Original Research Article

DETERMINATION OF ANTIPLASMODIAL ACTIVITY OF SOLVENTS EXTRACT OF *SENNA OCCIDENTALIS* LEAVES

Abstract

The high rate of resistance to antimalarial drug suggest the importance to discover new compounds with potential antimalarial activities.This research work is aimed at evaluating the antimalarial activity as well as characterisation of potential bioactive compounds.The *in vitro* antimalarial activity of the methanol, ethyl acetate, chloroform and hexane fraction of ESOL was carried against *Plasmodium falcifarum*, The antimalarial activity of the extracts was determined by calculation of the percentage elimination of the ESOL fractions after three day of incubation against *Plasmodium falcifarum*. Analysis for the detection of possible bioactive compound (s) in the ESOL was conducted using Gas chromatography Mass spectrometry (GCMS). Results showed that ECOL have antimalarial properties that were dose dependent. Furthermore, there was a significant increase ($p<0.05$) in mean percentage elimination of all the extract when compared with placebo (normalsaline). All the fractions of ESOL shows an activity less than the conventional drug, Artemisinin Combination Therapies(*ACT),*with the chloroform extract showing highest antiplasmodial activity of 94.64% at 5000 µg/ml. Chloroform fraction of ECOL was found to contain containUrs-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 have potential anti-plasmodial activity.

Keywords: anti-plasmodial activity GCMS and Senna occidentalis

1.0 INTRODUCTION

Malaria is estimated to kill more than 1 million people annually, the majority of whom are young children. Together with pneumonia, diarrhoea, measles and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries (Unicef, 2000).Malaria is mostly a disease of 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 (WHO, 2017). Most malaria cases and deaths occur in sub-Saharan Africa. Ninety per cent 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% outpatient visits to health facilities, 30% childhood mortality and 11% maternal deaths (WHO, 2014). 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 bites (WHO, 2014).

Senna occidentalis is widely distributed and commonly used plant. *Sennao ccidentalis*, 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 (Alhassan *et al.,* 2017). *Senna occidentalis* have been reported to have many pharmacological effects including antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, antimutogenic, antidiabetic, wound healing, hepatoprotective, anti-inflammatory, analgesic, antimalarial ,antipyretic and other effect. The plant is widely used

by the local people of Hausa-Fulani tribe in northern Nigeria for the prevention and treatment of various diseases liver and kidney diseases inclusive (Alhassan *et al.,* 2017).

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 phytoactive chemicals that may support its numerous applications in folk medicine (Francis, 2002). The whole plant is useful as a purgative and as a tonic. The seeds and leaves are used as cure for cutaneous diseases (Yadava, 2011). 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 (Usha and Katsuri 2007). 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 (Reeta *et al.*, 2013).

2.0 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.* (2006). Leaves of *Senna occidentalis* was 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 orbital shaker (Gallenkamp 5A-4131, England) at room temperature for 72 hours. The leaf extract was then filtered through cotton cloth and subsequently with a filter paper (12.5 cm size). The process was repeated until the leaves dark brown was exhausted and rotary evaporator was used to concentrate the extract. The concentrated crude extract was collected and stored in 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 dimethylsulphoxide (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 parasiteamia of *Plasmodium*falciparum were collected from the Department of Haematology, 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 centre of a clean glass slide at least 2mm from one end. 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 15minutes. Geimsa's stain was dropped on each smear, and allowed to stay for about 10minutes. Excess stain was washed with clean tap water. The smears were dried in air by hanging the glass slides inverted in a rack. Each dried smear was microscopically observed under a high power objectives (x100) using oil immersion. An average parasitaemia was determined using the reading of 3 microscopic fields (Hanne *et al.*, 2002).

2.7 Preparation of Culture Medium for parasite cultivation

Blood sample (2ml) from a healthy rabbit were withdrawn using a disposable 5ml syringe (BD 205WG). The sample was defibrinated by allowing it to settle for atleast 45minutes. The defibrinated blood sample was further centrifuged at 2500rpm for 10minutes. 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 μ g/ml gentamicin sulphate (Trager, 1982). 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 (Devo *et al.*, 1985).

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% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fractions at concentration of 500, 1000, 2000, and 5000µg/ml was determined under 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 being atmospherically inert (about 5% O_2, 2% CO_2 and 93% nitrogen gas) as demonstrated by Mukhtar *et al.*, (2006).

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

3.0 RESULTS AND DISCUSSIONS

3.1 Results

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

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
Colour of extract	Dark brown
Texture of extract	Sticky

Antiplasmodial properties of the methanol of *Senna occidentalis* leaf extract, including Positive and negative control on were presented in the 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 exponential decrease in parasite count with giving the highest curative activity of 97.05%. However, the negative control showed no decrease in parasite count.

