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Trado-Medical uses, Chemical Constituents and Biological Activities of *Newbouldia laevis* (Bignoniaceae): A Review

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Abstract

Newbouldia laevis (P. Beauv.) Seem. (Family, *Bignoniaceae*), commonly known as tree of life, is a purple-flowering plant that is widely distributed in many parts of Africa. Different parts of the plant, including the leaves, flower, stems and roots are prevalently used in African traditional medicine for the management of many diseases and conditions like diabetes, hypertension, skin diseases, ulcer, tumors, pains, infectious diseases, inflammation, dysentery, sickle cell disease and impotency. This review discusses the trado-medical uses, chemical constituents, and biological activities of *N. laevis*. Based on information generated from scientific investigations deposited in PubMed and SCOPUS, the chemical constituents of the plant include glycosides, anthraquinolones, volatile oils, tannins, steroids, alkaloids, flavonoids, terpenoids and sterols. Extracts different parts of the plant and compounds isolated from them have been reported to have several health-promoting potentials such as antioxidant, antimalarial, trypanocidal, antimicrobial, anthelmintic, analgesic, anti-inflammatory, antidiabetic, anti-arthritic, anti-thrombotic, cytoprotective, anti-hypertensive, central nervous system modulatory, male reproduction enhancing and oxytocic properties. These scientific investigations have led credence to the ethnobotanical uses of the plant in folkloric practice. In addition, the presence of phytochemical constituents in the plant might be responsible for the wide biological potentials.

***Key words**: Newbouldia laevis*; Phytochemistry; Ethnobotanical uses; Biological activities; Tree of life; *Bignoniaceae*

Introduction

Researches on medicinal plants are growing due to increasing human recognition of the need to rely on natural resources around him and to exploit the traditional medicinal knowledge as a source of new drugs.[1] Furthermore, a greater population of the world resides in low-income countries where economic limitations render them incapacitated from accessing quality healthcare and affording effective chemical drugs; hence, their reliance on natural products with history of potency in disease management.[2] Natural products, from plants and marine origin have been traditionally used in treating diseases from time immemorial.[3] In addition, a good number of effective drugs currently used in treating malaria, cancer, and many other diseases were derived from plants.[2,4-6] *Newbouldia laevis* (P. Beauv.) Seem. (Syn. *N. pentandra* (Hook.) is widely distributed in many parts of Africa and is commonly known as Tree of life, African nut tree, and Fertility tree in English, *Oke-ogirishi* or *Ogilishi* in Igbo, *Akoko* in Yoruba, *Aadurukuu* in Hausa languages of Nigeria. The plant is locally known as *Faangum* in Cameroon, *Moquiquiri* in Mali, *Din-a-lah* in Liberia, *Akan-asante* in Ghana, *Fula-fulfulde* in Ivory Coast, *Dupwan, Kpatsima* or *Avahi* in Togo, *Bulom* in Sierra Leone, *Sukunde* in Guinea and Gambia, and *Sibompol* in Senegal.[7,8] It is a small tree of about 7-20 m tall depending on the region where it was found and the stem grows vertically with few branches. The leaves are shiny-green and produce purple flowers that harbour sweet liquid. Several classes of compounds have been identified in different parts of the plant and include phenolics, glycosides, anthraquinolones, volatile oils, tannins, glycosides, steroids, alkaloids, flavonoids, and terpenoids.[9,10] *Newbouldia laevis* is widely used in trado-medical practice for the management of diseases and conditions like skin infection, tooth and stomach aches, pains, diabetes, hypertension, tumor, malaria and sickle cell anaemia.[7,11-16.] Extracts and compounds isolated from this plant have been reported to possess several biological activities such as antioxidant, antimalarial, anti-inflammatory, antiulcer, hepato-renal protection, anti-trypanosomal, anticancer, antidiabetic, anti-hypertensive, central nervous system modulatory and oxytocic properties.[17-24] This review summarizes, findings of the trado-medical uses, chemical constituents, and biological properties of *N. laevis*.

Trado-Medical Uses

The trado-medical uses of *N. laevis* widely depend on the ethnic location of the plant and the part of the plant used. The Togolese and Nigerians use the leaves prepared as a decoction

alone or in combination with other plants and administered orally for the treatment of malaria and fever.[8,15,25-26] Also, the Tiv people of North-Central Nigeria prepare a polyherbal decoction of the leaves mixed with leaves of *Crossopteryx febrifugg* and *Morinda lucida* and taken orally to treat malaria.[27] Inhabitants of Omo Forest Reserve in Western Nigeria boil the leaves in combination with leaves of *Mangifera indica* and *M. lucida* and taken orally to treat malaria and fever.[28] Similarly, in Southern Nigeria, the Ikwere people use the leaves, stem bark and roots for treating migraine, skin infections, fever, malaria, stomach ache, epilepsy and conjunctivitis.[29] Furthermore, the Ijebu people of Southwest Nigeria use the leaves decoction in combination with *Momordica charantia*, *Vernonia amygdalina* and *Ocimum gratissimum* leaves to treat measles, while the stem bark boiled with sugar cane juice is used in the treatment of dysmenorrhea by the Ibibio of Southern Nigeria.[30-31] In other reports, Ghanaians, Cameroonians and Nigerians use the bark, roots and leaves for the treatment toothache, stomachache, diarrhea, dysentery, malaria, fever, breast cancer, sexually transmitted diseases (STDs), anemia, ulcer, arthritis, rheumatism, hemorrhoids, constipation cardiovascular diseases, diabetes, cough, elephantiasis and urinogenital tract infection.[1,3,11-14,32-39] The ethnomedicinal uses of various parts of the plant were summarized in Table 1.

Chemical constituents

Alkaloids were the first class of compounds isolated from the root bark of *N. Iaevis*. Pyrazole alkaloids (withasomnine (**1**), 4'-hydroxywithasomnine (**2**), 4'-methoxywithasomnine (**3**) newbouldine (**4**), 4'-hydroxynewbouldine (**5**), and 4-methoxynewbouldine (**6**)) were isolated from the plant.[40,41] Also, three phenylpropanoid glycosides (verbascoside (**7**), martynoside (**8**) and newbouldioside) were isolated from the roots using a combination of chromatographic and spectroscopic methods.[42] Further spectroscopic analysis of newbouldioside revealed that the compound was actually three related compounds named as newbouldiosides A-C (**9**-**11**).[43] Also, isolated from the root bark includes naphthoquinone-anthraquinone (newbouldiaquinone (**12**), 2-acetylfuro-1,4-naphthoquinone (**13**), 2-methyl-9,10-anthracenedione (**14**), canthic acid (**15**) and lapachol (**16**).[35] Purification of the methanol root extract led to the isolation of chrysoeriol (**17**) a flavonoid, quinones (newbouldiaquinone (**12**), 2-acetylfuro-1,4-naphathoquinone (**13**), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (**18**) and lapachol), sterol (β-sitosterol-3-O-β-D-glucopyranoside (**19**)), triterpenes (oleanolic acid (**20**) and canthic acid), ceramide

(newbouldiamide (**21**)) and one phenolic derivative (2-(4-hydroxyphenyl)-ethyltriacontanoate (**22**).[44] Recently, a German researcher isolated three new phenylethanoid glycosides identified as newbouldiosides D-F (**23**-**25**) from the stem bark.[45] Natural compounds belonging to different classes of phytochemical constituents isolated from the stem bark of this plant include furanonaphthoquinones like 5-hydroxy-dehydroiso-α-lapachone (**26**), 2-acetyl-5-hydroxynaphtho[2,3-β]furan-4,9-dione (**27**), 2-isopropenylnaphtho[2,3-β]furan-4,9-dione (**28**), 2-(1'-methylethenyl)-5-hydroxynaphtho[2,3-β] furan-4,9-dione (**29**), 2-(1'-methylethenyl)-7-hydroxy-naphtho[2,3-β] furan-4,9-dione (**30**), β-resorcylic acid (**31**), atraric acid (**32**); benzofuran like 2-(1'methylethenyl)-6-hydroxy-2,3-dihydrobenzo[β]furan (**33**), 2,3-dimethoxy-1,4-benzoquinone (**34**) and 2-(4-hydroxyphenyl) ethyl triacontanoate (**22**).[46] Newbouldiamide, oleanolic acid, β-sitosterol (**35**) and β-sitosterol glucopyranoside were also isolated from seeds of *N. laevis*.[47] Dereplication study of the aqueous extract of *N. laevis* leaves using high-performance liquid chromatography coupled with diode array detector (HPLC-DAD) analysis revealed presence of phenolic compounds like epicatechin (**36**), gallic acid (**37**), chlorogenic acid (**38**), caffeic acid (**39**), isoquercitrin (**40**), kaempferol (**41**), quercitrin (**42**) and quercetin (**43**).[48] In addition lapachol derivatives such as β-lapachone (**44**), 3-hydroxydehydroiso-α-lapachone (**45**), 5-hydroxydehydroiso-α-lapachone (**26**), 3,8-dihydroxydehydroiso-α-lapachone (**46**) and 5,7-dihydroxydehydroiso-α-lapachone (**47**), phytosterols and triterpenoids such as stigmasterol (**48**), ursolic acid (**49**), β-sitosterol-3-O-β-D-glucopyranoside, β-sitostenone (**50**) and stigmasterol glucoside (**51**), as well as phytosphingosine like hexadecadihydrosphingosine (**52**), a quinolone alkaloid (evocarpine (**53**)), chrysarobin (**54**) and harmalol (**55**) were isolated from the leaves, and other parts of *N. laevis*[44] (Figure 1). Other phytochemicals isolated from the plant include 5-hydroxy-7-methoxydehydroiso-α-lapachone[49] and 2-(1-hydroxyethyl)-2-acetyl-naphtho[2,3-β]furan-4,9-dione[50] from the roots.

Biological Activities

Anticancer activity

Despite the traditional use of *N. laevis* in treating cancer in Cameroon and other countries, Kuete *et al*.[51] investigated the cytotoxicity of methanol extracts of the leaves and barks against multidrug resistance leukemia (CCRF-CEM) cells and reported that the extracts had weak inhibitory effects (<40% inhibition of proliferation) compared to >85% by doxorubicin.

The leaf extract was, however, more active than the bark extract. Perhaps the effectiveness of this plant in traditional settings is due to its use in drug-sensitive species and in addition to its use in combination with other plants. Meanwhile, the cytotoxicity of a naphthoquinone (2-acetylfuro-1,4-naphthoquinone) isolated from *N. laevis* root bark was tested against pancreatic MiaPaCa-2, breast cancer CCRF-CEM, CEM/ADR5000 cells, leukemia PF-382, leukemia HL-60, pancreatic Capan-1, breast MCF-7, colorectal SW-680, renal carcinoma 786-0, human brain glioblastoma U87MG, lung A549, colon melanoma colo-38, cervix HeLa, cervix Caski cancer and normal liver AML12 cells.[51] The compound exhibited good to excellent antiproliferative activities against these cancer cells with IC_{50} values of 9.41±2.79, 16.75±0.42, 1.81±0.02, 0.57±0.06, 4.81±0.77, 3.81± 0.96, 1.66±0.09, 3.55±0.02, 6.81±0.61, 1.94±0.18, 5.49±0.35, 0.67±0.18, 0.40±0.10, 0.17±0.03 and >20 µg/mL, respectively. Based on the IC_{50} values, the isolated compound was more active than doxorubicin against PF-382, CEM/ADR5000, Capan-1, Caski and Colo-38 cancer cells. The naphthoquinone also suppressed the growth of blood capillaries in angiogenic inhibitory assay using chorioallantoic membrane of quail eggs as a model. This demonstrates that the compound is very beneficial in preventing tumor progression and spreading. Other compounds isolated from the stem bark of this plant with anticancer activity include 5-hydroxydehydroiso-α-lapachone, 2-acetyl-5-hydroxynaphtho [2,3-β]furan-4,9-dione, 2-isopropenylnaphtho[2,3-β]furan-4,9-dione, 2-(1'-methylethenyl)-5-hydroxynaphtho[2,3-β]furan-4,9-dione, 3,8-dihydroxydehydro-iso-α-lapachone and 2-(1'-methylethenyl)-6-hydroxy-2,3-dihydrobenzo[β]furan.[46,52]. Previously, Azuine et al.[53] (1995) showed that *N. laevis* extract also has anticancer activity in grafted cancer mouse models. These reports generally indicate that natural products derived from *N. laevis* are potential candidates that demand further exploration for cancer treatment.

