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ASSESSMENT OF CYTOTOXICITY AND ANTIPLASMODIAL ACTIVITIES OF *Prosopis africana* LEAF EXTRACTS

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ABSTRACT
Malaria is a major health concern in Sub Saharan Africa and there are few effective treatment options. Nigeria has diverse flora with potent antimalarial phytochemicals and high ethnomedicinal plants uses. This study investigated the phytochemicals, Cytotoxicity and in-vitro antiplasmodial activity of ethnomedicinal plant which is Prosopis africana. Crude ethanol extract and macerated fractions from the ethnomedicinal plant were screened for major classes of antiplasmodial phytochemical compounds i.e., terpenoids, alkaloids, flavonoids, anthraquinones and Steroids. The antiplasmodial assay was conducted at 5% parasitaemia for 24 and 48 hours, against P. falciparum. Artemether-Lumefantrine was used as a positive control while 0.5% DMSO in RPMI 1640 medium was used as negative control. Moreover, all plant extract and fractions of P. africana were found to be effective in vitro for antiplasmodial activity, they demonstrated remarkable bioactivities at all concentrations; Methanol fraction (Pa-05) shows the highest activity with percentage elimination of 83.9% at 625µg/ml, 87.5% at 1250µg/ml, 92.9% at 2500µg/ml and 96.4% at 5000µg/ml. However, all the extract fractions have shown a good activity against the Brine Shrimp nauplii larvae. Crude Ethanol extract (PA-01) and n-Hexane fraction displayed the highest activity (LC_{50} 58.482µg/ml and 75.462 µg/ml) respectively. However, the results suggested that extracts of P. africana showed the most curative anti-plasmodia effect in infected blood which may be attributed to the presence of phytochemical constituents such as a alkaloids, flavonoids, and terpenoids.
Keywords: Ethanol extract, Fractions, Phytochemicals, Cytotoxicity and Antiplasmodial

INTRODUCTION

Medicinal plants and human beings have unique relationship since time immemorial. Man's vital interest in plants, primarily as a source of food, shelter, and clothing, dates back to the very origin of human civilization. Even before primitive hunter, gatherers developed Agriculture; there have been some people who have made a point to understand how to harness the power of the mysterious chemicals found all around. Thus, natural products chemistry was born (Arnold and Lixin, 2005). In the past two centuries, natural products chemistry has come into its own as a bounteous source of interesting and useful chemicals. Modern scientists have succeeded in treating many of the diseases of humanity by utilizing naturally occurring chemicals. As our knowledge of the intimate workings of chemicals and cell structures has grown, natural products chemistry continues to contribute to drug development (Arnold and Lixin, 2005).

Malaria is a parasitic disease that is transmitted to human beings by female *Anopheles* mosquitoes. There are four main malaria causing parasites namely: *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium falciparum*. Additionally, in some parts of Southern Asia there are reports of *P. knowlesi* (the simian *Plasmodium* species) infecting human beings, (White, *et al*., 2013; Kantele and Jokiranta, 2011).

In Nigeria, the burden of malaria is well documented and has been shown to be a big contributor to the economic burden of disease in communities where it is endemic and is responsible for annual economic loss of 132 billion Naira (Onwujekwe, *et al.,* 2000). It is estimated that 300,000 deaths occurring each year, 60% of outpatient visits and 30% hospitalizations are all attributable to malaria (Teklehaimanot, *et al*., 2008).

Medicinal values of Prosopis species have been mentioned in ancient literatures (Rajvanshis and Garg, 2015). An early report by Kirtikar, *et al*., (1935), mentioned that all parts of Prosopis spp. are traditionally used by indigenous people for curing various ailments (Khejra, 2001).

Water extracts of leaves and bark are traditionally used to cure mouth and throat infections, as well as bronchitis and ulcers; internal diseases including parasites and urinary diseases; and skin parasitic infections as well as dermatitis (Pasiecznik, 1999). This research is aimed at screening of the plant extract for cytotoxicity and antiplasmodial activity.

