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Isolation and Characterization of Anti-Plasmodial Compounds from *Sterculia setigera* Delile

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Abstract: *Dry stem bark powder (200g) of Sterculia setigera was percolated with ethanol (1L) and the crude extract was macerated with petroleum ether, n-hexane, ethyl acetate, chloroform, methanol and water. The fractions were screened for phytochemicals and anti-plasmodia activity using standard procedures. The phytochemical analysis revealed the presence of tannins, reducing sugars, glycosides, flavonoids, alkaloids, steroids and absence of saponins. The anti-malaria assay on the ethanol (0.1cm ^{3}crude) extract of S.setigera showed remarkable activity on Plasmodium falciparum, with the highest percentage elimination of malaria parasites at 10µg/ml. Assay-guided column chromatography on the ethanol extract (20g) of S.setigera resulted in the isolation of three flavonoids but when tested separately showed less effect on malaria parasites compared to the crude extract. The work provides a scientific basis for using stem bark of S.setigera as anti-malaria source in northern Nigeria.*

Key words: *Anti-plasmodial , Sterculia setigera , phytochemicals , flavonoids*

INTRODUCTION

Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Sirivastave *et al.,* 1996). The interest in the scientific investigation of these local and common medicinal plants from Nigeria is based on the popular use of these plants to combat many diseases including malaria fever. Therefore, research into the effects of these local medicinal plants is expected to enhance the use of these plants against malaria fever. However, most of the plants used in folk medicine have not been adequately studied (Kapoor *et al.,* 1969).

Malaria is a borne infectious disease caused by a protozoan parasite of the genus *Plasmodium.* The disease is widely distributed in tropical and sub-tropical Africa and South-west Asia. The species capable of infecting human includes only four; *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax and Plasmodium malariae,* (Kubmarawa *et al.,* 2007).

The malaria parasite, *Plasmodium falciparum*, because of its genetic diversity, has demonstrated an almost uncanny ability to evade the unfavorable conditions imposed by drug therapy. Hardy genotypes escape unharmed and pass along their resistance to progeny as sensitive organisms die off. Thus, to date, the vast majority of antimalarial therapies widely used have lost their usefulness over time (WHO, 2004). There comes the need to investigate the promising reported local medicinal plants around us, basically used as food, to counter the threat imposed by genetically specific *P. falciparum* of the same environment around us.

Resistance to antimalarial drugs has increased the global cost of controlling the disease. Therapeutic failure necessitates consultation at a health facility for further diagnosis and treatment, resulting in loss of working days for adults and absence from school for children. Studies in East Africa suggest that ineffective treatment causes anaemia, which renders children's health more fragile. In Central Africa, the appearance of chloroquine resistance led to an increase in hospital admissions because of severe attacks of malaria. Similarly, increasing mortality trends were found at the community level in Senegal. The impact of drug resistance can also be illustrated by the changes in the proportion of *P. falciparum* relative to other species of malaria parasites. For example, in India since the advent of drug resistance, *P. falciparum* accounts for more than 50% of all malaria attacks, instead of the previously reported 23%. (WHO, 2004). Malaria claims approximately 515 million people each year most of which are children, this is a large number compared to HIV/Aids.

Sterculia setigera Del. (*Sterculia*ceae) is used in trado-medicine by various indigenous communities. For instance, the Yorubas of Nigeria use a black soap prepared from black powder obtained from burnt mixture of the fruits and seeds in dermatosis. In Sudan, dried bark hot water extract is used for jaundice and dried stem bark for treating wounds. Stem bark decoction is used to treat diarrhoea by the Igedes, its bark as a mixture is macerated and used against dysentery by some tribes in central Nigeria (Wikipedia, 2013). The research study reports on the active compounds present in *Sterculia setigera* Del. responsible for its activity against malaria parasite.

MATERIALS AND METHODS

Sample Collection

Stem bark of Sterculia setigera were collected at Bomala area of Akko Local Government, Gombe State on 1st June 2011. The edible fruits, leaves, roots and stem bark were identified at the Department of Biological Science, Bayero University, Kano.

