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Evaluation of Antiplasmodial Activity of Solvents Extract of *Senna occidentalis* Leaves

Muhammad Ibrahim Usman [a*] and Abubakar Bilyamini Maazu [a]

[a] *Department of Biochemistry, College of Medical Sciences, Yobe State University Damaturu, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The high rate of resistance to an antimalarial drug suggests the importance to discover new compounds with potential antimalarial activities. This research work is aimed at evaluating the antimalarial activity as well as characterization of potential bioactive compounds from extracts of *Senna occidentalis* leaves (ESOL). The *in vitro* antimalarial activity of the methanol, ethyl acetate, chloroform, and hexane extracts was carried against *Plasmodium falciparum*. The antimalarial activity of the extracts was determined by calculation of the percentage elimination of the ESOL after three days of incubation against *Plasmodium falciparum*. Analysis for the detection of possible bioactive compound(s) in the ESOL was conducted using Gas chromatography Mass spectrometry (GCMS). Results showed that ESOL has antimalarial properties that were dose-dependent. Furthermore, there was a significant increase ($p<0.05$) in the mean percentage elimination of all the extracts when compared with placebo (normal saline). All the extracts show an activity less than the conventional drug, i.e Artemisinin Combination Therapies(ACT), with the chloroform extracts showing the highest anti-plasmodial activity of 94.64% at 5000 µg/ mL. Chloroform extracts were found to contain Urs-12-en-3-ol, acetate (3 beta), (Alpha-amyrin), Lup-20(29)-en-3-ol, acetate and 12-Oleanen-3-yl acetate, (Beta-amyrin acetate) as possible bioactive compounds. This study suggests that ESOL has potential anti-plasmodial activity.

Keywords: Anti-plasmodial activity; GCMS; Senna occidentalis.

**Corresponding author: E-mail: ibrahimmuhd@yahoo.com;*

1. INTRODUCTION

"Malaria is estimated to kill more than 1 million people annually, the majority of whom are young children. Together with pneumonia, diarrhea, measles, and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries" [1]. "Malaria is mostly a disease of the hot climate. In 2017, 91 countries and territories had ongoing malaria transmission. According to the latest WHO estimates, released in December 2017, there were 216 million cases of malaria in 2017 and 445000 deaths" [2]. Most malaria cases and deaths occur in sub-Saharan Africa. Ninety percent of malaria cases in the world occur in Africa south of the Sahara.

"In Nigeria, malaria is endemic throughout the country accounting for up to 60% of outpatient visits to health facilities, 30% of childhood mortality, and 11% of maternal deaths" [3]. "Malaria is a vector-borne disease, caused by protozoan parasites of the genus Plasmodium. It is transmitted from the blood of an infected person and passed to a healthy human by a female anopheles' mosquito bite" [3].

"Senna occidentalis is a widely distributed and commonly used plant. *Senna occidentalis*, commonly called 'Dora rai' in Hausa, 'Akidiogbara' in Igbo, 'Abo rere' in Yoruba, and 'Coffee senna' in English has been reported to contain many phytochemicals including alkaloids, anthocyanosides, phenolics, proteins, phlobatannins, steroids, tannins, flavonoids, anthroquinone, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars and cardiac glycosides" [4]. "*Senna occidentalis* has been reported to have many pharmacological effects including antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, anti-mutagenic, anti-diabetic, wound healing, hepato-protective, anti-inflammatory, analgesic, antimalarial, antipyretic and another effect. The plant is widely used by the local people of the Hausa-Fulani tribe in northern Nigeria for the prevention and treatment of various diseases liver and kidney diseases inclusive" [4].

"It is a tropical plant that grows on wastelands in villages and towns and on roadsides. The seeds are the primary material of interest though the leaf and roots are also used. The seeds are roasted and used as a coffee substitute. The plant's tissues contain a host of phyto-active chemicals that may support its numerous applications in folk medicine" [5]. "The whole plant is useful as a purgative and as a tonic. The seeds and leaves are used as a cure for cutaneous diseases" [6]. "The roasted seeds are used to manage hypertension in Ghana. It is used for fever, menstrual problems, tuberculosis, diuretic anemia, liver complaints, and as a tonic for general weakness and illness" [7]. "Leaves of *S. occidentalis* are externally applied for wound healing, itching, bone fracture, ringworm, skin diseases, and throat infection. An infusion of the bark is used in folklore medicine for diabetes" [8].