MATERIALS AND METHODS

General Procedure

All glassware used were thoroughly washed with detergent, water and dried before used. Analytically grade solvents and Reagents used in this research include; Ethanol, Methanol, n-hexane, Chloroform, Ethyl acetate and DMSO (Sigma Aldrich and Qualichem). Ferric chloride, Potassium iodide, Bismuth (II) nitrate, Hydrochloric acid, Acetic anhydride, and Sulphuric acid.

Collection of the Plant Samples

A small branch of *Prosopis africana* (BUKHAN 0193), was collected from Tamu in Kurfi Local Government Area of Katsina State on 12th April, 2018. The plant sample was identified and authenticated at the Plant Biology Department, Bayero University Kano.

Solvent Extraction of the Plant Materials

Each powdered sample (500g) percolated using Ethanol (2L) for 72 Hours (i.e. Three days) with constant agitation of about 20 to 30 times a day. The weight of each extract was recorded after the solvent was evaporated using Rotary Evaporator at 40^0C (Ajaiyeoba *et al.,* 2006, Musa *et al*., 2015 and Haruna *et al.,* 2018).

Maceration of the Crude Extracts

The crude Ethanol Extracts were macerated sequentially using n-Hexane, Chloroform, Ethyl acetate and Methanol. The fractions of these solvents were dried by exposing them to air at room temperature (Sofowora, 1984).

Qualitative Phytochemical Screening

Extracts of *Prosopis africana* leaf was screened for the presence of phytochemicals such as Alkaloids, Saponins, Tannins, Terpenoids, Flavonoids, Steroids and Anthraquinones using the methods of Clulci, (1994); Sofowora, (1984); Tor-anyin, (2003); Harborne (1998) and Adoum *et al*. (2016).

Assay for Cytotoxicity

Brine shrimp Lethality bioassay was carried out to investigate the cytotoxicity of the plant extracts. Fifty gramme (50mg) of *Atemia salina* (leach) eggs were introduced into a hatching chamber containing ocean/seawater (75ml). The hatching chamber was kept under an inflorescent bulb for 48 hours for the eggs to hatch into shrimp larvae. However 20mg of each test fractions Pa-01, Pa-02, Pa-03, Pa-04 and, Pa-05 of *Prosopis africana* Leaves were separately dissolved in 2ml of extract's solvent (ethanol, n-hexane, chloroform, ethyl acetate, and methanol) respectively to give final concentration of 10mg/ml (10,000µg/ml). From this, an aliquot of 5.0, 50, and 500µL of each solution of extracts were measured and transferred into a clean 5ml marked test tubes by the use of micro-pipette of which was allowed to evaporate, thereafter, one drop of Dimethyl Sulphuroxide (DMSO) was added before adding the shrimp to facilitate the solubility of test materials, followed by 2-3 drops of seawater, each dosage was tested in triplicate corresponding to 1000, 100, and 10µg/ml respectively, the positive control contains $K_2Cr_2O_7$, and negative only contains see water. To each test tube, ten (10) larvae of *Atemia salina* were added and the final volume of each solution was adjusted to 5ml with seawater immediately after adding the shrimps. The shrimps were allowed for 24 hours after which the number of dead shrimps in each test tubes were counted (Adoum, 2015). The LC_{50} values for each fraction was determined at 95% confidence level or intervals by analyzing the data on a computer loaded with a "Finney programme". Finally, the LC_{50} values of the brine shrimps obtained were recorded (Adoum, 2015).

In vitro Antiplasmodial Assay

Determination of antiplasmodial Activity of the Extract

After 24 and 48 hours of Incubation, an aliquot of the culture medium was dropped on a microscopic slide, stained, and viewed under oil immersion. The average percentage elimination of the erythrocytes that appeared as blue discoid's cells was determined using the formula as follows:

$$\% \text{ Elimination} = \frac{N}{Nx} \times 100$$

% = percentage activity of the extracts,

N = Total number of cleared red blood cells (RBC),

Nx = Total number of parasite (RBC) [Muktar *et al.,* 2006].

