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ARTICLE

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Full Length Research Paper

Phytochemical and acute toxicity studies of methanolic extracts of selected antimalarial plants of Nupeland, north central Nigeria

Usman Idris Nda-Umar^{1*}, Mohammed Gbate¹, Abdulkadir Nda Umar¹, Yahaya Masaga Alfa¹ and Abdullahi Mann²

¹Department of Science Laboratory Technology, Federal Polytechnic, P. M. B. 55, Bida, Niger State, Nigeria.

²Department of Chemistry, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria.

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Malaria remains one of the most prevalent diseases throughout tropics and subtropical areas and its effective chemotherapy remains a problem. Hence the need to continue to explore plants for their therapeutic potentials. In Nupeland, *Lannea barteri* (monkey's faru), *Piliostigma thonningii* (camel's foot tree or monkey's bread), *Clerodendrum aculeatum* (haggar bush or garden quinine) and *Crateva adansonii* (Three leaved-caper) are some of the plants commonly used in the treatment of malaria. This study evaluated the phytochemical constituents and the acute toxicity of the methanolic extracts of these plants using standard procedures. Results of the qualitative phytochemical analysis showed that all the methanolic extracts of the plants tested positive to alkaloids, saponins, tannins, terpenoids and glycosides. While only *P. thonningii* shows the presence of anthroquinones. The analysis also shows the presence of flavonoids in all the plants extracts except *C. adansonii* root. Acute toxicity study of the extracts of *L. barteri* and *P. thonningii* leaves revealed an oral LD₅₀ > 2500 mg/kg body weight, *C. aculeatum* leaf has LD₅₀ >1500 mg/kg body weight and *C. adansonii* root has LD₅₀ >2000 mg/kg body weight in mice. The presence of some of the phytochemicals in the plants' extracts and the values of the LD₅₀ recorded could explain their use traditionally for the treatment of wide array of illness including malaria.

Key words: Malaria, *Lannea barteri*, *Piliostigma thonningii*, *Clerodendrum aculeatum*, *Crateva adansonii*, phytochemical, acute toxicity.

INTRODUCTION

Malaria is a parasitic disease caused by *Plasmodium* species and it remains one of the most prevalent

diseases throughout the tropics and subtropical areas and is responsible to a great extent for poverty and under

*Corresponding author. E-mail: uindaumar@gmail.com Tel: +2347038683835.

development in sub Saharan Africa (WHO and UNICEF, 2003). The World Health Organization reports that there were 198 million cases of malaria worldwide in 2013. Out of which estimated 548,000 deaths occurred. 90% of these deaths occurred in African region and the most vulnerable group is children under the age of 5 years (WHO, 2014).

Chemotherapy with effective antimalarial drugs remains the main method to control malaria in the absence of a suitable vaccine treatment (WHO, 2011). Unfortunately, the malaria parasites have developed resistance against many of the antimalarial drugs including insecticides couple with the fact that the newly produced effective anti-malarial drugs are expensive, especially for most poor Nigerians to afford. These have resulted in therapy failure and resurgence in transmission of malaria. Hence the need to continue to explore indigenous knowledge of traditional medicine and therapeutic potential of plants through pharmacological research, bioprospecting and drug discovery.

In an earlier ethnobotanical survey of medicinal plants used for the treatment of malaria in Nupeland, Nda-Umar et al. (2014) documented ninety three plant species belonging to thirty-nine families from one hundred and six ethnomedicinal recipes. The families mostly encountered during the survey include *Caesalpiniaceae*, *Asteraceae*, *Euphorbiaceae*, *Poaceae*, *Malvaceae*, *Rubiaceae* and *Solanaceae*. It was also discovered that the application of the plants ranged from use of whole plants to use of parts like leaves, stem, root, stem bark, root bark and floral parts.

Several extracts of the plants documented above have been investigated for their phytochemicals and antimalarial activities (Ndjakou et al., 2006; Titanji et al., 2008; Jigam et al., 2010; Adumanya et al., 2012).

However, no detail investigations or scientific explanation on the rationale for the use of some of these plants have been reported in spite of the rich historical and educational antecedent of the Nupe medicinal plants. The dearth of this information coupled with the increasing need for a safer, cheaper and available antimalarial drugs necessitates detail study of *Lannea barteri*, *Piliostigma thonningii*, *Clerodendrum aculeatum* and *Crateva adansonii* commonly used in Nupeland.

This study is aimed at evaluating the phytochemical constituents and the acute toxicity profile of the methanolic extracts of *L. barteri*, *P. thonningia*, *C. aculeatum* leaves and *C. adansonii* root.