Antidiabetic activity

In Mexico, *N. laevis* is well-known in folkloric for its use in controlling hyperglycemia associated with diabetes.[54] This inspired Bosha *et al*.[55] to examine the effect of methanol extract of the leaves on experimental diabetic rats induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate. It was reported that after 24 h of administration, the extract (250 mg/kg) suppressed fasting blood glucose level by 60.2% compared to 51.5% by glibenclamide (2 mg/kg). To investigate the possible mechanism of hypoglycemic properties

of the plant´s leaves, Kolawole *et al.*[56] observed that ethanol leaves extract significantly reduced postprandial glucose levels and inhibited pancreatic α-amylase activity in diabetic rats at 500 mg/kg (IC_{50} = 58.7 µg/mL) relative to 92.3 µg/mL of acarbose (50 mg/kg). The authors further assessed the inhibitory effects of the extract on baker's yeast and rat intestinal α-glucosidases and rat pancreatic α-amylase, *in vitro*. The extract was reported to significantly inhibit both baker's yeast and rat intestinal α-glucosidases with IC_{50} values of 2.2 µg/mL and 43.5 µg/mL, respectively compared to 3.8 µg/mL and 62.7 µg/mL, respectively by acarbose. These results showed that the extract acts by inhibiting the two enzymes that play the key roles in increasing blood glucose level. Based on the reported antidiabetic activities of the plant leaves, Mbagwu *et al.*[23] evaluated the inhibitory effects of ethanol extract of the leaves on α-amylase activity and reported that the extract potently inhibited the enzyme with IC_{50} value of 102.91 mg/mL. Apigenin isolated from the methanol fraction of the leaves dichloromethanol-methanol extract exhibited antidiabetic property.[57] Treatment of adrenaline-generated hyperglycemic rats with apigenin (25 and 50 mg/kg) dose-dependently suppressed the glucose level more than glibenclamide (5 mg/kg). In addition, apigenin (50 mg/kg) returned the glucose level to normal within 2 h compared to 4 h by glibenclamide. Flavonoids such as rutin and apigenin have been demonstrated to protect rats' pancreatic β-cells from diabetogenics like streptozotocin in addition to their inhibition of hyperglycemia provoked by this pancreatic toxicant,[58-59] partly through antioxidant mechanisms.[60] Two caffeic acid glycosides, newbouldasides A and B isolated from the plant strongly inhibited α-amylase activity with IC_{50} values of 4.95 and 4.44 mg/mL, respectively relative to reference drug, acarbose (IC_{50} = 4.05 mg/mL).[23] This result supports the earlier reports that inhibition of α-amylase activity is one of the mechanisms through which the extract exhibits its hypoglycemic effect and further adds that newbouldiosides A and B are among the compounds in the plant leaves responsible for its antidiabetic property. Other compounds isolated from this plant with reported antidiabetic activity include 9-(4-nonyl-phenyl)-non-8-enoic acid, β-sitosterol and β-sitosterol glucopyranoside.[47,61.] In addition to the above, the effects of solvent extracts of *N. laevis* roots[62], leaves[63-70], flowers[71] and stems[72] on glucose regulation in normal and diabetic conditions have been documented in several reports. The plant extracts were shown to improve insulin secretion and recognition by its receptors, increase glucose uptake and improvement of pancreatic function[73], inhibition of key enzymes involved in glucose metabolisms such as α/β-glucosidases and α-amylase[55,61], boosting antioxidant status[74], lipid peroxidation and glycosylation of proteins[64], oxidative

stress and gastric emptying[70]. These findings have demonstrated that *N. laevis* leaves and its active compounds are potential candidates for managing hyperglycemia and insulin resistance associated with type-2 diabetes. Hence, clinical trials on the plant and compounds isolated with them with good hypoglycemic effects are strongly encouraged as they might emerge as new antidiabetic agents.

Antimicrobial activity

In an *in vitro* study, aqueous-ethanol bark extract of *N. laevis* showed weak to moderate activity against selected bacteria species with minimum inhibitory concentrations (MICs) of 25, 12.5, 25, 25, 6.25 and 25 mg/mL corresponding to *S. typhi, S. aureus, S. pneumoniae, B. subtilis, K. pneumoniae,* and *P. aeruginosa*. Similarly, *n*-hexane and chloroform leaves extracts were reported to have very good antifungal activities with inhibition zone diameter (IZD) of 36.5 and 71 mm for the *n*-hexane extract against *C. albicans* and *A. fumigatus*, respectively; 18.8 and 31 mm for chloroform extract against *C. albicans* and *A. fumigatus*, respectively. Chukwujekwu *et al.*[75] reported that petroleum ether, dichloromethane and ethanol root extracts showed moderate antibacterial activities against Gram-positive bacteria (*Bacillus subtilis, S. aureus* and *Micrococcus luteus*) and Gram-negative bacterium (*Escherichia coli*) with MIC values ranging from 0.39-6.25 mg/mL. The ethanol extract was more active against *E. coli* and *B. subtilis* (MIC values of 0.39 and 1.56 mg/mL, respectively) while dichloromethane extract was more active against *S. aureus* (MIC = 0.78 mg/mL). However, all the extracts were inactive against *K. pneumonae,* a Gram-negative bacterium. Lapachone-type naphthoquinones (6-hydroxydehydroiso-α-lapachone, 7-hydroxydehydroiso-α-lapachone, 5,7-dihydroxydehydroiso-α-lapachone, dehydroiso-α-lapachone, 3-hydroxy-5-methoxydehydroiso-α-lapachone, 5-hydroxydehydroiso-α-lapachone, 5-methoxydehydroiso-α-lapachone, 3,8-dihydroxydehydroiso-α-lapachone and 3-hydroxydehydroiso-α-lapachone) isolated from dichloromethane root extract exhibited moderately antifungal (*C. albicans*) and antibacterial (*Cladosporium cucumerinum, B. subtilis* and *E. coli*) activities *in vitro*.[76] The antimicrobial activities of the root extract could also be attributed to the presence of quinones, sterol, triterpenes, ceramides, alkaloids (quinine, lunamarine, sparteine and ribalidine), flavonoids (epicatechin, anthocyanin, resveratrol, flavonones and rutin) and phenolics isolated from the root.[47,77]

Flavonoids and flavonoid-rich extracts have been demonstrated to have good antibacterial activities, potentially by inhibiting keys enzymes in their metabolic pathways, inhibiting membrane components synthesis and their assembly in addition to modification of membrane permeability and its other functions, halting DNA synthesis and formation of biofilms[78-79]. Using four bacterial isolates, crude aqueous extract of *N. laevis* leaves was reported to have higher antibacterial activity with respective MICs of 1.25 mg/mL against *S. typhi* and 0.62 mg/mL each against *K. pneumonia, E. coli* and *Shigella spp*. compared with 0.31 mg/kg against *K. pneumonia* and 1.25 mg/kg each against *S. typhi, E. coli* and *Shigella spp* by hydro-ethanol extract[80]. On the other hand, methanol extracts of the leaves were reported to be weakly active against *B. anthracis, Corynebacterium pyogenes, S. epidermidis* and *S. faecalis,* moderately active against *E. coli* and strongly active against *B. subtilis, Clostridium sporogenes* and *K. pneumonia,* all of which were lower in activity compared to streptomycin[81]. Nandita and Nanja[82] earlier reported that stem bark methanol extract exhibited significant antibacterial activities against *Clostridium tetani, C. perfringens, S. faecalis, Nocardia osteriodes, P. mirabilis, Serratia marcescens, K. sp* and *P. aeruginosa* while acetone and aqueous extracts showed weaker activities against the bacteria tested. The antibacterial activities by the methanol extract against the tested bacteria are slightly lower than gentamycin and tetracyclin that served as reference antibacterial drugs. In another study, crude aqueous-ethanol extract and its basic metabolites were reported to show significant activity against *S. typhi, S. aureus* and *Coliform bacilli*[83], as well as against *P. aeruginosa* and *C. albicans*[37]. These reports are similar to those recorded by other researchers on the leaves[84-92], root[93] and stem bark[94-95].

Based on the studies reviewed, it can be inferred that a wide range of bacterial and fungal species that are susceptible to *N. laevis* extract and compounds isolated from it, supporting the view of the plant as a source of broad-spectrum antimicrobials. The ability of the plant extractants to be active against drug-resistant strains further strengthens the opinion that further investigations are needed to exploit the potential of the plant, especially the leaves as a source of antimicrobial agents.

Antimalarial activity

A combined aqueous extract of *Morinda lucida, Phyllantus amarus, Vernonia amygdalina and Newbouldia laevi*s leaves were subjected to 4-day suppressive and 9-day curative tests

against chloroquine sensitive *Plasmodium berghei* NK65 in mice. The combined extract was reported to suppress the parasite growth by 9.8% (200 mg/kg, *p.o.*) and significantly decreased the parasite density in the curative test after 9 days treatment.[96] In another *in vivo study*, the aqueous ethanol extract (200 mg/kg, *p.o.*), and its solvent fractions (*n*-hexane, ethyl acetate, aqueous and *n*-butanol) (400 mg/kg each, *p.o.*) respectively inhibited *P. berghei* NK65 growth in mice by 47.34, 67.68, 79.85, 66.54 and 76.43% comparable to 86.50% by pyrimethamine (1.2 mg/kg, *p.o.*) in the prophylactic test.[97] Similarly, the extract and its fractions respectively suppressed plasmodial growth by 37.31, 73.80, 89.47, 70.64 and 88.89% relative to 92.73% by artesunate (5 mg/kg, *p.o.*) in 4-day suppressive test. In addition, treatment of parasitized mice with the extract and its solvent fractions for 9 days respectively gave 84.77, 90.09, 75.72 and 87.75% chemosuppression compared to 90.95% by artesunate (5 mg/kg, *p.o.*) in curative test. It can be inferred that ethyl acetate fraction gave the highest activity against the parasite. The authors attributed the antiplasmodial activities to the presence of alkaloids, saponins, glycosides, tannins, terpenes, and flavonoids in the extract based on the previous reports of antimalarial activities of such phytochemicals.[98]

A recent *in vivo* study showed that methanol extract of *N. laevis* leaves (200 mg/kg, *i.p.*) weakly suppressed malaria parasitemia in mice (30.14%) compared to chloroquine phosphate (5 mg/kg, 78.89%). The authors further reported in acute toxicity assay that the extract has an LD_{50} value of 471.43 mg/kg, *i.p.*[99] The variations between these results can be attributed to differences in dose, duration and route of administration. However, the crude extracts and their solvent fractions showed lower activities against the parasites assayed relative to standard drugs used. In a SYBR-GREEN fluorescence *in vitro* antiplasmodial assay, of *N. laevis* against selected malaria parasites based on the traditional use in treating malaria in Cote d'Ivoire.[100] It was reported that among solvent extracts, only methanol extract gave significant inhibition of chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains alongside four chloroquine-sensitive *P. falciparum* field strains (W535, W539, W552 and ANK02) isolated from infected Ivorians. The antiplasmodial activity of the plant extract was generally lower than chloroquine for the chloroquine-sensitive strains and quinine/artesunate for the Dd2 strain. This methanol extract was partitioned by solvent-solvent method using *n*-hexane, ethyl acetate and *n*-butanol and the fractions tested against the parasites. It was reported that all the fractions were active against Dd2 strains and two strains of the field isolates (W639 and A149) with ethyl acetate fraction being more active against Dd2 and

