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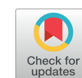
Differential constituents in roots, stems and leaves of Newbouldia laevis Thunb. screened by LC/ESI-Q-TOF-MS



Affo Dermane [a], Kafui Kpegba [b], Kodjo Eloh [c,*], Dorcas Osei-Safo [d], Richard K. Amewu [d], Pierluigi Caboni [c]

[a] *Department of Pharmaceutical Sciences, University of Lomé, 01 BP 1515 Lomé 01, Lomé, Togo*
[b] *Laboratoire de Chimie Organique et des Substances Naturelles (Lab COSNat), Département de Chimie, Faculté Des Sciences, Université de Lomé, 01 BP 1515 Lomé 01, Lomé, Togo*
[c] *Department of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, 09124 Cagliari, Italy*
[d] *Chemistry Department, University of Ghana, P.O. Box LG 56, Legon, Ghana*

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ABSTRACT

Newbouldia laevis (P. Beauv.) Seem. or "Boundary Tree" is a medium sized angiosperm in the Bignoniaceae family. It is native to tropical Africa, especially used in West Africa to mark property boundaries in rural areas. Despite its intensive use in folk medicine, the plant chemical composition is under investigated. The study is aimed at identifying the chemical constituents of *N. laevis* crude plant extracts by high-performance liquid chromatography (HPLC) on line with an untargeted high-resolution mass spectrometry. The used HPLC method showed to be suitable for the determination of withasomnine, newbouldine, and lapachol derivatives together with other known bioactive compounds like phytosterols and triterpenoids. The importance of these chemical constituents is discussed with respect to the role of *N. laevis* in West African ethnomedicine.

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1. Introduction

Approximately two-thirds of the world biological diversity is found in tropical zones, mainly developing countries. *Newbouldia laevis* (P. Beauv.) Seem. (Bignoniaceae) is a valued herb in tropical Africa medicine, and as such was used and grown for centuries in Togo (West Africa). It is a medium sized angiosperm that grows to a height of about 10 m. From the six most used plant extracts to treat life-threatening disease malaria in Togo, *N. laevis* was reported in a study to be the most active [1]. At 12.6 μg/ml, *N. laevis* inhibited 50% growth of the deadliest unicellular protozoan parasite of humans; *Plasmodium falciparum* that causes malaria. Furthermore, *N. laevis* is frequently cited to treat several diseases including diarrhea, dysentery, some sexually transmitted diseases or used as anthelmintic [2].

Medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. Recently, Kuete et al. reported ten compounds isolated from the methanolic root bark extract of *N. laevis* that demonstrated strong antimicrobial activity against twenty-one microorganisms bacterial species as well as three yeasts [3]. Another study identified furanonaphthoquinones, atraric acid and a benzofuran derivative in the plant stem barks [4]. More, alkaloids, saponins, flavonoids, glycosides, tannins and cyanides are regularly reported to be present in *N. laevis* extracts [5–7]. Despite liquid chromatography (LC) and mass spectrometry (MS) have become a common tool for investigating quantity, quality and chemical diversity of plant metabolites, data on *N. laevis* constituents LC-MS profiles are still lacking. To speed up future studies involving *N. laevis*, such as metabolite characterizations, updating metabolites database with relative retention times and mass spectra is crucial.

Over the past few years, liquid chromatography coupled with mass spectrometry (LC–MS) applications in natural products analysis have been growing dramatically because of the ever more improved separation and detection capabilities of LC–MS apparatuses. In particular, novel high-resolution hybrid instruments linked to high-performance LC and the hyphenations of LC–MS with other separation or analytical techniques greatly lead to unequivocal identification and highly sensitive quantification of natural products at trace concentrations in complex matrices [8,9]. LC-MS proved to be a very useful tool and is largely applied to the characterization of plant secondary metabolite.

In West Africa, medicinal plants recipes are mostly prepared as decoction, infusion, maceration in water, ethanol and methanol or sauce [10]. More, varying amounts and combinations of compounds present in the different parts of the plant, roots, stems and leaves could explain the various therapeutic effects. Thus, understanding the bio-distribution of these secondary metabolites in roots, stems and leaves is important to understanding pharmacological efficiency. Therefore, it is mandatory to determinate most used medicinal plants chemical composition from different organs using different solvent extractions (different polarity). In our ongoing activities in medicinal plants chemical characterizations

* Corresponding author at: Via Ospedale 72, 09124 Cagliari, Italy.
E-mail address: kodjoeloh@outlook.com (K. Eloh).

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[11,12], this current study was carried out to compare the chemical composition of *Newbouldia laevis* leaves, stem and root barks found in West Africa by an LC-MS metabolomics platform. Column chromatography and fractionation methods were performed to optimize LC-MS identification of some major components. Identified compounds were discussed accordingly to reported pharmacological effects of *N. laevis*.

2. Material and methods

2.1. Plant material

N. laevis leaves, roots and stem barks were harvested at Tsévié (Togo), in December 2014. Plant material was identified by one of the authors and confirmed by a botanist, Koffi Akpagana (Botany Department, University of Lomé). A Voucher specimen was deposited in the department Herbarium Centre under the number 233.

2.2. Chemicals and reagents

Ethanol, methanol, petroleum ether, acetone, formic acid and $CDCl_3$ were of analytical grade and acquired from Sigma Aldrich (Milan, Italy). Analytical standards were gently provided by the laboratory or synthetic chemists.

2.3. Sample extraction

2.3.1. Hydroethanolic plant extracts

In the face of the reports on the most popular extraction method used, *N. laevis* hydroethanolic extract was investigated. Different parts were washed free of dust, dried in laboratory conditions and reduced to powder with a Thomas Muler mill (4 series, reference 3375 E20). Plant powders were subsequently macerated with an ethanol-water (80/20, v/v) mixture for 72 h. Following proportions were used: 450 g of leaves in 3 l of solvent mixture; 448 g of stem barks in 3 l; and 429 g of root barks in 2.5 l. Supernatants were collected through cotton before filtration using 0.40 μm filter papers. Filtrates were evaporated to dryness under vacuum at 40 °C and kept in dark falcons at −20 °C for fractions, supernatants and pellets preparations.

