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ANTI-PLASMODIAL SCREENING OF SELECTED MEDICINAL PLANTS USED IN THE TREATMENT OF MALARIA AMONG THE UKAMBANI TRIBES OF KENYA

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ABSTRACT

Malaria is one of the top neglected tropical diseases affecting mostly African sub-Saharan region, coupled with the high spread of drug resistant *Plasmodium falciparum* and lack of effective vaccines. These have posed for an urgent demand for the development of a novel compound with an antiplasmodial activity from plant origin that will be therapeutic against the lethal strain of *Plasmodium* parasites. In this study, eight plants from Kenyan ethnomedicine were selected. The choice of these plants was affected by their use in the management of malaria in a traditional way. The plant were extracted for their bioactives using polar and non polar solvent and the result showed that polar extract of *Rhamnus prinoid* and *Ceasalpinea volkensii* had the highest percentage yield of 21.14% and 20.41%, while the non polar extract of *Croton macrostachyus* and *Macroglossia pyrifolia* had the least percentage yield recovery with 4.14% and 2.93%, respectively. The extract were further subjected to a qualitative phytochemical analysis and the result showed the presence of the following phytocompounds; alkaloids, phenols, flavonoids, anthroquinones, saponins, coumarins, essensial oils and terpenes. The extract were finally subjected to *in-vitro* antiplasmodial analysis and the result revealed that *Ceasalpinea volkensii* methanol extract, *Vernonia lasiopus* methanol extract and *Macroglossia pyrifolia* methanol extract had the IC_{50} values of 2.37±1.70 µg/ml, 4.50±1.75 µg/ml, 2.39±1.95 µg/ml, with an excellent antiplasmodial activity. While, the non polar extract of *Rhamnus pyrifolia* dichloromethane, *Albizia gumifera* dichloromethane with IC50 of 16.45±0.55 µg/ml and 8.20±1.45µg/ml with a promising antiplasmodial activity. While the extract of *Croton macrostachyus dichloromethane*, *Ceasalpinea volkensii* and *Albizia gumifera methanol* were all found to be in-active even at higher concentration. The antiplasmodial activity of the extracts was all found to have a strong correlation in all the polar extract and at variance with that of the non polar solvent extract. It can be concluded, that polar extract of *Vernonia lasiopus*, *Ceasalpinea volkensii*, *Rhamnus prinoid*, *Clausina anesatata* and *Croton macrostachyus* could be utilized as a potential source for the novel anti-malarial drug.

Key words : Malaria, Alkaloids, Solvent, *In-vitro*, Plasmodium and Polar.

Introduction

Malaria is an infectious disease that is caused by *Plasmodium* parasite of the genus *Protozoa*, it is transmitted through a bite of a female *Anopheles* mosquito from one infected person to another (Rowe *et al.*, 2006). Centre for disease control and prevention Kenya (CDCP-K) in 2021 reported that, there were approximately 10,700

