



SCREENING OF *CASSIA SINGUAENA*, *COMMIPHORA KERSTINGII*, *KHAYA SENEGALENSIS* FOR BRINE SHRIMP LETHALITY AND ANTIPLASMODIUM ACTIVITY

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ABSTRACT

Extracts of *Cassia singueana*, (twigs), *Commiphora kerstingii* (stem) and the bark of *Khaya senegalensis* were screened for antimalaria activity. Brine shrimp lethality assay was also employed as activity guide. Chloroform extracts of *C. singueana* was found to be the most active in both brine shrimp lethality (BST) and anti-malaria assay. The aqueous extract of *K. senegalensis* and *Commiphora kerstingii* were found to be in-active against brine shrimp larvae. The phytochemical analysis of crude plants under investigation showed presence of saponins, tannins, glycosides and sterols.

Keywords: Screening of *Cassia singueana*, *Commiphora kerstingii*, *Khaya senegalensis*, brine shrimp test.

INTRODUCTION

Medicinal plants contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1982; Basile *et al.*, 1999). Africa in general and Nigeria in particular where malaria fever is one of the most prevalent diseases still rely on traditional medicine as an alternative medicine. *C. singueana* Del. (Fabaceae), *C. kerstingii* Engl. (Burseraceae) and *K. senegalensis* Desr. (Meliaceae), grow in the savanna regions to humid forests of Northern Nigeria. The plants are found in Kano, Sokoto, Bauchi and Plateau States of Nigeria (Blench, 2005).

The aerial parts of *C. singueana* "Runfu" in Hausa, together with leaves of *Tamarindus indica* are boiled together in a form of concoction for the treatment of pyrexia and malaria.

C. kerstingii ('Arrabii' Hausa) is used for the treatment of diarrhea and dysentery in Hausa folk medicine. The methanol extract of the stem bark of the plant exhibited remarkable antimicrobial activity due to presence of flavonoids and alkaloids (Ajoku *et al.*, 2007).

The bark of *K. senegalensis* "Madachi" in Hausa is bitter and is commonly used as anti-malaria and stomachache reliever agents. The plant is also used in the indigenous system of medicine in Burkina Faso to treat inflammatory diseases (Lombo, 1993), fever and gastrointestinal pain (Kerharo and Adams, 1974). Traditional medical practitioners in some parts of Nigeria use the seed oil of *K. senegalensis* to treat diabetes mellitus (Ayo *et al.*, 2007). The water, methanol, chloroform, and petroleum ether extracts of the plant showed some degree of antibacterial activity (Umeh *et al.*, 2005), while the ethanol extract of the stem bark was found to be associated with antioxidant activity (Darius, 2007). The limonoides components which were isolated from the stem bark of *K. senegalensis* were found to have insecticidal properties (Amit and Sheilendra, 2007).

Scopoletin, β -quercitin, and rutin were also isolated from the leaves of the plant (Olmo *et al.*, 1997).

C. singueana, *C. kersitingii*, and *K. senegalensis* were employed in this paper to study the antiplasmodium activity as well as the cytotoxicity in brine shrimp larvae (BST) of the three plants.

MATERIAL AND METHODS

Plant Materials

The plant materials used in this study were collected in September, 2007, from Yako village in Kiru Local Government Area of Kano State, Nigeria. The plants were identified by Mallam Baba Ali Garko (Former Staff of Bayero University Herbarium, Kano) and authenticated by Dr. B.S. Aliyu, Department of Biological Sciences, Bayero University, Kano.

Extraction and Fractionation

Air-dried and ground plant materials were extracted and fractionated as described by Fatope *et al.*, (1993). Each plant sample (200g) was percolated with absolute ethanol and the residue (F₁) was solvent partitioned to give chloroform (F₂), ethylacetate (F₃), and water (F₄) soluble fractions. The chloroform soluble, fraction of each plant was further partitioned between n-hexane and methanol to give n-hexane (F₅) and methanol (F₆) soluble fractions. All the fractions were concentrated in vacuum, weighed, and stored in a freezer until tested.

Brine Shrimp Lethality Test (BST)

Each plant extract was screened against brine shrimp larvae of *Artemia salina* according to protocols described by Mayer *et al.*, (1982) and Fatope *et al.*, (1993). In this test, sea water obtained from Lagos Beach was used to cultivate the larvae. Dimethylsulphoxide (DMSO) was added to test and control vials to enhance the solubility of test materials.

Antimalaria Assay**Collection of Blood Sample**

Human blood samples positive for *Plasmodium falciparum* were collected from Nassarawa Hospital, Kano and transported to the laboratory in potassium edentate (K₃EDTA) in a thermocool flask at 4°C as demonstrated by Dacie and Lewis (1968). The blood samples were kept in a freezer until use.