2.3.2. Methanolic plant extracts

For a comprehensive metabolomics phytochemical analysis, 3 g of each plant part powders previously obtained were extracted with 30 ml of methanol in 50 ml falcons. Extraction was optimized by ultrasonication for 30 min then kept for 24 h at room temperature. Methanol supernatant was then filtered by 0.40 μm microfilter after centrifugation at 2000 rpm for 10 min. Crude extracts concentrations were calculated by evaporating an aliquot of 200 μl of each extract under a gentle nitrogen stream. Diluted samples to 5 mg/ml were analyzed by LC/ESI/QTOF.

2.3.3. Supernatants and pellets preparation: fractionation by precipitation in cold ethanol

Hydroethanolic extracts of *N. laevis* stem and root barks were used to obtain two fractions of pellet and supernatant. To 20 g of each extract, 200 ml of ethanol-water 75/25 (v/v) mixture was added and kept at 4 °C. After 24 h, the mixture was centrifugated at 1500 rpm for 15 min. The supernatant was then collected and the resulting pellet dissolved in the same volume of solvent ethanol-water mixture, cooled at 4 °C to be treated under the same conditions as above. The two supernatants were mixed and evaporated under vacuum at 40 °C as well as the pellet to obtain the supernatant and pellet fractions. Samples were kept in freezer at −20 °C until chemical analysis.

2.4. Column chromatography fractionation

Hydroethanolic root and stem barks of *N. laevis* extracts were separated by column chromatography. Following solvent systems were used to elute the different compound classes: petroleum ether, acetone, ethanol and methanol. Aliquots of 20 g of extracts were applied on the top of a 60 × 5 cm column, containing silica gel (reference 60GF254, 0.040–0.060 mm, activated at 150 °C for 3 h) packed with petroleum ether. As eluent, we used approximately 250 ml of every mixture of petroleum ether: acetone (2.5:97.5) then raising progressively the gradient to 75:25 followed by pure ethanol and finally pure methanol. The collected fractions were submitted to thin-layer chromatography (TLC), using as mobile phase a mixture of petroleum ether, acetone and methanol. Similar fractions based on their TLC profile were mixed for chemical analysis.

2.5. Liquid chromatography coupled to mass spectrometry LC-MS/ESI/QTOF

Plant extracts were analyzed by reverse-phase liquid chromatography on an Agilent 1200 series LC system using a Kinetex EVO C18, 100 Å, 5 μm, 150 × 2.1 mm (Phenomenex, Castel Maggiore, Italy). The LC conditions were as follows: flow rate: 0.3 ml/min; solvent A: 0.1% formic acid in bi-distilled water; solvent B: methanol; and gradient was from 10% to 100% B over 10 min and kept at this level for 10 min. Four microliters of every sample were then analyzed by Electrospray ionization in positive mode using an Agilent 6520 Quadrupole (Q)-Time of Flight (TOF) mass spectrometry (MS). Mass spectral data were acquired in the m/z range of 100–1500 amu with an acquisition rate of 1.35 spectra/s, averaging 10,000 transients. The source parameters were adjusted as follows: drying gas temperature 250 °C, drying gas flow rate 5 L/min, nebulizer pressure 45 psi. Based on the original acquisition files, we performed a pre-processing step with MetAlign software used for automated baseline correction and alignment of all extracted mass peaks across all samples. Results were stored as CSV file. ESI/QTOF MS data were then analyzed using the molecular feature extraction algorithm of the MassHunter Workstation software (version B 03.01 Qualitative Analysis, Agilent Technologies, Santa Clara, CA, USA). The molecular feature extraction algorithm took all ions into account exceeding 1000 counts with a charge state equal to one. Blank runs showed maximum 8 features with intensity threshold at 1000 counts. Isotope grouping was based on the common organic molecules model. Features were compared to reported compounds from *N. laevis* and in the Metlin database [13].

On the Q-TOF-MS/MS scan dependent; MS/MS autoswitch experiments were performed sequentially on the two main ions recorded by LC/ESI/MS. The combination of quadrupole ion-selectivity with the full scan sensitivity of the TOF analyzer allowed the on-line recording of autoswitch MS/MS experiments using an energy of 40 eV. In this study, up to two different co-eluting precursor ions could be selected for further MS/MS experiments. Nitrogen was used as collision gas. For accurate mass measurements in MS/MS, the $[M + H]^+$ precursor ion or a characteristic fragment ion of some selected compounds were used as lock mass.

2.6. Nuclear magnetic resonance (NMR)

Identification of a main compound of TLC single spot sample from the column chromatographic analysis was carried using nuclear magnetic resonance (NMR) spectroscopy. One and two-dimensional NMR spectra were recorded at 298 K using a VARIAN Spectrometer (model Mercury Plus) operating at 500 MHz for the ^1H nucleus and the 125 MHz for the ^{13}C nucleus using the standard pulse sequences available in the Varian software. NMR spectra were measured as solutions in $CDCl_3$ in 5 mm o.d. tubes. Chemical shifts (d) were expressed in parts per million.

Table 1
Different studied solvent extracts with respective abbreviations.

Extraction	Abbreviation
SBME	Stem bark methanolic extract
SBS	Stem bark hydroethanolic extract supernatant
RBP	Root bark hydroethanolic extract pellet
RBME	Root bark methanolic extract
RBS	Root bark hydroethanolic extract supernatant
RBETOH	Root bark ethanolic extract
RBWE	Root bark water extract
SBP	Stem bark hydroethanolic extract pellet
SBWE	Stem bark water extract
LTE	Leaves hydroethanolic extract
LME	Leaves methanolic extract

2.7. Melting point

Melting point of the isolated compound was determined on a Koffler melting point apparatus for samples contained in an open capillary tube in an electrically heated metal block apparatus.

3. Results and discussions

Mass spectrometry has become a primary tool for metabolite identification and quantification in plant research. However, both the size and the complexity of the data produced by this experimental technique impose great computational challenges for data analysis. In recent years, numerous web-based tools aimed at supporting mass-spectrometry-based metabolite profiling and data sharing between analytic laboratories [14–16]. This work applied available data analysis tools like online XCMS [14], Metlin [15] and MetaboAnalyst 3.0 [16] to explore chemical composition of a widely used tropical medicinal plant, *N. laevis* different solvent crude extracts (Table 1) and fractions by mass spectrometry coupled to quadrupole time of flight detector. Using this method, high-resolution mass spectrometry data was uploaded online for potential structure identification. Different evidences were used to propose probable structures herein. Desirable additional evidence such as chromatographic retention behavior for available compound standards were checked. When important, automatic MS/MS experiments for fragments and ionization behavior were made to confirm candidate structures [17].

