hexane, methanol, and a mixture consisting of methanol and methylene chloride in a 1:1 ratio. The solution will undergo filtration using Whatman paper prior to concentration using a Rotavapor system (BÜCHI Labortechnik AG, Switzerland) until complete evaporation and the formation of viscous residues. The crude extracts obtained will be stored at a temperature of 4°C for subsequent utilization.

Aqueous extraction. The method described in [25] was used to obtain aqueous extracts. After adding 100 grams of the necessary powdered plant ingredients to clean, 1000-milliliter beakers with labels, 600 milliliters of distilled water was added. To assist extraction, the mixes were carefully put into beakers and covered with aluminum foil. The beakers were then heated at 80°C for 1.5 hours. The mixes were freeze-dried for 48 hours after Whatman's No. 1 filter sheets. The

percentage yields of dry and lyophilized samples were transferred to sterile, pre-weighed universal bottles. These bottles were securely frozen at -20°C till use.

Organic extraction. Following reference [25], dichloromethane and methanol were used for consecutive extraction. In summary, 600 ml beakers with a total volume capacity of 1000 ml macerated the botanical components for 48 hours at 25°C. The mixtures were vacuum reduced at 40°C using a rotary evaporator after filtration with double-layer Whatman's number one filter papers. After dichloromethane extraction, the leftovers will be macerated in 1000 ml beakers with 600 ml methanol. The liquid was filtered and condensed under reduced pressure in a rotating evaporator at 56°C. The anticipated extract volumes were placed in weighed sterile glass containers. The bottles were then frozen at -20°C until use.

Anti-plasmodial assays

Preparation of stock drugs. Stock solutions were prepared in a sterile laminar flow hood using deionized water and 0.22 m membrane filters to provide aseptic conditions. The water-insoluble extracts were dissolved in a solution containing 0.02% dimethyl sulphoxide (DMSO) and subsequently diluted with sterile deionized water to obtain the necessary concentrations [12]. The stock drugs were stored at a temperature of -20°C.

Culture of malaria parasites. The Kenya Medical Research Institute (KEMRI) Malaria Laboratories in Nairobi provided this study with P. falciparum strains that were resistant to antimalarials. The culture medium was produced by adding 25 mM N-2 hydroxyethylpiperazine-N-2, 10% human serum, 25 mM NaHCO3, 50 mg/ml gentamycin (0.5 ml), and 25 mM HEPES to RPMI 1640. The experiment used 28-day-old human type O+ erythrocytes. The cultures were kept at 37°C in 3% CO_2, 5% O_2, and 92% N_2.

In Vitro antiplasmodial assays. The extracts' ability to prevent [G-3H]-hypoxanthine incorporation into the malaria parasite was assessed using an *In Vitro* semi-automated microdilution test [26]. The experiment involved uniformly distributing 25 µL aliquots of culture media over 96-well flat-bottomed microculture plates, except for row B. The experiment diluted each sample two-fold with a titertek motorized hand diluter. This study established a 64-fold concentration range from 200 g/ml (100%) to 3.125 g/ml (1.56%). The 50-liter test solutions were carefully poured three times onto row B. All rows except rows R9-R12, which included non-parasitized erythrocytes, were subjected to suspensions (200 l, 1.5% v/v) of parasitized erythrocytes (0.4% parasitemia) in the culture media. The plates were incubated at 37°C, 3% CO2, 5% O2, and 92% N2. After 48 hours, the plates were incubated for 18 hours. After that, 25 liters of culture media with 0.5 Curie of [G-3H]-hypoxanthine was added to each well. The contents of each plate were collected, cleaned with distilled water, and dried on glass fiber mats. Then, liquid scintillation was used to measure each sample's radiation. The beta counter data was uploaded to Oracle and imported into Excel 2016 to calculate IC50 values. Interpolating the logarithmic transformation of concentration and counts per minute (CPM) data yielded the medicine concentration (IC50) that inhibits [G-3H]-hypoxanthine absorption by 50%:

$$IC_{50} = \text{antilog}\left(\text{Log}\, X_1 + \frac{(\text{Log}\, Y_{50} - \text{Log}\, Y_1)(\text{Log}\, X_2 - \text{Log}\, X_1)}{(\text{Log}\, Y_2 - \text{Log}\, Y_1)}\right)$$

The concentrations and CPM values for data points above and below the CPM midpoints are denoted as X1, Y1, X2, and Y2, respectively. Additionally, Y50 represents the CPM value that lies midway between parasitized and non-parasitized control cultures.

In Vitro cytotoxicity assays. Three-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) fast calorimetric tests assessed the extracts' cytotoxicity [25]. A mitochondrial dehydrogenase enzyme splits the tetrazolium ring of the light yellow MTT molecule in the experiment. This reaction forms dark blue formazan crystals that cannot cross the cell membrane. Formazan synthesis in non-diseased cells correlated with viable cell count [25]. The African Green Monkey's renal tissue-derived Vero (E196) cell line was grown in Eagle's Minimum Essential Media (MEM) with 10% fetal bovine serum. A 100 µL solution evenly distributed 20,000 cells in 96-well plates. After 24 hours at 37°C in 5% CO2, the cell culture reached 90% confluence. After 24 hours of incubation, 1000 g/ml control and test extracts were added. Next, 100 g/ml chloroquine was added as a positive control. After exposing cells to test extracts for 48 hours, 10 µl of MTT assay reagent (10 mg/ml) was added to each well. The cells were incubated for 4 hours under the same conditions. The media was removed from the plate, and 100 liters of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plates were then spectrophotometrically analyzed. The optical density (OD) per well for each drug concentration was measured to record the data.

Data management and analysis. Quantitative data was tabulated using MS Excel 2016 and afterwards exported to IBM SPSS software version 25. The data was represented using the standard error of the mean (SEM) and subjected to descriptive statistical analysis. The evaluation of acute toxicity and cytotoxicity was conducted by determining the CC50 values, whilst the assessment of *In Vitro* antiplasmodial activity was performed by determining the IC50 values. The logarithmic transformation was employed in the computation of toxicity and cytotoxicity.

Ethical considerations. Ethical approval for this study (Protocol: MMUST/IERC/015/2021) was given by the Institutional Ethical Review Committee of Masinde Muliro University of Science and Technology. The study has been granted clearance by the National Commission for Science, Technology, and Innovation (NACOSTI) in Nairobi, Kenya, with reference number NACOSTI/P/21/14450. In order to ensure confidentiality, the data was safeguarded through the use of password protection, with exclusive access granted solely to the principal investigator.