W639 while aqueous fraction was more active against A149. To evaluate the safety of the solvent fraction of the methanol extract, erythrocyte hemolytic assay was conducted using 100 and 200 µg/mL of each fraction. It was reported that all the fractions gave ≤ 1% hemolytic activity compared to 100% hemolytic activity by Triton X100, showing high safety profile at the above concentrations.[101] This observation agrees with an earlier study by Togolese researchers that ethanol extract of *N. laevis* leaves inhibited *P. falciparum in vitro* with IC_{50} value of 12.6 µg/mL as well as 43-77% inhibition by ethanol extract and its ethyl acetate, ethanol, *n*-hexane and chloroform fractions (0.5-5.0 mg/mL) reported by Nigerian researchers.[102-104]

Antiparasitic* (*Trypanocidal, Onchocercidal and anti-Leishmanial*) *activities

The antiparasitic effects of *n*-hexane, methanol and ethyl acetate extracts of *N. laevis* leaves were investigated against wild strains of *Trypanosoma* such as *T. brucei* Lister s427 (WT) and *T. congolense,* and drug-resistant (diamidine-resistant strain (*Tb*AT1-KO), multidrug resistant strain (B48), Cpd A-resistant strain (NPD-001) and wild type *Leishmania mexicana* M379 IL3000).[68] Ethyl acetate extract gave the highest activity against *T.b.*s427 and *T.b.*B48 cells with EC_{50} values of 5.4 and 4.2 µg/mL respectively, compared with EC_{50} values of 0.000117 µg/mL and 0.000354 µg/mL, respectively against *T.b.*s427 and *T.b.*B48 cells of pentamidine, the reference trypanocidal drug. The authors isolated pheophytins A and B from ethyl acetate extract and amongst the two compounds pheophytin B gave strong inhibition against s427 (WT), B48, NPD-001, *Tb*AT1-KO and R0.8 (cAMP phosphodiesterases inhibitors- TbrPDEB1 and B2-resistant strain) with EC_{50} values of < 3 µg/mL whereas pheophytin A gave weak inhibitory activity with EC_{50} ranging from 22.8-28.8 µg/mL.[68] The extracts also exhibited weak activity against *L. Mexicana* M379 IL3000; with only *n*-hexane and ethanol extracts showing remarkable activity with EC_{50} values of 50 and 100 µg/mL. Thus, it can be shown that the leaves extract of the plant poses trypanocidal activity to both drug-sensitive and drug-resistant strains and that among the two compounds isolated from the plant, only pheophytin B showed good trypanocidal effect. A recent investigation by Eyong et al.[50] subjected 13 compounds isolated from the chloroform-methanol extract of the plant's root to antiparasitic analyses against *Onchocerca ochengi*, a parasite that causes onchocerciasis. Among the isolated compounds, lapachol and 2-(1-hydroxyethyl)-2-acetyl-

naphtho[2,3-β]furan-4,9-dione completely inhibited the parasite growth at 5 µg/mL after 5 days.

Antioxidant activity

Using several radical scavenging, reducing and antioxidant models, Habu and Ibeh (2015) reported that crude ethanol extract of the leaves inhibited radicals such as DPPH (85.85%), ferric reducing antioxidant power assay (FRAP) (64.01%), lipid peroxide (91.85%), HOCl (62.10%), singlet oxygen (96%), peroxynitrite (62.10%), nitric oxide (80.08%), iron chelating (90.11%), superoxide anion (81.11%) and hydroxyl (76.10%). The chemical constituents of the extracts were also reported to be ascorbic acid (515.53 IU/100g), vitamin E (26.46 IU/100g), saponins (6.2 mg/100g), alkaloids (2.20 mg/100g), cardiac glycosides (1.48 mg/100g), steroids (8.01 mg/100 g), amino acids, vitamin A (188.28 IU/100g), tannins (0.09 mg/100g), terpenoids (3.42 mg/100g) and flavonoids (1.01 mg/100g). The presence of these diverse antioxidant chemicals in the extract could be responsible for some of the observed biological activities. The crude methanol extract of the leaves was reported to have lower antioxidant activity against DPPH radicals with IC_{50} value of 176.28 µg/mL relative to ascorbic acid (IC_{50} value of 76.9 µg/mL) that served as standard.

Methanol extract of *N. laevis* stem bark demonstrated good radical scavenging activities in superoxide anion, hydrogen peroxide and DPPH radical scavenging assays. The scavenging activities of the extract were higher than butylated hydroxyanisole (BHA) except in DPPH assay where BHA gave two-fold higher activity.[69] The extract was further shown to significantly inhibit β-carotene oxidation as well as exhibiting good FRAP.[70] In an *in vitro* study using various antioxidant assays, 75% methanol extract of the stem bark exhibited moderate antioxidant activity by scavenging hydroxyl (41.09%), nitric oxide (26.57%) and DPPH radicals (24.86%). The extract also inhibited lipid peroxidation (51.91%) and showed good FRAP (0.27%). The antioxidant activity was reported to be proportional to the total phenolic contents (139.17 mg/L GAE) and total flavonoids content (76 µg/mL QE).[71] In another study, ethanol extract of the stem bark was reported to exhibit good FRAP with EC_{50} value of 2.80 mg/mL compared to 18.18 mg/mL of vitamin C which served as reference. This result showed that the extract demonstrated about 6-fold higher FRAP than vitamin C. DPPH radical scavenging assay, the extract (0.1-3 mg/mL) was examined with *n*-propyl gallate (0.01-0.3 mg/mL) and α-tocopherol (0.01-0.3 mg/mL) as positive standards. It was reported

that the extract showed concentration-related scavenging activity that was lower than that of α-tocopherol which was also lower than *n*-propyl gallate. The antioxidant property of the extract is reported to correlate positively with the total phenolic content.[11] The above reports demonstrate that different parts of *N. laevis* have antioxidant and radical scavenging properties and the activities of the parts vary from another with the leaves consistently showing higher activity compared to the stem bark and other parts. It is also interesting to know that the antioxidant potentials of different solvent extracts of the plant were shown to be proportional to the phenolic and flavonoid contents of the extracts[92].

Analgesic and anti-inflammatory activities

Based on the traditional use of different parts of *N. laevis* in treating pain and inflammation, Usman *et al.*[110] assessed the analgesic effect of the ethanol extract of the flowers on acetic acid-provoked stomach constriction response in mice as well as carrageenan-induced rat paw edema (acute inflammation). The extract at doses of 50, 100 and 200 mg/kg, *p.o* dose-dependently suppressed acetic acid-generated writhing's by 63.41, 66.67 and 83.74%, respectively relative to 83.74% by ketoprofen (10 mg/kg, *p.o.*). Similarly, the extract at the same doses respectively elicited 30.68, 33.14 and 38.70% inhibition of carrageenan-evoked paw edema. This activity is, however, lower than 59.72% inhibition by the reference drug, ketoprofen. Udeozo *et al.*[10] also reported that ethanol extract of the flower, which is rich in flavonoids, tannins, terpenes, steroids, alkaloids and cardiac glycosides, inhibited acetic acid-mediated writhing's and formalin-generated nociception. The authors further demonstrated that the extract has a moderate safety profile with LD_{50} value of 1264.9 mg/kg, *i.p.* Collectively, these results demonstrate that the extract has good analgesic effect and moderate protection against acute inflammation.

Kolawole and Akanji[111] investigated the effects of ethanol extract of *N. laevis* leaves on diabetic (60 mg/kg streptozotocin, *i.p*)-generated oxidative stress and inflammation. It was reported that just like glibenclamide (5 mg/kg, *i.p*), the crude extract (500 mg/kg, *p.o* for 28 days) improved the antioxidant status by increasing the levels of GSH and activities of CAT, GPx and SOD by 53.1%, 21.5%, 20.2% and 33%, respectively in the liver of diabetic rats compared with untreated-diabetic rats. Reduction in nitric oxide (NO), pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) and chemokines production are known indicators of anti-inflammation. It was recorded that the

extract at the same dose reduced the level of NO, TNF-α and IL-1β in serum of both diabetic and normal rats. TNF-α and IL-1β, in addition to interferon-γ (INF-γ) are upstream activators of inducible nitric oxide (iNOS) which generates NO from L-arginine, increasing inflammation and destruction of pancreatic Islet cells[112].

Among the phenylpropanoid glycoside isolated from *N. laevis*, verbascoside (100 μM) was reported to possess anti-inflammatory property by suppressing *i*NOS activities and down-regulating its gene expression, as well as antioxidant property by reducing radical production and boosting SOD, CAT and GPx activities in LPS and IFN-γ-activated THP-1 cells.[113] In an *in vivo* model of colitis, verbascoside has been shown to inhibit bowel inflammation by suppressing the activities and gene expression of matrix metalloproteinases and pro-inflammatory cytokines which are involved in inflammation as well as inhibiting oxidative stress.[114] Recently, Mun *et al*.[115] investigated the anti-inflammatory activities of atraric acid, one of the furanonaphthoquinones isolated from the stem bark of *N. laevis* by Gormann *et al*.[45] using both *in vitro* and *in vivo* models. The Korean researchers demonstrated that atraric acid (300 μM) inhibits inflammation in LPS-activated Raw macrophages by suppressing the level of inflammatory mediators such as pro-inflammatory cytokine (36.3, 93.3 and 80.9% inhibition of IL-1β, IL-6 and GM-CSF, respectively), nitric oxide (69% of inhibition) and prostaglandin E2 (100% inhibition) and dose-dependently inhibit *i*NOS and cyclooxygenase-2 (COX-2) gene expression.[115] The authors further reported that the compound regulated the expression of activated forms of ERK and NF-κB signaling pathway, *in vitro.* Furthermore, it was reported that atraric acid suppressed LPS-generated inflammation in mice and reduced pro-inflammatory cytokine levels in both mouse peritoneum and serum, and attenuated organ damage associated with LPS-generated inflammation. These findings have shown that atraric acid might be responsible for the anti-inflammatory properties of *N. laevis,* and have, at least in part, provided the molecular mechanism of anti-inflammatory activities of *N. laevis*, supporting its traditional use.

Antipyretic activity

To examine the potential antipyretic properties of *N. laevis*, in relation to the traditional use of boiled leaves for treating fever, the inhibitory effects of *n*-hexane, ethyl acetate, and aqueous extracts of the leaves (25, 50 or 100 mg/kg, *p.o* for each extract) on baker's yeast-provoked pyrexia was evaluated by Bafor *et al*.[116]. It was reported that ethyl acetate and

aqueous extract showed significant and dose-dependent antipyretic activity, whereas as *n*-hexane extract was inactive. Among the two active extracts, aqueous extract produced higher activity than ethyl acetate extract; however, the aqueous extract had lower activity compared to paracetamol (20 mg/kg, *p.o.*) that served as reference antipyretic drug. In an *in vivo* study, Ghanaian authors reported that 70% ethanol extract of the leaves (30, 100 and 300 mg/kg, *p.o.*) significantly and dose-relatedly inhibited carrageenan-provoked chick paw edema and carrageenan-generated mechanical hyperalgesia at levels comparable with reference diclofenac (10, 30 and 100 mg/kg, *i.p.*).[117] Similarly, at the same doses in high temperature-induced pain, the extract was reported to dose-dependently increase the latency time at last phase by 66.12%, with no significant effect on the early phase. Diclofenac (10, 30 and 100 mg/kg, *i.p.*) which served as standard, inhibited both acute and chronic (biphasic) phases of hyperthermal-originated pains. In formalin-provoked pains, the extract inhibited both phases (54.47 and 83.62%, respectively) of pain, significantly and dose-dependently unlike diclofenac that inhibited the first phase (60.89%) only. Another standard, morphine (1, 3 and 10 mg/kg) significantly and dose-relatedly inhibited both phases of pains (42.48 and 60.38%, respectively).[117] Similar to the above report on ethanol extract of the leaves, Ainooson *et al.*[38] reported that ethanol extract of the stem bark (at the same doses as above) significantly inhibited both phases of formalin-induced nociception with ED_{50} values of 33.15 mg/kg and 28.41 mg/kg compared with standard drugs, morphine (1, 3 and 10 mg/kg) which acted on both phases (1.90±0.63 and 7.63±4.67 mg/kg) and diclofenac (10, 30 and 100 mg/kg, *i.p.*) that acted on only second phase (34.87±10.20 mg/kg). In general, the extract has been shown to have potent analgesic and anti-inflammatory properties. Although the result supports the traditional application, further study to isolate the specific components of the extract that elicits these biological activities is needed.

