

CHOLINESTERASES INHIBITION AND ANTIOXIDANT POTENTIALS OF NEWBOULDIA LAEVIS AND FICUS EXASPERATA VAHL LEAF EXTRACT

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Abstract

Cholinesterases inhibition and *in vitro* antioxidant potentials of *Newbouldia laevis* and *Ficus exasperata* leaf extracts were studied using standard methods. Preliminary screening of the leaf extract showed the presence of alkaloids, tannins and saponin and terpenoid. 4.26mg/GAEq, 0.24mg/QEq and 11.313mg/GAEq, 0.124mg/QEq were the Total phenolic and flavonoid content for *N. laevis* and *F. exasperata* respectively. Both extracts showed DPPH scavenging ability above 50% at lower concentrations of the extract. The extracts showed good ferric-cyanide reducing antioxidant power. Hydroxyl radical scavenging ability was more 80%, while nitric oxide radical scavenging activities of the extracts were more 50%. Also, 81 and 113 percentage inhibition of acetylcholinesterase and butrylcholinesterase was obtained for *N. laevis* leaf extract, while *F. exasperata* leaf extract had 92.30 % inhibition of acetylcholinesterase and 77% of butrylcholinesterase respectively. GC-MS and FT-IR identified the presence of diverse compounds and functional groups in both plant extracts.

Keywords: Oxidative stress, antioxidant, Cholinesterases Inhibition, *Newbouldia laevis*, *Ficus exasperata*, Leaf Extract.

Introduction

In developing countries Nigeria inclusive, many plants materials are important resources used in the treatment of infectious diseases and other ailments. Other functions of plant extracts include antidiabetic and antihyperlipidemic (Ibrahim, Ali, Saleh, El nagger, Abd & Seham 2015), food supplement (Francescato, Debenedetti, Schwanz, Bassani, Ame´ lia & Henriques 2013) and component of functional foods (Oniszczuk, Olech, Oniszczuk, Wojtunik-kulesza & Wojtowicz 2016). One of such plants is *Newbouldia laevis*. It has been used in the treatment of syphilis and dysentery (Usman & Osuji, 2007) and wound dressing (Iwu, 2000). Also, the plant extract has been reported to possess other pharmacological activities such as antibacterial (Akerle, Ayinde & Ngiagah 2011; Ejele, Duru, Ogukwe & Iwu 2012) and antioxidant activity (Ogunlana & Ogunlana, 2008). These pharmacological properties could be attributed to the phytochemicals present (Omeje, Omeje & Asomadu, 2018a), which are responsible for the color, flavor and taste of plants (Densie, 2013). Anaduaka, Ogugua, Egba & Apeh (2013) earlier reported the presence of flavonoids and alkaloids in the leaf extract of *N. laevis*.

Plants play essential roles in our daily activities, ranging from its use as food, medicine and in postharvest storage of some agro commodities. One of the major problems facing farmers is postharvest storage. Though there are many insecticides used against pests, this does not happen without its attendant problems of poisoning and toxicity related illnesses in animals when consumed. Hence, the use of plant materials for this purpose becomes necessary. One of the plants used for this purpose is *Ficus exasperata*.

Ficus exasperata is commonly known as sand paper tree (Enogieru, Charles, Omoruyi, Momodu & Ezeuko 2015). It has been reported to possess many pharmacological potentials such as hepato-protection (Enogieru et al., 2015) and anti-androgenic potential (Usang, Ibor, Owolodun, Eleng, Ujong & Udoh 2015). Hence, in this study detailed antioxidant and phytochemical potentials of chloroform extracts of *N. laevis* and *F. exasperata* were reported.

Methods

Plants materials

Leaves of *N. laevis* and *F. exasperata* collected from their natural habitats at Edem-ani, Nsukka LGA, Nigeria and identified by a taxonomist. Freshly collected green leaves were air-dried in a shade, before pulverizing it into fine powder using an electric blender. The powdered samples were subjected to extraction with chloroform at room temperature for 72 hours, after which the whole mixtures were filtered and concentrated.

Chemicals

Acetylthiocholine iodide, butyrylthiocholine iodide, 5,5' dithio-bis [2-nitrobenzoic acid] (DTNB). 2, 2-Diphenyl-1-picrylhydrazyl, Acetylcholinesterase and Butrylcholinesterase were products of Sigma-Aldrich (Germany). Other chemicals were of analytical grades.

Extraction Method

The extraction processes involved grinding the leaves of *N. laevis* and *F. exasperata* into fine powder and soaked with 80 % chloroform in a glass container. The extraction set up was left to stand for 72 hrs with intermittent shaking. On the expiration of 72 hr., the solution was filtered with calico cloth and No. 1 Whatman filter paper, before concentrating the extract using Soxhlet extraction apparatus at 45 °C. The concentrate was stored at 4 °C for further analysis.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of the extract was carried out using AOAC method (2010).

Total Flavonoid Content Determination of the samples:

Total flavonoid content of the leaf extracts was determined using the method described by Kumaran & Karunakaran (2007). TFC of the extract was expressed as quercetin equivalents.

Determination of Total Phenolic Content

The quantification of total phenolic in the plant samples was carried out using the Folin-Ciocalteu method as described by Singleton, Orthofer & Lamuela-raventos (1999). The Gallic acid standard curve was used to quantify total phenolic contents as mg of Gallic acid equivalent (GAE)/g of dried extract.

Acetylcholinesterase and Butrylcholinesterase inhibitory activity Assay

Acetylcholinesterase and butrylcholinesterase inhibitory activity of the plant extracts was assayed

as described by Ellman, Courtney, Andres & Featherson (1961). Acetylthiocholine iodide and butyrylcholine iodide were used as substrates. Each extract concentration was mixed with enzyme solution (500 µl) and incubated at 37°C for 30 min. The absorbance was taken at 410 nm after adding Ellman's reaction mixture (3.5 ml; 0.5 mM acetylcholine and butyrylthiocholine, 1 mM DTNB respectively) in 50 mM sodium phosphate buffer (pH 8.0).

The percentage of enzyme inhibition was calculated using the following formula:

$$\left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100$$

Determination of DPPH radical scavenging activity

The *in vitro* antioxidant activity by DPPH assay was assessed using the stable free radical DPPH. To 1 ml of various concentrations of the plant extracts, 1 ml of DPPH (0.1 mM) was added in the test tube. After incubation for 30 min in the dark at room temperature, absorbance was recorded at 517 nm. The percent DPPH radical scavenging was calculated with the equation: DPPH radical scavenging (%) = $\left[\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \right] \times 100$. Ascorbic acid was used as the standard.