MATERIALS AND METHODS

Plant species

L. barteri, commonly known as 'monkey's faru' ('Yinchi' in Nupe, 'Faru' in Hausa and 'Ekika' in Yoruba) is a deciduous tree with a spreading crown. It is a savannah specie that spread across Guinea to Uganda areas. It grows from 5-18 m tall. It is an important source of a red brown dye that is used in traditional

dyeing in Africa and also supplies food, medicines and materials (Neuwinger, 2000).

P. thonningii, commonly known as 'camel's foot', 'monkey's bread' or 'foot tree'. It is known in Nupe as 'Bafin', 'Kalgo' in Hausa, 'Abafe' in Yoruba and 'Okpoatu' in Igbo. It is a leguminous and perennial plant that belongs to the family Fabacea. It is a savannah specie found across inter tropical Africa. It produces flowers around November and December. It bears hairy flat pod fruit that turns light brown and woody on maturity (Jimoh and Oladiji, 2005).

C. aculeatum, commonly known as 'haggar bush' or 'garden quinine'. It is a deciduous tree with a spreading crown widely distributed in the tropics from the Central African Republic to Nigeria and Uganda. It is an important source of red brown dye that is used in traditional dyeing in Africa and also supplies food and medicines (Cobett et al., 2003). It is known as 'Yawo lawogi yiwo' in Nupe.

C. adansonii, commonly known as 'three leaved-caper'. It is widely distributed in tropical Africa especially in the Sudan zone. It is a tree 3-10 m high with trunk 30-50 cm in diameter. The leaves are compound trifoliate and alternate. Inflorescence is a corymbs form panicles occurring on top of branches. Corymbs bear 15-20 flowers, which are white and only visible when the tree is defoliated. Fruit is berry, spherical with diameter of 3-8 cm on pedicle 5-6 cm long (Mann et al., 2003). It is known as 'Kulanchi' in Nupe, 'Gudai' or 'Ungududu' in Hausa and 'Oto' or 'Eegun oru' in Yoruba.

Collection of plant materials

All the parts of the plant samples used in this work were collected in Bida, Niger State, Nigeria in June, 2015. They were identified and names authenticated by Taxonomy section of the Biology unit of Science Laboratory Technology Department, Federal Polytechnic Bida. The plants were deposited with the following herbarium numbers: *L. barteri* (SLT/BFHB/11), *P. thonningia* (SLT/BFHB/50), *C. aculeatum* (SLT/BFHB/248) and *C. adansonii* (SLT/BFHB/56).

Animals

Fifty-two (52) adult Swiss albino mice of either sex were used for this study (including the control). They were obtained from National Institute for Medical Research (NIMR), Lagos and were acclimatized for three (3) weeks in the Biological garden of Department of Science Laboratory Technology, Federal Polytechnic, Bida. The mice were housed in ventilated plastic cages, fed with pellets and have access to potable water throughout the period of the study.

Preparation of the crude extract

The fresh leaves samples of *L. barteri*, *P. thonningii*, *C. aculeatum* and fresh root of *C. adansonii* were dried at room temperature (27 – 29.5°C) for four weeks. The dried samples were pounded separately using mortar and pestle into powdered form. 150 g of the plant powder was soaked in 600 ml of methanol for 72 h and filtered with Whatman filter paper No. 1. The filtrates were dried using a rotary evaporator at 35°C for 48 h. The extracts obtained were kept under refrigeration and away from light prior to further processing and use (Mann, 2007).

Phytochemical screening

Phytochemical screening was performed using standard methods as described in Parekh and Chanda (2007), Adebayo and Shola

(2009), Chugh and Bharti (2012) as follows:

Test for alkaloids

To 2 ml of the plant extract, 0.2 ml of dilute HCl (1%) was added in a test tube. Then few drops of Dragendroff's reagent were added. Brownish-red or orange precipitate indicates the presence of alkaloids.

Test for flavonoids

To 3 ml of the plant extract, 2 g of magnesium powder was added and few drops of concentrated HCl added. Formation of orange, pink, red to purple colours indicates presence of flavonoids.

Test for saponins

To 5 ml of the plant extract, 10 ml of distilled water was added in a test-tube. The solution was mixed by shaking vigorously and was observed for stable persistent froth indicating presence of saponins.

Test for tanins

To 5 ml of the plant extract, 10 ml of warm distilled water was added in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the 2 ml of the filtrate. Observation of blueblack-green or blue-green precipitate indicates the presence of tannins.

Test for terpenoids

5 ml of the plant extract was added to 2 ml of chloroform. 3ml Concentrated H₂SO₄ was carefully added to form a layer. A reddish brown coloration interface indicates the presence of terpenoids.

Test for glycoside

0.5 ml of the extract was shaken with 2 ml of Chloroform in a test tube and few drops of concentrated Sulphuric acid were added. The formation of reddish brown steroid ring indicates the presence of glycoside.

