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Phytochemical Screening, Acute Toxicity and Analgesic Properties of Aqueous Extract of *Flueggea virosa's* Root in Rats

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Abstract

Introduction: *Flueggea virosa* (Roxb. ex Willd.) belongs to the family of Euphorbiaceae and grows wild in tropical Africa and most parts of the world; but can also be domesticated. Different parts of the plant have been claimed to have many folkloric usages for treatment of diverse ailments. Therefore, the aqueous extract of the *Flueggea virosa's* root was investigated to ascertain its total yield, phytochemical components, acute toxicity and analgesic activity in groups of Wistar rats. **Materials and Methods:** Extraction was by the locally employed decoction method. Phytochemical screening was done by standard methods. Acute toxicity was evaluated by the modified Lorke's method in one phase and analgesic activity was tested using *Tail-Flick* and *Formalin* models using rats. **Results:** The percentage yield of the extract was 9.1%. Tannins, saponins, terpenoids, steroids, cardiac glycosides, and reducing sugars were evident. Acute toxicity tests indicated that it is generally safe; with piloerection observed at high doses only. The extract showed a statistically significant ($p<0.05$), dose-dependent inhibition of pain in formalin test and some level of non-statistically significant and non-dose dependent pain reduction in tail-flick test at 100-400mg/kg of body weight given orally. **Conclusion:** *Flueggea virosa* root produces modest yield of extract and possesses potent phytochemicals, some measure of acute safety and significant analgesic properties.

Keywords: acute toxicity, analgesic, *Flueggea virosa*, phytochemicals, rats, root.

Introduction

Flueggea virosa is of the family of Euphorbiaceae which grows wild in tropical Africa, Arabian Peninsula, tropical Asia, Japan, Australia and Polynesia but can also be domesticated. Different parts of the plant have many folkloric usages for diverse ailments including pain, fever, malaria, sexual dysfunction, diabetes, epilepsy, antiarrhythmic,

snakebites, venereal diseases, rheumatism, sterility, contraception, rashes, diarrhoea, pneumonia, impotence, cough and HIV-related illness with the root being claimed to be the most potent part and the ripe fruits can be eaten by humans, animals and birds (1-7). Its common names include white-berry bush, Chinese water berry (China), Mukwamba or Mteja (Kenya), Omukarara (Uganda) and common bushweed (8). All plant parts were found to contain indolizidine alkaloids (9). Other compounds isolated from the leaves are the isocoumarine, bergenin, gallic and ellargic acids and the quercetin and rutin flavonoids (9). The stem bark contains the triterpenes friedelin and friedelinol. The twigs contain about 8% tannins; the root bark 0.4 – 0.6% alkaloids and the entire root 0.04% alkaloids (9). The root and root bark's methanol and water extracts of the plant have been reported to possess a host of activities as follow: bergenin component with a dose dependent *in vitro* anti-plasmodial activity (10,11) and significant *in vivo* trypanocidal, anti-arrhythmic activity in rats; lipid lowering and atherogenic index decrease in hyperlipidaemic rats (12-14); a transient fall in arterial blood pressure in dogs (12); a slightly depressive action on the isolated intestine of rabbits, which rapidly normalized (15); a weak haemolytic activity, a slight activity in the oral glucose tolerance test in rabbits but did not lower blood glucose below fasting levels in either the fed or fasted state (16).

