

Phytochemical Characterization and *in-vivo* Anti-Malaria Activity of *Lantana camara* Leaf Extract

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Abstract: The characteristic multi-drug resistant strains of *Plasmodium* to the existing anti-malaria has increase the global threat of malaria, couple with the wide medicinal application of *Lantana camara* in different countries and the literature of its use as anti-microbial, antifungal and insecticidal agents, and the quest for alternative anti-malaria this work look into the phytochemical screening, characterization and the *In-vivo* anti-malaria activity of the *Lantana camara* leaf extracts. The leaf extracts from plant were screened for phytochemicals and further FTIR characterized to correlate the routine qualitative phytochemical screening. *In-vivo* anti-plasmodia screening was carried out using 24 Swiss albino mice under laboratory conditions. The effectiveness of the aqueous and ethanolic extracts from the plant (*L. camara*) was compared with standard drug Chloroquine. The result indicated the presence of Alkaloids, Cardiac glycosides and Saponins in the Petroleum ether, aqueous and ethanolic extracts. Carbohydrate in both aqueous and ethanolic extracts, while Flavonoids and steroids in petroleum ether and ethanolic extracts. Tannins and terpenoids were present in aqueous and ethanolic extracts respectively. The identified phytochemicals correlate with FTIR spectral analysis from the identified characteristic functional groups. *In-vivo* study showed that Chloroquine was 83% effective in clearing the parasites, while aqueous and ethanolic extracts were 38 and 76% effective respectively. The curative capacity indicated possible concentration and time-dependency. The ethanolic extract showed higher level of anti-malaria potential and has indicated some level of protection.

Key words: Anti-Malaria activity, FTIR spectral, *Lantana camara*, phytochemicals

INTRODUCTION

Malaria is a disease caused by infection with single cell parasites of the genus *Plasmodium*. It is characterized by periodic bouts of severe chills and high fever and may result in death if left untreated (Malaria, 2009). In Africa More than a million people died of the disease annually. According to World Health Organization (WHO), malaria is wide spread in North America and other temperate region (WHO, 1978; Malaria, 2009). Today, malaria occurs mostly in tropical and subtropical regions particularly in sub-Saharan African and south Asia (Oyewole *et al.*, 2008; Malaria, 2009). Public health officials had hoped to exterminate malaria and wipe it out during the 20th century. However, malaria has developed defenses against many anti-malaria drugs, which makes the drugs less effective in its treatment.

Malaria therefore, remains a global threat to humanity and all effort today focus on its control. More so, development of malaria vaccine was confronted with

difficulties such as evolvement of variants plasmodia parasites due to its genetic transformation, coupled with hundreds of different strategies for evading human immune system, many of which mechanisms are yet to be well comprehended due to its complex nature (Malaria, 2009). However, considerable progresses have been made in vaccine development (Palaniswamy *et al.*, 2008).

Malaria threat to humanity is compounded by the prevalence of the multi-drug resistant strains of *Plasmodium* and adverse reaction of individuals to available anti-malaria drugs. The mortality and morbidity level due to malaria lead to the urgent need for an alternative anti-malarial therapy that are effective and affordable to people that are prone to this disease and are economically backward with difficult access to modern health facilities (Oyewole *et al.*, 2008). Attention is focused on the natural heals; due to the presence of the phytochemicals which have been proved to have many curing ability to many disease (Oyewole *et al.*, 2008).

World Health Organization (WHO, 1978) estimate indicated that about 4 billion people (80% of the world population) are presently inclined to the use of herbal

medicine as a primary health care. In addition, 119 plants, about 74% derived pharmaceutical medicine are in use in modern medicine in a way correlated directly with their traditional application thereby reflecting their native culture.

