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## Antipyretic, phytochemical and toxicological investigations of ethanol extract of the leaves of *Baphia pubescens* Hook.F (Family: Leguminosae)

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### Abstract

The root, stem and leaves of *Baphia pubescens* is claimed by the people of Ogidi in Idemili Local Government area of Anambra State, Nigeria to have anti pyretic, analgesic and antimicrobial properties. The decoction of the leaves in alcohol is used to treat wounds, running stomach (diarrhea), aches and pains as well as fever. This investigation was carried out to ascertain the veracity of these claims. The leaves of *Baphia pubescens* was collected and dried at ambient temperature and pulverized. Exactly 200 g of the powdered drug was extracted with 400ml of ethanol using the cold maceration technique for 24hours with occasional shaking. This was filtered and the procedure repeated with the marc. The combined filtrates were concentrated under reduced pressure with rotary evaporator. The preliminary phytochemical tests were carried out using standard methods. The antipyretic activity using brewer's yeast to induce pyrexia was conducted. The acute toxicity test of the extract was determined using the Lorke's method. The leaves of *Baphia pubescens* exhibited antipyretic activity by lowering the temperature induced by brewer's yeast in rats. Alkaloids, flavonoids, saponins terpenoids carbohydrates, steroids, tannins, reducing sugar and proteins were found. Preliminary studies support the claim that the leaves of *Baphia pubescens* possess antipyretic properties.

### 1. INTRODUCTION

Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria<sup>1</sup> Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs. However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis<sup>2</sup> This work is therefore a preliminary work to prove that there is scientific evidence to the use of leaves of *Baphia pubescens* in the treatment of pyrexia.

One major problem of herbal medicine practice is that there is no official standard and / or local monograph<sup>3</sup>. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health<sup>4</sup>.

Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin<sup>5</sup>. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials<sup>6</sup>

### 1.1 *Baphia Pubescens* as a Medicinal Plant

Taxon: *Baphia pubescens* Hook.F.

Genus: Baphia

Family: Leguminosae - Papillonoideae

Tribe: Sophoreae

Synonym: *Bahia bancoensis* Aubrev

Common names:

English names: Benin camwood

Other names: Awewi, urohun, Maajigi

### 1.2 Description of the Plants

A tree about 20 ft. high, with trunk 20cm in diameter the ultimate branches slender, terete, densely brown-silky. Petioles 3/8– 1/2 in. long, slender, ferruginous; leaves oblong or narrow-obovate, 3–4 in. long, acuminate, the base cuneate or slightly rounded, subcoriaceous, under surface ferruginous on the veins when young. Pedicels 1–4 together from the main branches, 1/4 in. long, erecto-patent, ferruginous-downy. Bracteoles minute, rounded. Calyx 1/4 in. deep, finely ferruginous-downy. Corolla twice as long as the calyx, white; standard roundish, 1/2 in. broad. Pod straight, 3 in. long, 3/4 in. broad, membranous, rigid, glabrous, brown, polished, narrowed to both ends (WJ Craig 1999).



Fig.1



Fig.2



Fig.3

### 1.3 Geographical Distribution

Benin camwood (*Baphia pubescens* Hook.F) - It has a distribution similar to that of *Baphia nitida*, It's main geographical area is Africa found in countries including Nigeria, Zaire, Congo, Ivory Coast, Benin, Cameroon, Gabon, Ghana, Liberia<sup>7</sup>.

Habitat: Guineo-Congolian forest; Guinea Congolia/Sudania regional transition zone forest<sup>8</sup>.