Pain is an unpleasant sensory and emotional experience (17) that is mediated by specific nerve fibres to the brain where its conscious appreciation may be modified by various factors. The word "unpleasant" comprises the whole range of disagreeable feelings from being merely inconvenienced to misery, anguish, anxiety, depression and desperation to the ultimate core of suicide (18,19). But pain is a protective mechanism telling us that the body is not well. Drugs that relieve pain especially due to multiple causes are called analgesic drugs e.g. paracetamol, morphine but those that relieve pain due to a single cause or specific pain syndrome only, e.g. migraine (ergotamine); neuralgias (carbamazepine); angina pectoris (glyceryltrinitrate) and pain of inflammation of any cause are not classified as analgesics. Analgesics are classified as narcotics when they act in the central nervous system and cause drowsiness e.g. opioids; and non-narcotics when they chiefly act peripherally e.g. diclofenac. Acute pain is managed primarily but not invariably by analgesic drugs. Painful stimuli consist of direct stimulation of pain receptors by various means. Three methods are generally used to induce the required pain stimuli in mice and rats for evaluation of analgesic properties of a natural extract and they include physical, thermal and chemical methods (20). There were popular claims of traditional usage of this plant in the treatment of pain but there were no reports of studies in the literature in some countries of East African region to confirm or dispute that this plant possesses the property of pain relief, hence this work was done to fill this existing gap.

Materials and Methods

Preparation of Plant Extract

Fresh roots of *Flueggea virosa* were then collected on 9th of February, 2010 from several trees growing in Rabai area of Mombasa Kenyan coast in the morning hours during rainy season identified by a local herbalist from the area. The taxonomy of *Flueggea virosa* plant was identified by a botanist from Kampala International University-Western Campus (KIUWC) Ishaka, Bushenyi, Uganda. A voucher specimen of the plant was deposited in the Herbarium of the Pharmacognosy Unit of School of Pharmacy KIUWC, Ishaka, Bushenyi, Uganda. The roots were washed off sand and particles, dried under a shade and then reduced to powdery form. A decoction method was used to prepare the extract by weighing 500g of the powder and suspending it in 2,500ml of distilled water. This was then boiled for 5 minutes, allowed to cool, filtered twice with some cotton wool and then filtrate evaporated over a water bath at 50°C and dried in the hot air oven at 40°C to make *Flueggea virosa* extract (FVE). The harvested yield was then weighed and percentage calculated.

Preliminary Phytochemical Screening

Qualitative phytochemical screening tests were conducted on the *Flueggea virosa* aqueous extract with previously published standard methods (21,22).

Laboratory Animal Acquisition and Maintenance

Male and female Wistar rats weighing 100g and more were used. These animals were bred and housed in the Animal Facility Centre of the School of Pharmacy, KIUWC. The animals were separated and kept for ten days for acclimatization in a cage lined with wood shavings, maintained at room temperature having adequate ventilation and naturally illuminated environment with 12 h of light and 12 h of darkness. They were fed on standard diet (Nuvita® Animal Feed Ltd, Jinja Uganda) and allowed access to clean drinking water *ad libitum*. The animal experiments were conducted according to the National Institute of Health Guide for the care and use of laboratory animals (23) and ethical guidelines for investigation of experimental pain in con-

scious animals (24).

Acute Toxicity Determination

The Lorke's method (25) was used with modifications (rats instead of mice were used, procedure was conducted in one phase instead of two and a control group of rat was used in addition to the experimental groups). In this one phase, geometric doses of 100mg/kg, 500mg/kg, 2500mg/kg and 5000mg/kg of body weight of FVE were administered respectively to the rats in each of the 4 groups of three rats per group (n=3) while the control group (n=3) received distilled water. All the rats were kept under the same conditions and observed for toxicity signs for six hours after drug administration and were scored for mortality and general behaviour after 24 h. The arithmetic mean of the smallest dose that killed a rat and the highest dose that did not kill a rat was taken as the mean lethal dose (LD50) of the extract.

Analgesic Tests

In this study, two types of painful stimuli were used to induce pain in the rats by direct stimulation of pain receptors and they included: thermally (tail-flick test) and chemically (formalin) induced pain.