malaria mortality, with 3.5 million outpatient clinical cases. [Centre for disease control and prevention Kenya (CDC, 2021)]. The parasites initiate the blood stage infection of the erythrocyte blood cell which is the key behind the basis of the ailment (Dvorak *et al.*, 1975). The failure of chloroquine and sulphadoxime pyrimethamine in the treatment of malaria has lead to a heavy reliance on *Artemisin* based combination therapy (ACT) as the only first line treatment (Dondorp *et al*., 2009). In all the malaria endemic nations, *Artemisin* combination therapy (ACT) are supplied only in few public health facilities, the cost of the purchase and lack of the national health care insurance policies are some of the numerous challenges faced by the stake holders in the management of malaria infection (Amin *et al*., 2009). This has paved ways for scientists/ researchers for a further collaboration on the discovery of novel compound with a promising anti-plasmodial activity. The choice of this plant for the study was affected by their ethnobotanical application. Their roots, stems, leaves and fruits have been used by the local community in the treatment of malaria and other related ailment. The development of novel and promising compound from the plant source is a research intensive science. This has grown to embrace more of the discoveries on the plant medicinal expertise. The medicinal potential of all plant species are attributed to the presence of the different phytochemical constituent contained in each of them (Tiwari *et al*., 2011). The phytoconstituent are referred to as secondary metabolite, because they are the by product of the reaction of the primary metabolite (carbohydrate, protein and vitamin) and they are responsible for the medicinal value and protection of the plant against all the biotic and abiotic factors (Milliken, 1997). The continuous use of chloroquine in the treatment of malaria infection has lead to the high resistance of the parasite (White, 2007). The *Artemisin* combination therapy (ACT) has remained the only viable options as most of the quinoline and its analogues have been reported to be toxic and the parasites have developed a high resistance to them (Machini *et al*., 2006). Kenyan Government under the presidential malaria initiatives (PMI) have made several effort in the fight against the malaria mortality using the following strategies in combating the ailments; entomological monitoring, insecticide management, public enlightenment on the use of insecticide treated net and indoor residual spraying (PMI, 2018). Reviews from the recent literature revealed that plant phytocompound may vary from one plant to another with certain factors such as specie type, genetic variation, geographical location and other abiotic factors (White, 2007). *Vernonia lasiopus* have been reported to have a various medicinal value, its recipe have been use in the treatment of malaria infection (Dharani *et al*., 2010). The organic fraction of the *Vernonia lasiopus* have been reported to possess a sedative, analgesic and membrane stability of the red blood cell (RBC) (Kokwaro, 1976). The use of *Vernonia lasiopus* have been varied among the different Kenyan communities. The Kikuyus in central region use it in the treatment of malaria, Ukambani of the lower eastern use it in the treatment of venerable disease, LuoNyanza in the Southern region use it for the treatments of scabies, while the Maasai and Samburu tribe use it in the treatment of sores (Dharani *et al*., 2010). The most common compound isolated from *Vernonia lasiopus* include *lasiophalides*, *elemanoids* and *epivernodalol* (Dharani *et al*., 2010). *Albizia gumifera* (*leguminosae*) is a common medicinal plant that have been used in the treatment of different ailment in Kenya. These includes bacterial and parasitic infection (Rukunga and Waterman, 2001). One of the most important compound isolated from *Albizia gumifera* is the spermine alkaloid (Geyid *et al.*, 2005). The methanol extract of *Albizia gumifera* has been isolated for the antimicrobial, anti-parasitic and anti-trypanosomial activity (Helen *et al*., 2013). A number of ethnobotanical survey have been documented on some number of plant species used by the Kenyan locals in the treatment of malaria, the result revealed that there is no combined and extensive studies on their phytochemical analysis and extensive *in-vitro* screening of these plant. Hence, only limited number of these plant with claimed phytochemical have been subjected to antiplasmodial and phytochemical analysis (Muthura *et al*., 2007).

Here, we analyzed these plant; *Rhamnus prinoid*, *Croton macrostachychus*, *Macroglossia pyrifolia*, *Albizia gumifera*, *Clausina anesatata*, *Caesalpinia volkensii*, *Vernonia lasiopus* and *Sienna didymobotrya* for the presence of their bioactive molecule and *in-vitro* anti-plasmodial screening using the SYBR green dye 1 fluorescence assay in order to validate their use in malaria remedy and to utilize them as the bedrock for the development of novel anti-malarial drug from Kenyan ethno-medicine.

Materials and Methods

Plant materials and preparation of the samples

The fresh plant for the study were collected in the month of January- February, 2021 from their natural habitat in Machakos county, Kenya. Where they grew naturally (Fig. 1). The plant samples were furnished to an acknowledged taxonomist in Botany Department, Jomo Kenyatta University of Agriculture and Technology,

Fig. 1 : Images of, the selected Kenyan anti-malarial plants used for the study. **A.** *Rhamnus prinoid*, **B.** *Croton macrostachychus*, **C.** *Microglossa pyrifolia*, **D**. *Albizia gumifera*, **E.** *Clausinaanesata*, **F.** *Caesalpinia volkensii*, **G.** *Vernonia lasiopus*, **H.** *Sienna didymobotrya*.

Kenya for botanical authentication. The voucher specimen were deposited in the herbarium for future reference. The collected plant sample were all washed with distilled water, cut into small pieces and dried under shade for two weeks according to the method described by Sofowora *et al*. (2013) with a slight modification.

Extraction

The plant bio-active were all extracted using the cold maceration technique, using polar and non polar solvent was according to the method describe by Hijazi *et al*. (2009). The solvent of high performance analytical grade and were all purchased from Sigma Aldrich and British Drug House.