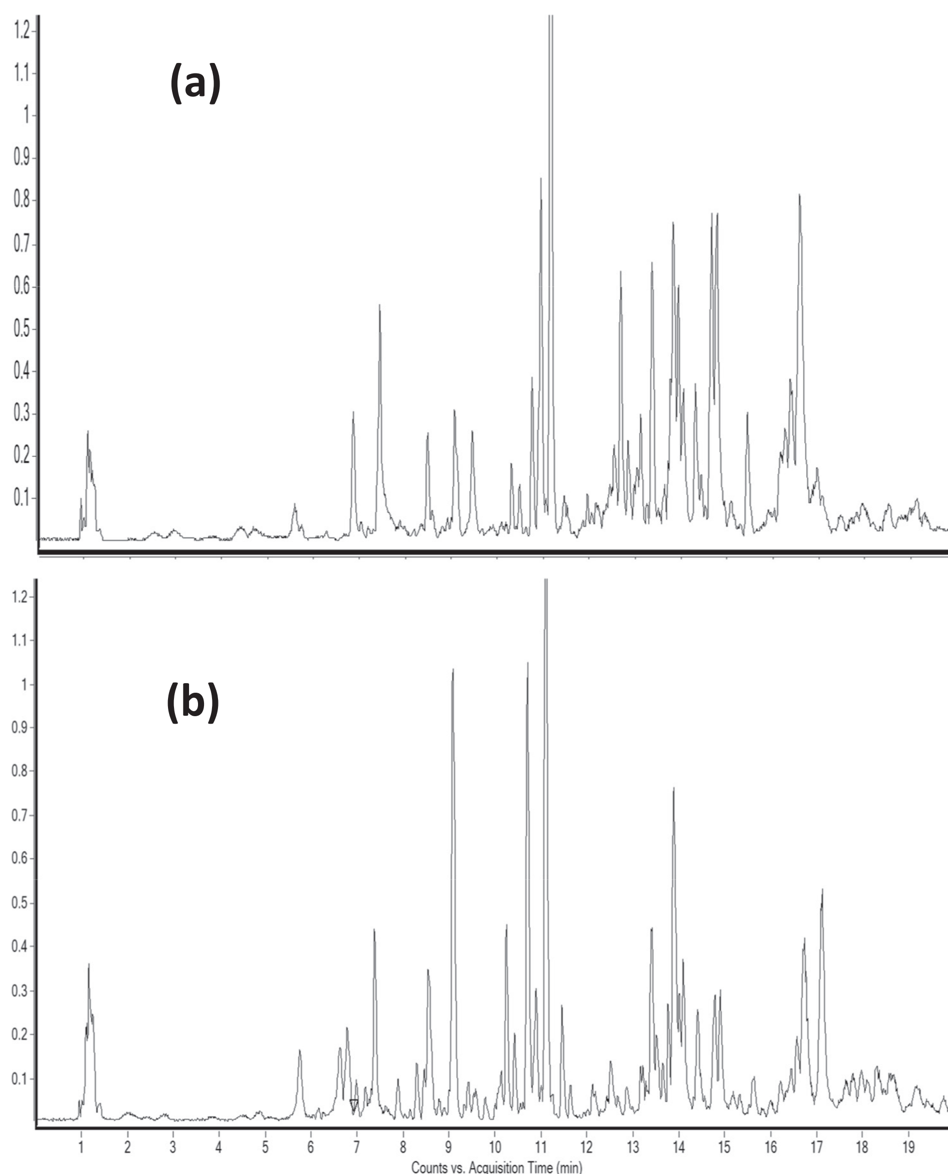

Fig. 1. LC-MS chromatograms of *N. laevis* stem bark pellet (a) and root pellet (b).

Working with water and methanol gradient mobile phase, twelve crude extracts were analyzed for their potential bioactive secondary metabolites determination by high-performance chromatography. Sample chromatograms are reported on Figs. 1–5. In 20 min, more than fifty metabolites were successfully separated and detected in each solvent extract after an electrospray ionization. Fig. 1 shows chromatograms obtained from *N. laevis* root and stem barks hydroethanolic pellets. Similar metabolites may be present in those two extracts with different levels. *N. laevis* root and stem barks pellets share similar bioactivity and may be used alternatively for the treatment of some ailments. Unlike pellets, stem and root barks hydroethanolic supernatants (Fig. 2) were different either qualitatively and quantitively in respect to chemical composition. For instance, filtering hydroalcoholic extracts before uptake might eliminate some metabolites with positive or negative effects on patients. Chromatograms on Figs. 3 and 4 compare ethanolic and water extracts of *N. laevis* root and stem barks. Fewer components were detected compare to hydroethanolic extracts. For a better therapeutic synergistic effect of the plant, hydroethanolic extraction could be a better choice over aqueous or ethanolic extract.

After the establishment of technologies for high-throughput protein analysis (proteomics), gene expression analysis (transcriptomics) and DNA sequencing (genomics), metabolomics is now evolving. Metabolomics is the term used for essentially comprehensive, nonbiased, high-throughput analyses of complex metabolite mixtures typical of plant extracts [18]. Herein, *N. laevis* leaves chemical composition was confronted to stem and root barks by a metabolomics study. Methanol was used because it is a good solvent for a holistic extraction [19]. Extraction was assisted with an ultrasonicator prior to mass spectrometry analysis. Fig. 5 illustrates chromatograms of the plant leaves, root and stem barks while Fig. 6 shows the quantitative chemical compositional variation of the three extracts under study. The first four identified compounds in stem and root barks extracts showed level differences but the

