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Phytochemical and Anti-inflammatory Activities of the Methanol extract and Fractions of *Dalbergiella welwitschii* Baker (Baker .f) leaves

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ABSTRACT

To investigate the Phytochemical and antiinflammatory activites of the methanol extract, petroleum ether and ethyl acetate fractions of *Dalbergiella welwitshii* Baker (Baker.f) leaves. The Phytochemical screening was carried out using standard procedures. The *in vivo* and *in vitro* antiinflammatory activities of the methanol extract, petroleum ether and ethyl acetate fractions were studied using egg-albumin induced rat paw edema test and protein denaturation models at different doses (50, 100 200 mg/kg body weight and 2000µg/ml, 1000µg/ml, 500µg/ml, 250µg/ml) respectively. The extract and fractions of *Dalbergiella welwitschii* exhibited significant anti-inflammatory activities at the doses tested in the *in vivo* (50, 100 200 mg/kg body weight) and *in vitro* (2000µg/ml, 1000µg/ml, 500µg/ml, 250µg/ml) models. These results justifies the use of *D. welwitshii* leaves in folklore treatment of arthritis and rheumatism.

Keywords: *Dalbergiella welwitschii*, methanol, hexane, ethyl acetate, anti-inflammatory, protein denaturation, *in vivo*, *in vitro*

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INTRODUCTION

Dalbergiella welwitschii Baker (Baker f.) (Fabaceae) is one of the two (2) species of the genus *Dalbergiella*. It is commonly known as the West African blackwood and elemosoo (Yoruba, SW Nigeria). [1]

D. welwitschii is a shrub or climber commonly found in the dry deciduous and riverine forests particularly from Guinea through Cameroun to Angola. [2] The folklore uses of the leaves of *D. welwitschii* include its use in the treatment of cutaneous and subcutaneous parasitic infection while the whole plant is used for the treatment of arthritis, rheumatism, stomach troubles and as abortifacient. [2] In addition, *D. welwitschii* is employed as purgatives, treatment for bronchial ailments, menstrual disorder as well as anthelmintic. [1]

The aim of this study is to investigate the anti-inflammatory effect of the leaves of *D. welwitschii* using the *in vivo* and *in vitro* models.

METHODOLOGY

Plant Collection

The leaves of *Dalbergiella welwitschii* Baker (Baker f) were collected in August 2012 from Ibadan, Oyo State. The plant was authenticated at the Forestry Research Institute of Nigeria (FRIN) with a voucher number FHI 1096647. Voucher specimens were deposited at the herbaria of the Forestry Research Institute of Nigeria and the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu. The leaves were air dried, milled into powder and stored for further analysis.

Phytochemical Analysis

The Phytochemical screening of the leaves were carried out on the leaves of *D. welwitschii* using standard procedures. [3, 4]

Extraction

The dried leaves of *D. welwitschii* were macerated in 80% methanol for 72 hours with intermittent shaking. The filtrate was then concentrated under reduced pressure in a rotary evaporator (Gallenkamp) and made aqueous before partitioning successively with hexane and ethyl acetate to yield the hexane and ethyl acetate fractions.

Anti-inflammatory Study.

In vitro Model

The anti-inflammatory study was carried out using the method adopted from Sangita et al., 2012. [5] 0.2 ml of egg albumin from fresh egg was mixed with 2.8 mls of phosphate buffer



saline (PBS, pH6.4) and 2 mls of the test sample or standard drug. The mixture was then incubated at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 15 minutes after which it was boiled in a temperature regulated water bath at 70°C for 5 mins. The mixtures were cooled and their absorbance measured at 660 nm in a UV-spectrophotometer. Five doses of the test samples (2000 $\mu\text{g}/\text{ml}$, 1000 $\mu\text{g}/\text{ml}$, 500 $\mu\text{g}/\text{ml}$, 250 $\mu\text{g}/\text{ml}$ and 125 $\mu\text{g}/\text{ml}$) and the reference drug (Diclofenac) were assayed and Distilled water was used as control. Each of the assays was carried out in triplicates.

STATISTICAL ANALYSIS

The % inhibition of the protein denaturation was calculated by

$$\% \text{ Inhibition} = 100 \times 1 - V_t / V_c$$

V_t = absorbance of test sample; V_c = absorbance of control

In vivo Study

Animals

Wistar albino rats obtained from the animal house, University of Ibadan weighing between 80g and 150 g were used for the assay. The assay was performed according to International guiding principles concerning the care and use of laboratory animal.

The *in vivo* anti-inflammatory study was evaluated using egg albumin induced edema (Phlogistic agent) model. [6, 7] 0.1 ml of egg albumin from fresh egg was subcutaneously injected into the right hind paw of the rats. The rats were administered the test sample and reference drug 30 minutes before inducing inflammation. The rat paw circumference were measured at intervals of 0 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min and 24 hrs.

