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Alkaloids and Anti-malarial Activity of *Psidium guajava* Leaves Extract

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ABSTRACT

The potential use of plants as nontoxic, safe and alternative sources for malaria management and treatment by pregnant women has been investigated. The extraction of these plants is essential in isolating antimalarial agents with the aim of understanding their role in the treatment of malaria. Therefore, this study was designed to identify the presence of alkaloid in relation to antimalarial activity present in *P. guajava* leaves against *P.falciparum*. The crude extract was obtained after adjusting the pH of the aqueous mixture from 3.2 to 11.2 before (DCM:H_2O) liquid-liquid partitioning and concentration under reduced pressure to obtain the desired DCM crude extract. The antimalarial test was carried out at 24 and 48 hours incubation using a blood sample containing 5% parasitaemia. This was used to determine the antiplasmodial activity of the plant leaves extract. Qualitative phytochemical screening on all plant leaves tested positive for the presence of alkaloids. All the different concentrations of the plant leaves extracts; 10 $mgml^{-1}$, 5 $mgml^{-1}$, 2.5 $mgml^{-1}$ and 1.25 $mgml^{-1}$ tested positive for antimalarial activity when screened. The highest concentration (10 $mgml^{-1}$) showed the most elimination of the malaria parasite at both 24 and 48 hours. The leaves extracts of *Psidium guajava* showed 58% elimination of *Plasmodium falciparum*. The results of the antimalarial activity are promising and show that *Psidium guajava* could be used in the management of malaria by pregnant women during their first trimester.

Keywords: Antimalarial, alkaloids, *Psidium guajava* leaves, antimalarial drugs.

INTRODUCTION

The use of natural products especially plants for the treatment of ailments has been the longest medical practice globally [1, 2]. With herbalists prescribing variety of plants to treat and manage diseases, the plants are either given whole or as concoctions [3, 4]. Some plants such as

artemisia annua were traditionally used as concoctions to treat as several ailments. In the 1990's researchers in China embarked on a research to find antimalarial treatments for the soldiers and eventually isolated artemisinin as the active ingredient present in *artemisia annua* to treat malaria [5, 6].

Malaria is a treatable and avoidable disease caused by five *Plasmodium* parasite species, two species *P. falciparum*, *P. vivax* pose the greatest treat [7]. The most prevalent and deadliest *Plasmodium* parasite in the African continent is the *P. falciparum* which results in 96 % deaths of the 620,000 deaths caused by malaria globally in 2021 [7]. Quinine originally discovered from *Cinchona* tree and its derivatives were initially used in the treatment of malaria. However, rise in antimalarial resistance resulted in the search for alternatives [8]. The discovery and isolation of artemisinin as a potent antimalarial agent provided a solution for antimalarial resistance. In order to reduce cases of resistance, partial resistance and reduced efficacy; artemisinin are mainly used as a combination with other drugs (Artemisinin-based combination therapy (ACT)) [7, 9]. Though ACTs are largely used to treat malaria these antimalarials cannot be administered to pregnant women within the first trimester of their pregnancy due to severe birth defects associated with ACTs [10]. Currently, the use of oral quinine plus clindamycin is the recommended first line treatment administered to pregnant women during their first trimester [11]. However, resistance and easy access to these medications restricts low income families.

Psidium guajava leaves have been used traditionally by pregnant women to aid with pregnancy, treat gastrointestinal, malaria and microbial infections [12]. Several phytochemicals such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid have all been identified and isolated from the plant [13].

Therefore, the present study was designed to investigate the presence of alkaloid in *P. guajava leaves* and the antimalarial activity observed was tested.

MATERIALS AND METHODS

Plant material collection and identification

Leaves from *Psidium guajava* leaves were collected from Sabon-gari area in Daura, Katsina state, Nigeria. The leaves were then authenticated as *Psidium guajava* at the Biology Department of Umaru Musa Yar'adua University (UMYU), Katsina State, Nigeria.

