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Pharmacognostic profiles, evaluation of analgesic, anti-inflammatory and anticonvulsant activities of *Newbouldia laevis* (P. Beauv.) Seem. ex Bureau leaf and root extracts in Wistar rats

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ABSTRACT

Ethnopharmacological relevance: Newbouldia laevis is a popular medicinal plant whose leaves and roots are used in Nigeria as ethnomedicinal prescriptions for pain, inflammation, convulsion, and epilepsy. These claims have not been scientifically verified prior to this study.

Aim of the study: To determine pharmacognostic profiles of the leaves and roots and evaluate the analgesic, anti-inflammatory, and anticonvulsant activities of methanol leaf and root extracts in Wistar rats.

Material and methods: The pharmacognostic profiles of the leaves and roots were determined using standard procedures to serve as fingerprints for the plant. The methanol leaf and root extracts of *Newbouldia laevis* were tested for acute toxicity using the OECD's up and down method at the maximum dose of 2000 mg/kg (orally) in Wistar rats. Analgesic studies were carried out in acetic acid-induced writhing in rats and tail immersion. The anti-inflammatory activity of the extracts was evaluated using carrageenan-induced rat paw-oedema and formalin-induced inflammation in rats' mode. The anticonvulsant activity was determined using strychnine-induced, pentylenetetrazol-induced, and maximal electroshock-induced rat convulsion models. For each of these studies, the extracts doses of 100, 200 and 400 mg/kg were administered to the rats following the oral route.

Results: The pharmacognostic profiles showed that the leaves possessed deep-sunken paracytic stomata (5-**8**-16 mm^2; adaxial, 8-**11**-24 mm^2; abaxial epidermis), vein islets (2-**4**-10 mm^2; adaxial), vein terminations (10-**14**-18 mm^2; adaxial), palisade ratio (8.3-**12.5**-16.4 mm^2; adaxial, 2.5-**6.8**-12.2 mm^2; adaxial), covering unicellular trichome (8–14; adaxial), spheroidal calcium oxalate crystals (3–5 μm), and oval-shaped striated starch grain with no hilum (0.5–4.3 μm). The transverse section of the leaf showed the presence of spongy and palisade parenchyma as well as a closed vascular bundle. The root powder showed the presence of brachy sclereid, fibers without lumen, and lignin. All physicochemical parameters fall within the acceptable limits, phytochemical contents showed mainly glycosides, alkaloids, and steroids while acute oral toxicity (LD_{50}) of the parts for 14 days did not produce any toxicity signs or mortality in the rats. The extracts produced dose-dependent (100–400 mg/kg) analgesic involving opioid receptors, anti-inflammatory, and anticonvulsant activities in the rats which were significant ($p \leq 0.05$) when compared to the standard drugs. The leaf extract possessed the most potent analgesic and anti-inflammatory effects in the rats, while the most anticonvulsant effects were observed in rats

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treated with the leaf extract. Both extracts showed elevated levels of protection against strychnine-induced, pentylenetetrazol-induced, and maximal electroshock-induced seizure in rats.

Conclusion: Our study revealed some pharmacognostic profiles of *Newbouldia laevis* leaves and roots that are vital for its identification from closely related species often used for adulteration in traditional medicine. The study further showed that the leaf and root extracts of the plant possessed dose-dependent analgesics, anti-inflammatory and anti-convulsant activities in rats, thus, justifying its use for the treatment of these diseases in Nigerian traditional medicine. There is a need to further study its mechanisms of action towards drug discovery.

Abbreviations

LD_{50}	Median lethal dose
NSAIDs	Non-steroidal anti-inflammatory drugs
SEM	Scanning electron microscope
TLC	Thin layer chromatography
GC	Gas chromatography
MSD	Mass spectrum detector
NMR	Nuclear magnetic resonance
FTIR	Fourier transform infra-red
CNS	Central nervous system
GABA	Gamma-aminobutyric acid
i.p.	Intraperitoneal
b.w.	Body weight
ANOVA	Analysis of variance
SPSS	Statistical package for social sciences

1. Introduction

Medical plants are now been to considered as important sources for managing, treating and preventing different types of diseases (Yadav Abhishek and Samanta Krishanu, 2021). All plants contain important phytochemicals that can be used as drug agents that act as lead compounds in the development of various types of orthodox medicines (Jones et al., 2006). Nowadays, many less developed or developed countries of the world are patronizing medicinal plants or their products for some important aspects of human healthcare such as treatment of diseases like malaria, cancer, inflammation, pains, ulcer, diabetes, infections, etc. These medicinal plants include garlic, gingko, aloe, graviola, ginseng and *Catharanthus*, among others (Yadav Abhishek and Samanta Krishanu, 2021). Ethnomedicinal uses of these medicinal plants for decades have shown some prospects in future due to wide distribution of over 5000 species of plants throughout the world occurring in both terrestrial and aquatic habitats. Most of these plants have not been evaluated ethnopharmacogically and their biological activities could be useful in the treatment of some difficult or incurable diseases in the future (Yadav and Samanta, 2021).

Currently, ethnomedicinal uses of plants have been improved upon by various research in an aspect of pharmacy termed pharmacognosy (Alamgir, 2017; Jones et al., 2006). Pharmacognosy has been defined as the scientific study of drug of natural origin as well as their ethnobotany, methods of collection, modes of preparation, standardization techniques, ethnopharmacology, cultivation, conservation, and commerce. Pharmacognosy as an aspect of natural science consist of various disciplines such as phytochemistry, ethnopharmacology, economic botany, toxicology, systematics, ethnobotany, taxonomy, microbiology, pharmaceutics, and biotechnology. Since about 80% of the rural population in the world depends of drugs from natural sources for their primary healthcare, pharmacognostic profiles of these drugs are of utmost important for quality control and standardization of herbal medicines (Jones et al., 2006). Evaluation of the pharmacognostic profiles of herbal drugs will greatly help to ensure the efficacy, safety and quality of the medicinal plants' products (Lalthanpuii and Lalchhandama, 2020; Ukwubile et al., 2019; Zhang et al., 2019).

Presently, several important orthodox medicines such as artemisinin, vincristine, vinblastine, quinine, morphine, galanthamine, atropine, among others were derived from bioactive compounds isolated from medicinal plants for the treatment of vasrious diseases pains, inflammations, convulsions, cancer, malaria, etc. (Alamgir, 2017; Jones et al., 2006; Mohammed Golam Rasul., 2018; Nonglang et al., 2022; Prior et al., 2005). For instance, ailment such as pain is a sign in within the CNS indicating certain abnormality in the body. It is often characterized by symptoms like ache, tingle, burn, or prick which may be short-lived or lasting. In most cases, pain can be a sign for underlying disease or certain pathological condition of an organ (Pandey et al., 2020). Currently, treatment of pains is done using various types of analgesics or NSAIDs with some levels of adverse effects in the body. Because of these undesirable side effects resulting from the intake of these pain-killers, research into the treatment of pains (acute or chronic) using medicinal plants have gained much attention due to the numerous advantages of drugs from plants over conventional medicines (Alam et al., 2020). The use of plants to treat pains have successfully yielded the desired results especially on chronic pains experienced by older adults (Sharma et al., 2020).

