

Pharmacy and Drug Development

Research Article

Bioactive Compounds in Ethanol Stem Bark Extracts of *Newbouldia leavis* Adopting Gas Chromatography – Mass Spectrometry (GC-MS) analysis

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Abstract

Newbouldia leavis is a potent herbal plant which holds a deep place in traditional medicine. Its strength is not only in leaf and roots but the chemistry of its stem bark. The stem bark extract contains several phyto-compounds known to possess anti-inflammatory, antitumor, cytotoxic, gastro-protective, anticancer, dermato-protective, anti-helminthic, antioxidant, antidiabetic, cardio-protective, immune-stimulatory, hepato-protective amongst others. GC-MS analysis of bioactive compounds in ethanol stem bark extracts of *Newbouldia leavis* revealed a total of 36 phyto-compounds representing 96.57 %. The stem bark was dominated by Humulene (16.85 %), gamma-Terpinene (16.11 %), 9, 17-Octadecadienal (12.53 %), p-Cymene (10.59 %), Linoleic acid ethyl ester (9.37 %), 4-Amino-3-hydroxybutanoic acid (8.85 %) and Caryophyllene (7.51 %). A synergy between these compounds heals, nourish and improves the overall vitality of the body.

Keywords: Antimicrobial, Anti-inflammatory, Antioxidants, Herbs, Extracts, Phyto-compounds

Introduction

Newbouldia leavis is an evergreen multipurpose herbal plant belonging to the family Bignoniaceae widely distributed in Africa and some parts of Asia [1]. The plant contains unique profile of phyto-compounds with therapeutic properties. In folk medicine, it is used for treating dysentery, pyrexia, gastrointestinal disorder, skin infections, cough, arthritis, tooth ache, bleeding and sexually transmitted diseases [2]. The stem bark and leaves of *Newbouldia leavis* contains unique profile of flavonoids and phenolic compounds, nature's compound known to trigger oxidative stress and disrupts cancer cells [3], neutralize free radicals and protects cell membrane from oxidative damage [4]. The stem bark also contains tannins and alkaloids with therapeutic potential that calms, cleanse and restores the body [5]. *Newbouldia leavis* leaves are rich in minerals like calcium, magnesium and phosphorus capable of supporting bone strength and electrolyte balance [6].

Ethanol extract from the stem bark of *Newbouldia leavis* have been reported to inhibit *Staphylococcus* spp, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella* spp and *Escherichia coli* which are the common bacteria behind wound and intestinal infections [7]. Root infusions are used to treat gastrointestinal discomfort, respiratory issues and sexually transmitted diseases [8]. In preclinical studies, extract from *Newbouldia leavis* leaves, root and stem bark have shown antitumor potential and reduce cancer cell proliferation [9]. Methanolic extract from *Newbouldia leavis* leaves helps to reduce insulin response and glucose metabolism [10]. Bark decoction are used to treat acute diarrhea, bleeding and inflamed mucus membrane [11-12].

Previous research by [13] have shown that *Newbouldia leavis* methanolic leaf extract contained 9, 12-Octadecadienoic acid (Linoleic acid) (15.35 %), Oleic acid (14.68 %), Stigmasterol (11.12%), 13-Octadecenal (10.98 %), Phytol (6.90 %) and

n-Hexadecanoic acid (5.17 %) as predominant phyto compounds. Chemical constituents present in methanol extract of *Newbouldia leavis* stem bark revealed the presence of Maltol (9.64 %), 2-(3-Bromo-4-methoxy-4 methyl cyclohexyl) propionitrile (8.46 %), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (2.25 %) and Benzoic acid, 4-hydroxy-3,5-dimethoxy- (2.06 %) as major bioactive compounds [14]. However, there have been inconsistencies in result due to variation in plant age, location, processing method, storage and specie [11]. Therefore, this study was carried out to examine the bioactive compounds in ethanol stem bark extracts of *Newbouldia leavis* by GC-MS analysis.

Materials and Methods

Experimental location

The experiment was conducted at the Biochemistry Department, Gandhi College of Agriculture, Rajasthan, India.

Collection of *Newbouldia leavis* stem bark and extraction

Fresh and mature stem bark of *Newbouldia leavis* was collected from Gandhi Teaching and Research Farm Rajasthan, India in the month of December, 2023 and sent to the Biological Science department, Sumitra Research Institute, Gujarat for proper identification before it was assigned a voucher number SG/08C/2023H. The samples were cut into smaller pieces, air dried for 12 days and powdered using an elec-

tric blender. 250 g of *Newbouldia leavis* stem bark powder was macerated into 1000 mL ethanol for 48 hours at room temperature with intermittent stirring. It was then filtered with Whatman filter paper and concentrated under rotary evaporator at a temperature of 45°C before it was transferred immediately to the laboratory for further analysis.

Gas chromatography – mass spectrometry analysis of *Newbouldia leavis* stem bark extract

GC/MS analysis of *Newbouldia leavis* stem bark extract was carried out using Claudus 5006 GC-MS Auto Sampler (China) equipped with two silica capillary columns, interfaced with a quadrupole detector (single quadrupole acquisition Method-MS parameters report), source temperature 230°C, Quadrupole temperature 150°C; the temperature program was 60°C for 2 min, 60-240°C at 3 °C/min, then kept at 240 °C during 8 min; injector temperature, 240°C. The mass spectrometry transfer line temperature, 250 °C; carrier gas, helium at a flow rate of 0.7 ml/min; injection type, split, 20:1; ionization voltage, 70 eV; electron multiplier 1000 eV; scan range 33-400 amu; scan rate, 1.56 scan/s.