Anti-thrombotic activity

The anti-thrombotic activity of methanol extract of *N. laevis* leaves was assessed in both *in vivo* and *in vitro* studies. The extract (100 and 200 mg/kg, *p.o.*) significantly increased the clothing time in a manner similar to heparin (0.75 and 1.5 mg/kg, *p.o.*) and aspirin (1.0 and 2.0 mg/kg, *p.o.*) in rabbits. In *in vitro* studies, the extract, in a concentration-related manner, increased the coagulation time of normal rabbit blood as well as thrombin-induced clotting of human blood.[118] The extract was further shown to contain some phytochemicals, including alkaloids, tannins, flavonoids, and phenols, and was reported not to provoke any sign of

toxicity within three days of oral administration up to 2000 mg/kg, suggesting a high safety profile.

Aphrodisiac activity

The effect of aqueous extract of *N. laevis* leaves on enzymes associated with erectile dysfunction was examined by Akomolafe *et al.*[47]. The extract (20.27-90.10 µg/mL) significantly and concentration-dependently suppressed phosphodiesterase-5 (PDE-5) activity with IC_{50} value of 44.25 µg/mL compared to that of PDE-5 inhibitor, sildenafil citrate (IC_{50} = 2.78 µg/mL); moderately inhibited arginase activity with IC_{50} value of 134.92 µg/mL, compared to that of arginase inhibitor, N-hydroxy-L-arginine (IC_{50} = 0.75 µg/mL). Also, the extract strongly inhibited acetylcholinesterase (AChE) activity with IC_{50} value of 0.53 mg/mL, which was higher than the AChE inhibitor, prostigmine (IC_{50} = 2.44 mg/mL). The extract also, had weak inhibitory effect on angiotensin I-converting enzyme (ACE) activity (IC_{50} = 130 µg/mL), which was lower compared to the inhibitory activity of an ACE inhibitor, lisinopril (IC_{50} = 0.22 µg/mL). This enzyme inhibitory activity could be linked to the phenolic acids and flavonoids in the extract. Plant-derived flavonoids and phenolic acids have been reported to have inhibitory activities on enzymes linked with erectile dysfunction and has been shown to improve erectile status in living systems.[119-121]

Antihypertensive activity

The antihypertensive activity of methanol extract of *N. laevis* leaves were examined by Enye *et al.*[122] It was reported that the extract (10, 20, 50, 80 and 100 µg, *i.v.*) significantly and dose-relatedly reduced pentobarbitone (40 mg/kg, *i.p.*) induced hypertensive response in cats. At 80 and 100 µg, the extract gave similar activity to acetylcholine (20 µg, *i.v.*) and higher activity than adrenaline (10 µg, *i.v.*). It was also observed that the effects of the extract are short-lived compared to the positive standards, suggesting that the anti-hypertensive compound(s) in the extract has short half-life. This result calls for further investigation into the potentials of deriving antihypertensive agents from the plant. Due to poor bioavailability and short half-life, the incorporation of the bioactive constituents, when isolated into biocompatible carriers to increase the delivery at therapeutic doses in target sites, is recommended.

Cardioprotective activity

Based on the traditional use of both the leaves and roots for treating cardiovascular diseases, Nigerian researchers evaluated the cardioprotective potentials of *N. laevis* roots and leaves against carbon tetrachloride (CCl_4)-provoked cardiomyopathy in rats.[18] The water-ethanol roots and leaves extracts dose-dependently (200-800 mg/kg) attenuated CCl_4-induced cardiotoxicity as characterized by lower activities of creatine kinase, lactate dehydrogenase (LDH) and AST and level of cardiac troponin I, markers of cardiac status in serum of herbal drugs-pretreated rats compared to placebo. During myopathies, these intracellular components of the cardiac cells are released into circulation, increasing their serum levels and activities. The ability of the extracts to maintain lower levels of these markers in cardio-intoxicated rodents is an indication of cardio protection. Considering the fact that dyslipidemia is a risk factor to cardiovascular diseases, it was reported that the extract ameliorated CCl_4-provoked dyslipidemia as shown by dose-related reduction in T. Chol, LDL and TAGs levels with concomitant elevation in HDL level compared to placebo. Carbon tetrachloride induces cardiotoxicity partly by suppressing antioxidant status and increasing lipid peroxidation in cardiomyocytes. The authors ascribed the cardioprotective activities of the extracts to the presence of different classes of phytochemicals such as alkaloids, tannins, saponins, flavonoids, anthraquinones, terpenoids, and cardiac glycosides in both extracts.[18] However, the specific pharmacological principles responsible for the cardioprotective activities of the extracts as well as the mechanisms through which the extracts exert their cardio protection are unknown. They are hence, subjects of further investigation.

Anti-ulcer activity

Aqueous-ethanol extract of *N. laevis* bark and its *n*-hexane, ethyl acetate, and aqueous fractions were examined against ethanol-induced acute stomach ulceration. It was reported that pretreatment of rats with the extract and its solvent fractions (100 mg/kg) significantly inhibited ulceration by 78, 80, 73, and 70%, respectively by extract, aqueous fraction, ethyl acetate fraction and *n*-hexane fraction relative to 78% inhibition by cimetidine (100 mg/kg), a positive control that act by antagonizing H2 receptors.[20] This observation infers that the plant is a potential source of anti-ulcer and gastroprotective compounds; hence, further investigations are needed to exploit, isolate, and characterize these compounds.

Hepatoprotective activity

In an *in vivo* study, aqueous-ethanol extract of *N. laevis* leaves was reported to exert good protection against a hepatotoxin, CCl_4 that liver microsomal enzymes convert to its radical metabolites that alkylates cellular macromolecules and provoke peroxidation of polyunsaturated fatty acids in the membrane of the hepatocytes, causing liver damage.[9] The pretreatment with extract (100, 200, and 300 mg/kg) prevented hepatic necrosis and steatosis, lipid peroxidation, oxidative stress/reduction in antioxidant status and dyslipidemia associated with CCl_4 intoxication comparable with silymarin (100 mg/kg). Similarly, treatment with aqueous extract of *N. laevis* leaves (200 mg/kg) for 30 days showed attenuation of the histological characteristics associated with cadmium-mediated testicular and hepatic toxicities, both in the preventive and curative intervention models.[18] However, the molecular mechanism of the tissue protection was not clearly defined by the above studies. Also, the study designs of the above study, except Enye *et al*.[19], did not add any reference drug. The inclusion of a study group that will be pretreated with a known hepatoprotective drug that has antioxidant and renoprotective properties such as silymarin will have made these studies complete.

Oxytocic effect

Based on the ethnomedicinal use in Southeastern Nigeria to induce labour, Bafor and Sani[123] examined the effects of scalar concentrations of aqueous and ethanol extracts of *N. laevis* leaves (0.05×10^{-2}-200×10^{-2} mg/mL) on isolated uterus of rat intraperitoneally pretreated with diethylstilboesterol to induce oestrus. The two extracts significantly promoted uterine contraction with higher impact on the frequency than amplitude. It was also reported that ethanol extract was more active than aqueous extract, whereas both extracts showed relatively lower uterine contractility compared with acetylcholine, a reference oxytocic and a muscarinic receptor agonist. The extracts were suggested to have the same mode of uterine contraction with acetylcholine due to the related contraction profile observed. This study has supported the traditional use of the plant as a natural labour-inducer in Southeastern Nigeria.

To investigate the mechanism of oxytocic effects of the plant extract with higher contractility (ethanol extract), Bafor et al.[124] went further to examine the effects of smooth muscles contraction antagonists (phentolamine (4.09 and 40.91 nM), diphenhydramine (4.45 and 44.47 nM), atropine (1.18 and 11.91 nM), and verapamil (2.03 and 20.35 nM) on the contractility of the extract at different concentrations and compared the effect with oxytocin.

An α-adrenergic and serotonin receptors blocker, phentolamine has no effect on the uterine contractility of the extract, implying that the extract does not induce contraction via adrenergic and serotonin receptors. Meanwhile, histamine H1 and muscarinic receptors blocker (diphenhydramine) and a cholinergic receptor blocker (atropine) had concentration-dependent suppressive effect on the uterine contraction induced by the extract. This inhibitory effect is suggestive of surmountable antagonism as evidenced by significant increase in the EC_{50} of extract without affecting E_{max}. Additionally, the ability of verapamil, an L-type Ca^{2+} channel blocker to elevate the EC_{50} of extract and reduced the E_{max} significantly, an indication of non-surmountable antagonism, suggest that the extract act via activation of L-type Ca^{2+} channel, leading to increased influx of Ca^{2+}, depolarization of the myometrial smooth muscles and inducing contraction. Aside from verapamil, other antagonists have no effect on oxytocin-induced uterine muscle contraction. Generally, this observation suggests that, similar to oxytocin, the extract exerts its uterine contractility by activation of L-type Ca^{2+} channel; the extract, in addition, also act as agonist of cholinergic, histamine H1 and muscarinic receptors.

Effects on central nervous system

Based on the traditional use in treating disorders of the central nervous system in different parts of Africa[125-128]. Olajide and co-workers[129] previously showed that methanol extract of the stem bark at doses of 100-400 mg/kg body weight dose-dependently inhibited carrageenan-generated oedema in rats and prevented yeast-induced pyrexia, leptazol-induced seizures and acetic acid-induced writhing in mice, and enhanced phenobarbitone sleeping time in mice, suggesting that the plant has potential benefits in managing central nervous system abnormalities. In another investigation, Igwe and Nwobodo[126] investigated the potential protection of mice subjected to metrazol test (leptazole (70 mg/kg)-generated) and electric shock-provoked convulsion by combined aqueous extract of *N. laevis* root and stem barks (2.5-80 mg/kg intraperitoneally administered 30 minutes prior to induction). It was reported that the dose of the extract that kills 1% (LD_1) and dose of the extract that can protect 99% of the mice (ED_{99}) were 43.7 and 30.2 mg/kg, respectively, by intraperitoneal administration. In the same vein, the LD_{50} and ED_{50} of the extract were reported to be 489.8 and 5.6 mg/kg, respectively. From these results, the safety factor (LD_1/ED_{99}) and the therapeutic index (LD_{50}/ED_{50}) were determined to be 1.45 and 87.2, respectively in the drug-produced convulsion. It was also reported that the extract was more effective against drug-

generated convulsion than electric shock-induced convulsion, which has ED_{50} value of 63.1 mg/kg. In general, these findings showed that extracts of the root and stem of *N. laevis* have potential application as a source of anticonvulsant candidates. However, the study did not use any reference anticonvulsant drug such as valproate, gabapentin, acetazolamine and carbamazepine as a standard control, making the study incomplete. Again, the active compound(s) responsible for the anticonvulsant activity as well as the mechanism of action of the extract is unknown and hence, subject to further investigation. The effects of the extract and/or its isolated compounds on experimentally-generated convulsion in the presence and absence of sodium channel agonists (amiloride, aconitine, and veratridine) or γ-aminobutyric acid receptor blockers (such as tiagabine, gabitril, diacomit, and stiripentol) will help in understanding the mechanism of convulsion-protection of the extract and/or its isolate.