Test for anthraquinones

5 ml of the plant extract was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% ammonia solution was added to the filtrate. A pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

Acute toxicity test

Acute toxicity test of the plants extracts was carried out using the method of Lorke (1983) as described in Latha and Reddy (2009). In phase 1 of the experiment, the animals were randomly grouped into three of three mice each and were orally treated with the plant extract of 10, 100 and 1000 mg/kg body weight respectively. The mice were placed under observation for 24 h to monitor their behavior and any mortality. In phase 2, the animals were grouped into three of one animal each and were orally treated with higher

doses of the plants' extracts at 1500, 2000 and 2500 mg/kg body weight. The animals were also observed separately for 24 h for signs of toxicity and mortality. This procedure was repeated for all the plants' extracts.

RESULTS AND DISCUSSION

The results of qualitative phytochemical screening of the methanolic extracts of *L. barteri*, *P. thonningii*, *C. aculeatum* leaves and *C. adansonii* root are shown in Table 1. The result revealed the presence of alkaloids, saponins, tannins, terpenoids and glycosides in all the plants extracts tested. While only *P. thonningii* shows the presence of anthroquinones. The screening also shows the presence of flavonoids in all the plants' extracts except *C. adansonii* root. The presence of variety of phytochemicals in the present study gives the indication that the plants, extracts could be used for curative activity against pathogens and therefore could explain their use traditionally for the treatment of wide array of illness including malaria (Anaduaka et al., 2013). Similarly, a number of researchers have linked the presence of certain phytochemicals as responsible for the treatment of specific diseases. Tannins and flavonoids are known to be present in extracts used as antibacterial and antioxidant (Cook, 1996). Flavonoids and glycosides are also known to prevent cardio-vascular diseases and ulcers (Swigio and Tyrakwoska, 2003). The presence of alkaloids in plant extracts are also used for wide range of pharmacological activities including antimalarial, antiasthma, anticancer, etc (Kittakoop et al., 2014). Recent studies also showed that tannins containing extract was used to treat haemorrhoids (Njoku and Akumufula, 2007), as antiviral (Lu et al., 2004) and antiparasite (Kolodziej et al., 2005). Sofowora (1986) also indicated that the presence of these secondary metabolites in plants produces some biological activities responsible for their potential use as drugs. This may be responsible for the use of these medicinal plants for local therapy.

Tables 2 and 3 shows the result of acute toxicity test of the plants' extracts. In phase 1 of the study as indicated in Table 2, there was no any sign of adverse effect noted on the mice treated with the methanolic extracts of the plants at 10, 1000, 1000 mg/kg body weight. However, in the phase 2 of the study as indicated in Table 3, when the animals were treated with the methanolic extracts of the plants at 1500, 2000, 2500 mg/kg body weight, observable signs of toxicity were noticed. The animals treated with the methanolic extract of *L. barteri* leaf at 1500 and 2000 mg/kg body weight became weak and inactive but later became normal after 1 - 2 h, while animal treated with 2500 mg/kg body weight of the methanolic extract became inactive and was shivering but later became normal after 3 h. However, there was no mortality noticed hence the LD₅₀ of *L. barteri* is > 2500 mg/kg body weight. There was no adverse effect noticed

Table 1. Phytochemical constituents of the plants' extracts.

Plants	Parts used	Alkaloids	Flavonoids	Saponins	Tannins	Anthroquinones	Terpenoids	Glycosides
<i>Lannea barteri</i>	Leaf	+	+	+	+	-	+	+
<i>Piliostigma thonningii</i>	Leaf	+	+	+	+	-	+	+
<i>Cleorodendrum aculeatum</i>	Leaf	+	+	+	+	+	+	+
<i>Crateva adansonii</i>	Root	+	-	+	+	-	+	+

+ = present, - = absent.

Table 2. Acute toxicity studies of the plants' extracts in mice (Phase 1).

Plants	Parts used	Dose (mg/kg bw)	No of animals used	Mortality	Toxicity signs
<i>Lannea barteri</i>	Leaf	10	3	0	No observable sign of toxicity.
		100	3	0	No observable sign of toxicity.
		1000	3	0	No observable sign of toxicity.
<i>Piliostigma thonningii</i>	Leaf	10	3	0	No observable sign of toxicity.
		100	3	0	No observable sign of toxicity.
		1000	3	0	No observable sign of toxicity.
<i>Cleorodendrum aculeatum</i>	Leaf	10	3	0	No observable sign of toxicity.
		100	3	0	No observable sign of toxicity.
		1000	3	0	No observable sign of toxicity.
<i>Crateva adansonii</i>	Root	10	3	0	No observable sign of toxicity.
		100	3	0	No observable sign of toxicity.
		1000	3	0	No observable sign of toxicity.

