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Analgesic Activities and Phytochemistry of Aqueous Leaf Extract of *Greenwayodendron suaveolens* (Engl. and Diels) Verdc.

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ABSTRACT: This study examines the analgesic activities and phytochemical constituents of aqueous leaf extract of *Greenwayodendron suaveolens* (Engl. and Diels) Verdc. Analgesic activities were evaluated by means of acetic acid-induced writhing reflex and hot plate nociception models. The extract was also screened for presence of secondary metabolites. The plant extract caused 4.21%, 14.00%, 21.10% and 19.10% inhibition to acetic acid induced writhing in a graded dose dependent manner of 100, 200, 400 and 800 mg/kg respectively. It also protected the mice significantly ($P<0.05$) against thermal induced pain stimulus at a temperature of 55±1 ^{0}C. The activity of the extract appears to be mediated by both peripheral and, especially, central routes. Phytochemical screening revealed the presence of saponins, phenols and flavonoids, which may be responsible for the activities of the extract. The study validates the therapeutic use of the plant in the management of pain.

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Greenwayodendron (Annonaceae) is a monophyletic rainforest tree genus endemic to Tropical Africa (Piñeiro *et al*., 2016). Most taxonomic treatments recognise two species, namely, *G. oliveri* in Western Africa, and *G. suaveolens* in Central and Eastern Africa including Nigeria. There are recent evidences for the recognition of six distinct species (Lissambou *et al*., 2018). *Greenwayodendron suaveolens* (Engl. and Diels) Verdc is a deciduous medium-sized to a large tree, up to 35–45 m tall. Some of its vernacular names in Nigeria include 'ewai' (Edo), 'eleku' (Isekiri), 'osharo' (Urhobo) and 'agudugbu' (Yoruba) (Keay, 1989). The leaves vary in shape but usually have an elegant elongated leaf tip with a narrow elliptic shape and mostly glabrous.

Various uses have been reported on different parts of *G. suaveolens*. The wood is used for house construction, joinery, mine props, furniture and stakes for yam cultivation, rafters and shafts of spears. It is suitable for flooring, interior trim, railway sleepers, toys, agricultural implements, vats, draining boards, food containers, turnery, veneer and plywood. Root, leaves and barks of this plant are used in traditional medicine for the treatment of fever, oedema, swollen glands, headache, constipation, hernia, and facilitation of child birth, fertility, anthelmintic, aphrodisiac, stomach ache, rheumatism and pains (Tafokou, 2011). The study examines the analgesic activities and phytochemical properties of aqueous extract of the leaves of *G. suaveolens*.

MATERIALS AND METHODS

Collection and Preparation of Plant material: Fresh leaves of *G. suaveolens* were collected from matured stands located in the Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. The plant specimen was authenticated by Dr. H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, with the voucher number UBH_G347 deposited in the herbarium of the department. The leaves were air-dried before reducing to coarse powder with an electric milling machine. About 400 g of the powdered leaves were macerated in 5 L of boiled distilled water. After 24 hours, it was filtered through a clean, colourless mesh. The filtrate was concentrated over a water bath to obtain a semi-solid paste of *G. suaveolens* leaf extract (GSLE), which was collected in a clean container, sealed and stored in a refrigerator until further use.

Experimental Animals: Albino mice (16-29 g) of either sex were obtained from the Animal Unit of the Department of Biochemistry, University of Benin, Benin City, Nigeria.

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They were allowed 14 days of acclimatization, maintained under standard nutritional and environmental conditions of normal relative humidity, room temperature, and 12 hour light and 12 hour dark cycle. Standard food pellets and tap water were provided *ad libitum.* The feed was withdrawn only during the experimental hours. Experiments were conducted in accordance with the ethical guidelines of the Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

Acetic Acid-Induced Writhing Test: The analgesic effect of extract was evaluated by the acetic acid-induced mouse writhing test (Koster *et al*., 1959). Adult mice were allotted randomly to six groups of 5 mice each. The extract (100, 200, 400 and 800 mg/kg), acetyl salicylic acid (aspirin) (100 mg/kg) and distilled water (10 ml/kg) were administered orally to the respective groups of animals 30 minutes prior to intraperitoneal injection of acetic acid (10 ml/kg of 0.7% v/v). Immediately following acetic acid administration, each mouse was closely monitored for writhes within a 30 minutes period. The numbers of writhes were counted and expressed as a percentage inhibition of abdominal constrictions between the control and treated groups.