Tail-Flick Test: The method of D'Amour and Smith (26) as subsequently modified for rats using hot water bath (27) was adopted with minor modification as follows: The rats were initially screened for the test by immersing about 2cm of their tails into water heated in a water bath and maintained at 55 ± 1°C. Twenty five rats that lifted their tail within 5 seconds were selected for the study. The baseline tail reaction response time was used to group the rats into five groups of five animals each (n=5). Tail reaction response time was done with 2cm of the tail initially marked from the tail tip immersed in the water bath at 55± 1°C. The FVE at doses of 100, 200, and 400mg/kg of body weight was administered orally to three groups of experimental rats; one group was given aspirin (100mg/kg) and the control group was administered distilled water at 10ml/kg of body weight. Tail reaction response was then taken at 30 and 60 minutes after drug treatment. A cut-off time of 6 seconds was used in order to prevent damage to the tail.

Formalin Induced Pain Test: The modified method of Dubuisson and Dennis (28) modified by Tjolsen (29) was adopted as follows: The animals were grouped into five groups of five animals each (n=5) based on their weights. The FVE at 100, 200, and 400mg/kg of body weight was administered orally to three groups; aspirin 100mg/kg of body weight was administered to the fourth group while the control group was administered distilled water 10ml/kg of body weight. After 30 minutes of treatment, 0.05ml of 2.5% formalin was injected subcutaneously into the subplantar surface of the left hind paw of rats and observed in two phases. The first phase was every two minutes for the first ten minutes post formalin (representing the early phase referred to as aphasic pain) and the second phase was every 5 minutes starting from the 15th minute post formalin to the 60th minute (representing the late phase referred to as tonic pain). Scoring was done by observing whether the rat was walking normally on the paw (0), or light resting on the paw (1), elevating the injected paw (2) or licking or biting or grooming the injected paw (3).

Statistical Analysis

Results were expressed as mean values ± Standard Error of Mean (SEM) and statistical comparison of data was performed using descriptive statistics and Analysis of Variance (ANOVA)-repeated measures. All levels of significance were set at $p < 0.05$.

Results

Harvested Yield

500g of dry powder of *Flueggea virosa* yielded 9.13% of extract (45.63g).

Phytochemical Analysis

The phytochemical screening tests on aqueous rxtract of the root of *Flueggea virosa* were positive to tannins, saponins, steroids, terpenoids, cardiac glycosides and reducing sugars but negative to phlobatannins and flavonoids.

Acute Toxicity Tests

No significant toxic effects were observed except that mild piloerection was observed at high doses of FVE at 2,500 and 5,000mg/kg body weight. Oral administration of *Flueggea virosa root* aqueous extract to rats up to 5,000 mg/kg resulted in no death of any of the test rats after 24 h. Hence, the LD_{50} of FVE in rats was estimated to be greater than 5,000 mg/kg (Table 1).

Analgesic Tests

The FVE showed some analgesic activity in both tests as described below.

Tail-Flick Test: Tail-flick test showed insignificantly non-dose dependent inhibition of pain down the different dose groups when compared with the control group. This is re-

flected in Table 3. Here, after 30 mins from the time of administration of extract, the response times recorded for the test groups were 2.0±0.2, 1.9±0.2, 1.9±0.2 seconds at respective doses of 100, 200 and 400mg/kg of body weight as compared with aspirin group of 1.9±0.3 seconds at 100mg/kg and distilled water group of 1.9±0.1 seconds at 10ml/kg of body weight. There were higher values of pain inhibition at 60mins than 30mins after administration of extract though the same pattern was followed in both time durations. The higher values of tail responses recorded at 60mins duration for extract groups after extract administration were 2.1±0.1, 2.1±0.3 and 2.40±0.10 seconds at doses of 100, 200, and 400mg/kg of body weight while those recorded for aspirin and distilled water groups were lower (2.0±0.1 and 1.7±0.2) seconds at 100mg/kg and 10ml/kg of body weight respectively. Table 3 also shows that the difference between the tail response time of rats in each group after administration of the extract (T_1 - T_0 (30mins)) and (T_2 - T_0 (60mins)) and their tail response at time zero (T_0) before administration of the extract was not statistically significant. The difference followed non-dose dependent trend for both 30mins and 60mins durations as follows: 0.6±0.1, 0.7±0.1; 0.5±0.2, 0.7±0.3; and 0.5±0.1, 1.1±0.1 seconds respectively at doses of 100; 200; and 400mg/kg of body weight as compared with aspirin group of 0.4±0.2, 0.5±0.4 seconds at 100mg/kg and distilled water group of 0.6±0.2, 0.4±0.1 seconds at 10ml/kg of body weight.