Determination of hydroxyl radical scavenging activity

The hydroxyl radical (-OH) scavenging activity was measured by the method of Jin, Cai, Li & Zhao (1996). The scavenging activity on -OH was calculated by the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs. sample} - \text{Abs. blank}}{\text{Abs. so} - \text{Abs. blank}} \times 100$$

where Abs. so is the absorbance of the deionized water instead of H₂O₂ and sample in the assay system.

Anti-hemolytic assay

Anti-hemolytic activity was determined using the method as described by Baydar, Ozkan & Yasar (2007).

Tannic acid was used as standard.

$$A_{\text{Control}} - A_{\text{Test}} \text{ Calculation} = A_{\text{Control}}$$

Ferric cyanide (Fe³⁺) reducing antioxidant power assay

Reducing power of the extracts was measured by the direct reduction of Fe³⁺(CN⁻)₆ to Fe²⁺(CN⁻)₆ by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition

of excess Fe³⁺ (Oyaizu, 1986), and the absorbance measured spectrophotometrically at 700 nm.

Nitric oxide radical scavenging

The nitric oxide radical scavenging capacity of the fractions was measured by Griess reaction Sangermeswaran, Balkrishnan, Deshraj & Jayakar (2009). Ascorbic acid was used as reference standard.

Percentage of inhibition = $\left[\frac{A_0 - A_1}{A_0} \right] \times 100$, Where A₀ is the absorbance of sodium nitroprusside in PBS (without extracts and ascorbic acids) and A₁ is the absorbance in the presence of the fractions and ascorbic acid.

Methods for GC-MS and FT-IR

One of the efficient methods in the identification of organic compounds is the use of Gas Chromatography-Mass Spectroscopy (GCMS-QP2010 plus Shimadzu, Japan). The unknown organic compounds in the chloroform leaf extract mixture found in the leaf were matched with the National Institute of Standards and Technology (NIST) library. The Fourier transform infrared spectroscopy (FT-IR-8400S Shimadzu, Japan) was used. The leaf was dried in the oven at 60°C and ground into fine powder, using electric blender. From the prepared sample, two milligrams of the sample was weighed and mixed with 100 mg KBr (FT-IR grade) before compressing it to prepare a salt-disc (3 mm diameter). The disc was immediately transferred to the sample holder and FT-IR spectra were recorded in the absorption range between 500 and 4000⁻¹cm.

Results

Qualitative phytochemical analysis of *Newbouldia laevis* and *Ficus exasperata*

Qualitative phytochemical analysis of *Newbouldia laevis* detected the presence of alkaloid, tannin and saponin, while glycoside, flavonoid, resin, steroid and terpenoid were not detected. Similarly, tannin and terpenoid were detected in the chloroform leaf extract of *Ficus exasperata*.

Quantitative phytochemical analysis of *Newbouldia laevis* and *Ficus exasperata*

Also, quantitative phytochemical analysis carried out on *Newbouldia laevis* showed the composition the total phenolic content and the total flavonoid content. The total phenolic content was obtained as 4.62mg/GAEq while the total flavonoid content was obtained to be 0.24mg/QEq. For *F. exasperata*, 0.124 mg/ QEq and 11.313 mg/GAEq were obtained as

the total flavonoid and phenolic contents respectively.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Free radical (DPPH) scavenging ability of *Newbouldia laevis* and *Ficus exasperata* were carried out and their results were presented in figure 2. The % inhibition was calculated at different concentrations of 10, 20, 30, 100 and 200 µg/ml, with corresponding % inhibitions of 59, 56, 45, 40 and 12 respectively. Also, % inhibition of 78.43, 68.74, 82.70, 48.55 and 25.67 were observed for *F. exasperata* at the same concentration. The result shows that inhibition decreased as the concentration of the extracts increased.

Ferric-cyanide (Fe²⁺) reducing antioxidant power assay of *N. laevis* and *F. exasperata* leaf extract

The ferric-cyanide reducing power was obtained and the results are presented in Fig. 1. There was increase in the absorbance of the reaction mixture as concentration of the extracts increased. This indicates that both leaves have reduction capabilities.

Nitric Oxide radical scavenging

The nitric oxide radical scavenging abilities of the chloroform leaf extracts of *N. laevis* and *F. exasperata* were analyzed and the result shown in Table 1. *N. laevis* extract gave more than 50 % nitric oxide inhibition at all the concentrations of 10, 20, 30, 100 and 200 µg/ml studied. *F. exasperata* leaf extract nitric scavenging ability was observed to be less than 0 % as shown in Table 1.

Hydroxyl radical scavenging and Metal (Fe²⁺) chelating activity assay

The hydroxyl radical scavenging ability of *Newbouldia laevis* leaf and *F. exasperata* were calculated to be 94 and 98.7 % respectively. The metal (Fe²⁺) chelating ability of the chloroform extract of *Newbouldia laevis* and *F. exasperata* were obtained as 5668 and 2065 respectively.

Anti-hemolytic assay

The anti-hemolytic assay was performed across various concentrations and the % inhibitions for both leaf extracts obtained are presented in the Table 2. At the concentrations studied, more than 50 % anti-hemolytic inhibition was observed. While *F. exasperata* leaf extract gave 51.72, 73.56 and 62.56 % at 100, 200 and 300 µg/ml respectively. It was observed that the % inhibition decreased as the

extract concentration increased to 500 and 600 µg/ml.

Enzyme activity assay

The percentage inhibition of cholinesterases by *N. laevis* and *F. exasperata* leaf extract was studied. 81 and 113% inhibition were obtained for acetylcholinesterase and butyrylcholinesterase respectively by *N. laevis* while 92.30 and 77 % inhibitions was produced for acetylcholinesterase and butyrylcholinesterase by *F. exasperata*.

Gas chromatography- Mass spectroscopy (GC-MS) analysis on *Newbouldia laevis*

A total of thirteen (13) compounds were identified in the chloroform leaf extract of *Newbouldia laevis* as shown in Table 6. Similarly, the chromatogram of the leaf extract of the *Ficus exasperata vahl* showed seventeen (17) compounds as presented in Table 5.