Similarly, inflammation is an immunological response by the body due to invasion of microorganisms like viruses and bacteria often resulting in swelling, redness, heat, and pain in the affected part of the body. It may be acute or chronic inflammation condition which is associated with diseases like asthma, cancer, diabetes, Alzheimer's disease, and heart problems (Lee et al., 2019; Nainwani et al., n.d.). Treatment of inflammations is usually done using NSAIDs which have been reported to have caused several side effects in the body. Many plant extracts have been successfully used in traditional medicine for treating inflammations, examples include *Vernonia puaciflora*, *Bidens pilosa*, *Spondias venulosa*, etc., (U. C. Anes, 2015; Mohammed Golam Rasul., 2018; Pandey et al., 2020).

On the other hand, disease such as convulsion occurs because of involuntary contraction of muscles especially in children, although it occurs also in adults. It is very common in epileptic seizure and can also occur due to high fevers, brain traumas and infections (Dighe and Barve, 2019). Convulsion can be localized in a particular part of the body or the whole body resulting in seizures, though not all seizures cause convulsion (Akhigbemen et al., 2019). Factors such as sudden rise in fever, certain epileptic seizure, hypoglycemia, and tetanus are often tagged symptoms of convulsion. Many plants have been researched upon to be very effective in the treatment convulsion in various parts of the world. Some of these plants include thyme, cannabis, cloves, ginger, turmeric, tetrapleura, flowers of *Newbouldia laevis* and cowhage. These plants many bioactive compounds that were potent of convulsion and epileptic seizures (Akhigbemen et al., 2019; Dighe and Barve, 2019; Olatokunboh et al., 2009; Shelar et al., 2018; Shinde et al., 2018; Yeddes et al., 2022).

The plant *Newbouldia laevis* is a rapid-growing tropical tree of 3–8 m high in western and about 20 m high in eastern and northern Nigeria belonging to the Family Bignoniaceae. It commonly called African hyssop, '*Ogirishi*'' in Igbo, '*Àdùrúúkù*'' in Hausa, and ''*Akoko*'' in Yoruba (Nigeria). The plant is widely distributed in Nigeria, Senegal, Ghana, DR Congo, Sierra Leon, Benin republic and South American countries like Brazil. All the parts of the plant have been used to treat various diseases

in traditional medicine such as malaria (stembarks), diabetes, pile (stembarks), epilepsy (flowers), and juice (Ndidi et al., 2020). In Nigeria, the leaf, stembark, and root decoctions are used as analgesic, anti-inflammation and anticonvulsant or epileptic agent in traditional medicine (Iwu, 2014). Although, some of these claims on the uses have not been verified scientifically, yet, the use of these plant parts as ethnomedicinal prescription for pain, inflammation (reducing swellings) and convulsion (or epilepsy) have gained more acceptance in most rural communities in South east Nigeria (Iwu, 2014). The bole is cylindrical up to 90 cm in diameter, often used for marking boundaries and fences in Nigeria, besides its ethnomedicinal uses. The juice from its flowers contain significant amount of sugar, and has become very attractive to children who lick the flowers (Iwu, 2014; Pages et al., 2013). The leaves of the plant contain tannins, alkaloids, saponins, cardiac glycosides, flavonoids and anthraquinones (Dermane et al., 2020).

Therefore, this present study was carried out to determine some important pharmacognostic profiles of leaves and roots of *Newbouldia laevis* with a view to identifying possible adulteration from closely related species, and evaluate the analgesic, anti-inflammatory and anticonvulsant activities of methanol leaf and root extracts induced Wistar rat models.

2. Materials and methods

2.1. Collection, identification, and authentication of plant material

Fresh leaves and roots of *Newbouldia laevis* was collected in early morning hours from a forest in Nsukka, Enugu State, Nigeria. The identification and authentication of the plant was done at the herbarium unit of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Nigeria, where a voucher specimen number UMM/FPH/BIN/001 was deposited for the plant at the herbarium unit.

2.2. Preparation of plant extracts

The collected fresh leaves and roots of *N. laevis* were carefully rinsed in water to remove unwanted debris and dirt. They were shade-dried for two weeks in free air. The shade-dried plant materials were then ground into fine powders using electrical blender (model: BLG 1500 PRO, Binatone, Nigeria) and weighed. Powdered samples of leaves and roots weighing 1000 g each were extracted with 100% methanol (Sigma Aldrich, St Louis, Mo, US) using cold maceration technique for 72 h. The extract was each evaporated to dryness in a rotary evaporator (Buch, UK) to obtain greenish and brownish jelly-like extracts of leaves (16.4% w/w yield) and roots (8.4% w/w yield) respectively. The extracts were weighed and stored in a refrigerator at 10 °C for further use.

2.3. Pharmacognostic studies on N. laevis leaf and root powders

To standardize the leaves and roots of the plant, we carried out various aspects of pharmacognostic studies to serve as profiles (fingerprint) for proper identification of the plant.

2.3.1. Quantitative microscopy of leaves and roots

Some important qualitative microscopic features of the leaf such as stomatal number, stomatal type, stomatal index, vein islet number, vein termination number, palisade ratio, starch grains, trichomes, calcium oxalates, and transverse section as well as other profiles of leaves and roots were determined following previously described procedures (Dodiya and Jain, 2017). The nature of stomata of the leaf was observed using Phenom desktop SEM (ThermoFisher Scientific, MA, USA).

2.3.2. Physicochemical evaluation of powdered leaves and roots

The physicochemical parameter of leaves and roots were determined using the procedures previously described (C. Anes et al., 2023; Dodiya and Jain, 2017), to evaluate parameters such as moisture contents, ash value, extractive values, etc.

2.3.3. Phytochemical analysis of extracts

Phytochemical contents of methanol leaf and roots extracts were evaluated using the standard methods to test for the presence of certain metabolites (Bijauliya et al., 2021; Kumar et al., 2015; Olaleye et al., 2021; Ukwubile, 2017).

