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Qualitative phytochemical evaluation and antispasmodic studies of the stem bark of *Boswellia dalzielii* on rabbit jejunum

Umar Tanko Mamza[1*], Hannah Alim Madziga[2], James Yakubu[3], Olufunke A Sodipo[4], Fannah Inna Abdulrahman[5], Irfan Z Khan[6]

[1, 3, 5, 6] Department of Pure and Applied Chemistry, University of Maiduguri, Borno State, Nigeria

[2] Department of Veterinary Physiology and Biochemistry, University of Maiduguri, Nigeria

[4] Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, Borno State, Nigeria

Abstract
This study investigated the phytochemical constituents and antispasmodic evaluation of crude methanolic stem-bark extract of *Boswellia dalzielii.* Phytochemical analysis revealed the presence of alkaloids, flavonoids, cardenolides, cyanogenic glycosides, higher fatty acids, saponins, tannins, steroids, cardiac glycosides, aloes, terpenoids and carbohydrates. The antispasmodic effect of the extract on isolated smooth muscle of the rabbit's jejunum of acetylcholine (ACH) and barium chloride ($BaCl_2$) induced contractions have indicated a dose-dependent inhibition of the jejunal contraction from 18.9% to 79.4% and 34.1% to 84.2% with the bath concentration of (0.53-2.67 mg/ml) respectively. This study suggests that *B. dalzielii* extract interfered with the mechanism of jejunal rhythmic activity of the smooth muscle and hence could serve as good antidiarrhoeal and gastrointestinal remedy. The presence of tannins, saponins and flavonoids could be responsible for the remarkable antispasmodic activity observed from the study. This justifies the ethno-medicinal use of this plant as anti-diaarhoeal agent.

Keywords: phytochemical evaluation, antispasmodic, *Boswellia dalzielii*, stem-bark, rabbit jejunum

Introduction
Medicinal plants have the richest bioresource of drugs of traditional systems of medicine, modern medicine, nutraceuticals, intermediates and chemical entities for synthetic drugs [1]. The phytochemicals have made significant contribution in maintaining human health. The significant of drugs derived from plants cannot be over emphasized with the recent trend of high percentage of chronic and infectious diseases globally [2].

Gastrointestinal problems are among the most frequently encountered diseases in human. The intestinal functional disorders that are part of these problems correspond to chronic digestive symptoms that point to a dysfunction of the lower part of the digestive tract, particularly the small intestine and the colon and that cannot be explained by any organic abnormality [3]. They are characterized by persistent or recurrent excruciating pain in the abdomen [4]. These syndromes are frequently observed worldwide and can affect between 15 to 20% of the population [3].

Several medicinal plants are used for the treatment of gastrointestinal diseases such as intestinal cramps, diarrhoea and transit disorders. *Boswellia dalzielii* is one of these important medicinal plants that is widely used in the treatment of digestive tract diseases and stomachaches [5]. Different studies have revealed its pharmacological properties such as antimicrobial [6, 7, 8], anti-inflammatory [9,10], antiarthritic [9, 11], Antiseptic [12], antitrypanosomal [13], antihepatitis, anti-HIV/AIDS [14, 15, 16], anticancer [16], antimalaria [12] and as immune modulatory activity [17].

B. dalzielii is a tree from a family: Burseraceae. It is commonly known as frankincense tree that grows up to 13m high and is found mainly in the Savannah region of West Africa [5]. The tree has a characteristic pale papery bark that is peeling and ragged. The plant is locally called "arrarabi" in Hausa; "Kaafikafi" in Kanuri; "debro" in Babur/Bura; "mofu" in Marghi all in Nigerian languages. The stem bark has been found to contain phenolic compounds such as protocatechuic acid, gallic acid and ethylgallate as well as a diterpenoid - incensole and triterpenoids - boswellic acid derivatives [5, 7, 18]. Antispasmodic agents have smooth muscle relaxation property and are used to decrease gastrointestinal motility, inhibit gastric acid secretion and to relieve pain associated with diarrhoea and other gastrointestinal disorders [5, 19]. Natural products will continue to play a crucial role in meeting this demand through the expanded investigation of the world's biodiversity, much of which remains unexplored.

In this study, the phytochemical evaluation and the antispasmodic activity of the methanolic extract of the stem bark of *B. dalzielii* was investigated.

Materials and Methods
Plant Collection, Identification and Extraction
Fresh sample of the stem-bark of *B. dalzielii* Hutch was collected from Bagale village (Long. 12º 34' E; Lat. 9º 18' N), Gieri Local Government Area of Adamawa State, Nigeria. The plant material was identified and authenticated by a plant taxonomist from the Department of Biological Sciences, University of Maiduguri where a voucher specimen number (#340) was prepared and deposited at the Post Graduate Research Laboratory, Department of Pure and Applied Chemistry, University of Maiduguri, Nigeria.

The plant material was cleaned, air-dried under shade for seven days and pulverized into coarse powder using mortar and pestle. 250 g of the powdered material was cold macerated with 2.5 litres of methanol for 78 hr. The product was filtered and concentrated with rotary evaporator to dryness at 45 ^{o}C and kept for further analysis.