Discussion

Phytochemical screening of *Newbouldia laevis* showed the presence of alkaloids, tannins and saponins in the chloroform extract of the leaves. The detection of alkaloids and saponins in the leaf extract was in line with the report of Anaduaka et al. (2013), in their work in which ethanol was used as solvent. Also, they reported the presence of tannins, steroids, glycosides and terpenoids which were not detected in this study. This could be attributed to the difference in the solvents used. Usman & Osuji (2007) and Azando, Hounzangbe-adote, Olounlade, Brunet, Fabre, Valentin & Hoste (2011) detected the presence of tannins, terpenoids, flavonoids, steroids and cardiac glycosides in the leaf extract of *Newbouldia laevis*. Similarly, Usman & Osuji (2007) did not detect the presence of alkaloids and saponins in their study. However, Dandjesso, Klotee, Dougnon, Segbo, Gbaguidi, Fah, Fanou, Loko & Dramane (2012) reported the absence of alkaloids, flavonoids, saponins and steroids on the leaf extract. The report of Ayoola, Yusuf & Oki (2016) detected the presence of flavonoid, terpenoid, tannin, alkaloid, phytic acid, trypsin inhibitor, phenol, antioxidants, carotenoid, oxalate and cyanide. Josiah & Bartholomew (2015) also detected alkaloids, saponins, tannins, cardiac and steroidal glycosides, flavonoids, other metabolites were amino acids and vitamins A, C and E. The variations in the reports could be attributed to the differences

in solvents used, geographical locations and season or time of plant material harvest.

Phytochemicals are chemicals produced by plants through primary or secondary metabolism (Molyneux, Lee, Gardner, Panter & James, 2007), which are known to have antioxidant, anti-inflammatory, antibacterial, anticarcinogenic and antimutagenic properties (McGuire, 2011). Alkaloids have many beneficial properties such as anti-inflammatory, antioxidant, protects from diabetes, hyperlipidemia and obesity (Li, Lo, Pan, Lai & Ho, 2013). The presence of these phytochemicals could be responsible for the pharmacological activities observed in the use of *Newbouldia laevis* in folk medicine.

Also, qualitative analysis of the chloroform extract of the *Ficus exasperate vahl* leaf, showed the presence of tannin and terpenoid. In the work of Ughachukwu, Ezenyeaku, Ezeagwuna & Anahalu (2012), flavonoids, glycosides, phenolic acids, saponin, alkaloids and steroids were detected, though the solvent used was ethanol. So the difference in the phytochemicals detected could be attributed to the solvent of extractions used. A report by Kazeem, Oyedapo, Raimi & Adu (2013) showed the presence of different phytochemicals detected in the leaf extract of *Ficus exasperata vahl* when different solvents were used for extraction. Similarly, chloroform extract of *Ficus exasperata vahl* leaf showed the presence of tannin, flavonoids, saponin, steroid, cyanogenic glycosides, triterpenes and xantho proteins (Olaniran et al., 2017). From the above experiments, organic solvent might have an effect on the qualitative phytochemical analysis of the *Ficus exasperate vahl* leaf extract.

Newbouldia laevis showed a total phenolic content of 4.62mgGAE/g. The total phenolic content of *N. laevis* as reported by Tuo, Béourou, Touré, Ouattara, Meité, Ako, Yao, Koffi, Coulibay, Coulibaly & Djaman (2015) was 6.32mgGAE/g, which is higher than the report of this research. While Ayoola et al., (2016), reported total phenolic content of 2.86 mgGAE/g which is lower to the value obtained.

The total flavonoid content obtained was 0.24mgQE/g. According to Ayoola et al. (2016), the flavonoid content obtained was 0.15mgQE/g. Tuo et al., (2015) reported total flavonoid content for *N. laevis* as 3.85mgQE/g. The variations could be attributed to the differences in the solvents used by

the researchers and the season of plant material harvest.

Furthermore, total flavonoid contents of the chloroform extract of *Ficus exasperate vahl* leaf extract was 0.124mg/QEq, which is high when compared to the 0.062mg/QEq in the chloroform leaf extract of the same plant as reported by Olaniran et al.(2017) and low when compared to 46.63mg/QEq reported by Saheed, Taofeeq, Taofik, Abduhakeem & Nurain (2015). Ethanolic extract of the same leaf yielded 35.00 mg /REq (Shoib and Shahid, 2015).

The total phenolic content of the chloroform extract of *Ficus exasperata vahl* leaf was 11.313mg/GAEq, which is low when compared to 68.40mg/GAEq (Saheed et al., 2015) and 45.17 mg/GAEq. The concentration is low when compared to the value obtained in the ethanolic extract of *Ficus exasperata vahl* leaf (Shoib and Shahid, 2015). The flavonoid and phenolic concentration of plant material could correlate positively to its antioxidant potentials. Many researchers have opined that these phenolic compounds are capable of scavenging free radicals, hence the presence of these compounds in the plant extracts suggest they have the potentials to chelate these free radicals.

The free radical scavenging potential of the leaf extracts at various concentration so f 10, 20, 30, 100 and 200 µg/ml. The result was presented as percentage inhibition as follows 59, 56, 45, 40 and 12% respectively. The chloroform extract of *Newbouldia laevis* extract showed a strong inhibition of DPPH at low concentration (10µg/ml), which decreased as concentration of the plant extract increased as shown in figure 2. This result suggests that the chloroform extract of *Newbouldia laevis* may possess significant pro-oxidant or free radical generating activities with increase in concentration of the extract, which could be attributed to the low concentration of flavonoids in the extract.

The results of the research conducted by Nithya & Balakrishnan (2011) to determine the free radical scavenging potential of some medicinal plants such as *Hyptissua veolens* (98.06%), *Alpina calcarata* (97.4%), *Ocimum basillicum* (95.08%), and *Passiflora edulis* (95.68%). These leaves, when compared to *Newbouldia laevis*, have a strong inhibition of DPPH radical.

The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry (Huang, Ou & Prior, 2005). The DPPH radical scavenging ability of the chloroform extract of *Ficus exasperata vahl* leaf extract was conducted using different concentrations with 30µg/ml giving the highest percentage inhibition of 82.71. Earlier, Olabukola, Owumi & Oluwatosin (2014) reported that *F. exasperata* extract scavenged DPPH radical by 10.5% at 10µg/ml and 58.7% at 750µg/ml. Ethanol extract of the plant showed a percentage inhibition of 77% at 1.0mg/ml (Uwemedimo et al., 2016).

Ferric-cyanide (Fe³⁺) reducing antioxidant power (FRAP) assay

Ferric-cyanide reducing power of *Newbouldia laevis* leaf extracts were determined using FRAP assay and the result was represented in fig. 1. This method is based on the reduction of ferric complex (Fe³⁺) to blue-colored ferrous complex (Fe²⁺) by the action of electron donating antioxidants (Dudonné, Vitrac, Coutière, Woillez & Mérillon, 2009). It was observed that there was an increase in the absorbance of the reaction mixture as concentration was increased. This suggests that *Newbouldia laevis* has a great reduction capability.