Phytochemical screening

The prepared extract was used to test for the presence of the different phytochemical constituent. The following reagents (Wagner, Ferric, Bontrager's, Sodium hydroxide (NaOH), Ferric chloride, Iodine, Gelatin, Salkowski's, Fluorescence and Froths) test was used according to the method described by Edeoga *et al.* (2005) with a slight modification.

Anti-plasmodial screening

Parasites Culture

2.5g of Sodium bicarbonates ($NaHCO_3$) was dissolved in a 1000 ml distilled water and was left to stir a magnetic stirring bar for two hours. The solution was supplemented with Ross-well park memorial institute (RPMI 1640) media, glucose 5ml (50% w/w in distilled water). Synergistic brand (SYBR) green dye 3ml (30mg/ml in 1M Sodium hydroxide (NaOH) L-glucotamine 15 ml (0.395 ml in distilled water) and Albumen 30ml (30% w/v in Ross-well memorial park institutes (RPMI 1640) IM to form the completee medium. The PH was adjusted to (7.30-7.35) neutral. The volume of culture medium was adjusted to 500ml with distilled water and was sterilized and labeled for a further use.

Blood sample preparation

The blood sample (O+) was prepared in line with the protocols described by Moon *et al.* (2013). Shortly the blood sample was centrifuged at 1750 rpm for 10 minutes and was washed twice and stored at 5% in (Ross-well park memorial institute (RPMI 1640).

Maintenance of the parasites culture

The parasites were maintained at 3.5 hematocrits. The volume of the packed cells was measured and transferred into the sterile flask. The required volume of the gas (97% N_2, 3% CO_2 and 6% O_2) and was incubated at 39^0C according to the protocol described by Ribacke *et al*. (2013).

Parasites seeding

96 well plates were used for the seeding of the parasites and the plants extracts for the in-vitro anti-malarial assays. The well plate labeled (a-h), (i-j) and (k) were all seeded with the synchronized parasites culture. 2% parasites, 3% hematocrits were all pi-petted 200ml in to each of the duplicate wells. An aliquot 30 µ liter of the various concentrations of (1000, 500, 250, 125, 62.5, 31.25, 15.25 and 7.15µg/ml) of each extract in the respective wells in duplicates. The wells of (i-j) received 10ml of the Chloroquine and Dihydroartemisin as the positive control, while the column wells [k-l] was left empty with only the seeded parasites, which served as the negative control for the experiments. The plates were covered and kept in the gas chamber and was maintained

at (97% N_2, 3% CO_2 and 6% O_2) and left incubated at 39^0C for 48-72 hrs following the incubation of the parasites. It was harvested and the fluoresence dyes as the results of the formation of the chelates with the Deoxyribonucleic acid (DNA) of the parasites and the florescence emission was measured at 520nm wavelength and the Inhibition maximal concentration (IC_{50}) values was determined using this formula;

(IC_{50}) = anti log (log X_1+[logY_{50}-logY_1)* (logX_2-logX_1]/(logX_2-logX_1]/logX_2-log Y_1

Where, Y_{50} – The count per minute (CPM) values in a mid-way between parasitized and non-parasitized cultures (X_1, Y_1, X_2 and Y_2) are the concentrations and fluorescence emission values respectively for the data point below fluorescence range (Sixsmith *et al.*, 1984).

Statistical analysis

The entire *in-vitro* screening assay was carried out in the duplicate and the numerical data were analyzed using the Microsoft Excel 2013 using the non-linear regression aided determination of the maximal inhibition concentration (IC_{50}). One way analysis of variance (ANOVA) was used for the analysis of the counts with the comparison of the survival time among each of the groups with reference. The *p* values of less than 0.005 was considered to be the statistically significant different.

