JOURNAL OF PHARMACOLOGICAL SCIENCES AND EXPERIMENTAL THERAPEUTICS
(Official Journal of the Nigerian Society for Pharmacological Sciences and Experimental Therapeutics)

ISSN 3115 - 6487

Seizure-modulating compounds identified in the leaves of *Newbouldia laevis*

Oyetunji T. Kolawole[1], Emmanuel A. Soetan[2], Oluwatosin M. Adewole[2], Adetunji A. Habeebat[3], Akeem A. Ayankunle[4], Olayemi K. Wakeel[1], Oluwaseyi O. Adeyeba[5]

[1]Department of Pharmacology and Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[2]Department of Pharmacology, College of Health Sciences, Bowen University, Iwo, Osun State, Nigeria
[3]Department of Chemistry, College of Physical Sciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[4]Department of Pharmacology and Therapeutics, College of Health Sciences, Osun State University, Osogbo, Nigeria
[5]Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

ARTICLE HISTORY

Received 15th August 2025
Revised 8th September 2025
Accepted 10th September 2025

Published 15th September 2025

Keywords:
Seizures,
Newbouldia laevis,
Picrotoxin,
Anticonvulsant,
Fractions,
Bioactive
compounds

***Corresponding author**
Oyetunji T. Kolawole,
Department of Pharmacology and Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
E-mail: otkolawole@lautech.edu.ng
Tel. +234-803-932-8444

ABSTRACT

Background: Convulsions in epilepsy and other ailments often lead to disability and diminished quality of life. Identification of bioactive compounds in medicinal plants may lead to development of useful anticonvulsant drugs. In this study, crude extract of *Newbouldia laevis* leaves and its chromatographic fractions were evaluated for anticonvulsant activity in mice and screened for bioactive compounds.
Method: Crude extract of the leaves (N-Crude) was obtained by Soxhlet extraction and subjected to chromatographic fractionation. Three main fractions, N-Green, N-Pink, and N-Yellow were obtained. Anticonvulsant effects of N-Crude and the fractions were evaluated by sodium barbital-induced hypnosis (45 mg/kg b.w i.p), picrotoxin-induced convulsion ((10 mg/kg b.w i.p), and strychnine-induced convulsion (2 mg/kg b.w i.p) tests. The samples were screened for bioactive compounds using gas chromatography - mass spectrometry (GC-MS) analysis.
Results: In sodium barbital hypnosis test, N-Crude, N-Green, and N-Pink caused significant dose -dependent decrease in sleep latency and prolongation of sleep duration. In picrotoxin-induced convulsion test, they significantly prolonged seizure latency and decreased seizure duration. N-Crude and the fractions did not cause any significant difference between the control and treated groups in strychnine-induced convulsion test. They protected mice against picrotoxin-induced mortality, except in N-Yellow-treated group where 100 % mortality was recorded. GC-MS analysis of N-Crude and the fractions revealed the presence of bioactive compounds. The most abundant of these compounds are benzoic acid methyl esters, neophytadiene, phytol, isophytol, cyclohexane, Isophorone, and 2(4H)-benzofuranone.
Conclusion: The results showed that *N. laevis* is rich in bioactive compounds which possess anticonvulsant property. Synergistic and additive effects of these compounds are likely responsible for its anticonvulsant activity.

INTRODUCTION

Some neurological disorders such as epilepsy are characterized by recurrent convulsion (seizures) which involves part of the body or the entire body[1]. During an episode of convulsion, there are excessive electrical discharges in the brain cells which alter consciousness, movement, and actions. These often lead to disability and diminished quality of life[2]. It is estimated that epilepsy with its characteristic episodes of convulsion accounts for more than 0.6 % of global disease burden. Recent report also shows that epilepsy affects as many as 50 million people

worldwide making it one of the common neurological disorders[3]. Seizures may be classified as partial (focal) or generaliized. Each type of seizure is characterized by a specific pattern of events, as well as different pattern of motor or sensory manifestation. Partial seizures arise from a localized area in the brain and cause specific symptoms. A partial seizure can spread to the entire brain and cause generalized seizure[4]. Partial seizures include simple seizures where consciousness is not impaired or Jacksonian seizures - a focal seizure that begins with an uncontrolled stiffening or jerking in one part of the body such as finger, mouth, hand or foot, which may progress to generalized seizure[5]. Generalized seizures include tonic-clonic, myoclonic, and absence seizures. Manifestation of a generalized tonic-clonic seizure include alternate contraction (tonic phase) and relaxation (clonic phase) of muscles, a loss of consciousness, and abnormal behavior[6]. Myoclonic seizures involve sudden forceful contractions involving the musculature of the trunk, neck, and extremities. Absence seizures previously referred to as petit mal seizures are characterized by brief loss of consciousness during which physical activities ceases. The seizure typically last a few seconds, occuring many times a day, and may go unnoticed by others[7]. Seizures has also been categorized as idiopathic or acquired. Idiopathic seizures have no known cause; acquired seizure disorders are known to arise from high fever, electrolyte imbalance, uremia, hypoglycemia, hypoxia, brain tumors and some drug withdrawal reactions. Once the cause is removed, the seizure theoretically ceases[8].

Most anticonvulsant drugs have specific uses, that is, they are of value only in the treatment of certain types of seizure disorders. These drugs possess the ability to depress abnormal neural discharges in the central nervous system, resulting in an inhibition of seizure activity. Examples of such drugs are barbiturates, benzodiazepines, hydantoins, oxazolidinediones, and succinimides[9]. In spite of these treatment options, there is a need to search for more anticonvulsant drugs that are more effective for different seizure types. In this regard, attention has now shifted to medicinal plants that are traditionally used for convulsion. Bioactive compounds from such plants may possess anticonvulsant properties. Identification of such compounds may lead to development of more anticonvulsant drugs. One medicinal plant that is traditionally used as a remedy for convulsion is *Newbouldia laevis*. It is a fast-growing evergreen tree that belongs to the Bignoniaceae family. Its common names are Fertility tree, Tree of life, and African Border tree. It is known as Akoko in Yoruba, Aduruku in Hausa, and Ogirisi in Igbo. There are preliminary reports on the anticonvulsant effects of *N. laevis* leaves[10]. In this study, the crude leaf extract of *N.laevis* and its chromatographic fractions were evaluated for anticonvulsant activity and screened for bioactive compounds.

MATERIALS AND METHODS

Drugs and Chemicals

The following drugs and chemicals were used for the study: picrotoxin (Sigma, USA; CAS No:124-87-8), strychnine (Sigma, USA; CAS No:57-24-9), diazepam (Sigma, USA), sodium barbital (Sigma, USA; CAS No:144-02-5), Dimethyl sulfoxide (DMSO CAS No: 67-68-5), Ethanol (Sigma, USA; CAS No: 64-17-5).

Collection of plant

Leaves of *Newbouldia laevis* were collected from the premises of Bowen University College of Health Sciences, Ogbomoso, in August. The leaves were identified and authenticated by a taxonomist in the Department of Biological Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. A voucher specimen was deposited in the herbarium of the Department and the voucher number (LHO 839) was assigned to the sample.

Ethical approval

Ethical approval (ERC/FBMS/041/2024) was obtained from Faculty of Basic Medical Sciences Ethical Committee and all procedures were carried out in accordance with the approved research protocols of Ladoke Akintola University of Technology, Ogbomoso.

Preparation of ethanol crude extract of *N. laevis*

The leaves were washed thoroughly with clean water to remove dust and debris that may contaminate the samples. The washed leaves were spread under the shade and air-dried for 7 days. The dry leaves were then pulverized by a grinding machine. The pulverized was then extracted in 80% ethanol using Soxhlet extractor. The resulting ethanol extract was concentrated at 40 ^{o}C in a rotary evaporator (Yield = 8.2%). The ethanol extract (N-Crude) was kept in an air-tight container and stored in a refrigerator at -20 ^{o}C[11].

Chromatographic fractionation of the crude extract

Dry ethanol extract of *N. laevis* was subjected to a preliminary test using thin layer chromatographic plate to determine the best solvent for column separation. Many solvents were used including butanol, n-butanol, ethyl acetate, methanol, n-hexane, hexane, cyclohexane, and ethyl ether. N-hexane and ethyl acetate in the ratio 9:1 has the best spot on the thin layer chromatographic plate, hence, n-hexane and ethyl acetate at 9:1 was found to be the

most suitable solvent for column chromatography separation. For the column chromatographic separation, silica gel was used as stationary phase and n-hexane and ethyl acetate in the ratio 9:1 was the mobile phase. After pooling eluent fractions, three main fractions were obtained. They were named N-Pink, N-Green, and N-Yellow.

