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ANALGESIC, PHYTOCHEMICAL AND TOXICOLOGICAL INVESTIGATIONS OF ETHANOL EXTRACT OF THE LEAVES OF *BAPHIA PUBESCENS HOOK.F* (FAMILY *LEGUMINOSAE*)

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ABSTRACT

Baphia pubenscens plant 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 is used to treat, running stomach (diarrhea), aches and pains as well as fever. This investigation was carried out to ascertain the truth of these claims.

The leaves of *Baphia pubenscens* 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 pubenscens* exhibited analgesic activity by elongating endurance time of rats on hot plate. Alkaloids, flavonoids, saponins terpenoids carbohydrates, steroids, tannins, reducing sugar and proteins were found.

Preliminary studies support the claim that the leaves of *Baphia pubennscens* possess antipyretic properties.

Keywords: *Baphia Pubenscens*, Marc, Endurance Time, Hot Plate.

INTRODUCTION

Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria[1] Even the World Health Organization[2] 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[3]. However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonised by majority of allopathic medical practitioners as it is considered to have no scientific basis[4]. 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. 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[5].

Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin[6]. 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[7].

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BAPHIA PUBESCENS AS A MEDICINAL PLANT

Taxon: *Baphia pubescens* Hook.F.
Genus: *Baphia*
Family: *Leguminoseae -papillonoideae*

Tribe: Sophoreae
Synonym: *Baphia bancoensis* Aubrev
Common names:
English names: Benin camwood
Other names: Awewi, urohun, Maajigi

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.

Figure 1: The leaves of*Baphia pubescens*

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, Zarie, Congo, Ivory Coast , Benin, Cameroon, Gabon, Ghana, Liberia[8].

Habitat: Guineo-Congolian forest; Guinea Congolia/ Sudania regional transition zone forest[9].

Medicinal Uses

The leaves or leaf juice are applied against parasitic skin diseases. A leaf infusion is drunk to cure enteritis and other gastrointestinal problems[10].

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[11].

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[12].

An extract of young leaves with some salt and red pepper is used as nose drops against headache[13]

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[14]

In Sierra Leone a bark decoction is drunk to cure cardial pain and bark and leaves are prepared as an enema to treat constipation[15].

In Nigeria and Ghana the pounded dried root, mixed with water and oil, is applied to a ringworm-like fungus attack. In Côte d'Ivoire a leaf extract of camwood and *Senna occidentalis* (L.) Link is drunk against asthma.

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.

In the leaves saponins, flavonoids, glycosides and true tannins are present. 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

Leaf extracts of *Baphia nitida* have also been found to show analgesic effect in mice[16]

MATERIALS AND METHOD

DRUG, CHEMICALS AND SOLVENTS

Ethanol, tween-80, Paracetamol, ibuprofen and Aspirin tablets.

MATERIALS

Test tubes, test tube rack, syringe and needle 1ml, 2ml, 3ml), Electronic weighing balance (Gulfes Mediqaland scientific, England), measuring cylinder, conical flask, beakers (10ml, 25ml, 50ml, 500ml capacities)Miller (Thomas Laboratory Mill, UK) ,glass rod, hand gloves, rotary evaporator, Brewer's yeast,

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.

PREPARATION OF ETHANOL EXTRACT FOR PHARMACOLOGICAL STUDY

200g of powered plant sample was macerated in 400mls of ethanol (analytical grade) for 48 hours after which it was filtered with muslim 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^{O}C.

Phytochemical Screening

The tests carried out ware based on procedures outlined by Harbourne[17] and Evans[18]

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 Dragendorffs and Wagner's reagents. Turbidity or precipitations with either of the reagents indicated the presence of alkaloids in the extract.

Test for tannins

To 0.5g of the extrac, 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

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

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.

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 flavoniods.

(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

TEST FOR STERIODS 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.

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.

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 filterate was added equal volumes of fehing'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.

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.

