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Phytochemical and anti-diarrheal studies of *Bauhinia rufescens* Lam

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Abstract

Bauhinia rufescens Lam, family Leguminosae – Caesalpinoideae, an economic, ornamental and medicinal plant with fruits edible to human and animals, was subjected to phytochemical and anti-diarrheal studies. The phytochemical screening of the leaves, roots and stem bark showed the presence of saponins, tannins, cardiac glycosides, flavonoids, sterols and terpenes. The methanol extract of the leaves showed 100 percent protection at dose levels of 125 and 250mg/kg in castor oil-induced diarrheal study in mice. This activity was comparable to 5mg/kg of loperamide. The extract at bath concentration of 0.04 – 0.32 mg/ml produced a relaxation of the rabbit-jejunum and median lethal dose (LD_{50}) of 1265 mg/kg intraperitoneally in mice. This result supports the traditional use of the plant as anti-diarrheal agent.

Keywords: *Bauhinia rufescens*, Anti-diarrheal activity, Phytochemical constituents.

Introduction

The genus Bauhinia, family Leguminosae–Caesalpinoideae, commonly known as the orchid tree is generally used for ornamental, hedges and carpentry purposes. *Bauhinia rufescens* is widely distributed in tropical Africa and India. It is a much-branched shrub or small tree reaching the height of 5-8m. It is called "kulkul" in Arabic, "Nammare" in Fulani and "Jirga" or "Matsagi" in Hausa. The leaves, roots and stem bark are used in traditional medicine for the treatment of disease conditions such as fever, chest complaints, venereal diseases such as syphilis, leprosy, dysentery, diarrhea, chicken-pox, measles and eye infections (Dalziel, 1956; Oliver, 1960; Burkill, 1995). The plant is also used superstitiously in cases where the stem bark is worn as a charm, while the leaves, along with the pods, are mixed, boiled with corn porridge and butter, used as "Magannin Karfe" meaning medicine against spear, knife or arrow (Burkill, 1995). Polycyclic aromatic compounds, such as benzopyran–8, 9–diol, have been isolated from the root bark of the plant (Maillard *et al*., 1991). Flavonoids such as quercetin and kaempferitrine (lespedin) have also been isolated from a Brazilian species of Bauhinia – *B. forticata* (Silva *et al*., 2000). This study was carried out to investigate the phytochemical constituents of the different parts of the plant used in traditional medicine

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and to study the anti-diarrheal activity of the leaf extract.

Experimental

Plant materials. The leaves, roots and stem bark of *Bauhinia rufescens* were collected in Samaru village, Zaria, in the month of September, 2007. The plant parts were authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a specimen with voucher number 427 has been deposited. The plant parts were air-dried and powdered using mortar and pestle.

Extraction process. The powdered plant materials (200g each) were weighed and extracted in a soxhlet extraction apparatus using 1 litre of petroleum ether 60 – 80^{o}C) for 24 hours separately. The marcs obtained thereof were air-dried and re-extracted using 1 litre of methanol for 48 hours separately. The filtrates obtained were evaporated under reduced pressure to afford solid residues which were weighed and coded as PEL, MEL, PER, MER, PESB and MESB respectively for petroleum ether and methanol extracts of leaves, roots and stem bark of the plant.

Phytochemical study. The solid residues obtained from the extraction process were subjected to standard tests for the presence of secondary metabolites such as saponins, tannins, flavonoids, cardiac glycosides, alkaloids, anthraquinones, sterols and terpenes (Evans, 1989; Sofowora, 1993; Silva *et al*., 1998).

Experimental animals. Swiss albino mice of both sexes weighing 25-30g and adult male rabbit weighing 4.5kg were obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The mice were maintained on standard laboratory animal feeds and water *ad libitum*, and housed in clean plastic cages at room temperature throughout the study. These studies were carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as acceptable internationally.

Acute toxicity study. Acute toxicity study of the methanol extract of the leaves of *B. rufescens* was carried out according to the method of Lorke (1983). Thirteen mice were used for the study which involves two phases. In the first phase of the study, three doses of the extract (10, 100, 1000 mg/kg) were administered intraperitoneally to three groups containing three mice each. The second phase of the study involved three groups of one mouse each given *i.p.* 1600, 2900 and 5000 mg/kg respectively. One mouse was used as the control and given normal saline *i.p.* In each phase, the mice were observed for lethality for a period of 0-24 hours. The LD_{50} of the extract was calculated as the geometric mean of the lowest lethal dose and the highest non-lethal dose.