RESULTS AND DISCUSSION

Fractions of *P. africana* Leaf Extracts Recovered

The ground fine powdered *Prosopis africana* leaves (500g) was percolated and yielded 86.42g of Ethanol extract, which gives 17.28%. From the crude extract, 76.42g was macerated with different solvents (Table 1) yielded n-Hexane fraction 10.85g (14.20%, black and sticky), Chloroform fraction 1.55g (2.03%, black and sticky),

Ethyl acetate fraction 6.45g (8.44%, black and sticky), Methanol fraction 53.60g (70.14%, black and sticky) and Water fraction 3.01g (3.94%, white crystalline solid).

This is in consistent with reported findings of Abdurrahman *et al*., (2016) of which, the air-dried sample (200 g) was percolated using ethanol and the crude extract was further macerated using n-hexane, chloroform, ethylacetate and methanol respectively. The above result is also in order, buttressing studies conducted by Akintayo et al., (2017) and Apampa *et al*. (2019).

Phytochemical Contents *P. africana* Leaf Extracts

The phytochemical analysis carried out on the leaf extracts of *Prosopis africana* plant reveals the presence of the following secondary metabolites: Alkaloids, Saponins, Tannins, Flavonoids, Terpenoids, and Anthraquinones from the crude and macerated fractions.

Table 2, shows the presence of only Saponins in all the fractions, Alkaloids are present in Crude extract, n-Hexane, Chloroform and Ethyl acetate fractions, Flavonoids however present in all with the exception of n-Hexane fraction,Tannins and Anthraquinones are absent only in Chloroform fraction, whereas, Terpenoids are absent in n-Hexane and Ethyl acetate fractions.

However, Rwang *et al*. (2016) conducted research and found that, the phytochemical screening of the plant extracts (water and methanol) used revealed the presence of tannins, Saponins, anthraquinones, cardiacglycosides, carbohydrate and steroids while terpenes were absent, of which alkaloids and flavonoids were not included in their findings. Hence, the results presented in Table 2 above is in consistence with Rwang *et al*. (2016) findings, even though only water and methanol extracts were used in their studies.

Antiplasmodial Activity of *P. africana* Leaf Extract (Fractions)

The antiplasmodial bioassay was carried out on all the fractions and the Crude extract. The antiplasmodial activity of *Prosopis africana* leaves extract and fractions however shows significant percentage elimination of the parasites. As shown in Table 3, the fractions and the crude extracts demonstrated a remarkable bioactivities at all concentrations; Methanol fraction (Pa-05) shows the highest activity with percentage elimination of 83.9% at 625µg/ml, 87.5% at 1250µg/ml, 92.9% at 2500µg/ml and 96.4% at 5000µg/ml.

However, the literature comparison of this result is found in the research conducted by Amoa *et al*. (2013), Adoum (2015) and Abdurrahman *et al*. (2016) of which their reported findings were effectively in consistent with the present studies.

Cytotoxicity of the *P. africana* Extracts

All the extracted fractions have shown a good activity against the Brine Shrimp nauplii larvae. Crude Ethanol extract (Pa-01) and n-Hexane fraction (Pa-02) displayed the highest activity (LC_{50} 58.482µg/ml and 75.462 µg/ml) respectively, while the Ethyl acetate fraction is the least active with LC_{50} value of 89.427 µg/ml as shown in Table 8. This result is consistent with reported findings of Adoum *et al.* (1997).

Table 1: Physical Properties of Macerated Fractions of *Prosopis africana* Leaves Extract.

Solvent Extraction	Code of Fraction	Texture	Colour	Weight (g)
Ethanol (Crude)	Pa-01	Sticky	Black	76.42
n-Hexane	Pa-02	Sticky	Black	10.85
Chloroform	Pa-03	Sticky	Black	1.55
Ethyl acetate	Pa-04	Sticky	Black	6.45
Methanol	Pa-05	Sticky	Black	53.60
Water	Residue	Crystalline	White	3.01

KEY: Pa-01: Ethanol (Crude Extract), Pa-02: n-Hexane Fraction, Pa-04: Ethyl acetate Fraction, Pa-03: Chloroform Fraction Pa-05: Methanol Fraction