RESULTS

Percentage plant yields. Three solvents water, methanol and dichloromethane were utilized in the extraction of the selected plants. Overall, water gave the highest percentage yield of the plant extracts. Dichloromethane gave the lowest percentage yield of the three solvents. In the case of the selected plants, from plants of the *Leguminosae* family produced the highest yield *Senna didmobotrya* (12.6%) and *Senna occidentalis* (11.6%) in water. Additionally, *Senna didmobotrya* gave the highest percentage yield in methanol solvent of 6.3% followed by *Lantana trifolia L* 6.2%. *Trichilia emetic* and *Spathodea campanulata* produced the highest percentage yield when dichloromethane was the solvent of 2.7% and 2.4% respectively. The summary of the overall percentage yields from the three solvent is shown in Figure 1.

A total of 16 plants were selected for *In Vitro* antiplasmodial and cytotoxic analyses. The summary of botanical and local identification as well as the percentage yields per solvent is summarized in Table 1. Majority of the plants utilized in Kakamega County for malaria treatment are from the family *Lamiaceae*. These included *Ajuga integrifolia*, *Fuerstia africana*, *Ocimum kilimandscharicum* and *Rotheca myricoides*. Plants in this family gave fairly high percentage yields across all the solvents with water being the better solvent in terms of yield. Conversely, plants in the families *Compositae* and *Canellaceae Acmella caulirhiza* and *Warbugia ugandensis* respectively gave the least percentage yields across the three solvents.

In Vitro antiplasmodial activities of selected plants

The *In Vitro* antiplasmodial activities of the selected plants were determined. This study adopted the interpretation of antiplasmodial activities from previous studies as follows: high activity (IC_{50} of ≤10 µg/ml), moderate activity (IC_{50} of 11-49.9 µg/ml), low activity (IC_{50} of 50-100 µg/ml), and inactive (IC_{50} of ≥100 µg/ml)(Gathirwa *et al*., 2011; Waiganjo *et al*., 2020). The *In Vitro* antiplasmodial activities were assessed based on the three solvents water (aqueous), methanol and dichloromethane (organic). The plant extracts were tested against both wild-type chloroquine sensitive 3D7 and mutant chloroquine resistant W2 *P. falciparum* strains.

Aqueous plant extracts of *Acmella caulirhiza*, *Rotheca myricoides* and *Ficus thonningii* exhibited high antiplasmodial activity IC_{50} of ≤10 µg/ml against 3D7 *P. falciparum* strains whilst *Trichilia emetic, Fuerstia africana* and *Ficus thonningii* showed high antiplasmodial activity against W2 *P. falciparum* strains. Antiplasmodial activities of selected plants against 3D7 *P. falciparum* strains are summarized on Table 2 and those of W2 are summarized on Table 3. Most of the aqueous plant extracts showed moderate antiplasmodial activity (IC_{50} of 11-49.9 µg/ml) against both 3D7 and W2 *P. falciparum* strains. Ten of the sixteen selected plants exhibited moderate antiplasmodial activity against 3D7. These include *Lantana trifolia*, *Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Fuerstia africana*, and *Ocimum kilimandscharicum*. Nine out of 16 showed moderate antiplasmodial activity against W2. They include: *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Senna didmobotrya*, *Senna occidentalis*, *Clausena anisata*, *Rotheca myricoides*, and *Ocimum kilimandscharicum*.

Two plants exhibited low antiplasmodial *In Vitro* activity against 3D7 strains. These plants are: *Carissa edulis* and *Warbugia ugandensis*. Low antiplasmodial activity against W2 was only exhibited by *Spathodea campanulata*. *Senna occidentalis* was the only plant among the selected that was inactive (IC_{50} of ≥100 µg/ml) against 3D7 strains. On the other hand, *Zanthoxylum gilletii, Warbugia ugandensis* and *Ajuga integrifolia* were inactive against W2 strains.

Organic extracts were done using methanol and dichloromethane. Methanol extracts of 6 out of the 16 selected plants *Acmella caulirhiza*, *Lantana trifolia*, *Ajuga integrifolia, Spathodea campanulata, Clausena anisata* and *Fuerstia Africana* produced high antiplasmodial activity against 3D7 strains. *Lantana trifolia* methanol was the only based plant extracts that exhibited high antiplasmodial activity against the mutant strain W2. Similarly, 6 of the 16 selected methanol extracted plants showed moderate antiplasmodial activity against 3D7. These plants include: *Solanum incanum, Zanthoxylum gilletii*, *Rotheca myricoides*, *Senna didmobotrya Ocimum kilimandscharicum*, and *Ficus thonningii*. Additionally, majority of the methanol extracted plants 11 out of 16 produced moderate antiplasmodial activity against W2. These plants are: *Acmella caulirhiza*, *Carissa edulis*, *Solanum incanum*, *Senna didmobotrya*, *Senna occidentalis*, *Clausena anisata*, *Trichilia emetic*, *Rotheca myricoides*, *Fuerstia africana*, *Ocimum kilimandscharicum* and *Ficus thonningii*.

Low antiplasmodial activity against 3D7 was exhibited by the following three methanol extracted plants; *Carissa edulis*, *Senna didmobotrya* and *Warbugia ugandensis*. Also, the same was shown in *Ajuga integrifolia* and *Spathodea campanulata* against W2 *P. falciparum* strains. Absence of antiplasmodial activity was exhibited by *Senna occidentalis* methanol extracted against 3D7, while methanol extracted *Zanthoxylum gilletii* and *Warbugia ugandensis* showed no antiplasmodial activity against W2 strains.

The other organic solvent dichloromethane was used as a solvent. Antiplasmodial activities of dichloromethane-based plant extracts were determined against both 3D7 and W2. Of the 16 plants, 6 exhibited high antiplasmodial activities against 3D7 strains. These plants are: *Solanum incanum*, *Spathodea campanulata*, *Clausena anisata*, *Ocimum kilimandscharicum*, *Ficus thonningii* and *Lantana trifolia*. Four of the sixteen plants including *Carissa edulis*, *Senna occidentalis*, *Rotheca myricoides* and *Ficus thonningii* showed high antiplasmodial activity against W2. Moderate antiplasmodial activity was shown by *Carissa edulis*, *Senna didmobotrya*, *Ajuga integrifolia*, *Trichilia emetic* and *Zanthoxylum gilletii* against 3D7. Moreover, a majority *Lantana trifolia*, *Solanum incanum*, *Senna didmobotrya*, *Ajuga integrifolia*, *Spathodea campanulata*, *Trichilia emetic*, *Rotheca myricoides*, *Fuerstia africana* and *Ocimum kilimandscharicum* produced moderate activity against W2.

