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Research Article

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Potential Application of the West African frankincense, *Boswellia dalzielii* Hutch, for Drug and Perfumery Products

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Abstract

Boswellia dalzielii is the West African species of the frankincense producing genus (B. carterii, B. frereana and B. serrata are the more popular congeners). Its ethnobotanical uses include the treatment of rheumatism, venereal diseases and gastro-intestinal disorders, among others.Scientific investigations were carried out to evaluate the biological properties relevant to their ethnomedical uses and to better understand the chemistry of the plant. This is with a view to identifying possible applications for medicinal, cosmetic and industrial purposes. The stem bark was subjected to solvent extraction and activity-directed fractionation to isolate bioactive compounds. The isolated compounds were characterised using joint spectroscopic techniques, including 2-D NMR and Mass Spectrometry. The gum resin was steam-distilled to obtain volatile oil, which was analysed by GC-MS. Another portion of gum resin was also extracted by organic solvent and fractionated by column chromatography. From the results obtained; the antimicrobial/antioxidant activity of the stem bark was accounted for by isolated compounds – protocatechuic acid, gallic acid and ethyl gallate with minor contribution from a novel stilbene glycoside and a cembranediterpenoid (incensole). The extracts also demonstrated antifungal, anti-inflammatory, cytotoxic and hypoglycemic effects. The gum resin (frankincense) showed anti-inflammatory activity and yielded volatile oil consisting mainly of monoterpenes (fragrant essence). The gum resin extract yielded incensole and 3-O-acetyl-11-keto-boswellic acid (AKBA). The spectrum of biological activities observed justifies the ethnomedical uses and suggests great potential for further drug development. The essential oil can be employed in perfumery products and in related industry.

Keywords: Boswellia, Frankincense, Incensole, Boswellic acid, Gum resin

Introduction

Boswellia is the frankincense-producing genus, belonging to the family Burseraceae. The main species that account for the commercially available frankincense (olibanum) are usually *B. sacra, B. carteri, B. frereana, B. papyrifera* and *B. serrata*. These are found in North-East Africa, through the Middle East, to India [1,2]. The species found in the West African Sudan savannah is *Boswellia dalzielii* Hutch. It is a deciduous tree, exuding a whitish fragrant resin (frankincense) [3].

In ethnomedicine, the gum resin of *B. dalzielii* is used as a stomachic and for treating venereal diseases. The fragrant resin of *B. dalzieliiis* burned alone or with other fragrant resins to fumigate clothing/rooms to drive out flies and mosquitoes [4]. The aqueous (dialyzed) extract of the dried resin from Cameroon has been shown to possess anti-inflammatory activity in male rats [5]. In northern Nigeria, the stem bark is used to treat several ailments including rheumatism, septic sores, venereal diseases and gastrointestinal conditions [4]. However, in spite of its numerous applications in ethnomedical practice in the sub region, the West African species is less valued commercially and less studied compared to its more popular congeners known for their resin (frankincense).The present study is therefore expected to provide a rationale, if any, for the folkloric uses of the plant and contribute to the knowledge of its chemistry. This is believed would further enhance its value as a potential source of compounds for medicinal, cosmetic and other purposes.

Materials & Methods

Plant material

The stem bark of *B. dalzielii* was collected in Jos, Nigeria during the dry season between December and March.The plant was authenticated by comparison with specimens in the herbarium collections of Forestry Research Institute (FRIN) Ibadan, Nigeria. Voucher specimen (Number: UJ/PCG/HSP/89B13) has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.The gum resin (frankincense) of *B. dalzielii* was the exudate collected by making incisions in the plant's stem bark. The gum was allowed to harden before being separated from the bark. The bark was chopped into small pieces, air-dried and pulverized by pounding in a wooden mortar.

Extraction

The dried pulverized bark of *B. dalzielii* was extracted with 50% aqueous ethanol at room temperature to obtain the crude extract. The stem bark extract of *B. dalzielii* was partitioned between

water and dichloromethane, ethyl acetate and n-butanol to give the corresponding fractions.The gum resin was extracted with diethyl etherand acidic fraction prepared from it using the method of Corsano and Nicoletti modified [4]. Another portion of the gum resin was subjected to hydrodistillation to obtain the volatile oil.