The ferric cyanide reducing power assessment of *Newbouldia laevis* and *Ficus exasperate vahl* leaf extract in chloroform showed increasing absorbance as the concentration increased. Olabukola et al. (2014) and Wonder, Abotsi, George, Ainooson, Ama & Amo-barimah (2010) reported increased absorbance of methanolic extract of *Ficus exasperate vahl* leaf which is in accordance with the result of this study. These results are indicative of the ability of the leaf extract of *Ficus exasperata vahl* to reduce ferric to ferrous state, thereby reducing its reactivity.

Nitric oxide radical scavenging

The results of nitric oxide scavenging activity are given in Table IV. All the concentrations studied (10, 20, 30, 100 and 200 µg/ml) gave more than 50 % nitric oxide radical scavenging ability. Rozina, Sukalayan & Pijush (2013) reported less than 50 % NO scavenging activity of *Triumfetta homboidae* at low concentrations, with high concentrations giving about 50 % NO radical scavenging ability. Josiah & Bartholomew (2015) reported lower NO scavenging activity for *Newbouldia laevis*. For *F. exasperata*, it

was observed that the chloroform extract does not have nitric oxide radical scavenging ability, since all the concentrations studied gave less than 0 % of nitric oxide radical scavenging ability as shown in Table 1.

Hydroxyl radical scavenging activity assay

The percentage inhibition of the hydroxyl radical scavenging activity of *Newbouldia laevis* and *Ficus exasperata vahl* are 94 and 98.7 %. The result obtained is in line with that of Josiah & Bartholomew (2015). They reported more than 50 % scavenging potential of the extract at concentrations above 100 µg/ml. At 300µg/ml, percentage inhibition of the ethanol, butanol and ethyl acetate extracts of *Ocimum americanum* against hydroxyl radicals was 67.95, 66.67 and 46.15% as reported by Aluko, Oloyede, Afolayan (2013). This observation suggests that the extract of *Newbouldia laevis* could be used as an alternative remedy in combating the oxidative activity caused by hydroxyl radical.

The chloroform extract of *F. exasperata vahl* leaf scavenged hydroxyl radical by 98.7% which makes it a powerful antioxidant when compared to the work of Olubukola et al.(2014) that reported the same plant to have scavenged hydroxyl radical by 72.5%. The plant could have phytochemicals with the potentials of mobbing up the destructive specie. Antioxidants possess great potential in ameliorating ROS- induced diseases (Rocha et al., 2014).

Metal (Fe²⁺) chelating ability assay

The result of this study differs from the result obtained by Josiah & Bartholomew (2015). The metal chelating ability of *N. laevis* and *F. exasperata vahl* were obtained as 5668 and 2,065 respectively. The chelating ability of these extracts was high when compared to 909.91 reported by Chandra, Balamurugan, Thiripura & Rekha (2012) on *Kalanchoe pinnata* leaf. One of the mechanisms of action of antioxidants is to chelate and deactivate transition metal thereby preventing such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalyzed reaction.

Anti-hemolytic assay

The magnitude of hemolysis was appeared to be much more overwhelming, when red blood cells were exposed to any toxicant like hydrogen peroxide (Naim, Gestetner, Bondi & Birk, 1976). Anti-

hemolytic assay performed on *Newbouldia laevis* across various concentrations showed that *Newbouldia laevis* might have coagulant properties.

Enzyme activity assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are enzymes that catalyze hydrolysis of acetylcholine and butyrylcholine respectively. Inhibition of these enzymes results in an increase in the levels of acetylcholine and butyrylcholine in the brains, as well as a corresponding increase in cholinergic functions (Colović, Krstić, Lazarević-pašti, Bondžić & Vasić, 2013).

Newbouldia laevis was tested for its acetylcholinesterase inhibitory effect at 40µg/ml, which gave 81% inhibition. The percentage of inhibition as reported by Shahat, Abeer, Essam & Mansour, (2015) was similar to the one obtained. From the result, it could be observed that *Newbouldia laevis* was able to inhibit acetylthiocholine iodide hydrolysis by the enzyme; hence it has a strong activity against acetylcholinesterase.

The chloroform extract of *F. exasperate vahl* leaf showed an inhibitory ability of 92.3 and 77% on acetylcholinesterase and butyrylcholinesterase respectively, suggesting that some of the phytochemicals present in the extracts of *Ficus exasperata vahl* leaf could have anticholinesterase ability. Also, the inhibition of cholinesterases by plant extracts could be phenomenally responsible for the action of *F. exasperate vahl* leaf extract against pests in postharvest storage. This inhibition results in build-up of acetylcholine and prolonged transmission of nerve impulse leading to death by respiratory failure (Robert et al., 2012). The result of the butyrylcholinesterase inhibitory activity was 93.3% at 40µg/ml. This result is higher when compared to the result of *Spondia smombin* (52.66%) by Elufioye, Obuotor, Agbedahunsi & Adesanya (2017).

Gas chromatography-mass spectroscopy analysis (GC-MS)

According to Shibula & Velavan, (2015), Gas chromatography-mass spectroscopy (GC-MS) is an important technique for the identification and quantification of organic compounds in the extract of *N. laevis*. The spectra of this study showed the

identification of thirteen peaks that were confirmed by their retention time, percentage area, molecular weight and formula. The identified compounds are: Hexanal dimethyl acetal (0.49%), alpha-Ethyl-beta-propylacrolein (0.40%), Lauraldehyde, dimethyl acetal (0.92%), Pentadecanoic acid (15.20%), n-Hexadecanoic acid (11.50%), 11-Octadecenoic acid (11.44%), Octadecanoic acid (12.75%), Oleic acid (27.53%), Octadecanoic acid (8.72%), 9,12,15-Octadecatrienoic acid (2.95%), Pentadecanoic acid, 2-hydroxy-1 (hydroxymethyl)ethylester (1.16%), 7,10-Hexadecadienoic acid (4.75%), 9-Octadecenal (2.19%) as shown in Table 6.

