Full Length Research Paper

Anti-*Plasmodium falciparum* activity of *Aloe dawei* and *Justicia betonica*

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Malaria is a fatal disease caused by different *Plasmodium* species of parasites and has remained the major killer of humans worldwide especially the children under five years of age and pregnant women. In this study, the anti-Plasmodia activities of the crude leaf ether extracts of *Aloe dawei* (AD) and *Justicia betonica* (JB) on *Plasmodium falciparum* were investigated, with chloroquine diphosphate as a positive control. The results showed that ether extracts of JB had EC_{50} of 13.36 (95% CI: 8.032 to 22.23) µg/ml and AD had 7.965 (95% CI: 3.557 to 17.84) µg/ml. The chloroquine diphosphate had EC_{50} of 24.86 (95% CI: 9.239 to 66.89) µg/ml. The qualitative phytochemical analysis of the ether extract showed that JB contains steroids and triterpenoids, alkaloids and saponins while AD contained steroids and triterpenoids, anthraquinolones, alkaloids and saponins. The results provides evidence that JB and AD contain compounds with anti-*P. falciparum* activity and hence their use by the traditional herbalist and local communities in treatment of malaria.

Key words: Anti-*Plasmodium falciparum*, activity, EC_{50}, *Aloe dawei*, *Justicia betonica*.

INTRODUCTION

Malaria is a life-threatening parasitic disease caused by various protozoan species of *Plasmodia* organisms including *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium falciparum*; with *P. falciparum* being the most deadly parasite accounting for most malaria cases in humans worldwide (World Health Organization (WHO), 2012; Kaiser Family Foundation (KFF), 2013). Globally, according to the latest WHO estimates, it is reported that malaria accounted for about 219 million cases with more than 660,000 death in 2010, and in sub-Saharan Africa, it accounted for 174 million (81%) of the total malaria cases with 596,000 (90%) death and mostly the vulnerable groups like

children under the age of five years contributing to 85% and pregnant mothers (WHO, 2012; KFF, 2013). Malaria is reported to cost US$ 12 billion annually in Africa and in some African countries, it may cause up to 1.3% growth reduction per year as compared to countries without malaria (WHO, 2012; KFF, 2013).

In Uganda, malaria ranks among the third top killer diseases in the country (MCP, 2010; UMoH, 2010; UMoH and UNICEF, 2010). It is endemic in 95% of Uganda and it accounts for 40% of Ugandan public health expenditure (MCP, 2010; WHO, 2012). Malaria remains the major public health problem in the country with annual estimates of 10 million cases and 70,000 to 110,000

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annual deaths of which 91% are children below 5 years of age, and annually it accounts for approximately 30 to 50% of outpatient care, 15 to 50% of hospital admissions and 9 to 14% of inpatient deaths (Nankabirwa et al., 2009; UMoH, 2010; UMoH and UNICEF, 2010; WHO, 2012). However, due to the poor and fake antimalarials drugs (Newton et al., 2010; Nayyar et al., 2012; Björkman-Nyqvist et al., 2013), on the market together with the high cost of the available effective antimalarial drugs, many people especially the local communities and traditional herbalist in rural areas use medicinal plants to treat malaria cases (Elujoba, 2005; Pierre et al., 2011; Stangeland et al., 2011).

Traditional herbal medicines have been used for thousands of years to treat malaria worldwide, and about 1,277 medicinal plants from 160 families with antimalarial activities have been reported (Willcox and Bodeker, 2004; Karou et al., 2011; Stangeland et al., 2011; WHO and MSH, 2012). The two modern antimalarial drugs (Artemisins and Quinine) in use currently are derived from herbal medicines (Brunton et al., 2013). It is also reported that the proportion of people who use herbal medicines to treat malaria worldwide rages from 0 to 75%, with an overall average of 20% and this is because they are cheap and easily accessible to most rural people in various countries (Willcox and Bodeker, 2004; WHO and MSH, 2012). Many medicinal plants such as *Justicia betonica* and *Aloes* species have been reported to have anti-malarial activities and are used locally by the traditional herbalist and local communities in various part of Uganda to treat malaria (CITES, 2003; Lamorde et al., 2008; Lubia et al., 2008; Rutebemberwa et al., 2009; Stangeland et al., 2011; Guianas, 2013). The *J. betonica* (Kanchanapoom et al., 2004; Subbaraju et al., 2004) and *Aloes* species (CITES, 2003; Lubia et al., 2008; Karou et al., 2011; Guianas, 2013) have been reported to have various compounds with medicinal properties. However, the efficacy and safety of these medicinal plants is not known and this is coupled with the lack of standard dosages to be administered to malarial patients. This study therefore investigated the anti-*P. falciparum* activity of ether extract of *A. dawei* (AD) and *J. betonica* (JB) medicinal herbs that are locally used by various communities in Uganda to treat various conditions like malaria.

