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Saponins and Polyphenolics of Methanol Leaf Extract of *Boswellia dalzielii* Hutch

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The aim of this study was to investigate saponins and polyphenolic constituents of methanol leaf extract of *Boswellia dalzielii* Hutch.The qualitative phytochemistry was based on using reagents known to produce a particular color change which is distinct to that compound, while gas chromatography flame ionization detector (GC-FID) was used for the quantitative phytochemistry. The result revealed 24 polyphenolic compounds with protocatechuic acid (32.08%), catechin (23.77%), quercetin (8.35%), luteolin (20.15%) and kaempferol (12.79%) as the predominant constituents, and 11 phytocompounds of saponin with sapogenin (89.98%) and saponine (0.09%) being the predominant constituents. The experimental results indicate that the methanolic extract of *Boswellia dalzielii* Hutch leaves could be a potent antioxidant agent.

Keywords: Boswellia dalzielii Hutch; saponins; flavonoids; gas chromatography; antioxidant; phytochemistry.

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1. INTRODUCTION

Medicinal plants represent a growing alternative to clinical medicine in the management of health challenges and diseases. Plant exhibits wide biological and pharmacological activities such as anti-inflammatory, diuretic, laxative, anti-spasmodic, anti-hypertensive and anti-microbial functions due to the presence of pharmacological bioactive phytoconstituents available in these plants. Flavonoids are natural polyphenolic compounds characterized by the presence of two benzene rings linked together with a heterocyclic pyran or pyrone ring [1] and play significant role in reducing the susceptibility to most disease [2]. Recent studies on saponins have shown anti-cancer activity, such as anti-proliferation, anti-metastasis, anti-angiogenesis, and reversal of multi-drug resistance (MDR) effects [3], suggesting that saponins are promising prospect for anti-cancer research and development. Hence, the need to investigate the saponins and polyphenolic phytoconstituents of methanol leaf extract of *Boswellia dalzielii* hutch.

The tree *Boswellia dalzielii* Hutch known as the "frankincense tree" is a savannah forest plant which belongs to the family of *Burseraceae* [4]. The leaves are used medically as a diaphoretic, an expectorant prophylactic against fever [5]. Uzama *et al* [6] reported presence of moisture 12.24%, ash 7.43%, crude fibre 32.85%, crude lipids 20.41%, crude protein 1.00% and carbohydrate 26.07% in the leaf, and moisture of 8.51%, ash 14.23%, crude fibre 42.86%, crude lipid 14.23%, crude protein 0.40% and carbohydrate 19.56% in the tree bark [6]. The plant has been extensively studied for anti-microbial [7], antispasmodic [8], hepatoprotective [9], anti-cataract [10], mosquitocidal [11]. Therefore, *B. dalzielii* is a potential therapeutic plant, however only the quantitative phytochemistry of essential oils of *B. dalzielii* leaves have been investigated [4]. No study has been reported on the quantitative phytoconstituents of saponins and polyphenols of this plant hence this study was designed to determine the saponins and polyphenolic constituents present in *B. dalzielii* leaves.

2. MATERIALS AND METHODS

The plant *Boswellia dalzielii Hutch* was obtained from Maitunku hill, Bambam, Dadiya district of Gombe state. Plant was identified by Mr.Thlama Daniel Mshelbwala, Forestry Technology Department, Federal College of Forestry, Jos and confirmed by Dr. Ekeke Chimezie, University of Port-Harcourt Reference Herbarium for Research and Germplasm Conservation, Department of Plant Sciences and Biotechnology, University of Port-Harcourt, Nigeria; where it was given the voucher number UPH/V/1247.

2.1 Methanol Extraction

The pulverized plant samples were extracted according to the method reported by Marita et al. [12]. The extract obtained was stored in an air tight plastic can.

2.2 Phytochemical Screening

2.2.1 Qualitative phytochemical analysis

Standard methods were used for the qualitative determination of saponins [13,14] and flavonoids [15]. This method involved using reagents known to produce a particular color change which is distinct to that particular compound.

2.2.2 Quantitative analysis of phytochemical constituents of flavonoids

2.2.2.1 Instrumentation

Hewlett Packard 6890 Flame Ionization detector Gas Chromatography system powered with ChemStation Rev.A09.01 [1206] software was used.

2.2.3 Quantitative analysis of phytochemical constituents of flavonoids

Extraction was carried out according to the method reported by Kone-Millogo et al. [16].The gas chromatography conditions for flavonoid detection were; HP INNOWax column with (30 m x 0.25 mm x 0.25 µm) dimension, split injection with split ratio of 20:1, nitrogen was the carrier gas with an inlet temperature of 250°C. The oven operated initially at 50°C. First ramping at 8°C/min for 20 min and maintained for 4 min, second Ramping at 12°C/min for 4 min and maintained for 4 min. A detector temperature of 320°C, hydrogen pressure of 22 Psi and compressed air of 35 Psi were used.

2.2.4 Quantitative analysis of phytochemical constituents of saponins

The extraction was carried out according to the modified method reported by Kone-Millogo et al. [16]. The extract was filtered and concentrated to l.0 ml in the vial for gas chromatography analysis

and lµl was injected into the injection part of the gas chromatography. The gas chromatography conditions for this analysis were; Capillary DB-225 MS column with dimension of (30 m x 0.25 mm x 0.25 µm), carrier gas was nitrogen, split injection with split ratio of 20:1 and an inlet temperature of 250°C were used. The oven program started with an initial temperature of 60°C for 5mins, first ramping at 12°C/min for 18 mins and the second ramping at 15°C/min for 5 mins. The detection temperature was 32°C, hydrogen pressure was 28 psi and compressed air was 40 psi.