Lantana camara is a small perennial shrub of Verbeanceae family with various common names based on different ethnic groups. The names include Trinidad (*wild sage*), Prickly lantana, *Ewon adele* (Yoruba), *Anya nnuu* (Igbo) and *Kimba mahalba* (Hausa). It is a low erect or sub-scandent vigorous shrub with stout recurved prickles and strong odour of black currants. It grows to 1.2-2.4 meters. It is a significant weed with 650 different varieties, in over 60 countries (Day *et al.*, 2003; Craig, 2006). The plant is documented to be used traditionally in the treatment of intestinal worms, eye diseases and male fertility problem and also to facilitate child birth in different countries (Invan, 1981 in Day *et al.*, 2003). The leaf extracts were reported to exhibit antimicrobial, fungicidal, insecticidal and nematocidal activities (Sharma *et al.*, 1999; Begum *et al.*, 2003).

Medicinal plants are potential natural healers and their significance in communities cannot be over emphasized. Their activities in treatment of ailments lie in the phytochemical composition. Looking at the wide medicinal application of *Lantana camara* traditionally in different countries and the literature of its use as antimicrobial, antifungal and insecticidal agents, this work look into the phytochemical characterization and the anti-*Plasmodia* activity of the *Lantana camara* leaf extracts.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *Lantana camara* plant were collected from Tudun wada campus of Kaduna Polytechnic, Kaduna between March and April 2009. The sampled leaf was duly authenticated at herbarium section of Ahmadu Bello University, Zaria in the Botany Section of the Department of Biological Science. The leaves were collected and washed thoroughly with water and air dried in a shady area at room temperature (25°C) for about two weeks. The dried leaves were pulverized using pestle and mortar. The processed leaves were then stored in a brown bottles away from sunlight until required.

Preparation of plant crude extracts: 50 g of the powdered plant samples were extracted with 300 cm³ of petroleum ether for 7 h using Soxhlet extractor. The extract obtained was concentrated by steam evaporation. The residue obtained was dried and used for further ethanolic and aqueous extraction following continuous extraction method. 50 g of the powdered plant residue obtained was soaked in 300 cm³ of distilled water for 48

h with intermittent shaking. The resultant mixture was filtered with cheese cloth and the filtrate was concentrated by steam evaporation on water bath.

Alcoholic extraction was carried out using Soxhlet extractor. 50g of the powdered sample residue obtained after the extraction with petroleum ether were extracted using 300 cm³ of ethanol for 18 h. The extract obtained was concentrated by heating on water bath steaming.

The percentage yield was calculated by = (Mass of the extract/Mass of the sample)×100

Phytochemical screening: Phytochemical screening for the major constituents was undertaken using standard qualitative method as described by Sofowara, 1993. The plant extract was screened for the presence of Alkaloids, Anthraquinones, Cardiac glycosides, Carbohydrates, Flavonoids. Also included are steroids, phlobotannins, Tannins, saponnin and terpenes.

Characterization procedure: The routine procedure for the infrared analysis of lantana camera leaf (Egwaikhide and Gimba, 2007) was adapted using Fourier Transformed Infrared (FTIR) spectrophotometer model 8400s. The extracts were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the FT IR and scanned through the IR region.

In-vivo test:

Parasite inoculation: The *Plasmodium berghei* was obtained from National Pharmaceutical Research Institute Abuja through the Biochemistry Department, Federal University of Technology Minna and maintained by blood transfer in albino Swiss mice of body weight (21-24 g) of the same sex.

The *Plasmodium berghei* was prepared and inoculated as described by (Okonkon *et al.*, 2008). The blood was prepared by determining the percentage parasitemia and the erythrocytes count of the donor mouse and further diluting the blood with normal saline in proportion such that 0.2 cm³ of the blood contains 1.0 × 10⁷ *P. berghei* parasitized erythrocytes. 0.2 cm³ of the infected blood was inoculated intra-peritoneally to each mouse on day 0.

Animal grouping: Albino Swiss mice of weight 21-24 g of the same sex were obtained from Animal house of Ahmadu Bello University Zaria. They were maintained on standard animal pellets and enough water. The animals were grouped into six groups of 4 mice each identified as group A, B, C₁, C₂, D₁ and D₂.