Experimental animals

Healthy male Swiss mice weighing 20 ± 5 g were obtained from the Animal House of the College of Health Sciences, LAUTECH, Ogbomoso. The mice were kept in polypropylene cages in a well-ventilated laboratory at room temperature. The mice were fed standard commercial animal feed (Ladokun Feed Ltd, Ibadan, Nigeria). They were also allowed free access to clean water. All procedures were followed as outlined in the Guide for the Care and Use of Laboratory Animals published by the National Research Council[12]

Experimental Procedure

N-Crude, N-Pink, N-Green, and N-Yellow were each dissolved in 1% DMSO and used for the following experiments:

Sodium barbital-induced hypnosis

Twenty-five mice were fasted overnight and assigned to 5 groups (n = 5). Group 1 and 2 were pretreated orally with 1% DMSO (10 ml/kg b.w) and diazepam (2 mg/kg b.w)[10], respectively. Groups 3, 4, and 5 were pretreated orally with 150, 300, and 600 mg/kg b.w of N-Crude, respectively. Thirty minutes after the pretreatment, sodium barbital (45 mg/kg b.w)[10] was administered intraperitoneally in all the groups. Onset of sleep (sleep latency) and duration of sleep (time interval between loss and gain of righting reflex) were noted[10, 13,].

In separate experiments, effects of the fractions: N-Green, N-Yellow, and N-Pink on sodium barbital – induced hypnosis were also investigated using the same method.

Picrotoxin-induced convulsion

Effect of N-Crude on picrotoxin-induced seizures was investigated by the method used previously[10, 14]. Mice were assigned to 5 groups (n = 5) and treated orally using as follows:

Group I: 1% DMSO (10 ml/kg b.w)
Group II: Diazepam (2 mg/kg b.w)
Group III: N-Crude (150 mg/kg b.w)
Group IV: N-Crude (300 mg/kg b.w)
Group V: N-Crude (600 mg/kg b.w)

All drugs were administered using oral cannula 30 minutes before intraperitoneal administration of picrotoxin (10 mg/kg b.w)[10]. Thereafter, each mouse was placed separately in a transparent observation chamber. The animal was observed for 30 minutes after picrotoxin administration. Seizure latency and seizure duration were recorded. Percentage death within 24 hours was also recorded. In separate experiments, the effects of N-Green, N-Yellow, and N-Pink on picrotoxin-induced seizures were also investigated using the same method and doses.

Strychnine-induced convulsion

In this model, mice were assigned to 5 groups (n = 5) and treated as follows:

Group I: 1% DMSO (10 ml/kg b.w)
Group II: Diazepam (2 mg/kg b.w)
Group III: N-Crude (150 mg/kg b.w)
Group IV: N-Crude (300 mg/kg b.w)
Group V: N-Crude (600 mg/kg b.w)

All drugs were administered orally using oral cannula 30 minutes before intraperitoneal administration of strychnine (2 mg/kg b.w)[10, 13]. Each mouse was then placed separately in a transparent observation cage, and observed for 30 minutes after administration of strychnine. Onset of seizure and seizure duration were recorded. Percentage death within 24 hours was also recorded[15]. In separate experiments, the effects of N-Green, N-Yellow, and N-Pink on strychnine – induced seizures were also evaluated using the same method.

GC-MS analysis

Samples were analyzed using an Agilent 8860 Gas Chromatogram equipped with a 5977B Mass Spectrometry Detector System (MSD), fitted with Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30x0.25 µm ID x 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1ml/min, and an injection volume of 1 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 300 °C, the ion-source temperature was 250 °C, and the GC oven temperature was programmed from 110 °C (1 min), then ramped at 15 °C/min to 310 °C (2 min), mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min[16].

Statistical analysis

Data obtained were expressed as mean ± standard error of mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's *Post hoc* test. A level of $P < 0.05$ was considered significant. GraphPad Prism version 5.0 for windows was used for these statistical analyses (GraphPad software, San Diego California USA)

RESULTS

Effects of *N. laevis* Crude extract and fractions on sodium barbital-induced hypnosis

The effects of crude extract and the fractions of *N. laevis* on sodium barbital-induced hypnosis are presented in Tables 1. In the control group, onset of sleep (sleep latency) and duration of sleep were 14.63 ± 2.55 min and 49.46 ± 7.41 min, respectively. The crude extract and the fractions caused dose-dependent decrease in sleep latency. Compared with the control, the decrease was significant ($p<0.05$) with N-Crude and N-Green at 300 and 600 mg/kg b.w. Administration of 600 mg/kg b.w, of N-Crude and N-Green significantly ($p<0.05$) decreased sleep latency from 14.63 ± 2.55 to 4.85 ± 0.21 and 5.32 ± 1.46 min, respectively. At 600 mg/kg b.w, N-Pink also significantly reduced ($p<0.05$) sleep latency to 6.43 ± 0.65 min. Likewise, at 600 mg/kg b.w, N-Crude, N-Green and N-Pink caused a significant prolongation of sleep duration ($p<0.05$) compared to the control.

Table 1: Effect of crude extract and chromatographic fractions of *N. laevis* on sodium barbital hypnosis in mice

Treatment	Dose	Sleep latency(min)	Duration of sleep (min)
Control	10 (ml/kg)	14.63 ± 2.55	49.46 ± 7.41
Diazepam	2 (mg/kg)	4.41 ± 0.82*	107.42 ± 14.60*
N-Crude			
N-Crude	150 (mg/kg)	11.20 ± 1.61*	68.44 ± 6.30
N-Crude	300 (mg/kg)	6.82 ± 0.94*	72.00 ± 10.05*
N-Crude	600 (mg/kg)	4.85 ± 0.21*≠	96.72 ± 12.11*≠
N-Green			
N-Green	150 (mg/kg b.w)	8.74 ± 2.40*≠	60.40 ± 8.04*≠
N-Green	300 (mg/kg b.w)	7.50 ± 1.21*≠	84.33 ± 5.53*
N-Green	600 (mg/kg b.w)	5.32 ± 1.46*	98.07± 10.21*
N-Pink			
N-Pink	150 (mg/kg b.w)	10.54 ± 1.04	54.26 ± 4.11
N-Pink	300 (mg/kg b.w)	9.22 ± 1.12	67.76 ± 6.12
N-Pink	600 (mg/kg b.w)	6.43 ± 0.65*	82.56 ± 5.33*
N-Yellow			
N-Yellow	150 (mg/kg b.w)	13.34 ± 2.10	46.24 ± 3.32
N-Yellow	300 (mg/kg b.w)	12.52 ± 1.50	42.66 ± 3.14
N-Yellow	600 (mg/kg b.w)	16.14 ± 1.42	56.01 ± 5.20

Values represent mean ± SEM (n = 5); *$p<0.05$ compared with the control. ≠$p>0.05$ compared with diazepam. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract, N-Green = *Newbouldia laevis* green fraction, N-Pink = *Newbouldia laevis* pink fraction, N-Yellow = *Newbouldia laevis* yellow fraction

Effects of *Newbouldia laevis* on chemoconvulsant-induced convulsion and mortality

Effects of N-Crude, N-Green, N-Pink, and N-yellow on seizure duration, seizure latency, and mortality after induction of convulsion with picrotoxin and strychnine are summarized below:

Effects of *Newbouldia laevis* fractions on seizure latency and seizure duration

In picrotoxin-induced convulsion test, the seizure latency was significantly increased in mice treated with 300 and 600 mg/kg b.w of *N. laevis* crude extract (N-Crude) and the green fraction (N-Green) compared with mice treated with 1% DMSO (control). In the control group, the seizure latency was 62.44 ± 8.01 sec. After the administration of 300 and 600 mg/kg of N-Crude, seizure latency was significantly prolonged to 84.65 ± 10.11 sec and 92.71 ± 6.10 sec respectively. Following administration of N-Green at 300 and 600 mg/kg b.w, seizure latency was also significantly increased ($p<0.05$) to 87.60 ± 8.41 sec and 98.24 ± 6.55 sec respectively. The effect of N-Crude and N-Green is comparable to that of diazepam (2 mg/kg b.w) which prolonged seizure latency to 96.56 ± 10.61 sec. While 300 and 600 mg/kg b.w of N-Crude reduced seizure duration from 56.73 ± 6.42 sec to 30.33 ± 4.55 sec and 18.40 ± 6.40 sec respectively, N-Green at 300 and 600 mg/kg b.w significantly reduced seizure duration to 36.92 ± 4.43 and 26.35 ± 2.73 respectively. Compared with control, administration of 6oo mg/kg b.w of N-Pink prolonged seizure latency significantly ($p<0.05$) to 80.31 ± 8.25 sec. It also significantly reduced seizure duration from 56.73 ± 6.42 sec to 31.90± 7.33 sec. N-Yellow did not significantly prolong seizure latency at any of the

doses administered. The results are presented in Tables 2.

