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The Effect of *Newbouldia laevis* Root and Stem Bark Extract on Testosterone Induced Prostate Hyperplasia in Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. The study was conceived and designed by authors KNB and AUP. The experiment, under the supervision of author AUP, was carried out by authors KNB, MGK and NIG. The data was curetted & analyzed by authors NIG, KNB and AKV; while the manuscript was drafted by authors KNB and AUP. All of the authors have read the work and agreed to have it published in the Journal of Complementary and Alternative Medical Research. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: Benign prostatic hyperplasia (BPH) is a non-malignant tumor of the prostate gland, common among the elderly men, and has been treated in the past with natural product of plant. *Newbouldia laevis (N. laevis)* is a medicinal plant that has been utilized in the treatment of

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various diseases but not prostate tumors. The purpose of this study was to evaluate the therapeutic impact of *Newbouldia laevis* root and stem bark extract on testosterone induced prostate hyperplasia in albino rats.

Experimental Procedure: Twenty male albino rats were divided into 4 groups (N=5): HA (Negative control), HB (model hyperplasia), HC (high dose extract treatment), and HD (low dose extract treatment). The experimental animals were induced for BPH, and thereafter treated with 1000 mg/ kg body weight (HC) and 500 mg/kg body weight (HD). Samples were collected from the animals for experimental analysis.

Results and Conclusion: There was significant increase in prostate index, epithelial proliferation, PAS positivity, Ki67 expression, serum IL-6, total protein and testosterone in the model hyperplasia group. All these recorded changes are significantly ($P<0.05$) reversed among *Newbouldia laevis* extract treated groups. GCMS analysis of the plant extract revealed important bioactive substances including antioxidant, anti-inflammatory and antitumor agents. Toxicity study revealed an oral lethal dose of over 5000 mg/kg body weight. This study shows that N. laevis root - stem extract has the propensity to alleviate prostate tumors possibly through anti-inflammatory, antitumor, antioxidant, and serum testosterone down regulation mechanisms.

Keywords: Phytochemical; antioxidant; benign prostate hyperplasia; histopathology; biochemical.

1. INTRODUCTION

Benign prostatic hyperplasia (BPH), remains a serious public health challenge among the aging global male population [1,2]. The male ubiquitous disease of the elderly occurs at about 70% of men above the age of 70 [3]. BPH is a progressive condition marked by bothersome lower urinary tract symptoms (LUTs) such as frequent urination, urgency, nocturnal urination, diminished and intermittent stream force, and the sense of incomplete bladder emptying [4]. Cellular proliferation at the glandular/and stromal levels is a typical histological hallmark of this disease, resulting in enlargement of the prostate gland and consequently lower urinary tract symptoms(LUTs), potentially due to urinary blockage [5]. Although BPH is not a life-threatening condition, it has a significant impact on a person's quality of life [6].

There are no clear cut causes of BPH known yet, but factors such as age, hereditary, lifestyle, diet, physical activity, and alcohol have been associated with the condition [7]. several partially overlapping and complementary theories about BPH have been proposed, including embryonic re-awakening, stem cell defects, hormone imbalance signaling, and, more recently, chronic inflammation. Inflammation, which is one of the most common causes of prostatic diseases can be initiated by oxidative stress [1,7]. Oxidative stress is a byproduct of reactive oxygen species (ROS), and can be formed when oxygen is not completely reduced during aerobic metabolism [8]. Superoxide anions (O^{-}_{2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) are some examples of ROS. The absence/deficiency of the complementary antioxidants to mop up the free radicals could lead to inflammation. Inflammation involves the secretion of pro inflammatory mediators, including interleukin 6(IL-6) which is common in prostate tumors [9,10].

Chronic inflammation, according to Prajapati et al. can initiate genomic instability, which can lead to DNA damage, oncogene activation, or tumor suppressor gene impairment [11]. Furthermore, inflammation is connected to androgen receptor (AR) over expression and can be caused by a variety of factors, including viral, environmental, and even nutritional factors [1]. According to Kruslin et al., androgen receptor (AR) over expression is a typical feature of the prostate micro-environment in both benign and malignant tumors, which may be related to the elevated levels of testosterone and androgens in prostate tumors [12].

Traditional BPH treatments, which are dominated by 5 alpha reductase inhibitors and alpha 1-receHtor antagonists, are frequently associated with negative side effects. Such side effects might include gynecomastia, headache, dizziness, chest pains, upper respiratory infectious disease, loss of libido, erectile dysfunction, and male infertility due to decreased sperm count [13,14]. Finding a BPH treatment that works effectively and has a low complication rate over the long term is necessary.

Patients are increasingly turning to natural products of plants, for relief from their ailments. Some of the plant products have been proven to

decrease tumor development, increase apoptosis, or modify certain signaling pathways implicated in tumors [13,14,15]. *Newbouldia laevis* (*N.laevis*) is an African medicinal plant that has been used widely for the treatment of various kinds of diseases [16,17,18]. The plant is an angiosperm of the Bignoniaciaceae family and also common to African countries such as Nigeria, Senegal, Cameroon, Gabon and Angola [19]. It is a common practice in traditional herbal medicine practice to use different plant parts for different diseases, and in some cases a mixture of the parts is utilized. Phytochemical analysis of the root and stem of *N.laevis*, by Igwe and Nwobodo revealed the presence of alkanols, flavonoids, glucosides, saponins, and tannins [20]. The plant's root and stem barks parts share similar bioactivity and contains anti tumor agents as recorded by Dermane et al. [21]. Therefore, the current study was aimed to investigate the impact of *Newbouldia laevis* root and stem extract on chemically induced prostatic hyperplasia. This study might contribute to the development of new BPH prevention or treatment medicines by serving as an experimental foundation.

2. MATERIALS AND METHODS

2.1 Plant Sample Authentication and Preparation

At the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, the *Newbouldia laevis* root and stem bark used in the study was verified. A sample of the plant material was taken and placed at a herbarium at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, and was given the voucher number MOUAU/ZEB/HERB/016.After washing, the plant's root and stem (root-stem) bark was chopped into small pellets and allowed to air dry in the shade for 28 days before being processed into powder in a mill that was made locally. A mass of 200 grams of the powdered material were, macerated in 1.5 liters of ethanol for 48 hours prior to filtering. They were then filtered twice: once through a sieve and once through a whatman filter press. The resulting filtrate (extract in solution) was concentrated to dryness at 40ºC in a hot air oven to obtain a pasty dark brown extract which weighed 8.2 grams and represented 4.1% extract yield. The extract was preserved in a refrigerator at -4ºC temperature until needed.