2. MATERIALS AND METHODS

2.1 Collection and Processing of *Senna occidentalis* Leaves

Fresh Leaves of *Senna occidentalis* leaves were collected from Bayero University Kano, Kano State, and identification was done by a Botanist at Herbarium in the Department of Plant Biology, Bayero University Kano. A sample with accession number BUK/HAN/ 0073 was deposited at the herbarium of the Department.

2.2 Extraction of *Senna occidentalis* Leaf

Methanol extraction of the leaves was done according to the method described by Veeramuthu *et al* [9]. Leaves of *Senna occidentalis* were air-dried under shade and grounded using a grinding mill. The powdered leaf material weighing 100g was macerated with 1000 mL methanol in an Erlenmeyer flask and placed on an orbital shaker (Gallenkamp 5A-4131, England) at room temperature for 72 hours. The leaf extract was then filtered through a cotton cloth and subsequently with a filter paper (12.5 cm size). The process was repeated until the leaves' dark brown was exhausted and a rotary evaporator was used to concentrate the extract. The concentrated crude extract was collected and stored in a polythene bag.

2.3 Fractionation of the Crude Methanol Leaf Extract

The fractionation was done by partitioning the methanol extract on the order of increasing polarity, starting with n-hexane (index 0.1), chloroform (index 4.1), and finally ethyl acetate (index 4.4).

2.4 Preparation of Test Solution

A stock solution (10,000µg/mL) was prepared by dissolving the extracts (20mg) obtained from

Senna occidentalis leaves in dimethyl sulphoxide (DMSO) (2mL). The following concentrations; were made by serial dilution as follows:

- 500μg/mL (0.05mL of stock solution + 0.95mL DMSO)
- 1000μg/mL (0.1mL of stock solution + 0.9mL DMSO)
- 2000μg/mL (0.2mL of stock solution + 0.8mL DMSO)
- 5000μg/mL (0.5mL of stock solution + 0.5mL DMSO)

2.5 Sourcing of Malaria Parasite

Malaria parasites of infected blood samples containing a parasitemia of *Plasmodium* falciparum were collected from the Department of Hematology, Bayero University Clinic, Kano. 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.

2.6 Determination of *Plasmodium falciparum* (Positive Blood Samples) by Thin Smear Method

Using a clean capillary tube, a small drop of each blood sample was placed at the center of a clean glass slide at least 2mm from one end. Smears were formed by moving the coverslip 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. The excess stain was washed with clean tap water. The smears were dried in the air by hanging the glass slides inverted in a rack. Each dried smear was microscopically observed under a high power objective (x100) using oil immersion. An average parasitemia was determined using the reading of 3 microscopic fields.

2.7 Preparation of Culture Medium for Parasite Cultivation

Blood samples (2mL) from a healthy rabbit were withdrawn using a disposable 5mL syringe (BD 205WG). The sample was defibrinated by allowing it to settle for at least 45minutes. The defibrinated blood sample was further centrifuged at 2500rpm for 10 minutes. The supernatant layer was collected and sterilized. The separated sediment was centrifuged further 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 RPMI 1640 salt medium and sterilized 50 $\mu g/mL$ of gentamicin sulfate [10]. The composition of the RPMI 1640 (Roswell Park Memorial Institute medium) salt is as described below; KCl 5.37mM, NaCl 10.27mM, $MgSO_4$ 2.56mM, $NaHPO_4$ 17.73mM, $Ca(NO_3)_2$ 0.42mM, $NaHCO_3$ 2.5mM and glucose 11.0mM as demonstrated by [11].

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

A tested solution (0.1mL) of 500, 1000, 2000, and 5000μg/mL and the culture medium (0.2mL) were added into a test tube containing 0.1 mL 0f 5% parasitemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fraction at a concentration of 500, 1000, 2000, and 5000μg/mL was determined microscopically at 37^{o}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 was atmospherically inert (about 5% O_2, 2% CO_2, and 93% nitrogen gas) as demonstrated by Mukhtar et al*.* [12].

2.9 Determination of Activity of Parasitemia Clearance of the extract

At the end of each incubation period, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Leishman'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 each incubation period, using the formula below;

$$\% = \frac{\mathrm{N}}{\mathrm{Nx}} \times 100$$

Where, % = Percentage inhibition of the parasites
N = Total number of cleared RBC
Nx = Total number of parasitized` RBC

3. RESULTS AND DISCUSSIONS

3.1 Results

The physical properties of the methanol extract of *Senna occidentalis* (Table 1) yielded 12.5% after 100g of the powdered leaves. The extract was observed to be dark brown with a sticky texture.