Table 2: Effect of crude extract and chromatographic fractions of *N. laevis* on picrotoxin-induced convulsion

Treatment	Dose	Seizure latency (sec)	Seizure duration (sec)
Control	10 (ml/kg)	62.44 ± 8.01	56.73 ± 6.42
Diazepam	2 (mg/kg)	96.56 ± 10.61*	24.33 ± 7.05*
N-Crude			
N-Crude	150 (mg/kg)	76.34 ± 6.42	45.81 ± 7.20
N-Crude	300 (mg/kg)	84.65 ± 10.11*≠	30.33 ± 4.55*≠
N-Crude	600 (mg/kg)	92.71 ± 6.10*≠	18.40 ± 6.40*≠
N-Green			
N-Green	150 (mg/kg b.w)	72.14 ± 8.30	48.50 ± 6.30
N-Green	300 (mg/kg b.w)	87.60 ± 8.41*	36.92 ± 4.43*
N-Green	600 (mg/kg b.w)	98.24 ± 6.55*	26.35 ± 2.73*
N-Pink			
N-Pink	150 (mg/kg b.w)	66.06 ± 5.70	54.22 ± 6.24
N-Pink	300 (mg/kg b.w)	70.93 ± 8.07	48.00 ± 5.84
N-Pink	600 (mg/kg b.w)	80.31 ± 8.25*	31.90± 7.33*
N-Yellow			
N-Yellow	150 (mg/kg b.w)	55.20 ± 3.51	61.58 ± 7.40
N-Yellow	300 (mg/kg b.w)	58.20 ± 5.52	50.86 ± 6.23
N-Yellow	600 (mg/kg b.w)	67.85 ± 9.43	44.64 ± 6.06

Values represent mean ± SEM (n = 5); *p<0.05 compared with the control. ≠p>0.05 compared with diazepam. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract, N-Green = *Newbouldia laevis* green fraction, N-Pink = *Newbouldia laevis* pink fraction, N-Yellow = *Newbouldia laevis* yellow fraction

In strychnine-induced convulsion, there were no significant differences between the control and groups of mice treated with the crude extract, the fractions, and diazepam (P>0.05). The results are presented below (Tables 3).

Table 3: Effect of crude extract and chromatographic fractions of *N. laevis* on strychnine-induced convulsion

Treatment	Dose	Seizure latency (sec)	Seizure duration (sec)
Control	10 (ml/kg b.w)	43.31 ± 4.70	12.54± 2.42
Diazepam	2 (mg/kg b.w)	62.20 ± 6.22	14.40 ± 2.13
N-Crude			
N-Crude	150 (mg/kg b.w)	46.42 ± 5.65	14.06 ± 2.22
N-Crude	300 (mg/kg b.w)	39.74 ± 3.35	18.93 ± 3.54
N-Crude	600 (mg/kg b.w)	40.82 ± 4.10	16.11 ± 2.45
N-Green			
N-Green	150 (mg/kg b.w)	36.80 ± 6.12	17.41 ± 3.22
N-Green	300 (mg/kg b.w)	42,77 ± 8.40	16.25 ± 2.13
N-Green	600 (mg/kg b.w)	48.63 ± 6.84	14.86 ± 2.55
N-Pink			
N-Pink	150 (mg/kg b.w)	33.45 ± 5.52	10.40 ± 2.50
N-Pink	300 (mg/kg b.w)	28.20 ± 3.00	9.81 ± 1.74
N-Pink	600 (mg/kg b.w)	36.63 ± 4.30	12.55± 2.11
N-Yellow			
N-Yellow	150 (mg/kg b.w)	28.15 ± 3.04	8.66 ± 1.42
N-Yellow	300 (mg/kg b.w)	38.12 ± 5.23	16.10 ± 2.32
N-Yellow	600 (mg/kg b.w)	46.72 ± 7.02	10.54 ± 2.63

Values represent mean ± SEM (n = 5); *p<0.05 compared with the control. ≠p>0.05 compared with diazepam. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract, N-Green =

Newbouldia laevis green fraction, N-Pink = *Newbouldia laevis* pink fraction, N-Yellow = *Newbouldia laevis* yellow fraction

Effects of *Newbouldia laevis* fractions on mortality

In picrotoxin-induced convulsion, mortality within 24 h after the experiment was 100% in the control group and 40% in the group treated with diazepam. Administration of 150, 300 and 600 mg/kg b.w of N-Crude caused 20, 0, and 0 percent mortality, respectively, and N-Green produced the same results. N-Pink at 150, 300 and 600 mg/kg b.w caused 100%, 80% and 80% mortality respectively. N-Yellow did not protect mice against picrotoxin-induced mortality. Mortality was also 100% in all the groups including the diazepam-treated group in the strychnine-induced convulsion. The results are shown in Tables 4

Table 4: Effect of crude extract and chromatographic fractions of *N. laevis* on mortality after 24 hours of seizure induction

Treatment	Dose	Picrotoxin (%)	Strychnine (%)
Control	10 (ml/kg b.w)	100	100
Diazepam	2 (mg/kg b.w)	40	100
N-Crude			
N-Crude	150 (mg/kg b.w)	20	100
N-Crude	300 (mg/kg b.w)	0	100
N-Crude	600 (mg/kg b.w)	0	100
N-Green			
N-Green	150 (mg/kg b.w)	20	100
N-Green	300 (mg/kg b.w)	0	100
N-Green	600 (mg/kg b.w)	0	100
N-Pink			
N-Pink	150 (mg/kg b.w)	100	100
N-Pink	300 (mg/kg b.w)	80	100
N-Pink	600 (mg/kg b.w)	80	100
N-Yellow			
N-Yellow	150 (mg/kg b.w)	100	100
N-Yellow	300 (mg/kg b.w)	100	100
N-Yellow	600 (mg/kg b.w)	100	100

Values represent mean ± SEM (n = 5); Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract, N-Green = *Newbouldia laevis* green fraction, N-Pink = *Newbouldia laevis* pink fraction, N-Yellow = *Newbouldia laevis* yellow fraction

GC-MS Analysis

GC-MS analysis of the *N. laevis* extract and the fractions revealed a total of 60 compounds. Analysis of N-Crude revealed the presence of 26 compounds. The most abundant of these compounds are benzoic acid methyl esters, and neophytadiene. Others are phytol and isophytol. Seventeen major bioactive compounds were found in N-Green fraction of *N. laevis*. The most abundant of these are neophytadiene, phytol, and cyclohexane. Seven compounds were detected N-Pink. Isophorone and 2(4H)-benzofuranone are the most abundant of the compounds. N-Yellow contains 10 compounds, the most abundant of which is isophorone . The compounds identified in the crude extract, and the tree fractions are shown in Tables 5-8 below. GC-MS chromatograms are also shown in Figures 1-4.

Table 5: List of compounds identified in the crude extract (N-Crude) of *N. laevis* leaves