Three doses each of the crude extract and fractions were assayed. Five rats ($n = 5$) were used per group.

Group 1 – Rats received 200 mg/kg of test sample

Group 2 - Rats received 100 mg/kg of test sample

Group 3 - Rats received 50 mg/kg of test sample

Group 4 - Rats received 1 ml of water

Group 5 - Rats received 5 mg/kg of reference drug

The % inhibition of the edema was determined

$$\% \text{ In hibition} = l_o - l_i / l_o \times 100$$

l_o = change in paw circumference in control group, l_i = change in paw circumference in treated group.



STATISTICAL ANALYSIS

Data are presented as Mean \pm SEM , while the Statistical analysis was analysed using the one way Analysis of Variance (ANOVA).

RESULTS

The Phytochemical screening of the leaves of *D welwitschii* in this study revealed the presence of flavonoids, tannins and saponins. Table 1

The methanol extract, hexane and ethyl acetate fractions of *D welwitschii* exhibited a dose dependent activity in the *in vitro* anti-inflammatory assay. The methanol crude extract was the most active with a percentage decrease in protein denaturation of 44%, 32.1%, 22% and 16% at the doses of 2000 μ /ml, 1000 μ g/ml, 500 μ g/ml and 250 μ g/ml respectively.

In the *in vivo* anti-inflammatory model, the effect of the extract and fractions of *D welwitschii* on acute inflammation exhibited a dose dependent effect at a dose of 200 mg/ml and 100mg/ml by significantly reducing the paw edema when compared to the untreated control group at the same time. The anti-inflammatory activities exhibited by the extract and fractions at 100 and 50 mg/ml were not significantly different from each other. The statistical analysis also indicated that the activities of the extract, fractions and reference drug (Diclofenac, 5mg/ml) were not significantly ($p < 0.05$) different from each other.

DISCUSSION

Inflammation is a physiological reaction responsible for the development of diseases such as cancer and type 2 diabetes. [8] It is also considered to perform the function of limiting damage and initiating the healing process. [9]

Inflammation occurs in three phases with the early phase mediated by histamine in the first two hours, the intermediate involves the activity of the bradykinin and the late phase involves the synthesis of prostanoid. [10, 11]

Several anti-inflammatory models are employed in determining the anti-inflammatory activities of natural products, in this study, the protein denaturation and egg albumin-induced models were used to investigate the anti-inflammatory activity of *Dalbergiella welwitschii*. This is because these models used to investigate the anti-inflammatory activities are considered reliable.

The Phytochemical study of the leaves of *D welwitschii* revealed the presence of flavonoids, saponins and tannins. Table 1



In the *in vitro* study, the activity of the hexane and ethyl acetate fractions were similar however, the activity exhibited by the methanol extract was more pronounced in the protein denaturation. Fig. 1

In the *in vivo* study, the activities of the methanol extract, hexane and ethyl acetate fractions at all the doses tested were comparable and more pronounced than the effect of the reference drug (Diclofenac, 5 mg/ml). Table 2 – 4

The statistical analysis ($p < 0.05$) shows that there were no significant differences between the activities of the extract, fractions and the reference drug at all doses tested.

Furthermore, this study reveals the ability of the methanol extract, hexane and ethyl acetate fractions of *D welwitschii* to effectively suppress the early and later phases of inflammation, this is because the reduction in the paw swelling volume in edema is useful as an index in determining the anti-inflammatory activity of natural products [12].

The anti-inflammatory activities exhibited by the leaves of *D welwitschii* may be due to the presence of flavonoids and tannins. Several plants rich in flavonoids have been reported to exhibit anti-inflammatory activities. [8, 12, 13] These flavonoids rich plants have also been reported to inhibit prostaglandin synthesis, exhibit analgesic effects, anti-inflammatory effects and antioxidant activities. [14]

The extract and fractions of *D welwitschii* could therefore be exerting anti-inflammatory activities by the presence of flavonoids thereby creating a decrease in the progression of lipid oxidation and mitigating inflammatory processes [15,16,17] by acting as antioxidants in scavenging free radicals or by reducing the generation of excessive nitric oxide and cytokinase.

This study therefore suggests the protective effect of *D welwitschii* against inflammation because of the presence of flavonoids. Finally, this study justifies the use of *Dalbegiella welwitschii* in folklore medicine in the treatment of arthritis and rheumatism.

Table 1 Phytochemical analysis of *Dalbergiella welwitschii* leaves.