Phytochemical Screening

The crude methanolic stem-bark extract of *B. dalzielii* was phytochemically screened for the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, higher fatty acids, steroids, terpenoids, cardiac glycosides, anthraquinones and cardenolides using standard procedures [20, 21, 22, 23].

Animals and Tissue Preparation

Five healthy adult rabbits (*American chinchilla*) weighed between 3.8 to 4.5 kg of both sexes were obtained from the Veterinary Research Institute, Vom, Plateus State, Nigeria. They were acclimatized for 7 days, fed with standard animal feeds and clean water *ad libitum*, maintained at normal environmental temperature (26-28°C) and deprived of food 24 hours before commencement of the experiment.

The rabbits were sacrificed by a blow on the head and exsanguinated after which the abdomen was cut open and segment of the jejunum dissected and trimmed to remove adhering mesentery, divided into 2 cm segments. The fresh tissue was then kept in a freshly prepared physiological solution (Tyrode solution) with a pH of 7.4, aerated with 95% oxygen and 5% CO_2 and maintained at 37°C for further analysis.

Ethical Consideration

All the animals in this study were handled in line with the International Guiding Principles for Biomedical Research Involving Animals and it was certified by the ethical committee on animals of the University of Maiduguri, Nigeria.

Antispasmodic Studies on Isolated Rabbit Jejunum

The method described by Schlemper [24] was adopted in this study. Five adult rabbits which had free access to food and water were starved overnight prior to the experiment. The animals were sacrificed by a blow on their head, exsanguinated and their abdomen cut open. Segments of their jejunum about 2.0 cm long were placed separately in 75ml organ baths containing Tyrode's solution, well aerated and maintained at 37°C. The tissues were equilibrated for 60mins before use. Dose-responses for acetylcholine (0.04mg/ml bath concentrations) and barium chloride (0.4mg/ml bath concentrations) were obtained. The contractile responses of the spasmogens were recorded on the kymograph paper by means of a frontal writing lever in Ugo Basile unirecorder 7050 (GMBH, German). The tissue was washed three times with the physiological solution and allowed to rest before the addition of the next spasmogen. The direct effect of the extract (0.53-2.67mg/ml bath concentrations) was investigated after allowing the tissues to rest for 30mins. Similarly, the effect of the extract (0.53-2.67mg/ml) was investigated on sub maximal dose of acetylcholine (0.04µg/ml) and barium chloride (0.4mg/ml) so as to study the effect of the extract on these spasmogens. The percentage inhibition of contraction induced by each dose of the extract against the specific spasmogen was calculated and recorded.

Statistical Analysis

Responses were express as mean ± SEM and analysed using student t-test. P values less than 0.05 (P<0.05) were considered to be statistically significant.

Results and Discussion

The results of phytochemical screening of the stem bark of *B. dalzielii* showed the presence of alkaloids, flavonoids, steroids, saponins, tannins, terpenes, higher fatty acids, cardiac glycosides, cardenolides, aloes and carbohydrates as presented in Table 1 which is in agreement with the results obtained by previous researchers [25,26].

The results of the antispasmodic effect of the extract are presented in Tables 2 and 3 respectively.

The relaxative effect of the extract on isolated smooth muscle was confirmed from its cumulative response on rabbit jejunum of acetylcholine (ACh) induced contraction in which there was a dose-depended inhibition of the jejunal contraction from 18.9%-79.4% (Table 2). The effects of the extract on barium chloride induced contraction of the isolated smooth muscle of rabbit jejunum also showed similar response to that of ACh but with greater contractile percentage inhibition from 34.1% - 84.2% (Table 3) which is in agreement with the results obtained from previous study [3, 25, 26, 27]. From the results obtained, it was observed that the extract was more sensitive to $BaCl_2$ on the rabbit jejunum compared to ACh which equally showed similar results obtained by Hassan *et al*. [25, 28, 29]. The methanolic extract of the stem bark of *B. dalzielii* inhibited both acetylcholine and barium chloride induced contractions significantly (P<0.05) of the rabbit jejunum at all bath concentrations.

The antispasmodic effect of the extract on the isolated tissue were as a result of the active constituents such as saponins, flavonoids and tannins which possess the ability to antagonize the activities of spasmogens such as acetylcholine (ACh) and barium chloride [25, 30, 31, 32]. The activities of spasmogens may be antagonized by the extract either through the muscarinic and histaminic receptor sites or other musclotropic routes such as the inhibition of calcium ion influx and outflux as demonstrated by the extract through the inhibition of acetylcholine and $BaCl_2$ induced contractions. There are also different mechanisms that are involved in gastrointestinal smooth muscle relaxation by plant extracts [33]. These include the blocking action on muscarinic M3, histaminic H1 and 5-HT receptors [3, 25, 34, 35]. The relaxant effect may also be induced by stimulating the nitric oxide [36, 37], purinergic [38], adrenergic [39], or GABAergic modulatory systems [40].