S/N	RT	Area%	Compound name	CAS#	Molecular Formula	MW (g/mol)
1	3.23	0.94	Cyclotetrasiloxane, octamethyl-	000556-67-2	C8H24O4Si4	296.61
2	3.963	2.53	2-Ethylacridine	055751-83-2	C15H13N	207.27
3	4.02	1.08	4-Phenyl-3,4-dihydroisoquinoline	006187-58-2	C15H13N	207.27
			1H-1,2,4-Triazole-5(4H)-thione, 4-allyl-3-(3-furyl)-	013228-36-9	C9H9N3OS	207.25
			1H-Indole, 5-methyl-2-phenyl-	013228-36-9	C15H13N	207.27
4	4.707	3.34	Decane, 2-methyl-	006975-98-0	C11H24	156.31
			Tridecane, 3-methyl-	006418-41-3	C14H30	198.388
			Carbonic acid, eicosyl vinyl ester	1000382-54-3	C23H44O3	368.5937
5	4.815	25.88	Benzoic acid, methyl ester	000093-58-3	C8H8O2	136.1479
6	5.965	0.8	3-Chloro-4-ethyl-5-phenyl-1,2-oxazole	1000411-55-1	C12H10ClFN2O2	268.67
			Carbonic acid, methyl nonyl ester	1000314-62-1	C20H40O3	328.5298
			4,5-Dihydrooxazole-5-one, 4-chloromethylene-2-phenyl-	014848-36-3	C10H10ClNO	195.64
			trans-4-(2-(5-Nitro-2-furyl)vinyl)-2-quinolinamine	0847-10-9	C15H11N3O3	281.27
			Cyclotetrasiloxane, octamethyl-	000556-67-2	C8H24O4Si4	296.61
8	6.154	1.99	Allylamine, 3-chloro-N-isopropyl-2-methyl-, (E)-	023240-44-0	C7H14ClN	147.64
			1-(1-Propen-1-yl)-2-(2-thiopent-3-yl)disulfide	1000322-30-2	C6H10S2	146.3
			Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 1'-isopropyl-2,4'-dimethyl-11-(2-methoxyethoxymethoxy)-	1000157-52-8	C20H14O5	334.3
9	7.23	1.07	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-	000995-83-5	C10H30O4Si5	354.77
			2-Amino-4-nitrophenol, 2TBDMS derivative	1000333-46-7	C18H34N2O3Si2	382.6452
10	8.111	2.41	Benzonitrile, 2-amino-5-nitro-	017420-30-3	C7H5N3O2	163.13
			p-Phenylenediamine, N,N,N'-trimethyl-N'-[2-(N-methylanilino)ethyl]-	032869-56-0	C18H25N3	283.4
			sulfamide, N-[4-(diethylamino)phenyl]-N'-(1,1-dimethylethyl)-	1000397-49-7	C14H25N3O2S	299.43
11	8.352	0.8	2-Tetradecene, (E)-	035953-54-9	C14H28	196.3721
			1-Tridecene	002437-56-1	C13H26	182.35
			Carbonic acid, methyl tetradecyl ester	1000314-62-5	C16H32O3	272.4235
12	9.084	0.79	Pentasiloxane, dodecamethyl-	000141-63-9	C12H36O4Si5	384.8393
			benzoic acid, 4-[[(trimethylsilyl)oxy]methyl]-, trimethylsilyl ester	1000397-43-6	C13H22O3Si2	282.48298
			3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	##########	C18H52O7Si7	577.2
13	9.656	1.41	2,4-Di-tert-butylphenol	000096-76-4	C14H22O	206.32
			Phenol, 2,6-bis(1,1-dimethylethyl)	000128-39-2	C14H22O	206.3239
14	9.828	2.89	Ethyl 4-hydroxyphenylacetate, TBDMS derivative	1000071-82-5	C10H12O3	180.2
			Ethyl alpha-hydroxy-O-nitrocinnamate	1000243-02-8	C11H11NO5	237.21
			(E)-2-Fluoro-3-(4-N,N-dimethylaminephenyl)-propenoic acid, ethyl ester	110915-65-6	C13H16FNO2	237.27 g/mol
15	10.486	0.85	Hexahydro-1-oxa-cyclopropa[d]inden-2-one	037643-23-5	C9H12O2	152.19
			n-Nonadecanol-1	001454-84-8	C19H40O	284.5203
			n-Heptadecanol-1	001454-85-9	C17H36O	256.4671
16	11.35	1.89	Ethyl 4-hydroxyphenylacetate, TBDMS derivative	1000071-82-5	C10H12O3	180.2
			(E)-2-Fluoro-3-(4-N,N-dimethylaminephenyl)-propenoic acid, ethyl ester	110915-65-6		
			Trimethylsilyl 2-(trimethylsilyloxy)propaneperoxoate	1000368-57-7	C9H20O3Si2	232.42
17	11.51	1.25	Methoxyacetic acid, 2-tridecyl ester	1000282-04-5	C16H32O3	272.42
			Methoxyacetic acid, 2-tetradecyl ester	1000282-04-8	C17H34O3	286.4
			Methoxyacetic acid, 3-tetradecyl ester	1000282-04-9		

18	12.723	20.7	Neophytadiene	000504-96-1	C20H38	278.5
			1-Methoxy-3-(2-hydroxyethyl)nonane	070928-44-8	C12H26O2	202.33
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	000473-55-2	C10H18	138.2499
19	12.786	2.9	2-Pentadecanone, 6,10,14-trimethyl	000502-69-2	C18H36O	268.4778
			2(1H)-Naphthalenone, octahydro-4amethyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-	054594-42-2	C15H26O	C15H26O
			(4R*,5R*,9S*)-5,9-Dimethylspiro[3.5]nonan-1-one	063088-19-7	C11H18O	166.26
20	12.935	4.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	C20H40O	296.531
			9-Eicosyne	071899-38-2	C20H	278.5157
			1-Methoxy-3-(2-hydroxyethyl)nonane	070928-44-8	C12H26O2	202.33
21	13.095	9.68	Bicyclo[4.1.0]heptane, 3-methyl-	041977-47-3	C8H14	110.2
			3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	C20H40O	296.531
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	004795-86-2	C10H	138.2499
22	13.421	1.9	Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-, endo-	020536-40-7	C10H18	138.2499
			Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-	004443-51-0	C11H20O	168.2759
			2,3-Dimethyl-8-oxo-non-2-enal	1000186-82-6	C11H18O	182.26
23	13.524	2.49	Hexadecanoic acid, methyl ester	000112-39-0	C17H34O	270.4507
			Hexadecanoic acid, methyl ester	000112-39-0		
			Tetradecanoic acid, 12-methyl-, methyl ester, (S)-		C16H32O	256.4241
24	13.971	0.97	benzeneacetaldehyde, .alpha.-(methoxymethylene)-4-nitrotrans-	1000396-10-6	C10H9NO4	207.18
			2,3-Methylenedioxy-b-methyl-b-nitrostyrene	086029-47-2	C9H7NO4	193.16
			4H-1,2,4-triazole-3,5-diamine, N3-(4-fluorophenyl)-N5-methyl-	1000396-10-8	C8H9N5	175.19
25	15.012	4.65	Isophytol, acetate	1000374-86-2	C22H42O	338.5677
			Phytol	000150-86-7	C20H40O	296.531
			Pentyl triacontyl ether	1000406-42-5	C35H720	508.9
26	15.121	1.25	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	066884-74-0	C15H24O2	236.3499
			Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl	1000129-52-1	C11H13NO3	207.23
			benzeneacetaldehyde,.alpha.-(methoxymethylene)-4-nitro-	1000396-10-6	C10H9NO4	207.18

