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ANTIPLASMODIAL EVALUATION AND PHYTOCHEMICALS SCREENING OF EXTRACTS OF ERYTHRINA SENEGALENSIS (FABACEAE)

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ABSTRACT

Powered stem -bark of *Erythrina Senegalensis* was extracted with ethanol and macerated with *n*-hexane chloroform, ethylacetate, methanol and water. The extract and fraction were tested for Antiplasmodial activity. Phytochemicals analysis of the plant extract and fractions todetect the presence of secondary metabolic was also carried out. Sensitivity test result showed that both the extract and fractions were active against the malaria parasite. The Phytochemicals analysis revealed the presence of alkaloids, Saponins, tannins, Flavonoids, steroids and resins.

INTRODUCTION

Medicinal plants contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1982). Medicinal plants, depending on the ecological, historical and medicinal background of each community, provide the basic health care. Traditional medical practitioners, alongside the modern drugs researchers, are believed to have driven their basis for the usage of plant parts for the therapeutic purpose through observance of its effect on a particular target.

The discovery of active constituents from plants starts with the collection of

relevant plant part, usually based on ethno-medical claims; collection, drying and extraction of plant material.

Erythrina Senegalensis is mostly tropical or subtropical, moderately growing shrub about 5-7 inches high with greenish leaves (Gillman, 1997). It is locally used in the treatment of gastrointestinal disorders (Ajoku, 2003).

The predominantly common usage of the plant in the treatment of burnings, malaria, gastrointestinal and dermatological disorders attracts the attention for the need to screen and establish scientific facts about it.

The study was conducted with the motive and objective of determining the Phytochemicals constituents present in it, and the sensitivity of malaria parasite to its extract and fractions.

MATERIALS AND METHODS

Collection and identification of plant material;

The stem bark of *Erythrina Senegalensis* was collected from Rimin Gado Local Government area, Kano State, and identified by Baba Ali of the Biological Science Department, BUK. The stem bark was air dried and grounded into powder using mortar and pestle as described by Muktar and Tukur (1999).

EXTRACTION

The powdered plant material was subjected to ethanol extraction using technique described by Fatope and Hamisu (1993).

Ethanol Extract

The air-dried sample (200g) was percolated using ethanol (700ml) for a period of 14 days. The extract obtained was concentrated at 4-10 °C using a rotor evaporator machine. The resulting Extract was air-dried and labeled (ES1).

The crude (ethanol) (ES1) was macerated using extracts (10g) chloroform, n-hexane methanol, ethylacetate and water. The macerated fractions were dried by exposing to air at room temperature. (Sofowora et. al. 1986).

n-Hexane Fraction

Ethanol extract (10g) was first macerated with about 150ml of n-hexane for several macerations. The fractions (ES1) obtained was evaporated to dryness. The residue was further macerated with chloroform.

Chloroform Fraction

The residue from above was further subjected to maceration with about 100ml of chloroform for several times until the solvent no longer dissolves any of the residues. The solution was then air-dried for concentration at room temperature, and labeled (ES3)

Ethylacetate Fraction

The residue from above was further macerated with about 100ml of Ethyl acetate solvent. The same procedure involved in the above was used. The resulting solution was collected, air-dried, labeled (ES4) and kept.

Methanol Fraction

Solution obtained from dissolving the residue above using 100ml of methanol, was collected air-dried for concentration, labeled (ES5) and stored for further analysis.

Water Fraction

The last part of the residue from above was macerated with distilled water. More of the water was added to ensure possible dissolution of the residue. The resulting solution was concentrated at room temperature, labeled (ES6) and kept for further analysis.

Phytochemicals Screening

The fractions were subjected to Phytochemicals screening to determine the secondary metabolic present in the plant material.

Test for Alkaloids

To 1.0ml of each fraction in a separate test tubes, 2-3 drops of Dragendorff's and Meyer's reagents were added. An orange red precipitate with the Dragendorff's reagent or white precipitate with Meyer's reagent denotes the presence of alkaloids.

Test for Saponins

To 0.5g of the powder in a test tube, 0.5ml of distilled water was added and shaken vigorously. A persistent froth which last for at least 15 minutes indicates the presence of Saponins.

Test for Flavonoids

To 2ml of 4mg/ml of each fraction, a piece of magnesium ribbon was added followed by Conc. HCl in drops. Presence of Flavonoids is indicated by the appearance of a range of colour, crimson to magenta. (Sofowora, 1993).

Test for Steroids

Chloroform (3ml) was added to each extract solution (2ml), followed by addition of few drops of acetic acid anhydride, and concentrated HCl acid (5-6 drops), by means of pipette. A brown ring at the interface indicates the presence of steroids.

Test for reducing sugars

To 1ml of each fraction, 2.0ml of distilled water, followed by Fehling's solution, was added and the mixture warmed. Brick-red precipitates was formed at the bottom of the test indicates the presence of reducing sugars. (Muhammad, 1994).

Test of Tannins

Two to three drops of 5% ferric chloride (FeCl_3) solution was added to 2ml of each extract, diluted with distilled water. A green-black coloration denotes the presence of tannins (Sofowora, 1984).

Bioassay

A stock solution (10, 000 $\mu\text{g/ml}$) was prepared by dissolved the extract (20mg) obtained from *E. Senegalensis* in dimethyl Sulphoxide, DMSO, (2ml), using the dilution formula $C_1V_1 = C_2V_2$ from the stock solution, the following concentrations were made: 500 $\mu\text{g/ml}$, 1000 $\mu\text{g/ml}$ and 5000 $\mu\text{g/ml}$.

