



PHYTOCHEMICAL SCREENING AND SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ETHYLACETATE SOLUBLE EXTRACT OF *ERTHRINA SENEGALENSIS*

¹SANI, K.D., and ¹HARUNA M. I.

Department of Pre-ND Sciences, Audu Bako College of Agriculture, Dambatta, Kano, Nigeria.

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ABSTRACT

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Correspondence author:

e-mail:

kamalsani2007@yahoo.com

Phone:

+234-806-7040618;

.Crude ethanolic extracts of stem bark of *Erythrina senegalensis* was macerated with ethyl acetate, and the resulting fraction was labeled E₁. The fraction was screened to detect the presence of secondary metabolites. Alkaloids, flavonoids, steroids, saponins, tannins and reducing sugars were found. Column chromatographic analysis of the Extract was conducted using varying solvent ratios of petroleum ether, chloroform, ethylacetate and methanol. The chromatographic fractions of similar retention time (R_x) and TLC pattern were pooled and micro chromatographed. Bioassay of the pooled fractions was carried at different concentrations: 500µg/ml, 1000µg/ml, 2000µg/ml and 5000µg/ml. the pooled fraction with the highest percentage elimination of plasmodium parasite was recorded.

INTRODUCTION

The use of natural products in the traditional medicine has been enormously pronounced as traced back to human history. Some plants are general while some are specific with a fast tracking record in alleviating diseases, if not in preventing it. (Lonella, 1997). The Egyptian medicine written in about 1550 B.C. includes the use of so many plants, historical background, specifically described dosage and instructions for herbal remedies (Wikipedia, 2008). Natural products formed the bedrock foundation

in the development of drugs. Reports have shown that almost half of the best selling drugs were either of plant origin or their derivatives (Reuter/Zog by, 2000).

Malaria is caused by *Plasmodium falciparum*. It kills three out of every ten under the age of five, and one out every ten women during the first pregnancies (Ebong and Adiele, 2001). *Plasmodium falciparum* is considered the most transmissible parasitic diseases (Mosanya, 2001).

Aims and Objectives

The research work is aimed at screening the ethylacetate fraction of crude ethanolic extract of *Erythrina senegalensis* for detecting the presence of secondary metabolites and its anti plasmodial effect at various concentrations.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh stem bark of *Erythrina senegalensis* was collected from Rimin Gado Local Government Area of Kano State, Nigeria. The taxonomical identification of the plant was confirmed by a botanist in the Biological Sciences Department, Bayero University, Kano.

Preparation of Extract

The air-dried sample (200g) was percolated using ethanol (700ml) for two weeks. The crude extract was macerated using Ethylacetate.

The macerated fraction of ethylacetate was dried by exposing to air at room temperature (Sofowaraet. al, 1984).

Phytochemical Screening

The macerated fraction of ethylacetate obtained was subjected to phytochemical chemical screening to determine the presence of secondary metabolites (DJilani et al. 2006)

Preparation of Test Solution

A stock solution (100,000µg/ml) was prepared by dissolving the extract (200mg) obtained in DMSO (2ml). The following concentrations; 500, 1000, 2000 and 5000 µg/ml were made by serial dilution (Hanne, 2002)

Source of Malaria Parasite for Assay

In-vitro Assay of the Activity of the extract (Ethyl acetate) on *Plasmodium falciparum* culture was carried out.

The sensitivity of the parasites to the tested fraction at concentrations of 500, 1000, 2000, and 5000µg/ml was determined microscopically at 37°C after 24 and 48hours of incubation. (Mukhtar et.al.2006).

Column Chromatography of Ethyl acetate Extracts.

The extract (10g) was thoroughly mixed with silica gel and loaded into a column. The column was run by eluting solvents of varying polarities, the collected fractions were allowed complete evaporation to dryness at room temperature.

Micro column Chromatography of Pooled Fractions

The fractions with similarities in Thin Layer Chromatography patterns and retention time were pooled together. The fraction with the largest amount was micro chromatographed by eluting solvents of different polarities.