Results and Discussion

Extraction and percentage yields of the plants extract

The result of the extraction and the percentage yield was influenced directly by the solvent of extraction and the nature of the extraction used for the study. The percentage yields of the extract (w/w-yield of the extract in g *100) of the different extract obtained (Table 1). The result showed that the solvent/ extractive method affected the nature of the percentage yield recovery. Among all the result obtained, the polar solvent had the highest percentage yield in order of SD-MeOH>CA-MeOH > CV-MeOH>RP-MeOH with the following values of 15.21%> 19.00%>20.96% >21.15%, while the non polar extract had the least percentage recovery yield of CM-DCM>AG-DCM>MP-DCM>RP-DCM with the recovery yield of 2.93%>4.21%>4.64%>8.10%, respectively (Table 1). Among the higher percentage yield obtained for the polar extract is attributed to the polar protic nature and higher dielectric constant of the methanol over the dichloromethane, which is the non polar extracts which enhanced the solubility of the non polar metabolites. But, in the contrary the results of the *Vernonia lasiopus* extract in respects of the type of solvent of extraction for both the polar and non polar solvent but the percentage yields obtained a high value of VL-DCM & VL-MeOH with 13.31% and 14.24% (Table 1) .The solvent was able to dissolve all the metabolite depending on the types of the constituent (Kokate *et al.*, 2005). The choice of solvent for the extraction of all the phytocompound is very crucial not only into for the percentage yield, but for the qualitative and quantitative composition of each phytocompound (Mukharjee, 2005). From the previous study, it was reported that the maximum extractive values were obtained using the polar extract. Polar solvent such as methanol have been commonly used in the extraction of polar metabolite where as the non polar solvent like dichloromethane have been used in the extraction of the non polar bio-active compound (Pandey and Tripathi, 2014; Sasidharan *et al.*, 2011; Altemimi *et al.*, 2017). In all the circumstances, the polar solvents due to their ability to dissolve and become miscible in organic solvent the bio-active to be extracted using must be soluble (Makundunmi, 2015). Based on the results of the percentage yields of SD- MeOH and SD-DCM (Table 1). There was a total significant difference in the results obtained ($p< 3.5$) (Table 1). The major reason for this variation is that polar extracts had the ability to dissolve all other bioactive compound in contrast to other non polar extract. Hence, it has the ability to have the highest percentage yields in comparison to the non polar extracts. The results of this study is in agreement with the findings of Kigondu (2007), which stated that polar extract have the highest extraction ability in comparison to other non polar solvent and was also supported by the findings of Korir *et al*. (2014).

Qualitative Phyto-chemical analysis of the plant extract

The medicinal plants used for the study are from the families of *Rubiaceae*, *Lugumeceae* and *Fabaceae*. They comprise of 95% tree and 5% shrub, most of the parts used are leaves and stem (Table 2). The result revealed that the plant extract are rich in the presence of Phyto-chemicals consisting of alkaloid, phenol, saponin, anthroquinone, essensial oil, flavonoid, coumarin and terpene (Table 2). Ninety five percent of all the plant extract tested contained the presence of alkaloid. .The high alkaloid in *P. guineense* is in congruent with the findings of Ajayeoba *et al*. (2006). That the efficacy of all the plants used in the treatment of malaria was due to the presence of presence of alkaloid. The medicinal plants that are moderately rich in alkaloids have the potential health benefits effects (Jigam *et al.*, 2010; Ikewuchi *et al.*, 2015). Saponins are found to be present in all the

Table 1 : Summary of the plants used, solvent, recovery in (g) and percentage (%), voucher specimen and parts used.

S. no.	Plants name/solvents	Abbreviation	Recovery (g)	Yield (%)	Voucher specimen	Parts used
1.	*R. prinoid* / dichloromethane	RP-DCM	16.20	8.10	IN/RP/JKUAT/001/2020	Leaves
2.	*R. prinoid* / methanol	RP-MeOH	42.38	21.14		Leaves
3.	*C. macrostachychus* / dichloromethane	CM-DCM	5.87	2.93	IN/CM/JKUAT/005/2020	Stem bark
4.	*Cr. macrostachychus* / methanol	CM-MeOH	20.53	10.21		Stem bark
5.	*M. pyrifolia* / dichloromethane	MP-DCM	9.28	4.14	IN/MP/JKUAT/007/2020	Stem bark
6.	*M. pyrifolia* / methanol	MP-MeOH	10.62	5.31		Stem bark
7.	*A. gumifera* / dichloromethane	AG-DCM	8.42	4.21	IN/AG/JKUAT/003/2020	Leaves
8.	*A. gumifera* / methanol	AG-MeOH	9.48	4.24		Leaves
9.	*C. anesata* / dichloromethane	CA-DCM	20.57	10.23	IN/CA/JKUAT/009/2020	Leaves
10.	*C. anesata* / methanol	CA-MeOH	38.00	19.00		Leaves
11.	*C. volkensii* / dichloromethane	CV-DCM	18.98	9.49	IN/CV/JKUAT/011/2020	Leaves
12.	*C. volkensii* / methanol	CV-MeOH	41.92	20.41		Leaves
13.	*V. lasiopus* / dichloromethane	VL-DCM	26.72	13.31	IN/VL/JKUAT/013/2020	Whole
14.	*V. lasiopus* / methanol	VL-MeOH	28.49	14.24		Whole
15.	*S. didymobotrya* / dichloromethane	SD-DCM	20.43	10.21	IN/ SD/ JKUAT/015/2020	Leaves
16.	*S. didymobotrya* / methanol	SD-MeOH	31.52	15.21		Leaves