Saponins and flavonoids have been shown to decrease ACh and histamine induced contractions of guinea pig and rabbit ileum [25, 27]. Tannins have been reported to possess an astringent property in gastrointestinal tract, reduces peristaltic movement and intestinal secretion [25, 26, 27].

The result of the study therefore suggest that the extract has the potential of being developed as an anti-motility agent as a remedy for gastrointestinal tract system-related problems [25]. The presence of the identified metabolites (alkaloids, flavonoids, tannins, saponins, terpenes) in the plant could be responsible for this remarkable antispasmodic effect revealed in this study [30, 31, 32, 41].

Table 1: Phytochemical constituents of crude Extract of the stem bark of *B. dalzielii*

S. No	Phytochemicals	Test	Results
1	Alkaloids	Dragendorff's	+
		Mayer's	+
		Wagner's	-
2	Aloes		+
3	Anthroquinones	Borntrager's	-
	Free Anthroq.	Borntrager's	-
	Combined Anth.	Borntrager's	-
4	Cardenolides	Legal's	+
		Keller-Kilianis	+
5	Cardiac glycosides	Salkwoski's	+
		Liebermann-Burchard	+
6	Terpenoids		+
7	Cyanogenic glycoside		+
8	Flavonoids	Shinoda's	+
		Ferric chloride	+
		Lead acetate	+
		NAOH	-
9	Higher fatty acids		+
10	Phlobatannins		-
11	Resins		+
12	Saponins	Frothing	+
		Fehling's	+
13	Tannins	Ferric chloride	+
		Lead acetate	+
		Gold beater's	+
14	Steroids	10% HCl	-
15	Carbohydrates		+
	i.General test	Molisch's	+
	ii.Monosaccharides	Barfoed's	+
	iii.Free reducing sugar	Fehling's	+
	iv.Combined reducing sugar	Fehling's	+
	v. Soluble starch		+
	vi. Ketoses		+
	vii.Pentoses	Salivanoff's	+

Key: + = present, - = Absent, CM =crude methanol extract

Table 2: Effect of methanolic extract of stem bark of *Boswellia dalzielii* on ACh (0.04mg/ml) induced contracted on rabbit jejunum (n = 5)

Volume added of extract (ml)	Bath concentration (mg/ml)	Amplitude of Ach (cm)	Amplitude of ACh and Extract (cm)	Difference in Amplitude of ACh and combined ACh and Extract (cm)	Percentage contractile inhibition
0.2	0.53	9.5 ± 0.1^{a}	7.7 ± 0.1^{b}	1.8	18.9
0.4	1.07	15.1 ± 0.1^{a}	4.9 ± 0.1^{b}	10.2	67.5
0.6	1.60	15.4 ± 0.3^{a}	3.9 ± 0.0^{b}	11.5	74.6
0.8	2.13	17.7 ± 0.1^{a}	3.8 ± 0.0^{b}	13.9	78.5
1.0	2.67	18.0 ± 0.1^{a}	3.7 ± 0.0^{b}	14.3	79.4

Key: Within rows, means with different superscripts are statistically significant ($P<0.05$) when compared with the amplitude of normal using one-way analysis of variance (ANOVA), Values are means ± SEM, ACh= acetylcholine

Table 3: Effect of methanolic extract of stem bark of *Boswellia dalzielii* on $BaCl_2$ (0.4mg/ml) induced contracted on rabbit0020jejunum (n = 5)

Volume added extract (ml)	Bath concentration (mg/ml)	Amplitude of $BaCl_2$ (cm)	Amplitude of $BaCl_2$ and Extract (cm)	Difference in Amplitude of $BaCl_2$ and combined $BaCl_2$ and Extract (cm)	Percentage contractile inhibition
0.2	0.53	12.6 ± 0.1^{a}	8.3 ± 0.1^{b}	4.3±0.0	34.1
0.4	1.07	7.3 ± 0.3^{a}	3.3 ± 0.0^{b}	4.0±0.0	54.7
0.6	1.60	4.9 ± 0.1^{a}	1.2 ± 0.1^{b}	3.7±0.1	75.5
0.8	2.13	2.8 ± 0.1^{a}	0.5 ± 0.0^{b}	2.3±0.1	82.1
1.0	2.67	1.9 ± 0.4^{a}	0.3 ± 0.2^{b}	1.6±0.2	84.2

Key: Within rows, means with different superscripts are statistically significant ($P<0.05$) when compared with the amplitude of normal using one-way analysis of variance (ANOVA), Values are means ± SEM, $BaCl_2$= Barium chloride

Conclusion

The methanolic stem-bark extract of *B. dalzielii* has showed the presence of alkaloids, aloes, flavonoids, cardiac glycosides, cardenolides, Cyanogenic glycoside, higher fatty acids, saponins, carbohydrates, tannins, terpenoids and steroids with a remarkable relaxative effect on the isolated smooth muscle of rabbit jejunum indicating dose-dependent inhibitory rhythmic activities. The antispasmodic effect of

this plant was due to the presence of the afore mentioned metabolites. The results of the study therefore scientifically prove that the extract shows the potential of an anti-diarrhoeal agent and as a remedy for GIT system related problems.

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