2.2 Gas Chromatography-mass Spectrometry Analysis of the Extract

The GC-MS analysis of the root-stem extract, was performed utilizing BUCK M910 BUCK M910 Gas chromatography furnished with HH-5MS section (30 m long × 250 µm in width × 0.25 µm in thickness of film). Spectroscopic identification by GC–MS included an electron ionization framework which used high energy electrons (70 eV). Unadulterated helium gas (99.995%) was utilized as the transporter gas with stream pace of 1 mL/min. The underlying temperature was set at 50 – 150°C with an expanding pace of 3°C/min and a holding season of around 10 min. At long last, the temperature was expanded to 300 °C at 10°C/min. One microliter of the pre-arranged 1% of the concentrates diluted with particular solvents was infused in a splitless mode. Relative amount of the compounds present in every one of the concentrates was communicated as rate dependent on the top region created in the chromatogram. The distinguishing proof of the constituents of *Newbouldia laevis* root- stem extract was accomplished on the premise of comparing the retention index of the mass spectral fragmentation patterns, with those found on the data base of the National Institute Standard and Technology (NIST). In each case the obscure spectra of the mass spectrum was compared with the known component of the NIST database.

2.3 Acute Toxicity Test

The acute toxicity test of the plant extract was carried out in accordance with a modified Lorke's method as was used by Orieke et al. [22]. A total of 21 albino rats weight range 145-253 were used. In the first phase of the test, 9 rats assigned to 3 groups (A, B and C) were administered 10, 100, 1000 mg/kg body weight of the extract respectively. Thereafter, the animals were observed within 24 hours for toxicity signs or death. With the observance of zero percent mortality within the period, the study proceeded to the second phase. In the second phase, another set of 9 rats also assigned to 3 groups (D, E and F) of 3 rats each were administered 1600, 2900 and 5000 mg/kg body weight of the extract. When zero percent mortality was also observed after 24 hours of treatment, the highest dose used (5000 mg/kg) was repeated on the last set of 3 rats as confirmatory test. This last set of test animals were observed within 24 hours and a further 7 days, yet no mortality was

observed, leading to a conclusion that LD_{50} value for the extract is >5000 mg/kg body weight.

2.4 Animals

Twenty mature male albino rats were obtained from the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike's laboratory animal house. The rats were housed in a brightly lit, well-ventilated environment. The rats were given a standard rat pellet diet (vital feeds Nigeria Ltd) and were given free access to tap water. Fourteen days after acclimatization of the laboratory animals, the animal investigation commenced in accordance with current laws of the land governing the use of experimental animals, as well as the University's Ethical Committee's ethical Permission.

2.5 Experimental Design

Twenty albino rats, weighing between 162 - 253 grams, were divided into four groups(N=5) at random and designated HA, HB, HC, and HD. HB group was the model hyperplasia group that was induced for prostate tumor, without plant extract treatment, whereas HA group was the negative control group and the animals here are only nourished on food and water. Animals in groups HC and HD were induced for prostate hyperplasia and thereafter, orally administered, respectively with, 1000 and 500 mg/kg body weight of the plant extract for two weeks. The induction of prostate hyperplasia was through subcutaneous administration of testosterone propionate (TP) (Biocar pharmaceuticals, Wuhan, China) 5 mg/kg body weight for 28 days. A day after the last extract administration, the animals were fasted overnight for sample collection. Under chloroform sedation, the animals were in turn dissected, and a blood sample collected through cardiac puncture for biochemical analysis. Prostate glands were also collected washed, weighed and fixed in neutral buffered formalin.

2.6 Biochemical Analysis

The total protein level of the sample was determined using the Biuret method (Ernest,1996), while the Interleukin-6 (IL-6) measurement was carried out using commercial ELISA kits (MH Biomedical, Ohio, USA) . While the Hormonal analyzer (Fs-113, China), was used to detect serum testosterone level. All of the analyses were carried out according to the manufacturer's instructions, utilizing serum samples from the test animals.

2.7 Histopathological Analysis

The prostate sample had been fixed for 24 hours in neutral buffered formalin before being processed and embedded. The prostate tissue samples were trimmed and sectioned at 5 microns thickness. Haematoxylin and eosin (H&E) were used to demonstrate general tissue architecture; while periodic acidic Schiff reveal PAS reactivity; and immuno expression of KI 67 will validate our findings. Additionally, utilizing an antigen-antibody reaction, ki 67 immunohistochemistry was carried out to confirm the presence proliferation of epithelial or stromal tissue. The microscopy was carried out with Leica microscope in collaboration with a consultant pathologist.

2.8 Prostate Weight (PW) and Body Weight Assessment

The weights of the animals were taken at the beginning of the research and towards the end of the experiment before sacrificing the animals. After excising the prostates of the rats, their weights were measured using chemical weighing balance. The prostate index (PI) was calculated as PW/BW 100 percent, and the mean PI ratios in each group were calculated.

2.9 Statistical Analysis

The data was expressed as mean ± standard deviation and analysis of variance was conducted with SPSS (version 21.0), Posthoc and the normality along with homogeneity of the data was determined using Turkey test. The cut off for statistically significant difference was $P< 0.05$.

3. RESULTS

Phytochemical profiling of ethanol extract of *Newbouldia laevis* root and stem bark by gas chromatography mass spectrometry in our study revealed the presence of 68 chemicals (suppl. Table 1 and Suppl Fig. 1). Some of the phytochemical compounds are known medicinal bioactive agents including p-cymne, farnesene, terpinene, carophyllene, humulene, uvaol, piperine, nerolidole, 3-carene and bisabolen. The acute toxicity of the plant extract in albino rats in our study has shown that oral lethal dose was over 5000 mg/kg body weight, as none of the

animals displayed any external symptom such as sluggishness, edema, or even death.

