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Phytochemical composition and anti-inflammatory activities of *Brachystegia eurycoma* seeds and stem bark

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ABSTRACT

Brachystegia eurycoma seeds and stem barks which are used as food and medicine in South Eastern Nigeria were analyzed for their phytochemical composition and anti-inflammatory activities. Phytochemical investigations showed the presence of bioactive compounds to include alkaloids (0.70-1.74%), flavonoids (3.72-4.99%), tannins (0.37-0.19%), saponins (0.07-1.94%) and phenols (0.03- 0.13%). The anti-inflammatory activities of the extracts at concentrations of 100 and 50 mg/kg body weights were evaluated in carrageenan-induced acute and formalin-induced chronic inflammatory models in 36 albino rats. Inhibition of acute inflammation of B. eurycoma seeds were 46.66% and 61.92% while the chronic inflammation gave 32.82% and 49.84%. The stem barks gave 54.27% and 66.34% inhibition of acute inflammation while inhibition of chronic inflammations were 44.82% and 55.45%. These results signify that the extracts from B. eurycoma possess marked anti-inflammatory activities which may be due to the presence of bioactive constituents in them.

Keywords: *Brachystegia eurycoma*, anti-inflammatory activity, phytochemical, medicinal plant.

INTRODUCTION

In Nigeria, various indigenous plants are used both as food and as medicine. Apart from the phytochemical constitutents of some of these plants, information on their bioprotective properties has not been fully studied and documented. One of such plants is *Brachystegia eurycoma.* The voracious uses of *B. eurycoma* in South Eastern Nigeria as a soup thickner and condiment and also as a medicinal plant necessitated a probe into its anti-inflammatory activity and phytochemical composition.

B. eurycoma grows mainly along the river banks or swamps in Western and Eastern Nigeria. It also grows on well-drained soils. It is a large tree with irregular and twisted spreading branches. The seed has a roundish flat shape with brown colour and hard hull. The fruit ripens from September to January and is released by explosive mechanism [1,2]. It flowers between April and May. The fruits occur as broad lathery dark purplish brown pods containing between four and six brown shiny flat disc-like seeds. The plant also possesses a rough fibrous bark, which peels off in patches and often gives out brownish buttery exudates [3,4].

In Nigeria, the main culinary use of the gum from *B. eurycoma* is in thickening soups. In the preparation of most soups thickeners are usually added as condiments. They are known to cause increased viscosity in soups, giving it more palatability and good mouth feel. The gum also impacts certain desirable functional properties when added in

other foods. The leaves from *B. eurycoma* make an excellent browse material for cattle, sheep, and goats. In agro-forestry, the tree is suitable as a shade tree and ornamental plant especially in the dry season when the trees produce masses of coloured young foliage. The fruit pods make good fuel wood. Timber sold in international market is also produced from *B. eurycoma* [3].

According to researchers, the seed helps in maintaining heat within the body when consumed, in other words, it helps in the control of the body temperature [5]. The seed also helps in softening bulky stools and have been associated with the protection against colon and rectal cancer [6]. This present work was aimed at investigating the phytochemical composition and anti-inflammatory activities of the ethanol extracts of the seeds and stem barks of *B. eurycoma*.

MATERIALS AND METHODS

B. eurycoma seeds were bought from Umuahia main market in Abia State, Nigeria. The plant seeds were identified and authenticated by Mr. I.K Ndukwe of the Taxonomy section, Forestry Department, Michael Okpara University of Agriculture, Umudike . Clean and wholesome seeds were selected. The seeds were weighed (1kg) and then decoated by soaking in water for 24 hours. The loosened hull was washed off with several changes of water. The dehulled seeds were air-dried before milling. The barks of *B. eurycoma* were harvested from the tree plant located at Umuovo village stream in Old Umuahia, Umuahia South Local Government Area of Abia State, Nigeria. 2 kg of the harvested barks were weighed and then dried on the laboratory bench for 30 days. The decoated seeds and barks of *B. eurycoma* were milled into a uniform and fine powder by a mechanically driven attrition mill. The seeds gave 777.66 g weight of flour while the bark gave 1851.88 g. The powdered plant materials were dried and kept properly for further use.