RESULTS AND DISCUSSION

In the preliminary phytochemical analysis it was shown that the secondary metabolites are present in different fractions containing; alkaloids, flavonoids, steroids, sugars (Table 1). The anti Plasmodial analysis of the ethyl acetate fraction revealed that the pooled fraction of Es_I-3-10 derived from the micro column of Es_I-02-17, demonstrated the highest percentage of plasmodium elimination. The presence of alkaloids in the fractions justified its Antiplasmodium activity on the malaria parasite.

Table 1: **Phytochemicals present in the Ethyl acetate fraction of the stem bark of *Erythrina senegalensis*.**

Fraction	Phytochemicals present
Ethyl acetate	Alkaloids, Flavonoid, Steroids, Sugars, Saponins and Tannins

Table 2: Column Chromatography Fractions of Ethylacetate Soluble Extracts of Stem Bark of *Erythrina senegalensis*.

Column Eluents	Pooled or single fractions	Pool code	Weight (g)
Petroleum ether/chloroform (1:1)	2-10	ES ₁ -02-10.34	
	11-14	ES ₁ -02-2	0.43
	15-16	ES ₁ -02-3	0.25
	17-18	ES ₁ -02-4	0.32
	19-25	ES ₁ -02-5	0.37
	26 alone	ES ₁ -02-6	0.12
	27-30	ES ₁ -02-7	0.31
Chloroform (100%)	31-34	ES ₁ -02-8	0.24
	35 alone	ES ₁ -02-9	0.54
	36-37	ES ₁ -02-10	0.43
	38-41	ES ₁ -02-11	0.21
	42 alone	ES ₁ -02-12	0.81
Chloroform/Ethylacetate (1:1)	43-66	ES ₁ -02-13	0.54
	67-68	ES ₁ -02-14	0.31
Ethylacetate (100%)	69-87	ES ₁ -02-15	0.41
	96-101	ES ₁ -02-16	0.32
Methanol/Ethylacetate (1:1)	89-95	ES ₁ -02-17	3.52
	96-101	ES ₁ -02-18	0.23
	102 alone	ES ₁ -02-19	1.02
	103-104	ES ₁ -02-20	1.03
Methanol (100%)	105-112	ES ₁ -02-21	0.87
	113-118	ES ₁ -02-22	0.52
	119-121	ES ₁ -02-23	1.04
	122-123	ES ₁ -02-24	0.72

Table 3: Micro column chromatography fractions of ES₁-02-17 (3.5g)

Colum Eluents	Pooled Fractions	Pool code	Weight
PE 100% & PE/CHCl ₃ (1:1)	1-8	ES ₁ -3-1	0.30g
PE/CHCl ₃ (8:2) & PE/CHCl ₃	9-14	ES ₁ -3-2	0.32g
PE/CHCl ₃ (7:3) & PE/CHCl ₃ (6:4)	15-20	ES ₁ -3-3	0.37g
PE/CHCl ₃ (1:1)	21-22	ES ₁ -3-4	0.12g
PE/CHCl ₃ (1:1) & PE/CHCl ₃ (4:6)	23-26	ES ₁ -3-5	0.34g
PE/CHCl ₃ (4:6)	27-28	ES ₁ -3-6	0.17g
PE/CHCl ₃ (3:7)	29-30	ES ₁ -3-7	0.14g
PE (3:7)	31-32	ES ₁ -3-8	0.27g
PE/CHCl ₃ (3:7) & PE/CHCl ₃ (2:3)	33-36	ES ₁ -3-9	0.48g
PE/CHCl ₃ & CHCl ₃ (100%)	37-44	ES ₁ -3-10	0.49g

Table 4:Antiplasmodium activity of the Pooled Fractions.

Pooled code	Percentage Elimination of Plasmodium Falciparum at 5000µg/ml
Es ₁₋₃₋₁	54.84
Es ₁₋₃₋₃	48.30
Es ₁₋₃₋₅	73.92
Es ₁₋₃₋₉	68.31
Es ₁₋₃₋₁₀	87.94

CONCLUSION

From the results, Ethylacetate soluble Fractions of *Erythrina senegalensis* showed a significant Antiplasmodium activity with fraction Es₁₋₃₋₁₀ having 87.94% parasitic elimination at a concentration of 5000µg/ml with fractions Es₁₋₃₋₁₀.

The phytochemical screening of the fraction revealed that the presences of secondary metabolites such as; Alkaloids, Flavonoids, Steroids, Sugars, Saponins and Tannins

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