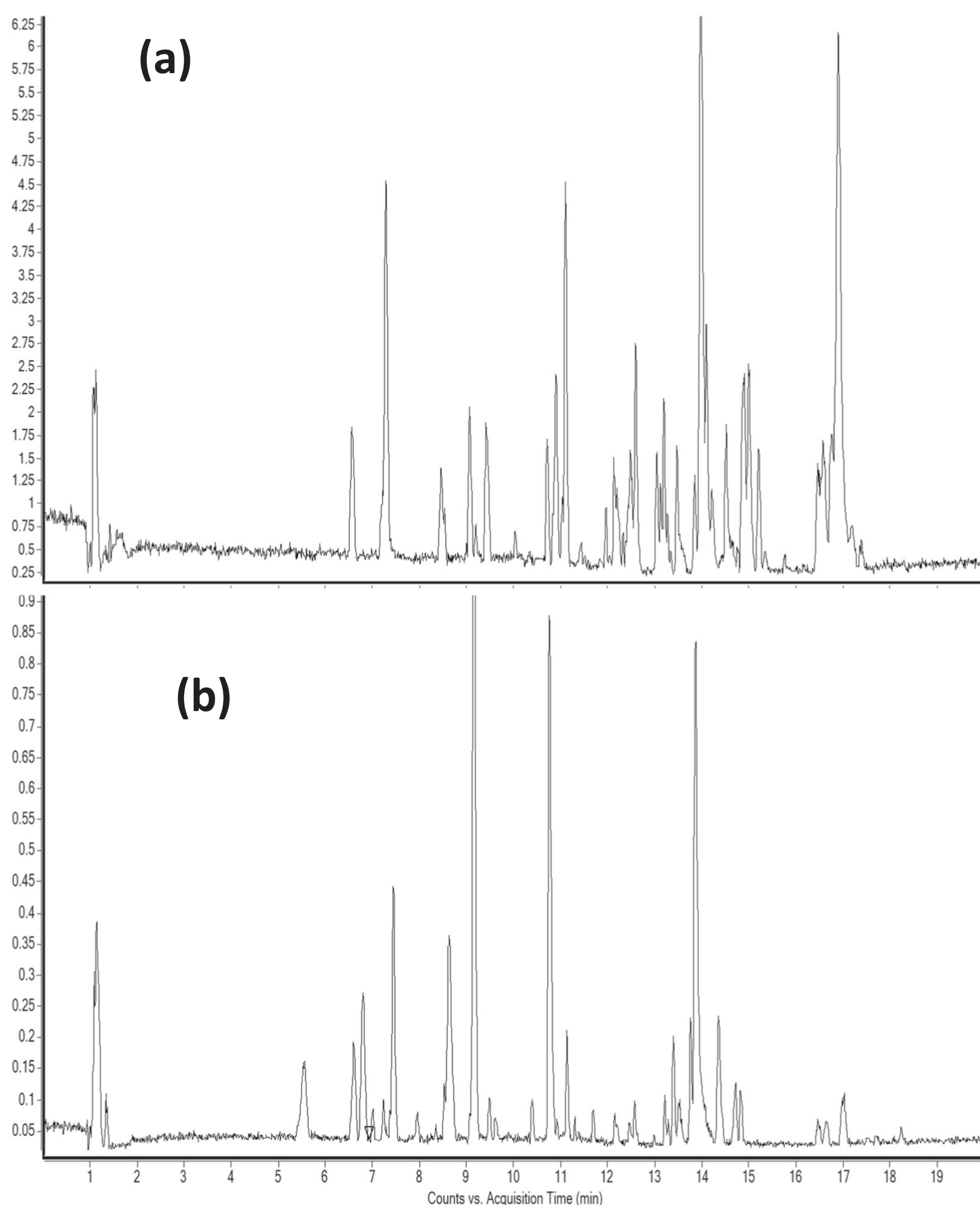

Fig. 2. LC-MS chromatograms of *N. laevis* stem bark supernatant (a) and root supernatant (b).

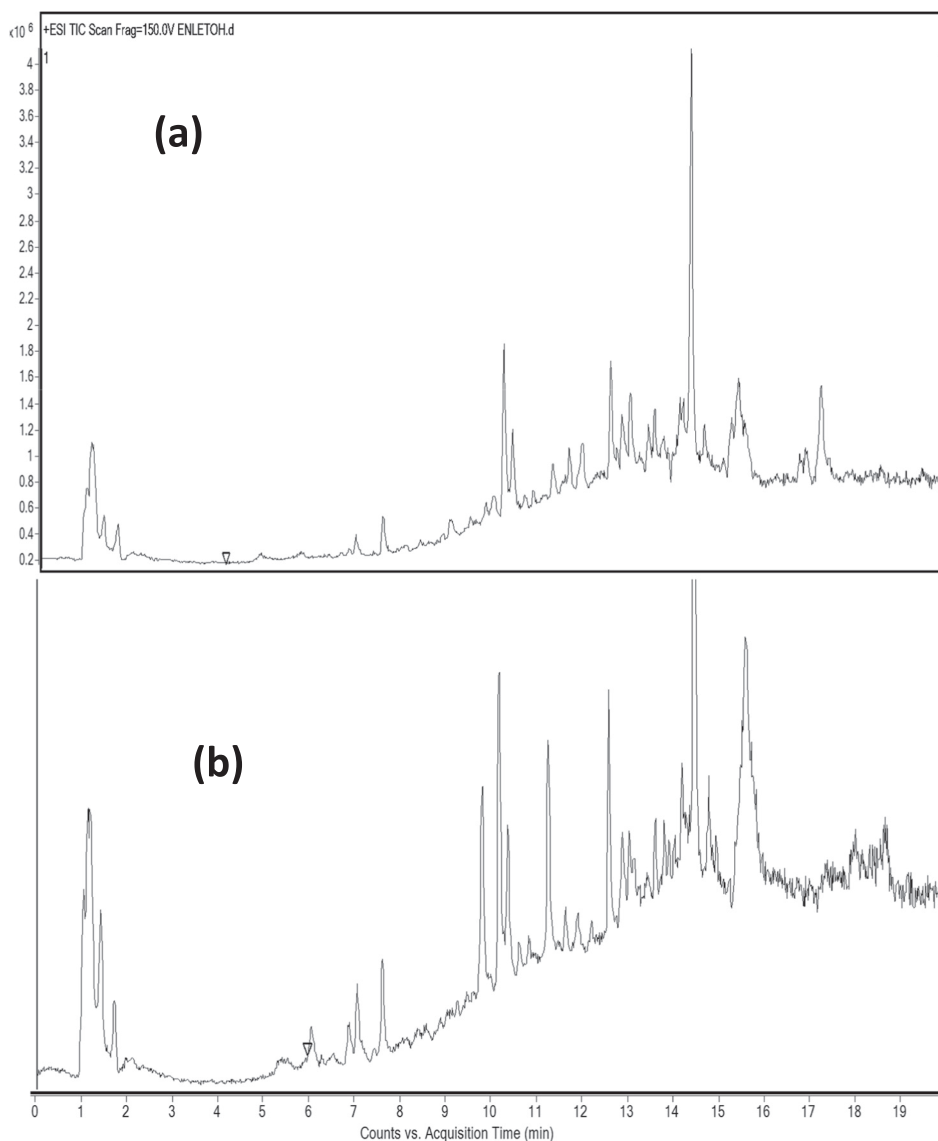

Fig. 3. LC-MS chromatograms of *N. laevis* stem bark ethanolic extract (a) and root ethanolic extract (b).

extracts demonstrated similar quantitative composition for the other components. Leaves extracts showed different levels of the all metabolites respect to stem and root barks. Many studies reported similar secondary metabolites levels in root and stem showed [20]. We report in supporting information (Figs. S_{4-23}) mass spectra and extracted ion chromatograms of the most important constituents detected.

