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Pharmacognostic and hypotensive evaluations of the stem bark of *Ficus exasperata* Vahl (Moraceae)

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Abstract

Elevated blood pressure is one of the non-communicable ailments whose control is attempted by the use of medicinal plants in ethnomedicine. In furtherance of our previous work on *Ficus exasperata* as a plant used in controlling elevated blood pressure, evaluations of the microscopy and other pharmacognostic parameters that could aid in the identification of the plant stem bark were carried out using standard methods. The probable effect of the methanol extract of the stem bark on anaesthetized normotensive rabbit blood pressure was also examined at doses of 5,10,20,30 and 40 mg/kg. The stem bark was observed to occur as flat and channelled pieces when fresh and single quill after drying. The powdered compound contained cork cells, cortical parenchyma, sclereids, fibres and prismatic calcium oxalate crystals as microscopic characters. The percentage weight loss on drying was 6.1 ± 0.15 while the water and alcohol extractive values were1.87 ± 0.04 and 0.59±0.03% respectively. The total ash value was 28.49±0.89 % while the water soluble and the acid insoluble ash values were 2.31± 0.19 and 9.93±0.2% respectively. The preliminary phytochemical results on the stem bark include saponins, tannins, flavonoids and no alkaloids and anthracene derivatives. At 5 mg/kg, the methanol extract of the stem bark produced 6.6±1.2 mmHg fall in mean arterial blood pressure with the effect more pronounced on the systolic than the diastolic blood pressure. At 10 mg/kg, the reduction in the blood pressure was 15.66±2.9 mmHg after which higher doses did not produce corresponding significant decrease in the blood pressure. Each dose administered was accompanied by corresponding reduction in the heart rate indicating the probable effect of the extract on the myocardium. The results showed that the stem bark of *F. exasperata* also possess hypotensive effect like the leaf reported earlier.

Keywords: *Ficus exasperata*: Pharmacognostic parameters; Hypotensive effects

Introduction

More than 10% of Nigerian population were reported to have high blood pressure (Mabadeje, 2002) while only about one tenth of the population were estimated to be able to afford seeking help at clinics and hospitals (Steyn and Walker, 2000). These observations are largely responsible for the wide use of herbal medicines in treating many ailments including high blood pressure. Among medicinal plants reportedly prescribed by some herbal medicine practitioners is *Ficus exasperata Vahl* (Moraceae). It is a plant characterized by its highly rough and abrasive leaves (Keay, 1989). Akah *et al* (1997) reported the anti-ulcer effects of the leaf extract while the hypotensive effects of the leaves have also been reported along with the

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pharmacognostic parameters to aid its identification in powder form, (Ayinde *et al*, 2007). This work further reports the pharmacognostic parameters and the effects of the stem bark on the anaesthetized normotensive rabbit blood pressure.

Experimental

Collection and preparation of plant material. The fresh stem bark of *F. exasperata* was collected in June 2008 within the University of Benin, Ugbowo Campus. After removing the unwanted materials, the stem bark was cut into pieces and air dried for a few days after which it was oven dried at 60^{o}C. Thereafter, the dried pieces of the stem bark were reduced to powder using electric grinding machine.

Drugs and chemicals. These include chloral hydrate, phloroglucinol, concentrated hydrochloric acid, urethane, methanol, ethanol, chloroform (Sigma Chemicals).

Macroscopic and microscopic features. Macroscopical and organoleptic features of the bark examined include the shape, colour (inner and outer surfaces), taste, odour, fracture, presence or absence of lichens. The microscopic evaluation of the powdered bark was carried out using the standard technique of clearing the powdered sample in chloral hydrate solution and mounting in glycerin (African Pharmacopoeia, 1986). The presence or absence of features like cork cells, sclereids, fibres, calcium oxalate crystals, cortical parenchyma, cortical collenchyma as well as vessels were noted. Abbe-type camera lucida (at a magnification of x 180) was used to examine and draw the observed elements.

Determination of the quantitative standards. The following quantitative parameters were determined on the powdered compound using standard methods (African Pharmacopoeia, 1986, British Pharmacopoeia, 1988) described as follows:

Moisture content (water loss on drying). About 2.5 g of the powdered drug was weighed into a clean crucible of known weight. After oven-drying at 105^{o}C for 5 hrs, the crucible was removed, cooled and weighed to determine the weight loss in the powdered drug. This was determined by subtracting the weight of powdered drug dried at 105^{o}C from the weight of air-dried powdered-drug. The average percentage weight loss with reference to the air-dried powdered drug was determined for four replicates.