3.1 Effects of Newbouldia Laevis Root-stem Extract on the Serum IL-6 in Albino Rats

The hyperplasia model group (HB) revealed a significant (P<0.05) increase in serum interleukins-6 concentration compared to the negative control group (HA), as well as the extract treated groups (HC &HD). In contrast, there is significant decline (P<0.05) in the serum IL-6 concentration in both high dose group (HD), and low dose group (HC) (Fig. 1 a).

3.2 Effects of *N. laevis* Root- stem Extract on Serum Total Protein Concentration in BPH Induced Albino Rats

Findings in our study revealed that the animals in HB (model hyperplasia) group demonstrated significant (P<0.05) elevation of serum total protein when compared with those in negative control group HA. On the other significant (P<0.05) decline in total protein in serum was revealed in the high dose treated group (HC), as well as low dose treated group, when compared with the model hyperplasia (Fig. 1b).

3.3 Effects of *N. laevis* Root Stem Extract on the Serum Testosterone Concentration

The HB group had a significant (P<0.05) increase in serum testosterone concentration, compared to the negative control group (HA).Conversely, the serum testosterone concentration significantly (P<0.05) declined in *N.laevis* high dose treated group as well low dose, when compared with the model hyperplasia group. (Fig. 1c).

3.4 Effect of *N. laevis* Root and Stem Bark Extract on the Prostate Index in BPH Induced Rats

Significant (P<0.05) increase in prostate weight was observed in HB group when compared with the rest of the groups. Accordingly, the prostate index of rats in the model hyperplasia group was significantly (P<0.05) higher in comparison to than the rest of the groups. On the other hand the animals treated with the extract *N.laevis* root -stem bark, demonstrated significant decline prostate index in both high and low dose groups when compared with the BPH induced group (Table 1, Fig. 1d).

3.5 Effects of *N. laevis* Root and Stem Bark Extract on Induced BPH in Rats

Hematoxylin and Eosin staining in this study revealed remarkable no remarkable morphological changes in the lining of the epithelium of animals in the negative control group (HA), when compared with prostate or acini of animals in the Model hyperplasia group (HB), in which there was substantial epithelial proliferation and increase in thickness. The epithelial growth into the lumen was suggestive of benign prostatic hyperplasia. The animals in the high dose treated group (HC), as well as low dose treated group (HD), showed reduction in the epithelial proliferation similar to those in negative control group (Fig. 2,a-d). The staining with periodic acid Schiff, supported significant epithelial proliferation among the animals in model hyperplasia group, with deep stain uptake(magenta color), which was not the same with that of either negative control group or *Newbouldia laevis* extract group (Fig. 2, e-h). IHC was used to identify epithelial proliferation in more detail. Comparing the model hyperplasia group (HB) to the other groups, which showed little uptake of stain (Fig. 2, i–l), the model hyperplasia group (HB) had considerable expression of the proliferation marker (ki 67) (Fig. 2, e- l).

4. DISCUSSION

The bioactive agents in our study are mostly alkaloids and terpernoids, and have been found to be antioxidant, anti-inflammatory, antineoplastic, as well as antioxidant [23,24,25,26,27]. The oral lethal dose of over 5000 mg/kg body weight in the work, is an indication that the extract could be safe when taken for medicinal purposes.

Prostatic hyperplasia is a prevalent disease of men with advanced age, often associated with urinary tract disease. By repeatedly inflaming the epithelial cells over time, testosterone promotes hyperplasia in simple epithelial cells, leading to discomfort in the urinary system [28]. The restoration of increased prostatic index and histological morphological alterations by *N.laevis* extract of root and stem, in this study has demonstrated that the ethanol extract may considerably suppress the development of testosterone-induced prostatic hyperplasia.

Compared to the animals in the model hyperplasia (HB) group, reduction in IL-6, total protein, and testosterone further suggest that the extract of *Newbouldia laevis* root and stem might constitute an effective drug for the effective management of BPH.