Determination of Phytochemicals

Alkaloids and phenols were determined according to the method pf Harborne [19] while tannin was determined using the method of Van-Burden and Robinson [20]. Saponin was determined using the method of Obadoni and Ochuko [21]. Flavonoids were determined according to the method of Boham and Kocipia [22].

Plant Extracts

The powdered plant materials were each packed into a soxhlet apparatus (2L) and extracted exhaustively with 1000 ml of ethanol for 24 hours. The ethanol extracts were concentrated using a rotary evaporator at 45^{o}C and in a hot air circulating oven to get the extracts.

Determination of Anti-Inflammatory Activity

Acute and chronic anti- inflammatory activities of extracts were evaluated by carrageenan-induced acute and formalin-induced chronic inflammatory models in albino rats. The extracts were administered orally.

i. Carrageenan-induced Paw Edema

18 male albino rats were divided into 6 groups of 3 animals in each group. In all groups, the inflammation was induced by single sub-plantar injection of 20µl of freshly prepared 1% carrageenan suspension in normal saline. Group 1 treated with carrageenan alone served as control. Group 2 and 3 received the *B. eurycoma* seed extract while Group 4 and 5 received the *B. eurycoma* stem bark extract, all at concentrations of 50 and 100 mg/kg body weight orally 1 hour before the carrageenan injection. The extracts were presolubilized in 0.2% dimethyl sulfoxide and a fine suspension was prepared in phosphate buffered saline. Group 6 was administered with reference drug diclofenac potassium (10 mg/kg body weight) also orally 1 hour before carrageenan injection. The paw thickness of animals in all groups were measured using vernier calipers before and 3 hours after carrageenan injection.

ii. Formalin-induced Paw Edema

18 male albino rats were also used in this model. Experimental procedure was the same as described above except that single dose of 0.02µl of formalin (2%) was used to induce inflammation. The extracts were administered once daily for 6 consecutive days.

In the above two models, the degree of edema formation was determined as increase in paw thickness. In the case of acute anti-inflammatory activity, paw thickness was measured once daily for 6 days. The increase in paw thickness and percent inhibition were calculated as follows:

Increase in paw thickness = $P_t - P_o$

% inhibition = $P_c - P_T / P_c$ X 100/1

Where:
P_t = Paw thickness at time, t.
P_o = Initial paw thickness
P_c = Increase in paw thickness of the control group
P_T = Increase in paw thickness of the treatment group

Statistical Analysis
All measurements were replicated three times and means and standard deviation determined. The student t-test at $P<0.05$ was applied to assess the difference between the means [23].

RESULTS AND DISCUSSION

Table 1 shows the quantitative determination of phytochemical constituents of *B. eurycoma* seeds and stem barks. High quantity of alkaloids, flavonoids and saponins were found in *B. eurycoma* seeds and stem barks. Tannins and Phenols content were not much.

Table 1: Phytochemical composition of B*rachystegia eurycoma* seeds and stem barks.

Phytochemical	seeds (%)	stem barks (%)
Alkaloids	1.74 ± 0.20^b	0.70 ± 0.10^c
Flavonoids	3.72 ± 0.11^a	4.99 ± 0.20^a
Tannins	0.70 ± 0.10^c	0.37 ± 0.03^c
Saponins	0.87 ± 0.10^c	1.94 ± 0.10^b
Phenols	0.03 ± 0.002^d	0.13 ± 0.01^d

Data are means ± starndard deviation of triplicate determinations. Means followed by the same superscript in each row are not significant (P<0.05).

The flavonoids content was very high in the two plant parts but the bark contained 4.99% while the seed contained 3.72% of flavonoids. Flavonoids are a group of polyphenolic compounds ubiquitously found in fruits and vegetables. They have multiple biological activities, including antioxidative, vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immune – stimulating, anti-allergic, antiviral and estrogenic effects, as well as being inhibitors of phospholipase A2, cycloxygenase, lipoxygenase, glutathione reductase and xanthine oxidase [7,8].The detection of high quantity of flavonoids in the seeds and stem barks of *B. eurycoma* attaches more nutritional and medicinal value to the plant. Another secondary metabolite constituent of *B. eruycoma* seeds and stem barks that was detected was saponin. The saponin cotent was more in the barks {1.94%} than the seeds (0.87%}. Saponins have been shown to possess antifungal properties and therefore help to protect the body from disease – causing organisms and infections. They have a characteristic ability to form foams in aqueous solution and may exhibit cholesterol binding properties [9].