From this preliminary analysis of those chromatograms, we noticed matrix effect or interference in response due to the presence of coelution of some main compounds. When using ESI for analyzing extracts of complicated matrices, although, LC–MS is one of the most sensitive and selective analytical techniques, it suffers from matrix effects. Matrix effects could cause either a loss in response (ion suppression) or an increase in response (ion enhancement). As a matter of fact, we performed a column chromatography on the hydroethanolic root and stem barks extracts to eliminate some invasive components. Using thin-layer chromatography, similar fractions were mixed to optimize LC-MS compound identification. A pure compound was successfully isolated and characterized by: ^{13}C and ^{1}H NMR spectroscopy (Table S_1 and Figs. S_{1-2}) and 2D NMR spectroscopic (Fig. S_3), melting point and high-resolution mass spectrometry. The pure isolated compound was subsequently identified as beta-sitosterol. The effect of beta-sitosterol on plasma insulin and glucose levels in normo- and hyperglycemic rats indicated an increase of the fasting plasma insulin levels with a corresponding decrease in fasting glycemia [21]. Beta-sitosterol is also reported for its multiple anti-tumor activities [22].

Furthermore, Fractions R_1-R_3 after analysis by LC-MS revealed compounds not detected in previous crude extracts. Principal groups of compounds identified in analyzed extracts (Table 1) are presented below. Structures of main specific secondary metabolites to *N. laevis* [23,24] are showed on Fig. 7.

3.1. Pyrazole alkaloids

From spectra analysis, we realized that *N. laevis* extracts contain a large amount of pyrazole alkaloids. Table 2 shows identified alkaloids with distribution in different solvent extracts (Table 1). Withasomnine and newbouldine (Fig. 7) derivatives were the main molecules and were readily present in every sample. A large number of structurally diverse natural compounds containing azole nucleus constitute an important class of biologically active heterocycles that are gaining more attention in the field of medicinal chemistry. Withasomnine was isolated from the roots of the Indian *Withania somnifera* that has been

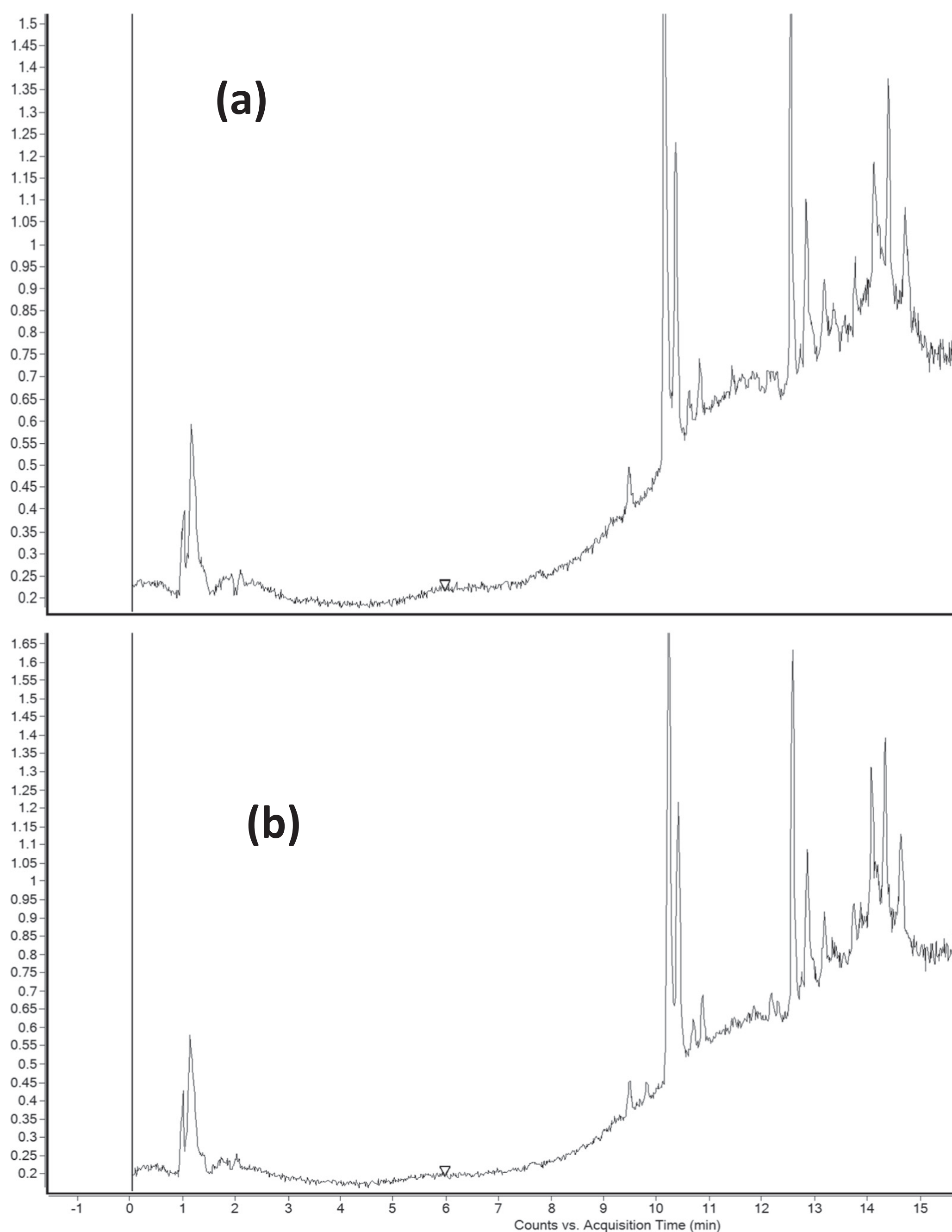

Fig. 4. LC-MS chromatograms of *N. laevis* stem bark water extract (a) and root bark water extract (b).

used in traditional Ayurvedic medicine for the treatment of enlarged spleen, migraines, and many infections and as an aphrodisiac. Withasomnine displays both Central nervous system and circulatory system depressant properties as well as being a mild analgesic [25]. Its analogues isolated from similar plant sources are described in more recent literature. More, from a theoretical study on pyrazole alkaloids of natural origin, withasomnine exhibited a strong antimalarial activity along with its derivatives para-hydroxy and -methoxy [26].