$$\mathrm{I}\ (\%) = \frac{\mathrm{WC} - \mathrm{WT}}{\mathrm{WC}} x\ 100$$

Where I = inhibition; WC = mean number of writes control; WT = mean number of writhes tested

Hot Plate Nociceptive Test: The method of Eddy and Leimbach (1953) was adapted. Fresh mice were divided into six groups of five (5) mice each. The negative and positive control groups received oral administration of 10 ml/kg distilled water and 100 mg/kg isobutylphenylpropionic (ibuprofen) respectively, while treatment groups received 100, 200, 400 and 800 mg/kg respectively of the plant extract. After 1 hour, animals were individually placed (for not more than 15 seconds, cut off time) on a hot plate maintained at a temperature of 55±1 ^{0}C. The time taken to flick the hind paw or lick or attempt jump from the hot plate was considered as the reaction time of a particular animal. The reaction time was recorded at 0, 30, 60, 90 and 120 minutes.

Phytochemical Screening: The extract was subjected to phytochemical screening for the identification of alkaloids (Dragendorff's, Mayer's, and Hager's tests), flavonoids (lead acetate, and alkaline reagent tests), saponins (frothing test) and phenols (ferric chloride test) according to Evans (2009).

Statistical Analysis: Data obtained from the study were subjected to statistical analysis using SPSS version 16.0 software. One way analysis of variance (ANOVA) was performed, while mean separation was by Duncan multiple range post hoc test. A value of P< 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Acetic Acid-Induced Writhing Reflex: Aqueous leaf extract of *G. suaveolens* at (100, 200, 400 and 800 mg/kg) reduced the mean number of writhes significantly (P< 0.05) compared to the negative control. The 400 mg/kg dose produced the highest (21.07%) inhibition index but did not compare with the activity of the standard (Table 1).

Table 1: Effect of aqueous leaf extract of *Greenwayodendron suaveolens* leaf on acetic acid-induced writhing reflex in mice.

Treatments	Doses (mg/kg)	Mean number of writhes ± SEM	Percentage inhibition
Distilled water	10 ml/kg	71.20±1.04^a	00.00
Aspirin	100	31.50±0.65^d	55.76
GSLE	100	68.20±1.20^b	04.21
GSLE	200	61.60±0.51^c	13.48
GSLE	400	56.20±1.78^c	21.07
GSLE	800	57.60±1.66^c	19.10

n =5; mean values with different superscripts within a column are significantly different (P< 0.05, Duncan multiple range post-hoc test); GSLE= *Greenwayodendron suaveolens* leaf extract

Acetic acid-induced writhing and the hot plate models are suitable for screening analgesic potentials of a substance (Bentley *et al*., 1983). Administration of acetic acid (i.p.) produces an abdominal writhing response due to sensitization of chemosensitive nociceptors by prostaglandins (Sutharson *et al*., 2007) as well as lipoxygenase products (Dhara *et al*., 2007) in the peritoneal fluid. Aqueous leaf extract of *G. suaveolens* significantly (P< 0.05) inhibited the numbers of writhes dose dependently in the present study. The highest inhibitory effect (21.07%) was conferred by the 400 mg/kg dose (Table 1). The activity of the extract was however not comparable to the standard drug, aspirin. Although, writhing response test is particularly sensitive for peripherally acting analgesics (Neves *et al*., 2007), it is also indicative for both central and peripheral acting analgesics (Trongsakul *et al.*, 2003). In the acetic acid-induced writhing test, the abdominal constriction is sensitive to drugs with analgesic activity similar to aspirin, antagonists of kinin receptors as well as the centrally and peripherally acting opioid analgesics (Barber *et al*., 1995).

Hot Plate Nociceptive Response: Figure 1 shows that GSLE significantly (P< 0.05) and dose dependently extended the latency time of mice to respond to thermal stimulus when compared with control. The

activities of the 200, 400 and 800 mg/kg dose were comparable to the standard at 60 minutes post-treatment, but only 200 mg/kg sustained the performance after 90 and 120 minutes.