Where, C_1 = initial concentration

V_1 = initial volume

C_2 = Final concentration

V_2 = Final volume

Sourcing of malaria parasite for Assay

Malaria parasite of infected blood sample containing a heavy parasiteamia of plasmodium falciparum were collected from the Department of Hematology, Malam Aminu Kano Teaching Hospital (AKTH). The samples were received in K3-EDTA coated disposable plastic sample bottles, with tightly fitted plastic corks,

and transported to the Microbiology Laboratory of Bayero University Kano.

Determination of plasmodium falciparum (position Blood samples) using thin smear method.

The method followed was as described by Decie and Lewis (1998). Using a clean capillary tube, a small drop of each blood sample was placed at the centre of a clean glass slide at least 2mm from one end. A cover slip placed at an angle of 45° in front of each drop and drew backward to make a contract with each was ensured. Smears were formed by moving the cover slip forward on each glass slide. The thin smears were immersed in methanol contained in a Petri dish for about 15 minutes. Giemsa's stain was dropped on each smear, and allowed to stay for about 10 minutes. Excess stain was washed with clean tap water. The smears were dried in air by hanging the glass slides inverted on a rack. Each dried smear was microscopically observed under a high power objectives (X100) using oil immersion. An average parasiteamia was determined using the reading of 3 microscopic fields (Hanne et.al, 2002). Separation of the Erythrocytes (5% Parasiteamia) from the serum of the Blood samples 50% dextrose solution (0.5ml) was added to each of the blood samples (5ml) and defibrinated, and then centrifuged at 2500rpm for 15 minutes in a spectra Merlin centrifugation machine. Supernatant layers were separated from their sediments. The latter were diluted with normal saline (Decie, 1968) and centrifuged at 2500rpm for 10 minutes further. The resulting supernatant was discarded. Sample with higher parasiteamia (above 5%) were diluted with fresh malaria negative erythrocytes (Hanne et.al, 2002).

Preparation of plasmodium falciparum culture medium

Blood sample (2ml) from the main vein of a healthy rabbit were withdrawn using a disposable 5ml syringe (BD 205WG). The sample was defibrinated by allowing settling for at least 45 minutes (Lewis, 1968). The defibrinated blood sample was further centrifuged at 2500rpm for 10 minutes.

The supernatant layer was collected and sterilized. The separated sediment was further centrifuged for about 5 minutes. The supernatant layer was added to the first one in a test tube. The sediments were discarded. The Serum was supplemented with the RPMI 1640 salt medium and sterilized with gentamacin sulphate (Trager, 1982).

The composition of RPMI salt is as described below:

KCl 5.37mm, NaCl 10.27mm, mgso4 2.56mm, NaH₂PO₄ 17.73mm, Ca(NO₃) 2 0.42mm, NaHCO₃ 2.5mm, and glucose 11.0mm (Dero et. al, 1985).

in-vitro Assay of the Activity of the Extract on plasmodium culture.

A tested solution (0.1ml) and the culture medium (0.2ml) were added into a test tube containing 5% parasitemia erythrocytes and mixed up thoroughly. The sensitivity of the parasites to each tested fractions at concentration of 500, 1000, 2000, and 500µg/ml was determined

Table 1

Plant Fraction	Fraction Code	Texture	Colour
Ethanol	ES1	Oily	Dark brown
Ethyl acetate	ES2	Crystal	Dark brown
Chloroform	ES3	Gummy	Greenish
Methanol	ES4	Solid	Yellow
n- hexane	ES5	Gummy	Whitish
Distilled water	ES6	gummy	Whitish

microscopically at 37°C after 24 and 48 hours of incubation. The incubation was carried out under a bell jar system with a lighted candle that ensured the condition being atmospherically inert (Muktar et. al, 2006)

Determination of the Activity

After 24 and 48 hours of incubation, an aliquot of the culture medium was dropped on a microscopic slide and stained with Gyms material. The average percentage elimination of the erythrocytes that appeared as blue discoid cells was determined using the formula below:

$$\% = N/Nx \times 100$$

Where, % = percentage activity of the extracts

N =total number of cleared red Blood cells (RBC)

Nx = Total number of Parasitized RBC

RESULTS

The extract of this plant, Erythrina Senegalensis, was found be only in texture, Dark brown in colour, and active against the parasite. The appearance of various solvent fractions and their activities against the malaria parasites is shown in table 1 and table 3 respectively as shown below. The presence of secondary metabolic in each fraction is shown in table 2.

Discussion

In the preliminary Phytochemicals analysis it is shown that the distribution of the secondary metabolisms was variant at different fractions tested, with the crude ethanolic extracts containing alkaloids, tannins, Flavonoids, steroids, and sugars. The plasmodia analysis of the different fraction obtained from the stem-bark of *Erythrina Senegalensis* showed an appreciable activity against the malaria parasite. At the concentrations of 2000 and 5000µg/ml, the ethyl acetate fraction

of the stem-bark exhibit the highest activity of 86.0% and 93.0% parasite elimination, respectively. The chloroform solution fraction has shown a remarkable antiplasmodial activity at the above concentration with 79.1% and 81.4% parasite elimination, respectively.

Conclusion

Erythrina Senegalensis extract and fractions were found to exhibit a significant Antiplasmodial activity on the malaria parasite positive blood sample tested.

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