3.2. Lapachol derivatives

Table 3 displays detected lapachol derivatives from *N. laevis* solvent extracts and were more present in root bark extracts. Lapachol derivatives are naturally occurring naphthoquinones compounds having cytotoxic properties that can be advantageous for treating some types of cancer. These compounds induce oxidative stress and nucleophilic alkylation. Lapachol antiviral, antimicrobial, anti-inflammatory, and antimalarial effects, as well as its significant effect on *Trypanosoma cruzi* (responsible of sleeping sickness) are reported. More, lapachol was reported to inhibit *Onchocerca ochengi* parasites [27]. In addition, more recent investigations have shown that lapachol is an effective reagent for preparing new bioactive substances [28]. It is believed that the antitumor activity of lapachol may be related to its interaction with nucleic acids [29]. The naphthoparaquinone β-lapachone potential as an anti-trypanosomal agent was also reported. Studies demonstrated that β-lapachone can directly target DNA topoisomerases and inhibit their activity, which results in cytotoxicity [30].

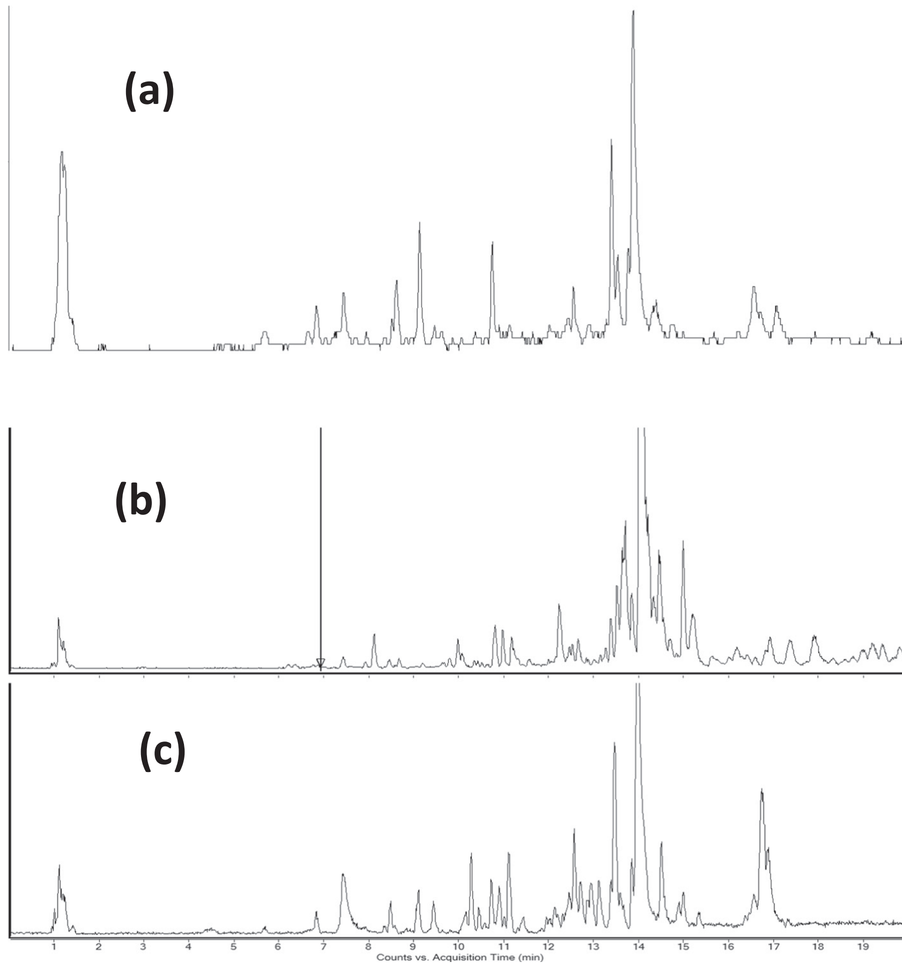

Fig. 5. LC-MS chromatograms of *N. laevis* root bark (a), leaves (b), and stem bark (c) methanolic extracts.

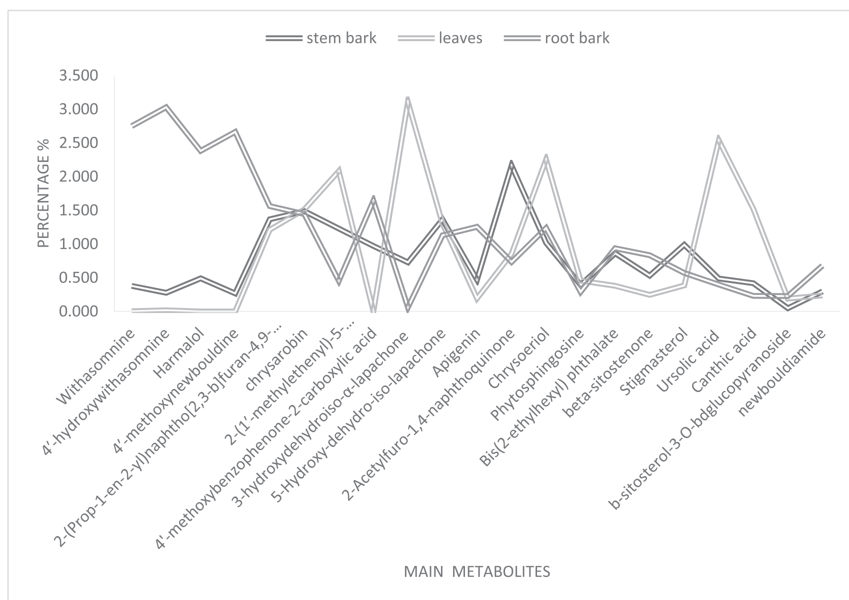

Fig. 6. Metabolomics analysis of *N. laevis* root bark, leaves and stem bark methanolic extracts. Major components are plotted on Y-axis and normalized chromatographic peak areas to 100 (%) on X-axis.