Extractive values. These include alcohol and water extractive values. For the alcohol extractive value, 4.0 g of the powdered drug sample were macerated in 100ml of alcohol (99 – 100% BDH,) in a closed volumetric flask for 24 hours with frequent shaking for 6 hours before being allowed to stand for 18 hrs. After 24 hrs, the mixture was rapidly filtered into a clean dry beaker. 25 ml of the filtrate was evaporated to dryness on a water bath using an evaporating dish of known weight. It was further dried at 105^{o}C for 6 hr. The dish was weighed after cooling. The weight of alcohol extractive was obtained by subtracting the weight of the dish from the weight of the dish containing dry alcohol extractive. The average of four alcohol soluble extractives was calculated with reference to the air-dried powdered drug.

Water-soluble extractive was carried out as stated above except that chloroform-water (1:400) was used.

Determination of Ash values

Total ash. 3 g of the powdered drug were weighed into a crucible previously ignited to obtain a constant weight. The content was incinerated in a furnace at 650^{o}C. After cooling, the crucible was weighed and the weight of ash obtained determined. The average percentage of ash with reference to the air-dried powdered drug was calculated using four replicates.

Acid-insoluble ash value. 25 ml of concentrated hydrochloric acid was added to the ash obtained as described earlier. The crucible with the contents was gently boiled for 5 minutes while covered with a watch - glass which was rinsed with 5 ml hot water and the rinsing added to the crucible. On cooling, the content was filtered through a previously dry ashless filter paper (Whatman No 1) of known weight. The residue on the filter paper was washed with hot water until the filtrate became neutral to blue litmus paper. The filter paper with the insoluble matter was dried to a constant weight at 105^{o}C. The weight of insoluble matter was determined by subtracting the weight of filter paper from the dry weight of the filter paper containing the insoluble ash. The percentage of the acid-insoluble ash with reference to the air- dried drug was calculated.

Water-soluble ash. 25 ml distilled water was added to the ash obtained as earlier described. After boiling gently for 5 minutes, the content of the crucible was filtered through previously weighed dry ash-less filter paper. After washing the residue with hot water, the filter paper was dried in an oven at 105^{o}C until a constant weight was obtained. The weight of the residue was obtained by subtracting the weight of the dry filter paper from the weight of the filter paper containing the residue. The weight of water-soluble ash was then obtained by subtracting the weight of the insoluble ash (i.e. the residue) from the weight of ash started with. The percentage of water soluble ash with reference to the air-dried powdered drug was calculated.

Phytochemical analyses. The presence or otherwise of phytochemical constituents of the medicinal plant like the saponins, tannins, alkaloids, anthracene derivatives, flavonoids, and steroidal glycosides were determined using standard methods (Evans, 1989; Sofowora, 1984).

Extraction of plant material. About 600 g of the powdered stem bark was extracted by maceration method using methanol (2 x 2 L). The container was shaken at 4 hours interval for 12 hours. The content was filtered after 72 hours. The filtrate obtained was concentrated on a rotary evaporator maintained at 40^{o}C. The semi-solid material obtained **(**6.89g; representing 1.15%**)** was kept in a refrigerator until required for further use.

Animal experiments. All the rabbits used were matured males purchased and maintained in the Animal House of the Department of Pharmacology and Toxicology with normal animal pellets (Livestock Feeds, Benin) and water *ad libitum* till required. Approval for use of the animals was obtained from the Faculty of Pharmacy Ethical Committee on the use of Animals for Experiments.

Each animal (1.1 to 1.3 kg) was anaesthetized with urethane (1,500 mg/kg) administered intraperitoneally. After cannulating the ear vein for drug administration, the carotid artery was cannulated and connected via a Bentley Physiological pressure transducer to two-channel Ugo Basile recorder (Gemini 7070) for recording blood pressure and heart rate. 0.5ml of normal saline was first administered into the animal to test the normalcy of the experimental set up. The methanol extract was dissolved in normal saline and administered at doses of 5, 10, 20, 30 and 40 mg/kg. The experiment was carried out in triplicate. The mean arterial pressure (MAP) was calculated before and after administration of the extract as

MAP = Diastolic pressure + 1/3 (Systolic –Diastolic) mmHg.

Statistical analysis. All data were expressed as mean ± SEM (standard error of mean). Where applicable, the data were compared using *Student t- test*, on Graph pad Instant ^{R} version 2.05 software (UK). The level of significance was from $P < 0.05$.