polar extract and absent in almost all the non polar extracts (Table 2). Saponins have carcinogenic activity and other health benefits. They play the vital role in anti-malarial activity of the plants (Adesokan and Akanji, 2010). The essential oil were found mainly in the non polar extract (Table 2). Generally, the essentially essential oil is known to exhibit antibacterial activity (Kamatou and Viljoen, 2005). In the recent studies, the essential oil of *C. anesatata*, estragole and anethole (Aulessi and Dongou, 2004) were reported as the major chemical compounds. It is necessary to point out any chemical compounds of any essential plats oil vary greatly which depends on certain factors such as geographical region, age of the plants, climatic changes and experimental conditions (Deferara and Ziogas, 2000; Jerkovic and Mastelic, 20001). The essential oil as important bio-actives molecules constitutes the mono-terpenes and sesqui-terpenes. These constituents have been reported to posses antibacterial property (Shane and Whylie, 1999). The mechanism of terpenes involved the disruption by the lilophilic compounds (Cowan, 1999). It has been reported that the anti-microbial and anti-malarial property of *C. anesata* is as the results of the major chemical constituents of alpha- pienne, which is known to have antimicrobial activity (Mellion and Stratis, 2007). The phytochemical constituents in every plants vary due to the genetic composition and their biodiversity in nature. The evolution of the plants cannot be attributed to the methods of the extraction (Ordenaz *et al*., 2006). Hence, the metabolites are responsible for the defence of the plants against the pathogens (Anjali and Sheetal, 2013). The indication of a high presence of phenol in polar extracts is in agreements with the findings of (Nagavani *et al*., 2010 and Oteng *et al*., 2012). Flavonoid consists of different range of substance that fights against the diseases in human (Harbone and William, 2010). The presence of flavonoids in all the polar extracts in comparison to the non polar extracts ($p<0.5$). This is in congruent of other researchers that the reported quantity of flavonoids as a bioactive in plants is responsible for all their anti-malarial and biological activities (Olorunsola *et al*., 2011). The highest content of flavonoids are recorded in all the plants extracts with only the exception of MP-DCM, MP-MeOH (Table 1), respectively. The polarity of the solvents is as the result of the different variation of the extracts metabolites. In the previous work of (Omoruyi *et al*., 2012) reported that the use of different solvent polarity ad dielectric constant in the determination of each bioactives. A significant amount of terpenes are observed in the non polar extracts of RP-DCM, CA-DCM & AG-DCM (Table 1). But they are generally different from each other . Some of the reported biological

activity of tannins is to inhibits the growth of the microbes (Bhumi and Savisthrani, 2014). Saponins are known for its frothing actions and is traditionally used as a detergents and pesticides (Bhumi and Savisthrani, 2014). The saponin were observed to be higher in all the extracts of polar extract (Table 1) in comparison to those of the non polar extract. Saponins plays a vital role especially in the fight against the microbial gents and because of the non sugar molecules t is regarded as a good antioxidant, Hence, these study is in the support of all. Coumarins as a phytocompounds that were observed in all the extracts have been reported to to posses the antitumor activity. In a recent study, it indicates that 7-hydroxy coumarin inhibits the releases of cyclin D1, which is expressed in different types of cancer (Chen and Walsh, 2001). The different analogues of coumarins have different therapeutic applications such as chemotherapy, anticancer and others (Pelkonen *et al*., 1997). Different researchers have reported that anthroquinones are the characteristic of hydroxyanthroquinone drugs (Marino *et al.*, 1998; Podolak *et al*., 1998). They contain the c- and o bond molecules of sugar moieties of aglycone of sugar is used in the chinese traditional medicine (Budzisk *et al*., 2013).

