Special Conference Edition, April, 2022
http://dx.doi.org/10.4314/bajopas.v13i1.58S

Bayero Journal of Pure and Applied Sciences, 13(1): 381 - 384
ISSN 2006 – 6996

SHORT COMMUNICATION

ANTI - MALARIAL ACTIVITY OF N-HEXANE EXTRACT OF *Sphenostylis stenocarpa*

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ABSTRACT
The whole plant n-hexane Extract of Sphenostylis stenocarpa was evaluated for its anti-malarial properties against plasmodium falciparum parasite. Bioactivity-guided column chromatography of the crude n-hexane extract led to the selection of a fraction labeled using 100% n-hexane. The fraction was tasted using arthemether – Lumefentrin as positive control against plasmodium falciparum parasite and demonstrated percentage elimination of 75, 68, 58 and 45 at 0.01, 0.00.5, 0.0025 and 0.00125 mg/cm³ respectively.
Keywords: Anti-malaria, plasmodium falcifarum, Sphenostylis stenocarpa and n-Hexane.

INTRODUCTION

Plant have always been considered to be a possible alternative and rich source of new drugs and most of anti-malarial drugs in use today such as guanine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compound as templates (Salawu, *et al.,* 2010)

Malaria is a disease caused by *plasmodium* parasites which is transmitted through bites of infected female anopheles mosquito. *Plasmodium falciparum* is the most virulent of all five *plasmodium* species that causes malaria has continued to be life threatening and thus it's danger to public health. Report of World Health Organization (2016), estimated that 3.2 billion people worldwide are at risk of malaria infection with sub-Saharan Africa alone accounting for 13% while the global cases of malaria infection in 2015 was 212 million with 429,000 death.

Artemisinin based combination therapies (ACTs) still presents new challenges as growing reports of resistance inn malaria endemic areas to these conventional anti-malaria emerges (handboonkunupakarn and white (2016) the several side effect caused by orthodox drugs like gastro intestinal tract (GIT) disturbance, vertigo, itchiness, skin rashes, nausea, vomiting e.t.c, and a tremendous rise in cost of medicines in Africa (Ogungbamigbe *et al,.* 2008), there is need for novel agents from plants that can overcome current problems of malaria therapy.

Sphenositylis stenocarpa is characterized by its fruit (Legume) and stipulated leaves. Popularly called Majingha in southern part, in Hausa is called kashinkaji. Three species of *Sphenostylis stenocarpa* under-exploited African yam bean (Azeke *et al.,* 2005)

MATERIALS AND METHODS

Collection and Authentication of Plant

Sphenositylis stenocarpa were whole plant was obtained from Otukpo Local Government of Benue state Nigeria on 10th sept, 2015. The plant was authenticated at the department of the Biological Sciences University of Agriculture, Makurdi, Benue State Nigeria. With voucher number is 2729.

Extraction of Plant Material

Sphenositylis stenocarpa whole plant were air dried and ground into fine powder. 1000g of the powder was then percolated with n-hexane (2.5l) and allowed to stand for 24 hours at room temperature. The percolate was then filtered and concentrated using rotary evaporation at 30^0c.

Column Chromatography

Exactly 4.9g of n-hexane crude extract was used for column chromatography and obtained the total of 149 fraction of $50cm^3$each. Further Purification led to the selection of fraction SS-1-32 which was then subjected to the anti-malaria bioassay.

Anti-Malaria Assay

Preparation of Stock Solution

A stock solution of 1000μg/cm^{3}was prepared by dissolving (20mg) of sample fraction dimethyl sulphoxide (DMSO), (2ml). 500, 1000, 2000 and 5000mg/cm^3 respectively prepared from stock solution by send dilution using dilution for male:

$C_1V_1 = C_2V_2$

Where:

C_1= Initial concentration, C_2= Final concentration, V_1 = Initial volume, V_2 = Final volume

Sourcing of Malaria Parasites

Malaria parasites infected blood samples containing heavy parasitaemia of Plasmodium falciparum were collected from N-TAWA Diagnostic laboratory opposite Murtala Muhammad Specialist Hospital, Kano.

