

Full Length Research Paper

Micromorphological, anti-nociceptive and anti-inflammatory investigations of stem bark of *Daniellia oliveri*

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Anatomical and powdered samples of stem bark of *Daniellia oliveri* were investigated for its micromorphological profile, while the aqueous extract was investigated for its anti-nociceptive and anti-inflammatory effects in mice and rats, respectively. The micromorphological study indicated the presence of characteristic bundles of phloem tissues, separated by medullary rays, abundant grains of starch in isodiametric parenchyma cells, prisms of calcium oxalate crystals, cork cells and cortex parenchyma. The extract showed a significant anti-nociceptive activity at the tested doses (50, 100, 200 mg/kg i.p.). The extract at the same doses showed a non-dose dependent anti-inflammatory activity. The effect was significant at doses of 100 and 200 mg/kg. These findings contribute to the preparation of a monograph for proper identification of the plant and also corroborate some of the traditional uses.

Key words: Acute toxicity, anti-inflammation, anti-nociceptive, chemomicroscopy, *Daniellia oliveri*, micromorphology, phytochemical screening.

INTRODUCTION

Daniellia oliveri (Rolfe) Hutch and Dalziel M. (Cesalpiniaceae) is a large Savannah tree growing up to 110 ft high (Hutchinson and Dalziel, 1954). The plant grows more characteristically in the forest and open country, whether in moist or dry situations. In Nigeria, it is called "iya" in Yoruba, "maje" in Hausa and "abwa" in Ibo (Dalziel, 1937). Traditionally, it is used in treating breast tumors, vestibule vaginal fistula, swellings and abscesses (Survey Report, 1998). A concoction from the stem bark is used against diabetes and sickle cell disorders (NCAC Policy and Operational Guidelines, 1992). The plant had wrongly been called Copaibalsam, as a substitute or adulterant. It contains brownish yellow oleoresin when

the bark is cut as deep as the wood. Diterpenoids including daniellic acid and oliveric acid had been isolated from the plant (Glasby, 1991). -sitosterol was isolated from the neutral fraction of the petroleum extract (Hauser, 1970). Insufficient documentation of information for the preparation of a monograph on the plant and lack of scientific basis for some of its traditional uses prompted this study.

MATERIALS AND METHODS

Plant material

The stem bark of *D. oliveri* was collected from a tree growing in Idu Industrial forest, Abuja in September 2003. The tree was authenticated by Jemilat Ibrahim, a Taxonomist with the Ethnobotany and Herbarium section of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The

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sample was given voucher specimen number NIPRD H5483 and was then deposited at the Herbarium.

Preparation of plant extract

Extract was obtained by percolating 100 g of powdered stem bark in 1 L of distilled water for 72 h. The resulting extract was concentrated using a water bath and allowed to dry up completely. The yield was 3.4%.

Experimental animals

Wistar rats (205.8 – 229.9 g) and Swiss albino mice (23.5 – 29.5 g) of both sexes were employed for the different investigations. They were obtained from the Animal Facility Centre (AFC), Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The animals were maintained under normal environmental conditions. They were fed *ad libitum* with standard feed (Ladokun Company, Ibadan, Nigeria) and water from Municipal Area Council, except when starvation was needed in the course of the study.

Drugs and chemicals

Aspirin (Sigma, USA), glacial acetic acid (Searle, Essex, England) were used for the animal investigations, while glycerol (BDH, Poole, England), hydrochloric acid (BDH, Poole, England), sulphuric acid (May and Baker, England), ferric chloride (Avondale, Oxon, England) were used for the chemomicroscopical investigation.

Micro- and chemo-microscopical investigation

Plant sample collected was separated into two parts, a small part mounted in a solution of glycerol for fresh micromorphological study. The other part was cut into small pieces, air-dried for 3 days, powdered using a pestle and mortar and stored in airtight sample bottles. Anatomical sections of the fresh samples were prepared for micromorphological study as outlined by the AOU/STRC (1986) and described according to the method of Wallis (1985). The same methods were adopted for the powdered samples. Chemomicroscopical investigations on the samples were carried out.

Phytochemical screening

The presence of various phytochemical constituents in the extract was determined using the standard screening tests as outlined by Trease and Evans (1983).

Acute toxicity study (LD₅₀)

The intraperitoneal (i.p.) acute toxicity of the extract was evaluated in Swiss albino mice by modifying the method of Lorkes (1983). In brief, this method involved the determination of LD₅₀ value in biphasic manner. The animals were starved of feed but allowed access to water 24 h prior to the study. In the initial investigatory step (phase I), a range of doses of the extract producing the toxic effects was established. This was done by intraperitoneal administration of widely differing doses of the extract (10, 100, 1000, 1500 mg/kg i.p.) to four groups of mice (of four mice each).