MATERIALS AND METHODS

An experimental study design was used to determine the activities of the ether leaf extracts of *A. dawei* and *J. betonica* on the *P. falciparum* parasites. These medicinal herbs have been reported to be used in various communities of Uganda to treat malaria (Lamorde et al., 2008; Lubia et al., 2008; Rutebemberwa et al. 2009; Stangeland et al., 2011).

Processing and extraction

The *A. dawei* (AD) and *J. betonica* (JB) were collected from Wakiso

district in Central Uganda. Botanical identification was carried out at the Makerere University herbarium. The leaves of both medicinal herbs were cleaned using water and then were dried in a shade until constant weight was obtained, in the Department of Pharmacology and Therapeutics laboratory, Makerere University College of Health Sciences. The dry leaves were then crushed by pounding in a wooden mortar into a fine powder. A total of 200 g of each medicinal herb powder was soaked in 500 ml of ether in Erlenmeyer flasks for 48 h. The mixture was filtered using a Whatman No.1 filter paper in a Buchner funnel. The filtrate was collected in a conical flask. The dry ether extract for both plants were obtained from the filtrate using a Heidolph model rotary evaporator (BUCHI Rotavapor R-205 model) and to obtain a complete dry ether extract, it was exposed at room temperature in dark sample bottles to prevent direct exposure to light that would cause oxidation of the compounds in the extracts for 24 h in order to allow complete evaporation of the ether solvent.

Qualitative phytochemical analysis of the ether extracts of *J. betonica* and *A. daweii*

The phytochemical compound in the ether extracts were determined using the following methods (Sofowora, 1993; Trease and Evans, 2002; Usman et al., 2009; Damodaran and Manohar, 2012).

Test for tannins

About 0.5 g each portion was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002; Usman et al., 2009; Damodaran and Manohar, 2012).

Test for anthraquinones

A few drops of 2% HCl were added to 1 ml of the extract. Appearance of the red color precipitate indicated the presence of anthraquinones (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Liebermann-Burchard test for steroids

To 0.2 g of each portion, 2 ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring that is, aglycone portion of cardiac glycoside (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Test for triterpenoids

A little of each portion was dissolved in ethanol. To it, 1 ml of acetic anhydride was added followed by the addition of conc. H_2SO_4. A change in color from pink to violet showed the presence of triterpenoids (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Test for terpenoids

A little of each portion was dissolved in ethanol. To it 1 ml of acetic

anhydride was added followed by the addition of conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Test for saponins

One gram of each portion was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 min. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Sodium hydroxide test for flavonoids

Few quantity of the each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002; Usman et al., 2009; Damodaran and Manohar, 2012).

Test for alkaloids

Few quantity of the each portion was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second, 1 ml Mayer's reagent was added and appearance of buff-colored precipitate will be an indication for the presence of alkaloids (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Fehling's test for free reducing sugar

About 0.5 g each portion was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars (Sofowora, 1993; Usman et al., 2009; Damodaran and Manohar, 2012).

Anti-P. falciparum activity study

The anti-P. falciparum activity of the JB and AD ether extracts was evaluated at the department of Microbiology, Makerere University, College of Veterinary Medicine, Animal Resources and Biosecurity, using standard methods (Moll et al., 2008; Kalra et al., 2006; WHO, 2001; Trager and Jensen, 1976).

Preparation of culture media and the P. falciparum organisms

The culture medium was prepared by dissolving 10.4 g of powdered Roswell Park Memorial Institute (RPMI) 1640 (Sigma-Aldrich Chemical Company, Munich, Germany) and 5.94 g of HEPES (N-2 hydroxyethyl piperazine-N-2-ethane sulphonic acid) (Sigma-Aldrich Chemical Company, Munich, Germany) in 1 L of sterile distilled water. The medium was filtered using 0.22 mm millipore filter, stored at 4°C ready to be used. The wash medium was prepared by adding 1.6 ml of 7.5 % (w/v) of $NaHCO_3$ (Sigma-Aldrich Chemical Company, Munich, Germany) to 43.4 ml of the medium above

(RPMI 1640 with HEPES) in 50 ml centrifuge tubes for use (Moll et al., 2008; Omoregie and Sisodia, 2012; Shujatullah et al., 2012). The wild P. falciparum organisms were obtained from the isolated parasites in blood samples with mono-infection in the Microbiology Laboratory which were obtained during the screening of patients with uncomplicated malaria after consent in Mulago hospital prior to malaria treatment. The P. falciparum organisms were cultured with the freshly prepared culture medium (Moll et al., 2008; Omoregie and Sisodia, 2012; Shujatullah et al., 2012).