Dichloromethane plant extracts that exhibited low antiplasmodial activities against 3D7 included *Acmella caulirhiza*, *Senna occidentalis*, *Clausena anisata* and *Fuerstia africana*. Low antiplasmodial activity against W2 was shown by *Acmella caulirhiza* and *Warbugia ugandensis*. Lack of antiplasmodial activity was shown by *Warbugia ugandensis* when tested against 3D7. Similarly, no antiplasmodial activity was detected when Zanthoxylum gilletii extracted in dichloromethane was tested against W2. Figures 2, 3, 4 and 5 are showing the actual inhibitory concentrations of the selected plants.

In Vitro cytotoxic activities of selected plants

Previous studies have described biological efficacies of plant extracts as not being as a result of *In Vitro* cytotoxicity if the selectivity index is ≥ 10 (28,29). Therefore, this study set low selectivity index at < 10 while high selectivity index was considered at ≥ 10. The selectivity indices were obtained by dividing IC_{50} of Vero cell lines with those of 3D7 and W2 *P. falciparum* strains. The analysis was done per solvent. Of the 16 selected aqueous plant extracts, 7 had a high selectivity index ≥ 10 when tested against 3D7. The plants included: *Acmella caulirhiza*, *Carissa edulis*, *Lantana trifolia*, *Solanum incanum, Senna occidentalis, Warbugia ugandensis* and *Spathodea campanulata*. The rest of the plants had a low selectivity index against 3D7 that is; *Zanthoxylum gilletii*, *Senna didmobotrya*, *Ajuga integrifolia*, *Clausena anisata*, *Trichilia emetic*, *Rotheca myricoides*, *Fuerstia africana*, *Ocimum kilimandscharicum* and *Ficus thonningii*. Aqueous selectivity indices against W2 *P. falciparum* strains were high for *Lantana trifolia, Solanum incanum, Warbugia ugandensis, Spathodea campanulata,* and *Rotheca myricoides*. The indices were low for *Acmella caulirhiza*, *Carissa edulis*, *Zanthoxylum gilletii*, *Senna*

didmobotrya, Senna occidentalis, Ajuga integrifolia, Clausena anisata, Trichilia emetic, Fuerstia africana, Ocimum kilimandscharicum and *Ficus thonningii*.

Organic extracts also generated diverse selectivity indices. For methanol extracts 3 out of 16 plants had high selectivity against 3D7 namely; *Carissa edulis, Warbugia ugandensis* and *Solanum incanum*. The remaining 13 plants had low selectivity indices against 3D7. These are; *Acmella caulirhiza, Lantana trifolia, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum* and *Ficus thonningii*. In the case of W2 strains, only 1 plant had a high index that is *Warbugia ugandensis* after methanol extraction. The rest, 15 selected plants had low indices against W2 namely; *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum* and *Ficus thonningii*. Selectivity indices for dichloromethane extracts for both 3D7 and W2 *P. falciparum* strains were high in *Warbugia ugandensis* only. The rest of the plants namely *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum* and *Ficus thonningii* had low selectivity indices.

DISCUSSION

The comparative analysis of the aqueous extract of Acmella caulirhiza leaves demonstrated a significantly higher percentage yield in comparison to the methanol and dichloromethane solvents. Both the aqueous and methanol extracts exhibited significant antiplasmodial action against the 3D7 strain. Nevertheless, the aforementioned extract exhibited a moderate level of antiplasmodial efficacy against the W2 strain. The antiplasmodial activity of the dichloromethane extract was found to be low against both 3D7 and W2 strains. The results suggest that the aqueous extracts of Acmella caulirhiza leaves have the potential to effectively combat the 3D7 and W2 strains of plasmodia. Furthermore, these extracts demonstrate superior efficacy as a solvent for extraction. The results are consistent with a previous investigation conducted in Nyanza, Kenya, which examined the medicinal plants commonly utilized by the Kuria and Luo people. Specifically, the dichloromethane (DCM) extract derived from Acmella caulirhiza exhibited significant antiplasmodial activity against both W2 and D6 strains. It is worth noting that the study exclusively employed DCM extracts for this evaluation [30]. The aqueous extract of Carissa edulis demonstrated the highest yield of extract in comparison to the methanol and DCM extracts. The present investigation aligns with the findings of a previous study conducted by [28], which indicated that the aqueous leaf extract of the plant exhibited the highest extraction yield, followed by methanol and dichloromethane (DCM). The leaf extract of Carissa edulis exhibited moderate antiplasmodial activity, whilst the aqueous and methanol extracts had poor antiplasmodial activities against the 3D7 strain. Nevertheless, the leaf extract of DCM exhibited a significant level of antiplasmodial activity, whilst the aqueous and methanol extracts displayed a modest level of antiplasmodial activity against W2. In contrast to the aforementioned findings, a study conducted by [28] revealed that the aqueous extract exhibited significant antiplasmodial activities. Conversely, both the DCM and methanol extracts shown relatively poor antiplasmodial activities. The medical significance of Carissa edulis is further substantiated by a study conducted within the Maasai culture in Kenya, whereby it was observed that the plant exhibits antiplasmodial properties [32]. The aqueous extract of Lantana trifolia demonstrates a higher yield of extract in comparison to the extracts obtained using methanol and dichloromethane (DCM). The methanol extract derived from the leaves of Lantana trifolia exhibited significant antiplasmodial action against both 3D7 and W2 strains. The DCM extracts exhibited significant antiplasmodial activity against the 3D7 strain, while displaying a moderate level of activity against the W2 strain. The plant's aqueous extracts exhibited limited antiplasmodial efficacy against the 3D7 strain and moderate antiplasmodial activity against the W2 strain. The findings imply that non-polar solvents are more effective in extracting active phytochemical compounds compared to polar solvents. The results align with previous research, indicating that the petroleum ether extract and chloroform extract exhibited a moderate level of antiplasmodial activity, whereas the ethanolic extract had a low level of antiplasmodial activity [33]. The antiplasmodial activity of the aqueous and methanol extracts obtained from the leaves of Solanum incanum exhibited a moderate level of effectiveness against the 3D7 strain, whereas the dichloromethane (DCM) extract shown a high level of antiplasmodial activity. Nonetheless, the aqueous, methanol, and DCM extracts derived from Solanum incanum leaves exhibited a moderate level of antiplasmodial efficacy. Despite not being the optimal solvent for extraction, DCM has proven to be the most favorable choice for extraction. The results are consistent with a study conducted on the DCM extract of Solanum incanum leaves, which shown a moderate IC50 >64 antiplasmodial activity against P. falciparum. However, it is important to note that the study only tested for both aqueous and methanol [34] extracts. The aqueous, methanol, and DCM extracts derived from Zanthoxylum gilletii exhibited a modest level of antiplasmodial activity against the 3D7 strain, while demonstrating no significant antiplasmodial activity against the W2 strain. The results obtained in this investigation contradict the findings of a previous study that demonstrated significant antiplasmodial efficacy of the aqueous extract derived from zanthoxylum gilletii [35]. The maximum yield of extract was obtained from the aqueous leaf extract of Senna didmobotrya, followed by the methanol and DCM extracts. The results of the experiments indicated that all extracts exhibited a modest level of antiplasmodial activity against both the 3D7 and W2 strains. The present investigation contradicts a previous study conducted in Embu, Kenya, which demonstrated that the aqueous and methanol leaf extracts of Senna didmobotrya had limited antiplasmodial activity against both 3D7 and W2 strains of malaria parasites. However, the dichloromethane extract displayed a considerable level of antiplasmodial activity (Waiganjo *et al*., 2020). The observed variance in the findings could perhaps be attributed to the varying geographical locations of the plants. This suggests that the phytochemical contents of a plant may be influenced by its geographical location, even if it is the same plant species. The maximum yield was obtained from the aqueous leaf extract of Senna occidentalis, followed by the methanol and DCM extracts. In contrast to a conducted investigation, the use of aqueous, methanol, and hexane extracts revealed that hexane exhibited the highest yield of 31.32%, followed by methanol with 12.29%, and aqueous with 8.9% [36]. In addition to the percentage yield, it is noteworthy that the hexane extract exhibits the most potent antiplasmodial activity, with an IC50 value of 3.47 µg/mL. This is followed by the methanol extract, which has an IC50 value of 3.79 µg/mL, and lastly, the aqueous extract with an IC50 value of 4.03 µg/mL [36]. The aqueous and methanol extracts of Warbugia ugandensis exhibited limited and

