

**ANTIPYRETIC AND PHYTOCHEMICAL EVALUATION OF THE ETHANOL EXTRACT OF THE LEAVES OF *DESMODIUM VELUTINUM***

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### **Abstract**

*Desmodium velutinum* has been reported in traditional medicine to have medicinal properties. One of the medicinal uses of *Desmodium velutinum* in Nigeria is in the treatment of fever. This research sought to establish this claim by determining the anti-pyretic activity of *Desmodium velutinum* leaves against pyretic-induced experimental rats. The leaves of *Desmodium velutinum* were dried, powdered and extracted with ethanol for 48hrs at room temperature with constant shaking using mechanical shaker. This was concentrated using rotary evaporator. The plant extract was screened for its phytochemical constituents, using standard procedures and its anti-pyretic activity, using brewer's yeast to induce pyrexia in rats. Phytochemical screening revealed the presence of, resins, tannins, flavonoids, saponins and glycosides. The extract showed significant anti-pyretic activity on experimental rats used. Ethanolic extract of the leaves of *Desmodium velutinum* therefore possesses anti-pyretic property.

**Key words:** Aspirin, hot plate, *Desmodium velutinum*.

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### **Introduction**

The use of herbs and medicinal plants as the first medicines is a universal phenomenon.<sup>[1, 2]</sup> Every culture on earth, through written or oral tradition, has relied on the vast variety of natural medicines found in healing plants for their therapeutic properties.<sup>[3, 4]</sup> All drugs of the past were substances with a particular therapeutic action extracted from plants. Thus, medicinal plants may be defined as any plant that can be put to culinary or medicinal use such as fox glove, opium poppy, and garlic. More researchers find that food and their individual constituents perform similar fashion to modern drugs and sometimes better without the dreaded side effects.<sup>[5- 9]</sup> The problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized.<sup>[10, 11]</sup> Coincidentally, the last decade witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine.<sup>[12- 14]</sup> Natural plants have been valuable sources of medicinal agents with proven potential of treating infectious diseases and with lesser side

effects compared to the synthetic drug agents.<sup>[15]</sup> Hence potentially useful drugs can often be recognized from their relative importance and use in folk medicine.<sup>[16, 17]</sup> Extracts of *Desmodium velutinum* are used traditionally in some disease conditions particularly headache. Hence *Desmodium velutinum* may be a source of a pharmacological active agent useful in the treatment of aches and pains.

### **Taxonomy of the plant**

#### Scientific classification

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Fabaceae
Genus:	<i>Desmodium</i>
Species:	<i>Desmodium velutinum</i>

### **Common names**

Chitkiboota - India

Latkan - India

### **Local names**

Ibo: Ikeagwuani

### **Description of plant**

*Desmodium velutinum* is a perennial, erect or semi-erect shrub or sub-shrub, up to 3 m high. Branches often dark red, yellow-brown when young, velutinous and short hooked-hairy. Leaves 1-foliolate, rarely 3-foliolate, ovate, ovate-lanceolate, triangular-ovate, or broadly ovate, 4-20 cm long and 2.5-13 cm wide, chartaceous to coriaceous, upper surface continuously oppressed-pubescent, lower surface densely velutinous. Inflorescence often dense, terminal or auxiliary, racemose or paniculate, 4-20 cm long, with 2-5 flowers at each node; flowers purple to pink. Pods narrowly oblong, 1-2.5 cm long, 2-3 mm wide, with dense yellow straight hairs intermixed with short hooked hairs, 5-7-jointed. Seeds ovate, flat, 1.3-1.6mm x 1.8-2.5mm, yellow when ripe. Depending on the genotype, there are 320,000-830,000 seeds per kg. *Desmodium velutinum* belongs to the botanical family Fabaceae. It is a perennial, erect or semi-erect shrub, up to 3m high. The plant is generally called Ikeagwani; the leaves are used for the control of non-specific diarrhoea. About 30 grams of whole leaves may be boiled in about 150-200ml of water and 20-50ml of the extract taken depending on the severity of the diarrhoea. The dose may be taken once but not

more than two doses may be taken in a day. Children may take lower doses. It is also claimed that the water extract of the leaves is used as an aphrodisiac.<sup>[18- 20]</sup>

### Geographical distribution of the plant

Sub- tropical Asia (China, Taiwan, India, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand, Vietnam) and tropical Africa.