2.3.4. Isolation and characterization of bioactive compounds from extracts

The crude methanol leaf and root extracts were separately subjected to silica gel column chromatography using gradient elution technique. A total of twenty fractions were collected from each extract and grouped into four groups A to D based on their profiles on TLC plates (Gel 60 F254 Merck, Chemtech Intl. Gujarat India). Fractions with most potent activity were purified using short column chromatography, purity was confirmed by single TLC spot (Eiceman et al., 1994; Kim et al., 1998). Characterization of bioactive compounds was done using an Agilent technologies GC (7890A) coupled to a MSD (5975C) with optimal operating conditions (Alam et al., 2020). Compounds were compared with those in the NIST library (Ukwubile, Ahmed, Katsayal, Ya'u et al., 2019).

2.4. Experimental animals

Adult Wistar rats of opposite sexes numbering one hundred and twenty (120) and weighing 100–150 g were purchased from PJ Rats Farm Ltd, Jos, Nigeria. The animals were acclimatized in the laboratory at 25 ± 4 °C, 10% humidity, 12 h light and dark light cycles for 7 days with free access to water and food. Approval for the use of the was obtained from the Animal in research ethical group of PJ Rats Farm Ltd with approval number: PJF/NG/JOS-035-2023.

2.5. Acute oral toxicity study

Five (5) Wistar rats of both sexes weighing between 100 and 120 g were administered maximum dose 2000 mg/kg extracts and monitored for signs of toxicity especially within the first 4 h. The rats were thereafter observed for 14 days for behavioral changes like redness of eyes, itching or mortality (Khalifa, 2022).

2.6. Analgesic activities of extracts

2.6.1. Acetic acid-induced writhing in rats

The analgesic activity of *N. laevis* leaf (NLE) and root extracts (NRE) were evaluated using acetic acid-induced writhing test in rats. Briefly, rats of opposite sexes weighing 100–150 g were grouped into five groups of rats per group. Group I (negative control) was administered 10 mL/kg distilled water, group II (positive control) was administered 100 mg/kg standard drug diclofenac sodium (Hovid Div. Phamatex Nig. Ltd), while groups III, IV and V were administered 100, 200, and 400 mg/kg extract doses of leaf and root intraperitoneal (i.p.). After 30 min, 0.5% acetic acid (10 mL/kg b.w.) was injected into the rats (i.p.). Five minutes after, the number of abdominal writhing was observed using magnifying handheld lens for 30 min in triplicate. The percentage inhibition of writhing was calculated from the formula below (Alam et al., 2020).

$$\% \text{ inhibition} = (\text{Nc-Nt/Nc})\ 100 \quad \text{(i)}$$

Where, Nc represents mean number of writhing in control group, and Nt is mean number of writhing in treatment groups.

2.6.2. Tail immersion test in rats

The rats were grouped into five groups of five rats per group. Food and water were withdrawn from the animals 2 h prior to the commencement of the experiment. Group I (negative control) received 10 mL distilled water, group II (positive control) received 50 mg/kg

pentazocine (Biopharma Nig. Ltd), while groups III, IV and V received extracts dose of 100–400 mg/kg (i.p.). After 1 h, 3 cm of the tail in each rat was immersed in water bath (Isotemp GPD 10, Fisher Scientific) containing hot water (50.5 °C). The reaction time was taken as time taken by each animal to withdraw or flick its tail from the hot water. A 20 s cut-off or no response time was noted, and reaction time was measured in 0, 15, 30, 45 and 60 min (Sharma et al., 2020).

2.6.3. Investigation of opioid receptors involvement in analgesic activity of extracts

The involvement of opioid receptors in analgesic activity of *N. laevis* leaf and root extracts was determined using a nonselective opioid receptor antagonist naloxone (Alpha Pharmacy & store Ltd). Briefly, Wistar rats weighing 100–130 g were randomly grouped into five groups of five rats. Group I received 10 mL/kg distilled water, group II received 2 mg/kg naloxone, group III received 50 mg/kg pentazocine, groups IV received 400 mg/kg extracts doses while groups V and VI received 400 mg/kg plus naloxone and 25 mg/kg pentazocine plus naloxone (i.p.). Fifteen (15 min) prior to administration of the samples to rats groups V and VI, naloxone was given to the animals (Hijazi et al., 2017).

2.7. Evaluation of anti-inflammatory activity of extracts

2.7.1. Carrageenan-induced paw-oedema test

To evaluate the anti-inflammatory activity of the extracts carrageenan-induced paw oedema rat model was used following previously described protocol (Pandey et al., 2020; Sharma et al., 2020). Briefly, twenty-five (25) Wistar rats grouped into five groups of five rats per group. Group I received 10 mL/kg distilled water, group II received 100 mg/kg standard drug diclofenac sodium (Phamatex Nigeria Ltd), group III, IV and V were administered 100, 200 and 400 mg/kg extract doses respectively. After 1 h, carrageenan (0.1% w/v dissolved in 0.1% normal saline) was given to the rats' sub-plantar area of the paw in right hind limb. The volume of paw oedema was measured at 0, 1, 3, and 5 h using plethysmometer (model: 37140, Ugo Basile). The percentage inhibition of inflammation was then calculated from the formula shown below:

$$\% \text{ Inhibition of oedema} = (PVc - PVt/PVc)100 \quad \text{(ii)}$$

Where, PVc denotes paw volume of control, and PVt denotes paw volume of treated.

2.7.2. Formalin-induced inflammation in rats

Twenty-five (25) rats were grouped as in carrageenan-induced paw oedema test above except that trats were induced using formalin following the method previously described (Sharma et al., 2020).

2.8. Evaluation of anticonvulsant activities of extracts

2.8.1. Strychnine-induced rat convulsion

Twenty-five Wistar rats of opposite sex were randomly grouped into five groups of five. Group I received 10 mL/kg distilled water, group II 4 mg/kg diazepam (Michelle Labs. healthcare), while groups I III, IVand V received extract doses 100, 200 and 400 mg/kg (i.p.). After 1 h, 2 mg/kg strychnine (Bio-Techne Ltd, UK) was injected (i.p.) into the rats. The rats were then observed for 30 min for the onset of convulsion or death (Mante et al., 2013).

2.8.2. Pentylenetetrazole-induced rat convulsion

Twenty-five Wistar rats were grouped as previously described (Mante et al., 2013). After 1 h of administering (i.p.) the test samples, 80 mg/kg pentylenetetrazole (Sigma-Aldrich, St Louis Mo, USA) was given to the animals. Observation for the onset of tonic-clonic convulsion or deaths of rats was made for 30 min period (Obese et al., 2021).

2.8.3. Maximal electroshock-induced rat convulsion

Rats were randomly grouped into five of rats per group following previously described protocol (Mante et al., 2013). Group I received 10 mL/kg distilled water, group II received 20 mg/kg phenobarbital (Emzor Pharm. Industries Ltd, Nig.), groups III, IV and V received extract doses 100, 200, and 400 mg/kg (i.p). After 1 h, the rats were exposed to electroshock at 150 mA, 0.2s using a pair of ear clip electrodes (Ugo Basile). The commencement of tonic-hind limb extension and its protection was recorded (Dighe and Barve, 2019).