negligible antiplasmodial efficacy against the 3D7 and W2 strains, respectively. The DCM leaf extract exhibited negligible antiplasmodial activity against the 3D7 strain and limited antiplasmodial activity against the W2 strain. The present investigation aligns with previous research that has shown a moderate antiplasmodial effect of the leaves extract of Warbugia ugandensis against P. falcipram [37]. In a separate investigation, it was observed that the chloroform leaf extract of Warbugia ugandensis exhibited significant antiplasmodial action against Plasmodium knowlesi and Plasmodium falciparum, with IC50 values of 3.14 µg/ml and 6.04 µg/ml, respectively [38]. Various extracts derived from Ajuga integrifolia leaves have exhibited varying degrees of antiplasmodial activity. The methanol extracts have demonstrated a significant level of antiplasmodial activity against the 3D7 strain, whilst the aqueous and DCM leaf extracts have exhibited a modest level of antiplasmodial activity against the same strain. In contrast, the extracts obtained from aqueous, methanol, and DCM solvents exhibited negligible, low, and moderate levels of antiplasmodial activity against the W2 strain, respectively. The present investigation corroborates previous research that has shown the modest antiplasmodial activity (IC50 = 29.04 µg/ml) of methanol leaf extracts derived from Ajuga integrifolia [36]. The findings of this study indicate that the extraction yields of aqueous, methanol, and DCM leaf extracts from Spathodea campanulata varied, with the aqueous extract demonstrating the highest yield. Furthermore, the extracts exhibited varying levels of antiplasmodial efficacy against the 3D7 and W2 strains. The aqueous extracts exhibited a moderate level of antiplasmodial activity against the 3D7 strain, however both the Methanol and DCM extracts demonstrated a high level of antiplasmodial activity against the same strain. Nevertheless, the antiplasmodial efficacy of the aqueous and methanol leaf extracts of Spathodea campanulata exhibited minimal activity against the W2 strain, whereas the dichloromethane (DCM) extract demonstrated a significant level of activity against the same strain. The findings indicate that the DCM extract exhibits superior properties as an extract and demonstrates antiplasmodial efficacy against both the 3D7 and W2 strains. The present discovery aligns with previous research indicating the presence of antiplasmodial properties in Spathodea campanulata. For example, the hexane and chloroform extracts derived from the stem bark exhibited notable inhibition of parasitemia, particularly at doses ranging from 100 to 400 mg/kg/day [39]. Similarly, the ethanol fraction has demonstrated significant antiplasmodial activity, with an IC50 value of 18.7±2.23µg/ml, against both chloroquine sensitive and resistant Plasmodium falciparum isolates [40]. The study's results indicate that the aqueous, methanol, and DCM leaf extracts of Clausena anisata exhibited antiplasmodial action against both the 3D7 and W2 strains. Despite the relatively low percentage yield, DCM exhibited the highest level of antiplasmodial activity, indicating that the phytochemical active components are more effectively extracted using DCM. The results are consistent with previous research indicating that the crude leaf extract of Clausena anisata exhibited a modest level of antiplasmodial action in an in-vitro study [41]. The aqueous, methanol, and DCM extracts of Trichilia emetic leaves have demonstrated a significant antiplasmodial efficacy against the 3D7 and W2 strains, on average. The findings of this investigation align with a previous study conducted in Côte d'Ivoire (Obbo *et al*., 2019), which indicated that the root bark extract of Trichilia emetic is one of the most effective plant extracts against Plasmodium falciparum, with an IC50 value of 8.36 g/ml. Furthermore, it has been observed that extracts derived from the leaves of Trichilia emetic aldehyde exhibit inhibitory effects on the growth of Plasmodium falciparum (with an IC50 value of 76 µM) as well as on the slow-proliferating breast cancer cells MCF7 (with an IC50 value of 78 µM). Additionally, these extracts have demonstrated strong inhibition of proliferation in S180 cancer cells (with an IC50 value of 7.4 µM) [44]. The aqueous, methanol, and DCM leaf extracts of Rotheca myricoides have demonstrated antiplasmodial efficacy against both 3D7 and W2 strains.The current study is aligned with a previous study conducted in Kakamega, namely in the East sub-county, which identified Rotheca myricoides as a frequently utilized medicinal plant for treating various infections, including malaria [24]. The aqueous, methanol, and DCM leaf extracts derived from Fuerstia Africana exhibited antiplasmodial efficacy against the 3D7 and W2 strains. The results are consistent with a previous investigation that shown significant antiplasmodial action of both petroleum ether and methanol, with IC50 values of 1.56 ± 0.00 µg/ml and 2.5 ± 0.4 µg/ml against D6 and W2 strains, respectively [45]. The aqueous, methanol, and DCM extracts of Ocimum kilimandscharicum leaves demonstrated a higher percentage yield compared to a previous study, which reported a yield of 3.8% for the extracts obtained from the leaves and twigs of Ocimum kilimandscharicum. Regarding the antiplasmodial activity, it is noteworthy that all of the plant extracts exhibited a moderate level of antiplasmodial activity. This finding aligns with a previous study conducted in Kenya, which focused on a selection of medicinal plants commonly utilized by the Luo and Kuria communities. The crude extracts of Ocimum kilimandscharicum leaves and twigs exhibited significant antiplasmodial action, with IC50 values of 1.5477±0.226 µg/ml and 0.8437±0.123 µg/ml against the 3D7 and W2 strains, respectively [30].