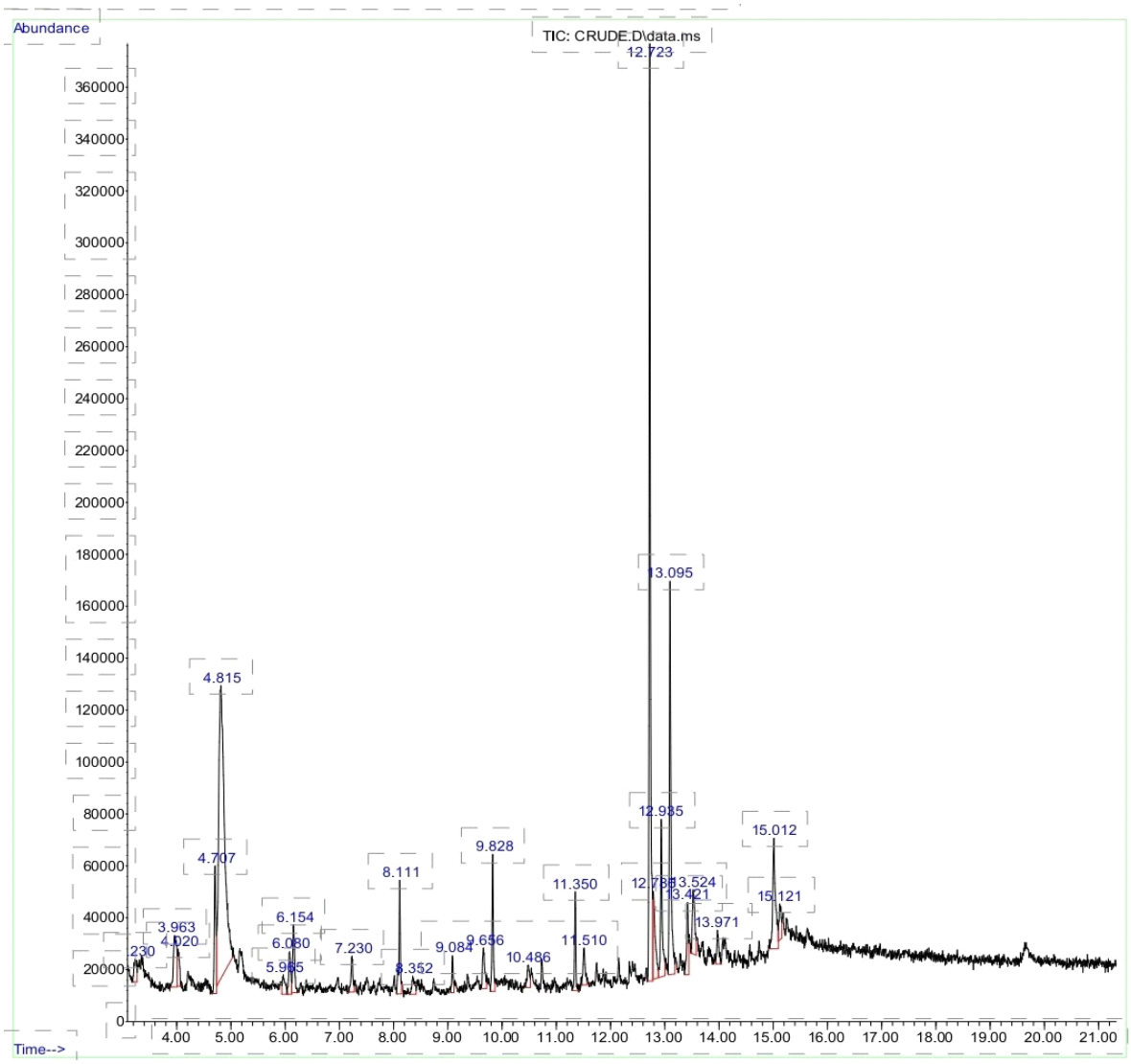

Figure 1: GC-MS chromatogram of N-Crude fraction of *Newbouldia laevis* leaves

Table 6: List of compounds identified in the green fraction (N-Green) of *N. laevis* leaves

S/N	RT	Area%	Compound name	CAS#	Molecular Formula	MW (g/mol)
1	9.358	1.39	Tridecane, 1-iodo-	035599-77-0	C13H27I	310.2579
			2-Bromotetradecane	074036-95-6	C14H29Br	277.28
			Carbonic acid, eicosyl vinyl ester	1000382-54-3	C23H44O3	368.5937
2	9.639	2.64	2,4-Di-tert-butylphenol	000096-76-4	C14H22O	206.32
			2,4-Di-tert-butylphenol	000096-76-4		
			2,4-Di-tert-butylphenol	000096-76-4		
3	11.739	1.71	Benzene, (1-pentylheptyl)-	002719-62-2	C18H30	246.4308
			Benzene, (1-pentylheptyl)-	002719-62-2		
			Benzene, (1-pentylheptyl)-	002719-62-2		
4	11.984	1.52	Benzene, (1-propylnonyl)-	002719-64-4	C18H30	246.4
			Benzene, (1-propylnonyl)-	002719-64-4		
			Benzene, (1-propylnonyl)-	002719-64-4		
5	12.122	1.98	Benzene, (1-ethyldecyl)-	02400-00-2	C18H30	246.4308
			Benzene, (1-ethyldecyl)-	02400-00-2		
			Benzene, (1-ethyldecyl)-	02400-00-2		
6	12.482	1.62	Benzene, (1-methylundecyl)-	002719-61-1	C18H30	246.4
7	12.631	2.56	Benzene, (1-pentyloctyl)-	2400-01-03	C19H32	260.5
8	12.717	34.21	Neophytadiene	000504-96-1	C20H38	278.5
			Bicyclo[3.1.1]heptane, 2,6,6-thyl-, (1.alpha.,2.beta.,5.alpha.)	006876-13-7	C10H18	138.25
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	000473-55-2		
9	12.774	3.38	Carbonic acid, pentadecyl prop-1-e n-2-yl ester	1000382-91-0	C19H36O3	312.4873

			7-Octen-2-one, 6-methyl-	035215-49-7	C9H16O	140.2227
			2-Pentadecanone, 6,10,14-trimethyl	000502-69-2	C18H36O	268.4778
10	12.809	1.92	Benzene, (1-propyldecyl)-	004534-51-4	C19H32	260.4574
			Benzene, (1-propylnonyl)-	002719-64-4	C18H30	246.4308
11	12.929	6.85	1-Hexadecyne	000629-74-3	$C_{16}H_{30}$	222.4094
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	000473-55-2	$C_{10}H_{18}$	138.2499
			Neophytadiene	000504-96-1	$C_{20}H_{38}$	278.52
12	13.032	1.33	Benzene, (1-ethylundecyl)-	004534-52-5	$C_{19}H_{32}$	260.4574
			Benzene, (1-ethyldecyl)-	002400-00-2	$C_{18}H_{30}$	246.4308
			Benzene, (1-ethylnonyl)-	004536-87-2	$C_{17}H_{28}$	232.4042
13	13.089	15.53	Cyclohexane, 1-methyl-4-(1-methyle thenyl)- , trans-	001124-25-0	C10H18	138.2499
			3,7,11,15-Tetramethyl-2-hexadecen- 1-ol	102608-53-7	C20H40O	296.531
			Cyclohexanol, 1-ethynyl-	000078-27-3	C8H12O	124.1803
14	13.386	1.2	Benzene, (1-methyldodecyl)-	004534-53-6	C19H32	260.4574
			Benzene, 1-methyl-3-propyl-	001074-43-7	C10H14	134.2182
15	13.449	2.39	1-Fluorononane	000463-18-3	C9H19F	146.2456
			1-Dodecanol, 2-hexyl-	110225-00-8	C16H34O	242.4406
			1-Decanol, 2-hexyl-	002425-77-6	C16H34O	242.4406
16	14.989	16.9	Phytol	000150-86-7	C20H40O	296.531
			Phytol, acetate	1000375-01-4	C22H42O2	338.5677
			Hexadecyl pentyl ether	1000406-41-8	C21H44O	312.5735
17	19.646	2.87	Bis(2-ethylhexyl) phthalate	000117-81-7	C24H38O4	390.5561
			Mono(2-ethylhexyl) phthalate	004376-20-9	C16H22O4	278.3435

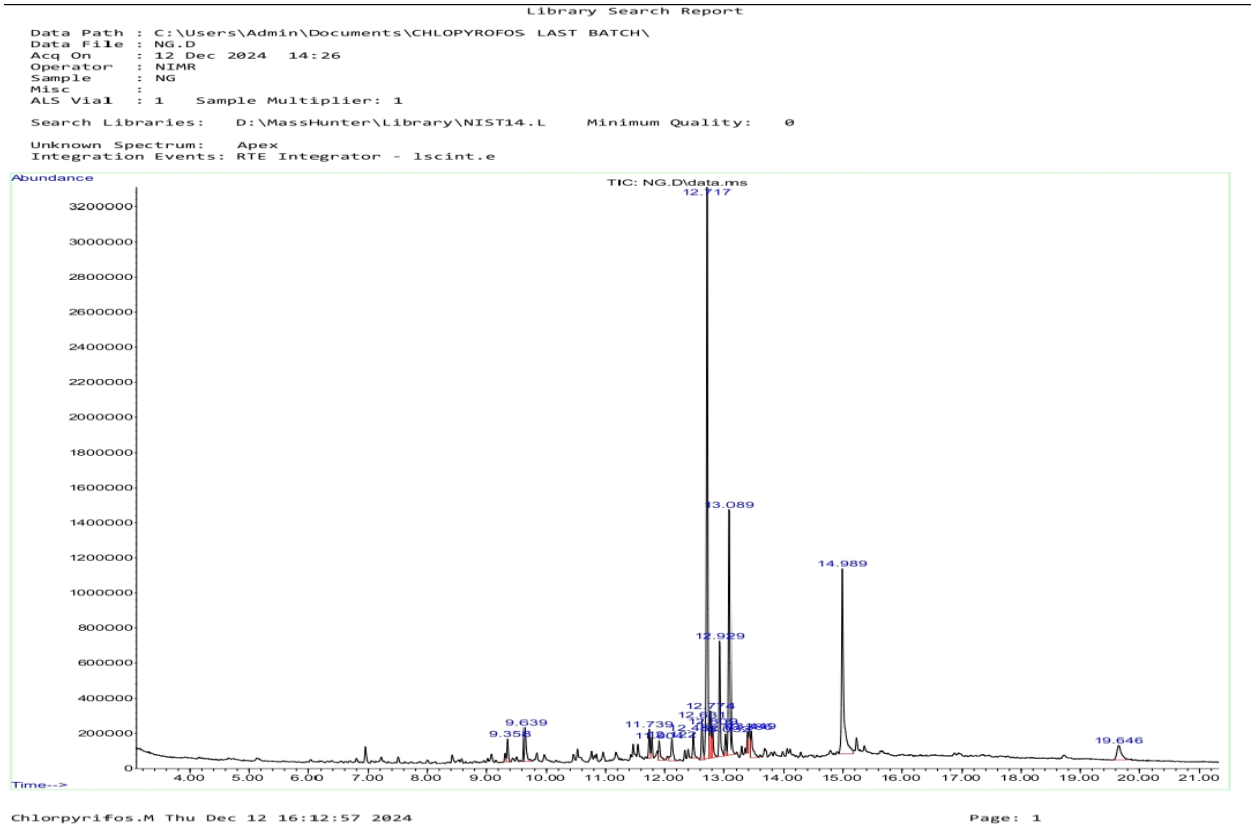

Figure 2 : GC-MS chromatogram of N-Green fraction of *Newbouldia laevis* leaves

Table 7: List of compounds identified in the pink fraction (N-Pink) of *N. laevis* leaves