Plant Preparation and Extraction

The leaves of *P. guajava* were washed with water then air-dried and pulverized to a powdered form. About 50 g of the ground guava leaves was macerated in 100 mL of ethanol for 72 h. The mixture was filtered and concentrated under reduced pressure to yield 5.5 g crude extract. About 100 mL of distilled water was added to the crude leaf extract to make a 100 mL crude mixture. The pH of the resulting mixture was adjusted from 3.2 to 11.2 by the addition of a few drops of concentrated sodium hydroxide solution. Liquid-liquid partitioning was carried out on the resulting mixture using 3 x 100 mL of Dichloromethane (DCM) both layers were collected. The DCM solution was concentrated under reduced pressure to afford a brownish residue.

Alkaloid Screening of DCM Extract

The presence of the phytochemical alkaloid was tested in the crude *P. guajava* leaves DCM extract previously obtained. All procedures were developed at room temperature. The extract was used for the subsequent qualitative analysis of metabolites using the method described by Junaid and Patil [14].

Antimalarial Screening of Crude DCM Extract

The antimalarial activity of the leaves extract of *P. guajava* was determined using blood sample with 5% parasitaemia collected from Bayero University Care Hospital Kano. Malaria parasite was identified by the ring shape of the immature trophozoites which retained blue colour of the stain. Parasitaemia levels and species parasites were recorded as described by Chotivanich *et al.* [15].

Separation of the Erythrocytes

Blood sample with 5% parasitaemia collected from Bayero University Care Hospital Kano was centrifuged at 2500 rpm for 15 min. After centrifugation, the supernatant (plasma) was discarded while the sediments (erythrocytes) were further centrifuged with normal saline at 2500 rpm for 5 min. The supernatant was then discarded and the erythrocytes were suspended in normal saline.

***In vitro* Anti-malarial Assay**

Psidium guajava leaf extract was screened for antimalarial activity against the *P. falciparum* strain. The *P. falciparum* strain was cultivated by a modified method described by Trager and Jensen [16]. The extracts were dissolved in DMSO. The final concentration of Dimethyl sulfoxide (DMSO) used was not toxic and did not interfere with the assay. Stock solution was prepared by dissolving 1 g of the extract in 1 ml of DMSO. Using serial doubling dilution, four different concentrations (10 $mgml^{-1}$, 5 $mgml^{-1}$, 2.5 $mgml^{-1}$ and 1.25 $mgml^{-1}$) of the extract were prepared. The antiparasitic effect of the compounds was measured by growth inhibition percentage as described by Carvalho and Krettli [17]. The culture media of 1 L of Roswell Park Memorial Institute (RPMI)-1640 liquid media was prepared by dissolving 10.4 g of the powdered RPMI 1640 into 1 L of distilled water and the autoclaving at 121 °C for 15 min. Exactly 0.5 mL of the extract solutions (10 $mgml^{-1}$, 5 $mgml^{-1}$, 2.5 $mgml^{-1}$, 1.25 $mgml^{-1}$) and 0.5 mL RPMI-1640 media were each transferred into their clearly labelled test-tubes. To each concentration of the extract, 0.1 ml of the malaria positive erythrocytes was added and shaken gently to ensure even distribution of the erythrocytes. The test tubes were transferred into a bell jar containing a burning candle. The cover of the bell jar was then replaced until the flame of the candle stopped burning. This supplied about 95% Nitrogen, 2% Oxygen, and 3% Carbon dioxide as described by Trager and Jensen [16]. The whole set up was transferred into an incubator maintained at 35 ^{o}C for 24 to 48 h. A control group consisting of culture media and positive erythrocytes (negative control) and culture media positive erythrocytes and anti-malarial agent Artemether (positive control) was also incubated along with the test concentrations. After 24 h of incubation, a thin smear from test tube was transferred onto clean glass slides and fixed in

absolute methanol (CH_3OH) then stained with Giemsa's stain. Each smear was observed under microscope using oil immersion to count the number of infected erythrocytes [16-18].

RESULTS AND DISCUSSION

Phytochemicals of the extracts of *P. guajava* leaves

The qualitative phytochemical analysis for alkaloid in the crude DCM extract showed the presence of alkaloids in the leaves of *P. guajava* plant. The pH of the crude extract which was initially 3.2 was adjusted to 11.2 to enable the efficient extraction of alkaloids.