The antiplasmodial activity of the aqueous leaf extracts of Ficus thonningii was shown to be significant, with an IC50 value of ≤10 µg/ml, against both the 3D7 and W2 strains of Plasmodium falciparum. Nevertheless, it is worth noting that both methanol and DCM extract demonstrated a modest level of antiplasmodial activity against both 3D7 and W2 strains of P. falciparum, with IC50 values ranging from 11 to 49.9 µg/ml. The aforementioned finding is consistent with recent studies that demonstrate the high and moderate antiplasmodial activity of methanol and hexane against both the K1 (multiresistant) and NF54 (sensitive) strains of P. falciparum, as indicated by their respective IC50 values of 5.3 ± 2.3 and 2.7 ± 1.6 µg/mL, and 21.1 ± 2.3 and 10.4 ± 1.6 µg/mL [46].

Table 1: Showing a summary of botanical and local identification as well as percentage yields per solvent

Voucher No	Family	Plant name	Local name	Yield (%)		
				H_2O	METH	DCM
KKA001	Compositae	*Acmella caulirhiza*	Shituti	3.12	1.78	0.74
KKA002	Apocynaceae	*Carissa edulis*	Shikata	9.14	5.82	2.22
KKA003	Verbenaceae	*Lantana trifolia*	Shimenenwa	9.37	6.25	0.93
KKA004	Solanaceae	*Solanum incanum*	Indalandalu	5.78	2.67	1.15
KKA005	Rutaceae	*Zanthoxylum gilletii*	Shikuma	8.19	3.76	1.53

KKA006	Leguminosae	*Senna didmobotrya*	Lubinu	12.62	6.32	1.68
KKA007	Leguminosae	*Senna occidentalis*	Imbindi	11.62	5.6	1.33
KKA008	Canellaceae	*Warbugia ugandensis*	Apachi	4.82	1.43	0.59
KKA009	Lamiaceae	*Ajuga integrifolia*	Imbuli yu mtakha	8.66	5.91	1.44
KKA010	Bignoniaceae	*Spathodea campanulata*	Mutsulio	7.35	4.77	2.38
KKA011	Rutaceae	*Clausena anisata*	Shihunya bukundu	5.11	2.86	1.22
KKA012	Meliaceae	*Trichilia emetica*	Munyama	9.01	5.28	2.67
KKA013	Lamiaceae	*Rotheca myricoides*	Shisilangokho	7.86	4.43	2.12
KKA014	Lamiaceae	*Fuerstia africana*	Muvesemu	8.03	3.23	1.68
KKA015	Lamiaceae	*Ocimum kilimandscharicum*	M'monyi	9.87	4.71	0.76
KKA016	Moraceae	*Ficus thonningii*	Mutoto	9.06	3.11	1.56

Data presented as percentages (%). Water, H_2O; Methane, METH; Dichloromethane, DCM.

Table 2: Showing antiplasmodial activities of based on different solvents against 3D7

Antiplasmodial activity ~ 3D7	**Solvent/Plant**		
	H_2O	**METH**	**DCM**
High activity (IC_{50} of ≤10 µg/ml)	*Acmella caulirhiza, Rotheca myricoides Ficus thonningii*	*Acmella caulirhiza, Lantana trifolia, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Fuerstia Africana*	*Solanum incanum, Spathodea campanulata, Clausena anisata, Ocimum kilimandscharicum, Ficus thonningii, Lantana trifolia*
Moderate activity (IC_{50} of 11-49.9 µg/ml)	*Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Fuerstia africana, Ocimum kilimandscharicum*	*Solanum incanum, Zanthoxylum gilletii, Rotheca myricoides, Senna didmobotrya Ocimum kilimandscharicum, Ficus thonningii*	*Carissa edulis, Senna didmobotrya, Ajuga integrifolia, Trichilia emetic, Zanthoxylum gilletii*
Low activity (IC_{50} of 50-100 µg/ml)	*Carissa edulis Warbugia ugandensis*	*Carissa edulis, Senna didmobotrya, Warbugia ugandensis*	*Acmella caulirhiza, Senna occidentalis, Clausena anisata, Fuerstia Africana*
Inactive (IC_{50} of ≥100 µg/ml)	*Senna occidentalis*	*Senna occidentalis*	*Warbugia ugandensis*

IC_{50}, Inhibitory concentration; Water, H_2O; Methane, METH; Dichloromethane, DCM

Table 3: Showing antiplasmodial activities of based on different solvents against W2

Antiplasmodial activity ~ W2	**Solvent/Plant**		
	H_2O	**METH**	**DCM**
High activity (IC_{50} of ≤10 µg/ml)	*Trichilia emetic, Fuerstia Africana, Ficus thonningii*	*Lantana trifolia*	*Carissa edulis, Senna occidentalis, Rotheca myricoides, Ficus thonningii*
Moderate activity (IC_{50} of 11-49.9 µg/ml)	*Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Senna didmobotrya, Senna occidentalis, Clausena anisata, Rotheca myricoides, Ocimum kilimandscharicum*	*Acmella caulirhiza, Carissa edulis, Solanum incanum, Senna didmobotrya, Senna occidentalis, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum, Ficus thonningii.*	*Lantana trifolia, Solanum incanum, Senna didmobotrya, Ajuga integrifolia, Spathodea campanulata, Trichilia emetic, Rotheca myricoides, Fuerstia Africana, Ocimum kilimandscharicum*
Low activity (IC_{50} of 50-100 µg/ml)	*Spathodea campanulata*	*Ajuga integrifolia, Spathodea campanulata*	*Acmella caulirhiza, Warbugia ugandensis*
Inactive (IC_{50} of ≥100 µg/ml)	*Zanthoxylum gilletii, Warbugia ugandensis Ajuga integrifolia*	*Zanthoxylum gilletii Warbugia ugandensis*	*Zanthoxylum gilletii*

IC_{50}, Inhibitory concentration; Water, H_2O; Methane, METH; Dichloromethane, DCM