Extraction and Fractionation

Fresh air dried sample (200g) was percolated using Ethanol (1.00L) for a period of 14 days. The extracts were drained and concentrated under reduced pressure using Rota vapor (R110 at 40^0). The ethanol extract was allowed to dry, weighted and labeled SS01. The crude (SS01) extracts were macerated with petroleum ether, n-hexane, ethyl acetate, chloroform, methanol and water as demonstrated by Tor-anyin *et al.* (2003)**.** All partitioned fractions

obtained were collected separately and sequentially, labeled SS 1-01 to SS 1-63 (Sofowora, 1984).

Chemical Analysis

Plant extract were phytochemically screened using standard techniques for the qualitative detection of Alkaloids, Flavanoids, Resins, Steroids, Sugars, Tannins and Saponins (El-olemy *et al*., 1994; Sofowora, 1984; Evans, 1995; Mudi, 2009).

Malaria Parasite Assay

Sourcing of Malaria Parasite for the Assay

Infected human blood Samples containing *Parasitaemia erythrocytes* were collected from Murtala Ramat Mohammed Specialist Hospital, Kano in K3-EDTA coated disposable plastic sample bottles tightly fitted with plastic corks.

Preparation of test solution

A stock solution (10^6µg/ml) was prepared by dissolving the extract (1g) obtained from *S. setigera* in dimethyl sulphoxide, DMSO (1ml). A sub-stock solution (10^4µg/ml) was prepared by adding 0.1cm^3 of stock solution to 0.9ml DMSO. The following concentrations; 1000µg/ml, 100µg/ml and 10µg/ml were made serial dilution.

Determination of *Plasmodium falciparum* (initial count) using thin smear method.

A small drop of blood sample was placed at the centre of clean glass slide and covered with slip at 45^{o}C and drawn backward to make contact. Smears were formed by moving the cover slip forward on glass slide and immersed in methanol in Petri dish for 15 minutes. Geimsa's stain was dropped on each smear and allowed for 10 minutes, dried and observed microscopically under a high power objectives (x 100) using oil immersion after which an average initial count of 46 was determined from 3 different counts (Hanne *et. al.,* 2002).

Preparation of *Plasmodium falciparum* culture medium: RPM 1640

Various concentrations of the following reagents/solutions were combined to form the culture media; KCl: 5.37mM, NaCl:10.27mM, $MgSO_4$: 0.4mM, $NaHPO_4$: 17.73mM, $Ca(NO_3)_2$: 0.42mM, $NaHCO_3$: 2.5mM, $C_6H_{12}O_6$: 1.0mM. The media were sterilized using 40µg/ml Gentamicin Sulphate (Devo *et. al.,* 1985).

In-vitro Bioassay on Plasmodium falciparum culture

Each plant sample (0.1cm^3) was mixed with 0.2cm^3 of the culture medium in a tube containing 0.1cm^3 of 5% *Parasitaemia erythrocytes* and the %elimination were determined microscopically after an incubation period of 48 hours at 37^{0}C in a bell jar (5% O_2 gas, 93% NO_2 gas) using the formula below.

$$\%\ elimination\ of\ parasitaemia\ red\ blood\ cells\ by\ a\ sample = \frac{N}{N_x}\ x\ 100\%$$

Where;

N = Total number of eliminated Parasitaemia red blood cells = final count.

N_x = Parasitized Parasitaemia red blood cells = 46.

Column Chromatography of *S. setigera* Crude Extract

The extract (20g) (SS-01) was thoroughly mixed with silica gel to a non-sticky powder. 500 grams fluka Aldrich mesh silica gel was de-fattened using n-hexane and Chloroform in 1:1 ratio. The column was run by eluting solvent systems of varying polarities as shown: n-hexane (100%), n-hexane /Chloroform (1:1), Chloroform (100%), Chloroform/ Ethyl acetate (1:1), Ethyl acetate (100%), Ethyl acetate/Methanol (1:1), Methanol (100%). A series of 63 elutes were collected in succession with respect to proceeding solvent systems in increasing polarity and further subjected to TLC technique to obtain pre-pure compounds which were later pooled in to fractions and purified using micro column, prep.TLC, 2D TLC and Vanillin/H_2SO_4 spray which aided separation visibility. The result of micro column of reduces the number of elutes to SS1 to SS13.