Results and Discussion

The macroscopic examination of the stem bark showed that the pieces occur as flat or channelled forms when fresh and as simple quill on drying. The outer part of the stem bark was dirty green while the inner part was brown in colour. The pieces break with short fractures with no fissures. The microscopic examination of the stem bark powder revealed the presence of highly lignified polygonal shapes cork cells. The cortical parenchyma was polygonal and plate like. The sclereids were up to 46.6± 2.7 µm long and 20.6 ± 1.9 µm wide. The fibres were 205.4± 6.3 µm. The calcium oxalate crystals were prismatic type. On the quantitative parameters, the stem bark powder was observed to have moisture content (weight loss on drying) of 6.1 ± 0.15 % while total ash value was 28.49±0.89 %. The acid insoluble ash was 9.93±0.2% while water soluble ash was 2.31± 0.19 %. The water extractive value was 1.87 ± 0.04% while the alcohol extractive value was 0.59±0.03% (Table 1). The powdered sample of the stem bark of *F. exasperata* was observed to contain saponins, flavonoids, tannins and steroidal compounds while it tested negative to alkaloids and anthracene derivatives.

The methanol extract of *F. exasperata* was observed to produce a relatively dose dependent effects in reducing the blood pressure in the normotensive rabbits. At a dose of 5mg/kg, the reduction in blood pressure was 6.6 ± 1.2 mmHg. The blood pressure was further reduced by 15.66 ± 2.9 mmHg at 10mg/kg after which the reductions in the blood pressure were no longer significantly different with further increase in the dose ($P > 0.05$) (Fig.1). The effect of the extract on the animal was well prolonged and sustained as indicated by the time it took the animals to recover to almost the former blood pressure before the administration of the extract. It was also observed that the extract exerted its effect more on the systolic than the diastolic blood pressure. At 5mg/kg, the extract did not have any effect on the systolic blood pressure, but subsequent administration of higher doses lead to corresponding significant decrease in the systolic pressure. On the other hand, the effects on the diastolic pressures were not marched with increase in doses administered (Fig.2). The reductions in the blood pressure exhibited by the extract were accompanied with corresponding decrease in heart rates. The dose of 10mg/kg reduced the heart rate by 17.02 times/min. It was further reduced by 76.6 and 80.85 at doses by 30mg/kg and 40mg/kg respectively (Fig.3).

Determining the quantitative parameters and the microscopic analyses of powdered samples of medicinal plants enabled the researcher obtain a set of values that can be used to differentiate and establish the proper identities of the plants even when they are closely related in terms of the constituents or pharmacologically. The weight loss on drying which is an indication of the moisture content of the air dried crude drug powder was observed to be within the range (14 %) specified by the African Pharmacopeia (1986) and was less than 9.84 ± 0.08 % observed for the leaf (Ayinde *et al*, 2007). The 6.1 ± 0.15 % observed for the stem bark shows that the plant material may not be prone to microbial contamination and deterioration or enzymatic activities usually associated with high moisture content. Such microbial spoilage or enzymatic activities may result in conversion of the pharmacologically active constituents of the medicinal plant to less potent or more toxic ones. As was observed for the leaf (30.68± 0.44%), the stem bark of *F. exasperata* had a total ash value of 28.49±0.89 % indicating the high concentration of calcium oxalate crystals.

This work has revealed the presence of tannins, saponins and flavonoids in the stem bark of *F. exasperata.* No alkaloids, and

anthraquinones were detected as earlier reported by Ijeh *et al* (2007). The variations in the results could be due to collection from different locations or that the plants were collected at different periods of the year or different time of the day.

The results obtained on the effects of the methanol extract on the rabbit blood pressure showed the potency of *F. exasperata* stem bark as a hypotensive drug plant. The extract exhibited a dose related response which can be attributed to the presence of various constituents of the plant material. At a dose of 10mg/kg, the rabbit arterial blood pressure was reduced by 15.66±2.9 mmHg. However, at 40mg/kg, the reduction was observed to be 18.22± 2.2 mmHg. These effects are products of the different actions of the extract on both systolic and diastolic blood pressure which were also observed to decrease with increase in doses. The reduction in the blood pressure was observed to be closely associated with corresponding reductions in heart rate as the doses increased. This suggests a probable direct effect of the extract on the myocardium as a kind of crude drug that induces negative inotropic and chronotropic effects on the heart. However, earlier reports on the hypotensive effects of the leaves indicated the probable stimulation of muscarinic receptors, coupled with the decrease in histamine release (*Ayinde et al, 2007).*