Identification of components Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology [15] having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Compounds	RT (min)	Molecular weight (g/mol)	Molecular formula	% Area
1-Hexadecanol	8.775	242.44	C16H34O	0.63
Pentadecane	8.901	212.41	C15H32	1.09
2,4-Di-tert-butylphenol	9.103	206.32	C14H22O	0.42
4-Heptafluorobutyryloxyhexadecane	10.40	438.5	C20H33F7O2	0.15
9,17-Octadecadienal	10.38	264.4	C18H32O	12.53
Palmitoleic acid	10.95	254.41	C16H30O2	0.06
4-Amino-3-hydroxybutanoic acid	12.30	119.12	C4H9NO3	8.85
9-Eicosenoic acid	12.57	310.5	C20H38O2	0.17
cis-Vaccenic acid	12.86	282.5	C18H34O2	0.13
n-Hexadecanoic acid	12.90	396.7	C26H52O2	0.02
2-methyl-Glutaric acid	13.12	146.14	C6H10O4	0.01
3-Isothiazolone	14.05	101.13	C3H3NOS	0.37
Cyclopentaneundecanoic acid	14.23	254.41	C16H30O2	0.11
Oleic acid	14.97	-	-	0.83

Linoleic acid ethyl ester	16.35	308.5	C18H32O2	9.37
Beta.-Famesene	16.90	204.35	C15H24	0.82
Nonane, 3-methyl	17.11	142.281	C16H32O2	1.24
2,6-Dimethyldecane	17.38	170.33	C12H26	0.32
2,3-Dibromopentane	17.60	229.94	C5H10Br	1.92
2-Ethylhexyl mercaptoacetate	18.93	247.347	C10H20O2S	2.55
Heptadecane, 2,6-dimethyl	20.38	268.521	C15H32	0.27
Hexane, 1-hexyloxy-5-methyl	20.67	200.36	C13H28O	0.56
2,2-Dichloroethanol	20.98	114.96	C2H4Cl2O	0.04
Methylene chloride	21.34	84.93	CH2Cl2	0.05
2-dimethyl-propionamide	21.56	101.15	C5H11NO	0.12
2,2-Dichloroethanol	22.08	114.96	C2H4Cl2O	0.81
p-Cymene	23.16	134.21	C10H14	10.59
Hexane, 1-hexyloxy-5-methyl	23.59	200.36	C13H28O	0.47
gamma-Terpinene	23.67	136.23	C10H16	16.11
Caryophyllene	24.55	204.35	C15H24	7.51
Trichloroacetic acid	25.32	345.7	C15H27Cl3O2	0.06
3-Heptafluorobutyroxy-pentadecane	25.93	310.39	C19H31F7O2	0.83
Ethyl palmitate	26.04	284.5	C18H36O2	0.15
10-Methylnonadecane	26.57	282.5	C20H42	0.44
Humulene	27.23	204.35	C15H24	16.85
Cyclopropane	28.02	42.08	C3H6	0.12
No. of compounds	36.00	-	-	-
Total				96.57

Table 1: Phyto-compounds in *Newbouldia leavis* stem bark extract by GC-MS analysis

Result and discussion

GC-MS analysis of *Newbouldia leavis* stem bark extract revealed a total of 36 phyto-compounds representing 96.57 %. The stem bark was dominated by Humulene (16.85%), gamma-Terpinene (16.11%), 9,17-Octadecadienal (12.53%), p-Cymene (10.59 %), Linoleic acid ethyl ester (9.37 %), 4-Amino-3-hydroxybutanoic acid (8.85 %) and Caryophyllene (7.51 %) which has been previously associated with antimicrobial, anti-inflammatory, cardio-protective and antioxidant properties [16]. Report by Ezealisiji and Nwoke [17]

have shown high concentration of 9,17-Octadecadienoic acid (15.35 %), Oleic acid (14.68 %), n-Hexadecanoic acid (5.17 %) compared to 12.53 %, 0.83 % and 0.02 % recorded in this study for *Newbouldia leavis* leaf extract. Similarly, Bright et al. [18] recorded a higher concentration of (6.35 %) for n-Hexadecanoic acid and 6.44 % (9,17-Octadecadienal acid) in methanolic extract of *Newbouldia leavis* stem bark. However, the result on 1-Hexadecanol (0.63 %), 2, 3-Dibromopentane (1.92 %), Cyclopentaneundecanoic acid (0.11 %)

and Humulene (16.85 %) observed in this study was similar to those reported by [19-21]. Geographical location, processing method and age of plant have been reported to influence the chemical composition of herbal plants [20-21]. The synergy between the major and minor phyto-compounds exerts numerous therapeutic properties, for instance, Oleic acid, 9-Eicosenoic acid, Linoleic acid ethyl ester and Palmitoleic acid have been suggested to possess antimicrobial properties [22-23], and they also protect the liver, kidney tissues from oxidative stress and reduce damage from toxins [24, 25]. Humulene have been identified to improve cardiovascular health and impede the growth of cancer cells [26-27-30]. 2, 4-Di-tert-butylphenol have been found to possess hypercholesterolemic and protects against neuro-degenerative diseases [28-29-31].

Conclusion

It was concluded that stem bark extracts of *Newbouldia laevis* contains numerous bioactive compounds with therapeutic properties. These compounds offers incredible healing power by its ability to fight, relieve pain and help to balance immune activities.

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