In vitro anti-malarial assay

Psidium guajava leaves extract was screened for its *in vitro* antimalarial activity against the *P. falciparum* strain using artemether as control. The anti-malarial activity of the extracts was tested after 24 h and 48 h incubation respectively to determine growth inhibition of the parasite. At 24 h the death of some *P. falciparum* was observed at all concentrations of the crude extract with 10 $mgml^{-1}$ showing the highest death of 6 parasites and 1.25 $mgml^{-1}$ showing the lowest death 1 parasite. At 48 h the death rate at each concentration increased with 10 $mgml^{-1}$ still showing the highest death of 9 parasites and 1.25 $mgml^{-1}$ showing the lowest death of 2 parasites. On average after 48 h death of parasites were observed at all concentrations; 10, 5, 2.5 and 1.25 $mgml^{-1}$. Whereas, for the standard control (artemether) after 48 h the death of all the parasites were observed at the different concentrations 10, 5, 2.5 and 1.25 $mgml^{-1}$ (Table 1).

Table 1: Raw data analysis at each concentration activity of the extract and standard control (Artemether) after incubation.

Parasites dead after incubation period	Concentrations of DCM extract $mgmL^{-1}$			
	10	5	2.5	1.25
24 h	6	4	3	1
48 h	9	6	5	2
Artemether (control)- All parasites were dead after 48 h at all concentrations				

The percentage elimination at the end of the incubation was determined for all the concentrations of the crude extract (Table 2). The results showed a 58% elimination of the *P. falciparum*

parasite by the crude leave extract while the standard control (artemeter) showed 2% elimination at the end of the incubation.

Table 2: Anti-plasmodial activity of the extract and standard control (Artemether) against the malaria parasites

	Crude DCM Extract (at all concentrations)	Artemether (Control)
Average number of parasites before incubation	62	62
Average number of parasites after incubation	36	2
Total number of parasites dead after incubation	26	60
Percentage of elimination at the end of incubation. $\%== \frac{N}{Nx} x100$	58%	2%

Where N is the total number of the parasites after incubation and Nx is the total number of the parasites before incubation.

The phytochemical analysis of *P. guajava* leaves extract showed the presence of alkaloids which is attributed with possessing some pharmacological activities traditionally observed from the plant. Organic extraction was also carried out based on the physicochemical properties of bioactive natural product(s) of interest by qualitative analysis and by adjusting the pH of the aqueous extract before partitioning with organic solvent. The phytochemicals of interest being alkaloids was successfully tested and observed after the pH was adjusted to 11.2, alkaloids are basic in nature with a pH value of 10.5 that possesses high retention factors based on their polarity and mostly appear in the form of neutral molecules when isolated [19].

The crude DCM extract was then subjected to antimalarial screening against *P. falciparum* and artemether as the positive control (Table 1). The Table shows different concentrations of the leaves extract tested against *P. falciparum* at 24 and 48 h after incubation. At all the concentrations parasitic death was observed suggesting that bioactive compound(s) present in the

crude extract are anti-malarial agents [20]. At 1.25 $mgmL^{-1}$ the dead of the parasites observed was very slow after both 24 and 48 h incubation. From 2.5 to 10 $mgmL^{-1}$ the number dead parasites gradually increases with their highest death observed after 48 h. From literature alkaloids such as quinines are good antimalarial agents we postulated that an alkaloid was the likely bioactive compound responsible [8].

When the percentage elimination of the extracts was compared with that of the artemether (control) (Table 2) a significant difference in their elimination was observed. Artemether is a potent antimalarial used to treat both complicated and uncomplicated malaria cases but are not recommended during the first trimester of pregnancy [21]. Therefore, using artemether as a control was to access the level potency of our leaf extract in relationship to the artemether. Malaria drugs such as quinine which are alkaloids are widely recommended for use for in the treatment of malaria during the first trimester of pregnancy. The potency of the crude DCM extract in eliminating malaria parasite suggests that the bioactive compound present could be used as malaria prevention agent [22-23].

CONCLUSION

The qualitative analysis of *P. guajava* leaves extract showed the presence of the alkaloids. The antimalarial activity of the extract was screened at different concentrations against *P. falciparum* carried out. The results showed that the higher the concentration of the extract the more the antimalarial activity observed. The potency of the bioactive compound is much lower than that of the control (artemether) suggesting *P. guajava* leaves can be used in the management of malaria and its possibility of it being used during the first trimester.

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