B. eurycoma stem barks contained more phenol (0.13%) than the seeds (0.03%). Phenolic compounds like flavonoids act as anti-inflammatroy and antimicrobial agents. For this reason, the presence of phenolic compounds in the seeds and stem barks of *B. eurycoma* indicates that this plant could be used as ant-inflammmatory and antimicrobial agent. Other secondary metabolite constituents of *B. eurycoma* seeds and stem barks detected are alkaloids and tannins. The seeds contained more alkaloids (1.74%} than the stem barks (0.70%} while tannin content was higher in the stem barks (0.37%} than the seeds {0.19%}. Alkaloids are plant bases which exhibit certain physiological properties when used in herbal medicine. A lot of them have anti-malaria and antimicrobial activities. An example is quinine and its derivatives. The presence of tannins in plant has been associated with ulcer management, wound healing, control of bleeding and burns in herbal medicine [10.]

Anti-Inflammatory Activity
The anti-inflammatory activities of the extracts from *B. eurycoma* seeds and stem barks on carrageenan and formalin- induced paw edema in 36 albino rats are shown in table 2. The ethanol extracts of *B. eurycoma* showed significant inhibitory effect against induced inflammation in both the experimental models. The carrageenan-induced acute and formalin-induced chronic inflammations were significantly inhibited by the extracts. The effect was evident from the inhibition of the paw edema (figure 1).

Table 2: Inhibition of Acute and Chronic Inflammation of *B. eurycoma* Seeds and Stem barks Extracts Administered orally (%).

Concentration mg/kg	*B. eurycoma* seed		*B. eurycoma* stem bark	
body weight	Acute	chronic	Acute	chronic
50	46.66 ±1.30^{C}	32.83±2.28^{d}	54.27±2.22^{b}	4 4.82±0.99^{c}
100	61.92 ±2.10^{a}	49.84±1.93^{c}	66.34±1.80^{a}	55.45±1.38^{b}

Data are means ± standard deviation of triplicate d eterminations. Means followed by the same superscript in each row are not significant ($p<0.05$).