Antiplasmodial screening of the plants extracts

The antiplasmodial activity of *V. lasiopus*, *A. gumifera* and *R. prinoid* have previously been studied, but to the best of our knowledge. This is the first time that *C. Macrostachychus*, *M. pyrifolia*, *C. anesata*, *C. volkensii* and *S. didymobotrya* are investigated for their anti-malarial activity. 16 extracts were prepared using the polar and non polar solvent of the crude drug were tested for the antiplasmodial activity (Table 2) using chloroquine and dihydroartemisin as the standard reference drug for the experiment (Table 2). The plants used for the study were all collected at the different places in their natural habitat in Machakos county of the lower eastern Kenya. Where they grew in their natural habitat and the local populace use them as the herbal recipes in the management of malaria and other related ailment. According to world health organisation WHO guideline and based on the previous studies (Jonville *et al*., 2008; Pink *et al*., 2005). The anti-plasmodial activity of any plants extracts are classified based on the followings; Highly active extracts with IC_{50}<5µg/ml, Promising active extract with IC_{50} values of 5-15µg/ml, moderate active extracts with IC_{50}>15µg/ml and inactive extracts with IC_{50}> 30µg/ml among all the 16 extract evaluated for the antiplasmodial activity against the strain of the extract of *C. volkensii* methanol CV-MeOH, *C. anesatata* dichloromethane CA-DCM and *C. macrostachychus* dichloromethane CM-DCM extract did not depicts the antiplasmodial activity even at a higher concentration. Despite of the long term claim of their efficacy in the malaria therapy by the locals (Table 2). Different studies have been characterized the antiplasmodial activity of most of the herbal drug extract. However, only few studies have been reported to have the long-term effect administration of the varying concentration of the herbal drugs against the malaria parasites (Amoah *et al*., 2015). This study was used to determine the IC_{50} values as well as long term effect of exposing the malaria parasites to the different doses of herbal recipe that could enhance the gametocyte production and the effectiveness against the Plasmodium parasites. The results is in agreement with the findings where the methanol extract of *M. oleifera* exhibited no activity against the *Plasmodium falciparum* strain (Kohlw and Jennet, 2012). After 48 hour of culture of the parasites in the presence of different extracts of varied concentration. The extract of *S. didymobotrya* methanol SD-MeOH, *S. didymobotrya* dichloromethane SD-DCM, *C. volkensii* dichloromethane CV-DCM and *V. lasiopus* methanol VL-MeOH with the following IC_{50} values of 5.00±0.35µg/ml, 2.37±19.70µg/ml, 2.53±0.48µg/ml, 4.50±1.7µg/ml exhibited high antiplasmodial activity (Table 2). The optimal increases in the gametocytes counts was accelerated by the gametocyte production in all the cultures treated with the herbal drugs, and this has been suggested to indirectly increases the gametocyte prevalence (Peterson *et al*., 2011). The phytochemical screening of where all the phytocompound of reveals the presence of the followings; alkaloids , flavonoids, phenols, anthroquinones, coumarins, terpenes, essensial oils and saponins (Tables 1 and 2). Due the variation in the percentage yields of each of the plants extracts, it was necessary it was necessary to repeat because of the species type (Solu *et al*., 2009) as well as parameters that could influence the phytochemical content of the plant. The medicinal plants collected in the different settings of geographical areas revealed to have the variations in their phytochemical content (Ntie-kang *et al*., 2014). Flavonoids and phenol bioactive compound have been identified in the study have been associate with the antiplasmodial activity (Odula *et al*., 1998) and this was the reason for the higher activity that was exhibited by the methanol extracts of *S. didymobotrya*, *C. volkensii* and *V. lasiopus* (Table 2).

Furthermore, the screening of *V. lasiopus* extract exhibited a high IC_{50} antiplasmodial values of. The chemistry of *Vernonia* species of such as *V. brachycalyx* and *V. amygdalina* have reported to posses the antiplasmdial activity (Oketch- Rabah, 1996). These

Table 2 : The summary if the results of the qualitative phytochemical screening of the plants under study.