2.9. Statistical analysis

Data were expressed as mean ± SD (n = 5). Significant difference between the control and treatment groups were considered at $p \leq 0.05$ using one-way ANOVA followed by Dunnett's post-hoc test. Statistical analysis was done using SPSS statistical software version 22.

3. Results

3.1. Evaluation of pharmacognostic parameters

3.1.1. Quantitative microscopy and histological features of leaf and root

Quantitative microscopic examination of *N. laevis* fresh leaf showed it contained a paracytic type of stomata that are numerous on the lower surface than the upper surface. Similarly, few numbers of glandular unicellular trichomes were observed on the upper surface. There are no epidermal cells resembling each other in the leaf epidermis (Table 1; Fig. 1).

The transverse section (TS) of the leaf showed it contain two parenchymatous cells (palisade and spongy parenchyma), closed vascular bundles without cambium, and moderately thick cuticle (Fig. 2a). In the root powder, there are lignin substance, prismatic calcium oxalate crystals, fiber sclereids with narrow lumen, and concentric vascular bundles, while the TS showed a wide pith and closed vascular bundle (Fig. 2b).

3.1.2. Physicochemical evaluations of leaf and root powders

The study showed that moisture content for leaf powder was 6.52 ± 0.01% w/w and 8.04 ± 0.10% w/w for root powder. The ash value for the leaf powder was the least with value of 4.24 ± 0.01% w/w while the root had the highest value of 12.06 ± 1.02% w/w. Furthermore, water extractive value of the leaf powder was the highest with the value of 10.28 ± 1.02% w/w while, the root powder had 6.20 ± 0.01% w/w. From the results, other parameters fall within the acceptable ranges (Table 2).

3.1.3. Phytochemical contents of leaf and root extracts

The phytochemical contents showed the presence of more metabolites in the leaf extract than the root. The results revealed the presence of tannins, flavonoids, alkaloids, triterpenes, saponins, and carbohydrates in the leaf extract while the root extract indicates the presence of flavonoids, alkaloids, cardiac glycosides, triterpenes and saponins

Table 1
Leaf surface data of *N. laevis* as determined using the microscope (40x).

Leaf Parameter	Quantity/mm^2	
	Upper surface	Lower surface
Stomatal number	8-**6.2**-14	12-**10**-22
Stomal index	12.14%	18.22%
Vein terminations number	10-**8.5**-16	4-**3.8**-10
Vein islets number	6-**5.1**-12	5-**3.4**-8
Palisade ratio	14.22	Na
Trichomes	5-**4.5**-7	Na

Numbers in bold are mean of repeated counts (n = 5), Na (not applicable). Glandular unicellular trichomes were observed on the upper surface of leaf.

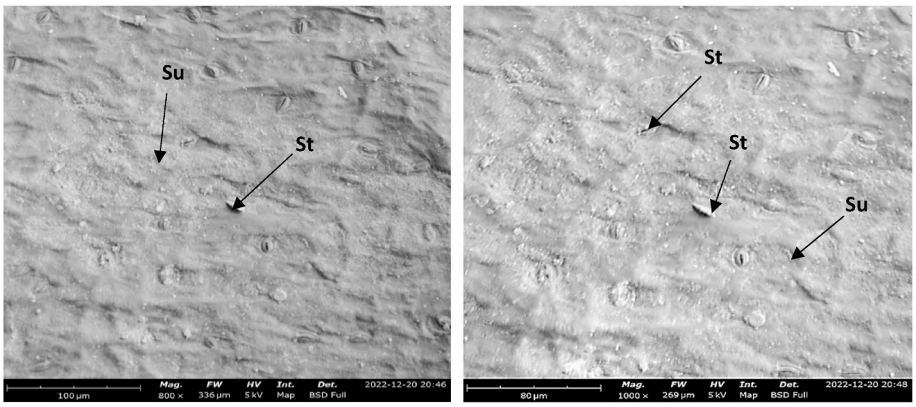


Fig. 1. Scanning electron microscopy (SEM) of *N. laevis* leaf (a) Upper surface (b) lower surface; 800x. St; stomata, Su; subsidiary cells.

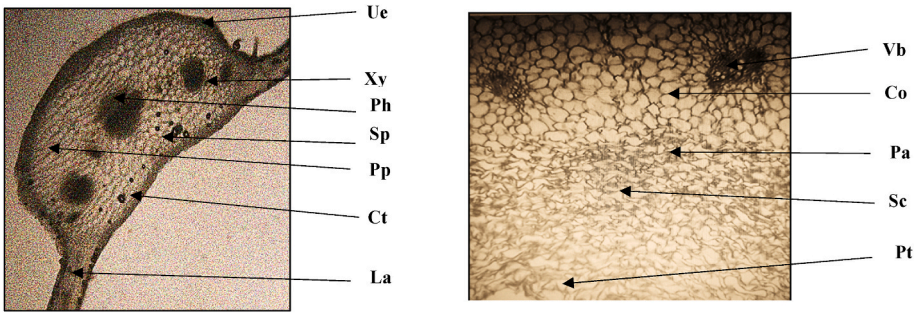


Fig. 2. The TS leaf and root of *N. laevis* showing various anatomical features. Ue; upper epidermis, Xy; xylem tissue, Ph; phloem tissue, Sp; spongy parenchyma, Pp; palisade parenchyma, Ct; cuticle, La; leaf lamina, Vb; vascular bundles, Co; collenchyma, Pa; parenchyma, Sc; sclerenchyma (with sclereid), Pt; pith, 40x.

Table 2
Physicochemical parameters of *Newbouldia laevis* leaf and root.

Parameter (% w/w)	Value (mean ± SD)	
	Leaf	Root
Moisture contents	6.52 ± 0.01	8.04 ± 1.10
Ash value	4.24 ± 1.01	12.06 ± 1.02
Total ash	8.58 ± 1.11	14.10 ± 1.04
Water soluble ash	2.02 ± 0.01	2.88 ± 0.01
Alcohol insoluble ash	0.80 ± 0.01	1.64 ± 0.02
Water extractive	10.28 ± 1.02	6.20 ± 0.01
Alcohol extractive	4.01 ± 0.01	8.44 ± 1.04
Dry matter	12.40 ± 2.02	10.14 ± 1.02

Results are mean ± SD (n = 3).

Table 3
Phytochemical contents of *Newbouldia laevis* leaf and root extract.