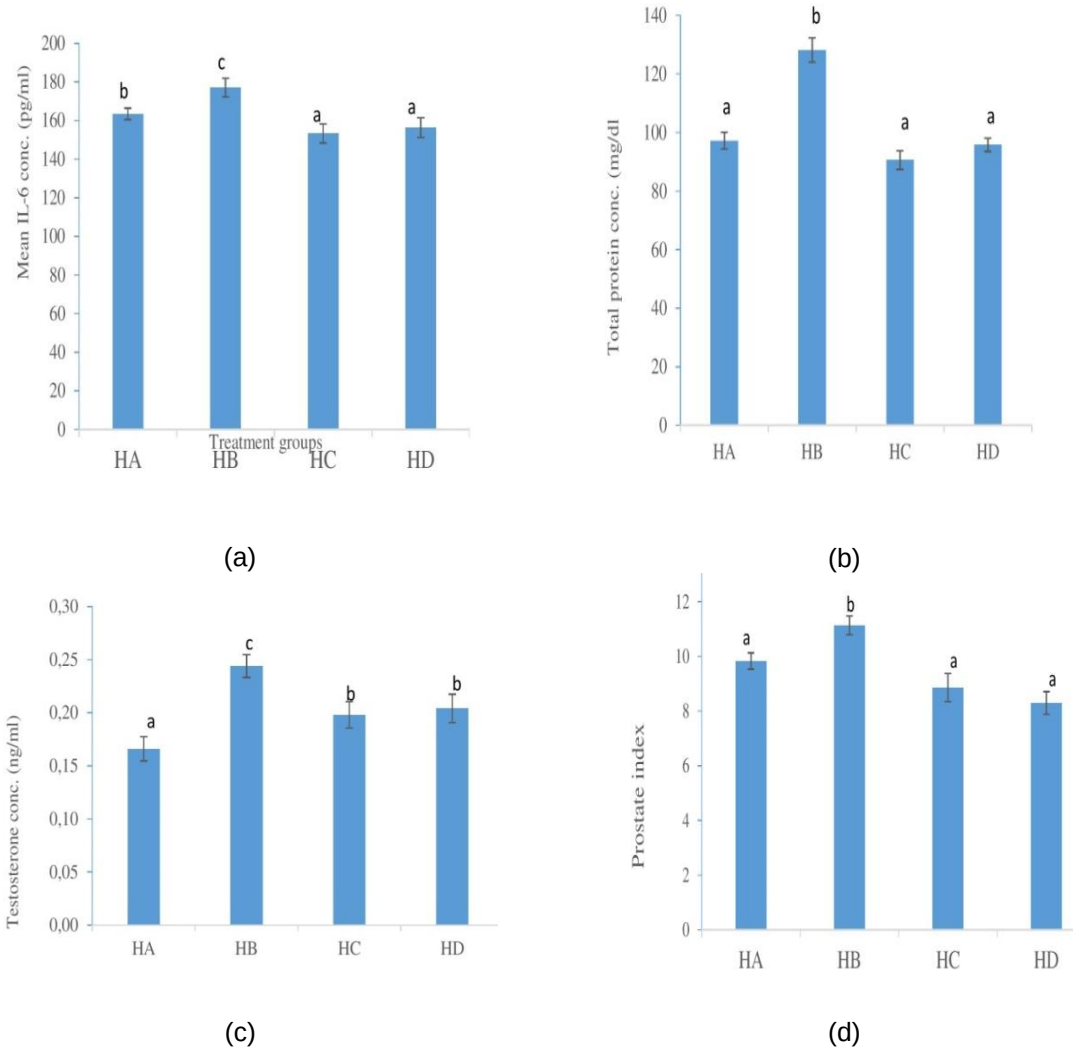

Fig. 1. The effects of *Newbouldia laevis* root and stem bark extract on the serum biochemical parameters and prostate index of BPH induced rats. *Bars* are Presented as mean ± standard deviation (n = 5). Bars with different letters superscripts are significantly different (P < 0.05). The data are expressed as mean ± SEM

Table 1. Prostate index

Treatment groups	Initial weight(g)	Final weight(g)	Prostate index	Prostate weight(g)
HA	167.00±2.55^{b}	261.00±16.45^{a}	9.19±1.81^{a}	2.40±0.51^{a}
HB	157.00±2.24^{a}	193.00±7.07^{a}	20.20±0.37^{c}	3.90±0.19^{c}
HC	158.00±8.86^{a}	188.00±9.19^{a}	18.64±0.54^{b}	3.50±0.07^{a,b}
HD	253.00±5.96^{a,b}	221.00±1.41^{ab}	18.10±0.38^{b}	5.00±2.18^{a}

Values are presented as mean ± standard deviation(n=5), and with different letter superscript are significantly different (P<0.05) from any paired mean within the column

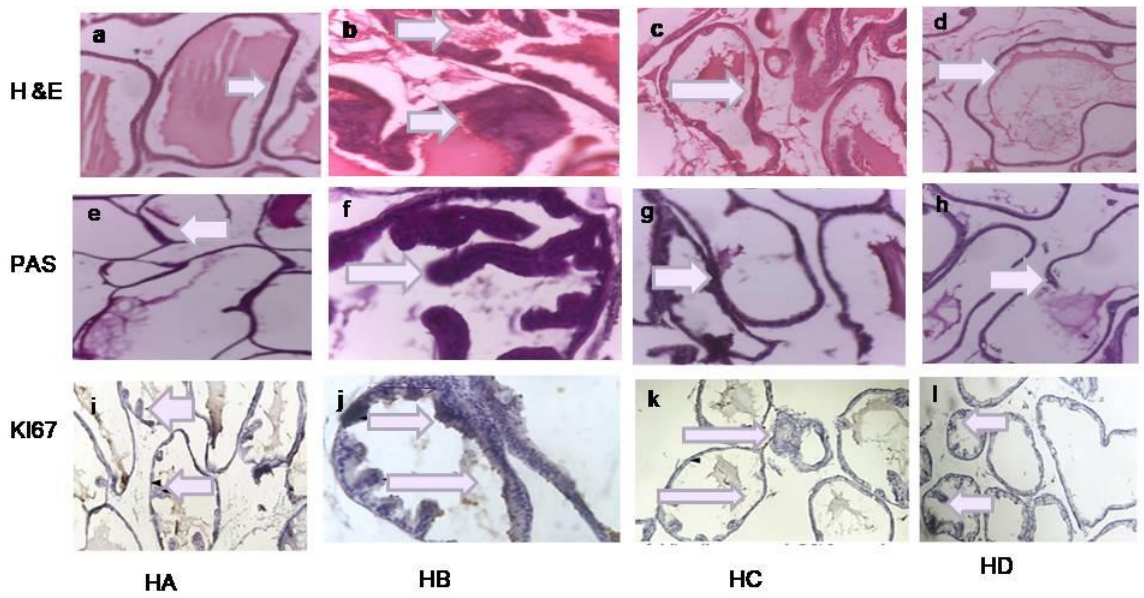

Fig. 2. Effects of *N. laevis* root-stem extract on the histological appearance of the prostate. HA.(a) section showing normal prostate acini and stroma,(e) PAS stain reactivity was mild,(i) low expression of KI 67.HB (b) Section shows remarkable epithelial proliferation(arrows), (f) intense PAS reactivity(magenta) observed 9arrow), (j) strong expression of KI 67 marker. HC (c) section showing areas of normal prostate, (g) PAS reactivity was mild,(k) mild KI 67 expression observed.HD (d) areas of apparent normal section observed, (h) mild PAS reactivity revealed, (l) scanty or weak expression of KI67. (X200)

Prostate index (PI) and histomorphological changes are important indicators of the development of prostate tumors such as benign prostatic hyperplasia, and has been used in testing of protective potentials of curative substances in the past [13,14]. PI is a marker of increased prostate weight. In the present study, the extract of root and stem of *Newbouldia laevis* reduced the PI and histomorphological abnormalities of testosterone –induced BPH rats consistently as related to previous studies [13,27,29]. This is an indication that the extract in our study can protect the prostate against tumor development.