Preparation of Plasmodium falciparum Culture Medium

Venous blood (2ml) from the main vein of white healthy rabbits pinnae was withdrawn using a disposable 5ml syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour (Dacie and Lewis, 1968). The defribranated blood was centrifuged at 1500rpm using Gellenkamp centrifuge for 10minutes and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500rpm for 5 minutes and the supernatant layer was added to the first test tube. The sediments were discarded and the serum collected was supplemented with the salt of RPMI 1600 medium (KCl 5.37mM, NaCl 10.27mM, MgS04 mM, NaHPO4 17.73mM, Ca(NO3)2 0.42mM, NaHCO3 2.5mM, and glucose 11.0 mM. (BDH ltd, UK) as demonstrate by Devo et al (1985). The medium was sterilized by 40μg/ml gentamicin sulphate (Trager, 1982).

***In-Vitro* Assay of the Activity of the Extracts on Plasmodium falciparum Culture**

Tested solution (0.1ml) and 0.2ml of the culture medium were added into a taste tube containing 0.1ml of 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the tested fractions was determined microscopically after incubation for 24 and 48 hours at 37°C. With serial concentration of 500, 1000, 2000 and 5000μg/ml. The incubation was undertaken in glass bell jar containing a lighted candle to ensure the supply of required quantity of CO_2 (about 5% 0_2 gas, 2% and about 93% nitrogen gas as demonstrated by Muktar et al, (2006).

Determination of the Activity

At the end of the incubation periods 24 and 48 hours, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and strained by Giemsa's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the average percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after incubation period – 24 and 48 hours, using the formula below;

Percentage Elimination =N/N_x x100 Where, N = Total number of cleared RBC , Nx = Total number of parasitized RBC Note: RBC = Red Blood Cells (Muktar et al., 2006).

RESULTS

Antiplasmodial Activity

Sphenostylis stenocarpa crude extract was tasted against *Plasmodium falcifarum* at concentration of 0.01 mg/cm^3 show a percentage elimination of 75% which is equivalent to a standard drug of Arthemather – Lumafentrin of the same concentration show 97% elimination. As the concentration is decreasing to 0.005, 0.00025 and 0.00125 mg/cm^3 of the crude extract of *Sphenostylis stenocarpa* the percentage elimination is decreasing to 68.5%, 58.7% and 45.7% respectively against the Parasite (Table 1).

Table 1: Anti-Malaria Sensibility Test of SS-1-32 against Erythrocytes

S/N	extracts	Concentration (mg/cm^3)	Initial count infected RBC/field	Total RBC per field	RBC with live parasite	RBC with parasite at end of incubation	Elimination at end of incubation (72 hrs.)
1	SS-1-32	0.01	92	212	23	69	75%
		0.005			29	63	68.5%
		0.0025			40	54	58.7%
		0.00125			50	42	45.7%
2	Artemethar & Lumefentrine 20/120mg tablet	0.01	92	212	2	90	97%
		0.005			4	88	95.7%
		0.0025			7	85	92.3%
		0.00125			11	81	88.0%

DISCUSSION

The *in vitro* antiplasmodial activity *Sphenostylis stenocarpa* n-haxane extract was investigated. The n-haxane fraction produce significant activity against *Plasmodium falcifarum* 75% elimination at end of incubation indicating that the fraction has more suppressive activity at higher dose. Although very few research studies has been devoted to this plant, some important studies have been conducted including one on how to relieve these health problems. If the yam bean could be grown in large quantities, this crop could be the important source of protein needed by the people of sub-Saharan Africa (Azeke *et al,.* 2005*).* It is also reported that use of the plant by the herbalist in Otukpo Local Government of Benue State in Nigeria for treatment of malaria. The proximate and amino acidsof dehulled African yam bean (*S. stenocarpa*) flour were analysed. The proximate composition showed that both protein and carbohydrate were found in high quantity (Oshodi *et al.*, 2009). HPLC profile of the hydrolysed *S.stenocarpa* sample revealed the presence of cysteine, methionine) and tryptophan amino acids (Landry and Delhaye, 1992). The study showed the protein hydrolysates derived from seed of *S. stenocarpa* had posses anti oxidant properties agaist a variety of physiologically relevent free radicals. Since the extract is n-haxane there is possiblity of presence of alkaloid terpenoids, terpenes and some steroids which impact their biological activity.

CONCLUSION

In light of the above, *Sphenostylis stenocarpa* showed a promising anti-plasmodia signs against *Plasmodium falciparum,* however, more studies are required to extensively explore its anti-malaria properties, further investigation for *in vivo* and phytochemical screening. Research activities is in progress to isolate more compounds.

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