Considering the reported relationships between central nervous system disorders such as anxiety and depression in convulsive patients[130-131], Murtala and Akindele[24] recently study investigated the effects of hydro-ethanol extract (25, 50, 100, and 200 mg/kg, *p.o*) of *N. laevis* leaves using hole-board (HB), elevated plus maze (EPM), light/dark exploration (LDE), open field (OF), social interaction (SI) tests for anxiolytic properties and forced swim (FS) and tail suspension (TS) tests for depression inhibition properties. It was reported that the extract exhibited significant antidepressant- and anxiolytic-like effects, which are, however, lower than that the effects by imipramine and diazepam that served as reference drugs, respectively. The authors evaluated the possible mechanism of anticonvulsant activities of one dose of the extract (200 mg/kg, *p.o*) using forced swim test in the presence and absence of catecholamine antagonists (administered 15 minutes prior to the extract). Aside a dopamine D2 receptor antagonist, sulpiride (50 mg/kg, *i.p*) which was reported to elevate the duration of immobility relative to mice administered extract alone, other catecholamine antagonists prazosin (α1 adrenoceptor antagonist), metergoline (5-HT2 receptor antagonist), and yohimbine (α2 adrenoceptor antagonist) had no significant effect on the anticonvulsant activity of the extract, confirming that the extract acts via activation of dopamine D2 receptor and enhancing dopaminergic system. To examine the safety of the extract by intraperitoneal administration, the LD_{50} of the extract was recorded to be 390 mg/kg. In addition, gas chromatography-mass spectrometric (GC-MS) analysis of the extract revealed that octadecanoic acid ethyl ester, (*E*)-9-octadecenoic acid ethyl ester, (*E*)-1,3-dosenoic acid, oleic acid, and phytol are the major constituents. The authors reported that the

anticonvulsant effects of the extract could be attributed to the phytoconstituents such as saponins, steroids, glycosides, flavonoids, phenols, alkaloids, and tannins detected in the extract, as well as the antioxidant properties of the extract.[132]

In animal models of schizophrenia-like psychosis by experimental induction using excitotoxin (methamphetamine (35 mg/kg b.w, *i.p*) and apomorphine (5 mg/kg b.w, *i.p*).)-induced stereotypy and haloperidol-induced catalepsy assays, Kolawole et al.[133] demonstrated that the plant leaf extracts have antipsychotic effects. This may provide an explanation for the folkloric use of the plant in the treatment of mental health conditions in some parts of Nigeria[128].

In another study, the activity of water and ethyl acetate extracts of *N. laevis* leaves and roots (200, 400, 600 and 800 mg/kg, *i.p*) against pentylenetetrazole (75 mg/kg, *i.p*)-provoked seizure was assessed. The extracts dose-dependently and significantly delayed the time of onset of seizure but also prolonged the duration of the seizure relative to induced-untreated. It was reported that at 800 mg/kg, aqueous extract of the leaves totally prevented the induction of seizure by pentylenetetrazole comparable to diazepam (10 mg/kg, *i.p*). Additionally, leaf extracts of the leaves were generally more active than the root extract, while aqueous extract gave higher activity compared to ethyl acetate extract. Furthermore, the extracts attenuated the suppression in serum Ca^{2+} and glucose levels associated with seizures.[134] In a thermal pain induced by hot plate- which is controlled by the central nervous system, the extracts significantly inhibited pain perception by delaying the time of reaction (latency time) when placed in hot plate. The aqueous leaves extract at 800 mg/kg was reported to demonstrate similar inhibition compared with reference analgesic, morphine (4 mg/kg). Interestingly, the variation in activities of the extracts was identical to the above; leaf extracts of the leaves were generally more active than the root extract while aqueous extract gave higher activity compared to ethyl acetate extract. The authors attributed the activities of these extracts to the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, terpenoids, and cardiac glycosides detected in both extracts[18], based on the antinociceptive activities of flavonoids, saponins, and tannins earlier reported[135-136].

Amos et al.[137] evaluated the effect of methanol extract of *N. laevis* leaves on central nervous system by monitoring its effects on spontaneous motor activity, exploratory behavior, and apomorphine-provoked climbing reaction in mice as well as pentobarbitone-generated

hypnotic behavior in rats. The extract (25, 50, and 100 mg/kg, *i.p*) was reported to dose-dependently and significantly reduced exploratory behavior, with 100 mg/kg being more active than the reference drug, nitrazepam (2 mg/kg, *i.p*). At the same doses, the extract (50 and 100 mg/kg, *p.o*) time- and dose-dependently suppressed spontaneous motor activity and ameliorated apomorphine (3 mg/kg, *s.c*)-provoked hyperactive climbing in mice. In addition, the extract increased the duration of pentobarbital (35 mg/kg, *i.p*)-generated sleep in rats with 100 mg/kg giving 2 fold higher activity compared with diazepam (1 mg/kg, *i.p*). These observations infer that the extract has sedative potentials. However, the mechanism of the sedative activity and the specific constituents of the extracts responsible for this activity is unknown, warranting further investigation.

Anthelmintic effect

In an *in vitro* study, Hounzangbe-Adote et al.[138] assessed the activities of extracts of four plants that are used in ethnomedicine for controlling GIT parasites, *Zanthoxylum zanthoxyloides, N. laevis, M. lucida* and *Carica papaya*) on three stages of *Haemonchus contortus* life cycle (the eggs, the infective larvae and the adult worms). It was reported that among the plants, *N. laevis* leaves extract demonstrated strong and the highest activity against the three stages of the parasite, justifying the folkloric use. In another study, Hounzangbe-Adote et al.[139] reported that at 75, 150, 300, 600, 1200, and 2500 µg/mL, 30% crude ethanol extract of *N. laevis* leaves concentration-dependently inhibited another major gastrointestinal parasitic nematode of ruminants, *Trichostrongylus colubriformis* egg hatching slightly lower than reference drug, oxfendazole (0.5, 1, 5 and 10 µg/mL). Similarly, the extract (150-600 mg/mL) strongly inhibited larval migration at capacities comparable with levamisole (15-60 µg/mL). In addition, after 24 h, the extract at 2500 µg/mL totally immobilized (100%) adult *T. colubriformis* worms compared to 18% by levamisole (1000 µg/mL).

Additionally, the activity of *N. laevis* leaves essential oil against the stages of the life cycle of the GIT nematode that causes strongyloidiasis in rats, *Strongyloides ratti* was investigated. It was reported that the essential oil had a good inhibitory effect on *S. ratti* egg hatching with IC_{50} value of 18.2 µg/mL compared to IC_{50} value of 2.5 µg/mL by thiabendazole which served as a reference drug. In addition, the essential oil also inhibited larva migration with IC_{50} value of 51 µg/mL relative to IC_{50} value of 36 µg/mL by levamisole, the reference drug. Interestingly, the essential oil demonstrated a good safety profile by having very low

cytotoxicity against Vero cells (IC_{50} >50 µg/mL). Furthermore, the essential oil was shown to contain majorly β-caryophyllene (36 %) and eugenol (5.8 %), suggesting that these two constituents might be responsible for the anthelmintic properties of the essential oil[140]. However, the activities of the two major components of the essential oil need to be tested in isolation to confirm their involvement in the anthelmintic activities of the essential oil. In a more recent study, Olounladé et al.[140] subjected crude aqueous-ethanol and aqueous-acetone extracts of *N. laevis* leaves to *Trichostrongylus colubriformis* larva migration assay and reported that the incubation (23°C) of the larva with both extracts (150, 300, 600, and 1200 µg/mL) for 3 h resulted in a significant reduction in larva migration, in a concentration-related manner, an indication of anthelmintic activity. To assess the role of tannins in the anthelmintic activity of the crude extracts, upon addition of polyvinyl polypyrrolidone (PVPP) which forms complexes with tannins and polyphenols and render them biologically inactivity, there was a slight reduction in inhibitory effect of the extracts on larva migration, suggesting the tannins partly contributes to the anthelmintic activity of the crude extracts.

Collectively, the above reports demonstrate that *N. laevis* leaves could be potential sources of candidates with broad-spectrum anthelmintic properties that act in all the stages of the parasites' life cycle. However, there is the need to examine the activities of the plant leaves on other nematodes of public health importance, isolate and characterize the specific compound(s) responsible for the anthelmintic properties through bioassay-directed isolation and investigate the molecular mechanism of action of extracts and compounds isolated from them.

Insecticidal effect

The potential of using *N. laevis* in controlling insects that infest maize during storage was assessed by Ogungbite and Oyeniyi[141]. The stem and root barks powder and their methanol extracts were reported to prevent maize grain weight loss associated with *Sitophilus oryzae* and *Sitophilus zeamais* infestation. The methanol extracts of the stem and root barks, which showed higher activity than their pulverized powder, were reported to significantly inhibit the development of the two insects. However, the root powder at a high concentration (0.5 g) achieved insecticidal activity against *S. oryzae* within 96 h. The root bark extract was also shown to be more active against *S. zeamais* than *S. oryzae* as well as more active than the stem bark extract. In a similar investigation, Ashawo and co-workers investigated the activity

of essential oils from different parts of the plant against *Sitrotoga cerealella*, a pest that affected the quality and quantity of rice in storage. They observed that at 4% concentration, all the plant part's *n*-hexane extract suppressed the development of the insect. Compared to other parts, the root back essential oil killed all the moths and prevented the emergence of adult insects within 3 days post-application[142], supporting the previous findings by Ogungbite and Oyeniyi[141] that the root of the plant contains the most effective insecticidal phytochemicals. Generally, these findings suggest that the root of *N. laevis* is a reservoir of promising natural, eco-friendly and cheap insecticidal compounds that demand further exploitation.

Effect on reproduction

In cadmium-induced ovarian dysfunction in rats, Oyewopo et al.[143] demonstrated that water extract of the leaves (200 mg/kg for 28 days) protected ovarian histological and functional alterations by suppressing the secretion of follicle-stimulating and luteinizing hormones. This suggests that the plant's leaves can be used to normalize ovarian malfunctions. In research designed to know the effects of aqueous extract of *N. laevis* leaves on selected male sex hormones, Egba et al.[144] reported that at 200 and 400 mg/kg, *p.o* for 21 days, the extract had no significant effect on serum levels of testosterone, follicle-stimulating hormone and luteinizing hormone. This suggests that polar compounds in *N. laevis* leaves may have a negligible effect on male fertility profile. However, there is a need to assess the effect of long-term consumption of such doses on male and female reproduction using hormonal profile and histology of male reproductive tissues, and as well, the specific components of the extracts responsible for such bioactivities. The biological activities of the plant extracts and some of the compounds isolated from them are summarized in Table 2.

Safety profile of *Newbouldia laevis*

To assess the chronic toxicity of *N. laevis*, Agbafor and Ezeali[145] administered water and ethyl acetate extracts of *N. laevis* leaves and roots (200-800 mg/kg) to rats for 21 days and determined the effect of the extracts on the kidney and liver. The extracts caused no significant change in the liver and kidney at 400 mg/kg and below but at higher doses (800 mg/kg), the extracts elicited slight hepato-renal toxicities as characterized by a small increase in serum activities of ALT, AST, GGT and ALP and levels of urea, creatinine and uric acid. Hence, it is strongly advised that the consumption of large doses of the herbal preparations of

N. laevis for longer duration should be avoided. Similarly, Obi *et al.*[146] exposed methanol extract of *N. laevis* to African green monkey kidney (Vero) cells and reported that it caused the death of 57.3% of the cells with IC_{50} value of 0. 045 µg/µL, suggesting that the plant might be harmful to normal primate cells. However, the study failed to specify the part(s) of the plant used in the research. In an *in vitro* study that employed *Allium cepa* root growth and standard Ames test for identification of changes in growth, chromosomal integrity, and DNA replication by mutagens in microbes (using *E. coli* in the study), it was reported that *N. laevis* aqueous leaves extract concentration-dependently suppressed mitosis and root growth length and induced signs of chromosomal mutations. This finding further supports the call for caution on using a high dose of the plant to avoid the induction of mutagenesis.[147] However, this observation needs to be assessed in an *in vivo* experiment using higher organisms such as nematodes and rodents to validate the above report. The safety profile of the plant was further buttressed in reports where no mortality was recorded up to 5000 mg/kg in short duration studies (24 h) while 500 mg/kg did not cause a significant change in morphological, behavioral, biochemical, and hematological status in long-term studies (30 days) for root and ethanol leaf extracts[148-149]. Specifically, after demonstrating a 95% achievement of wound healing and anti-ulcer properties of ethanol extract of the root-bark at 200 mg/kg, the extract was shown to have an LD_{50} value of 1296 mg/kg[150]. In addition, the leaves' aqueous extract was also shown to have a weak herbal drug interaction with anti-retrieval drugs as evidenced by low inhibitory activity against CYP2B6 enzyme, supporting its relative safety[151-152]. Furthermore, crude methanol extract of the leaves was subjected to cytotoxicity study using *Artemia salina* lethality assay, and it was observed that the extract has a good safety profile with LC_{50} value of 2690.3 µg/mL relative to 629.93 µg/mL by potassium dichromate that served as reference[153]. These results suggest that the safety of the plant varies based on the part of the plant, solvent of extraction, and type of organism used for toxicity testing. Despite these reports on the relatively low toxicity of the plant, it is recommended that robust studies using both cell lines and rodents are required to establish the toxicity doses of the plant over a long duration of administration as well as the toxicological status at the molecular level.