Inflammation is commonly present in BPH, and might cause tissue injury and the secretion of cytokines, which can drive angiogenesis and local growth factor production [8,9]. One of the pro inflammatory cytokines known to be involved in the prostate tumor pathogenesis is the interleukin-6(IL-6) [10]. IL-6 plays significant role in the development of prostate tumors. It is a pro inflammatory cytokine found in both the stromal and epithelial parts of the prostate, and has a role in the pathological alterations seen in BPH and prostate cancer [10,29]. In our study significant reduction of the serum level of IL-6, was observed among the plant extract treated groups compared to the model hyperplasia group. This might suggest that anti inflammation was involved in the mechanisms of the plant extract treatment of the BPH in this study. Further studies are still needed to substantiate the information.

Inflammation can reengineer the liver cells to produce acute phase proteins, such as C-reactive proteins and serum amyloid thereby elevating the serum protein level [2,29]. This explains the significant elevated serum total protein among the animals in HB group, and the alleviation of the inflammation in the study might be responsible for the normalization of blood total protein suggesting that plant extract may have had a role in the restorative effect.

Testosterone is the precursor of dihydrotestosterone (DHT) which is an important causative factor in the development of prostate hyperplasia [30]. DHT binds to the androgen receptors (ARs) to initiate its biological properties including cell proliferation, survivorship, transcription of insulin –like growth factor 1(IGF1), epidermal growth factor (EGF), and fibroblast growth factor(FGFs) [14,29]. In line

with our findings, the animals in the model hyperplasia group (HB) had considerably higher testosterone concentrations. On the other hand, both plant extract-treated groups (HC & HD) exhibited comparable effectiveness when it came to restoring a normal level of serum testosterone, which suggests that the plant extract had an impact on the condition.

This study's intrinsic flaw is that we did not conduct an experimental evaluation of the extract's effects on the male reproductive system in addition to the long-term treatment. The components of the plant extract responsible for the anti-BPH activity are yet to be known. Additionally, the precise signaling pathways required to fulfill the role of bioactivity are still unknown and understood. Last but not least, the rat model of BPH used in this study is distinct from humans, limiting the applicability of our findings to people. To further understand the underlying mechanisms of *N. laevis* root and stem extract in alleviating prostatic hyperplasia, we support more thorough studies that may potentially incorporate molecular analyses.

5. CONCLUSION

This study has demonstrated that the ethanol extract of *N. laevis* root and stem can lower the prostate index and safe guard the histomorphological characteristics of the prostate through possible anti-inflammatory, anti-tumor proliferation, anti-oxidant, and serum testosterone downregulation mechanisms. These mechanisms are attributable to the inherent phytochemical constituents of the plant extract which is relatively safe for medicinal purposes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Supplementary Table 1. GC-MS phytochemical components of ethanolic extract of root stem back of *Newbouldia laevis*

Peak number	Name of the compounds	Molecular formulae	Retention time	Area of peak	Ref number	Qual
		CH_2Cl_2.				
1	Methylene chloride	CH_2Cl_2.	5.310	0.26	1545	87
	Methylene chloride	CH_2Cl_2.			1543	87
	Methylene chloride	CH_2Cl_2.			1544	72
2	Methylene chloride	CH_2Cl_2.	5.547	0.33	1542	72
	Methylene chloride	CH_2Cl_2.			1545	64
	Methylene chloride	CH_2Cl_2.			1544	59
3	Methylene chloride	CH_2Cl_2.	5.723	0.23	1542	64
	Methylene chloride	CH_2Cl_2.			1545	58
	Benzene, 1,2,3-trimethyl-	$C_6H_3(CH_3)_3$.			9599	55
4	Methylene chloride	CH_2Cl_2.	6.298	0.28	1542	72
	Methylene chloride	CH_2Cl_2.			1545	64
	Methylene chloride	CH_2Cl_2.			1544	64
5	Benzene, 1,2,3-trimethyl	$C_6H_3(CH_3)_3$.	6.363	0.68	9595	86
	Benzene, 1,2,3-trimethyl	$C_6H_3(CH_3)_3$.			9592	83
	Benzene, 1,2,3-trimethyl	$C_6H_3(CH_3)_3$.			9599	81
6	Decane	CH_8CH_3.	6.496	0.87	19650	70
	Decane	CH_8CH_3.			19648	70
	Decane	CH_8CH_3.			19651	53
7	Acetic acid, chloro-, 1-methylbuty l ester	C7H13ClO2	6.774	0.54	35363	37
	N-(2,2-Dichloro-1-hydroxy-ethyl)-2, 2-dimethyl-propionamide	$C_7H_{13}Cl_2NO_2$			77338	33
	S-(Buthoxythiocarbonyl)thiohy droxy Lamine				35781	28
8	Benzene, 1,4-dichloro-	C 6 H 4 Cl 2	6.846	1.37	22510	97
	Benzene, 1,3-dichloro-				22507	95
	Benzene, 1,3-dichloro				22509	95
9	dl-Threitol	$C_4H_{10}O_4$	6.951	0.91	9879	18
	Ethanol, 2,2-dichloro-	**$C_2H_4Cl_2O$**			7164	16
	ClCH2C(O)OCH(CH3)2	**$C_5H_9ClO_2$**			16425	12
10	Acetic acid, chloro-, 1,1-dimethyl ethyl ester	**$C_6H_{11}ClO_2$**	7.132	0.60	24847	35
	N-(2,2-Dichloro-1-hydroxy-ethyl)-2 ,2-dimethyl-propionamide	**$C_7H_{13}Cl_2NO_2$**			77338	32
	Acetic acid, chloro-, 2-butoxyethy l ester	**$C_8H_{15}ClO_3$**			6017	25
11	p-Cymene	**$C_{10}H_{14}$ or $CH_3C_6H_4CH(CH_3)_2$**	7.199	0.7 9	15142	96
	o-Cymene	**$C_{10}H_{14}$**			15140	96
	Benzene, 1-methyl-3-(1-methylethyl)-	**$C_{10}H_{14}$**			15243	95
12	Undecane, 5,6-dimethyl-	**$C_{13}H_{28}$**	7.944	0.78	51424	43