Based on the results obtained, a phase II investigatory step was done by giving more specific doses (250, 300, 400, 500 mg/kg i.p.) to four other groups of mice.

The mice were observed for 24 h for such behavioural signs as nervousness, excitement, dullness, ataxia or death. The LD₅₀ was estimated from the geometric mean of the dose that caused 100% mortality and the dose which caused no lethality.

Acetic acid-induced writhing in mice

The anti-nociceptive investigation was carried out according to the method of Siegmund and Cadmus (1957), as was modified by Koster et al. (1959). The mice were divided into different groups (of four mice each). They were differently pre-treated with the extract (50, 100, 200 mg/kg i.p.), aspirin (100 mg/kg i.p.) and normal saline (10 ml/kg i.p.). 30, 60, 90 and 120 min after the treatment, 0.75% glacial acetic acid was administered to the mice (10 ml/kg i.p.). They were placed in a transparent observation box. 5 min after administration of acetic acid, the number of abdominal constrictions (writhes) made within 5 min of every mouse was counted. The results of the treatment groups were compared with those of normal saline pre-treated control. The percentage of the writhes was calculated as (Test mean/Control mean) X 100.

Anti-inflammatory study

The anti-inflammatory activity was evaluated using fresh egg albumin-induced oedema in rat paw. This is in accordance with the technique of Winter et al. (1962) as was modified by Akah and Nwambie (1994). Rats of either sex used for the investigation were fasted overnight. They were deprived of water during the experiment to ensure uniform hydration and to minimize variability in oedematous response (Winter et al., 1963). Administration of different doses of the extract (50, 100, 200 mg/kg i.p.), aspirin (100 mg/kg i.p.) and normal saline (10 ml/kg i.p.) was done on different groups of rats (of four each). 30 min later, the phlogistic agent (fresh egg albumin; 0.1 ml) was injected subcutaneously into the rats' subplantar surface of the hind paw. The measurement of the paw volume (cm³) was done on the principle of volume displacement using LETICA Digital Plethysmometer (LE 7500). The readings were taken before and at 20 min intervals after the injection of egg albumin for a period of 2 h. The oedema at each time was calculated in relation to the paw volume before the injection of the phlogogen.

Statistical analysis

The calculation of the average oedema for the anti-inflammation and percentage writhing reflex for anti-nociception were based on the expression of the numerical data as mean ± SEM. The statistical significance between groups was analysed using two-way analysis of variance (ANOVA), followed by student's *t*-test. P values < 0.05 were taken to be statistically significant.

RESULTS

Micromorphological investigation

A transverse section of the stem bark of *D. oliveri* revealed an outer and inner bark, the bulk of which is

Table 1. Result of the phytochemical screening of *D. oliveri* stem bark.

Test	Observation	Inference
Saponin: Extract vigorously shaken in a test-tube for 2 minutes	An horny comb-like frothing occurred	++
Tannin: Extract + few drops of FeCl ₃	An immediate green precipitate formed	++
Flavonoids: Extract + few drops of Magnesium chips + Conc. HCl	Slightly pinkish colouration obtained	-
Alkaloids: Extract + Drangadoff reagent	Brown precipitate obtained	++
Extract + Picric acid	Orange precipitate obtained	++
Terpenoids: Extract + Wagner's reagent	Brown precipitate obtained	-

++ = Present
- = Absent

made up of sclereids.

Outer bark: The outermost part consists of several layers of cork cells. They occur as regular rows of small slightly thick walled, flat polygonal cells, closely packed in radial rows. The cells contained a reddish-brown content in their lumen after bleaching with a 5% (w/v) sodium hypochlorite solution. The content gave a positive test for tannin on addition of 5% ferric chloride (Table 1). Parts of the cork layer are exfoliated. The cortex comprising of cortex parenchyma, sclereids and starch grains occur next to the cork cells, without any distinct separation from cork layer. Embedded in the cortex are large and small groups of abundant sclereids. They are nearly equal in size, rounded, square or occasionally oblong in shape. They possess thick striated cell walls with pits, narrow lumen appearing more distinctly as the layer next to the outermost layer of cork cells. The cortex parenchyma are non-tangentially arranged, being made up of spherical, thin walled cells, containing abundant starch grains. The sclereids get bigger in size and smaller in number as they gradually occur towards the inner surface of the bark. They now occur mostly as clusters, with the cells being more oblong than spherical. The cortex now appears more visible towards the inner cortex. The spherical parenchyma cells of the cortex differentiate into medullary rays, which are tube-like towards the inner part of the cortex.