Anthraquinone	Cyanogenetic glycosides	Alkaloids	Steroids	Cardiac glycosides	Flavonoids	Tannins	Saponins
-	-	-	-	-	+	+	+

- = Absent + = Present

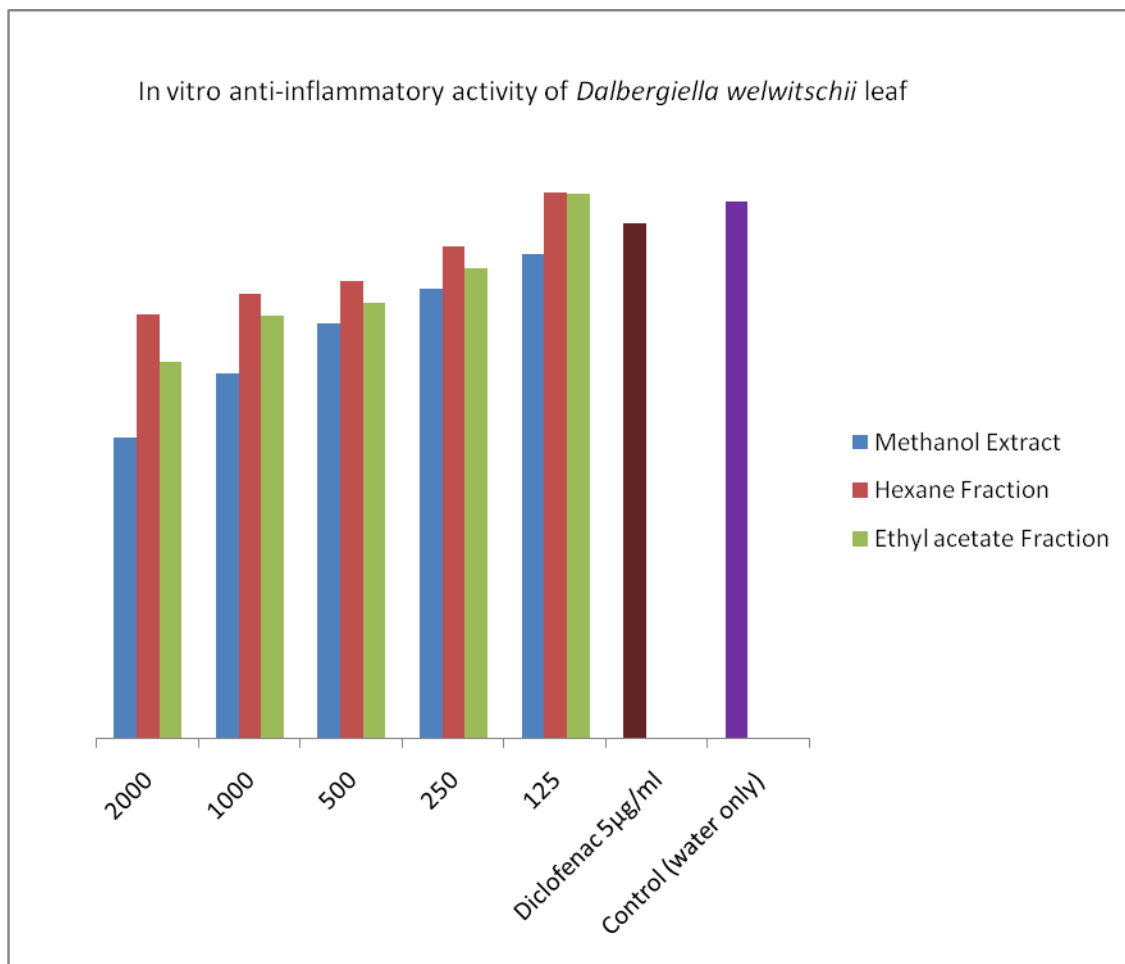


Fig 1: Effect of crude extract and fractions of *Dalbergiella welwitschii* leaves against Protein denaturation

Table 2: *In vivo* anti-inflammatory activity of methanol extract of *Dalbergiella welwitschii* leaves.

Dose	MEAN ± SDEV							
	0min	30min	60min	90min	120min	150min	180min	24hrs
200	2.87±0.45	2.60±0.40 19.2%* 9.4%**	2.37±0.31 25%* 17.4%**	2.06±0.12 31%* 28.2%**	1.97±0.21 30.3%* 31.4%**	1.90±0.26 28%* 33.8%**	1.83±0.15 26.8%* 36.2%**	1.60±0.06 17.7%* 40.8%
100	3.10 ±0.10	2.83±0.06 9.6%* 8.7%**	2.50±0.10 20.8%* 25.8%**	2.30±0.10 31%* 21.5%**	2.10±0.10 32.8%* 32.3%**	1.97±0.06 29.4%* 36.4%**	1.83±0.06 24%* 40.9%**	1.77±0.05 12.8%* 42.9% **
50	2.93±0.31	2.80±0.26 20%* 4.4%**	2.40±0.20 29.4%* 18.1%**	2.30±0.10 31%* 21.5%**	2.10±0.10 28.2%* 28.3%**	2.0±0.06 25.6%* 31.7%**	1.98±0.06 24%* 32.4%**	1.80±0.01 13.3%* 38.6%**
Water	3.37±0.21	3.13±0.12	3.16±0.06	3.00±0.10	2.83±0.15	2.65±0.15	2.50±0.10	2.03±0.10
Diclofenac 5mg/ml	3.4±0.15	3.1±0.15 8.8%*	3.0±0.13 5%* 11.8%**	2.60±0.36 13.3%* 23.5%**	2.4±0.38 16.9%* 29.4%**	2.35±0.38 18%* 32.4%**	2.30±0.26 18%* 32.4%**	2.0±0.15 3.9%* 41.2%**