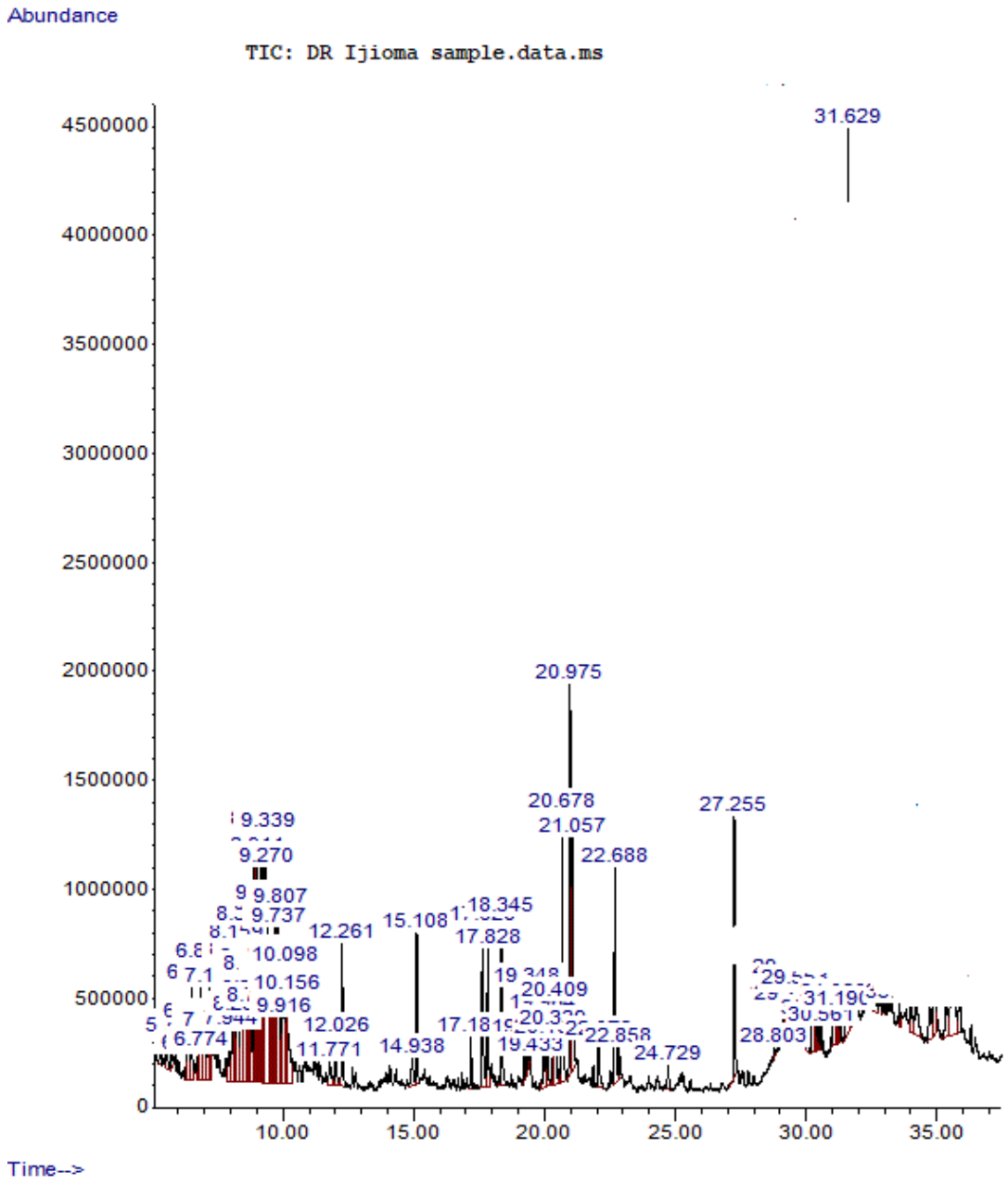
Peak number	Name of the compounds	Molecular formulae	Retention time	Area of peak	Ref number	Qual
	Octane, 4-ethyl-	$C_{10}H_{22}$			19654	38
	Pentane, 3-ethyl-2,4-dimethyl-	C_9H_{20}			13018	35
13	Oxalic acid, isobutyl nonyl ester	$C_{15}H_{28}$	8.112	1.75	132408	64
	Sulfurous acid, butyl octyl ester	$C_{20}H_{30}O_4$			111124	50
	Carbonic acid, isobutyl 2-ethylhex yl ester	$C_{13}H_{26}O_3$			93133	50
14	.gamma.-Terpinene	$C_{10}H_{16}$	8.159	1.38	16077	96
	.gamma.-Terpinene	$C_{10}H_{16}$			16078	93
	(+)-3-Carene	$C_{10}H_{16}$			16050	86
15	Decane, 3,6-dimethyl-	$C_{12}H_{26}$	8.256	0.92	40000	53
	Undecane, 2,7-dimethyl-	: $C_{13}H_{28}$			51421	53
	Undecane, 5,7-dimethyl	$C_{13}H_{28}$			51419	53
16	Decane, 3,4-dimethyl-	$C_{12}H_{26}$	8.380	2.29	40002	72
	Decane, 2,6,7-trimethyl-	$C_{13}H_{28}$			51452	59
	Dodecane, 4,6-dimethyl-	: $C_{14}H_{30}$			63642	53
17	Decane	$C_{10}H_{22}$	8.537	1.82	19649	72
	Decane	$C_{10}H_{22}$			19648	64
	Decane	$C_{10}H_{22}$			19651	53
18	Decane	$C_{10}H_{22}$	8.593	0.97	19648	72
	Undecane	$C_{11}H_{24}$			29354	72
	Oxalic acid, isobutyl nonyl ester	**C15H28O4**			132408	64
19	Oxalic acid, allyl nonyl ester	$C_{14}H_{24}O_4$	8.643	0.74	116960	64
	Tetradecane	**C14H30**			63623	59
	Decane, 3,7-dimethyl-	$C_{12}H_{26}$			39995	58
20	Hexane, 2,3,4-trimethyl-	C_9H_{20}	8.700	1.11	12990	70
	Decane, 4-ethyl-	$C_{12}H_{26}$			39977	64
	Undecane, 2,4-dimethyl-	$C_{13}H_{28}$			51423	59
21	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	8.787	0.73	127780	72
	Undecane, 4,7-dimethyl-	$C_{13}H_{28}$			51420	64
	Hexadecane	$C_{16}H_{34}$			89840	59
22	Undecane, 3-methyl-	$C_{12}H_{26}$	8.911	1.72	39984	59
	Undecane, 3,9-dimethyl-	$C_{13}H_{28}$			51434	59
	Undecane, 2,10-dimethyl-	$C_{13}H_{28}$			51444	59
23	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	8.959	3.66	76621	80
	Heptadecane, 2,6-dimethyl-	**C19H40**			128852	80
	Decane, 3,7-dimethyl-	$C_{12}H_{26}$			39995	72
24	Carbonic acid, nonyl vinyl ester	$C_{12}H_{22}O_3$	9.039	1.19	77841	72
	Undecane, 5-methyl-	$C_{12}H_{26}$			39989	70
	Heptane, 2,6-dimethyl-	C_9H_{20}			12957	58
25	Heptane, 2,4-dimethyl-	**C9H20**	9.119	1.42	12973	64
	Pentane, 2,2,3,3-tetramethyl-	C_9H_{20}			13015	59
	Nonane, 4-methyl-	$C_{10}H_{22}$			19665	59
26	Dodecane	**C12H26**	9.176	2.23	39972	87