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**	**IC_{50}** µg/ml
			24 hrs	**48 hrs**	**72 hrs**			
Control	ACT	34	1	0	0	1.00	97.05	480
	Negative	34	34	34	34	34	0	
	5000		21	21	20	20.66±0.12	30.48	

2000		26	27	27	26.70±0.16	21.47	
1000		30	29	29	40.57±0.14	18.86	
500		30	30	31	31.03±0.34	7.25	
5000		6	4	4	4.66±0.13	85.41	370
200		13	15	15	14.33±0.10	84.00	
1000		22	20	19	20.80±0.20	38.8	
500	34	28	27	27	27.30±0.16	19.90	

`

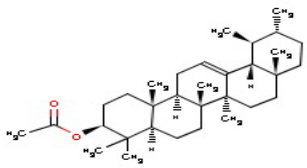
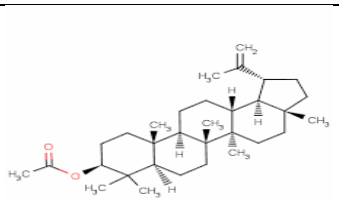
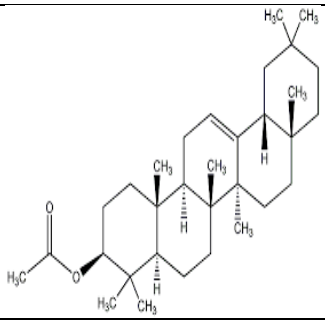
Antiplasmodial properties of *Senna occidentalis* leaf extract on *Plasmodium falciparum* were detected in all the partitioned fractions of the extract, namely chloroform Ethylacetate, and N-hexane partitioned fractions .Chloroform partitioned fractions gave the highest parasite clearance of 94.64.0% and 93.75 at 5000µg/ml and 2000µg/ml respectively. Least activity of 16.66% at the concentration of 500µg/ml was found in N-hexane partitioned fraction. Table 3 shows the antiplasmodial effect of the partitioned fractions of *Senna occidentalis* leaf extracts. Treatment with the leaf partitioned extract resulted in exponential decrease in parasite count with giving the chloroform partitioned extract with 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 parasite.

Table 3: Anti-plasmodial Properties of Solvents Fractions from Crude *Senna occidentalis*

Extracts	Conc. (µg/ml)	Initial	Average no. during			Average
no. %		counts	Incubation			After
Elimination	IC_{50} µg/ml					
			24hrs	48hrs	72hrs	
Ethylacetate	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	3.00
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	28.10
49.47						
	1000		32	29	27	29.33
47.20						
	500		49	47	44	46.66
6.66						

Table 4 shows some of the probable bioactive compounds from GCMS chromatogram of most active chloroform fraction. The spectra shows 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 .

Table 4: Summary of some possible compounds with identified in chloroform fraction extracts by GCMS techniques with possible antimalrial 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	$C_{32}H_{53}O_2$	

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. 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* 2017 on the effect of aqueous root extract of *Senna occidentalis* on acetaminophen induced hepatorenal toxicity rat model.

The search for drugs and dietary supplement from plants to treat and or manage malaria has accelerated in recent years which nessicate this research. *Senna occidentalis* is one of the plant with long history of traditional use in treatment of malaria, hepatitis, wound healing etc(

Alhassan *et al*., 2017).The antiplasmodial properties of fractions of *Senna occidentalis* leaf (Table 2 and 3) was observed during three days treatment and parasitaemia counts on the interval of 24 hrs was recorded. Treatment with the crude methanol extracts and the partitioned fractions of leaf extract resulted in exponential decrease in parasite count throughout the study period , with chloroform partitioned fraction showing the highest clearance activity, followed by Ethylacetate and least was N-hexane. Artismenia combine therapy gave the highest clearance activity by clearing almost all the parasite completely by the 3rd day of treatment. At concentrations of 5000, 2000 and 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% (Ramazani *et al*, 2010). 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 antiplasmodial 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, no information about the mechanism of the in vitro activity was reported. It is now shown that lupeol causes membrane shape changes of erythrocytes toward stomatocytic forms observable at concentrations below its IC50 value. The compound induced endovesiculation, which is characteristic of stomatocytogenic compounds (Hgersrend and Singer 1974). There is a good correlation between the lupeol concentration at which morphological changes in erythrocytes occur and the observed IC_{50} of many compounds.

This strongly suggests that the in vitro antiplasmodial activity of lupeol is indirect, being due to membrane modification of the host cell.

The structure of lupeol is reminiscent of that of cholesterol, and the compound is expected to be able to enter the cellular membranes. Due to the presence of a single hydroxy group and a large, apolar skeleton, lupeol acts as an amphiphile.