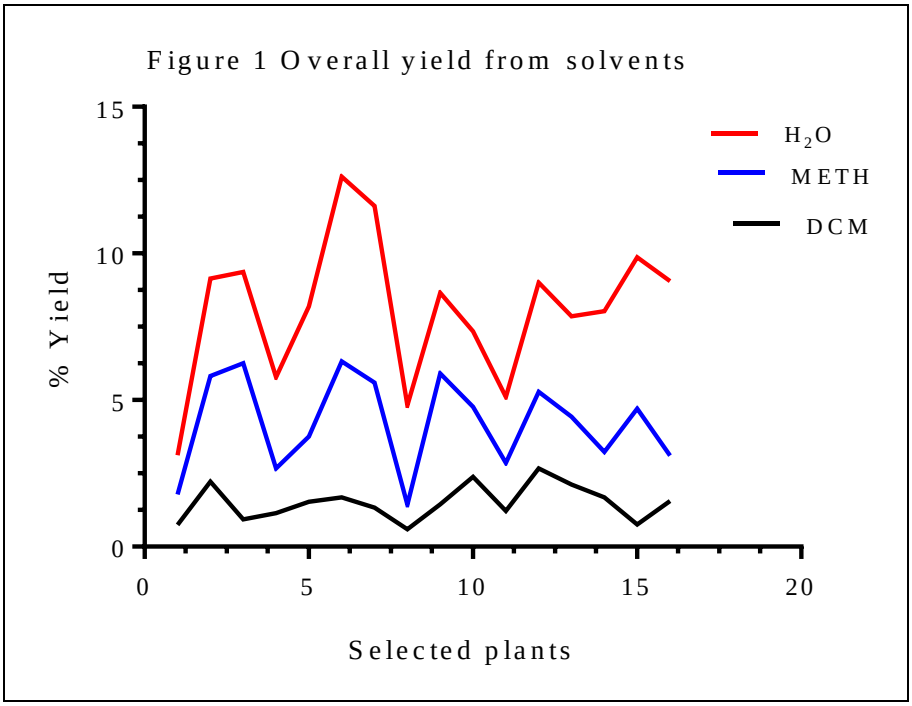


Figure 1: Showing percentage (%) yields of the solvents. Water, H_2O; Methane, METH; Dichloromethane, DCM

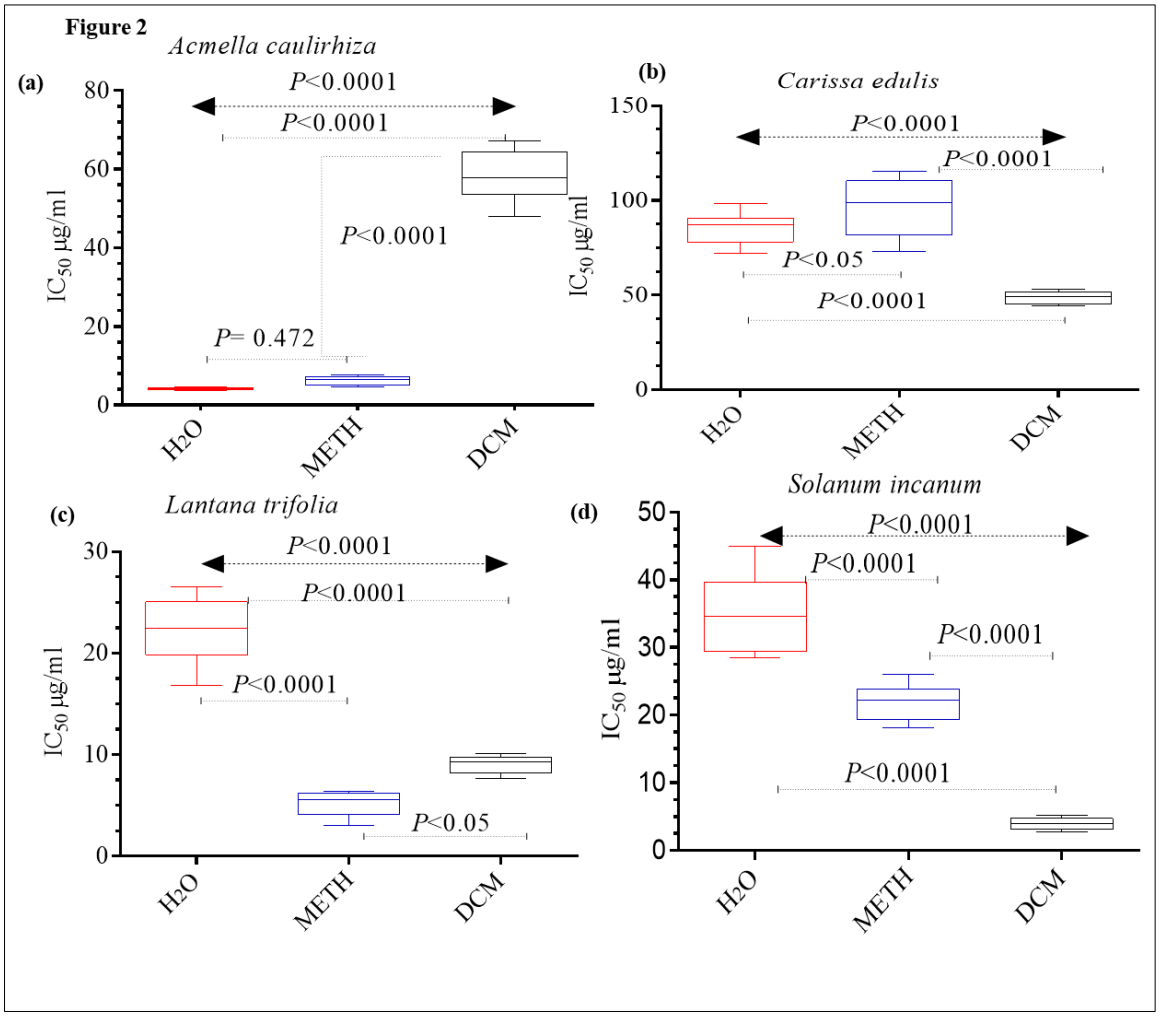


Figure 2: showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against 3D7. Water (H_2O), Methanol (METH) and Dichloromethane (DCM). Data compared across the groups (solvents) by ANOVA. *Post-hoc* analyses were done using Barnett's test. (a) *Acmella caulirhiza*; (b) *Carissa edulis*; (c) *Lantana trifolia*; (d) *Solanum incanum*.