Anti-plasmodial properties of the methanol of *Senna occidentalis* leaf extract, including positive and negative control on were presented in Table 2 The methanol of *Senna occidentalis* leaf extract gave the highest parasite clearance of 85.41.0% at 5000µg/mL, while least activity was found in the concentration of 500 µg/mL of the same extract. Treatment with the standard drug ACT resulted in an exponential decrease in parasite count giving the highest curative activity of 97.05%. However, the negative control showed no decrease in parasite count. Anti-plasmodial properties of *Senna occidentalis* leaf extract on *Plasmodium falciparum* were detected in all the partitioned extract, namely Chloroform, Ethyl acetate, and n-hexane partitioned extract. Chloroform partitioned extract gave the highest parasite clearance of 94.64.0% and 93.75% at 5000µg/mL and 2000µg/mL respectively. The least activity of 16.66% at the concentration of 500µg/mL was found in n-hexane partitioned extract.

Table 3 shows the anti-plasmodial effect of the partitioned fractions of *Senna occidentalis* leaf extracts. Treatment with the leaf partitioned extract resulted in an exponential decrease in parasite count giving the chloroform partitioned extract the highest curative activity. ACT was used as a standard drug though gave the highest parasite clearance of 97.05%. However, the negative control showed no decrease in the parasite.

Table 1. Physical properties of the extract of *Senna occidentalis* leaves

Property	
Weight of plant (g)	100
Weight of extract (g)	12.5
Percentage yield (%)	12.5
Color of extract	Dark brown
Texture of extract	Sticky

Table 2. Anti-plasmodial properties of methanol leaf extract of *Senna occidentalis*

Extracts	Conc.(µg/mL)	Initial count	Average no. during incubation			Average no. After Incubation	%elimination µg/mL	IC_{50}
			24 hrs	48 hrs	72 hrs			
Control	ACT	34	1	0	0	1.00	97.05	480
	Negative	34	34	34	34	34	0	
methanol	5000		21	21	20	20.66	30.48	
	2000		26	27	27	26.70	21.47	
	1000		30	29	29	40.57	18.86	
	500		30	30	31	31.03	7.25	
	5000	34	6	4	4	4.66	85.41	370
	2000		13	15	15	14.33	84.00	
	1000		22	20	19	20.80	38.8	
	500		28	27	27	27.30	19.90	

Table 3. Antiplasmodial properties of solvents fractions from crude *Senna occidentalis*

Extracts	Conc.(µg/mL)	Initial count	Average no. during incubation			Average no. After Incubation	%elimination µg/mL	IC_{50}
			24 hrs	48 hrs	72 hrs			
Ethyl acetate	5000	56	16	15	15	15.33	72.6	250
	2000		20	18	18	18.60	66.7	
	1000		28	28	28	28.30	49.3	
	500		35	35	35	35.00	37.5	
Chloroform	5000	56	3	0	0	3.0	94.64	430

	2000		4	4	3	3.50	3.75	
	1000		8	6	6	6.66	88.2	
	500		9	9	8	8.30	85.17	
n-hexane	5000	56	26	23	22	23.68	57.70	280
	2000		30	28	26		49.47	
	1000		32	29	27		47.20	
	500		49	47	44		6.66	

Table 4. Summary of some possible compounds identified in chloroform fraction extracts by GCMS techniques with possible antimalarial activity

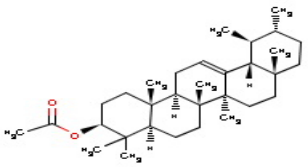
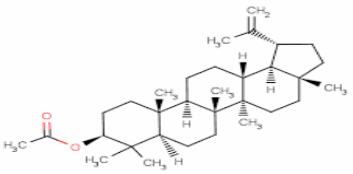
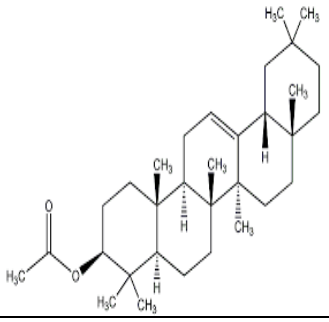
Compound	Peak#	R.time	Height%	Molecular formula	Derived structure
Urs-12-en-3-ol,acetate(3 beta) (alpha amyrin).	21	44.90	2.51	$C_{32}H_{52}O_2$	
Lup-20(29)-en-3-ol, acetate LUPENYL ACETATE	29	48.3	9.21	$C_{32}H_{52}O_3$	
12-Oleanen-3-yl acetate, (3.alpha) (beta amyrin acetate)	22	47.98	9.21	$C_{32}H_{53}O_2$	

Table 4 show some of the probable bioactive compounds from the GCMS chromatogram of the most active chloroform fraction. The spectra show the presence of 2-Hydroxymethylcyclopentanol (cis) 12-Oleanen-3-yl acetate (3.alpha) (Beta amyrin acetate), Lupeol Urs-12-en-3-ol, acetate (3-beta) (Alpha amyrin), Lup-20(29)-en-3-ol acetate 12-Oleanen-3-yl acetate.

