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

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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 IC50 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±1.45 µg/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.

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 IC50 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±1.45 µg/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.

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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 (Macchinie 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 lasiopalides, elemanoids and epiveronalol (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-trypanosomal 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,
Anti-plasmodial screening of selected medicinal plants used in the treatment of malaria

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 (NaHCO₃) 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% N₂, 3% CO₂ and 6% O₂) and was incubated at 39°C 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

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![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. Vernonias lasiopus, H. Sienna didymobotrya.](image-url)
at (97% N₂, 3% CO₂ and 6% O₂) and left incubated at 39°C for 48-72 hrs following the incubation of the parasites. It was harvested and the fluorescence dyes as the results of the formation of the chelates with the Deoxyribonucleic acid (DNA) of the parasites and the fluorescence emission was measured at 520nm wavelength and the Inhibition maximal concentration (IC₅₀) values was determined using this formula:

\[
(\text{IC}_{50}) = \text{anti log} \left( \text{log} X_1 + [\text{log} Y_{50} - \text{log} Y_1] \times \frac{\text{log} X_2 - \text{log} X_1}{\text{log} X_2 - \text{log} Y_1} \right)
\]

Where, \(Y_{50}\) – The count per minute (CPM) values in a mid-way between parasitized and non-parasitized cultures \((X_1, Y_1, X_2, \text{and} Y_2)\) 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 (IC₅₀). 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 extrac¸ts 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 *Rubiacaeae*, *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
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, 2000). 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- pinene, 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

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

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Plants name/solvents</th>
<th>Abbreviation</th>
<th>Recovery (g)</th>
<th>Yield (%)</th>
<th>Voucher specimen</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>R. prinoid / dichloromethane</td>
<td>RP-DCM</td>
<td>16.20</td>
<td>8.10</td>
<td>IN/RP/JKUAT/001/2020</td>
<td>Leaves</td>
</tr>
<tr>
<td>2.</td>
<td>R. prinoid / methanol</td>
<td>RP-MeOH</td>
<td>42.38</td>
<td>21.14</td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>3.</td>
<td>C. macrostachychus / dichloromethane</td>
<td>CM-DCM</td>
<td>5.87</td>
<td>2.93</td>
<td>IN/CM/JKUAT/005/2020</td>
<td>Stem bark</td>
</tr>
<tr>
<td>4.</td>
<td>Cr. macrostachychus / methanol</td>
<td>CM-MeOH</td>
<td>20.53</td>
<td>10.21</td>
<td></td>
<td>Stem bark</td>
</tr>
<tr>
<td>6.</td>
<td>M. pyrifolia / methanol</td>
<td>MP-MeOH</td>
<td>10.62</td>
<td>5.31</td>
<td></td>
<td>Stem bark</td>
</tr>
<tr>
<td>7.</td>
<td>A. gumifera / dichloromethane</td>
<td>AG-DCM</td>
<td>8.42</td>
<td>4.21</td>
<td>IN/AG/JKUAT/003/2020</td>
<td>Leaves</td>
</tr>
<tr>
<td>8.</td>
<td>A. gumifera / methanol</td>
<td>AG-MeOH</td>
<td>9.48</td>
<td>4.24</td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>9.</td>
<td>C. anesata / dichloromethane</td>
<td>CA-DCM</td>
<td>20.57</td>
<td>10.23</td>
<td>IN/CA/JKUAT/009/2020</td>
<td>Leaves</td>
</tr>
<tr>
<td>10.</td>
<td>C. anesata / methanol</td>
<td>CA-MeOH</td>
<td>38.00</td>
<td>19.00</td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>11.</td>
<td>C. volkensii / dichloromethane</td>
<td>CV-DCM</td>
<td>18.98</td>
<td>9.49</td>
<td>IN/CV/JKUAT/011/2020</td>
<td>Leaves</td>
</tr>
<tr>
<td>12.</td>
<td>C. volkensii / methanol</td>
<td>CV-MeOH</td>
<td>41.92</td>
<td>20.41</td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>14.</td>
<td>V. lasiopus / methanol</td>
<td>VL-MeOH</td>
<td>28.49</td>
<td>14.24</td>
<td></td>
<td>Whole</td>
</tr>
<tr>
<td>15.</td>
<td>S. didymobotrya / dichloromethane</td>
<td>SD-DCM</td>
<td>20.43</td>
<td>10.21</td>
<td>IN/SD/ JKUAT/015/2020</td>
<td>Leaves</td>
</tr>
<tr>
<td>16.</td>
<td>S. didymobotrya / methanol</td>
<td>SD-MeOH</td>
<td>31.52</td>
<td>15.21</td>
<td></td>
<td>Leaves</td>
</tr>
</tbody>
</table>
activity of tannins is inhibits the growth of the microbes (Blumi and Savisthriani, 2014). Saponins are known for its frothing activities and is traditionally used as a detergents and pesticides (Blumi and Savisthriani, 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 it 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 hydroxanthroquinone 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 ailments. 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\textsubscript{50}<5µg/ml, Promising active extract with IC\textsubscript{50} values of 5-15µg/ml, moderate active extracts with IC\textsubscript{50}>15µg/ml and inactive extracts with IC\textsubscript{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\textsubscript{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\textsubscript{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 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\textsubscript{50} antiplasmodial values of the. The chemistry of Vernonioa species of such as V. brachycalyx and V. amygdalina have reported to posses the antiplasmodial activity (Oketch- Rabah, 1996). These
Table 2: The summary of the qualitative phytochemical screening of the plants under study.

<table>
<thead>
<tr>
<th>Extracts/Phytochemical</th>
<th>Alkaloids</th>
<th>Phenols</th>
<th>Saponins</th>
<th>Anthraquinones</th>
<th>Essensial Oils</th>
<th>Flavonoids</th>
<th>Coumarins</th>
<th>Terpnes</th>
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<td>RP-DC</td>
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<td>VL-DCM</td>
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<td>VL-MeOH</td>
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<td>SD-DCM</td>
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<tr>
<td>SD-MeOH</td>
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</table>

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

Table 3: In-vitro antiplasmodial screening of the plants extracts.

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

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 IC50 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 IC50 4.21±0.85µg/ml, 4.95±0.34µg/ml. All the extract of these plant demands a further studies due to their unique IC50 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 (IC50 > 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 phyto compounds, 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 infrastructures. *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.

**References**


Centre for disease control and prevention, Kenya (2021).


of Ethiopia for their anti-microbial properties and chemical profiles. J. Ethnopharmacol., 97, 421-427.


Omonyi, B.E., Bradley G and Afolayan A.J. (2012). Antioxidant and phytochemical properties of Carpobrotus edulis (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC...