Anti-diarrheal study. Twenty-five albino mice fasted overnight prior to the commencement of the study were used. They were divided into five groups containing five mice each. The first group which served as the negative control received 10 ml/kg normal saline *i.p.* while the second, third and fourth groups received 250, 125 and 62.5 mg/kg *i.p.* respectively of the methanol extract of the leaves of *B. rufescens.* The fifth group (positive control) was given 5mg/kg loperamide *i.p.* Thirty minutes after the administration of drugs, 0.2ml of castor oil was administered to each mouse orally. The mice were placed in individual cages over clean filter paper. Three hours after the administration of castor oil, the filter papers were inspected for the presence of characteristic diarrhea droppings of the mice. Their absence was recorded as protection against diarrhea and percentage protection offered calculated (Akah and Offiah, 1992).

Isolated rabbit jejunum study. Adult male rabbit fasted overnight was sacrificed by a blow on the head. Segments of the jejunum about 3 cm long were removed and freed of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution. The tissue was mounted in 25ml organ bath containing Tyrode's solution maintained at 37^{o}C and aerated with air. An initial tension load of 0.5g was applied to the segment and an equilibration period was allowed while the physiological solution was changed every 15 minutes. Effects of graded bath concentration of 0.04 – 0.32 µg/ml of Acetylcholine and 0.04 – 0.32 mg/ml of methanol extract of leaves of *B. rufescens* were observed non-cumulatively. The contact time for each concentration was 1 minute followed by washing of the tissue three times. The tissue was allowed to rest before the addition of next dose. Responses were recorded on the micro dynamometer by the transducer (Agunu *et al*., 2005).

Results

The percentage yields of the solvents used for extraction – petroleum ether and methanol – of the different parts of the plant are shown in Table 1. The phytochemical study revealed the presence of saponins, flavonoids, tannins, cardiac glycosides, sterols and terpenes in the extracts of leaves, roots and stem bark of *B. rufescens* as shown in Table 2. The result of acute toxicity study of the methanol extract of the leaves (MEL) showed lethality in the second phase of the study while the groups in the first phase and control survived. The intraperitoneal LD_{50} of MEL was calculated as 1265mg/kg. The extract produced 100 percent protection against castor oil-induced diarrhea at dose levels of 125 and 250mg/kg which was comparable to 5mg/kg loperamide as shown in Table 3. The effect of MEL 0.04 – 0.32mg/ml on isolated rabbit jejunum was the relaxation of the spontaneous contraction of the jejunum as shown in figure 1. This effect was in contrast to that produced by graded concentration of acetylcholine (0.04-0.32 µg/ml) which was concentration – dependant contraction of the jejunum.

Discussion

The phytochemical study of the leaves, roots and stem bark of *Bauhinia rufescens* showed the presence of saponins, flavonoids, tannins, cardiac glycosides, sterols and terpenes. The extract coded MEL gave the highest percentage yield of 12.59 and was thus used for the anti-diarrheal study. The acute toxicity study of MEL revealed a wide margin of safety with LD_{50} of 1265mg/kg *i.p*. The MEL of *B. rufescens* showed anti-diarrhea activity against castor oil-induced diarrhea in mice. A dose level of 125mg/kg gave a 100 percent protection which was comparable to loperamide, a standard anti-diarrhea drug. Loperamide is widely used in the management of diarrhea disorders and it effectively antagonizes diarrhea induced by castor oil (Niemegeers *et al*., 1974).

Table 1: Percentage yield of extracts from leaves, roots and stem bark of *B. rufescens*

Plant part	Extract	Percentage yield
Leaves	PEL	0.6
Leaves	MEL	12.59
Roots	PER	0.3
Roots	MER	8.9
Stem bark	PESB	0.4
Stem bark	MESB	0.9

PEL = Petroleum ether extract of leaves MEL = Methanol extract of leaves
PER = Petroleum ether extract of roots MER = Methanol extract of roots
PESB = Petroleum ether extract of stem bark MESB = Methanol extract of stem bark

Table 2: Phytochemical constituents of the leaves, roots and stem bark of *B. rufescens*

Constituents	Plant extracts					
	PEL	MEL	PER	MER	PESB	MESB
Saponins	-	+	-	+	-	+
Flavonoids	-	+	-	+	-	+
Tannins	-	+	-	+	-	+
Cardiac glycosides	-	+	-	+	-	+
Sterols and terpenes	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

Table 3: Result of Anti-diarrheal Study of the methanol extract of leaves of *B. rufescens*