Table 1: Quantitative parameters observed for the stem bark powder of *Ficus exasperata.*

Quantitative parameters	Values obtained (%)
Weight loss on drying	6.1 ± 0.15
Total ash value	28.49±0.89
Water soluble ash value	2.31± 0.19
Acid insoluble ash value	9.93±0.2
Water extractive value	1.87 ± 0.04
Alcohol extractive value	0.59±0.03

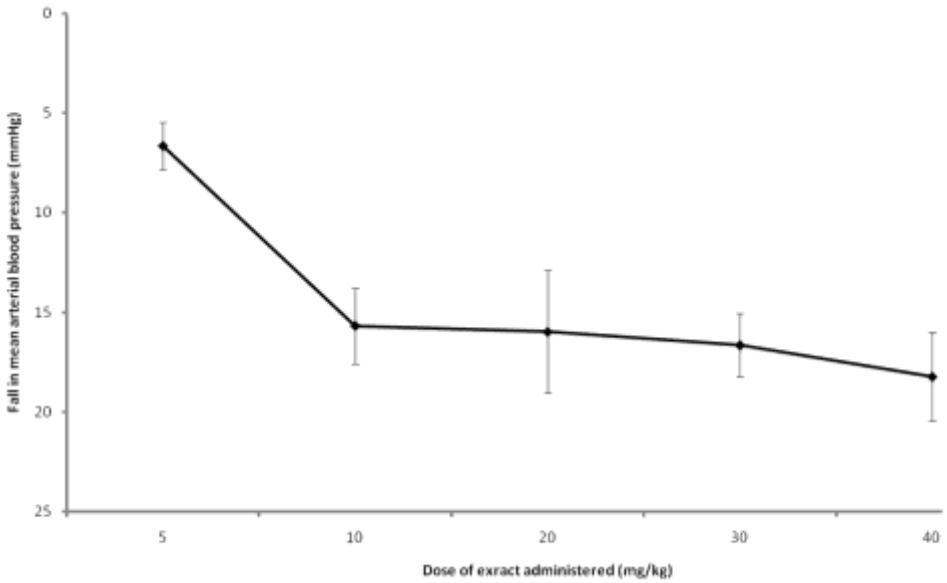

Fig 1: Effect of the methanol extract of *Ficus exasperata* stem bark on normotensive rabbit blood pressure.