Conclusion

Newbouldia laevis is widely used in West African traditional medicine and its biological properties have been investigated by researchers from different parts of the world. Several

biological activities such as antioxidant, antimalarial, trypanocidal, antimicrobial, anthelmintic, analgesic, anti-inflammatory, antidiabetic, anti-thrombotic, gastro-hepato-renoprotective, anti-hypertensive, properties have been reported on the plant. These biological activities are attributed, in part, to the secondary metabolites isolated from the plant. Despite the numerous reports on the biological activities of the plant, there have not been clinical trials on it, partly due to poor study design in some of the studies and the lack of in-depth mechanistic investigation. Hence, further investigations targeted at the cellular and molecular mechanisms of action of the plant extracts and compounds derived from them are highly advocated. In addition, there is a lack of information on the pharmacokinetic of the extracts and compounds isolated from them, warranting further investigation. Furthermore, some of the compounds isolated from the plant such as atraric acids, apegenin, withasomnine, verbascoside, martynoside and newbouldioside and others, which have been shown to possess good biological activities, and whose mechanisms of action and safety are already known, should be subjected to preclinical and clinical trials.

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Table 1: Ethnobotanical profile of *Newbouldia laevies*

Part of plant	**Place of use**	**Medicinal use**	**Ref**
Bark	Senegal	Paste made from the fresh bark is used for treatment of rheumatism and painful arthritis of the knees	7, 35
Stem bark decoctions	Ivory Coast and Nigeria	Decoctions is used for the treatment of epilepsy and convulsions in children	7, 35
Leaves and roots	Nigeria	Decoctions of leaves and boiled roots are used for fever	7, 35
Stem bark	Kumasi, Ghana	A decoction of fresh leaves and *Periploca nigrescens* Afzel are taken orally for 5-7 days to treat malaria	8
leaves, stem bark and roots	Ibadan, Southwestern Nigeria	boiled and drank for sickle cell anemia	11, 39
Leaves and roots	Ogurugu Community, Southeastern Nigeria	Boiled together and drank to treat malaria	15
Leaves	Plateau region of Togo	prepared as decoction and administered orally to treat malaria and fever	27
Leaves	Tiv people of North Central part of Nigeria	decoction of the leaves mixed with leaves of *Crossopteryx febrifugg* and *Morinda lucida* and taken orally for 5 days to treat malaria	28
Leaves	Omo Forest reserve in Western Nigeria	the leaves are boiled with leaves of *Mangifera indica* and *M. lucida* and taken orally to treat malaria fever	29

Leaves	Oyo State and other parts of South-western Nigeria	The decoction of leaves and *Alchornea laxiflora* bark and is taken orally to treat malaria	30
Leaves	Dutsin-ma metropolis of Katsina State, Nigeria	Decoction of the leaves is used for malaria	31
stem bark	Ikwere people of Port Harcourt, Southern Nigeria	Boiled and orally taken twice daily for six days for migraine and to prevent abortion in high abortion-risk pregnancy while the extract is bathed with for skin infections	32
leaves and roots	Ikwere people of Port Harcourt, Southern Nigeria	Boiled together and taken orally for treating fever, convulsion and epilepsy. The leaves alone are boiled and taken orally for malaria, stomachache and cough. The extract is also used to wash the eyes to treat eye pains, eye infection and conjunctivitis	32
Leaves	Ijebu people of Ogun State, Southwestern Nigeria	A cocktail of *N. laevis*, *Momordica charantia*, *Vernonia amygdalina* and *Ocimum gratissimum* leaves are boiled and drank thrice daily for measles. The leaves are also boiled with fresh leaves of *Momordica charantia* and taken orally twice daily for measles. Similarly, paste made from powdered *N. laevis* and *Elytraria marginata* leaves and *Elaeis guineensis* oil is applied topically for measles after bathing	32
Stem bark	Akwa Ibom State, Nigera	Boiled with sugarcane juice and taken one glass-cup thrice daily for 5-7 days for boil and dysmenorrhea	35
bark and leaves	Nigeria and Ghana	Applied topically and orally for treating breast cancers	35
Bark	Nigeria	masticated for toothache and boiled ones are taken for stomachache, dysentery and diarrhea	36
leaves, stem bark and roots	Nigeria and Cameroon	Preparations from these parts alone and in combination are used for diarrhea, dysentery, malaria, dental caries, sexually transmitted and cardiovascular diseases, and as worm expellant	37, 38

Leaves and stem bark	Ghana	Decoction is prepared and used for pain, different types of ulcer, arthritis, rheumatism, hemorrhoids and constipation	38
Leaves and roots	Bosomtwe and Sekyere East Districts of Ghana	Decoction of both parts is used for healing elephantiasis and convulsion. The parts are boiled together for treating malaria and pelvic pain.	41
		Fresh samples are ground and placed on wounds to stop bleeding and improve healing. A decoction is taken orally for cancers, diabetes, and muscle pains, cough and to increase libido and erection in males.	42
		Boiled leaves are used to treat microbial and parasitic infections of the urinogenital, respiratory and gastrointestinal tracts, diarrhea, malaria, and tooth pains.	
Roots, stem and leaves	Ghana	Boiled and drank to treat infection of the urinogenital tract	43

Table 2: Some of the biological effects of *Newbouldia laevis*

Substance tested/part used	Test model	Result	Ref
Hepatoprotective, Cardioprotective, Anti-ulcer Activities			
Aqueous-ethanol extract of the leaves	CCl_4-induced hepatotoxicity in rats	prevented hepatic necrosis and steotosis, lipid peroxidation, oxidative stress/reduction in antioxidant status and dyslipidemia associated with CCl_4 intoxication	9
Aqueous-ethanol extracts of the roots and leaves	CCl_4-induced cardiotoxicity in rats	Strongly protected the cardiac tissues against the cardiotoxin	18
Aqueous extract of the leaves	Cadmium-mediated testicular and hepatic toxicities	Protected and attenuated organ damage in both preventive and curative models	19
Aqueous-ethanol extract of the bark and its solvent fractions	Ethanol-induced acute stomach ulceration	Strongly inhibited the stomach and prevented lesion formation	20
Anticancer activity			
2-acetylfuro-1,4-naphthoquinone isolated from the root bark	Pancreatic MiaPaCa-2, breast cancer CCRF-CEM, CEM/ADR5000 cells, leukemia PF-382, leukemia HL-60, pancreatic Capan-1, breast MCF-7, colorectal SW-680, renal carcinoma 786-0, human brain glioblastoma U87MG, lung A549, colon melanoma colo-38, cervix HeLa and cervix Caski cancer cells	Good to excellent antiproliferative activities against the cancer cells; the compound was more active than doxorubicin against PF-382, CEM/ADR5000, Capan-1, Caski and Colo-38 cancer cells. the compound also elicited angiogenic inhibitory activity	51
Aqueous extract of leaves	Grafted cancer mouse model	Cytotoxic against the cancer cells leading to suppression of tumor mass and apoptosis	53

Antidiabetic activity			
Methanol extract of leaves	Alloxan-induced diabetes in rats	Suppressed fasting blood glucose level by 60.2%	55
Ethanol extract of leaves	Alloxan-induced diabetes in rats	Reduced postprandial glucose levels and inhibited pancreatic α-amylase activity in diabetic rats. The extract also inhibited both baker's yeast and rat intestinal α-glucosidases with IC_{50} values of 2.2 µg/mL and 43.5 µg/mL, respectively, and rat pancreatic α-amylase, *in vitro*	56
Different solvent extracts of the plant parts	*In vitro* enzyme inhibitory assays and normal and diabetic rats	Improved insulin secretion and its receptor's recognition, glucose uptake, pancreatic function, and antioxidant status, and inhibited α/β-glucosidases, α-amylase, lipid peroxidation, glycosylation of proteins, oxidative stress and gastric emptying.	55,56,61,64,67, 70,73,74
Antimicrobial activity			
Aqueous-ethanol, *n*-hexane and chloroform bark extracts	*S. typhi*, *S. aureus*, *S. pneumoniae*, *B. subtilis*, *K. pneumoniae*, and *P. aeruginosa*, and *C. albicans* and *A. fumigatus*	Inhibited the growth of the bacterial and fungal species	75
Lapachone-type naphthoquinones from the root	*C. albicans*, and *Cladosporium cucumerinum*, *B. subtilis* and *E. coli*	exhibited moderately antifungal (*C. albicans*) and antibacterial activities	76
Aqueous extract of the leaves	*S. typhi*, *K. pneumonia*, *E. coli* and *Shigella spp*	Strong cytotoxicity against the microbes	80
Flavonoids, flavonoids-rich extract and other solvent extracts of different parts of the plant	Different microbial isolates	good antibacterial activities by inhibiting peptidoglycan synthesis, and halting DNA synthesis and the formation of biofilms	78,79,86,87,88,89,90,91,92,95

Antimalarial activity			
Methanol extract of the leaves	*Plasmodium berghei*-infected mice	Weak antimalarial activity	99
Methanol extract of the leaves	Chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum*	Moderate antiplasmodial activity	100
Solvent extract of different parts of the plant	Drug resistant and drug-sensitive malaria parasites	Good antiplasmodial and antimalarial activities	102,103
Antiparasitic activity			
n-hexane, methanol and ethyl acetate extracts of leaves and pheophytins A and B from ethyl acetate extract	Wild strains of *T. brucei* Lister s427 (WT) and *T. congolense* as well as drug resistant strains	Ethyl acetate extract gave potent cytotoxic effects against the parasites; others gave low to moderate trypanocidal activity	104
Antioxidant activity			
Methanol extract of leaves	DPPH, lipid peroxide, peroxynitrite, HOCl, singlet oxygen, nitric oxide, superoxide anion and hydroxyl radicals scavenging, metal chelating and FRAP	Inhibited formation of radicals such as DPPH radical (85.85%), FRAP (64.01%), lipid peroxide radical (91.85%), HOCl (62.10%), singlet oxygen radical (96%), peroxynitrite radical (62.10%), nitric oxide radical (80.08%), iron chelating (90.11%), superoxide anion radical (81.11%) and hydroxyl radical (76.10%)	105
Methanol extract of Stem bark	DPPH radical, Superoxide radical and Hydrogen peroxide scavenging assays	Moderate scavenging of DPPH radical that was two-fold lower than buthylated hydroxyanisole (BHA) standard, an excellent concentration-related scavenging of superoxide anion ten-folds more than BHA, and a concentration dependent scavenging of H_2O_2 more than BHA	106
Methanol extract of stem bark	β-carotene oxidation and FRAP assays	The extract significantly inhibited β-carotene oxidation in a concentration-related manner and strong FRAP that is concentration dependent and is proportional to the phenolic content	107