S/N	RT	Area%	Compound name	CAS#	Molecular Formula	MW (g/mol)
1	5.124	19.76	Isophorone	000078-59-1	C9H14O	138.21
2	6.955	6.62	Hexadecane	000544-76-3	C16H34	226.44
			Octane, 2-methyl-	003221-61-2	C9H20	128.25
3	9.358	10.88	Octadecane	000593-45-3	C18H38	254.5
			Decane, 3-bromo-	030571-71-2	C10H21Br	221.18
			Undecane, 2,9-dimethyl-	017301-26-7	C13H28	184.36
4	9.65	12.61	2,4-Di-tert-butylphenol	000096-76-4	C14H22O	206.32
5	9.959	33.65	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	015356-74-8	C11H16O2	189.24
			2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	017092-92-1		
6	11.464	8.53	Heptacosane	000593-49-7	C27H	380.7335
			Octacosane	0630-02-04	C28H	394.7601
			2-methyloctacosane	1000376-72-8	C29H60	408.8
7	12.723	7.96	Tetradecanal	000124-25-4	C14H28O	212.3715
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	015356-70-4	C10H20O	156.2652
			Cyclohexene, 1,4-dimethyl-	002808-79-9	C8H	110.1968

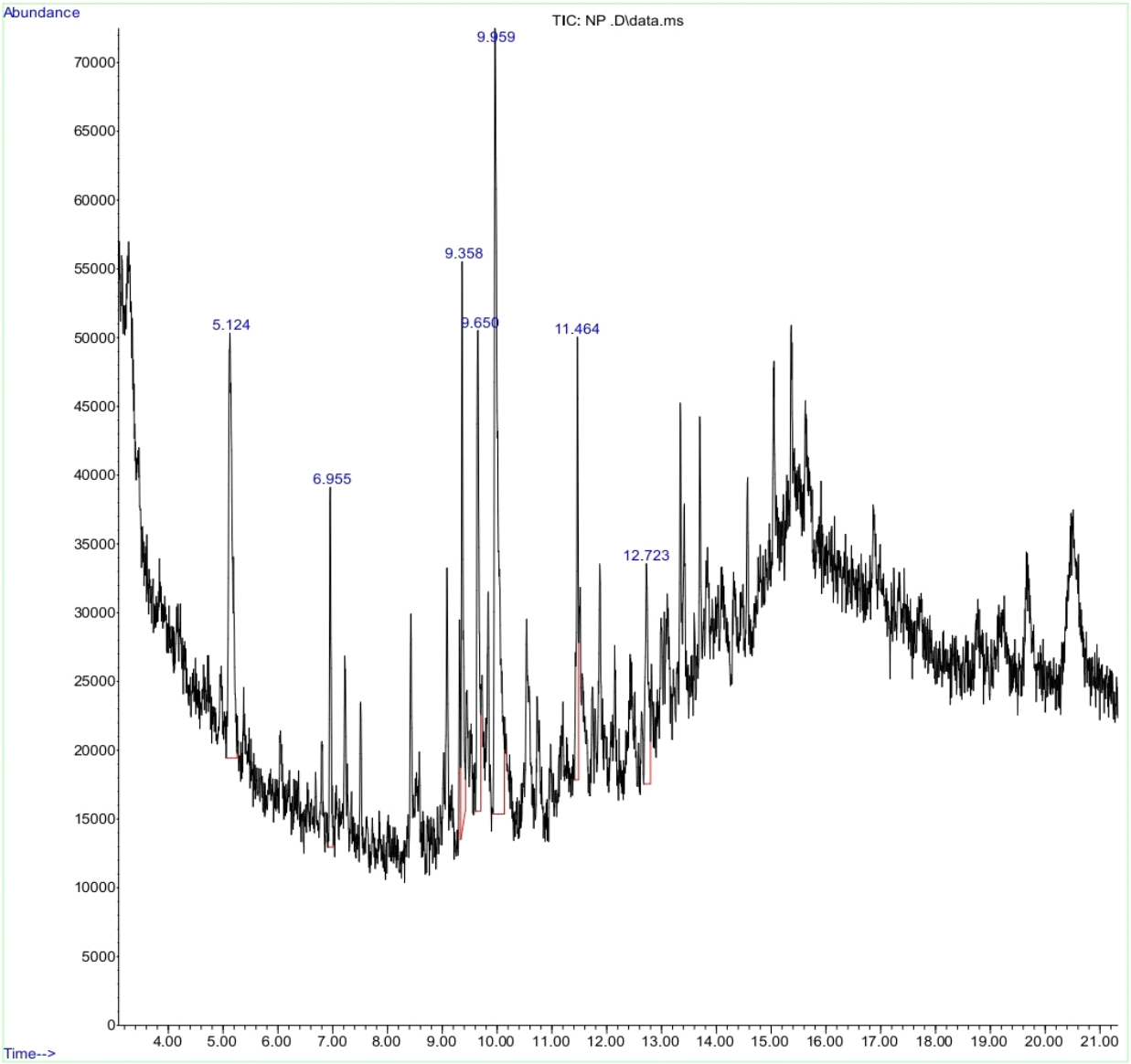

Figure 3: GC-MS chromatogram of N-Pink fraction of *Newbouldia laevis* leaves

Table 8: List of compounds identified in the yellow fraction (N-Yellow) of *N. laevis* leaves

S/N	RT	Area%	Compound name	CAS#	Molecular Formula	MW (g/mol)
1	5.113	30.64	Isophorone	000078-59-1	C9H14O	138.21
			Isophorone	000078-59-1		
			1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene	106251-09-6	C8H14N2	138.21
2	5.839	7.04	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	015356-70-4	C10H20O	156.2652
			Levomenthol	002216-51-5	C10H20O	156.2652
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,5. beta.)]-	002216-52-6	C10H20O	156.2652
3	6.955	8.24	Tridecane	000629-50-5	C13H28	184.3614
			Carbonic acid, eicosyl vinyl ester	1000382-54-3	C23H4403	368.5937
			Hexadecane	000544-76-3	C16H34	226.4412
4	8.42	6.07	10-Methylnonadecane	056862-62-5	C20H42	282.5475
			Pentadecane	000629-62-9	C15H32	212.4146
			Hexadecane	000544-76-3		
5	9.359	10.27	Heneicosane	000629-94-7	C21H44	296.5741
			Heptadecane	000629-78-7	C17H36	240.4677
			2-Bromotetradecane	074036-95-6	C14H29Br	277.28
6	9.645	8.04	2,4-Di-tert-butylphenol	000096-76-4	C14H22O	206.3239
7	11.464	10.2	Hexacosane	000630-01-3	C26H54	366.7
			Heneicosane	000629-94-7	C21H44	296.5741
			Heptadecane	000629-78-7	C17H36	240.4677
8	12.7	7.16	Benzene, (1-butyloctyl)-	002719-63-3	C18H30	246.4308
			Benzene, (1-butylpentyl)-	020216-88-0	C15H24	204.3511
			Benzene, (1-butylnonyl)-	004534-50-3	C19H32	260.4574
9	12.815	6.29	Benzene, (1-propyldecyl)-	004534-51-4	C19H32	260.4574
			Benzene, (1-propylheptadecyl)-	002400-03-5	C26H46	358.6434
			Benzene, (1-ethylundecyl)-	004534-52-5	C19H32	260.4574
10	13.398	6.04	Hexadecane, 1-chloro-	004860-03-1	C16H33Cl	260.886
			1-Octadecanesulphonyl chloride	1000342-70-4	C18H37ClO2S	353.003
			Octadecane, 1-chloro-	003386-33-2	C18H37Cl	288.939