### 1.4 Medicinal Uses

- a. The leaves or leaf juice are applied against parasitic skin diseases.
- b. A leaf infusion is drunk to cure enteritis and other gastrointestinal problems<sup>9</sup>.
- c. In Ghana, Côte d'Ivoire and Nigeria the leaves and bark are considered haemostatic and anti-inflammatory, and are used for healing sores and wounds<sup>10</sup>.
- d. In Côte d'Ivoire powdered leaves are taken with palm wine or food to cure venereal diseases, and leaf sap is applied as eye drops against jaundice<sup>11</sup>.
- e. An extract of young leaves with some salt and red pepper is used as nose drops against headache<sup>12</sup>.
- f. In Nigeria powdered heartwood is made into an ointment with shea butter (obtained from the seeds of *Vitellaria paradoxa* C.F.Gaertn.) which is applied against stiff and swollen joints, sprains and rheumatic complaints<sup>13</sup>.
- g. In Sierra Leone a bark decoction is drunk to cure cardiac pain and bark and leaves are prepared as an enema to treat constipation<sup>14</sup>.
- h. In Nigeria and Ghana the pounded dried root, mixed with water and oil, is applied to a ringworm-like fungus attack.
- i. In Côte d'Ivoire a leaf extract of camwood and *Senna occidentalis* (L.) Link is drunk against asthma.
- k. In Benin a decoction of the leaves is taken against jaundice and diabetes; in combination with leaves of *Morinda lucida* Benth. It is a treatment against female sterility and painful menstruation.
- l. An ointment made from the leaves showed anti-inflammatory activity in mice. Extracts of fresh leaves inhibited digestion in mice and rats, and showed anti-diarrhea.
- m. Leaf extracts of *Baphia nitida* have also been found to show analgesic effect in mice<sup>15</sup>.

## 2. MATERIALS AND METHOD

### 2.1 Drug, Chemicals and Solvents

Tween-80, Paracetamol (Emzor Nigeria), ibuprofen and Aspirin tablets (Emzor Nigeria).

### 2.2 Collection and Identification

The fresh leaves of *Baphia pubescens* were obtained from Ogidi, Idemili North Local Government Area of Anambra State, Nigeria in December 2013, during the dry season and was identified by Mr Ozioko, a Taxonomist with the Biosource Development and Conservation program (BDCP) Nsukka, Enugu State, Nigeria. The leaves were air-dried for 2 weeks in the Pharmacognosy laboratory. They were milled and 500g of the powdered plant material was obtained.

### 2.3 Preparation of Ethanol Extract for Pharmacological Study

200g of powdered plant sample was macerated in 400mls of ethanol (analytical grade) for 48 hours after which it was filtered with muslin cloth and further filtered using Whatman (No. 1) filter paper. The procedure was repeated with the marc. The combined filtrates were concentrated using rotary 45°C.

### 2.4 Phytochemical Screening

The tests carried out were based on procedures outlined by Harbourne<sup>16</sup> (1973) and Evans<sup>17</sup> (1996).

#### 2.4.1 Tests for alkaloids

To 0.5gm of the extract, 5.0 ml of 1% aqueous hydrochloric acid was added steamed on a steam bath and filtered. 1.0 ml of the filtrate was then treated with five drops of Mayer's reagent and a second 1.0 ml portion treated similarly with freshly prepared Dragendorff's and Wagner's reagents. Turbidity or precipitations with either of the reagents indicated the presence of alkaloids in the extract.

#### 2.4.2 Test for tannins

To 0.5g of the extract, 20ml of water was added, boiled and, filtered and used for the following test:

**(a) Ferric chloride test**

To 3ml of the filtrate, 2 drops of ferric chloride was added. Formation of a greenish black precipitate indicated the presence of tannins.

**(b) Lead acetate test**

To 3ml of the filtrate was added lead acetate solution. Formation of precipitate indicated the presence of tannins

**2.4.3 Test for saponins**

To 1.0gm of the plant extract, 5.0 ml of distilled water was added. The solution was shaken in a test tube and filtered. Frothing which persists on warming is a preliminary evidence for the presence of saponins. 10.0 ml of the filtrate was mixed with 5.0 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion which confirms the presence of saponins

**2.4.4 Test for proteins**

To 0.5g of the extract, 20ml of distilled water was added, shaken and, filtered and the filtrate was used for the following tests

**(a) Millon's test**

To a little portion of the filtrate in a test tube, two drops of millon's reagent was added. A white precipitate indicated the presence of proteins.