3. RESULTS

3.1 Qualitative Phytochemical Analysis

This study showed that the leaves of *B. dalzielii* contain saponins and flavonoids.

3.2 Quantitative Gas Chromatographic Analysis of Saponins

The Gas chromatography analysis of the air dried leaves of *Boswellia dalzielii* gave a total saponin in a yield of 1.46 mg/100 g of the methanolic extract. Eleven compounds were identified with Sapogenin (89.98%) and saponine (0.09%) being the predominant compounds. The identified saponin compounds along with their retention time and composition are presented in Table 1.

Table 1. Constituents of saponins in *B. dalzielii*

Constituents of saponin	Amount (mg/ 100 g) x 10^{-4}	Retention time (min)
Sapogenin	13176.9	22.59
Saponine	1451	26.28
Neochlorogenin	5.31	20.46
Diosgenin	2.51	19.51
Yanogenin	1.96	23.96
Tigogenin	1.66	20.11
Tribuloin	1.28	23.22
Conyzorgin	1.20	24.78
Hispogenin	1.04	17.35
Hecogenin	0.94	21.43
Salogenin	0.86	18.76

3.3 Quantitative Gas Chromatographic Analysis of Flavonoids

Analysis of polyphenolic constituents of the methanolic extract yielded a total of 13.54 mg/100g. Twenty four compounds were identified with protocatechuic acid (32.08%), catechin (23.77%), quercetin (8.35%), luteolin (20.15%) and kaempferol (12.79%) being the predominant compounds. The identified saponin compounds along with their retention time and composition were presented in Table 2.

Table 2. Polyphenolic constituents of *B. dalzielii*

Constituents of polyphenolics	Amount (mg/ 100g) x 10^{-3}	Retention time (min)
Protocatechuic acid	4346.11	11.37
Catechin	3220.37	13.81
Quercetin	1131.28	21.77
Luteolin	2730.27	17.31
Kaempferol	1732.68	18.53
Myricetin	351.48	25.16
Daidzein	9.98	15.89
Genistein	4.04	15.48
Butein	5.37	16.55
Epicatechin	4.10	19.50
Biochanin	2.03	17.07
Resveratrol	2.18	15.14
Gallocatechin	0.74	22.73
Robinetin	0.32	24.16
Epigallocatechin	0.61	20.63
Apigenin	0.14	16.01
Epicatechin-3-gallate	0.01	22.89
Isorhamnetin	0.08	23.35
(-)-Epigallocatechin-3-gallate	0.02	23.51
Eupalestin	0.05	25.39
Sinensetin	0.07	25.49
Biacalin	0.05	26.11

4. DISCUSSION

Identifying bioactive dietary constituents is an active area of scientific research that leads to new drug discovery. The main polyphenolic constituents of *Boswellia dalzielii* Hutch are protocatechuic acid, catechin, quercetin, luteolin and kaempferol. Protocatechuic acid (3,4-dihydroxybenzoic acid) is a phenolic acid which possess alleviating effect on inflammation, oxidation, hyperglycaemia, bacterial, cancer, ageing, tumour, asthma, ulcer and neuron [17-19]. Protocatechuic acid exhibited chemopreventive effect by inhibition of free radical generation, chelating metal transition

ions, scavenging free radicals via donation of hydrogen atom (H•) or electron (e) [20,21]. Reports have shown that catechins possess good alleviating properties for oxidation [22] and scavenging properties on varieties of free radicals such as reactive oxygen species *in-vitro* [23,24]. Kaempferol possess a double bond at C2-C3 in conjugation with an oxo group at C4, and the presence of hydroxyl groups at C3, C5 and C4, which are important structural features which makes it a potent anti-oxidant [2]. Furthermore, kaempferol is an inhibitor of the human monoamine oxidases (hMAO-A and hMAO-B) [25] and a potent superoxide scavenger, with an IC_{50} of 0.5 μM [26]. Quercetin has the ability to scavenge free radicals, bind to transition metal ions due to the presence of pharmacophores and a catechol group found in ring B hence regarded as the most potent scavenger of 2 O^{-}, and $ONOO^{-}$ among the flavonoids [27] and improves glutathione concentrations [28,29]. The flavonol group of flavonoids can react with a free radical, it donates a proton and becomes a radical itself, but the resulting unpaired electron is delocalized by resonance, making the flavonol radical too low in energy to be reactive [30]. Sapogenin fraction of *Euphorbia nerifolia* leaf showed antioxidant and radio-protective activities [31].

5. CONCLUSION

The overall experimental results indicate that the methanolic extract of *Boswellia dalzielii Hutch* leaves could be a potent antioxidant agent.

6. RECOMMENDATION

In view of the importance of herbal medicine to man, it is suggested that further study be carried out to elucidate the effect of these phytochemical constituents within the body and the degree and rate of absorption. The pathway of their metabolic conversions need to be identified and evaluated to accurately determine their effect in vivo and their effectiveness in preventing diseases arising from oxidative damage.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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