Constituents	Leaf	Root
Tannins	+	–
Flavonoids	+	+
Anthocyanins	–	–
Cyanogenic glycosides	–	–
Alkaloids	+	+
Cardiac glycosides	–	+
Triterpenes	+	+
Saponins	+	+
Carbohydrates	+	–

Note: + (detected), - (not detected).

(Table 3).

3.1.4. GC-MS profiles of leaf and root extracts

The GC-MS analysis of the isolated bioactive compounds from the leaf and root extracts showed the presence of mainly cyclic and aromatic compounds as well as unsaturated fatty acid esters. Similarly, some alkaloidal compounds were also revealed in the leaf such as pyridin-3-yl-ethanimidamide (m/z: 135.11 g/mol) and 2-N-butylacrolein (m/z:112.17 g/mol), while in the root extract only one alkaloidal compound 2-furanmethanamine (m/z: 97.12) was isolated (Tables 4 and 5).

3.1.5. Acute oral toxicity of leaf and root extracts

The study showed that at maximum dose of 2000 mg/kg body weight (b.w.) administered to the animals (oral), there were no signs of toxicity after two weeks of repeated administration of extracts. The LD_{50} was

Table 4
Isolated compounds from *N. laevis* leaf extracts analyzed by the GC-MS.

Compounds	Peak area (%)	Retention time (min)	m/z (g/mol)
5-Methyl-1-heptanol	3.19	5.56	129.15
2-Octanol-S-ester	4.98	8.99	130.02
Pyridin-3-yl-ethanimidamide	7.66	12.18	135.11
2-Ethylformanilide	3.61	13.92	149.23
Benzonitrile, 2-fluoro	4.35	14.05	121.04
N-Benzyl formamide	9.02	18.27	117.10
1,3-Benzoxazol-4-ol	12.98	17.85	135.12
N-Tridecan-1-ol	11.27	20.96	200.36
Melibiose	8.45	15.09	342.30
2-N-butylacrolein	6.06	25.68	112.17

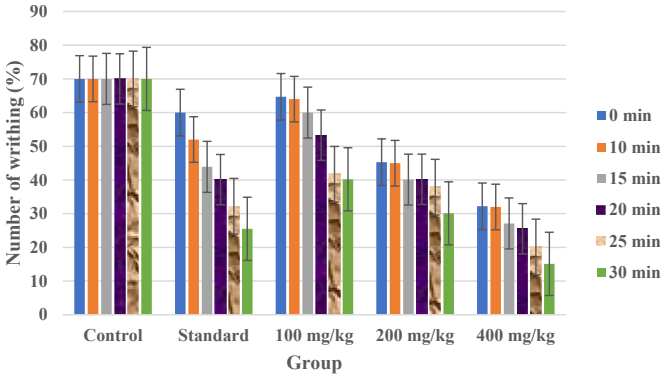
Table 5
Isolated compounds from *N. laevis* root extracts analyzed by the GC-MS.

Compounds	Peak area (%)	Retention time (min)	m/z (g/mol)
Benzoic acid, ethyl ester	55.53	5.22	150.10
1-Hexene, 3,5-dimethyl	1.67	8.96	112.21
2-Octanol, (S)-ester	4.98	8.99	130.02
1,6-Octadiene, 3,7-dimethyl-(S)	12.97	15.77	138.25
2-Furanmethanamine	2.79	16.14	97.12
1,10-Decanediol	4.47	16.42	174.28
Hydrazine, 1,2-dimethyl	4.44	17.83	60.09
3-Aminocrotononitrile	2.26	20.38	82.10
Fomepizole	11.49	34.94	82.11
2,6-Nonadienal, (E,E)-	1.86	15.06	138.21

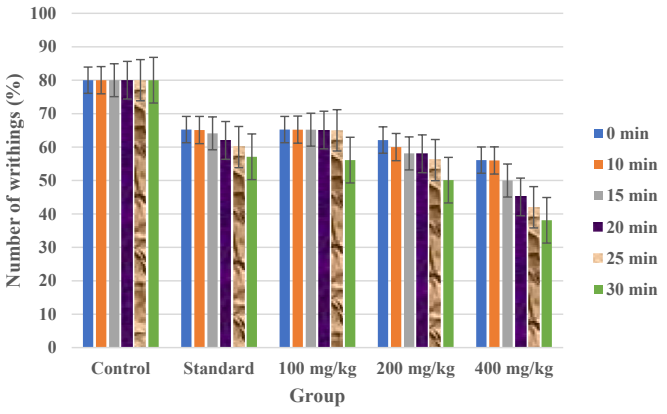
Table 6
Acute oral toxicity of *N. laevis* methanol leaf and root extract in rats.

Group (mg/kg)	Animal died/Animal survived	
	Leaf extract	Root extract
I (2000)	0/1	0/1
II (2000)	0/1	0/1
III (2000)	0/1	0/1
IV (2000)	0/1	0/1
V (2000)	0/1	0/1

LD_{50} > 2000 mg/kg b.w.

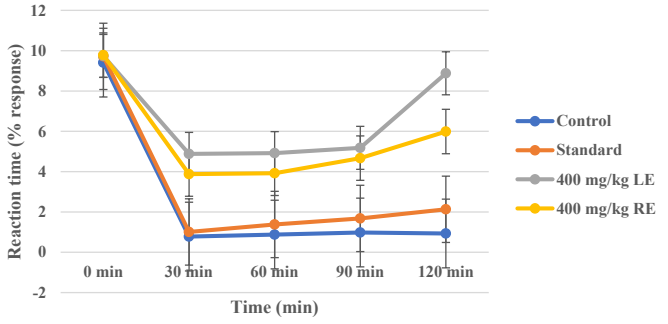


(a)

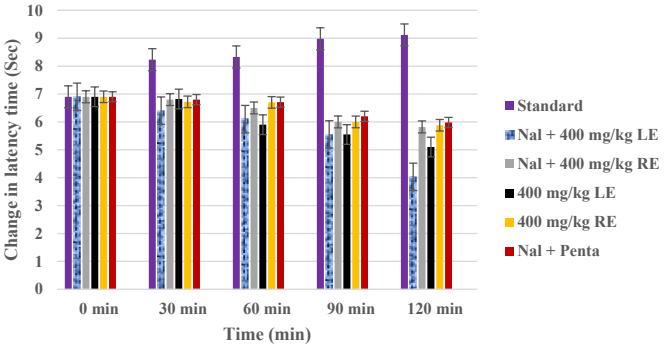


(b)

Fig. 3. Effects of *N. laevis* (a: leaf) and (b: root) extracts on abdominal writhing in rats. Results are mean ± SD (n = 5), $p \leq 0.05$ (one-way ANOVA followed by Duncan's multiple range test).