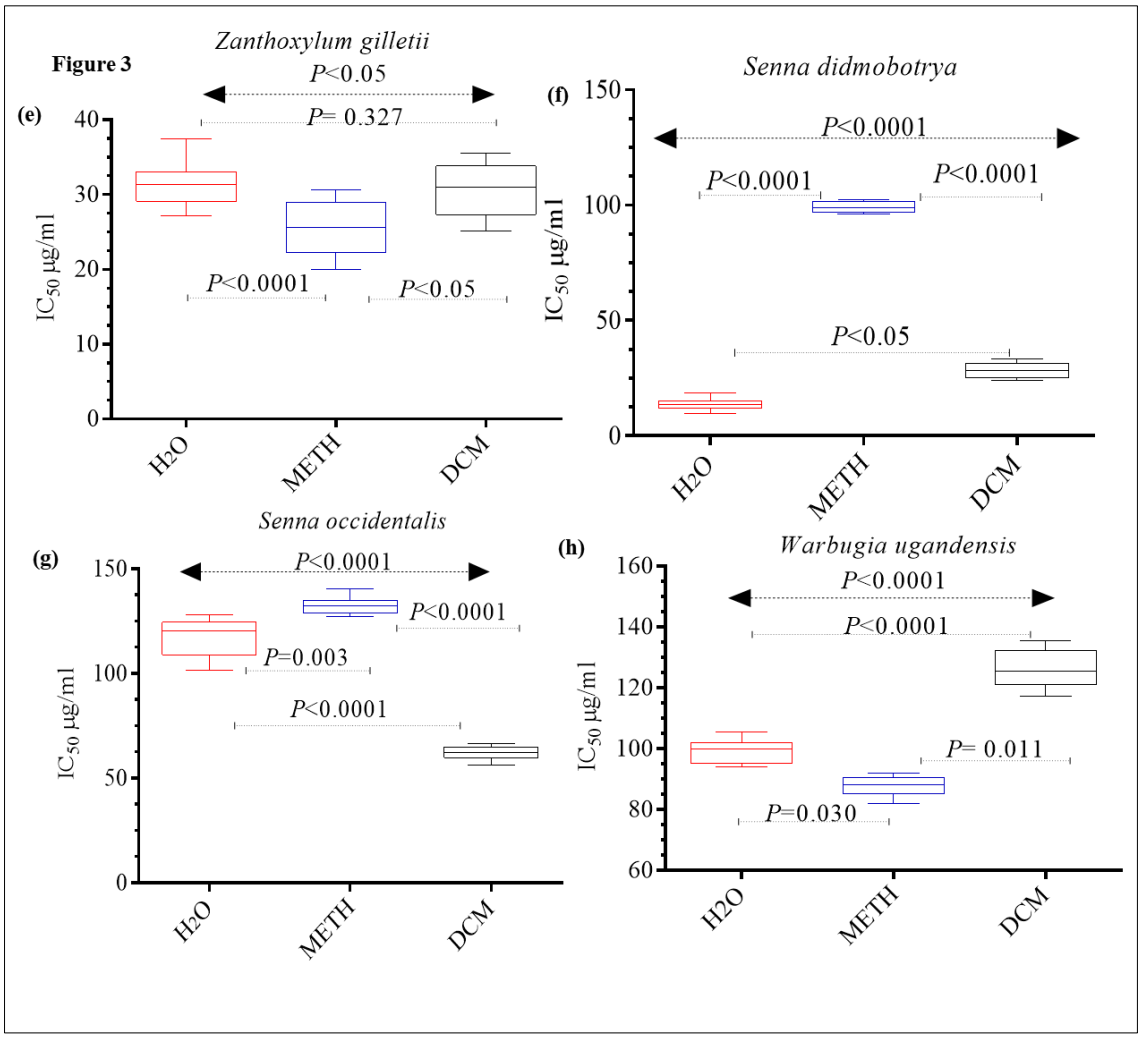
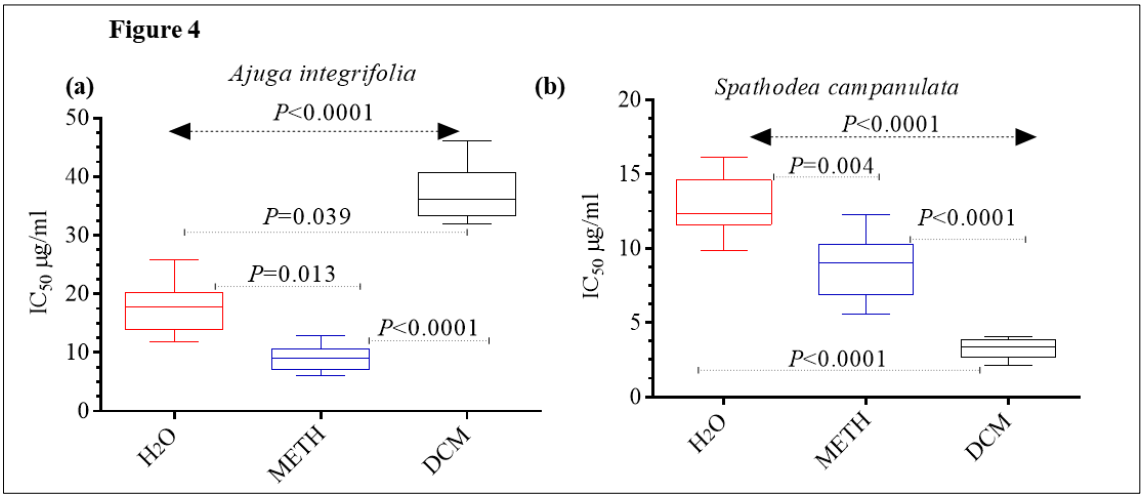


Figure 3: Showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against 3D7. Water (H_2O), Methanol (METH) and Dichloromethane (DCM). Data compared across the groups (solvents) by ANOVA. *Post-hoc* analyses were done using Barnett's test. (e) *Zanthoxylum gilletii*; (f) *Senna didmobotrya*; (g) *Senna occidentalis*; (h) *Warbugia ugandensis*.