Treatment	Dose	No of mice with diarrhea	% protection
Normal saline	10 ml/kg	5/5	0
MEL	62.5 mg/kg	2/5	60
MEL	125 mg/kg	0/5	100
MEL	250 mg/kg	0/5	100
Loperamide	5 mg/kg	0/5	100

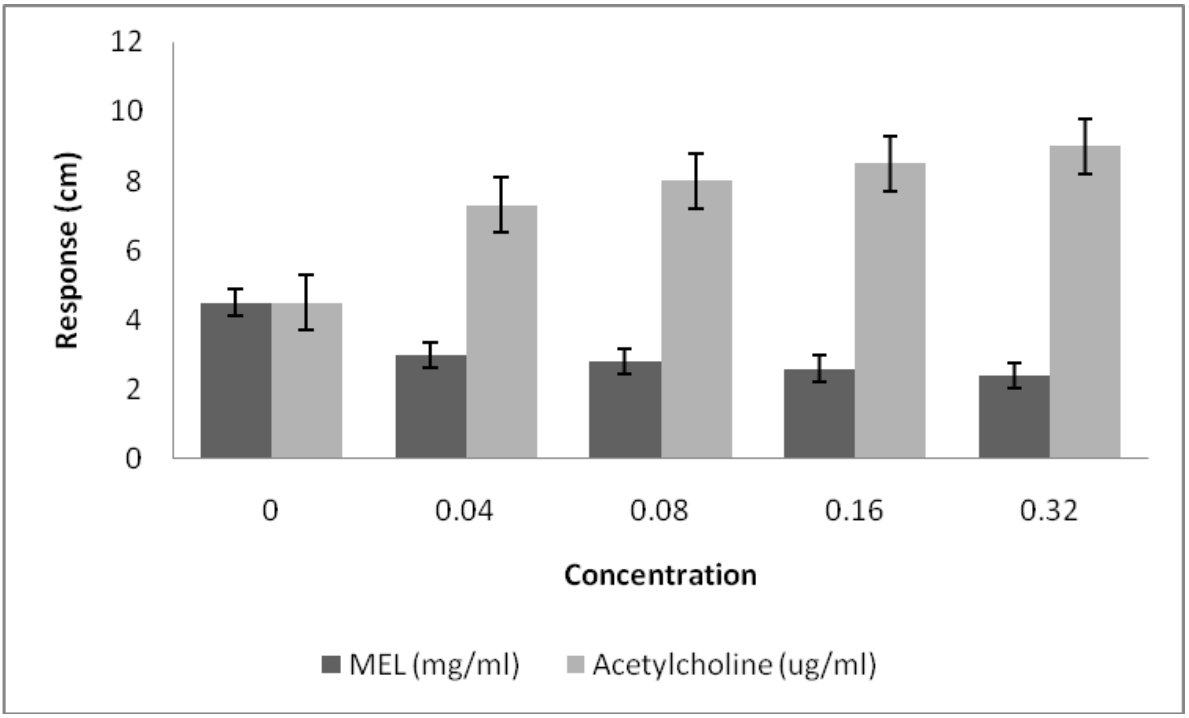

Figure 1: Effect of Acetylcholine (0.04-0.32µg/ml) and MEL (0.04-0.32mg/ml) on the spontaneous contraction of the rabbit jejunum.

Castor oil is made up of 90 percent ricinoleate which is then metabolized to ricinoleic acid, the active principle responsible for the diarrheal inducing property (Gaginella and Phillips, 1975). Ricinoleic acid stimulates the peristaltic activity in the small intestine leading to changes in the electrolyte permeability of the intestinal mucosa. It also stimulates the release of endogenous prostaglandins (Galvez *et al*., 1993). Studies have shown that saponins, flavonoids, tannins, sterols and

terpenes present in plants may produce anti-diarrhea effect by interacting directly with the intestinal mucosal (Longanga-Oushudi *et al*., 2000). It have also been suggested that anti-diarrhea effect of extracts may also be due to the inhibition of prostaglandins by flavonoids contained in such extracts (Galvez *et al*., 1993; Haruna *et al*., 1995). The relaxation effect of the methanolic extract of leaves of *B. rufescens* on rabbit jejunum further explains the ability of the extract to protect the mice against diarrhea through relaxation of the intestinal smooth muscle thus enabling the absorption of fluid by the intestinal mucosal (Heinrich *et al*., 2005). These results confirm the traditional uses of the leaves of *B. rufescens* in the treatment of diarrhea.

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