Chromatography

Fractionation of extracts was carried out using chromatographic methods to obtain purified fractions and isolated pure compounds. Thin-layer chromatography (TLC) was carried out using pre-coated plates (silica gel 60 F_{254} Merck) Spots were detected by viewing under ultraviolet light (254 and 366nm wavelength); and spraying with appropriate reagents. Accelerated Gradient Chromatography-AGC, a form of Medium Pressure Liquid Chromatography (MPLC), was carried out using silica gel 60, 0.040-0.063 mm particle size (Merck) [6]. Equipment for the AGC workstation was from BæckströmSeparo Ab, Lidingö, Stockholm.

Gas chromatography coupled with mass spectrometry (GC-MS) was carried out on the volatile oil obtained by steam distillation of *B. dalzielii* resin. The gas chromatogram was run using Hewlett-Packard 6890 GC series equipped with FID and HP-5 capillary column (cross linked 5% diPh, 95% dimethylpolysiloxane, 30m x 0.32mm i.d. x 0.25µm film thickness). The column temperature was programmed at 50-210°C at a rate of 3°C/min. The injector and detector temperatures were 220°C and 270°C, respectively. Samples (1µL of the oil solutions in chloroform, 2mg/mL) were injected by the splitless technique into nitrogen carrier gas (0.8 mL/min).

Bioassay

Screening of the extracts, fractions and pure compounds against typed organisms (*Bacillus subtilis* NCTC 8236; *Staphylococcus aureus* NCTC 6571; Escherichia coli NCTC 10418; Pseudomonas aeruginosa ATCC 10145 and *Candida pseudotropicalis* NCYC 6) was carried out using the agar diffusion cup-plate method. Other fungal species (*Candida albicans, Penicillium notatum, Aspergillus niger*) were also used [7]. The tests for spasmolytic activity of extracts and fractions were carried out on rabbit jejunum [4].

Test for anti-inflammatory activity was carried out on *Boswellia dalzielii* gum resin, the neutral and acidic fractions obtained from the resin. The method described for carrageenan-induced rat paw oedema was employed. The test for antioxidant/ radical scavenging properties of extracts, fractions and isolated compounds were carried out on TLC plates using two spray reagents: β-carotene (0.1%w/v in MeOH or EtOH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) [4].

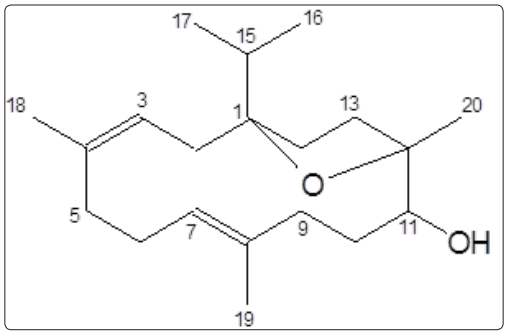
The hypoglycemic effect of the aqueous stem bark extract of *Boswellia dalzielii* was studied using male albino mice weighing between 15-40 g and was compared to that of chlorpropamide, a standard hypoglycemic agent [8].

Brine shrimp (*Artemia salina* Leach) test for cytotoxicity was carried out. Shrimp eggs were placed in saline water (1% w/v NaCl) in a soap dish. They hatched after 48 h incubation to produce larvae (nauplii). Ten nauplii were added to each test tube containing test samples at 1000, 100 and 10µg/mL. Each treatment was carried out in triplicate with saline water as control. After incubating for 24 h the number of dead nauplii in each test tube was counted and recorded. The data was processed using Finney Probit Analysis computer programme to calculate median lethal concentration (LC_{50}) values with 95% confidence intervals for statistically significant comparisons of potency. The bioactivity/ cytotoxicity of the extract, fractions and sub-fractions was monitored by the lethality estimate [9].