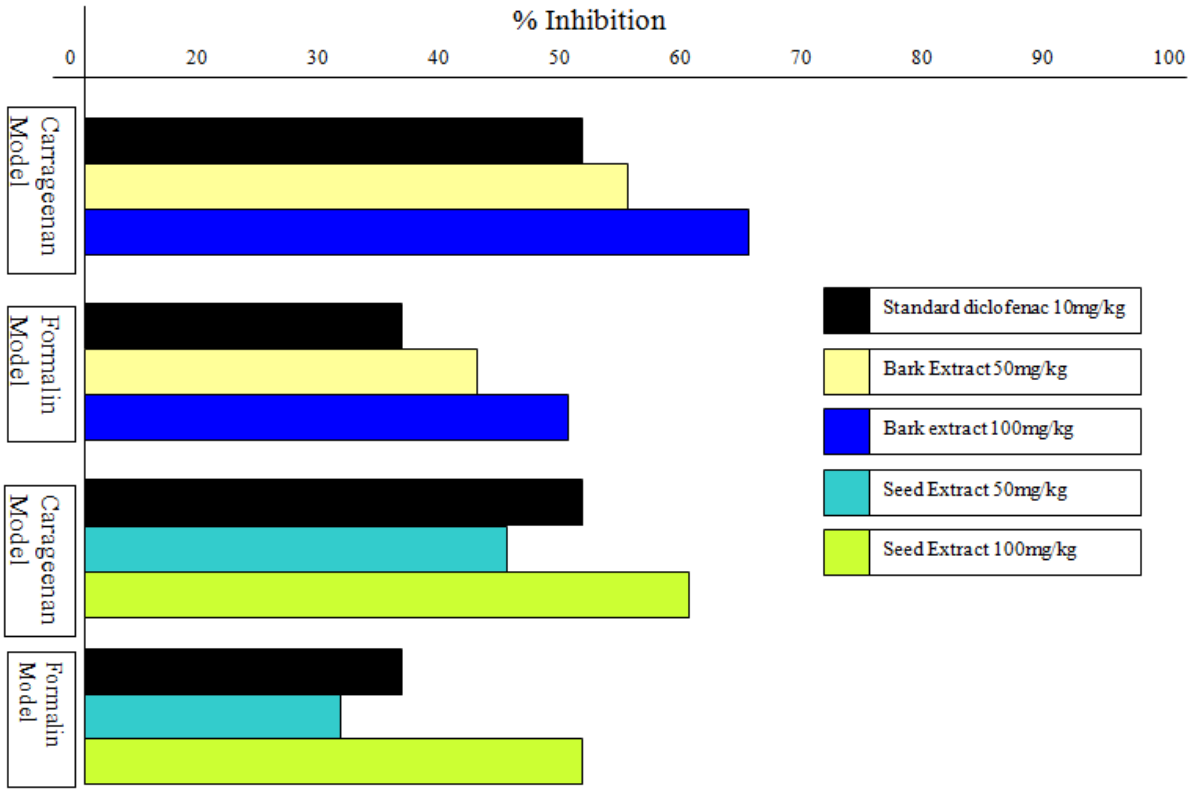

Fig 1: Inhibition of Acute and Chronic Inflammation of *Brachystegia eurycoma* seeds and stem bark Extracts Administered orally (%).

The effect was significantly high in 100 mg/kg body weight extract treated group compared with standard reference drug, diclofenac potassium. The carrageenan-induced paw edema was reduced by 61.92% and 46.66% with treatment of ethanol extract of *B. eurycoma* seeds at concentrations of 100 and 50 mg/kg body weight respectively compared to that of the control group. Whereas, the inhibitory effects were 49.84% and 32.82% for formalin-induced paw edema with the treatment of the extracts at 100 and 50 mg/kg body weight respectively. Standard reference drug, diclofenac potassium showed an inhibition of 50.12% and 37.48% of carrageenan and formalin-induced inflammations respectively (Figure 1)

The carrageenan- induced paw edema was reduced by 66..34% and 54.27% with treatment of ethanol extract of *B. eurycoma* stem barks at concentrations of 100 and 50 mg/kg body weight respectively compared to that of the control. Also, the inhibitory effects were 55.45% and 44.82% for formalin-induced paw edema with the treatment of the extracts at 100 and 50 mg/kg body weight respectively.

The anti-inflammatory activities of ethanol extracts of *B. eurycoma* are dose dependent. Carrageenan-induced acute inflammation in animals is one of the most suitable test procedures to screen anti-inflammatory agents [11]. The carrageenan-induced edema is mediated by activation of platelet activating factor (PAF) , prostaglandins and other

inflammatory mediators like histamine, 5-hydroxytryptamine and bradykinin [12] .The first phase is attributed to the release of prostaglandins [13,14]. Carrageenan also induces a protein rich exudate containing large number of neutrophills [15]. The extracts significantly inhibited the incidence of carrageenan-induced rat edema after 3 hours. Actually, the prostaglandin phase of the acute experimental inflammation is usually taken to include events that take place from 1 hour after carrageenan injection. This phase of inflammatory response was modified by *B. eurycoma* extracts, suggesting the involvement of prostaglandin in the anti-inflammatory property of the extract. The phase has been suggested to be sensitive to most clinically effective anti-inflammatory drugs [12] .

Formalin-induced paw edema is also one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembled human arthrities [11]. The nociceptine effect of formalin is also biphasic, an early neurogenic component followed by tissue mediated response [16]. The preliminary phytochemical analysis revealed that the major chemical constituents of the seeds and stem barks of *B. eurycoma* are flavonoids. Flavonoids have been reported to have high anti-inflammatory properties [7,8,17,18] . Thus flavonoids of the extracts could be responsible for the anti-inflammatory activities of the extracts.

The presence of phenols could also be responsible for this effect. From the phytochemical analysis, *B. eurycoma* stem barks contained more flavonoids (4.99%) and phenols (0.13%) than the seeds (which contained 3.72% flavonoids and 0.03% phenols). No wonder the degree of anti-inflammation was more pronounced in the stem barks than the seeds. From this study, the therapeutic use of *B. eurycoma* plant is revealed.

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