The concentration of oleic acid detected was higher when compared to the quantity (10.56%) detected in *Newbouldia laevis* reported by Iwu, Maureen, Onu & Rosemary (2018). Iwu et al., (2015) reported the presence of 2-phenoxy ethyl beta-2-phenoxy ethyl-3-phenyl propanoate, benzene-(1-methyl nonadecyl), 6-phenyl undecane, Eicosyne, hexadecanoic acid methyl ester, n-hexadecanoic acid, z-octadecanoic acid, 9-octadecanoic acid, phytol, oleic acid, octadecanoic acid, and squalene.

Table 7 showed the identities and percentage compositions of volatile compounds present in the chloroform leaf extract of *Ficus exasperata vahl*. A total of 17 compounds representing 100% of the total contents were identified as n-Hexadecanoic acid (40.52), 9, 12, 15-Octadecatrienoic acid (18.05) and oleic acid (25.33). However, a previous study by Sonibare et al., (2006) revealed that 1, 8-cineole (13.8%), (E)-phytol (13.7%) and p-cymene (11.4%) were the significant compounds in of the leaf oil of *Ficus exasperate vahl*.

Fourier transform infrared (FTIR) spectroscopy is employed in determining the functional groups present in a plant specimen (*Ficus exasperate vahl*) (Baker, Gazi, Brown, Shanks, Gardner & Clarke, 2008).

The FTIR analyses of chloroform extract of *N. laevis* leaf showed the presence of polychlorinated, nitramine, secondary and tertiary alcohol amino acid and ketones. Also, the chloroform extract of *Ficus exasperate vahl* leaf showed alkanes, sulphur compounds, halogen compounds, amides, carbonyl compounds functional groups which could be responsible for some of the pharmacological activities observed. This technique has been employed in studying the functional groups present

in some medicinal plants such as the ethanolic extract *Erythrina variegata* L. leaves which showed various chemical substituents such as alcohol, alkanes, aromatic carboxylic acid, halogen compounds and alkyl halide (Subrahmanian, Suriyamorthy & Devaki, 2017). Sulfonic acid, sulfoxide, alcohol, alkanes and charged amine were identified in *A. muricata* (Omeje, Ozioko, Opmeje 2018b). Subrahmanian *et al.* (2017) also screened the functional groups of ethylacetate extract leaves of *P. alatum* to be alcohol, phenol, alkanes, amides, carboxylic acids and was also reported to possess antioxidant property.

Conclusion

From the results obtained, it can be concluded that the extract of *Newbouldia laevis* possess some therapeutic purposes as a result of the phytochemicals present. The antioxidant activities *N. laevis* may be due to the presence of phenolic compounds containing the hydroxyl groups that confers the hydrogen donating ability. It also suggests that the extract of *N. laevis* is able to chelate free radicals. These properties could be exploited and used in the treatment of various diseases, including those associated with oxidative stress, such as Alzheimer's disease and cancer.

References

1. A.O.A.C. (2010). Association of Official Analytical Chemistry Methods of Analysis.
2. Akerele, J.O., Ayinde, B. A., & Ngiagah, J. (2011). Phytochemical and antibacterial evaluations of the stem bark of *Newbouldia laevis* against isolates from infected wounds and eyes. *Tropical Journal of Pharmaceutical Research*, 10(2), 211-217.
3. Aluko, B.T., Oloyede, O.I. & Afolayan, A.J. (2013). Polyphenolic contents and free radical scavenging potential of extracts from leaves of *Ocimum americanum* L. *Pakistan Journal of Biological Sciences*, 16(1), 22-30.
4. Anaduaka, E. G., Ogugua, V. N., Egba, S. I. & Apeh, V. O. (2013). Investigation of some important phytochemical, nutritional properties and toxicological potentials of ethanol extracts of *Newbouldia laevis* leaf and stem. *African Journal of Biotechnology*, 12(40), 5941-5949.
5. Ayoola, A. A., Yusuf, A. O. & Oki, D. G. (2016). Phytochemical screening and proximate analysis of *Newbouldia laevis* and *Allium sativum*. *Nigerian Journal of Animal Science*, 20 (1), 242 – 256.
6. Azando, E. V., Hounzangbe-Adote, M. S., Olounlade, P. A., Brunet, S., Fabre, N., Valentin, A. & Hoste, H. (2011). Involvement of tannins and flavonoids in the *in vitro* effects of *Newbouldia laevis* and *Zanthoxylum zanthoxyloides* extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. *Veterinary Parasitology*, 180 (3-4), 292- 297.
7. Baker, E. A., Gazi, E, Brown, M. D., Shanks, J. H., Gardner, P. & Clarke, M. W. (2008). FTIR- based spectroscopic analysis in the identification of clinically aggressive prostate cancer. *British Journal of Cancer*, 99, 1859-1866.
8. Baydar, N. G., Ozkan, G. & Yasar, S. (2007). Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Cont.* 18, 1131-1136.
9. Chandra, S. M, Balamurugan, V. S, Thiripura, S. S, & Rekha, R. (2012). Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe Pinnata*. *Journal of Chemical and Pharmaceutical Research*, 4(1), 197-202.
10. Colović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić A. M. & Vasić, V. M. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Current Neuropharmacology Journal*, 11(3), 315-335.
11. Dandjesso, C., Klotee, J.R., Dougnon, T.V., Segbo, J.M., Gbaguidi, F., Fah, L., Fanou, B., Loko, F. & Dramane, K. (2012). Phytochemistry and hemostatic properties of some medicinal plant sold as anti-hemorrhagic in Cotonou markets (Benin). *Indian Journal of Science and Technology*, 5(8), 3105-3109.
12. Densie, W. (2013). Phytochemicals' role in good health. *J Journal of Phtochemistry*, 15(9), 70.
13. Dudonné, S., Vitrac, X., Coutière, P., Woillez, M. & Mérillon, J.M. (2009). Comparative study of antioxidant properties and total

- phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, 57(5), 1768-1774.
14. Ejele, A.E., Duru, I. A., Ojukwe, C. E., Iwu, I. C. (2012). Phytochemistry and antimicrobial potential of basic metabolites of *Piper umbellatum*, *Piper guineense*, *Ocimum gratissimum* and *Newbouldia laevis* extracts. *Journal of Emerging Trends in Engineering and Applied Science*, 3(2), 309-314.
 15. Ellman, O. C., Courtney, K. D., Andres, V. & Featherson, R. M. (1961). A new and rapid determination of calorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7, 88-95.
 16. Elufioye, T.O., Obuotor, E.M., Agbedahunsi, J.M. & Adesanya, S.A. (2017). Anticholinesterase constituents from the leaves of *Spondias mombin* L. (Anacardiaceae). *Biologics: Targets and Therapy*, 11, 107-114.
 17. Francescato, L. N., Debenedetti, S. L., Schwanz, T. G., Bassani, V. L., Ame' lia, T. Henriques, A. T. (2013). Identification of phenolic compounds in *Equisetum giganteum* by LC-ESI MS/MS and a new approach to total flavonoid quantification. *Talanta* 105, 192-203
 18. Huang, D. J., Ou, B. X. & Prior, R.L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural Food and Chemistry*, 53, 1841-1856.
 19. Ibrahim, R. M., Ali, M. E., Saleh, D. O., El Nagger, E. B., Abd, E. O. & Seham, S. E. (2015). HPLC-DAD MS/MS profiling of phenolics from *S. securidaca* flowers and its antihyperglycemic and hyperlipidemic activities. *Revista Brasileira de Farcognosia* 25, 134-141.
 20. Iwu, I.C., Maureen, C. O., Onu, U.L. & Rosemary, U. (2018). GC-MS, phytochemical and antimicrobial analysis of the leaf of *Newbouldia laevis* (P. Beav). *Journal of Innovative Research and Development*, 7(7), 9-10.
 21. Iwu, M. M. (2000). Handbook of African medicinal plants. CRC press Inc. London. Pp19.
 22. Jin, M., Cai, Y., Li, J. & Zhao, H. (1996). 1,10-Phenanthroline-Fe²⁺ oxidative assay of hydroxyl radical produced by H₂O₂/Fe²⁺. *Progress in Biochemistry and Biophysics*, 23, 553 - 555.
 23. Josiah, B. H. & Bartholomew, O. I. (2015). In vitro antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of *Newbouldia laevis* ethanolic leaf extract. *Journal of Biological Research*, 48, 16.
 24. Kazeem, M. I, Oyadepo, B. F, Raimi, O. G. & Adu, O.B. (2013). Evaluation of *Ficus exasperata vahl*. Leaf extracts in the management of diabetes mellitus in vitro. *Journal of Medical Sciences*, 13(4), 269-275.
 25. Kumaran, A. & Karunakaran, R. J. (2007). In vitro antioxidant activities of methanolextracts of five *Phyllanthus* species from India. *LWT - Food Science and Technology*, 40, 344-352.
 26. Li, S., Lo, C.Y., Pan, M.H., Lai, C.S. & Ho, C.T. (2013). Black tea: Chemical analysis and stability. *Food Function*, 4, 10-18.
 27. McGuire, S. (2011). Dietary guidelines for Americans. *Journal of Advanced Nutrition*, 2(3): 293-294.
 28. Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E. & James, L. F. (2007). Phytochemicals: The good, the bad and the ugly. *Journal of Phytochemistry*, 66(22-24), 2973-2985.
 29. Naim, M., Gestetner, B., Bondi, A. & Birk, Y. (1976). Antioxidant and antihemolytic activities of soyabean isoflavones. *Journal of Agricultural Food Chemistry*, 24(6), 1174-1177.
 30. Nithya, N. and Balakrishnan, K.P. (2011). Evaluation of some medicinal plants for their antioxidant properties. *International Journal of PharmTech Research*, 3(1), 381-385.
 31. Ogunlana, O. E. & Ogunlana, O. O. (2008). In vitro assessment of antioxidant activity of *Newbouldia laevis*. *Journal of Medical Research*, 2(8), 176 -179.
 32. Olabukola, O. A., Owumi, S. E. & Oluwatosin, A. A. (2014). In vitro studies to assess the

- antioxidative, radical scavenging and anti arginase inhibitory potentials of extracts from *Artocarpus altilis*, *Ficus exasperata* and *Kigelia Africana*. *Asian Pacific Journal of Tropical Biomedicine*, 4, 5492-5499.
33. Omeje, K.O., Ozioko, J. N. & Opmeje, H. C. (2018b) Pharmacological potentials, characterization and fatty acids profile of *Persea americana* Mill. (Avocado) seed oil using Gas Chromatography-Mass Spectroscopy. *Biochem Anal Biochemistry*, 7, 361. doi: [10.4172/2161-1009.1000361](https://doi.org/10.4172/2161-1009.1000361).
34. Omeje, K. O., Omeje, H. C. & Asomadu, R. O. (2018a). Structural elucidation of phytochemical constituents of *Annona muricata* leaf using gas chromatography-mass spectroscopy and fourier transform infrared techniques. *PharmacologyOnline*, 1, 50-56.
35. Oniszczyk, A., Olech, M., Oniszczyk, T., Wojtunik-Kulesza, K. & Wojtowicz, A. (2016). Extraction methods, LC-ESI-MS/MS analysis of phenolic compounds and antiradical properties of functional food enriched with elderberry flowers or fruits. *Arabian Journal of Chemistry*, <http://dx.doi.org/10.1016/j.arabjc.2016.09.003>
36. Oyaizu M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition*, 44, 307-315.
37. Rozina, P., Sukalayan, K.K. & Pijush, S. (2013). *In vitro* nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. *The Pharma Innovation Journal*, 1(12), 83.
38. Ruberto, G. & Barrata, M. (2000). Antioxidant activity of selected oil components in two lipid model system. *Food Chemistry*, 69, 167-174.
39. Saheed, S, Taofeeq, G, Taofik, O. S, Abduhakeem, O. S. & Nurain, O. I. (2015). Indomethacin-induced gastric ulceration in rats: Ameliorative roles of *Spondias mombin* and *Ficus exasperata*. *Pharmaceutical Biology*, 54(1), 180-186.
40. Sangermeswaran, B., Balkrishnan, B. R., Deshraj, C. & Jayakar, B. (2009). In vitro antioxidant activity of *Thespesian Lampes* Dalz and Gibs. *Pakistan Journal of Pharmacy Science*, 22, 364-372.
41. Shahat, A.A., Abeer, Y.I., Essam, E. & Mansour, S.A. (2015). Acetylcholinesterase inhibition and antioxidant activity of some medicinal plants for treating neurodegenerative disease. *African Journal of Traditional Complementary Alternative Medicine*, 12(3), 97-103.
42. Shibula, K. & Velavan, S. (2015). Determination of phytocomponents in methanolic extract of *Annona muricata* leaf using GC-MS technique. *International Journal of Pharmacognosy and Phytochemistry Research*, 7(6), 1251-1255.
43. Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.
44. Subrahmanian, H., Suriyamoorthy, P. & Devaki, K. (2017). Fourier transform infra-red spectroscopy analysis of *Erythrina variegata* L. *Journal of Pharmaceutical Sciences and Research*, 9(11), 2062-2067.
45. Tuo, K., Béourou, S., Touré, A., Ouattara, K., Meité, S., Ako, A.B., Yao, S.S., Koffi, D., Coulibay, B., Coulibaly, A. & Djaman, A.J. (2015). Antioxidant activities and estimation of the phenols and flavonoids content in the extracts of medicinal plants used to treat malaria in Ivory Coast. *International Journal of Current Microbiology and Applied Sciences*, 4(1), 862-874.
46. Ughachukwu, P. O., Ezenyeaku, C. C. T., Ezeagwuna, D. A. & Anahalu, I. C. (2012). Evaluation of anti-bacterial properties of ethanol extract of *Ficus exasperata* leaf. *African Journal of Biotechnology*, 11 (16), 3874-3876.
47. Usang, A. U., Ibor, O. R., Owolodun, O. A., Eleng, I. E., Ujong, U. P., Udoh, P. B. (2015). Reproductive toxicological evaluation of *Ficus exasperata* ethanolic extract in male albino rats. *International Journal of*