Methanol/water (4:1) extract of stem bark	Deoxyribose, nitric oxide scavenging, DPPH radical scavenging, lipid peroxidation inhibition and ferric reducing potential assays	The extract exhibited good antioxidant activity by inhibiting scavenging hydroxyl radical (41.09%), nitric oxide radical (26.57%), DPPH radical (24.86%), and lipid peroxidation (51.91%) and FRAP (0.27). The antioxidant activity is proportional to the total phenolic content (139.17 mg/L GAE) and total flavonoid content (76 µg/mL QE)	108
Analgesic, anti-inflammatory and antipyretic activities			
Ethanol extract of the flowers	Acetic acid-induced writhing's in mice and carrageenan-induced rat paw edema	Potently inhibited writhing's and edema in a dose-dependent manner	110
Ethanol extract of the leaves	Streptozotocin-induced oxidative stress and inflammation in rats	Reduced the level of NO, TNF-α and IL-1β in serum of both diabetic and normal rats	111
Atraric acid from the leaves	LPS-activated Raw macrophages	Inhibited IL-1β, IL-6 and GM-CSF formation and reduced NO and prostaglandin E2 production by dose-dependently inhibiting *i*NOS and cyclooxygenase-2 (COX-2) gene expression. It also regulated the activation of ERK and NF-κB signaling pathway	115
Ethyl acetate and aqueous extracts of the leaves	Baker's yeast-provoked pyrexia	Dose-dependently inhibited pyrexia	116
70% Ethanol extract of the leaves	Carrageenan-provoked chick paw edema and carrageenan-generated mechanical hyperalgesia, hyperthermal-induced and formalin-induced pains	Inhibited edema and both phases of pains	117
Anti-thrombotic and antihypertensive activities			

Methanol extract of the leaves	Thrombin-induced clotting of human blood *in vitro*, and clotting time in rabbit	increased the coagulation time and thrombine-induced clotting of human blood	118
Methanol extract of the leaves	Pentobarbitone-induced hypertension in cats	Reduced blood pressure in the hypertensive cats	122
Oxytocic effect			
Aqueous and ethanol extracts of the leaves	Diethylstilboesterol-induced oestrus	promoted uterine contraction by the activation of L-type Ca^{2+} channel and cholinergic, histamine H1 and muscarinic receptors	123
Effects on central nervous system			
Extracts of the root and stem	Leptazol-induced seizures and electric shock-induced convulsion in mice	Inhibited convulsion	126
Methanol extract of the stem bark	Yeast-induced pyrexia, leptazol-induced seizures, acetic acid-induced writhings, and phenobarbitone sleeping time in mice	Inhibited pyrexia, seizures and writhings but enhanced sleeping time	129
Leaf extracts	Methamphetamine and apomorphine-induced stereotypy and haloperidol-induced catalepsy in mice	Inhibited stereotype and catalepsy, demonstrating antipsychotic effects	133
Aqueous extract of the leaves	Pentylenetetrazole-provoked seizure and thermal induction of pains	Prevented induction of seizure and inhibited hot-plate induced pain	134
Methanol extract of the leaves	Pentobarbital-generated sleep and apomorphine-provoked hyperactive climbing in mice	Sedative potentials by increasing sleeping time and reducing hyperactivity	137

n-hexane extracts of all the plant parts	Cytotoxicity against *Sitrotoga cerealella*	Cytotoxic against the parasite	142
Methanol extracts of the stem and root barks	Insecticidal activities against *Sitophilus oryzae* and *Sitophilus zeamais*	Inhibited the growth and maturity of the insects	141
Antioxidant activities			
Ethanol extract of stem bark	FRAP, DPPH radical scavenging assay and inhibition of lipid peroxidation	6-fold higher FRAP (EC_{50} = 2.80 mg/mL) than vitamin C (EC_{50} = 18.18 mg/mL). The extract also showed concentration-related DPPH radical scavenging activity and moderately inhibited lipid peroxidation of rat brain although lower than α-tocopherol and *n*-propyl gallate.	133
Methanol extract of leaves	DPPH radical scavenging	Moderate concentration-related scavenging activity with IC_{50} value of 176.28 µg/mL lower than ascorbic acid (IC_{50} value of 76.9 µg/mL) that served as standard	153

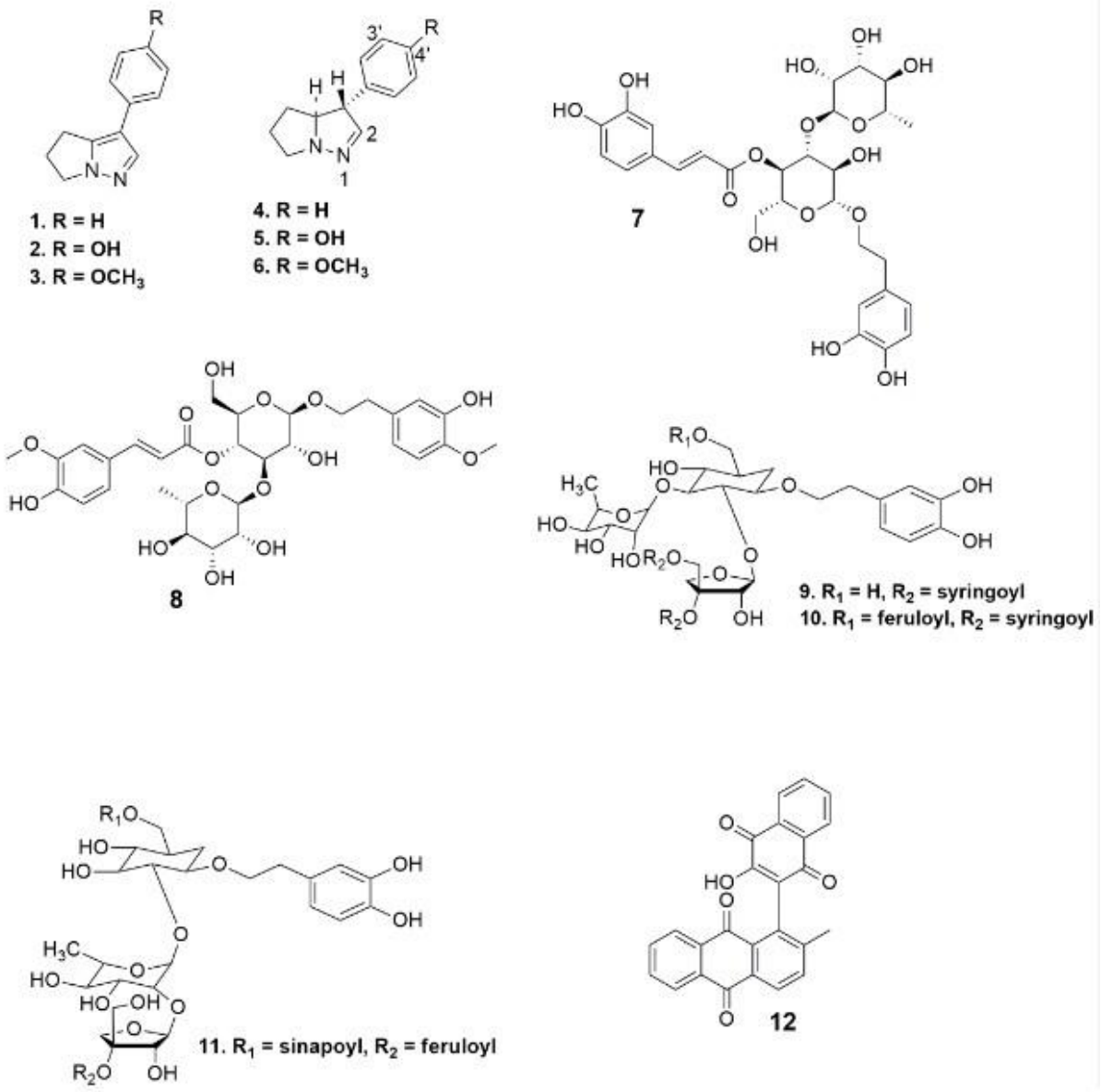


Figure 1: Chemical Structures isolated from different parts of Newbouldia laevis. Withasomnine (1), 4'-hydroxywithasomnine (2), 4'-methoxywithasomnine (3), newbouldine (4), 4'-hydroxynewbouldine (5), 4-methoxynewbouldine (6), verbascoside (7), martynoside (8), newbouldiosides A-C (9-11), newbouldiaquinone (12).

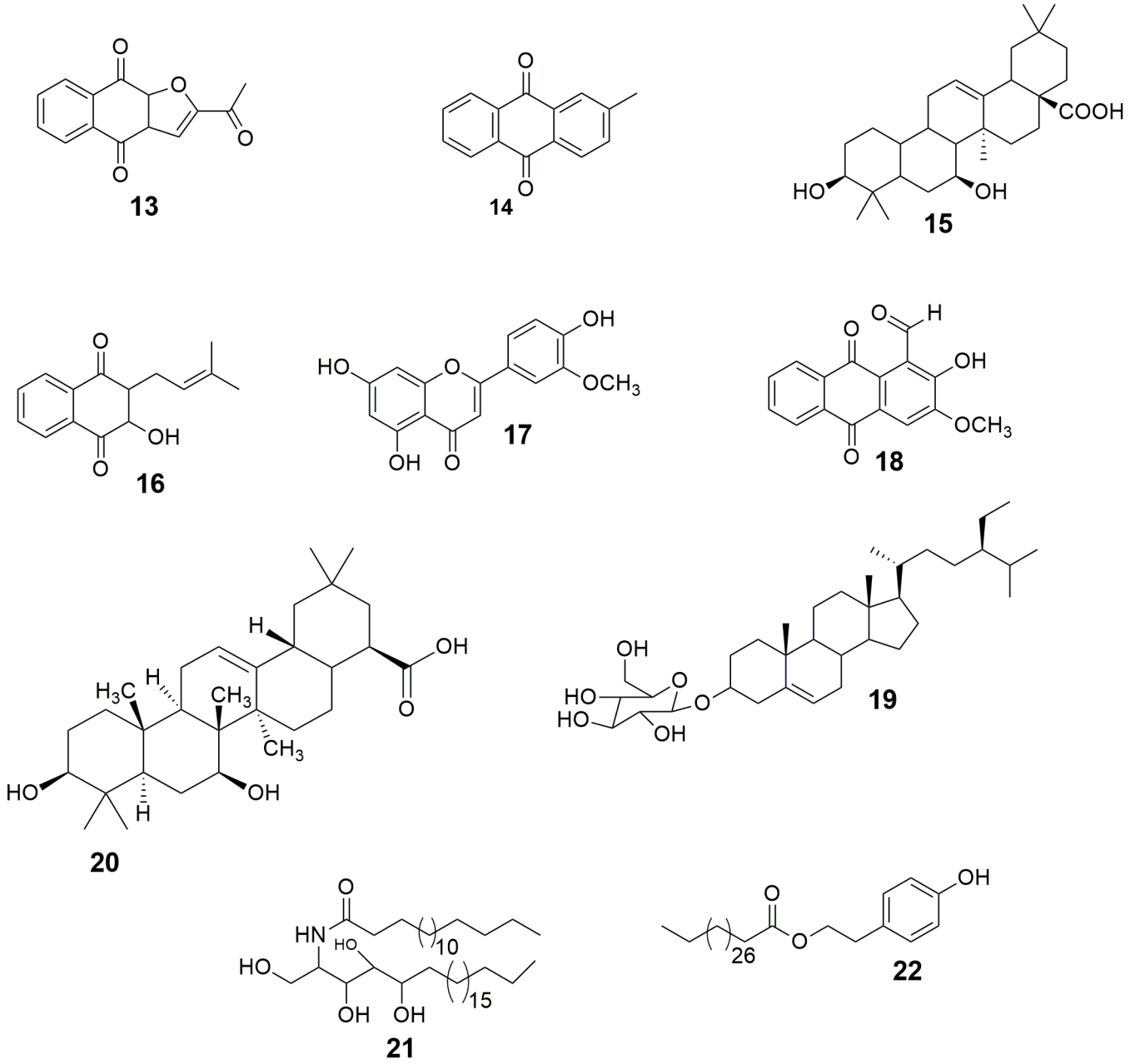
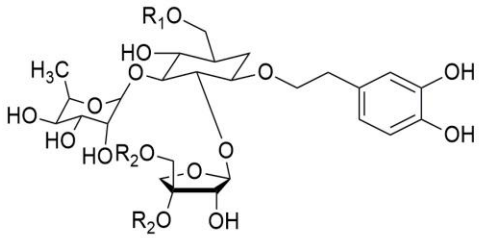
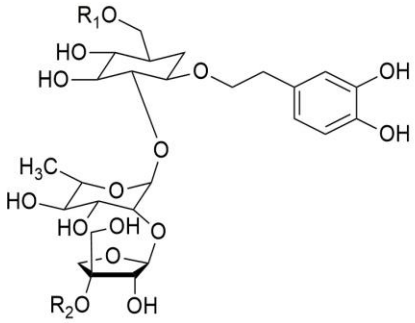


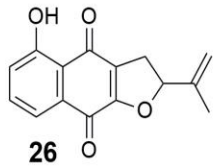
Figure 1 Continues: 2-acetylfuro-1,4-naphthoquinone (13), 2-methyl-9,10-anthracenedione (14), canthic acid (15), lapachol (16), chrysoeriol (17), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (18), sterol (β-sitosterol-3-O-β-D-glucopyranoside (19), oleanolic acid (20), newbouldiamide (21), 2-(4-hydroxyphenyl)-ethyltriacontanoate (22).