According to the bilayer hypothesis (Shertz and Singer, 1974), stomatocytes are generally formed when a lipophilic compound is incorporated into and expands the inner layer of the lipid membrane. Such changes appear to be more prohibiting with respect to parasite growth than incorporation of an amphiphile into the outer layer, as in case of echinocytogenic compounds. It is demonstrated that the inhibition of parasite growth does not require the presence of lupeol in the growth medium, since erythrocytes preincubated with lupeol proved to be unsuitable for parasite cultivation this strongly suggests the permanent incorporation of lupeol into the lipid bilayer. The presence of an excess of extracellular merozoites in a culture employing erythrocytes pretreated with lupeol suggests that the invasion of the erythrocytes has been impaired also. 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 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 (Dluzewski *et, al.,* 1983). No studies of incorporation of lupeol into erythrocytes and its effect on parasite

proliferation have been reported prior to this work. A recent report of Vidaya *et al* (2000) showing that erythrocytes of rats fed with lupeol exhibit altered osmotic fragility is compatible with this findings.

Although the exact mechanism by which stomatocytosis makes the erythrocytes unfavorable for *P. falciparum* invasion and growth has yet to be elucidated, the present findings are of interest for drug discovery programs based on natural products. Lupeol and other pentacyclic triterpenes and sterols with related structures are very common constituents of plants and are thus frequently encountered in plant extracts used for screening. Many synthetic drug candidates may also act 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.

4.0 CONCLUSION

It may be concluded that methanol extract of *Senna occidentalis* possess the potential for use in the treatment of malaria. The results shows that chloroform partitioned have the highest anti-plasmodial activity. The chloroform extracts was 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.

Reference

Alhassan A.J, Muhammad I.U, Imam A.A, Shamsudden Z.H. Nasir A, Alexander I. (2017). Effect of aqueous root extract of *senna occidentalis* on acetaminophen induced hepatorenal toxicity rat model, *Saudi Journal of Biomedical Research.*2:1-11

Devo, T.E., Akhouayri, I., Kisinza, W. and David, J. P. (1985).Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects. *Insect Biochemistry and Molecular Biol.,* 43: 407-416.

Dluzewski, A. R., K. Rangachari, W. B. Gratzer, and R. J. M. Wilson**. (**1983). Inhibition of malarial invasion of red cells by chemical and immunochemical linking of spectrin molecules. Br. J. Haematol. **55:**629–637

Francis J.K. (2002). *Senna occidentalis* (L.) Link Fabaceae Synonyms : *Senna occidentalis* L. Ditremexa occidentalis

Mukhtar M.D, Bashir M, Arzai A.H, (2006); Comparative.*In Vitro* Studies on Antiplasmodial activity of some Nigerian and Foreign brands of Chloroquine oral formulations marketed in Kano; *African Journal of Biotechnology*, 5(24):2464-2468.

Ramazani, A., Sardari, S., Zakeri, S. and Vaziri, B. (2010). *In vitro* antiplasmodial and phytochemical study of five *Artemisia* species from Iran and *in vivo* activity of two species. *Parasitol Res.*, 107:593–599.

Reeta M, Ravindra S. and Kasamarda (2013), The Ayurvedic approach. *Journal of Pharmaceutical and Scientific Innovation*. 2(2):2527.

Trager, G. (1982). "Targeting metastasis-initiating cells through the fatty acid receptor CD36".*Nature.*doi*:*10.1038/nature20791. Retrieved 7 December 2016*.*

UNICEF (2000). With the cooperation of the World Health Organization

UNICEF (2000). With the cooperation of the World Health Organization

Usha K, Kasturi G.M (2007) Hemalatha P. Hepatoprotective effect of Hygrophila spinosa and *Senna occidentalis* on carbon tetrachloride induced liver damage in experimental rats. *Indian Journal of Clinical Biochemistry*. ; 22(2):132-135. 7.

Veeramuthu, N., Verhaeghen, K., Van Bortel, W., Roelants, P., Marcotty, T., Baza, D., D'Alessandro, U. and Coosemans, M (2006). A significant increase in ***kdr*** in *Anopheles gambiae* is associated with an intensive vector control intervention in Burundi highlands. *Trop Med Int Health*, 13:1479-1487.

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

World Health Organization (2014). Chronic conditions: The global burden. Retrieved 2 December, from: http://www.who.int/chronic_conditions/burden/en/

World Health Organization (WHO) (2017).*World Malaria Report* ISBN 978-92-4-156515-8 WHO World Malaria Report (2008). Geneva: WHO; 2016. [Online] Available from: www.who.int/malaria/wmr2008. (Accessed on 2009 October 12).

Yadava, R.N, Satnami D.K. (2011). Chemical constituents from *Senna occidentalis* Linn. Indian Journal of Chemistry. 50B. 2011, 1112-11186.