Secondary phloem tissues: The phloem fibres are unicellular, lignified, slightly thickened on the cell wall possessing narrow lumen and pits. The medullary rays occur distinctly as a fine network of multi-seriate nearly straight cells. They are thin walled in 3 to 4 rows separating dark bands of phloem tissues. Prisms of calcium oxalate crystals occur abundantly in association with phloem fibres. The cells about the fibres contain single prism per cell. Spherical and thin walled phloem parenchymas alongside medullary rays contain starch

grains forming a sheath to each fibre group. The starch grain occur less in the inner bark as compared to the outer bark. The medullary rays tend to converge as they approach the inner stem as nearly parallel lines. The phloem fibres are arranged in tangential bands alternating with sieve fibres and phloem parenchyma. Separate and numerous bundles of secondary phloem tissues separated by medullary rays make up the inner bark (Table 2).

Acute toxicity study (LD₅₀)

Mice treated with doses 500 mg/kg i.p. were dull, panted, showed occasional abdominal stretching and died within 24 h of treatment. These adverse signs and deaths were however, not seen at doses below 500 mg/kg i.p. The LD₅₀ was estimated to be 447.21-mg/kg i.p.

Acetic acid-induced writhing in mice

The aqueous extract of *D. oliveri* stem bark (50, 100, 200 mg/kg i.p.) exhibited a significant ($P < 0.05$) anti-nociceptive activity against acetic acid-induced writhing in mice. 50 and 100 mg/kg i.p. doses exhibited a dose-dependent anti-nociception that progressively reduced over a period of 90 min post-treatment. However, at 120 min, the reduced anti-nociceptive activity increased again at these doses. The dose of 200 mg/kg i.p. on the other hand, caused a total anti-nociception up to 120 min. These results compared favourably with those of aspirin (100 mg/kg i.p.; Table 3).

Anti-inflammatory study

The study revealed that the extract (50, 100, 200 mg/

Table 2. Result of chemomicroscopical investigation of powdered sample of *D. oliveri* stem bark.

Test reagent	Observation	Inference
Phloroglucinol + Conc. HCl	Red colouration observed on the walls of cork cells; sclereids and fibres	Lignin (++)
N/5 Iodine solution	Blue-black colouration observed on abundant grains in the cortex parenchyma and medullary rays	Starch grains (++)
5% Ferric chloride	Greenish-black colouration observed in the lumen of cork cells and some sclereids	Tannins (++)
80% H ₂ SO ₄	The bright prismatic crystals abundant in the sample disappeared on the addition of the reagent	Calcium oxalate crystals (++)
Picric acid solution	No yellow colouration was observed	Protein (-)
Sudan IV	Red colouration was observed on the walls of the phloem fibres and sclereids	Cutin (++)

++ = Present
 - = Absent

Table 3. Effect of aqueous extract of *D. oliveri* stem bark on acetic acid-induced writhing in mice.

Treatment (mg/kg i.p.)	Number of writhes			
	30 min	60 min	90 min	120 min
Normal saline (10 ml/kg i.p.)	8.00 ± 1.4	10.00 ± 1.2	17.00 ± 2.2	24.00 ± 1.8
<i>D. oliveri</i> :				
50	1.67 ± 1.4*	2.67 ± 1.3*	3.75 ± 2.8*	0.50 ± 0.5*
100	0.00 ± 0.0*	0.50 ± 0.5*	2.50 ± 1.7*	1.00 ± 0.6*
200	0.00 ± 0.0*	0.00 ± 0.0*	0.00 ± 0.0*	0.00 ± 0.0*
Aspirin: 100	0.00 ± 0.0*	0.00 ± 0.0*	0.00 ± 0.0*	0.00 ± 0.4*

* Significant ($P < 0.05$) anti-nociceptive activity; two-way ANOVA; student's *t*-test

kg i.p.) reduced egg albumin- induced oedema in rats. However, the reduction was not dose- dependent and was significant ($P < 0.05$) only at doses of 100 and 200 mg/kg i.p. There was no significant ($P > 0.05$) effect of time on the anti-inflammatory activity of the extract at the tested doses (Figure 1).

DISCUSSION

The micromorphological result show diagnostic features that revealed a characteristic pattern of arrangement of the cellular components of the stem bark of *D. oliveri*. The most important diagnostic features include the abundance and thickness of the sclereids, starch grains, phloem bundles and medullary rays. These features are of diagnostic value in identifying the plant. The confirmation of presence of alkaloids, which has been indicated in antitumor and anti-inflammatory activities, support the local use of the plant. The determination of the nature of the alkaloids is the next stage of the study. The revelation of the median lethal dose (LD₅₀) of the

extract of *D. oliveri* stem bark to be 447.21 mg/kg i.p. is probably an indication that the extract may not be relatively safe. According to Lorkes (1983), estimated LD₅₀ value > 1 g is considered safe. This is of importance because a cumulative toxic effect could occur if the extract is taken over time. According to some studies already done, sub-chronic data may be sufficient to predict the hazard of long term, low dose exposure to a particular compound (McNamara, 1976).