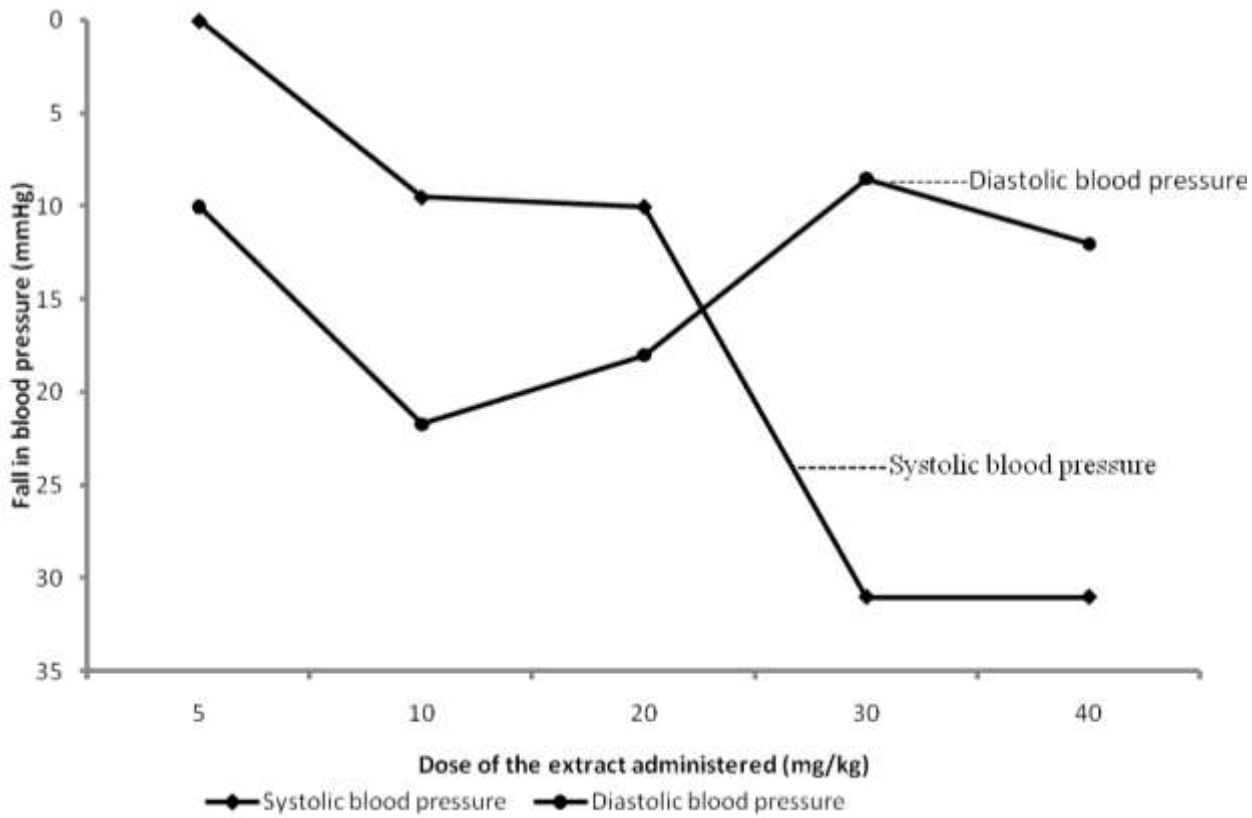

Fig 2: Effect of the methanol extract of *Ficus exasperata* stem bark on rabbit systolic and diastolic blood pressure.

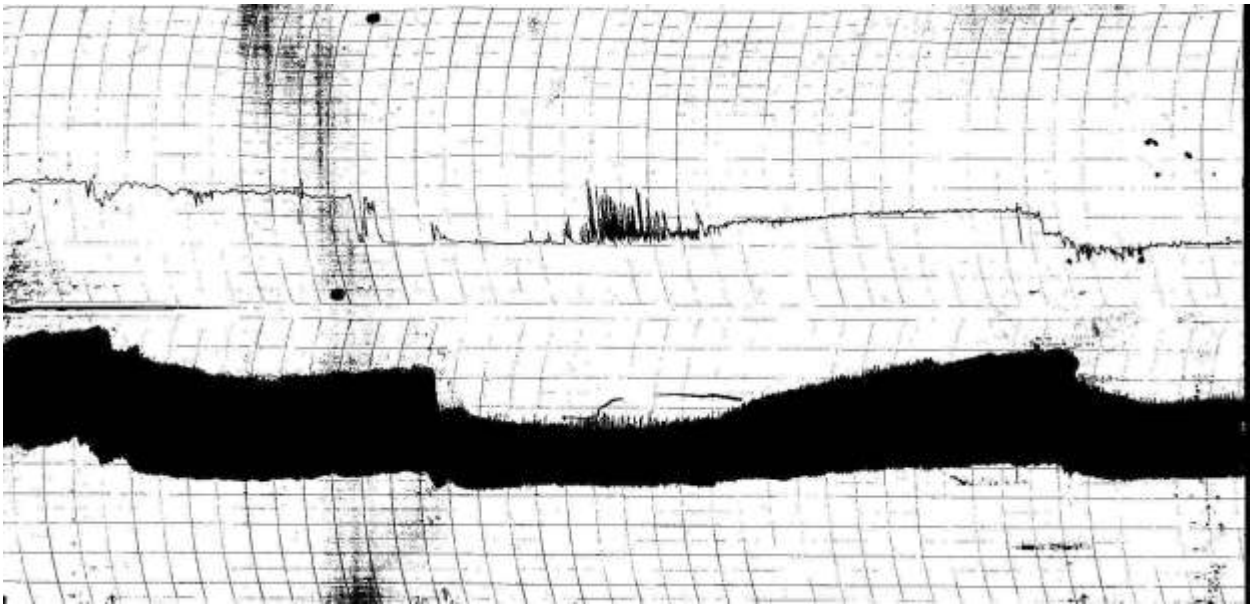

Fig 3: Tracing showing the effects of the methanol extract of *F. exasperata* stem bark on the rabbit blood pressure. The extract showed a sustained reduction in both the blood pressure and the heart rate.

Although, the actual group of constituents responsible for the observed hypotensive effect of *F. exasperata* stem bark is yet to be known, it is possible that the phytochemical constituents present in the plant material act synergistically to potentiate one another. The fact that the stem bark dose dependently reduced the blood pressure confirmed its use traditionally in reducing blood pressure.

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