Vol. 2 no. 2, pp 137-140 (September 2005)
http://www.ajol.info/journals/jpb

Journal of
PHARMACY AND
BIORESOURCES

Brine shrimp toxicity of acidic fractions of *Boswellia dalzielii* gum resin

Taiwo E. Alemika* and Stephen O. Ojerinde

Department of Pharmaceutical Chemistry, University of Jos, Jos. Nigeria

Received 10th May 2005; Accepted 2nd August 2005

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, swellings/ growths on the skin, among other things. The anti-inflammatory, anti-hyperlipidemic and immunomodulatory activities of *B. serrata* gum resin have been established as being due to triterpenoic (boswellic) acid derivatives such as β-boswellic acid, 3–*O*-acetyl–β–boswellic acid, 11–keto-β-boswellic acid and 3–*O*-acetyl–11-keto-β-boswellic acid. It was therefore considered pertinent to study the cytotoxic activity of the acidic fraction of *B. dalzielii* gum resin. The gum resin of B. dalzielii was extracted with diethyl ether and partitioned into acidic and neutral/basic fractions. These fractions had earlier been shown to have significant anti-inflammatory activity. The acidic fraction was fractionated into four sub-fractions (A-D) using Accelerated Gradient Chromatography (AGC). The fractions and sub-fractions, when tested for brine shrimp lethality, showed very high activity (LC_{50}<50μg/mL). Also the acidic fraction had a significantly higher activity over the neutral fraction. Being the most active, sub-fraction D (LC_{50} = 0.0013μg/mL) was subjected to AGC on silica. This afforded two pure compounds which, from preliminary chemical tests, were shown to be triterpenoids.

Keywords: *Boswellia dalzielii;* Brine shrimp; Gum resin; Triterpenoids

Introduction

Boswellia dalzielii Hutch (family Burseraceae) is a common deciduous tree in the Sudan savanna, growing up to 12m high. It is known by the local name of "*ararrabi*" (Hausa) in Northern Nigeria. Its stem bark is pale brown and smooth, peeling off in thin ragged papery patches. The slash is reddish brown, exuding a whitish fragrant resin. It is the West African species of the frankincense-producing plants - *B. carteri*, *B. frereana* and *B. serrata*. It is used, among others, to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailments (Burkill, 1985; Evans, 1989). However, in spite of its numerous applications in traditional medical practice in the sub region, the West African species is less known and studied compared to its more popular congeners. Phytochemical screening of the plant revealed lack of alkaloids (Baoua *et al.*, 1976) while saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenes were shown to be present (Alemika and Oluwole, 1991;

* Corresponding author. *E-mail address*: alemikat@unijos.edu.ng
ISSN 0189-8442

Adelakun *et al.*, 2001). In addition, the aqueous (dialyzed) extract of the dried gum resin from Cameroon has been shown to possess anti-inflammatory activity in male rats (Duwiejua *et al.*, 1993). The methanol and aqueous extracts also showed broad-spectrum antibacterial and antifungal activities (Ntiejumokwu and Alemika, 1991; Adelakun *et al.*, 2001). Further studies have shown that the antimicrobial/ antioxidant activities of the stem bark can be accounted for mainly by phenolic compounds – protocatechuic acid, gallic acid and ethyl gallate; as well as a diterpenoid – incensole (Alemika 2001; Alemika *et al.*, 2004). The present study therefore aimed at investigating *B. dalzielii* gum resin for bioactive compounds, and specifically for cytotoxic constituents of the acidic fraction. This is especially relevant since the plant is employed by traditional healers in treating swellings and growths on the skin (Azija, 1998). In addition focus has been on the acidic fraction because this has proved to be the source of active compounds found in the gum resin.

Experimental

General procedures. Thin-layer Chromatography (TLC) was carried out on Si gel 60 F_{254} Merck®. Accelerated Gradient Chromatography - AGC (Bæckström, 1993), a form of Medium Pressure Liquid Chromatography (MPLC) was carried out on columns packed with Si gel 60, 0.040-0.063mm Merck®. The MPLC workstation was from Bæckström Separo Ab, Sweden.