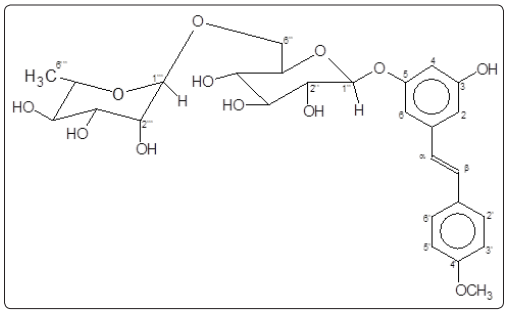
Results

Following the extraction of *B. dalzielii* stem bark, the crude extract was further fractionated by solvent partitioning. The ethyl acetate, which showed the best antimicrobial activity, was further fractionated. Judicious use of TLC and repeated column chromatography yielded several compounds. These include ethyl gallate, gallic acid, protocatechuic acid and β-sitosterol [10]. These constituents have been reported as common to many other plant species [4].

More importantly, one compound, incensole, was isolated and found to be unique to Boswellia. Incensole is a cembrane diterpenoid contributing significantly to the fragrant essence of frankincense. Incensole was characterized from the combined spectroscopic data generated by the isolated compound [11]. Another unique compound isolated from the stem bark extract is a stilbene glycoside named 4'-methoxy-(*E*)-resveratrol 3-*O*-rutinoside. This was also characterised from spectroscopic data [10].



Incensole

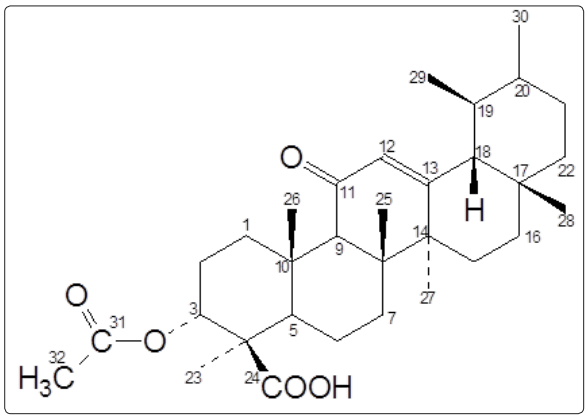


4'-methoxy-(*E*)-resveratrol 3-*O*-rutinoside

The volatile oil obtained from steam distillation of the gum resin (frankincense) of *B. dalzielii* was analysed by GC-MS. The volatile oil consisted mainly of monoterpenes, with α-pinene (57.9%) and camphene (35.4%) making up the bulk. other monoterpenes identified include, β-pinene (0.9%), camphor (0.7%), myrcene (0.6%) and limonene (0.6%). From the dichloromethane extract of the gum resin, incensole (a diterpene) was isolated.

Further proof of its close similarity to frankincense of other established species is seen in the isolation of 3-*O*-Acetyl-11-keto-β-boswellic acid (AKBA) from *B. dalzielii* gum resin. This compound was also isolated from the dichloromethane extract. The compound

was characterised using ^{1}H &^{13}C NMR along with EIMS data [4]. AKBA has been previously reported from the gum resin of the Indian frankincense *B. Serrata* [12]. AKBA is known to have anti-inflammatory properties.



3-*O*-Acetyl-11-keto-β-boswellic acid (AKBA)

From the results of bioassay tests carried out on extracts, fractions and isolated compounds, it was observed that antimicrobial and antioxidant activities of the stem bark were largely accounted for by isolated compounds – protocatechuic acid, gallic acid and ethyl gallate with some contribution from a stilbene glycoside and incensole [10].

Extracts demonstrated antifungal, anti-inflammatory, cytotoxic, antiproliferative and hypoglycemic effects [4,7,8,13]. The gum resin (frankincense) showed anti-inflammatory activity and yielded volatile oil consisting mainly of monoterpenes (fragrant essence) [14].

Thus the spectrum of biological activities observed justifies the ethnomedical uses and suggests great potential for development of drugs to treat diseases of microbial origin, inflammatory conditions, cancer. The essential oil has a characteristic fragrance and could find application in the cosmetic industry.

Conclusion

The gum resin (frankincense) from *Boswellia dalzielii* has comparable constituents and properties as frankincense from better-known congeners and established sources of the commodity. The biological activities exhibited by the gum resin and stem bark extracts also validates many of the uses of *B. dalzielii* in ethnomedicine.

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