Pooled fractions combined on the basis of purity by 2D-TLC was analyzed and the purity of all elutes were confirmed and tagged as SS 1-01 to SS 1-13, and some fractions was then tested against the malaria parasite (SS 1-07, SS 1-09 and SS 1-11) on the basis of high purity, abundance and appearance.

In-vitro Bioassay of SS 1-01 to Ss 1-13 on Plasmodium falciparum culture

Each fraction (0.1cm^3) was mixed with 0.2cm^3 of the culture medium in a tube containing 0.1cm^3 of 5% *Parasitaemia erythrocytes* and the %elimination were determined microscopically after an incubation period of 48 hours at 37^0C in a bell jar (5% O_2 gas, 93% NO_2 gas).

RESULTS AND DISCUSSION

The various extracts/fractions obtained were labeled and their weights and appearances were recorded (Table 1).

Table 1: Weights of various macerated fractions of *Sterculia setigera.*

Fractions	Coloration	Weight
A + Methanol	Reddish brown	4.50
A + $CHCl_3$	Dark brown	2.50
A + Ethyl acetate	Yellowish brown	3.00
A + n-C_6H_{12}	Brown	1.00
A + Pet-Ether	Yellowish brown	0.80
A + H_2O	Light brown	3.68

The phytochemical screening result showed the distribution of the presence of various secondary metabolites in the extract with absolute absence of saponin (Table 2).

Table 2: Phytochemical screening results of the stem-bark of *Sterculia* setigera.

Fraction	Tannins	Reducing Sugars	Glycoside	Flavonoids	Steroids	Saponins	Alkaloids
Ethanol	+	-	-	+	+	-	+
Methanol	+	-	+	+	+	-	-
Ethyl acetate	-	+	-	+	+	-	-
n-hexane	+	+	-	+	+	-	+
Petroleum ether	-	-	-	+	+	-	-
Chloroform	+	-	+	+	+	-	-
Aqueous solution	-	+	+	-	-	-	-

The results of antimicrobial activity of the extract are shown in Table 3. Various concentrations of the plant extract showed significant activity on the malaria parasite with the highest percentage elimination at 10µg/ml (Table 3).S Assay-guided column chromatography on the ethanol extract of S.setigera resulted in the isolation of three flavonoids but when tested separately showed less effect on malaria parasites compared to the crude extract (Table 5).

Table 3: Anti-plasmodium activity results of crude extract of stem-bark of *Sterculia setigera*

***Sterculia* setigera (SS01):**
10^3µg/ml: Final count = 12
N_x = 46
N = 46 - 12 = 34
(N/N_x) x 100% = 34/46 x 100% = 73.9%
10^2µg/ml: Final count = 28
N_x = 46
N = 46 - 28 = 18
(N/N_x) x 100% = 18/46 x 100% = 39.1%
10µg/ml: Final count = 43
N_x = 46
N = 46 - 43 = 3
(N/N_x) x 100% = 3/46 x 100% = 6.5%

Table 4. Weights of various Pooled fractions from column chromatography of stem-bark of *Sterculia setigera*.

Solvent System	Pooled or Single Fraction	Pooled Code	Mass in grams
n-hexane:$CHCL_3$	8	SS 1-01	0.3
--	9	SS 1-02	0.7
--	11-12	SS 1-03	0.57
--	13	SS 1-04	0.8
--	14-20	SS 1-05	1.9
$CHCL_3$:Et(OAc)	21-24	SS 1-06	0.9
--	25-28	SS 1-07	0.8
--	29-32	SS 1-08	1.3
Et(OAc)	33-34	SS 1-09	0.4
--	35	SS 1-10	0.1
--	36	SS 1-11	0.4
--	37-40	SS 1-12	0.7
--	41-42	SS 1-13	0.5

Table 5:. Antimalarial activity of the(active) pure compounds obtained from chromatographic analysis of stem-bark of *Sterculia setigera.*

Pure compound	Percentage elimination of malaria parasite at 1000(10^3)µg/ml
SS 1-07	64.55%
SS 1-09	38.64%
SS 1-11	49.90%
Combined SS 1-07+ SS 1-09+ SS 1-11	Percentage elimination of malaria parasite at 100(10^2)µg/ml 70.88%

CONCLUTION

Conclusively this work provides a scientific basis for using stem bark of S.setigera as anti-malaria source in northern Nigeria.

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