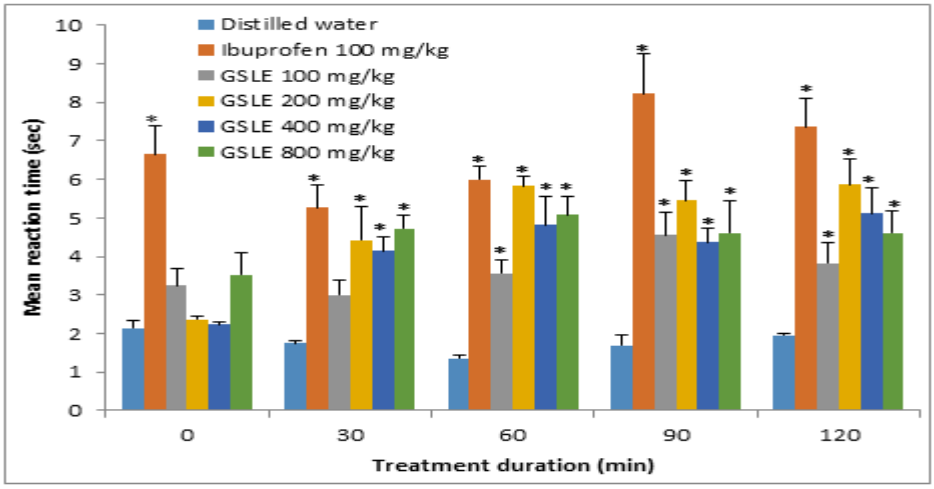

Fig 1: Effect of aqueous leaf extract of *Greenwayodendron suaveolens* (GSLE) on thermal pain latency in mice.
*P< 0.05 as compared to the negative control; n =5 for each group

Hot plate test is one of the most common tests of nociception that is based on a phasic stimulus of high intensity (Mandegary *et al*., 2004). Thermal-induced pain model is suitable for centrally mediated nociception (Parkhouse and Pleuvry, 1979). The present study revealed that the extract significantly (P< 0.05) increased the latency period of response to thermal pain stimulus in the experimental mice in a dose dependent manner (Figure 1). However, unlike the standard drug, the extract did not provide any effective protection within the earliest phase (0 minute) of the test. Similarly, the lowest test dose (100 mg/kg) was not active until the 3rd phase (60 minutes). It was the 200 mg/kg dose that maintained the most effective protection, comparable with the standard drug across the 3rd and 5th (60 and 120 minutes) phase of the test, while 400 and 800 mg/kg doses compared with the standard at the 3rd phase. The significant activities recorded in both models suggests that the extract may be acting either on visceral receptors (peripherally mediated action), or inhibition at the central level of transmission of painful messages.

Preliminary Phytochemistry: Saponins, phenols and flavonoids are present in the aqueous leaf extract of *G. suaveolens*, but alkaloid was not detected (Table 2).

Table 2: Phytochemical constituents of aqueous *Greenwayodendron suaveolens* leaf extract.

Test	Observation
Saponins	+
Alkaloids	-
Phenols	+
Flavonoids	+

+ = present; - = not detected

The vast and versatile pharmacological effects of medicinal plants are basically dependent on their phytochemical constituents. Some saponins as well as flavonoids have analgesic properties (Hussein; El-Anssary 2018). The analgesic activity of *Capsicum* spp. is due to the presence of capsaicinoids, which are simple phenolic compounds (Spiller *et al*., 2008). Similarly, the anti-inflammatory analgesic activity of *Filipendula ulmaria* was attributed to the action of simple phenolics (Hoffmann, 2003). The present study revealed the presence of saponins, flavonoids and phenols in the extract of *G. suaveolens* (Table 2). These phytochemicals may be acting synergistically, or singly to produce the analgesic activity recorded in this study. However, the method employed here is qualitative and therefore the results obtained presently are preliminary. Further phytochemical studies are required to isolate the individual secondary metabolites present in the plant extract.

Conclusion: The aqueous leaf extract of *G. suaveolens* possesses significant analgesic activities, which may explain its use in the traditional management of pain. The secondary metabolites present in the extract could be responsible for the activity.

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