Plant material. The gum resin of *Boswellia dalzielii* was collected around Jos, Nigeria during the dry season, between December and March. The plant was authenticated by comparing with voucher specimens deposited at the Herbaria of the Forestry Research Institute of Nigeria (FRIN), Ibadan and Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. A cutlass was used to make incisions on the bark to enable it exude the oleo-gum resin. The gum was dried and the bark removed from it.

Extraction, Fractionation and Isolation. The extraction of the gum resin and preparation of the acidic fraction was carried out according to the method of Corsano and Nicoletti (1967). The dried gum resin was extracted by refluxing with diethyl ether for 3 hours, yielding a yellow extract. The extract was shaken with 2M barium hydroxide and the aqueous layer separated. This was neutralized by addition of dilute sulphuric acid and then extracted with diethyl ether to give the acidic fraction (Fig. 1). The acidic fraction was chromatographed on Si gel (AGC) employing hexane-EtOAc-MeOH gradient. Four sub-fractions (A, B, C and D) were obtained. The extract, acidic and neutral fractions as well as the four sub-fractions were all subjected to brine shrimp test. Sub-fraction D, being the most active was further chromatographed to give two pure compounds. The isolated compounds were tested using ferric chloride and Liebermann-Burchard reagents (Stahl, 1969).

Brine shrimp (cytotoxicity) test. Brine shrimp (*Artemia salina* Leach) eggs were placed in saline water (1% $^{w}/_{v}$ NaCl) in a soap dish. After 48 h incubation at room temperature the hatched larvae (nauplii) were attracted to one side of the vessel by a light source and collected with a pipette. Samples were suspended in saline water in test tubes and ten nauplii were added to each test tube. The volume was made up to 5mL in each test tube giving final sample concentrations of 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 (Meyer *et al*, 1982; McLaughlin and Rogers, 1998).

Results and Discussion

From 1 kg of dried gum resin, 7.86g of crude extract was obtained, indicating a yield of 0.786%. Results of the brine shrimp lethality test (Table 1) show that the acidic fraction had lower LC_{50} values compared to the neutral/ basic fraction. This indicates that the acidic fraction is more active than the neutral fraction. In other words the activity of the gum resides largely in the acidic fraction. The acidic fraction was therefore subjected to column chromatography on silica (AGC) to give four major sub-fractions A, B, C, and D.

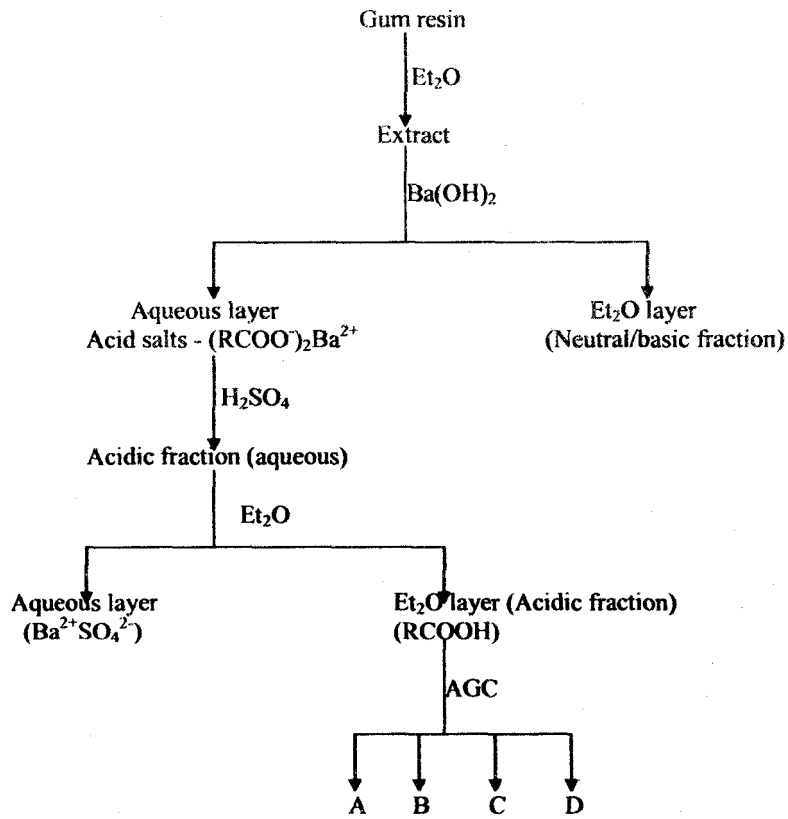

Fig. 1: Extraction/ Fractionation scheme

Table 1: Brine shrimp lethality (LC_{50}) of *Boswellia dalzielii gum* extract and fractions.