3.2 Discussion

Methanol was used in the extraction of *Senna occidentalis* leaf due to the fact that methanol can extract both polar and non-polar compounds to some extent. A yield of 12.5% signifies that methanol was good in the extraction of the phytochemicals from the dry leaves of *Senna occidentalis*. This is in agreement with that of Alhassan *et al* [4] on the effect of aqueous root extract of *Senna occidentalis* on an acetaminophen-induced hepato-renal toxicity rat model.

The search for drugs and dietary supplement from plants to treat and or manage malaria has accelerated in recent years which necessitate

this research. *Senna occidentalis* is one of the plants with a long history of traditional use in the treatment of malaria, hepatitis, and wound healing [4]. The anti-plasmodial properties of fractions of *Senna occidentalis* leaf (Table 2 and 3) were observed during three days of treatment and parasitemia counts on the interval of 24 hrs were recorded. Treatment with the crude methanol extracts and the partitioned fractions of leaf extract resulted in an exponential decrease in parasite count throughout the study period, with chloroform partitioned fraction showing the highest clearance activity, followed by Ethyl acetate and least was n-hexane. Artemisinin combine therapy gave the highest clearance activity by clearing almost all the parasites completely by the 3rd day of treatment. At concentrations of 5000, 2000, 1000 and 500 µg/mL, chloroform partitioned extracts produced the highest curative activity. From literature, an extract is regarded as highly active if the percentage clearance is greater than 50% [13]. It can be seen that the average number of parasites increases as the `concentration of the extract decreases. This indicates that higher concentrations of the extracts were found to be more effective on the parasites.

The antimalarial activities of *Senna occidentalis* may be linked Alterations of the erythrocyte shape were also observed with lupeol, and other triterpenoids (Urs-12-en-3-ol, acetate (3 beta) and 12-Oleanen-3-yl acetate, (3.alpha) (beta amyrin acetate) (alpha amyrin). The major anti-plasmodial constituent isolated from the plant *R. ilicifolia*. It has previously been described that lupeol exhibits inhibitory activity on *P. falciparum* growth in vitro but lacks in vivo activity in mice infected with *P. berghei*. However, there was no mention of the in vitro activity's mechanism. It has now been established that lupeol promotes erythrocyte membrane shape changes toward stomatocytosis develops at concentrations below its IC_{50} value. The substance caused endovesiculation, which is a stomatocytogenic chemical feature. The observed IC50 of several substances exhibits a strong association with the lupeol concentration required to cause morphological alterations in erythrocytes. This strongly shows that lupeol's indirect anti-plasmodial effect in vitro is caused by host cell membrane alteration.

Lupeol's structure is similar to that of cholesterol, and it is anticipated that the substance will be able to infiltrate cellular membranes. Lupeol functions as an amphiphile because it has a single hydroxy group and a big, apolar skeleton. The bilayer theory states that the expansion of the inner layer of the lipid membrane caused by the incorporation of a lipophilic chemical results in the formation of stomatocytes. When compared to the integration of an amphiphile into the outer layer, as in the case of echinocytogenic chemicals, such alterations appear to be more restricting in terms of parasite growth. Since erythrocytes preincubated with lupeol proved to be unsuitable for parasite cultivation, it is shown that the presence of lupeol in the growth medium is not necessary for the inhibition of parasite growth. This strongly suggests that lupeol has been permanently incorporated into the lipid bilayer. When using erythrocytes that have been pretreated with lupeol in a culture, an abundance of extracellular merozoites indicates that the invasion of the erythrocytes has also been compromised. In an inverse experiment relative to that described above, a parasite culture was treated with lupeol and subcultured with untreated erythrocytes. “In an experiment, the time of pre-incubation had to be limited to 3 to 6 h; otherwise, the parasites would die. In spite of the pretreatment with lupeol, the parasites grew normally in untreated cells after the removal of lupeol. Thus, the ability of the parasites to invade and grow in fresh erythrocytes was not impaired by the initial exposure to lupeol. Previous studies have demonstrated that alterations of the erythrocyte membrane such as cross-linking of spectrin, changes in deformability, spherocytosis, and modification of the cytoskeletal proteins have inhibitory effects on invasion” [14]. No studies of the incorporation of lupeol into erythrocytes and its effect on parasite proliferation have been reported prior to this work. A report by Vidaya et al [15] shows that “erythrocytes of rats fed with lupeol exhibit altered osmotic fragility and are compatible with this finding”.