Formalin Induced Pain Test: The FVE exhibited statistically significant dose dependent reduction of oedema in both phases 1 (early phase) and 2 (late phase) of formalin induced pain. In the early phase, pain inhibition achieved was 14.6, 22.0, and 29.3% at respective doses of 100, 200 and 400mg/kg which compared favourably with the standard drug, aspirin at pain inhibition of 17.0% at 100mg/kg. In the late phase, the same trend of significant pain inhibition (13.3, 16.0 and 26.7%) was achieved at the same dose levels which recorded higher pain inhibition than aspirin (5.3%) at 100mg/kg and lower pain inhibition than in the early phase (Table 3).

Discussion

The yield obtained from the extraction was moderately reasonable. This perhaps could be due to the short period of 5 minutes during which decoction was carried out. However, this is the method being used by the locals in extracting from the root of the plant and that is our reference point. It may be pertinent to note that the roots of this plant should preferably be harvested between the months of February and April (during the short rainy season) and between September and December (during the long rainy season) of any year for future work on the roots of this plant in this sub-region of Africa.

The phytochemical screening tests on FVE showed the presence of tannins, saponins, terpenoids, steroids, cardiac glycosides and reducing sugars. Tannins and saponins are perhaps responsible for the plant's noted analgesic properties. It can be recalled that the main therapeutic action of tannins is astringent effect because of its propensity to bind to albumin present in the skin and mucous membranes of the body to form an insoluble protective layer resistant to disease. Tannins also have been found to possess healing properties whereby they protect the affected areas treated with them while reducing inflammation at the same time. This is the more reason why herbs containing them can be utilised in compress for cuts and wounds, haemorrhoids, varicose veins and in medicine for diarrhoea, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract (21). Saponins are glycosides found widely among medicinal plants which form bubbles like soap when mixed

Table 1. Result of Acute Toxicity Test of Aqueous Extract of the Root of *Flueggea virosa* in Rats

Groups	Increased motor activity	Sedation	Pilo-erection	Appetite loss	Death
100 mg/kg	-	-	-	-	-
500 mg/kg	-	-	-	-	-
2500 mg/kg	-	-	+	-	-
5000 mg/kg	-	-	+	-	-
Control	-	-	-	-	-

Control: Distilled water 10ml/kg ; (+) means Mild effect; (-) means Zero effect

Table 2. Effect of the Aqueous Extract of *Flueggea virosa* Root on Thermally Induced Pain in Rats (Tail-Flick Response Time).

Treatment	Tail response time (Seconds)			
	30 min	60 min	T_1-T_0 (30min)	T_2-T_0 (60min)
Aspirin 100mg/kg	1.89±0.25	1.95±0.07	0.43±0.21	0.49±0.36
FVE 100mg/kg	2.04±0.20	2.10±0.19	0.62±0.12	0.68±0.09
FVE 200mg/kg	1.85±0.19	2.07±0.25	0.46±0.22	0.68±0.26
FVE 400mg/kg	1.86±0.18	2.40±0.10	0.50±0.13	1.05±0.12
Control	1.93±0.11	1.70±0.19	0.36±0.09	0.36±0.09

*Control: Distilled water 10ml/kg ; n=5, values presented as mean ± standard error of mean (S.E.M). *: p<0.05 was considered significant when compared with control, (ANOVA repeated measures). Values were statistically significant.*

Table 3. Effect of the Aqueous Extract of *Flueggea virosa* Root on Chemically Induced Pain in Rats (Formalin Test).