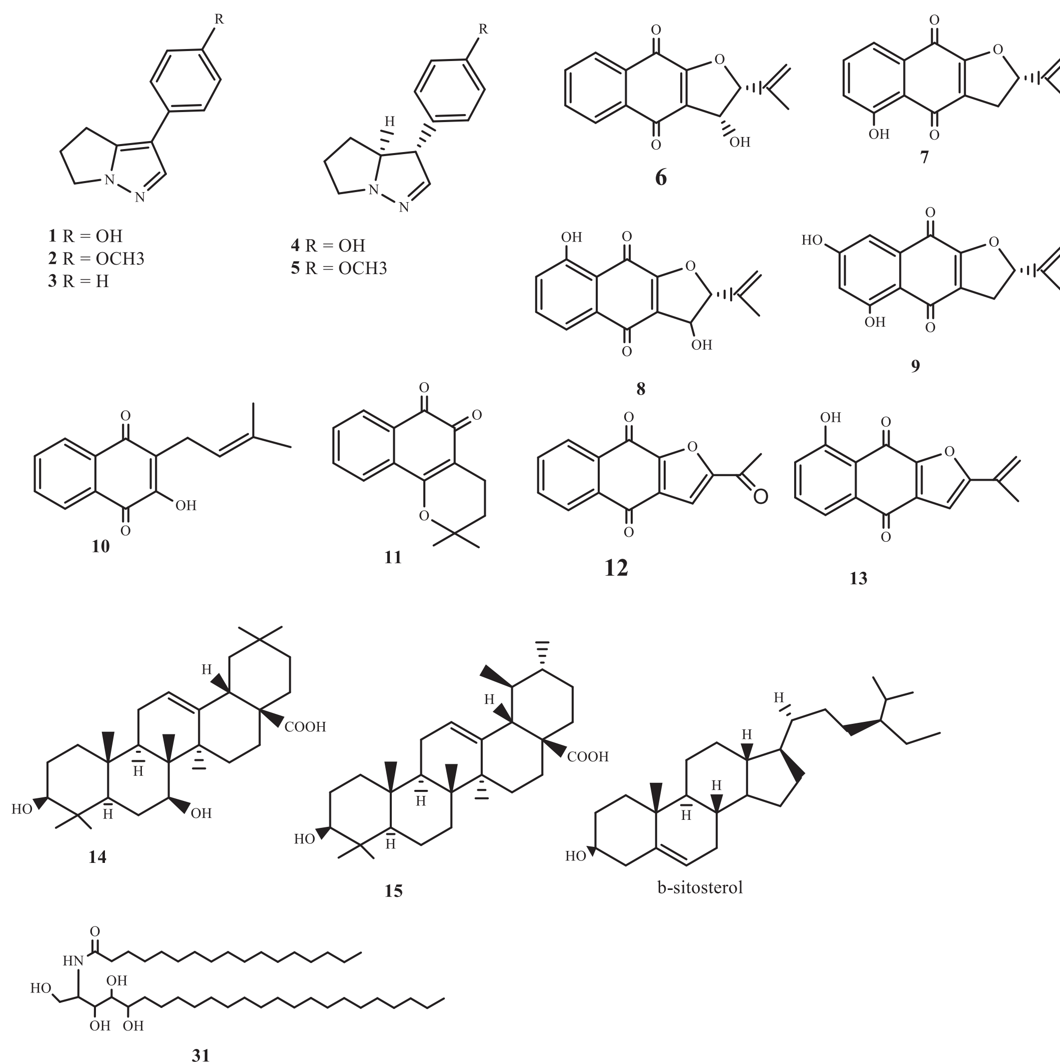

Fig. 7. Structures of main identified components of *N. laevis* different solvent extracts.

3.3. Triterpenoids

Table 4 presents the multiple triterpenoids and phytosterols identified in some *N. laevis* extracts. Triterpenoids were among the most abundant compounds in *N. laevis* plant extracts. Triterpenoids are widely distributed in the vegetable kingdom. Because of their ability to modulate the activity of several signaling networks, triterpenoids and phytosterols seem to be particularly promising for the prevention or treatment of various pathological states in terms of cardiovascular complications, tumor and cell proliferation, inflammation or hepatotoxicity. They are highly multifunctional and the antitumor activity of these compounds is measured by their ability to block nuclear factor-κB activation, induce apoptosis, inhibit signal transducer, and activate transcription and angiogenesis [31]. According to the available evidence, triterpenoids provide an excellent base from which to develop new agents that are markedly more potent. The fact that humans have safely been ingesting significant amounts of structurally related triterpenoids compounds as long as they have been consuming for instance olives (rich sources of triterpenoids) suggests that triterpenoid platform might be a relatively safe one for the design of new drugs [32].

Ursolic acid present here in *N. laevis* (Table 4) demonstrated antitumor and anti-inflammatory properties and has been investigated for its hepatoprotective effects. The mechanism of effect includes suppression of enzymes that play a role in liver damage such as cytochrome P450, cytochrome b5, CYP1A and CYP2A, and an increase in antioxidant substances such as glutathione, metalothionein, zinc, glutathione-S-transferase and glucuronosyltransferase, with simultaneous protective effects on liver mitochondria [33]. From Ghosh et al. work, stigmasterol

Table 2
Retention time, M + H adduct precise mass and extracts in which pyrazole alkaloids were detected.

No	Compound name	RT	M + H	Extracts
1	4′-Hydroxywithasomnine	9.12	201.0998	SBP, SBME, SBS, RBP, RBME, RBS, RBETOH, RBWE
2	4′-Methoxywithasomnine	10.61	215.1183	RBME, RBS, RBP
3	Withasomnine	10.73	185.1073	SBP, SBME, SBS, RBP, RBME, RBS, RBETOH, RBWE
4	4′-Hydroxynewbouldine	6.87	203.1212	RBETOH, SBWE, RBETOH, RBWE
5	4′-Methoxynewbouldine	10.08	217.1284	RBETOH, SBWE, RBETOH, RBWE

Table 3
Retention time, M + H adduct precise mass and extracts in which lapachol derivatives were detected.