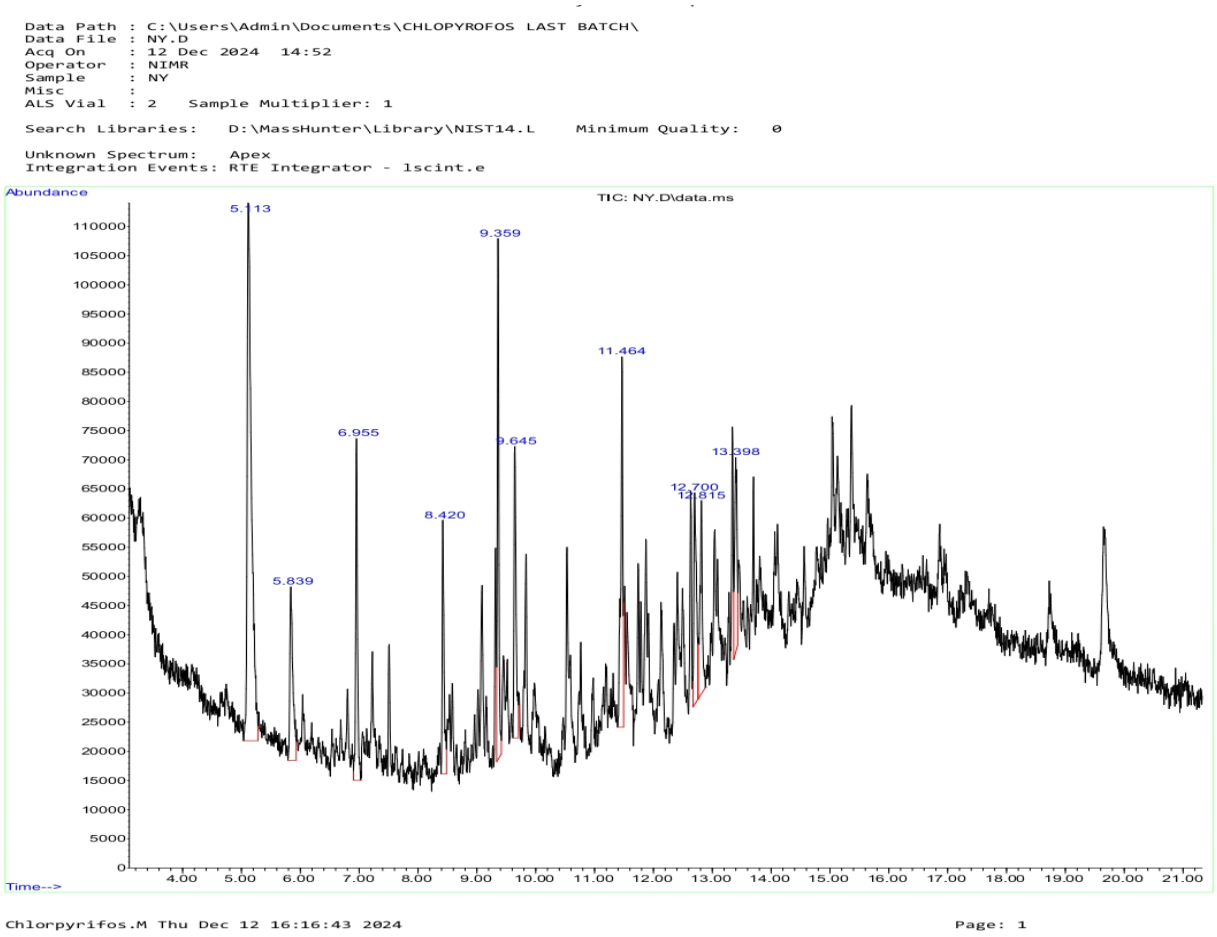

Figure 4: GC-MS chromatogram of N-Yellow fraction of *Newbouldia laevis* leaves

DISCUSSION

Findings from this work indicate that the leaves of *Newbouldia laevis* contain bioactive compounds which possess anticonvulsant and sedative properties. Over the years, screening medicinal plants for biological properties has become popular among researchers in the search for new lead molecules for drug development. *Newbouldia laevis* is a plant herbalists believe has medicinal properties and is frequently used as herbal remedies for various health problems, including epilepsy. Many of its therapeutic benefits have been validated by scientific investigations. In this study, anticonvulsant effects of the ethanol crude extract of *Newbouldia laevis* leaves and the three fractions obtained from its chromatographic separation were evaluated. The crude extract and the fractions were also subjected to GC-MS analysis to identify the bioactive compounds present in the leaves of the plant.

The significant reduction in sleep latency and the prolonged sleep duration caused by the crude extract and the fractions in sodium barbital hypnosis test indicate that *N. laevis* has depressant and sedative effects. This agrees with the previous report on the sedative effect of crude extract of *N. laevis* leaves[10]. N-Crude, N-Green, and N-Pink likewise increased seizure latency and reduced seizure duration in picrotoxin-induced convulsion. This suggests that they possess anticonvulsant properties[17]. Previous studies have also shown that the crude extract of leaves *Newbouldia laevis* has anticonvulsant effects[18]. The anticonvulsant effects observed with N-Crude, N-Green, and N-Pink might be due to interaction of bioactive compounds present in *N. laevis* with gamma aminobutyric acid (GABA) receptors. GABA is an inhibitory neurotransmitter. It is present in many parts of the brain. Activation of GABA receptors results in inhibition of neuronal firing, while inhibition of the receptors lead to enhanced neuronal firing and excitatory activities[19]. Activation of GABA receptors by the compounds in *N. laevis* may be the mechanism by which N-Crude, N-Green, and N-Pink produced their sedative and anticonvulsant effects[20]. This also likely explains the protection against mortality provided by *N. laevis* extracts. The extracts failed to protect mice against strychnine-induced convulsion and death. This indicates that they were unable to modulate glycine receptors which are the binding sites of strychnine[21].

GC-MS analysis of N-Crude revealed the presence of 26 compounds. Benzoic acid methyl ester, neophytadiene, phytol, isophytol, and octamethylcyclotetrasiloxane (D4) are the more abundant compounds among those identified.

Neophytadiene is a diterpene which interacts with GABA receptors to produce sedative, anxiolytic and anticonvulsant effects[22, 23]. The high percentage of neophytadiene present in N-Crude and N-Green possibly explains their anticonvulsant activities observed in picrotoxin-induced convulsion. Neophytadiene was also reported to have antioxidant property[22]. This may also contribute indirectly to its anticonvulsant effect[24]. Oxidative stress has been reported to contribute to pathogenesis of some seizure types, and in such cases, antioxidants have demonstrated the ability to reduce seizures[25].

GC-MS analysis did not reveal the presence of neophytadiene in N-Pink. However, it contains high level of 2(4H)-benzofuranone. It is in fact the most abundant compound in N-Pink. In the literature, compounds with benzofuran nucleus were reported to possess anti-inflammatory, antioxidant, and anticonvulsant properties[26]. The presence of 2(4H)-benzofuranone in the leaves of *N. laevis* probably accounts for the anticonvulsant effect of N-Pink.

Analysis of N-Yellow showed that it contains neither neophytadiene nor 2(4H)-benzofuranone. The most abundant compound identified in N-Yellow is isophorone. Lack of significant anticonvulsant effect of N-Yellow suggests that isophorone and other compounds in the fraction do not possess anticonvulsant property.

Another bioactive compound present in appreciable quantity in the leaves of *N. laevis* is 1-pentadecanol, 2,6,10,14-tetramethyl, also known as phytol. This compound has been reported to exhibit anticonvulsant activity by modulating neurotransmitter systems[27, 28]. Phytol also possesses antioxidant, anti-inflammatory, and anxiolytic properties[29]. Phytol and isophytol were detected in N-Crude and N-Green. These compounds could also have contributed to the anticonvulsant and sedative effects of the fractions.

CONCLUSION

The results of this work showed that the leaves of *N. laevis* are rich in bioactive compounds which possess anticonvulsant and sedative properties. Synergistic and additive effects of neophytadiene, 2(4H)-benzofuranone, Phytol, and other phytoconstituents in the leaves of *N. laevis* are likely responsible for its sedative and anticonvulsant activities.