Curative schizontal activity of the extract: The evaluation of anti-plasmodia activity of the extracts was carried out using the curative method described by Ryley and Peters, 1970 with the following modification. Each mouse was inoculated on the first day (0) intraperitoneally with 0.2 cm³ of infected blood containing

about 1.0×10^7 *P. berghei* parasitized RBCs. Seventy two hours later Group A animals were administered Chloroquine orally at 10 mg/Kg/day. Equivalent volume of distilled water (Negative control) was given to Group B animals. Group C and D were orally administered with aqueous and ethanolic extracts respectively using different concentrations of extracts, 25 mg and 40 mg/Kg/day denoted as C1 C2 and D1 D2, respectively. The drug and the extracts were given once daily for 5 days. The parasitemia level was monitored using a prepared thin film and Giemsa stained obtained from the mice tail daily for 5 days. The mean survival time for each group was determined by arithmetic means (Average survival time/day) of the mice after inoculation in each group over a period of 15 days (day 0 to day 14) as described by (Okonkon *et al.*, 2008).

Percentage parasitaemia: Percentage parasitaemia was calculated before and after treatment from:

$$\% \text{ parasitaemia} = (\text{Number of infected Rbcs} / \text{Total number of Rbcs}) \times 100$$

From the thin films made from the tail blood of each mouse, parasitaemia level was determined by counting the number of parasitized RBCs out of 200 erythrocytes in random fields of the microscopes. Average percentages (%) were then calculated.

Physical parameters such as body temperature, heartbeat rate and body weight of the animals were examined, before and after treatments.

RESULTS

Percentage yield of different solvent extracts of *lantana camara* leaf: The percentage yield for *L. camara* leaf extracts using different solvents were 31.46, 40.49, and 36.68% for petroleum ether, ethanol and aqueous extracts respectively. The percentage yield of the extracts was highest for ethanol (Table 1).

Phytochemical screening of different extracts: The results showed the presence of Alkaloids, cardiac glycosides, and saponins in all the extracts (petroleum ether, ethanolic and aqueous extracts). Carbohydrates were detected in both ethanolic and aqueous extracts,

Table 1: Percentage yield of different solvent extracts of *Lantana camara* leaf

Solvents/Extract	Percentage yield (%)
Petroleum ether extract	31.46
Ethanol extract	40.46
Aqueous extract	36.68

Table 2: Phytochemicals detected in different extracts of *L. camara* leaf

Phytochemicals	Petroleum ether extract	Aqueous extracts	Ethanolic extracts
Alkaloids	+	+	+
Anthraquinones	-	-	-
Carbohydrates	-	+	+
Cardiac Glycosides	+	+	+
Flavonoids	+	-	+
Phlobotannins	-	-	-
Saponins	+	+	+
Steroids	+	-	+
Tannins	-	+	-
Terpenoid	+	-	-

+: present; -: absent

while flavonoids and steroids were present in both petroleum ether and ethanolic extracts. Tannins and terpenoids were detected in aqueous and petroleum ether extracts respectively (Table 2).

The infrared spectra analysis of the different extracts of *Lantana camara* showed several absorption bands and sharp peaks at the finger print region of 1200 - 400 cm⁻¹. The results of the spectra analysis of the petroleum ether extract showed that the region of the finger prints is within 1229.66 - 500 cm⁻¹ (Fig. 1). The absorption band at 3421.83 cm⁻¹ is due to O-H stretching and 2917.43 - 2843.96 is due to C-H stretching which correlates with that of Williams and Fleming (1980). Absorption bands at 1739.83 - 1710.92 are due to C = O stretch or C = R stretching from an ester and bonding (Fig. 1). The spectra analysis correlate with the routine qualitative phytochemical screening which identified alkaloids and cardiac glycosides as the main characteristic functional groups which include, C = C, C = N, C = R, C = O and C-OH (Fig. 1). Although slight deviations were observed from the spectral of the pure functional groups the result is valid as the deviations are as a result of impurities that are likely to be associated with the crude extracts. Flavonoids have aromatic nucleus with C-OH (Phenols), while steroids are complex lipid which are invariably esters with C = R, C = C, C = O and C-OH as the contributing functional groups. These functional groups were confirmed by the absorption bands in the spectra of the petroleum ether extract (Fig. 1).