in animal treated with 1500 mg/kg body weight of the methanolic extract of *P. thonningii* leaf. However, animals treated with 2000 and 2500 mg/kg body weights of the extract became inactive and were shivering but later became normal after 3 h. Since no mortality was noticed the LD₅₀ of the plant > 2500 mg/kg body weight. The animal treated with 1500 mg/kg of the methanolic extract of *C. aculeatum* leaf also exhibited weakness and was inactive but later became normal after 3 h. While animals treated with 2000 and 2500 mg/kg body weights of the methanolic extract result to death of the animals indicating that the LD₅₀ of the plant extract is < 2000 mg/kg body weight. For animals treated with the methanolic extract of *C. adansonii* root at 1500 and 2000 mg/kg body weight resulted to shivering, inactive and loss of consciousness but became normal after 3 – 4 h. While the animal treated with 2500 mg/kg body weight result into death, therefore the LD₅₀ of the plant is < 2500 mg/kg body weight.

Organization for Economic Cooperation and Development (OECD) recommended chemical labeling and classification of acute toxicity LD₅₀ based on oral

administration as follows: very toxic ≤5 mg/kg; toxic >5≤50 mg/kg; harmful >50≤500 mg/kg; and no label >500≤2000 mg/kg (OECD, 2001). It follows from the foregoing classification that most of the plants' extracts under investigation are relatively safe since their LD₅₀ is above 1500 mg/kg body weight. LD₅₀ of *L. barteri* and *P. thonningii* leaves extracts is > 2500 mg/kg body weight, that of *C. aculeatum* leaf extract is >1500 mg/kg body weight and *C. adansonii* root extract is >2000 mg/kg body weight in mice. Similar and different findings have been reported on other plants. Sha'a *et al.* (2014) reported that mice fed with up to 3000 mg/kg body weight of the ethanolic extract of *Anacardium occidentale* (cashew) showed sign of weakness but later became active and the authors concluded that the plant extract is safe. In some other toxicological studies, results revealed that dosage up to 5000 mg/kg body weight of ethanolic extracts of *Newbouldia laevis* (Anaduaka *et al.*, 2013), aqueous leaf and root extracts of *Cymbopogon citrates* (Arome *et al.*, 2016) and crude hydroalcoholic extracts of *Embelia schimperi* (Debebe *et al.*, 2015) are safe. There are other studies that revealed toxicity of the extracts of

Table 3. Acute toxicity studies of the plants' extracts in mice (Phase 2).

Plants	Parts used	Dose (mg/kg bw)	No of animals used	Mortality	Toxicity signs
<i>Lannea barteri</i>	Leaf	1500	1	0	Weak and inactive but normal after an hour.
		2000	1	0	Inactive but became normal after 2 h.
		2500	1	0	Shivering and inactive but became normal after 3 h
<i>Piliostigma thonningii</i>	Leaf	1500	1	0	No observable sign of toxicity,
		2000	1	0	Shivering and inactive but became normal after 3 h.
		2500	1	0	Shivering and inactive but became normal after 3 h.
<i>Cleorodendrum aculeatum</i>	Leaf	1500	1	0	Shivering and inactive but became normal after 3 h.
		2000	1	1	Death
		2500	1	1	Death
<i>Crateva adansonii</i>	Root	1500	1	0	Shivering and inactive but became normal after 3 h`
		2000	1	0	Loss of sensitivity and consciousness, shivering and inactive but became normal after 4 h.
		2500	1	1	Death.

plants at a lower dose than the findings of this work. Usman et al. (2008) revealed that ethanolic extract of flower of *Newbouldia laevis* is moderately toxic because the LD₅₀ in mice was found to be 1264.9 mg/kg body weight when administered intraperitoneally. While Hilou et al. (2006) findings showed that *Amaranthus spinosus* and *Boerhaavia erecta* extracts have low toxicity with LD₅₀ at 1450 and 2150 mg/kg respectively.

Conclusion

The results obtained from the phytochemical and acute toxicity studies of *L. barteri*, *P. thonningii*, *C. aculeatum* leaves and *C. adansonii* root generally indicated that the plants contain the secondary metabolites tested and are relatively safe below 2000 mg/kg body weight which may be responsible for their use for treatment of malaria by local medicine practitioners. Further studies will reveal the antimalarial potentials of the plants extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The background of the entire page is a photograph of a hand holding two ripe, red, oval-shaped tomatoes on their green stems. The tomatoes are surrounded by lush green leaves, some of which have small holes, suggesting they are from a garden. The lighting is bright, highlighting the texture of the tomatoes and the vibrancy of the green leaves.

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