(a)



(b)

Fig. 4. Effects of *N. laevis* extracts on reaction time (a) and change in latency time in induced rats. Results are mean ± SD (n = 5), 400 mg/kg LE: leaf extract, 400 mg/kg RE: root extract, Nal: naloxone, Penta: pentazocine. There was statistical significance difference when compared to control ($p \leq 0.05$; one-way ANOVA followed by Dunnett's post-hoc test). The standard drug used was pentazocine.

estimated to be greater than 2000 mg/kg b.w. leaf and root extract (Table 6).

3.2. Evaluation of analgesic effects of extracts

3.2.1. Acetic acid-induced writhing test

The results from acetic acid-induced writhing in rats revealed that *N. laevis* extracts displayed dose-dependent reductions in number of abdominal writhing in rats. These decreases were witnessed more in the leaf extract at 400 mg/kg dose in 30 min duration than the root extract. These results were significance ($p \leq 0.05$; one-way ANOVA) when compared to the control groups (Fig. 3 a and b).

3.2.2. Effect of N. laevis extracts on rats' tail flick responses

From the results obtained, there was significant decrease in reaction time of responses by the animals after administration of 400 mg/kg dose of leaf and root extracts orally. These results were statistical significance ($p \leq 0.05$) when compared to standard drug pentazocine (Fig. 4 a and b).

3.3. Evaluation of anti-inflammatory effects of N. laevis extracts

3.3.1. Carrageenan-induced rat paw oedema

The results showed that carrageenan-induced paw oedema decrease paw diameter of rats in dose-dependent fashion. However, the leaf extract showed greater potency on paw oedema volume reduction than the root extract in both carrageenan and formalin-induced paw oedema experiments (Fig. 5 a and b). These results were also signficant when compared to control ($p \leq 0.05$).

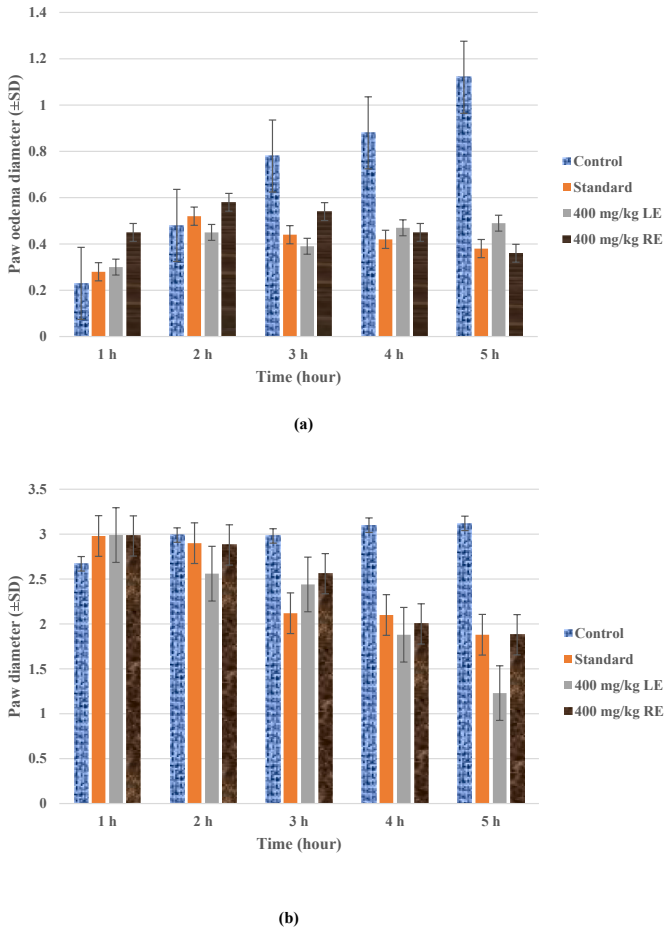


Fig. 5. Effects of *N. laevis* extracts on: (a) carrageenan and (b) formalin-induced rat paw oedema. Results are mean ± SD (n = 5). Statistical significance difference between control and treated was determined at $p \leq 0.05$ (one-way ANOVA followed Duncan's multiple range test).

Table 7
Anticonvulsant effects of *N. laevis* extracts on strychnine-induced seizure in rats.

Group dose	Time to seizure onset (s)	Latency of deaths (s)	% protection
10 mL distilled water	60.23 ± 2.11	82.14 ± 4.22	0
2 mg/kg diazepam	no seizures	no seizures	100
100 mg/kg LE	102.10 ± 2.02	128.02 ± 2.01	40
200 mg/kg LE	116.14 ± 4.012*	132.24 ± 4.02*	60
400 mg/kg LE	no seizures	no seizures	100
100 mg/kg RE	65.18 ± 1.01	76.12 ± 1.01	0
200 mg/kg RE	80.34 ± 2.04*	102.22 ± 4.02*	60
400 mg/kg RE	no seizures	no seizures	100

Results are mean ± SD (n = 5),*statistical significance at $p \leq 0.05$ (one-way ANOVA followed Dunnette's post-hoc), LE: leaf extract, RE: root extract.

3.4. Evaluation of anti-convulsant effects of N. laevis extracts

3.4.1. Strychnine-induced convulsion in rats

The results showed that at lower dose of extract administration to the rats 40% protection was offered to the animals by leaf extract while the root extract does not offer any protection. More protection from seizure was offered at 400 mg/kg dose of leaf and root extracts. These results were significantly different ($p \leq 0.05$) when compared to the control groups (Table 7).

Table 8
Anticonvulsant effects of *N. laevis* extracts on pentylenetetrazole (PTZ)-induced seizure in rats.

Group dose	Latency (min)	Recovery time (min)	Death/total (% mortality)
10 mL distilled water	0.82 ± 0.01	82.14 ± 4.22	4/5 (80; protection:20%)
2 mg/kg diazepam	44.11 ± 2.14*	1.64 ± 0.02*	0/5 (0; protection: 100%)
100 mg/kg LE	52.08 ± 1.15	8.02 ± 0.01	0/5 (0; protection:100%)
200 mg/kg LE	60.02 ± 2.01*	12.08 ± 1.01*	3/5 (60; protection 40%)
400 mg/kg LE	25.22 ± 2.01*	10.01 ± 0.01*	0/5 (0; protection:100%)
100 mg/kg RE	33.08 ± 2.02	23.00 ± 2.01	0/5 (0; protection:100%)
200 mg/kg RE	38.64 ± 3.01*	16.24 ± 1.01*	4/5 (80; protection:20%)
400 mg/kg RE	30.12 ± 2.01*	12.02 ± 1.01*	0/5 (0; protection:100%)

Results are mean ± SD (n = 5),*statistical significance at $p \leq 0.05$ (one-way ANOVA followed Dunnette's post-hoc), LE: leaf extract, RE: root extract.