23. R_1 sinapoyl, R_2 = syringoyl
24. R_1 sinapoyl, R_2 = H



25. R_1 = sinapoyl, R_2 = H



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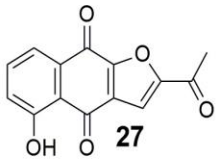

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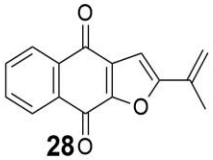

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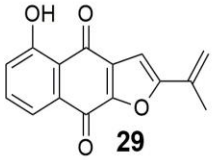

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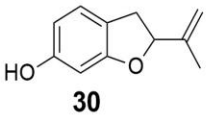

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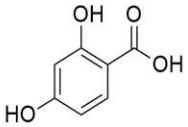

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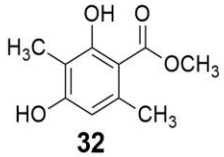

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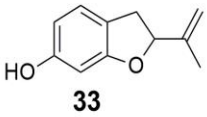

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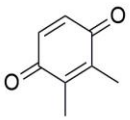

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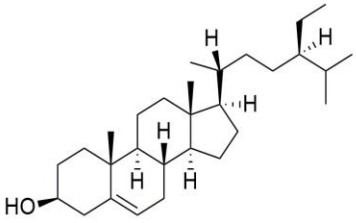

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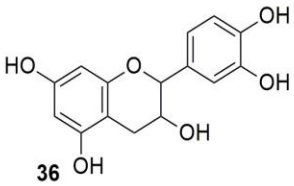

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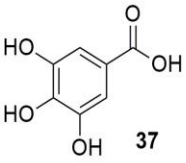

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Figure 1 Continues: newbouldiosides D-F (23-25), 5-hydroxy-dehydroiso-α-lapachone (26), 2-acetyl-5-hydroxynaphtho[2,3-β]furan-4,9-dione (27), 2-isopropenylnaphtho[2,3-β]furan-4,9-dione (28), 2-(1'-methylethenyl)-5-hydroxynaphtho[2,3-β]furan-4,9-dione (29), 2-(1'-methylethenyl)-7-hydroxy-naphtho[2,3-β]furan-4,9-dione (30), β-resorcylic acid (31), atraric acid (32), 2-(1'methylethenyl)-6-hydroxy-2,3-dihydrobenzo[β]furan (33), 2,3-dimethoxy-1,4-benzoquinone (34), β-sitosterol (35), epicatechin (36), gallic acid (37).

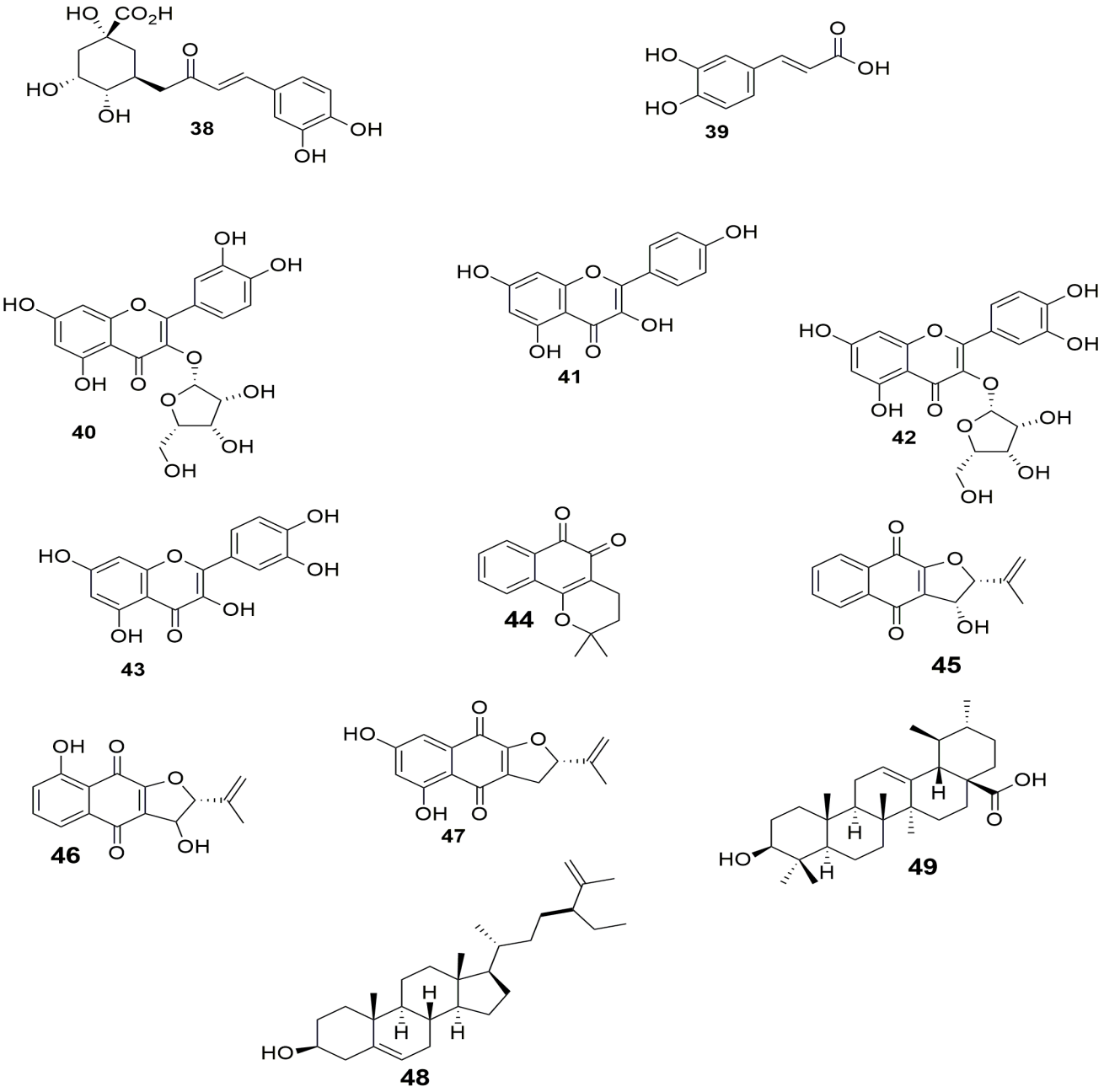

Figure 1 Continues: chlorogenic acid (38), caffeic acid (39), isoquercitrin (40), kaempferol (41), quercitrin (42), quercetin (43), β-lapachone (44), 3-hydroxydehydroiso-α-lapachone (45), 3,8-dihydroxydehydroiso-α-lapachone (46) and 5,7-dihydroxydehydroiso-α-lapachone (47), stigmasterol (48), ursolic acid (49).

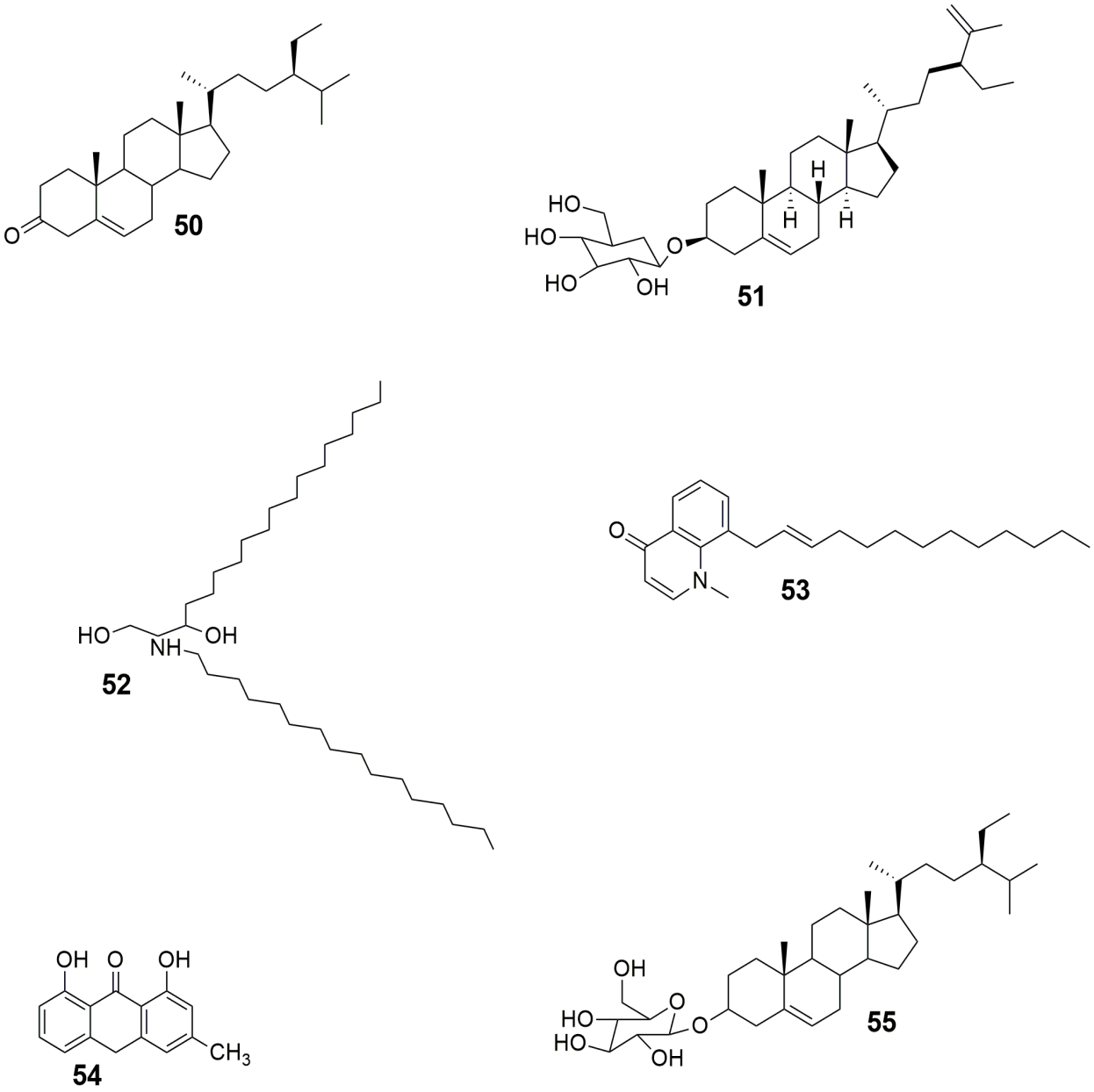

Figure 1 Continues: β-sitostenone (50), stigmasterol glucoside (51), hexadecadihydrosphingosine (52), evocarpine (53), chrysarobin (54), harmalol (55).