Antimalarial activity and phytochemical analysis of aqueous leaf extract of Vernonia amygdalina

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ABSTRACT

Blood samples contaminated with Plasmodium spp (Malaria positive blood samples) were obtained from the Microbiology laboratory, Specialist hospital Sokoto and confirmed for the presence of this parasites before the analysis. Also, the leaves of Vernonia amygdalina were collected from villages around permanent site of Usmanu Danfodiyo University Sokoto and taken to the herbarium for authentication. Aqueous extract of the leaves were made at different concentration ranging from 20 mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml. It was observed that the extract showed a higher zone of inhibition (15.0±0.58) at 100mg/ml concentration on Plasmodium malariae and (14.00± 1.00) on Plasmodium falcifarum. Plasmodium ovale showed a moderate zone of inhibition of (14.00±0.58) while Plasmodium vivax showed the least zone of inhibition (13.00±0.57). The analysis was controlled with Coartem (14.3±0.58). The phytochemical screening of the aqueous leaf extract of Vernonia amygdalina was investigated which showed the presence of Saponins, Tannins, Alkaloids, Cardiac glycosides, Glycosides, Steroids and Anthraquinones which have an antimalarial activity on Plasmodium parasites.

Key words: Blood samples, Plasmodium spp, Vernonia amygdalina, Concentrations, Zone of inhibition and Phytochemical analysis.

INTRODUCTION

Malaria is a mosquito borne infections disease caused by a protozoan parasite or eukaryotic protist of the genus Plasmodium. It is widespread in tropical Africa and Sub tropical regions including part of America (22 countries), Asia and Africa [12]. Approximately 350-500 million cases of malaria are reported each year, killing about one to three million people the majority of whom are young children in sub Saharan Africa [12]. Ninety percent of malaria related death occur in sub Saharan Africa. Malaria is commonly associated with poverty which is a major hindrance to economic development [3].

Malaria parasite are members of the genus Plasmodium, phylum Apicomplexa within the order Coccidida, sub order Haemosporina, which comprises of various parasites found in the blood of reptiles, birds and mammals [4]. Five species of Plasmodium parasite can infect human, the most serious form of the disease are caused by Plasmodium falcifarum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae. The fifth specie Plasmodium knowlesi, is a zoonosis that caused malaria in Macaque but can also be transmitted to humans[11]. A circle variety of drugs of anti malarial drugs are available to treat malaria. In the last 7years, treatment of Plasmodium falcifarum infection in endemics countries has been transformed by the use of combination of drugs containing an Artemisinin derivatives. Severe infection of malaria is treated with intravenous or intramuscular quinines or increasingly, the Artemisinin derivatives, Artesunate [2] . Over the years the parasites have developed resistance to some of the
conventional antimalarial drugs must notably chloroquine[15], thus the re-emergence of traditional herbal medicinal formulations in the treatment of malaria [13].

Vernonia amygdalina is commonly used in traditional medicine. It’s leaf decoction is used to treat fever, malaria, diarrhea, dysentery, hepatitis and cough as a laxative and as a fertility inducer [5]. They are also used as medicine for scabies, headache, and stomach ache. Root extract are also use as treatment against malaria and gastrointestinal disorders. In Nigeria, leaves are placed on a wound as a substitute for iodine [5]. One of the most common medicinal uses of V. amygdalina is as a treatment against intestinal nematode. It is also useful as a control agent against disease in plant. The ash from burnt branches is used to control seed-borne fungi (Aspergillus, Fusarium and penicillium spp). Thus ameliorating seed viability and germination capacity, it has also been used for brewing beer as a substitute for hops. It is also a well known bee plant [14]. So, in traditional practices, Vernonia amygdalina (bitter leaf) is used in the management of parasitic infection most importantly, Malaria fever which is the most rampant of the parasitic infection. Therefore, this work is carried out to conform the rationale for the traditional use of this herbal medicinal plant.

MATERIALS AND METHODS

Sample Collection
Malaria positive blood samples were collected from Specialist Hospital Sokoto and confirmed for the presence of the different specie of malaria parasites. Fresh leaves of Vernonia amygdalina were collected from villages around permanent site of Usmanu Danfodiyo University Sokoto and were authenticated in the herbarium sections of the department of biological sciences of same institution. The leaves were shade dried and pulverized into powdered form [8].

Extraction of Plant component
Hundred gram (100g) of powdered leaf of Vernonia amygdalina was dissolved in 2000ml of distilled water for 24 hours at room temperature for extraction of plant component and subsequently vortexing the mixture. The mixture was filtered using a muslin cloth, the filtrate was dried using hot plate for two days at 45°C. The substrate was allowed to stand in the air for water to be evaporated completely so as to obtain the dried plant extract [13].