3.4.2. Pentylenetetrazole (PTZ)-induced convulsion in rats

In Table 8 below, 100% protection of the rats was achieved when the animals were given low dose (100 mg/kg) and high dose (400 mg/kg) of extracts while the medium dose (200 mg/kg) does not offer 100% protection on the animals. A similarly results was obtained when 2 mg/kg diazepam (standard drug) was given to the animals (i.p.) where 100% protection was produced.

3.4.3. Maximum electroshock (MES)-induced convulsion in rats

There was dose-dependent decrease in onset of convulsion and duration of convulsion in rats exposed to maximal electroshock. However, much delayed onset and duration of convulsion was witnessed at medium dose (200 mg/kg) root extract. The values obtained were significant ($p \leq 0.05$) when compared to control group (Fig. 6).

4. Discussion

In recent times, pharmacognosy has rapidly improved the use of medicinal plants for treating various diseases because of much advancement in technology such as chromatographic processes (like thin layer, paper, column, gas, liquid and high-performance chromatography), microscopy, isolation and purification techniques. These processes have made it possible the rapid isolation of bioactive compounds that were thought to be difficult to isolate in their pure forms previously. Pharmacognosy as an aspect of ethnobotany, pharmacology, biological sciences, biochemistry, and medicinal chemistry has also combined other spectroscopic techniques like NMR and FTIR to accurately elucidate the structures of isolated compounds from medicinal plants (Ukwubile et al., 2019b). These is very crucial in quality assurance of ethnomedicinal prescriptions from plants. It is important to note

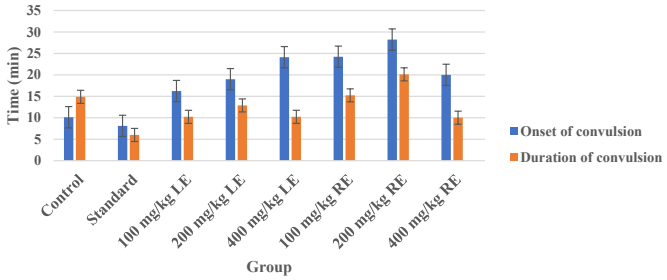


Fig. 6. Effects of *N. laevis* extracts on maximum electroshock-induced convulsion in rats. Results are mean ± SD (n = 5), LE: leaf extract, RE: root extract.

these processes developed have also helped to establish authentic fingerprints of medicinal plants' products which is aimed preventing and detecting adulteration of crude drugs. Furthermore, most of these isolated bioactive compounds have been used as lead compounds for the development of various known conventional medicines such as anticancer agents, antimalarials, antibiotics, antihypertensive agents, nutraceuticals, and antidiabetics (Belwal et al., 2014). Thus, medicinal plants have continued to be major source of raw materials for most essential synthetic drugs globally, and more than 100 medicinal plants are used worldwide for therapeutic purposes (Rosandy et al., 2013).

In the current study, pharmacognostic studies were carried out on the leaf and root of *Newbouldia laevis* plant, and other biological activities such as analgesic, anti-inflammation and anticonvulsant activities of the extracts from the leaf and root of the plant were determined in Wistar rats. The quantitative microscopical evaluation of the leaf showed that it contains paracytic type of stomata that are hypostomatic (i.e., deeply situated) as viewed using the scanning electron microscope (Fig. 1, Table 1). These stomata were numerous at the upper surface of the leaf than the lower surface. This uneven distribution of stomata in leaves of plants is of taxonomic and adaptation significance in plants. This is because, high number of stomata found on the upper surface help to facilitate rapid transpiration of water from leaves thereby, preventing the cell from undergoing cellularly imbalance. This adaptive strategy observed in this plant in the current study indicate that the plant is able to survive drought and desiccation withing the environment (Jones et al., 2006). Trichomes carryout several functions in plant such as maintaining still air on leaf surface and secretory of certain substances that are of importance in pharmaceutical industries. In the present study, the glandular unicellular trichomes observed in the leaf may serve same function especially secretory of sugary substances as seen in the plant parts like the stem, flowers, and fruits.

Our study also revealed that the transvers section of the leaf and root (Fig. 2 a and b) showed that the leaf contain palisade and spongy parenchyma, thin cuticle, calcium oxalate, and fiber sclereids with narrow lumen in root. The presence of palisade and spongy parenchyma is of taxonomic significance in that it helps to distinguish the plant from closely related species from other plant families or even the same family (Bignoniaceae). Moreover, palisade parenchyma has been reported as storage for sugar and starch while the spongy parenchyma helps to facilitate gaseous exchange in plants (Dodiya and Jain, 2017). These roles played by parenchyma is similar in this current study because of the ability of the plant to withstand long drought and desiccation.

Physicochemical evaluations are very crucial in pharmacognostic profiling of medicinal plants. This is because it helps to establish accurate fingerprints for crude drugs that could be use as important tool for creating monographs on drugs (Kunle, 2012). In this study, moisture contents were 6.25 $\pm$ 0.01% w/w and 8.04 $\pm$ 0.10% w/w for leaf and root respectively (Table 2). It showed that root powders are likely to be exposed to microbial attack than the leaf powder, thus, it should be properly stored in dry and clean environment to prevent microbial contamination. Similarly, water extractive value of leaf (10.28 $\pm$ 1.02% w/w) was greater than that of the root (6.20 $\pm$ 0.01% w/w). This results was similar to previous report obtained other researchers where it was reported that water extractive value was greater than alcohol extractive values (Bijauliya et al., 2021). The present phytochemical study further revealed that tannins, flavonoids, alkaloids, triterpenes and saponins were the major secondary metabolites detected in leaf extract while the root extract also contain cardiac glycosides in addition to other phytoconstituents (Table 3). It is an established fact that these phytochemicals play crucial roles in various aspects of human healthcare system such as anticancer, antimalarials, antidiabetics, antibiotics, antihypertensive, and anti-inflammatory agents (Omoregie et al., 2010). Their roles in the present study were not different from the previously reported ones. Many of the family Bignoniaceae have been reported to contain various types of flavonoids and triterpene that are used as treatment for inflammation and malaria respectively in traditional medicine (Bairagi and Sadiq, 2016).