Groups	Phase 1	Inhibition (%)	Phase 2	Inhibition (%)
Aspirin 100mg/kg	8.50 ± 0.29*	17.03	17.75 ± 0.63*	5.33
FVE 100mg/kg	8.75 ± 0.85*	14.63	16.25 ± 0.85*	13.30
FVE 200mg/kg	8.00 ± 0.91*	21.95	15.75 ± 1.31*	16.00
FVE 400mg/kg	7.25 ± 0.48*	29.27	13.75 ± 0.85*	26.67
Control	10.25 ± 0.48	-	18.75 ± 1.65	-

*Control: Distilled water 10ml/kg ; n=5, values presented as mean ± standard error of mean (S.E.M). *: p<0.05 was considered significant when compared with control, (ANOVA repeated measures). Values were statistically significant.*

with water. They have wide variety of therapeutic actions in the body including anti-inflammatory, expectorant, diuretic, anti-malarial and haemolytic effects on red blood cells (toxic) when injected into the blood stream and comparatively harmless when taken orally (21). Terpenoids are hydrocarbons which can extend to their oxygenated derivatives found in volatile plant origin possessing analgesic, antiseptic, stimulant, carminative, diuretic, anthelminthic, anti-rheumatic and counter-irritant properties (21), (22).

Acute toxicity studies are designed to determine the dose that will produce mortality or serious toxicological effects when given once or over a few administrations. They also serve to provide information regarding doses that should be used in subsequent studies. They can also give an early indication of the possible target organs. Acute toxicity test results are displayed in Table 2. Acute toxicity test on FVE in rats established a high LD_{50} suggesting that the aqueous extract of the leaves of *Flueggea virosa* may be generally regarded as safe with a remote risk of acute piloerection at high dose of about 5,000mg/kg body weight (Category IV classification) with no death (30).

The extract did not show statistically significant reaction time in tail flick test when compared with the control. The tail flick test is a standard method for the investigation of nociception and analgesia (26). This method measures a response to a brief, noxious stimulus appearing to emanate

from reflexes of the spinal cord which are modulated by supraspinal inhibitory mechanisms (31). The test is indicative of the morphine-like effect correlating to the activity of selective centrally acting analgesics. The test is also selective for peripherally acting analgesics indicative of non-steroidal anti-inflammatory drugs effect by inhibiting cyclooxygenase in peripheral tissues thereby interfering with the mechanism of transduction in primary afferent nociceptors (31).

The results of formalin-induced pain test are shown in Table 3. The extract showed significant ($p<0.05$) dose dependent analgesic activity (inhibition of pain in the formalin test) by causing inhibition of pain in both the neurogenic and aphasic phases of formalin test at 100, 200, and 400mg/kg to the order of 14.6 and 13.3%, 21.95 and 16% and 29.27 and 26.6% respectively.

Formalin test is biphasic, and measures pain of both neurogenic (first phase) and of inflammatory origin (second phase). Agents that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase. The ability of the extract to inhibit both phases of the formalin test indicates its involvement in both central and peripheral mediated activity, probably by prostaglandin synthesis inhibition as well as central inhibition mechanism. The first phase (0-10 minutes) is as a result of direct stimulation of nociceptors and measures centrally mediated effects and is insensitive to anti-inflammatory agents. The second phase (15-60 minutes) is dependent upon peripheral inflammation and changes in central procession due to chemical mediators released from damaged cells. This test, therefore, measures the response to a long lasting nociceptive stimulus similar to clinical pain and is recommended as a tool in basic pain research for studying the mechanisms of analgesic agents because of its connection to tissue injury (29).

In conclusion, *Flueggea virosa* root has a moderately reasonable yield in hot aqueous medium and possesses potent phytochemicals including tannins, saponins, terpenoids, steroids, cardiac glycosides and reducing sugars with some measure of acute safety and significant analgesic properties shown by the aqueous extract of the root of *Flueggea virosa*.

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