**Figure: 1** *Desmodium velutinum* - Typical appearance.

### Other species of *Desmodium* include;

1. *Desmodium lasiocarpum* (P. Beauv.) DC
2. *Desmodium latifolium* (Roxb. ex Ker Gawl.) DC
3. *Hedysarum lasiocarpum* (P. Beauv.)
4. *Hedysarum latifolium* Roxb. ex Ker Gawl.
5. *Hedysarum velutinum* Wild.
6. *Meibomia lasiocarpa* (P. Beauv.) Kuntze

In Ghana, native doctors mix the roots of *Desmodium lasiocarpum* with some hot peppers and use it as enema to cure blood in urine. In Nigeria, it is used as food for horses.

### Materials and method

Aspirin, ethanol, miller (Thomas Laboratory Mill, U.K), mechanical weighing balance (Ohaus, Poland), electronic weighing balance (Gulfes Medial and Scientific, England), filter paper (Wattman No. 1), white clean handkerchief (as porcelin cloth), rotary evaporator (Fulton, china), oven (Harris, England), mechanical shaker (Surgifrend, England), incubator (Genlab, U.K), culture plates, hot plate, autoclave (Health Team Instrument, England), beakers (10ml, 25ml, 50ml, 500ml capacities), cotton wool, hand gloves, syringes and needle (1ml, 2ml, 5ml)

**Plant material - collection and identification**

Young fresh leaves of *Desmodium velutinum* were collected from Ogidi, Idemili North Local Government Area of Anambra State in July 2011, during the rainy season and were identified by Dr. Ezugwu, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, and Nsukka.

**Extraction**

Fresh leaves of *Desmodium velutinum* were dried at ambient temperature until their weight which was measured at intervals was about the same. The dried leaves were pulverized using laboratory miller; 250g of the powder was macerated in 500ml of ethanol, placed on a mechanical shaker for 48 hours. The extract was filtered using clean white handkerchief. The filtrate was further filtered using No.1 Wattman filter paper. The filtrate was concentrated using rotary evaporator. The extract was stored in the refrigerator for future use.

**Animals:** Fifteen albino rats obtained from the Madonna University's animal house were freely fed with standard animal feeds and water and allowed to acclimatize.

**Phytochemical analysis of extract**

The extract was subjected to phytochemical screening by testing for alkaloids, saponins, resins, tannins, steroidal nucleus, glycosides, proteins and flavonoids using standard procedures.<sup>[21]</sup>

**Test for alkaloids**

About 20ml of 30% sulphuric acid in 50% ethanol was added to 2g of the extract and heated on a water bath for 10 minutes, cooled and filtered. 2 ml of the filtrate was tested with a few drops of Mayer's reagent (potassium mercuric iodide solution (1%). The remaining filtrate was placed in 100ml separating funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and the extract was tested with few drops of Mayer's, Wagner's, Dragendoff's reagent and picric acid solution. Alkaloids give milky precipitate with Mayer's reagent and reddish brown precipitate with the other indicator.

**Test for saponins**

About 20 ml of distilled water was added to 0.25g of the extract and boiled on a hot water bath for two minutes. The mixture was filtered while hot and allowed to cool and filtrate was used for the following test.

**Emulsion test:**

About 5 ml of the filtrate was diluted with 15 ml distilled water, two drops of olive oil was added and shaken vigorously. The formation of emulsion indicates the presence of Saponins.

**Fehling's test:**

To 5 ml of the filtrate was added 5 ml of Fehling's solution (equal parts of I and II) and the contents were heated on a water bath. Reddish Precipitate which turns brick red on further heating with sulphuric indicated the presence of saponins.

### **Test for tannins**

About 1g of the powdered material was boiled with 20 ml of water, filtered and used for the following test.

**Ferric Chloride Test** - To 3 ml of the filtrate few drops of Ferric chloride were added .A greenish black precipitate indicated the presence of tannins.

### **Test for flavonoids**

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

### **Ammonium Test**

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

### **1% Aluminium Chloride Test**

Another 4 ml of the filtrate was shaken with 1 ml of 1% Aluminum Chloride solution. The layers were allowed to separate. A yellow colour in aluminum chloride layer indicates the presence of Flavonoids.

### **Test for resins**

About 0.2 g of the extract was shaken with 15 ml of 96% ethanol. The alcoholic extract was then poured into 20 ml of distilled water in a beaker. A precipitate occurrence indicates the presence of resins.

### **Colour Test**

About 0.2 g of the extract was extracted with chloroform and the extract was concentrated to dryness. The residue was redissolved in 3 ml of acetone and another 3 ml of conc. HCL acid was added .The mixture was heated in a water bath for 30 minutes. A pink colour which changes to magenta red indicates presence of resins.