**(b) Xanthoproteic test**

5ml of the filtrate was heated with few drops of concentrated nitric acid. A yellow colour which changed to orange on addition of an alkali (dilute sodium hydroxide) indicated the presence of protein.

**2.4.5 Test for Flavonoids**

10ml of ethyl acetate was added to 0.2g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate was used for the following tests

**(a) Ammonium test**

4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate. A yellow colour in the ammoniacal layer indicated the presence of flavonoids.

**(b) 1% Aluminium chloride solution test**

4ml of the filtrate was shaken with 1ml of 1% aluminium chloride solution and the layers were allowed to separate. The formation of yellow colour in the aluminium chloride layer indicated the presence of flavonoids.

**2.4.6 Test for Steroids and Terpenoids**

9ml of ethanol was added to 1g of the extract and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath. 5ml of hot water was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5ml of chloroform using separating funnel.

To 0.5ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface showed the presence of steroids.

Another 0.5ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

**2.4.7 Test for Carbohydrates**

**Molisch test**

To 0.1g of the extract 2ml of water was added, boiled, and filtered. To the filtrate, two drops of naphthol solution in ethanol (Molisch reagent) was added. Concentrated sulphuric acid was gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

**2.4.8 Test for reducing sugars**

0.1g of the plant extract was shaken vigorously with 5ml of distilled water and filtered. The filtrate was divided and used as follows

**Fehling's test**

To a 1ml portion of the filtrate was added equal volumes of Fehling's solution 1 and II and boiled on a water bath for a few minutes. A brick red precipitate indicated the presence of reducing sugars.

## 2.5 Animals

White male albino rats (150-250kg) obtained from the animal house of the Department of Pharmacology and Toxicology of Madonna university Elele Campus River state were used for this study. All the animals were housed under standard environmental conditions where they have free access to food and water.

## 2.6 Acute toxicity test

The LD50 was carried out using the method employed by Dentrich Lorke<sup>18</sup> (1983).

It involves a total of thirteen rats. This test was carried out in two phases. phase one employed a total of nine rats they were grouped into three ,i.e. three rats per group, group one received 10mg/kg of the extract, group two received 100mg/kg, while group three received 1000mg/kg. All the administration was by intra-peritoneal route. The animals were constantly monitored for the next four hours, then intermittently for the next 6hrs then over a period of 24hrs, the number of dead animals were noted. From the result got in the first phase, the second phase was carried out. In this phase a total of four rats were used they were grouped into four groups of one rat per group. Group1 received 1600mg/kg; group 2 received 2900mg/kg, group 3 received 5000mg/kg, group 4(control) received 1ml of tween 80. The animals were monitored for another 24hrs for any death.

### Statistical Analysis

Results were expressed as mean  $\pm$  S.E.M. The data were analyzed using one way analysis of variance followed by Dunnett's post hoc test.

## 2.7 Antipyretic Activity

Brewer's yeast induced hyperpyrexia method: - twenty albino rats of either sexes were divided into four groups of five rats each. The normal body temperature of each rat was taken rectally at one hour interval for seven hours. The antipyretic activities of the extract were evaluated using the method described by Turner (1965). Hyperthermia was induced in all the four groups by subcutaneous injection of brewer's yeast (w/v) suspended in 0.5 %(w/v) sodium chloride solution. After 18h of yeast injection the vehicle (tween80), standard drug (paracetamol 150mg/kg) and the ethanol extract (100mg/kg, 200mg/kg and 400mg/kg) were administered to different groups orally respectively. Rectal temperature was recorded using clinical thermometer at 0hr, 2hrs, 3hrs, 4hrs after drugs administration<sup>19</sup>

Statistical Analysis:-all procedures were carried out in triplicates and the results expressed as  $\pm$ standard error of mean (SEM). Differences in observation were determined by analysis of variance (ANOVA) using Dunnette analysis method.