- Toxicology and Pharmaceutical Research, 7(6), 281-28.
48. Usman, H. & Osuji, J. C. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of *Newbouldia laevis*. *African Journal of Traditional Complementary Alternative Medicine*, 4(4), 476 - 480.
49. Wonder, M. K, Abotsi, Eric, W, George, k, Ainooson, Ama, K, Amo-Barimah & Eric, B. (2010). Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* P. Beauv (Moraceae). *Pharmacognosy Research*, 2(2), 89-97.

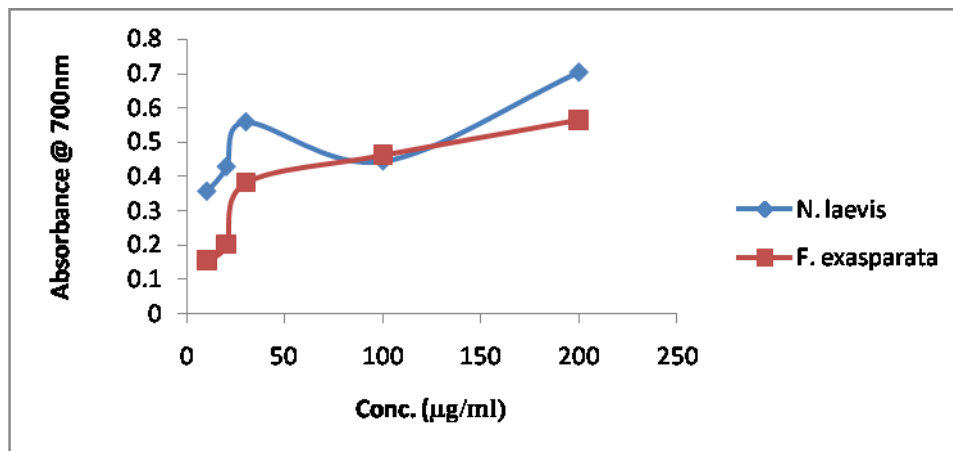


Fig. 1. Ferric cyanide reducing antioxidant power

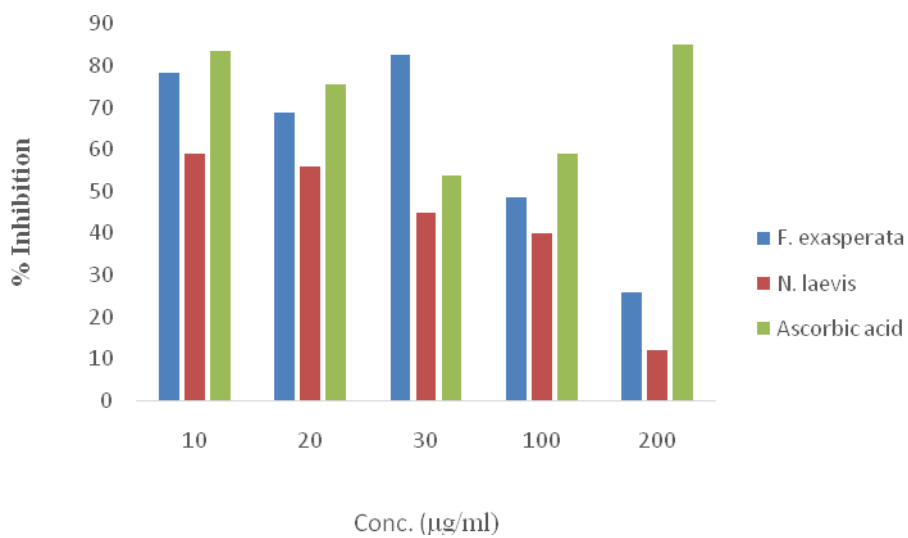


Fig. 2. DPPH Inhibition potentials of *N. laevis*

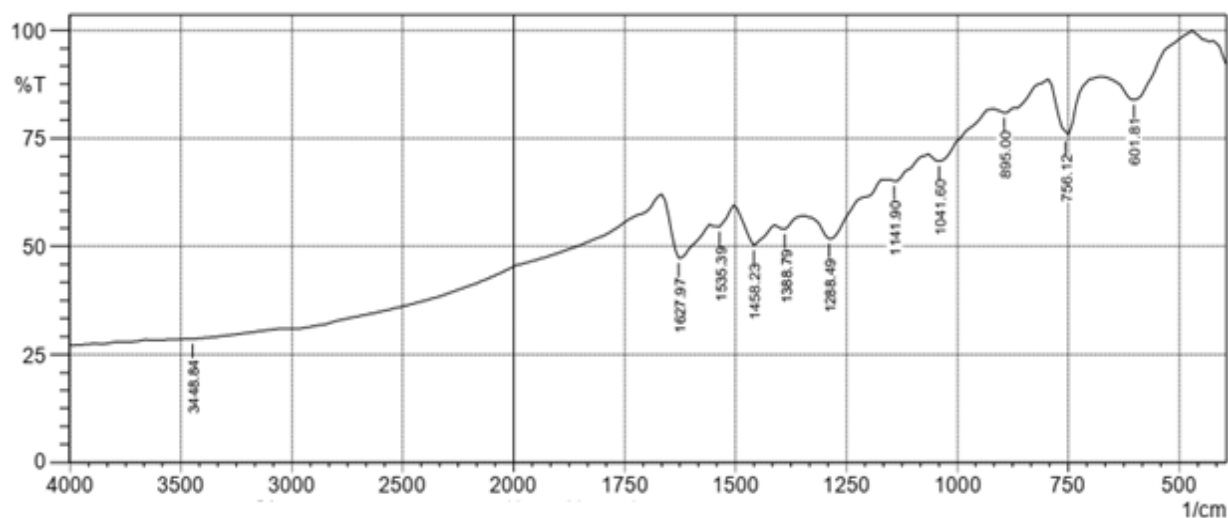


Fig. 3. FT-IR spectra of *Newbouldia laevis* leaf extract

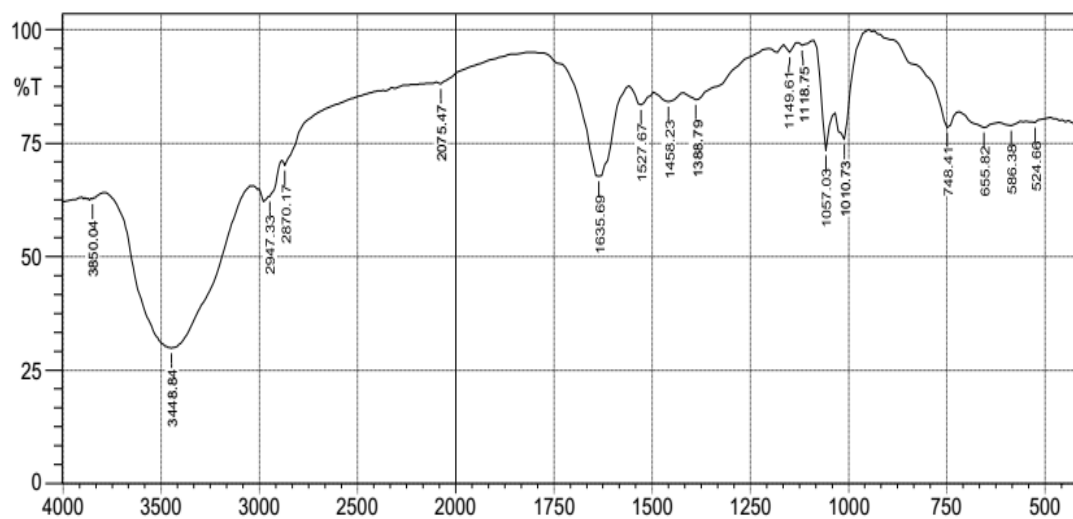


Fig. 4. FT-IR spectra of *Ficus exasperata* leaf extract

Table 1. FTIR analysis result of *F. exasperata* leaf extract

Peaks	Functional Groups
655.82	Sulphur compound (C-S str.)
748.41	equatorial halogen compound (C-Br str)
1010.73	monoflourinated halogen compound (C-F str.)
1057.03	diflourinated halogen compound (C-F str)
1458.23	alkane (C-H defasym)
1527.67	amides (N-H def plus C-N str)
1635.69	carbonyl compounds (N-H def plus C-N str)
2947.33	alkanes (C-H strsym)
3448.84	secondary amides (N-H str)

Table 2. FTIR analysis result of *N. laevis* leaf extract

Peaks	Functional Groups
756.12	polychlorinated (C-Cl str.)
895.00	nitramines (C-N vib.)
1041.60	secondary alcohol (C-O str.)
1141.90	tertiary alcohol (O-H Def.)
1288.49	secondary alcohol (O-H def.)
1388.79	alkanes (C-H def.)
1458.23	Nitrosamines (N=O str.)
1535.39	amino acid (NH ₃ ⁺ def.)
1627.97	ketones (C=O str.)