In Vitro Test

The assay was performed using RPMI 1640 as the culture medium used for cultivation of *P. falciparum* as demonstrated by Devo *et al.*, (1985). Dilution was prepared from each plant extract and the final concentrations prepared by dilution were 5000, 2000, 1000 and 500 µgml⁻¹. Controls were prepared without the plant extracts. Each test extract (0.1ml) and culture medium (0.2ml) were added into vial containing 0.1ml of 5% parasitemia erythrocyte and was then mixed thoroughly. The sensitivity of the parasites to the test fractions was determined microscopically after incubation for 24 and 48 hours at 37°C.

Phytochemical Screening of Plant Extracts

All the plants fractions obtained from *C. singueana*, *C. kerstingii* and *K. senegalensis* were screened for the presence of phytochemicals using methods described by Sofowora (1984).

RESULTS AND DISCUSSION

Preliminary screening of solvent partitioned extracts of *C. singueana*, *C. kerstingii* and *K. senegalensis* against brine shrimps showed that about 90% of the plant extracts were active in BST (Table 1). Only the aqueous extracts of *C. kerstingii* and *K. senegalensis* were inactive at LC₅₀ 1000 µgml⁻¹. The ethanol (F₁) and ethyl acetate (F₃) soluble fractions of *C. kerstingii* and *K. senegalensis* showed very high activity in BST both at LC₅₀ 1.7 µgml⁻¹ (See Table 1). While the cytotoxicity in BST of all the tested fractions of *C. singueana* were moderate. These results suggest potency in the plants extracts under investigation. The development of antimalaria drugs was facilitated when *P. falciparum* was successfully cultured (Trager and Johnson, 1976). Results (Table 2) shows a good antiplasmodium potency at high concentrations of all the fractions of the three plants.

Phytochemical screening result (Table 3) revealed the presences of saponins, glycosides and sterols in the targeted plants.

Table 1: The activity (in BST) of the various extracts form the plants under investigation

Plants Sample	Ethanol (F ₁) µg/ml	Chloroform (F ₂) µg/ml	Ethylacetate (F ₃) µg/ml	Aqueous (F ₄) µg/ml
<i>Cassia singueana</i>	16.14	10.58	12.50	31.52
<i>Commiphora kerstingii</i>	679.29	147.34	1.70	>1000
<i>Khaya senegalensis</i>	1.70	116.97	133.30	>1000

LC₅₀ is determined at 95% confidences interval

Table 2: The anti-plasmodium activity results (in percentage) for various fractions of *Cassia singueana*, *Commiphora kerstingii* and *Khaya senegalensis*.

Plants sample	Conc. µg/ml	Ethanol (F ₁) %	Chloroform (F ₂) %	Ethylacetate (F ₃) %	Aqueous (F ₄) %	n-hexane (F ₅) %	Methanol (F ₆) %
<i>Cassia singueana</i>	500	85.7	96.4	82.1	50.0	98.6	82.1
	1000	92.9	96.4	96.4	96.4	96.4	89.3
	2000	96.4	100	96.4	100	84.3	96.4
	5000	100	100	100	100	100	100
<i>Commiphora kerstingii</i>	500	82.1	82.7	75.0	75.0	82.1	82.1
	1000	82.1	89.9	89.3	89.3	82.1	82.1
	2000	96.4	100	89.3	100	92.9	92.9
	5000	100	100	96.4	100	100	96.4
<i>Khaya senelensis</i>	500	82.1	75.0	64.3	35.7	78.6	67.9
	1000	89.3	92.9	82.1	75.0	89.3	92.1
	2000	96.4	100	92.1	100	96.4	92.1
	5000	100	100	100	100	100	100

Table 3: Phytochemicals of crude plant extract of *Cassia singueana*, *Commiphora kerstingii* and *Khaya senegalensis*.

Phytochemicals components	<i>Cassia singueana</i>	<i>Commiphora kerstingii</i>	<i>Khaya senegalensis</i>
Saponins	+	+	+
Tannins	+	+	-
Flavonoids	-	-	+
Alkaloids	-	-	-
Glycosides	+	-	+
Sterols	+	-	+
Sugars	-	-	+

Key: + = present, - = absent

CONCLUSION

From the BST results, ethyl acetate and ethanolic soluble fractions of *C. kerstingii* and *K. senegalensis*, showed the highest activity in BST at LC_{50} $1.7 \mu\text{gml}^{-1}$ while 95% of the fractions of *C. singueana* showed moderate cytotoxicity in BST and anti-plasmodium activity. These results suggest that, further study

should be carried out on *C. singueana* to isolate and synthesize anti-malaria drugs. However, the phytochemical results predict some secondary metabolites such as tannins, glycosides, sterols and alkaloids which may be responsible for the activity of these plants especially *C. singueana*.

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