Confirmation of the Plasmodium Species
The malaria positive blood samples collected from Specialist Hospital Sokoto, were confirmed for the presence of Plasmodium rods. A drop of each blood was put on four different slides and a smear made. Leishman stain was used to stain the blood and after 2 min. a few drop of buffer solution of pH 6.5 was added and left to dry for 10 min. after which it was washed with distilled water and allowed to dry, a few drop of oil immersion was put on the slides and viewed under microscope using ×100 objective lens[9] Plasmodium falcifarum lactate dehydrogenase test (PLDH) was then used to confirm the different malaria parasites.

Media Used
Mueller Hinton agar medium and Salicin salt was used and it was prepared according to manufacturers instructions [9]. The inoculums were then inoculated by spread method using a sterile bent glass.

Antimalaria testing
Agar well method was used for the antimalarial testing, five holes were punched using a cork borer of 2mm in diameter and the different extract concentration were dispensed into each of the well respectively and allow to set for 1 hour, all the plates were incubated at 37°C for 24 hours. However, Coartem tablets steriley grinded into powdered form was used as a positive control.

Phytochemical Analysis
The test for Flavanoids, Tannins, Alkaloids, Saponins, cardiac glycosides, Glycosides, Volatile oils, Steroids, Arthraquines were carried out on the plant components as described by Oyeleke and Manga(2008).
RESULTS

The phytochemical screening of the plants extract showed the presence of alkaloids, tannins, saponins, glycosides, and cardiac glycosides, with arthraquines and steroids in trace amount. However, Volatile oils and Flavonoids were tested negative as indicated in Table 1. Table 2 showed the different diameters of the zone of inhibition as exhibited by the plant extracts at different concentrations. The largest zone of inhibition of the different concentrations on each of the different species of the Plasmodium parasites is recorded at the highest concentration (100mg/ml).With P. malariae (15.0±0.58), P. falcifarum (14.0±1.00), P. vivax (13.0±0.57), P. ovule (14.0±0.58) and the test control Coartem (14.3±0.58) showing no significant difference (P < 0.5) between the values of the zone of inhibition at different concentrations on the malaria parasites species.

Table 2. Antimalarial activity of Vernonia amygdalina at different concentrations.

<table>
<thead>
<tr>
<th>Malaria parasites</th>
<th>Concentration of extract in (mg/ml)</th>
<th>Zone of inhibition in (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20mg/ml</td>
<td>40mg/ml</td>
</tr>
<tr>
<td>P. falcifarum</td>
<td>6.0±0.58</td>
<td>10±1.58</td>
</tr>
<tr>
<td>P. vivax</td>
<td>5.0±1.52</td>
<td>8.0±1.0</td>
</tr>
<tr>
<td>P. malariae</td>
<td>7.0±0.57</td>
<td>8.0±1.64</td>
</tr>
<tr>
<td>P. ovule</td>
<td>8.0±1.50</td>
<td>9.6±0.58</td>
</tr>
<tr>
<td>Positive control</td>
<td>7.0±1.52</td>
<td>9.0±1.00</td>
</tr>
</tbody>
</table>

KEYS: The mean diameter of zone of inhibition is 2mm, therefore any zone of inhibition greater than 2mm indicates activity of extract and the values are mean ± standard deviation.

DISCUSSION

The result for antimalarial activity (Table 2) showed that the aqueous extract of Vernonia amygdalina leaves exhibit antimalarial activity on Plasmodium falcifarum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae even though some of these strains are resistant to conventional antimalarial drugs, they were susceptible to this herbal plant especially at higher concentration. as the activities of this extract increases with increase in their concentration. The aqueous extract of the plant is reported to be non-toxic to human health in a toxicity test carried out by [10] on the herbal plant. The antimalarial activity of Vernonia amygdalina was earlier reported by [1] in an in vivo antimalarial activity test of the plant against resistant P. berghei. In their findings, leaf extract produced 67 % suppression of parasitaemia in a four day test. The result of this work is in agreement with their result, though experimental methods differ. This work also agrees with [6], who reported the in vivo antimalarial activity of V.amygdalina . In his work, the aqueous extract of the plant was used to enhance the antimalarial effects of chloroquine in mice infected with chloroquine resistant and sensitive P. berghei strains.He revealed the potential of Vernonia to reverse chloroquine resistance when used as an adjuvant with chloroquine. This study validates the traditional use of V. amygdalina in the treatment of malaria in Nigeria.

The phytochemical analysis of the leaf extract of Vernonia amygdalina showed that the extract contains a significant amount of Saponins, Glycosides, Tannins, Cardiac glycosides, and steroids while sterol and anthraquinones are found in trace amount in the extracts. This is however in accordance to the work of [7]but part
of the analysis contradicts that of [10] that discovered the presence of flavonoids on the phytochemical screening carried out on the extracts of Vernonia amygdalina. It is therefore possible that the antimalarial activities of these leaves extracts are attributed to the presence of these phytochemical compounds which is higher at higher concentration.

REFERENCES