The ethanolic extract's spectral analysis revealed bands in the finger print region between 1261.49 - 500 cm⁻¹. Absorption bands at 3348.43 cm⁻¹ is due to OH stretching and 2925.15 - 2853.78 cm⁻¹ is due to C-H stretching. While 1654.98 - 1525.74 cm⁻¹ on the other hand is due to either C = C or C = O stretching.

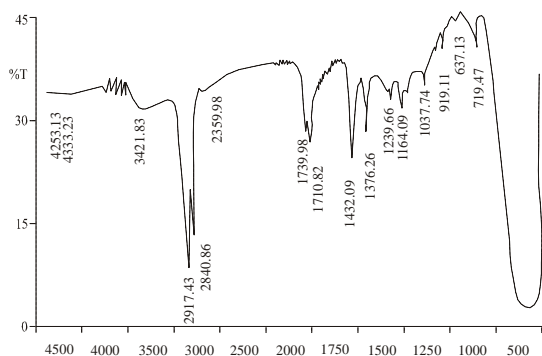


Fig. 1: Infrared analysis spectrum of petroleum ether extract of *Lantana camara* leaves showing the peak absorption bands of different functional groups

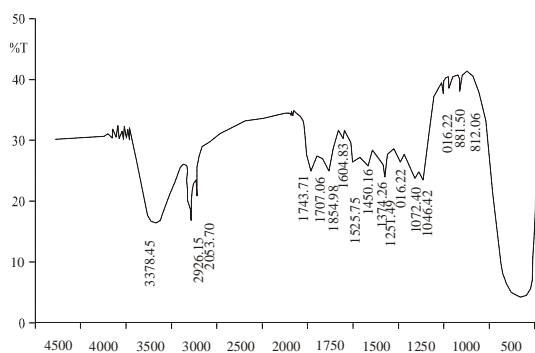


Fig. 2: Infrared analysis spectrum of ethanolic extract of *Lantana camara* leaves showing the peak absorption bands of different functional groups

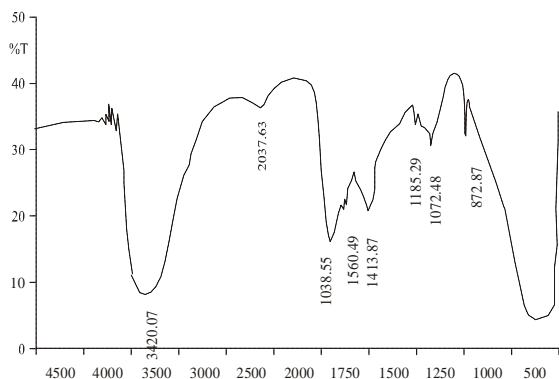


Fig. 3: Infrared analysis spectrum of aqueous extract of *Lantana camara* leaves showing the peak absorption bands of different functional groups

Absorption bands at $1743.71 - 1707.06 \text{ cm}^{-1}$ is due to $C = N$, $N = H$ or $C = R$ stretching. C-H or OH deformation showed absorption bands at $1460.16 - 1376.26 \text{ cm}^{-1}$ (Fig. 2). This is in correlation with the functional groups constituting the identified phytochemicals such as

alkaloids, flavonoids, cardiac glycosides and saponins in the extract. Carbohydrates are polyhydroxyl aldehydes and ketones which basically have $C = O$, $C-OH$ as contributing functional groups and are correlated in the spectral peaks (Fig. 2).