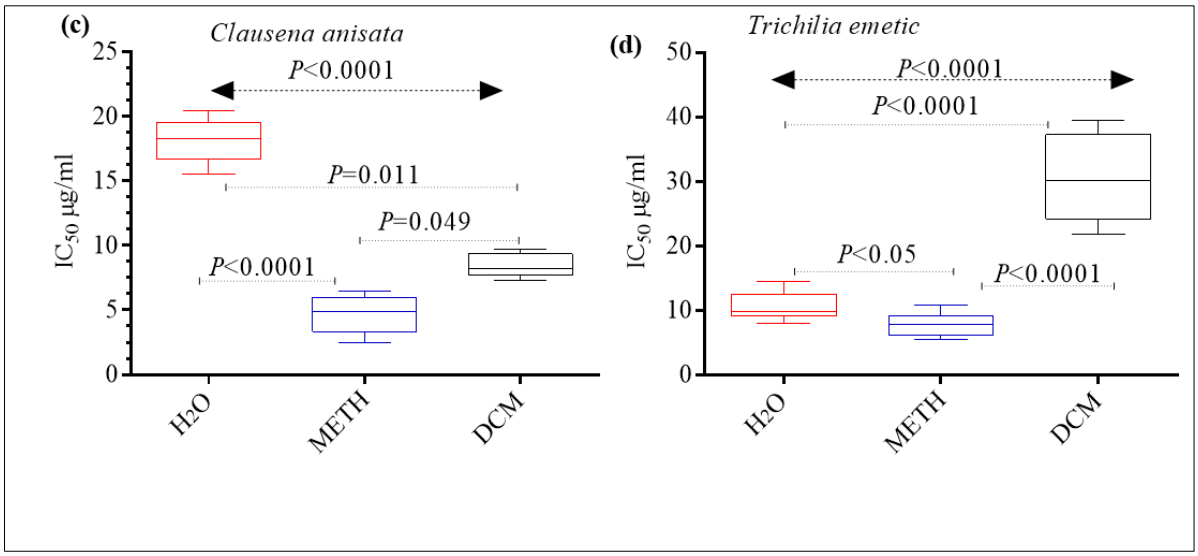


Figure 4: showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against 3D7. Water (H_2O), Methanol (METH) and Dichloromethane (DCM). Data compared across the groups (solvents) by ANOVA. *Post-hoc* analyses were done using Barnett's test. (a) *Ajuga integrifolia*; (b) *Spathodea campanulata*; (c) *Clausena anisata*; (d) *Trichilia emetic*.

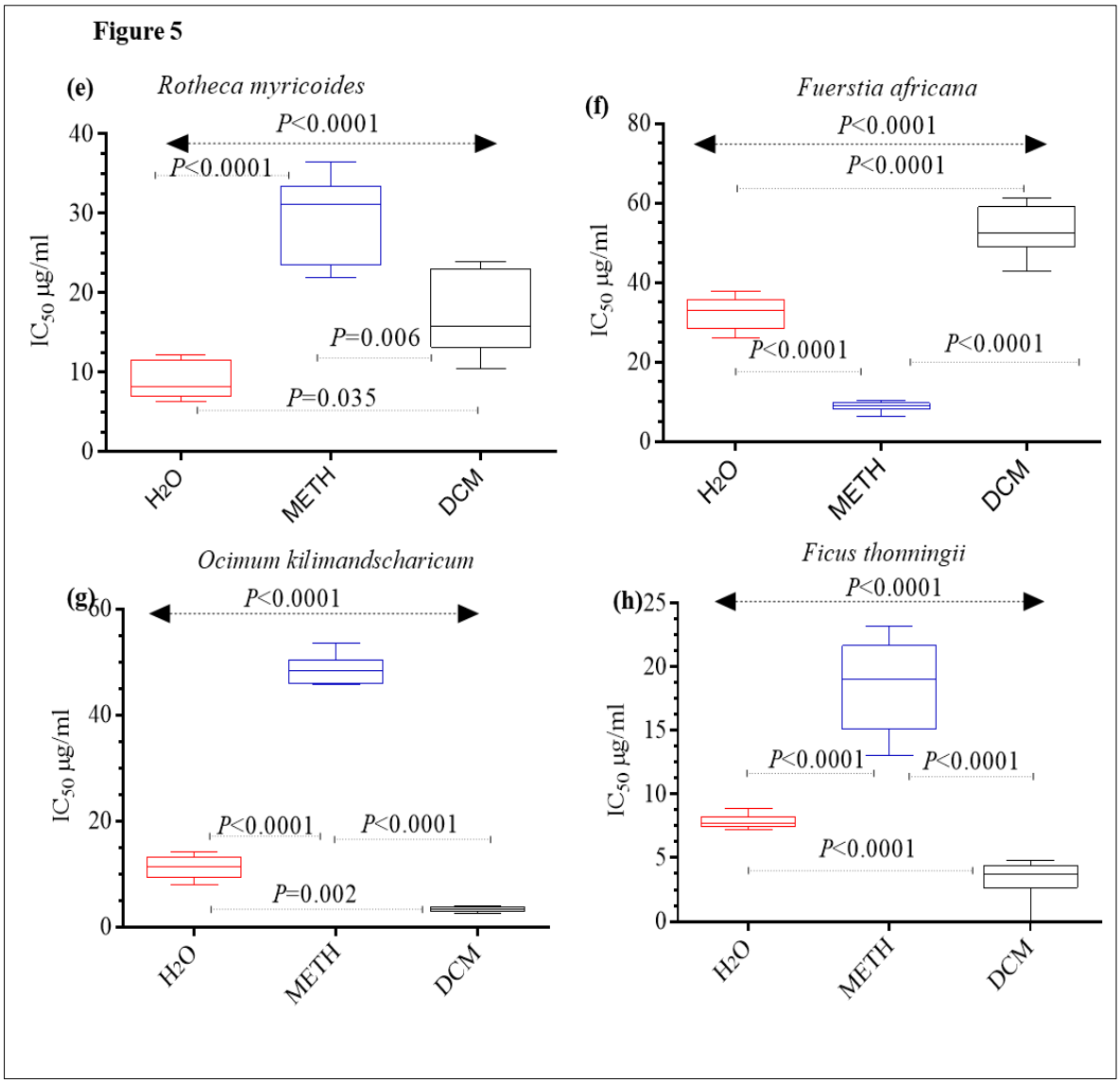


Figure 5: showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against 3D7. Water (H_2O), Methanol (METH) and Dichloromethane (DCM). Data compared across the groups (solvents) by ANOVA. *Post-hoc* analyses were done using Barnett's test. (e) *Rotheca myricoides*; (f) *Fuerstia Africana*; (g) *Ocimum kilimandscharicum*; (h) *Ficus thonningii*.

CONCLUSIONS

The aqueous leave extracts of the selected plants gave highest yield across all the plants followed by methanol and DCM. In addition, plant extracts with low IC50 (≤10 µg/ml) values could be potential sources for novel antiplasmodial compounds upon further development while Methanol and DCM extracts revealed high cytotoxic activities.

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Disclosure

None of the authors have a commercial relationship or financial conflict of interest as part of this study.

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