ACUTE TOXICITY TEST

Acute toxicity test- The LD50 was carried out using the method employed by Dentrich Lorke[19].

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 ,ie 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) received1ml of tween 80. The animals were monitored for another 24hrs for any death.

STATISICAL ANALYSIS

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

ANALGESIC ACTIVITY

Test for analgesic activity was carried out using in-vivo test method. A total of 15 adult albino rats were employed. The rats were grouped into four with 3 rats in each group. Each rat was individually placed on a hot plate at the temperature of 40^0C. The time the animal started licking it paws, or showing signs of discomfort was noted and it was taken as their normal endurance duration. Then the animals were treated with ethanol extract as follows:

Group 1 received 100mg/kg of ethanol extract
Group 2 received 200mg/kg of ethanol extract
Group 3 received 400mg/kg of ethanol extract
Group 4 received 0.5ml of tween 80
Group 5 received 150mg/kg of paracetamol which was used as standard drug. Readings were taken 30mins, 60mins, 90mins, 120mins, after oral administration of drugs. The duration of time each animal in each group can stay comfortably on the hot plate was taken, then the negative control group (4) was compared with other groups for significance in analgesic activity using analysis of variance.

RESULTS

PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT

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

Table 1: Phytochemical constituents

Constituents	Relative Appearance
Saponins	+
Tannins	++
Carbohydrates	++
Protein	+
Flavonoids	+
Reducing sugars	+
Alkaloids	+
Steroids	++

Table 2: Acute Toxicity (LD50) Of Ethanol Extract

Phase	Dose	No Of Deaths
1	10mg/kg 100mg/kg 1000mg/kg	0/3 0/3 0/3
II	1600mg/kg 2900mg/kg 5000mg/kg	0/1 0/1 0/1
Control	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

Table 3 - ANALGESIC ACTIVITY RESULT OF ETHANOL EXTRACT

Dose	Initial(sec)	30mins	60mins	90mins	120mins
100mg/kg	3.0± 0.1	3.0 ± 0.0	3.4± 0.02	4.8± 0.02	4. 8± 0.00
200mg/kg	3.0± 0.00	3.1± 0.05	5.5±. 0.00	6.0± 0.02	4.9± 0.05
400mg/kg	3.0± 0.12	4.4± 0.12	7.0± 0.11	6.4± 0.0	6.0± 0.12
Control	3.0±0.07	3.4±0.02	3.4±0.02	3.4±0.02	2.4±0.02
Standard	3.0± 0.02	3.5± 0.00	7.0± 0.00	6.5± 0.02	5.0± 0.02

DISCUSSION

Many reviews and articles reporting the biological activities of flavonoids[20], anthraquinones, polyphenols and phenols, and tannins[21], 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[22].

Alkaloids, phenols, flavonoids and glycosides have a number of biological activities and strong antibacterial potentials[23]. Alkaloids have exhibited promising activity against *H. pylori*[24] and a number of other bacterial strains[25]; The Result of phytochemical screening showed abundance of tannins, steriods, 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. From table the above all the animals have an initial endurance of 3secs. Group 1 animals were given 100mg/kg and their tolerance continued to increase as time increases reaching 4.8±0.02 at 90mins and reduced to 4.8±0.00 after 120mins when the effect of the drug decreases. This also applies to group 2 and 3 which were given 200mg and 400mg respectively. The rats in group 2 showed increase tolerance (3.1±0.0) to the hot plate after 30mins and reached 6.0±0.00 at 90mins reducing to 4.9±0.05 at 120mins when the effects of of the extract reduces. Group 3 rats had increase tolerance of 6.4±0.00 at 90mins which decreased to 6.0±0.12 after 120mins. This indicates that the duration of the analgesic activity of the extract could last for 1hr 30min thereafter start decreasing.

CONCLUSION

The ethanol leaves extract of *Baphia pubescens* exhibited analgesic activity, hence its use by the local community in Ogidi of Anambra State, Nigeria as analgesic drug.

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