The in vitro cultivation of P. falciparum isolates followed a modification of the standard culture techniques (Trager and Jensen, 1976). The culture medium consisted of RPMI 1640 (Sigma Aldrich), 2 g glucose, and 40 µg/ml gentamycin sulphate with supplemented 10% AB^+ serum. Culture medium was sterilised by filtration through a Millipore filter of 0.22 µm porosity and pH was adjusted to 7.4 by the addition of 4.2 ml of sterile 5% sodium bicarbonate. The plate was put in a candle jar and placed in the incubator set at 37.5°C for 24 to 30 h, depending upon development stage of the parasite. After 24 h incubation, a thin smear was prepared from the control well to see the mature schizonts, and if more than 10% schizonts were seen, it was considered to be valid; thick smears were prepared from each well by discarding the excess media with a micropipette (Moll et al., 2008; Omoregie and Sisodia, 2012; Shujatullah et al., 2012).

In vitro bioassay

The in vitro Micro-Test (MARK III) kit was used to determine the anti-P. falciparum activity of JB and AD ether leaf extracts (Moll et al., 2008; Omoregie and Sisodia, 2012; Shujatullah et al., 2012). The AD and JB ether extracts were each dissolved in a 0.2 ml of dimethylsulfoxide (DMSO) to facilitate the dissolution and topped up with distilled water to give a stock solution of concentration of 100 mg/ml. The chloroquine diphosphate was obtained from Sigma-Aldrich Chemical Company, Munich, Germany and was dissolved in 10% ethanol to a concentration of 1 mg/ml and diluted subsequently with culture medium to achieve the required concentrations required for the growth of the parasites. The preparation of ether and chloroquine diphosphate concentrations and the design of the test plates were based on the standard methods (Moll et al., 2008; Omoregie and Sisodia, 2012; Shujatullah et al., 2012). Standard drug (chloroquine) and extracts (at different concentrations of 1, 5, 10, 50, 100, 500, and 1000 µg/ml) were prepared in distilled water (chloroquine; Sigma) and DMSO (test extracts) and then serially diluted to achieve the required concentrations. All the culture plates with parasites and the ether extracts and chloroquine diphosphate were incubated at 37°C in a candle jar (5% CO_2, 17% O_2, 78% N_2) according to the method of Trager and Jensen (1976), for 18 to 48 h depending on the time taken by the parasite to develop to schizonts (Moll et al., 2008; Omoregie and Sisodia, 2012; Shujatullah et al., 2012). The synchronized culture with parasitaemia of 1.5 and 3% haematocrit were incubated in 96-well microtitre plate containing multiple concentrations of compounds/extracts for 48 h at 37°C in candle jar. Blood smears from each well were fixed in methanol, stained with Giemsa's stain and the numbers of infected red blood cells (RBCs) per 200 cells were counted. The parasite density was estimated as the number of mature schizonts per 200 white blood cells (WBC) from which the inhibitory concentrations of 50% of P. falciparum schizonts (EC_{50}) were determined using a log dose-response curve.

Determination of in vitro effective concentration (EC_{50}) values of the ether extract of JB and AD

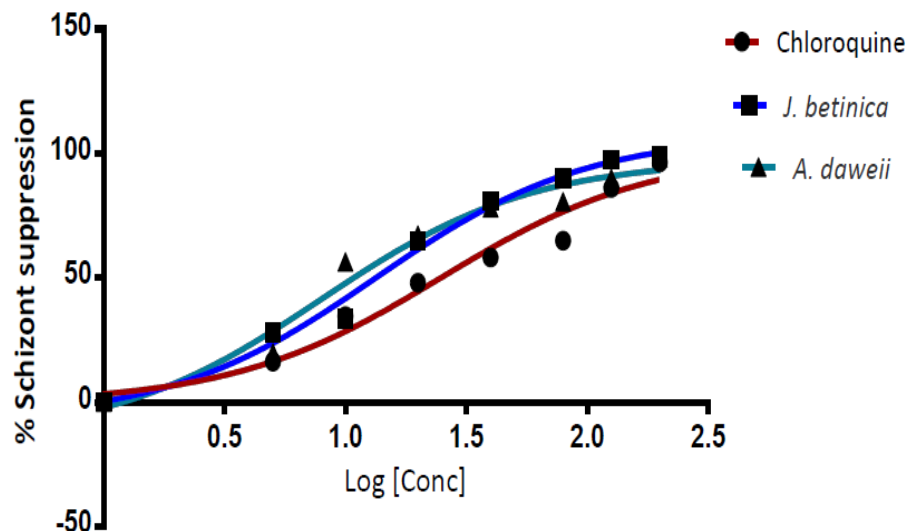The effective concentration (EC_{50}) defined as the drug concentration

Figure 1. Mean percentage schizonts suppression per 200 WBC against log concentration of the ether extracts of *Justicia betonica* and *Aloe dawei*

concentration required to suppress 50% of schizonts development were determined. The anti-malarial activities of the test extracts were expressed as 50% effective concentration (EC_{50}) determined from dose-response curve by non-linear regression analysis (curve-fit) using Graph Pad Prism (version 6) software at 95% confidence intervals (CI). All experiments were performed in triplicates and the results were expressed as percentage of *anti-Plasmodial* schizonts suppression. Crude extracts with EC_{50} values > 50 µg/ml were considered to be inactive (Kraft et al., 2003).