Values are presented as mean ± SEM; n=5

*test value compared to reference drug at the same time; **test value compared to 0 min

Table 3: *In vivo* anti-inflammatory activity of hexane fraction of *Dalbergiella welwitschii* leaves.

Dose	MEAN ± SDEV							
	0min	30min	60min	90min	120min	150min	180min	24hrs
200	3.30±0.26	2.87±0.31 8.3%* 13%**	2.67±0.31 15.5%* 19%**	2.27±0.32 24%* 31.2%**	2.13±0.29 24.7%* 35.5%**	1.97±0.15 25.7%* 40.3%**	1.90±0.10 24%* 42.4%**	1.77±0.06 12.8%* 46.4%
100	3.27 ±0.32	2.80±0.2 10%* 14.3%**	2.63±0.23 16.3%* 19.6%**	2.37±0.38 21%* 28.2%**	2.16±0.10 24.7%* 34.5%**	2.13±0.06 19.6%* 34.9%**	1.83±0.06 16%* 38.8%**	1.89±0.01 3.4%* 42.2% **
50	3.13±0.26	2.97±0.21 8.3%* 5.1%**	2.60±0.06 17.7%* 16.9%**	2.40±0.1 21%* 23.3%**	2.20±0.10 29.3%* 29.7%**	2.20±0.01 24.5%* 29.7%**	2.05±0.10 20%* 34.5%**	1.93±0.12 1.5%* 38.3%**
Water	3.37±0.21	3.13±0.12	3.16±0.06	3.00±0.10	2.83±0.15	2.65±0.15	2.50±0.10	2.03±0.10
Diclofenac 5mg/ml	3.4±0.15	3.1±0.15 8.8%*	3.0±0.13 5%* 11.8%**	2.60±0.36 13.3%* 23.5%**	2.4±0.38 18%* 32.4%**	2.35±0.38 18%* 32.4%**	2.30±0.26 18%* 32.4%**	2.0±0.15 3.9%* 41.2%**

Values are presented as mean ± SEM; n = 5

*test value compared to reference drug at the same time; **test value compared to 0 min

Table 4: *In vivo* anti-inflammatory activity of ethyl acetate fraction of *Dalbergiella welwitschii* leaves.

Dose	MEAN ± SDEV							
	0min	30min	60min	90min	120min	150min	180min	24hrs
200	2.97±0.21	2.77±0.21 15.1%* 3.6%**	2.50±0.10 20.9%* 15.8%**	2.30±0.29 31%* 24.9%**	2.23±0.29 30.3%* 24.9%**	2.13±0.29 28%* 28.3%**	2.03±0.21 26.8%* 31.6%**	1.87±0.02 17.7%* 37.0%
100	3.07 ±0.06	2.83±0.06 10.5%* 7.8%**	2.50±0.10 24.1%* 18.6%**	2.30±0.10 26.3%* 25.1%**	2.13±0.06 24.7%* 30.6%**	1.97±0.06 12.5%* 35.8%**	1.97±0.06 13.6%* 35.8%**	1.87±0.06 10%* 39.1%**
50	3.00±0.20	2.73±0.25 23.3%* 9%**	2.53±0.15 26.3%* 15.7%**	2.30±0.10 24.3%* 23.3%**	2.13±0.10 29.2%* 29%**	2.03±0.15 39%* 32.3%**	2.00±0.10 36%* 33.3%**	1.93±0.12 12.9%* 35.7%**
Water	3.37±0.21	3.13±0.12	3.16±0.06	3.00±0.10	2.83±0.15	2.65±0.15	2.50±0.10	2.03±0.10
Diclofenac 5mg/ml	3.4±0.15	3.1±0.15 8.8%*	3.0±0.13 5%* 11.8%**	2.60±0.36 13.3%* 23.5%**	2.4±0.38 16.9%* 29.4%**	2.35±0.38 18%* 32.4%**	2.30±0.26 18%* 32.4%**	2.0±0.15 3.9%* 41.2%**

Values are presented as mean ± SEM; n = 5

*test value compared to reference drug at the same time; **test value compared to 0 min



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