No	Compound name	RT	M + H	Extracts
6	3-Hydroxydehydroiso-α-lapachone	7.77	257.0829	SBP, SBME, SBS, LTE, LME
7	5-Hydroxydehydro-iso- α-lapachone	7.78	257.0832	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS
8	3,8-Dihydroxydehydroiso-α-lapachone	9.326	273.0757	R1
9	5,7-Dihydroxydehydroiso-α-lapachone	11.23	273.0766	R1
10	Lapachol	11.82	243.1041	R1
11	Beta-lapachone	8.63	243.1020	R1
12	Napabucasin	10.22	288.2887	SBP, SBME, LME, RBP, RBME, RBETOH, SBWE, RBETOH, RBWE
13	2-(1′-Methylethenyl)-5-hydroxynaphtho[2,3-*b*]furan-4,9-dione	11.48	255.0626	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS

decreased Ehrlich Ascites Carcinoma tumor volume in mice and increased life span of tumor bearing mice [34]. Canthic acid (Fig. 7) was first isolated from *Canthium dicoccum* [35] at a very poor yield. Our work determinate this acid in *N. laevis* with a relatively high concentration in methanol leaves extract. This will aid to isolate canthic acid from *N. laevis* leaves and evaluate its biological activity including anticancer activities.

3.4. Other compounds

Other compounds identified are shown in Table 5. Sphingolipids (SLs) were the main compounds and potentially contribute to *N. laevis* bioactivity. In recent years, there is more and more evidence that SLs function as key components in modulating cell responses and act as signaling and regulatory molecules. Sphingolipids represent a major class of lipids that are ubiquitous constituents of membranes in eukaryotes. Intensive research on SL metabolism and function has revealed members of the SL family as bioactive molecules playing roles from regulation of signal transduction pathways, through direction of protein sorting to the mediation of cell-to-cell interactions and recognition. SLs have also been reported to dynamically cluster with sterols to form lipid microdomains or rafts, which function as hubs for effective signal transduction and protein sorting [36].

The effect of evocarpine (EVO), a quinolone alkaloid isolated from *Evodiae fructus*, on Ca^{2+}-blocking activity has been examined. In the isolated rat thoracic aorta, evocarpine significantly inhibited the contraction induced by K^+ and that induced by external Ca^{2+} in the depolarized muscle [37]. Apigenin, a naturally occurring plant flavone, abundantly present *N. laevis*, is recognized as a bioactive flavonoid shown to possess anti-inflammatory, antioxidant and anticancer properties. Epidemiologic studies suggest that a diet rich in flavones is related to a decreased risk of certain cancers, particularly cancers of the breast, digestive tract, skin, prostate and certain hematological malignancies. It has been suggested that apigenin may be protective in other diseases that are affected by oxidative process, such as cardiovascular and neurological disorders [38]. Harmalol present here in fraction R_1 (Table 5), was reported to decrease heart rate and increased pulse pressure, peak aortic flow, and myocardial contractile force in dogs and could be useful to relieve cardiovascular pains.

4. Conclusion

In this work, the results of the first chemical investigation on secondary metabolites from all organs of the leaves, stem and root barks of *Newbouldia laevis* are reported. Beta-sitosterol was successfully isolated and identified using spectroscopy methods. More, high resolution mass spectrometry coupled with high pressure liquid chromatography was used to detect bioactive compounds from different chemical classes: pyrazole alkaloids, lapachol derivatives, triterpenoids, phytosterol and other chemical class compounds. Identified compounds were reported to be strong anticancer and antimalaria agents among other properties. This work is the first report combining a wholistic chemical study of all

Table 4
Retention time, M + H adduct precise mass and extracts in which triterpenoids and phytosterols were detected.

No	Compound name	RT	M + H	Extracts
14	Canthic acid	12.82	473.3674	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS, RBETOH, SBWE, RBETOH, RBWE
15	Ursolic acid	13.57	457.3619	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS
16	Stigmasterol	16.86	413.3779	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS, RBETOH
17	b-Sitosterol-3-O-bdglucopyranoside	16.93	577.4500	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS
18	Beta-sitostenone	15.58	413.3778	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS, RBETOH
19	Stigmasterol glucoside	17.02	592.4566 (M + NH_4)	RBETOH, SBWE, THWE, RBETOH, RBWE

Table 5
Retention time, M + H adduct precise mass and extracts in which the other compounds were detected.

No	Compound name	RT	M + H	Extracts
20	Hexadecadihydrosphingosine	10.11	274.2741	RBETOH, SBWE, RBETOH, RBWE
21	Evocarpine	10.33	340.2635	RBETOH, SBWE, RBETOH, RBWE
22	4,5-Di-*O*-methyl-8-prenylafzelechin-4beta-ol	11.42	387.1787	RBETOH, SBWE, THWE, RBETOH, RBWE
23	Chrysoeriol	10.76	301.0712	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS
24	Apigenin	10.82	271.0593	SBP, SBME, SBS, LME, RBP, RBME, RBS
25	Phytosphingosine	11.11	318.2995	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS, RBETOH, SBWE, RBETOH, RBWE
26	Chrysarobin	14.53	241.0847	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS
27	4′-Methoxybenzophenone-2-carboxylic acid	9.42	257.0808	SBP, SBME, SBS, LTE, RBP, RBME, RBS
28	Bis(2-ethylhexyl) phthalate	11.32	391.2852	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS, RBETOH, SBWE, THWE, RBETOH, RBWE
29	Diisodecyl phthalate	13.44	447.3469	R_2, RBETOH, SBWE, THWE, RBETOH, RBWE
30	Harmalol	8.35	201.1022	R_3
31	Newbouldiamide	18.59	670.6351	SBP, SBME, SBS, LTE, LME, RBP, RBME, RBS

N. laevis plant organs using soft and laboratory like extractions. Future studies could focus on isolation and bioactivity evaluation of newly detected components in *N. laevis*.

Author contributions

Affo Dermane: Conceptualization; Data curation and Formal analysis **Kafui KPEGBA:** Supervision and Conceptualization **Kodjo Eloh:** Writing - Original draft, Data curation and Formal analysis **Dorcas Osei-Safo:** Supervision and Methodology **Richard Amewu:** Supervision and Visualization **Pierluigi Caboni:** Resources and Writing - review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2020.100052.

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