The current findings are interesting for drug discovery programmes based on natural product research, even if the precise mechanism by which stomatocytosis renders the erythrocytes unfavourable for P. falciparum invasion and growth has yet to be clarified. Due to its widespread presence in plants, lupeol and other triterpenes and sterols with similar structures are typically found in plant extracts used for screening. Numerous potential synthetic drugs may also function as stomatocytogenic amphiphiles. The membrane alterations that inhibit parasite growth take place long before they can be detected by routine examination by

optical microscopy and thus care has to be exercised when *P. falciparum In vitro* growth inhibition results are interpreted.

4. CONCLUSION

It may be concluded that methanol extract of *Senna occidentalis* possesses the potential for use in the treatment of malaria. The results show that chloroform partitioned has the highest anti-plasmodial activity. The chloroform extracts were found to contain Lup-20(29)-en-3-ol, acetate, Urs-12-en-3-ol, acetate (3 beta) and 12-Oleanen-3-yl acetate, (3.alpha) which are reported to possess anti-malarial activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. UNICEF. With the cooperation of the World Health Organization; 2000.
2. World Health Organization (WHO).World Malaria Report ISBN 978-92-4-156515-8; 2017.

   WHO World Malaria Report. Geneva: WHO; 2016. [Online]; 2008.

   Available:www.who.int/malaria/wmr2008.

   (Accessed on 2009 October 12).
3. World Health Organization. Chronic conditions: The global burden; 2014.

   Available:http://www.who.int/chronic_conditions/burden/en/
4. Alhassan AJ, Muhammad IU, Imam AA, Shamsudden ZH, Nasir A, Alexander I. Effect of aqueous root extract of senna occidentalis on acetaminophen induced hepatorenal toxicity rat model, Saudi Journal of Biomedical Research. 2017;2:1-11.
5. Francis JK. *Senna occidentalis* (L.) link fabaceae synonyms : *Senna occidentalis* L. Ditremexa occidentalis; 2002.
6. Yadava RN, Satnami DK. Chemical constituents from *Senna occidentalis* linn. Indian Journal of Chemistry. 50B. 2011; 1112-11186.
7. Usha K, Kasturi GM. Hemalatha P. Hepatoprotective effect of *Hygrophila spinosa* and *Senna occidentalis* on carbon tetrachloride induced liver damage in experimental rats. Indian Journal of Clinical Biochemistry. 2007;22(2):132-135. 7.
8. Reeta M, Ravindra S, Kasamarda. The ayurvedic approach. Journal of Pharmaceutical and Scientific Innovation. 2013;2(2):2527.
9. Veeramuthu N, Verhaeghen K, Van Bortel W, Roelants P, Marcotty T, Baza D, D'Alessandro U, Coosemans M. A significant increase in kdr in *Anopheles gambiae* is associated with an intensive vector control intervention in Burundi highlands. Trop Med Int Health. 2006; 13:1479-1487.
10. Trager G. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature; 1982.

    DOI: 10.1038/nature20791. Retrieved 7 December 2016.
11. Devo TE, Akhouayri I, Kisinza W, David JP. Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects. Insect Biochemistry and Molecular Biol. 1985; 43:407-416.
12. Mukhtar MD, Bashir M, Arzai AH. Comparative. *In vitro* studies on antiplasmodial activity of some Nigerian and Foreign brands of Chloroquine oral formulations marketed in Kano; African Journal of Biotechnology. 2006; 5(24):2464-2468.
13. Ramazani A, Sardari S, Zakeri S, Vaziri B. *In vitro* antiplasmodial and phytochemical study of five Artemisia species from Iran and in vivo activity of two species. Parasitol Res. 2010;107:593–599.
14. Dluzewski AR, Rangachari K, Gratzer WB, Wilson RJM. Inhibition of malarial invasion of red cells by chemical and immunochemical linking of spectrin

molecules. Br. J. Haematol. 1983;55:629–637.

15. Vidaya L, Malini MM, Varalakshmi P. Effect of pentacyclic triterpenes on oxalate-induced changes in rat erythrocytes. Pharmacol. Res. 2000; 42:313–316.

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