RESULTS

Anti-*P. falciparum* activity

The results of the anti-*P. falciparum* activity of JB and AD ether leaf extracts using the chloroquine diphosphate as control, showed that these extracts had anti-Plasmodia activity with the 50% schizonts suppression per 200 white blood cell (WBC) (EC_{50}) values of 13.36 (95% CI: 9.03 to 22.24) µg/ml, 7.97 (95% CI: 3.56 to 17.85) µg/ml and 24.86 (95% CI: 9.24 to 66.9) µg/ml, respectively (Table 1 and Figure 1). The qualitative phytochemical analysis showed that JB ether extract contained various compounds including the steroids and triterpenoids, alkaloids and saponins while the AD ether extract had steroids and triterpenoids, anthraquinones, alkaloids and saponins (Table 2).

DISCUSSION

The observed anti-*P. falciparum* activity of the ether extracts of JB and AD leaves with EC_{50} values of 13.36 (95% CI: 9.03 to 22.24) µg/ml and 7.97 (95% CI: 3.56 to 17.85) µg/ml, respectively could be attributed to the

presence of the alkaloids, anthraquinolones, steroids and triterpenoids and saponins compounds in both the medicinal herbs. Previous studies have shown that JB and AD contain similar compounds that could be responsible for the anti-*P. falciparum* activity (Kanchanapoom et al., 2004; Subbaraju et al., 2004). Also some of the current antimalarial drugs used in the management and treatment of malaria globally are plant alkaloids derived from medicinal plants such as quinine from *Cinchona* tree of plants and the sesquiterpenes lactone endoperoxides from artemisinins from *Artemisia annua* or sweet wormwood (Willcox and Bodeker, 2004; Brunton et al., 2013). Many medicinal herbs have been reported globally to have anti-malarial activities and most of them have been reported to contain various compounds especially the alkaloids (Stangeland et al., 2011; WHO and MSH, 2012). These herbs have been used traditionally to treat malaria for many years worldwide and currently about 1,277 medicinal herbs have been reported to have *anti-Plasmodia* activities (Willcox and Bodeker, 2004; Karou et al., 2011; Stangeland et al., 2011; WHO and MSH, 2012). The observed low EC_{50} value of AD as compared to the JB could possibly be due to the presence of the high concentrations of alkaloids, steroids and triterpenoids, saponins and anthraquinolones in the herb.

However, since the compounds in the extracts were used in the crude form, their EC_{50} values were not comparable to the EC_{50} values of the chloroquine diphosphate which was in pure form. But also, the EC_{50} value of the chloroquine diphosphate in this study was slightly higher than those from previous studies and this could be due to the fact the *P. falciparum* organisms used in the study, were wild type from clinical isolates and

Table 1. *Anti-Plasmodia falciparum* activity values with 50% schizonts suppression per 200 WBC (EC_{50}) of ether extracts of *Justicia betonica* and *Aloe dawei*.

Medicinal herb	EC_{50} (95% CI) µg/ml
Justicia betonica (JB)	13.36 (8.032 - 22.23)
Aloe dawei (AD)	7.965 (3.557 - 17.84)
Chloroquine disphosphate	24.86 (9.239 - 66.89)

Table 2. Different classes of compounds in ether extracts of *J. betonica* and *A. Dawei*.

Medicinal herb	Various compounds in the ether extracts						
	Steroids and triterpenoids	Anthraquinolone	Alkaloids	Reducing sugars	Tannins	Saponins	Flavonoids
JB	++	-	+++	-	-	+++	-
AD	++	+++	+++	-	-	+++	-

Key: +++ strongly present, + present, - absent, JB - *Justicia betonica*, AD - *Aloe daweii*

possibly these were resistant to chloroquine disphosphate thus requiring slightly a higher concentration to suppress the schizonts development in culture. The study therefore concludes that JB and AD medicinal herbs have compounds with anti-*Plasmodia* activity though their pharmacological mechanisms of action is not known and hence their use by the traditional herbalists and local communities in the management and treatment of malaria in Uganda is justified.

Conclusion

The medicinal herbs *J. betonica* and *A.daweii* contain compounds with anti-*P. falciparum* activity and this could be the reason why they are used by traditional herbalists and local communities in Uganda to manage and treat malaria.

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