Sample	*LC_{50} ± SD (µg/mL)
Crude extract	429.6 ± 0.5
Acidic fraction	24.1 ± 2.9
Neutral fraction	467.7 ± 1.4
Sub-fraction A	0.5617 ± 0.82
Sub-fraction B	0.5617 ± 0.82
Sub-fraction C	0.4336 ± 0.52
Sub-fraction D	0.0013 ± 0.47
Control (saline water)	∞

*** Values computed by Finney computer programme for Probit Analysis**

Following the principle of bioactivity-driven fractionation, these sub-fractions, along with the major fractions and extract were tested in the brine shrimp lethality assay. From the results, sub-fraction D showed the highest activity, hence further chromatography was carried out on it for the isolation of pure compounds. This yielded two compounds, which were characterized by their reaction with spray reagents. The compounds proved to be non-phenolic, showing negative reaction to ferric chloride spray but tested positive to Liebermann-Burchard reagent which is indicative of triterpenoids (Stahl, 1969).

Conclusion

The results obtained from this study show that the gum resin of *Boswellia dalzielii* has potentially cytotoxic activity which resides largely in the polar portion of the acidic fraction. In addition the compounds responsible for this activity are believed to be triterpenoids.

References

Adelakun E.A., Finbar E.A.V., Agina S.E. and Makinde A.A. (2001): Antimicrobial activity of *Boswellia dalzielii* stem bark; *Fitoterapia* 72(7), 822-824.

Alemika T. E., Onawunmi, G. O. and Olugbade T. A. (2004): Isolation and characterization of incensole from *Boswellia dalzielii* stem bark

Alemika T. E. (2001): A study of *Boswellia dalzielii* Hutch. and *Steganotaenia araliacea* Hochst. as constituents of some traditional herbal remedies; Ph. D. Thesis, Obafemi Awolowo University, Ile-Ife. Nigeria.

Alemika T.O.E. and Oluwole F.S. (1991): An investigation of the potentials of *Boswellia dalzielii* and *Commiphora kerstingii* in the treatment of peptic ulcer; *W. Afr. J. Pharmacol. & Drug Res.*, 9/10, 91-94.

Azija O. (1998) – Personal communications

Bæckström P. (1993): Medium Pressure Liquid Chromatography; Proceedings of the workshop on modern separation methods; Bangkok, Thailand; pp. 1-9.

Baoua M., Fayn J. and Bassiere, J. (1976): Preliminary phytochemical testing of some medicinal plants of Niger; *Plant Med. Phytother.* 10, 251-266.

Burkill H.M. (1985): Useful plants of West Tropical Africa; vol. 1 White Friars Press Ltd., U.K.; pp.300-301.

Corsano S. and Nicoletti R. (1967): The structure of incensole; *Tetrahedron* 23, 1977-1984.

Duwiejua M., Zeitlin I.J., Waterman P.G., Chapman J., Mhango G.J. and Provan G.J. (1993): Anti-inflammatory activity of resins from some of the plant family burseraceae; *Planta Med.*, 59(1), 12-16.

Evans W.C. (1989): Trease and Evans' Pharmacognosy; 13th ed.; ELBS/Baillere Tindall, U.K.; pp.474-475.

McLaughlin J.L. and Rogers L.L. (1998): The use of biological assays to evaluate botanicals; *Drug Information Journal* 32, 513-524.

Meyer B.N., Ferrigni N.R., Putnam J.E., Jacobsen L.B., Nichols D.E. and McLaughlin J.L. (1982): Brine shrimp: a convenient general bioassay for active plant constituents; *Planta Medica* 45, 31-34.

Ntiejumokwu S. and Alemika T. O. E. (1991): Antimicrobial and phytochemical investigation of the stem bark of *Boswellia dalzielii; W. Afr. J. Pharmacol. & Drug Res.*, 9/10, 100-104.

Stahl E. (1969): Thin-layer chromatography. A laboratory handbook; 2nd Edition, 6th printing - 1990 (Translated by M. R. F. Ashworth); Springer-Verlag, Berlin; p.855.