Peak number	Name of the compounds	Molecular formulae	Retention time	Area of peak	Ref number	Qual
	Undecane, 4-methyl-	$C_{12}H_{26}$			39990	64
	Tridecane	**C13H28**			51391	59
27	Heptadecane, 2,6,10,14-tetramethyl	$C_{21}H_{44}$	9.270	2.31	155903	72
	2-Ethylhexyl mercaptoacetate	**C10H20O2S**			67867	64
	Dodecane, 2,7,10-trimethyl-	$C_{15}H_{32}$			76620	59
28	Decane, 2-methyl-	$C_{11}H_{24}$	9.339	3.82	29360	80
	Tridecane	**C13H28**			51391	80
	Hexadecane	**C16H34**			89840	72
29	Carbonic acid, nonyl vinyl ester	**C12H22O3**	9.481	0.97	77841	72
	Tridecane, 6-methyl-	$C_{14}H_{30}$			63638	72
	Decane, 5-ethyl-5-methyl-	$C_{13}H_{28}$			51471	64
30	Carbonic acid, nonyl prop-1-en-2-y l ester	$C_{13}H_{24}O_3$	9.541	1.06	91190	86
	Octane, 6-ethyl-2-methyl-	**C11H24**			29383	64
	Hexane, 3,3-dimethyl	C_8H_{18}			7784	59
31	Carbonic acid, nonyl vinyl ester	**C12H22O3**	9.636	1.01	77841	80
	1-Iodo-2-methylnonane	$C_{10}H_{21}I$			127780	64
	Decane, 2,4-dimethyl-	$C_{12}H_{26}$			39996	59
32	Octane, 2,3,7-trimethyl-	$C_{11}H_{24}$	9.693	1.29	29377	72
	Carbonic acid, nonyl vinyl ester	$C_{11}H_{24}$			77841	58
	Ether, hexyl pentyl	$C_{11}H_{24}O$			41574	53
33	Octane, 2,6-dimethyl-	$C_{10}H_{22}$	9.737	0.88	19689	59
	Decane, 2,6,7-trimethyl-	$C_{13}H_{28}$			51452	53
	Oxalic acid, isobutyl nonyl ester	**C15H28O4**			132408	52
34	Nonane, 3-methyl-	$C_{10}H_{22}$	9.807	2.48	19663	72
	Decane, 3,4-dimethyl-	$C_{12}H_{26}$			40002	72
	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$			76624	72
35	Decyl octyl ether	$C_{18}H_{38}O$	9.916	0.53	130950	64
	Heptane, 2,4-dimethyl-	C_9H_{20}			12973	53
	Oxalic acid, isobutyl pentyl ester	**C11H20O4**			79373	52
36	2,6-Dimethyldecane	$C_{12}H_{26}$	10.024	1.27	39979	83
	Undecane, 5-methyl-	$C_{12}H_{26}$			39989	70
	Octane, 3,4,5,6-tetramethyl-	$C_{12}H_{26}$			40012	64
37	Hexane, 2,3,5-trimethyl-	C_9H_{20}	10.098	1.00	12994	72
	Decane, 3,8-dimethyl-	$C_{12}H_{26}$			40006	64
	Octane, 2,3,3-trimethyl-	$C_{11}H_{24}$			29375	64
38	Undecane, 4,7-dimethyl-	$C_{13}H_{28}$	10.156	1.48	51420	64
	Carbonic acid, decyl vinyl ester	$C_{13}H_{24}O_3$			91176	59
	Nonane, 5-butyl-	**$C_{13}H2$**			51397	59
39	Naphthalene	**C10H8**	11.771	0.38	12197	97
	Azulene	$C_{10}H_8$			12191	96