For proper structural elucidation of isolated compounds from plant extract, the GC-MS has been recommended since it give mass-charge (m/z) number of the compounds (Mohamad et al., 2018). From the current study, methanol leaf extract of *N. laevis* contain important compounds such as pyridine-3-yl-ethaniminidamide (m/z:135.1 g/mol), melibiose (m/z: 342.30 g/mol), 2-N-butylacrolein (m/z:112.17 g/mol) and 2-ethylyformanilide (m/z: 149.23 g/mol) among other compounds (Table 4). The study also showed that methanol root extract contains 2-furanmethanamine (m/z: 97.12 g/mol), hydrazne-1,2-dimethyl (m/z: 60.09 g/mol), fomepizole (m/z: 82.11 g/mol) and 2,6-nonadienal (m/z: 138.21), among others (Table 5). These compounds play crucial roles in the body. For instance melibiose is a precursor for raffinose an important carbohydrate, pyridine-3-yl-ethaniminidamide and pyridine containing drugs are used as anticancer, antimicrobial, antioxidant, antiviral, antidiabetic, antihypertensive, anti-inflammatory and antimalarial agents, as well as antiprotozoal agent and psychopharmacological antagonists (Keskes et al., 2017). Also, hydralizine-1,2-dimethyl isolated from root extract has been reported also, as antihypertensive agent (lowering blood pressure), its roles in the current study was unknown. Similarly, fomepizole isolated from the root extract has been reported to be used antidote for removing poisons from ethylene glycol or methanol, and sometimes combined with hemo-dialysis to remove poisons from the body (Ukwubile et al., 2019).

Pharmacognosy as an aspect of natural product science has made it possible to assess the safety of ethnomedicinally used herbal preparations. In the current study, toxicity study showed that the extracts are safe at the dose of 2000 mg/kg administered to the rats (Table 6). There were no signs of toxicity witnessed in the animals after fourteen days of acute oral toxicity testing, thus, justifies its use in traditional medicine for treating various diseases such as analgesic, anti-inflammation and anticonvulsant agents. For instance, our study from the acetic acid-induced writhing analgesic test in rats revealed that the extracts significantly inhibited abdominal contraction induced by acetic acid in dose-dependent fashion with much inhibition witnessed in groups treated with leaf extract (Fig. 3 and b). This implies that the extracts might have displayed their analgesic activities using the peripheral nervous system as compared to the standard drug (pentazocine) a classical example of non-steroidal anti-inflammatory drugs (NSAIDs) used in this study. Similarly, in the tail-flick analgesic test, there was a dose-dependent reaction time response. The study showed that the combination of naloxone and 400 mg/kg leaf extract displayed significant delayed in reaction time when compared to naloxone and pentazocine group which indicates the involvement of opioid receptors (Fig. 4a and b). It implies that the extracts exhibited analgesic action mediated peripherally and centrally by involving the opioid receptors because naloxone was considered as a non-selective opioid receptor antagonist with a short duration of pharmacological actions (Hijazi et al., 2017).

From the results obtained in anti-inflammatory study by carrageenan-induced paw oedema in rats, the extracts showed dose-dependent inhibition of paw oedema with increasing experimental times. However, many reductions in paw volumes were obtained with rats treated with *N. laevis* leaf extract (Fig. 5 a and b). The use of carrageenan to induce inflammation in the rats triggered the release of mediator of inflammation such as serotonin, bradykinin and histamine which are released at the onset of inflammation followed by prostaglandins which are released in the later stage of inflammation (Pandey et al., 2020). Similarly, a dose-dependent reductions in paw oedema were obtained in formalin-induced inflammation in rats (Fig. 5 b). The ability of the plant extracts to greatly inhibit the inflammatory action of other promotors of inflammation such as tumor necrosis factor-A, cyclooxygenase-2 (COX-2) and interleukins which further affirms their use as anti-inflammatory prescription in traditional medicine.

In the current study, strychnine-induced convulsion in rats showed dose-dependent protection of the animals and delayed onset of seizures (Table 7). Strychnine is an alkaloid which is isolated from dried ripped

seed extract of *Strychnos nux vomica*, a shrub found mainly in East Indies which has been used as CNS stimulant among other uses. In addition, the use of Pentylenetetrazole (PTZ), a GABA-A receptor antagonist conferred 100% protection on the animals especially 100 and 400 mg/kg leaf and root extract doses (Table 8). The plant extracts reverse the symptoms of convulsions and seizures even death which was caused by PTZ by modulation of glutamatergic neurotransmitter. The result was not different from what was obtained in the maximal electroshock (MES) model where there was significant delay in the onset of convulsion in animals given leaf extract than root extract (Fig. 6). The MES induced convulsion model activate the release of calcium and sodium pump channels, making the extracts to be able to prevent the flow of these ions which subsequently resulted in delayed onset of convulsion and seizures witnessed. In general, most anticonvulsant drugs that are used clinically today exert their actions by blockage of neuronal ion channels or facilitating the neurotransmission of gamma-aminobutryic acid (GABA) (Shinde et al., 2018). Our study showed that the anticonvulsant activity of leaf and root extract of *N. laevis* might have been exhibited following any of the mention mechanisms or both.

5. Conclusion

Our study revealed important pharmacognostic profiles that will help in both taxonomic identification of the plants' leaf and root, and pharmaceutical indices for building drug monographs. The study also showed that methanol leaf and root extracts of *N. laevis* showed dose-dependent analgesic, anti-inflammatory, and anticonvulsant activities. These activities are due the presence of certain phytochemicals and bioactive compounds in leaf and root extracts. The study further revealed that anticonvulsant activity of the plant was achieved by the blockage of neuronal ion channels or promotion of GABAergic neurotransmission release. Our study further affirmed the use of *N. laevis* as ethnopharmacological prescription for pains, inflammation and convulsion or epilepsy in traditional medicine in Nigeria.

Ethical approval

The rats used in the present study was approved for the use with approval obtained from the Animal in Research Ethical Group of PJ Rats Farm Ltd with approval number: PJF/NG/JOS-035-2023.

CRediT authorship contribution statement

Cletus Anes Ukwubile: Designed the project, performed the experiment, Formal analysis, and preparation of the manuscript. **Emmanuel Oise Ikpefan:** Performed the experiment, review the literature, and, supervised the project. **Musa Yusuf Dibal:** Performed the experiment, data analysis, and, literature review. **Vivian Amarachukwu Umeano:** Formal analysis, Data curation, Performed the experiment, data analysis and literature review. **David Nnamdi Menkiti:** Formal analysis, Writing – review & editing, Performed the writing – review and editing and performed the experiment. **Clement Chidi Kaosi:** Performed the experiment and review the literature. **Simon Paul:** Formal analysis, Data analysis, and, Supervision, supervision of the project. **Ademola Clement Famurewa:** Performed the experiment, data analysis, and, Supervision, supervision of the project. **Henry Nettey:** Formal analysis, Supervision, supervision of the project, Writing – review & editing, Review and editing of the manuscript. **Timothy Samuel Yerima:** Formal analysis, Review of manuscript, and, Supervision, supervision of the project, All authors approved the final manuscript before submission.

Declaration of competing interest

We have none to declare.

Data availability

Data will be made available on request.

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