Aqueous extracts have the region of finger prints to be within $1158.29 - 461.97 \text{ cm}^{-1}$. The absorption bands indicated OH stretching at 3420.87 cm^{-1} , C - H stretching at 2917.68 . Either $C = C$ or $C = O$ stretching is indicated at $1638.58 - 1560.46 \text{ cm}^{-1}$. OH or C - H deformation is at 1413.87 cm^{-1} (Fig. 3), all of the specified peaks are supported by the preliminary qualitative phytochemicals identified in the extract (Table 4). Tannin particularly identified with this extract is characterized with $C = R$, C - OH aromatic nucleus and phenols (Treace and Evans 1989) which are correlated with the spectral (Fig. 3).

Variation in the physical parameters: The mice in all the groups showed variations in the average rate of heart beat, body temperature and the body weight before and after treatment. The results indicate a general increase in the body temperature in all the groups between 33°C and 36°C which indicated possible manifestation of the *Plasmodium species*. That was followed by subsequent fall in the temperature between 32 to 30°C of the treated groups. The temperature of the control group increases up to 40°C (Table 3). There was progressive increase in the average heartbeat rate between 60 and 65 pulses per minute after infection, before treatment. However, during and after the treatment a remarkable decrease was observed the heartbeat rate between 60 and 58 pulses per minute was observed, however the control group remain progressively high up to 64 pulses per minute, which is an indication of progress in malaria ailment (Table 3). There was no significant average weight loss within the control group. However the effect of the infection cannot be ruled out base on the fact that the proportional growth rate within the period retarded and the growth of the animals was maintained within the same range as obtained before and after treatment (Table 3).

In vivo anti-malarial activity: The group of mice treated with Chloroquine phosphate showed almost total clearance of about 83% at day 7 (Table 4) and showed close to 75% protection from the parasites after treatment (Table 5). The group of mice that were left as control and treated with distilled water group B showed daily increase in the average parasitemia from 6% (day 2) to 20% (day 5) to 25% (day 7) and a total mortality at the end of the experiment. The group C mice treated with aqueous extract also showed daily increase from 3% (day 1) to 13% (day 3) and suddenly reduced to 12% (day 4)

Table 3: Variations in some physical parameters of the mice before and after treatment

Chloroquine / extracts	Groups	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
		*Ave Temp °C	Ave. Temp °C	Ave. heart beat ave. Pulse/min	Heart beat Pulse/min	Ave. body weight (g)	Ave. body weight (g)
Chloroquin	A	35	32	61	53	22.1	22.0
Distilled water	B	36	40	62	64	23.1	19.2
Aqueous extract	C	34	32	60	58	24.3	24.1
Ethanol extracts	D	33	30	65	60	21.9	20.8

*Ave: average

Table 4: Means percentage parasitemia per day (%)

Groups days	0	1	2	3	4	5	6	7	% Clearance after treatment (%)
A	0±0.00	2.80±0.44	6.20±0.25	12.43±00.10	10.20±00.15	07.15±00.19	04.13±00.15	02.13±0.15	83
B	0±0.00	3.15±0.13	6.30±0.79	14.13±00.15	16.15±00.19	20.13±00.92	4.58±00.102	5.55±0.06	00
C ₁	0±0.00	3.05±0.66	6.00±0.98	13.10±00.12	12.55±00.06	12.18±00.15	11.18±00.24	10.58±0.98	20
C ₂	0±0.00	3.85±0.26	6.50±1.10	13.48±00.05	11.55±00.06	10.10±00.12	09.13±00.15	08.40±0.27	38
D ₁	0±0.00	2.90±0.34	5.70±1.10	12.38±00.25	11.40±00.12	11.45±00.10	11.18±00.24	10.58±0.05	15
D ₂	0±0.00	3.20±0.83	6.80±0.38	13.23±00.26	11.50±00.82	08.25±00.21	04.30±00.22	03.18±0.24	76

Data showed the means and standard deviation of parasitaemia samples taken in quadruplicates