Peak number	Name of the compounds	Molecular formulae	Retention time	Area of peak	Ref number	Qual
	1H-Indene, 1-methylene-	**C10H10**			12199	95
40	1-Tridecene	**$C_{13}H_{26}$**	12.026	0.45	49686	80
	Cyclopropane, nonyl-	**$C_{12}H_{24}$**			38293	72
	2-Dodecene, (E)-	**$C_{12}H_{24}$**			38286	70
41	Dodecane	**C12H26**	12.261	1.19	39973	94
	Dodecane	**C12H26**			39974	91
	Undecane	**C11H24**			29355	83
42	Naphthalene, 1-methyl-	**$C_{11}H_{10}$**	14.938	0.36	19726	96
	Naphthalene, 2-methyl-	**$C_{11}H_{10}$**			19729	95
	Benzocycloheptatriene	**$C_{11}H_{10}$**			19722	95
43	Tridecane	**$C_{13}H_{28}$**	15.108	1.24	51392	93
	Dodecane	**$C_{12}H_{26}$**			39973	80
	Undecane	**C11H24**			29355	80
44	1-(3,3-Dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	**C12H18**	17.182	0.41	33552	27
	Hexadecane, 2,6,11,15-tetramethyl-	**$C_{20}H_{42}$**			142256	22
	Oxalic acid, allyl nonyl ester	**C14H24O4**			116960	22
45	1-Hexadecanol	**C16H34O**	17.628	1.61	104424	72
	3-Tridecene, (Z)-	**$C_{13}H_{26}$**			49688	64
	4-Tetradecene, (Z)-	**$C_{14}H_{28}$**			61857	64
46	Tetradecane	**$C_{14}H_{30}$**	17.828	1.14	63625	90
	Tridecane	**$C_{13}H_{28}$**			51392	86
	Tridecane	**$C_{13}H_{28}$**			51393	86
47	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	**$C_{15}H_{24}$**	18.345	1.59	68786	58
	Caryophyllene	**C15H24**			68512	58
	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	**C15H24**			68667	55
48	Humulen	**$C_{15}H_{24}O$**	19.243	0.27	68480	96
	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	**$C_{10}H_{16}$**			16174	72
	1,3,7-Octatriene, 3,7-dimethyl-	**$C_{10}H_{16}$**			16136	72
49	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	**$C_{15}H_{24}$**	19.348	0.60	68665	94
	(E)-.beta.-Famesene	**$C_{15}H_{24}$**			68594	94
	(E)-.beta.-Famesene	**$C_{15}H_{24}$**			68601	53
50	Heptadecane, 2,6,10,14-tetramethyl	**$C_{21}H_{44}$**	19.433	0.0	155903	59
	Undecane	**C11H24**			29357	58
	Octane, 5-ethyl-2-methyl-	**$C_{11}H_{24}$**			29382	52
51	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.	**C15H24**	19.964	0.75	68946	97

Peak number	Name of the compounds	Molecular formulae	Retention time	Area of peak	Ref number	Qual
	alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-					
	(+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$			68646	95
	.beta.-copaene	$C_{15}H_{24}$			68520	94
52	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	$C_{15}H_{24}$	20.102	0.50	68904	98
	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$			68891	93
	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1RZ,9S*)]-	$C_{15}H_{24}$			68786	87
53	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	**C15H24**	20.339	0.49	68925	50
	(E,Z)-.alpha.-Farnesene	$C_{15}H_{24}$			68624	49
	Spiro[2.2]pentane-1-carboxylic acid, 2-cyclopropyl-2-methyl-	**C10H14O2**			36373	45
54	Pentadecane	**C15H32**	20.409	0.81		93
	Pentadecane	**C15H32**				90
	10-Methylnonadecane	$C_{20}H_{42}$				90
55	.beta.-Bisabolene	$C_{15}H_{24}$	20.678	2.52	68576	86
	beta.-Bisabolene.	$C_{15}H_{24}$			68561	83
	(E)-.beta.-Famesene	$C_{15}H_{24}$			68600	83
56	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	20.975	4.04	70634	96
	2,4-Di-tert-butylphenol	**C14H22O**			70632	91
	Phenol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$			70657	
57	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	21.057	2.47	68741	95
	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$			68734	89
	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	**C15H24**			68740	86
58	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	22.073	0.55	85747	81
	Nerolidol	**C15H26O**			85684	81

Peak number	Name of the compounds	Molecular formulae	Retention time	Area of peak	Ref number	Qual
	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	**C15H26O**			85759	58
59	1-Nonadecene	**C19H38**	22.688	1.93	126869	91
	4-Heptafluorobutyryloxyhexadecane	**C20H33F7O2**			253009	90
	Oxalic acid, allyl do decyl ester	**C17H30O4**			157473	90
60	Hexadecane	**$C_{16}H_{34}$**	22.858	0.28	89840	93
	10-Methylnonadecane	**$C_{20}H_{42}$**			142242	90
	Nonadecane	**C19H40**			128834	86
61	Aromandendrene	**$C_{15}H_{24}$**	24.729	0.82	68524	53
	Neoisolongifolene, 8-bromo-	**$C_{15}H_{23}Br$**			141667	52
	Uvaol	**C30H50O2**			254739	47
62	E-14-Hexadecenal	**$C_{16}H_{30}O$**	27.255	2.92	100553	91
	9-Octadecene, (E)-	**$C_{18}H_{36}$**			113637	87
	4-Heptafluorobutyryloxyhexadecane	**C20H33F7O2**			253009	87
63	Piperine	**C17H19NO3**	28.803	0.23	145056	98
	Glutaric acid, decyl 2-hexyl ester	**$C_{21}H_{40}O_4$**			210364	38
	Tetracyclo[2.2.1.0(2,6).0(3,5)]heptane-7-spiro-2'-cyclopropene	**C_9H_8**			8574	38
64	Piperine	**C17H19NO3**	29.148	0.61	145056	99
	3(2H)-Isothiazolone, 2-methyl-	**C_4H_5NOS**			7854	35
	Glutaric acid, 2-ethylhexyl 2-ethylbutyl ester	**C4H5NOS**			186342	35
65	Piperine	**C17H19NO3**	29.214	0.71	145056	98
	1H-Inden-1-one, 2-diazo-2,3-dihydro-3-methyl-	**C10H10O**			41510	38
	Glutaric acid, dodecyl 2-hexyl ester	**$C_{23}H_{34}Cl_2O_4$**			229666	35
66	Piperine	**C17H19NO3**	29.294	0.47	145056	99
	Glutaric acid, isobutyl octadecylester	**$C_{13}H_{24}O_4$**			253902	35
	Glutaric acid, butyl 4-methylpent-2-yl ester	**C15H28O4**			132544	35
67	Piperine	**C17H19NO3**	29.495	1.17	145056	90
	Glutaric acid, 2-ethylhexyl 2-decyl ester	**$C_{25}H_{48}O_4$**			229722	35
	Glutaric acid, dec-2-yl 2-octyl ester	**$C_{24}H_{42}O_4$**			229683	35
68	Piperine	**C17H19NO3**	29.553	1.10	145056	99
	Glutaric acid, hept-2-yl 2-ethylbutyl ester	**$C_{18}H_{34}O_4$**			173124	43
	Glutaric acid, 4-methylpent-2-yl octyl ester	**$C_{20}H_{38}O$**			186322	43



Supplementary Fig. 1. Phytochemical profiling of ethanol extract of Newbouldia laevis root and stem bark by gas chromatography mass spectrometry

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