Extracts/ Phytochemical	Alkaloids	Phenols	Saponins	Anthroqui-nones	Essensial oils	Flavonoids	Coumarins	Terpenes
RP-DC	++	++	++	++	++	++	++	++
MRP-MeOH	++	++	++	++	++	++	++	–
CM-DCM	++	++	–	–	–	–	–	++
CM-MeOH	++	++	++	++	++	++	++	++
MP-DCM	++	++	–	–	–	–	–	–
MP-MeOH	++	–	++	++	–	–	–	–
AG-DCM	++	++	++	++	++	++	++	++
AG-MeOH	–	–	–	–	–	–	++	–
CA-DCM	++	++	++	++	++	++	++	++
CA-MeOH	++	++	–	–	–	++	–	–
CV-DCM	++	++	++	++	++	++	++	++
CV-MeOH	++	++	++	++	++	++	++	++
VL-DCM	++	++	++	++	++	++	++	–
VL-MeOH	++	++	++	++	++	++	++	++
SD-DCM								
SD-MeOH								

Keys: (++)- Present (--)- Absent.

Table 3 : *In-vitro* anti plasmodial screening of the plants extracts.

Extracts	IC50 *P. falciparum* (µg/ml ± SD)
SD-MeOH	5.00 ±3.5
SD-DCM	2.37±1.70
CV-MeOH	2.53 ±1.48
CV-DCM	**
CA-MeOH	9.50±2.89
CA-DCM	**
CM-MeOH	6.60 ±1.53
CM-DCM	**
AG-MeOH	8.20 ±1.45
AG-DCM	4.50±1.75
VL-MeOH	8.10±3.05
VL-DCM	6.55±8.15
MP-DCM	2.39±1.95
MP-MeOH	16.45±0.55
RP-DCM	10.34±0.28
RP-MeOH	321 ±0.25
Ref. Drugs CQ DHA	15.28±0.19

Keys: **CQ**-*Chloroquine* **DHA**- *Dihydroartemisin* **- No effects

findings are consistent with the result of of the current finings in which the best activity are those of the methanol and dichloromethane extracts with the IC_{50} values of 4.50±1.75µg/ml and 8.10±3.05µg/ml, respectively. It would be interesting to investigate that plant further for the novel compound with a distinct antiplasmodial principle. *Rhamnus prinoid* exhibited a high antiplasmodial activity of IC_{50} 4.21±0.85µg/ml, 4.95±0.34µg/ml. All the extract of these plant demands a further studies due to their unique IC_{50} values which is classified as highly promising extract. Out of 16 extracts from 8 different plants species only three of them that did not depicts the antiplasmodial activity even at a higher concentration (IC_{50}> 30µg/ml) (Table 3). The lack of antiplasmodial activity in the aforementioned plants may not be the same as in the case of the *in vivo* screening since the compound may either acts as a prolong drug may need to undergo some metabolic reactions required for the desired activity. Apart, from the presence of the phytocompounds, it depends on other variables such as season, age in-tra species, variation, parts collected soil types, climatic factors and other non abiotic factors the lack of *in-vitro* screening of these plants does not imply that the plants is inactive as antimalarial plants, while the plants with inactive in-active to the in-vitro assay, may be against the in-vivo screening activity (Gessler *et al*., 1995). It is therefore important to undertake the in-vivo screening to validate the result of the antiplasmodial assay before drawing a final conclusion on the efficacy of their antiplasmodial potentials. Different

synthetic bioactives have prove to show the chloroquine sensitivity in resistant *Plasmodium falciparum*. However, little work was done on the reversal of the chloroquine sensitivity using medicinal plant (Rasoanaivo *et al.*, 1992). The recent study on the medicinal plants among the Akamba people of eastern Kenya have shown that alkaloids flavonoids and other bioactive compounds promotes the chloroquine action *in-vitro* studies.

Conclusion

After a thorough investigation on the selected medicinal plants. The phytochemical screeching and the *in-vitro* assay. It can be concluded that 95% of all the extract have exhibited an excellent antiplasmodial activity and these has justified the use of this these plant as antimalarials remedies. These plants could also be recommended as a template for the malaria therapy and administration in all Kenyan ethnomedicine especially in the rural areas where there is no conventional antimalarial drugs, health care infrastractures. *Vernonia lasiopus*, *Rhamnus prinoids* and *Sienna didymobotrya* could be recommended as chloroquine potentiation. The biochemical analysis of the plant could lead as the bedrock for the synthesis and development of a novel antimalarial drug candidates from the plants sources.

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Conflict of interest

The authors declares no conflict of interest.

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