Acknowledgment

The authors acknowledge the assistance provided by the technical staff of the Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso

REFERENCES

1. Anwar H, Khan QU, Nadeem N, Pervaiz I, Ali M, Cheema FF. Epileptic seizures. Discoveries (Craiova) 2020; 8(2):e110. doi: 10.15190/d.2020.7
2. Strzelczyk A, Aledo-Serrano A, Coppola A, Didelot A, Bates E, Sainz-Fuertes R, Lawthom C. The impact of epilepsy on quality of life: Findings from a European survey. Epilepsy Behav. 2023; 142:109179. doi: 10.1016/j.yebeh.2023.109179
3. Liu J, Zhang P, Zou Q, Liang J, Chen Y, Cai Y, Li S, Li J, Su J, Li Q. Status of epilepsy in the tropics: An overlooked perspective. Epilepsia Open 2023; 8(1):32-45. doi: 10.1002/epi4.12686
4. Pfisterer U, Petukhov V, Demharter S, Meichsner J, Thompson JJ, Batiuk MY, Asenjo Martinez A, Vasistha NA, Thakur A, Mikkelsen J, Adorjan I, Pinborg LH, Pers TH, von Engelhardt J, Kharchenko PV, Khodosevich K. Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis. Nat Commun. 2020;11(1):5038. doi: 10.1038/s41467-020-18752-7
5. Martz GU, Bertram EH. Secondary Generalization of Focal Onset Seizures. In: Atlas of Epilepsies Panayiotopoulos, C.P. (eds) Atlas of Epilepsies. 1st ed. London Springer, 2010. p. 219-223
6. Beghi E. The Epidemiology of Epilepsy. Neuroepidemiology 2020; 54(2):185-191. doi: 10.1159/000503831.-191
7. Orsini A, Valetto A, Bertini V, Esposito M, Carli N, Minassian BA, Bonuccelli A, Peroni D, Michelucci R, Striano P. The best evidence for progressive myoclonic epilepsy: A pathway to precision therapy. Seizure 2019;71:247-257. doi: 10.1016/j.seizure.2019.08.012
8. Sen A, Jette N, Husain M, Sander JW. Epilepsy in older people. Lancet 2020; 395(10225): 735-748 doi: 10.1016/S0140-6736(19)33064-8
9. Löscher W. and Klein P. The Pharmacology and Clinical Efficacy of Antiseizure Medications: From Bromide Salts to Cenobamate and Beyond. CNS Drugs 2021; 35(9):935-963. doi: 10.1007/s40263-021-00827-8
10. Kolawole OT, Adeyeba OA, Awodele O, Wakeel OK, Ayankunle AA. Protective Effects of Crude Extract and Fractions of Newbouldia laevis Leaves in Chemoconvulsant-Induced Seizures. TJNPR 2021 5(1): 217-224. doi.org/10.26538/tjnpr/v5i1.30
11. Kolawole OT, Adeyeba OA, Awodele O, Wakeel OK, Ayankunle AA. Crude and Partition Extracts of Newbouldia laevis Leaves Attenuate

Excitotoxin-induced Stereotypy in Mice. EJMP 2020; 31(18): 29-36.
doi: 10.9734/ejmp/2020/v31i1830341
12. National Research Council. Guide for the Care and Use of Laboratory Animals. National Academies Press, 2011. p. 1-220
13. Vital-Lopez FG, Balkin TJ, Reifman J. Models for predicting sleep latency and sleep duration. Sleep. 2021; 44(5):zsaa263.
doi: 10.1093/sleep/zsaa263
14. Pinar N. Effects of Tempol on Epileptic Activity in Picrotoxin-Induced Epilepsy in Rats. Neurology India 2021; 69(2):426-429.
doi: 10.4103/0028-3886.314542
15. Umukoro S, Omogbiya IA, Eduviere AT. Evaluation of the effect of jobelyn(®) on chemoconvulsants- induced seizure in mice. Basic Clin Neurosci. 2013; 4(2):125-9.
16. Otuokere EI, Akoh OU, Echeme O, Nwadire FC, Nwankwo CI, Egbucha JN, Ammasai K. GC-MS Analysis and Molecular Docking Studies to Identify Potential SARS-CoV-2 Nonstructural Protein Inhibitors from Icacina trichantha Oliv Tubers. TJNPR 2022; 6(8: 1336-1342
doi: 10.26538/tjnpr/v6i8.29
17. Drafor G, Duah E, Ankamah NA, Kpene GE, Mante PK. Investigating the Anticonvulsant Properties of Aqueous Ethanolic Extracts of the Leaves, Roots, and Fruits of Jatropha gossypifolia L. (Euphorbiaceae). Adv Pharmacol Pharm Sci. 2021; 2021: 5547353.
doi: 10.1155/2021/5547353
18. Ukwubile CA, Ikpefan EO, Dibal MY, Umeano VA, Menkiti DN, Kaosi CC, Simon Paul C, Famurewa AC, Nettey H, Yerima TS. 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. J Ethnopharmacol. 2023; 5;314:116632.
doi: 10.1016/j.jep.2023.116632
19. Sears SM, Hewett SJ. Influence of glutamate and GABA transport on brain excitatory/inhibitory balance. Exp Biol Med (Maywood). 2021; 246(9):1069-1083.
doi: 10.1177/1535370221989263
20. AbdulWaris A, Ullah AM., Rafi UR, Rajdoula MR, Bello ST, Alhumaydhi FA. Phytotherapeutic options for the treatment of epilepsy: pharmacology, targets, and mechanism of action. Front Pharmacol. 2024; 24;15:1403232.
doi: 10.3389/fphar.2024.1403232
21. Hussein RA, Ahmed M, Hans-Georg B, Ulrike B. Modulation of Glycine Receptor-Mediated Pain Signaling in vitro and in vivo by Glucose. Front Mol Neurosci. 2019; 12:280.doi: 10.3389/fnmol.2019.00280
22. Gonzalez-Rivera ML, Barragan-Galvez JC, Gasca-Martínez D, Hidalgo-Figueroa S, Isiordia-Espinoza M, Alonso-Castro AJ. In Vivo Neuropharmacological Effects of Neophytadiene. Molecules. 2023; 28(8):3457.
doi: 10.3390/molecules28083457
23. Manalu LP, Adinegoro H, Yustiningsih N, Astuti LR, Maisaroh PW, Subandrio Pongtuluran OB, Atmaji P, Hidayat T, Henanto H, Asgar A, Nasori AS, Triyono A, Elya, B, Arif AB. Impact of Drying Methods on Bioactive Compounds and Antioxidant Properties of Kalanchoe ceratophylla. Scientifica (Cairo). 2025; 2025:7146758.
doi: 10.1155/sci5/7146758
24. Rajeswaran S, and Rajan DK. Neophytadiene: Biological activities and drug development prospects. Phytomedicine: 2025; 143:156872.
doi: 10.1016/j.phymed.2025.156872.
25. Ismy J, Soebadi A, Mangunatmadja I, Monica M, Sari TT, Yuliarti K. Role of antioxidants in reducing oxidative stress and seizure frequency in drug-resistant epileptic patients. Narra J. 2024; 4(2):e790.
doi: 10.52225/narra.v4i2.790
26. Miao YH, Hu YH, Yang J, Liu T, Sun J, Wang XJ. Natural source, bioactivity and synthesis of benzofuran derivatives. RSC advances 2019; 9(47).
doi: 27510-27540.10.1039/C9RA04917G
27. Costa JP, Ferreira PB, De Sousa DP, Jordan J, Freitas RM. Anticonvulsant effect of phytol in a pilocarpine model in mice. Neurosci Lett. 2012; 523(2):115-8.
doi: 10.1016/j.neulet.2012.06.055
28. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Shill MC, Karmakar UK, Yarla NS, Khan IN, Billah MM, Pieczynska MD, Gokhan ZG, Malainer C, Nicoletti F, Gulei D, Berindan-Neagoe I, Apostolov A, Banach AM, Yeung WK, El-Demerdash A, Xiao J, Dey P, Yele S, Jó?wik A, Strza?kowska N, Marchewka J, Rengasamy KRR, Horba?czuk J, Kamal MA, Mubarak MS, Mishra SK, Shilpi JA, Atanasov AG. Phytol: A review of biomedical activities. Food Chem Toxicol. 2018; 121:82-94.
doi: 10.1016/j.fct.2018.08.032
29. Islam MT, Ayatollahi S.A, Zihad SMNK, Sifat N, Khan MR, Paul A, Salehi B, Islam T, Mubarak MS, Martins N, Sharifi-Rad J. Phytol anti-inflammatory activity: Pre-clinical assessment and possible mechanism of action elucidation. Cell Mol Biol (Noisy-le-grand). 2020; 66(4):264-269. doi.org/10.14715/cmb/2020.66.4.3