3448.84	ketones (C=O overtone; C=O str.)

Table 3. GC-MS analysis result of *Ficus exasperata* leaf extract

Name of compound	Formula	Mol. Weight	Retention Time	Area (%)	
2,3-Butanedioldiacetate	C ₈ H ₁₄ O ₄	174	4.897	0.22	
2, 3- Butanedioldiacetate	C ₈ H ₁₄ O ₄	174	5.06	40.23	
1,3-Propanediol, diacetate	C ₇ H ₁₂ O ₄	160	5.824	0.49	
Ethanol,2-(2-methoxyethoxy)	C ₅ H ₁₂ O ₃	120	7.143	1.93	
Benzofuran	C ₈ H ₈ O	120	7.231	1.82	
Ethanol,2-[2-(2-ethoxyethoxy)ethoxy]	C ₈ H ₁₈ O ₄	178	8.057	0.74	
Ethanol,2-[2-(2-ethoxyethoxy)ethoxy]	C ₁₀ H ₂₂ O ₄	206	10.51	0.64	
3-(pHydroxyphenyl)-1-propanol	C ₉ H ₁₂ O ₂	152	11.52	0.80	
2-Tridecene	C ₁₃ H ₂₆	182	12.052	0.62	
Acetic acid	C ₁₁ H ₁₆ O ₄		212	14.622	2.48
Bicyclo[2.2.2]octane-1,4-diol	C ₁₀ H ₁₆ O ₃	184	14.780	2.01	
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂		256	18.132	40.52
11-Octadecenoic acid	C ₁₉ H ₃₆ O ₂		296	19.964	0.75
9,12,15-Octadecatrienoic acid	C ₁₈ H ₃₀ O ₂	278	21.211	18.05	
Oleic acid	C ₁₈ H ₃₄ O ₂	282	21.316	25.33	
2-Methyl-Z,Z-3,13-octadecadienol	C ₁₉ H ₃₆ O	280	24.250	2.17	
Di-n-octyl phthalate	C ₂₄ H ₃₈ O ₄		390	24.913	1.20

Table 5. Compound identified in chloroform extract of *Newbouldia laevis* by GC-MS.

Name of compound	Formula	Mol. Weight	Retention Time	Area (%)
Hexanal dimethyl acetal	C ₈ H ₁₈ O ₂	146	3.80	0.49
Alpha.-Ethyl-.beta-propylacrolein	C ₈ H ₁₄ O	126	4.11	0.40
Lauraldehyde, dimethyl acetal	C ₁₄ H ₃₀ O ₂	230	11.90	0.92
Pentadecanoic acid	C ₁₇ H ₃₄ O ₂	270	15.48	15.20
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	16.06	11.50
11-Octadecenoic acid	C ₁₉ H ₃₆ O ₂	296	17.23	11.44
Octadecenoic acid	C ₁₉ H ₃₈ O ₂	298	17.42	12.75
Oleic acid	C ₁₈ H ₃₄ O ₂	282	17.78	27.53