## 3. RESULTS

### 3.1 Phytochemical Analysis of Ethanol Extract

The result of the phytochemical analysis indicates the presence of saponins, tannins, carbohydrate, protein, flavonoids, reducing sugars, alkaloids and steroids in ethanol extract of *Baphia pubescens* and presence of tannins.

**Table 1: Results of preliminary phytochemical analysis**

Phytoconstituents	Relative Appearance
Saponins	+
Tannins	++
Carbohydrates	++
Protein	+
Flavonoids	+
Alkaloids	+
Steroids	++

### 3.2 Acute toxicity (LD50) of ethanol extract

The results of acute toxicity (LD50) of ethanol extract are summarized in Table 2.

**Table 2: Results of acute toxicity study**

Phase	Dose	No. of Deaths
I	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
II	1600mg/kg	0/1
	2900mg/kg	0/1
	5000mg/kg	0/1
<b>Control</b>	1ml of tween 80	0/1

From the result of the LD50, the ethanol extract is well tolerated even at the dose up to 5000mg/kg. So is safe for acute administration

### 3.3 Antipyretic activity result of ethanol extract

The results of Antipyretic activity result of ethanol extract are summarized in Table 3.

**Table 3: Results of Antipyretic activity**

Dose	Pre induction of fever	Post induction of fever	1hr	2hrs	3hrs	4hrs
100mg/kg	37.20±0.03	39.80±0.02	39.60±0.03	38.80±0.02	38.76±0.05	38.68±0.05
200mg/kg	37.10±0.07	39.67±0.05	39.0±0.85	38.7±0.15	38.6±0.15	38.6±0.16
400mg/kg	37.17±0.20	39.90±0.02	37.70±0.09	37.33±0.07	37.10±0.02	37.20±0.02
Control	36.60±0.33	39.43±0.07	39.50±0.07	39.50±0.07	39.50±0.07	39.50±0.03
Standard	37.33±0.03	39.70±0.05	38.70±0.1	38.20±0.03	37.20±0.07	36.5±0.25

## 4. DISCUSSION

Many reviews and articles reporting the biological activities of flavonoids<sup>19, 20</sup>, anthraquinones, polyphenols and phenols, and tannins<sup>21</sup>, have been published in recent years. Several phenol compounds have been identified and isolated from plants and they have shown promising bacterial inhibiting properties against specific and broad spectrum of cultured as well as clinical bacterial strains including Methicillin-Resistant *Staphylococcus aureus* (MRSA), and multi-drug resistant bacteria. The presence of alkaloids has been shown to demonstrate biological activity<sup>22</sup>.

Alkaloids, phenols, flavonoids and glycosides have a number of biological activities and strong antibacterial potentials. Alkaloids have exhibited promising activity against *H. pylori* and a number of other bacterial strains<sup>23,24,25</sup>; The Result of phytochemical screening showed abundance of tannins, steroids, and carbohydrates moderate availability of alkaloids, saponins, flavonoids, proteins and reducing sugars in the ethanol extract of *Baphia pubescens* and some of this secondary metabolites such as flavonoids and alkaloids have been reported to be responsible for analgesic and anti-inflammatory properties. Pyrexia was reduced and the most effective dose was 400mg/kg of the extract. The reduction in pyrexia is dose dependent. Increasing the dose, increases the effectiveness of the agent. It is evident therefore that ethanol extract of *Baphia pubescens* leaves has significant anti-pyretic activity.

## 5. CONCLUSION

The phytochemical analysis carried out on the plant extract revealed the presence of alkaloids, flavonoids, saponin, carbohydrates, steroids, proteins and tannins. Some of these phytochemicals have been implicated in the anti-pyretic properties of *Baphia pubescens*. Anti-pyretic activity observed in many plants was assumed to result from single or combined actions of these metabolites, which could be the same for the present study. The ethanol leaves extract of *Baphia pubescens* exhibited anti pyretic activity, hence its use by the local community in Ogidi of Anambra State, Nigeria as anti-pyretic drug.

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