Table 2: Phytochemical Contents of *Prosopis africana* Leaves Extract (Fractions)

Fraction	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins	Anthraquinones
Pa-01	+	+	+	+	+	+
Pa-02	+	-	+	-	+	+
Pa-03	+	+	-	+	+	-
Pa-04	+	+	+	-	+	+
Pa-05	-	+	+	+	+	+

KEY: + : Presence of Phytochemicals, - : Absence of Phytochemicals

Table 3: Antiplasmodial Activity of *Prosopis africana* Leaves Extract (Fractions)

Sample or fraction	Concentration (µg/ml)	Average number of parasite before incubation	Number of parasites in 24hrs incubation	Number of parasites in 48hrs incubation	Overall average number of parasites after 48hrs incubation	Percentage elimination at the end of incubation (%)
Pa-01	5000		8	5	6.5	88.4
	2500	56	10	7	8.5	84.8
	1250		14	9	11.5	79.5
	625		16	12	14	75.0
Pa-02	5000		12	9	10.5	81.3
	2500	56	13	11	12	78.6
	1250		15	14	14.5	74.1
	625		16	15	15.5	72.3
Pa-03	5000		12	11	11.5	79.5
	2500	56	14	13	13.5	75.9
	1250		16	15	15.5	72.3
	625		19	17	18	67.9
Pa-04	5000		5	3	4	92.9
	2500	56	7	6	6.5	88.4
	1250		10	9	9.5	83.0
	650		12	11	11.5	79.5
Pa-05	5000		3	1	2	96.4
	2500	56	5	3	4	92.9
	1250		8	6	7	87.5
	650		10	8	9	83.9
Coartem	0	56	0	0	0	100
Control						
-	0	56	56	56	56	0

Table 4: Brine Shrimp Lethality (BST) of the extracts

Sample Code	Concentration (µg/ml)	Replica	Total population	Survivors after 24 H	Total Death	LC_{50} (µg/ml)
Pa-01	1000	3	10	0,1,1	10,9,9	58.482
	100	3	10	3,4,3	7,6,7	
	10	3	10	9,9,8	1,1,2	
	Control +	3	10	0,0,0	10,10,10	
	Control -	1	10	10	0	
Pa-02	1000	3	10	1,0,0	9,10,10	75.462
	100	3	10	5,6,5	5,4,5	
	10	3	10	9,8,8	1,2,2	
	Control +	3	10	0,0,0	10,10,10	
	Control -	1	10	10	0	
Pa-03	1000	3	10	0,1,0	10,9,10	88.180
	100	3	10	5,6,6	5,4,4	
	10	3	10	9,9,8	1,1,2	
	Control +	3	10	0,0,0	10,10,10	
	Control -	1	10	10	0	
Pa-04	1000	3	10	0,0,1	10,10,9	89.427
	100	3	10	6,5,5	4,5,5	
	10	3	10	9,9,9	1,1,1	
	Control +	3	10	0,0,0	10,10,10	
	Control -	1	10	10	0	
Pa-05	1000	3	10	1,0,1	9,10,9	82.574
	100	3	10	5,5,6	5,5,4	
	10	3	10	9,8,8	1,2,2	
	Control +	3	10	0,0,0	10,10,10	
	Control -	1	10	10	0	

CONCLUSION

The phytochemical screening of *P. africana* leaf extracts shows secondary metabolites which include Alkaloids, Flavonoids, e.t.c. after percolation and maceration processes. The *Prosopis africana* leaf extracts, shows an effective antiplasmodial activity, Methanol fraction (Pa-05) shows the highest activity with percentage elimination of 83.9% at 625µg/ml, 87.5% at 1250µg/ml, 92.9% at 2500µg/ml and 96.4% at 5000µg/ml. Proofing the significance of the plant leaves in treatment of malaria fever and supporting its traditional usage in Nigeria and West Africa.

RECOMMENDATIONS

Further research on the whole plant of *Prosopis africana* should continue for *in-vivo* antiplasmodial activity in order to adequately buttress the effectiveness of the plant extracts in the treatment of malaria.

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