Table 5: Mean survival time of mice after 14 days of infection

Group	Initial no.	No. of death	Percentage survival (%)
A	4	1	75
B	4	4	-
C ₁	4	3	25
C ₂	4	2	50
D ₁	4	2	50
D ₂	4	1	75

down to 10% (day 7) for the mice treated with 25 mg/kg/day of the extract (Group C₁). Group C₂ animals treated with 40 mg/kg/day of the aqueous extract, showed increase in the percentage level of parasitemia from approximately 4% (day 1) to 13% (day 3). However, the level decreased from 11% (day 4) to 8% (day 7) (Table 4) and the extract showed a 25% to 50% protection for C₁ and C₂ respectively (Table 5).

In group D mice treated with ethanolic extract of different concentrations; D1 (25 mg/kg/day) group showed daily percentage increase from 3% (day 1) to 12% (day 3) then reduced to 11.4% (day 4) to 10.5% (day 7). D2 (40 mg/kg/day) group showed increase in the percentage parasitemia from 3% (day 1) to 13% (day 3) and reduced subsequently from 11% (day 4) to 3% (day 7) after treatment (Table 4). The extract showed 50 and 75% protection for D1 and D2, respectively (Table 5).

DISCUSSION

The results of this research indicated that phytochemicals identified from *Lantana camara* leaf include alkaloids, cardiac glycosides, saponins flavonoid, tannin, steroids carbohydrate and terpenoids. The identification of these phytochemicals perfectly correlated with the findings from the spectral analysis of the extracts. The analysis show the presence of functional groups C = R, C = C, C = O, C = N and C-OH that are associated with esters, aromatic nucleus and phenols identified with tannins (Treace and Evans 1989).

Phytochemicals such as alkaloids, steroid, tannins and terpenoids detected in this work support the earlier report on isolation of the same phytochemicals from the stem, root and dry leaf of *Lantana camara* (Chalchat *et al.*, 1993; Singh, 1996; Chavan *et al.*, cited in Day *et al.*, 2003).

The results of this present study shows that both aqueous and ethanolic extracts of the leaf of *L. camara* contains anti-*Plasmodia* substances with properties that showed curative effects on the established malaria parasites infection. The ethanolic extract however show more potential that is comparable to the standard drug, Chloroquine as demonstrated by the mean survival time of the mice after treatment (Table 5). The parasite clearance capacity demonstrated by the plant from the result is both concentration and time dependent. That is based on the fact that the initially, increase in parasitemia levels in all groups started progressively, but as the treatment started drastic reduction of parasitic level was recorded (Table 4). Moreover, the status of parasite clearance differs when the concentrations were considered. The clearance capacity for 25 mg/kg body weight was far below that of 40 mg/kg body weight of the extracts. In addition, less protection for the animal survival shows the same pattern of variation (Table 5). It is pertinent to note that ethanolic extract indicated higher potential for parasite clearance and protection capacity.

The anti-*Plasmodial* activity of the two extracts could be associated with the presence of Alkaloids, tannins, steroids terpenoids and flavonoids (Philipson, and Wright 1991; Christensen and Kharaz, 2001; Palaniswamy *et al.*, 2008). Although the mechanism of their action is yet to be elucidated, some of the known plant extracts are said to exhibit their anti-plasmodia activity by causing the oxidation of red blood cells (Etkin, 1997) or by parasite protein synthesis inhibition (Kirby *et al.*, 1989) depending on the phytochemical constituents. More so, the constituent compounds identify

may act singly or synergistically with one or more phytochemicals to achieve the desired activities as suggested in the literature (Kirby *et al.*, 1989).

CONCLUSION

This research has established the anti-malaria activity of *Lantana camara* leaf extract with certain level of protection. The effect is concentration dependent. However, further work is required to identify the lead anti-*Plasmodia* components.

ACKNOWLEDGMENT

The authors wish to record their appreciation for the assistance and facilities provided by National Institute for Chemical Technology (NARICT) Zaria, Kaduna State and Biochemistry Department, FUT Minna, Niger State Nigeria.

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