### **Test for steroids and triterpenoids**

About 9 ml of ethanol was added to 1 g of the extract it was refluxed for a few minutes and filtered. The filtrate was concentrated on a boiling water bath. 5 ml of hot distilled 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 2.5 ml of chloroform using separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1 ml of conc. H<sub>2</sub>SO<sub>4</sub> to form a lower layer. A reddish brown interface shows the presence of steroids. 0.5 ml of the chloroform was evaporated to dryness on a water bath and heated with 3 ml of the concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

**Test for proteins****Million's test**

To a little portion of the mix made in test tube. Two drops of Million's reagent was added. A white precipitate indicates the presence of protein.

**Test for glycosides****Hydrolysis**

5ml of the extract was boiled with 5ml of dilute sulphuric acid for 15 minutes, filtered and cooled. The filtrate neutralized with 20% potassium hydroxide and treated with Fehling's solution was again boiled for 15 minutes and observed.

**Pharmacological tests:****Acute Toxicity Test (LD50):**

The LD50 was carried out using the method employed by Dietrich Lorke (1983). It involves a total of 13 mice. This test was carried out in two phases. Phase 1 employed a total of 9 mice. They were grouped into 3 groups of 3 mice per group. Group 1 received 10mg/kg of the extract. Group 2 received 100mg/kg of the extract. Group 3 received 1000mg/kg of the extract. All the administration was through intra-peritoneal route. The animals were constantly monitored for 4 hrs, then for 6 hrs; and then over a period of 24 hrs. The numbers of the dead animals were noted. From the result gotten from the first phase, the second phase was carried out. In this phase, a total of 4 groups of 1 mouse per group were used. Group 1 received 2000mg/kg of the extract. Group 2 received 3000mg/kg, Group 3 received 4000mg/kg, and Group 4 received 5000mg/kg. The animals were monitored for another 24 hrs for any death.

**Anti pyretic test:**

The anti-pyretic study was carried out using Animal model. Total of Fifteen rats were involved. They were divided into 5 groups of 3 animals per group. The normal body temperature of each animal was taken using rectal thermometer. Pyrexia was induced by injecting 10mg/kg of 15% brewer's yeast in Normal saline, subcutaneously in the neck of each animal. After 18 hrs, their body temperatures were taken, in order to confirm pyrexia.

Group one was administered 0.5ml of distilled water orally. Group two was given 100mg/kg of aspirin orally. Group three was administered 50mg/kg of extract orally. Group four was administered 100mg/kg of extract, while Group five was administered 200mg/kg of extract orally.

At 30mins, 60mins, 90mins, and 120mins post treatment, their body temperatures were taken to monitor the antipyretic effect of the extract.

**Results**

**Table: 1 The phytochemical constituents of the ethanol extract of *Desmodium velutium***

Bioactive compound	Ranges
Alkaloids	-
Glycosides	+
Flavonoids	+
Tannins	+++
Resins	+
Saponin	+
Steroid	-
Proteins	-

**Key:** - means absent, + means mildly present, ++ means moderately present, +++ means strongly present.

**Table: 2 Acute toxicity test**

PHASE	DOSE	DEATH
1	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
2	2000mg/kg	0/1
	3000mg/kg	0/1
	4000mg/kg	0/1
	5000mg/kg	0/1

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

**Table:3 Results for anti pyretic activity (Mean± SEM)**

Group	Dose	Normal	Pyrexia at induction	30mins	60mins	90mins	120mins
1	0.5 ml distilled Water	36.8± 0.70	37.4 ±0.60	38.6 ± 0.05	38.1 ±0.45	38.6 ±0.05	38.7 ±0.00
2	100mg/ kg aspirin	38.1 ± 0.40	38.6 ±0.60	37.4 ± 0.20	37.0 ±0.30	36.8 ±0.35	36.6 ±0.40
3	50mg/Kg extract	38.1 ± 0.35	38.3 ±0.05	38.0 ± 0.00	37.6 ±0.00	37.3 ±0.10	37.1 ±0.05
4	100mg/kg Extract	37.3 ± 0.00	38.3 ±0.10	36.8 ± 0.20	36.7 ±1.10	36.8 ± 0.60	36.6 ±0.65
5	200mg/kg	36.0± 0.10	38 .0± 0.05	37.1 ± 0.70	36.0 ±0.25	36.1 ± 0.20	35.7 ±0.15

NS = Not significant ( $p > 0.05$ ), \* = slightly significant ( $p < 0.05$ ), \*\* = extremely significant ( $p < 0.01$ )

## Discussion

The phytochemical analysis revealed the presence of tannins, saponins, glycosides, resins, and flavonoids. Tannins are known to have several pharmacological activities including anti-inflammatory activities. The extract exhibited anti-pyretic property.

## Conclusion

The traditional use of the leaves of *Desmodium velitimum* to manage fever was supported by this work, as extract of leaves of *Desmodium velitimum* showed significant anti- pyretic activity in experimental rats. However more work is to be done to characterize the actual constituents that are responsible for this activity.

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