For the abdominal constriction (writhing) model adopted for evaluation of anti- nociception, the writhing response is thought to partly involve local peritoneal receptors (Bentley et al., 1983; Mat et al., 1997; Atta and Alkofahi, 1998). The ability of the extract to cause a significant reduction in the number of acetic acid-induced writhes in mice probably suggests an antinociceptive property. The use of abdominal constriction (writhing) model for detection of anti-nociceptive activity is known to be very sensitive when compared with other models such as tail-flick model (Collier et al., 1968; Bentley et al., 1981). This study also showed that anti- nociceptive effect of the extract that progressively reduced over a 90

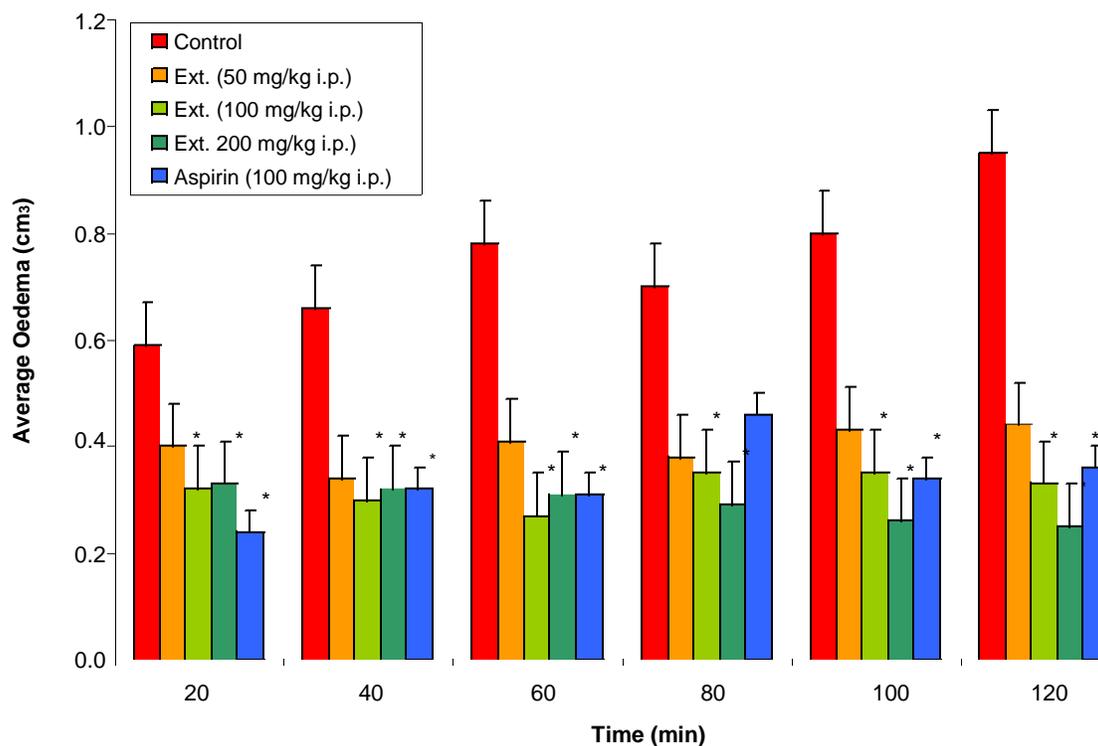


Figure 1. Effect of aqueous extract of *Daniellia oliveri* stem bark (50 -200 mg kg i.p.) on egg albumin-induced inflammation in rats. *Significant ($P < 0.05$) anti-inflammatory activity: two-way ANOVA; student t-test.

min period increased again at 120 min. It is possible that the metabolite of the extract also has anti-nociceptive activity. This is in line with some reports that some metabolites are as potent or even more potent in some activities as the parent compound (Curtis, 1996). This later observation could therefore mean that *D. oliveri* extract can have an elongated anti-nociceptive effect. The egg albumin-induced oedema model showed the anti-inflammatory property of the extract. This model has the capacity of detecting acute inflammation. Although the mechanisms of action for the anti-nociception and anti-inflammation are not yet elucidated, the combination of both effects can be taken advantage of, therapeutically. The combination of both properties is known to be exhibited by different non-steroidal anti-inflammatory drugs (NSAIDs) such as salicylates and their congenes (Reuse, 1978; Beuoist and Misse, 1979; Famaey, 1983). These two effects add to the justification for the traditional use of the plant extract in the treatment of vestibule-vaginal fistula (VVF), swellings and abscesses.

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