

Blood pressure lowering agent(s) in the leaves of a deciduous shrub-*Elaeophorbia drupifera*.

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Citation

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Abstract

The crude ethanolic extract was prepared from the leaves of *E. drupifera*. Lethality studies in mice showed a dose-mortality relationship with an LD₅₀ of 112.6mg/kg. mice i.p. The extract (2-260µg/kg.iv) was tested in graded doses on blood pressure of pentobarbital anaesthetized rats. In another group of rats, hypertension was induced using DOCA-salt. The extract (5-80µg/kg.iv) was administered and the BP and heart rate monitored.

In the normotensive group, the extract reduced the BP dose-dependently. A dose-response curve constructed gave a sigmoidal shape with an ED₅₀ of 134.9µg/kg.i.v. The maximum decrease produced by the extract (560µg/kg.iv) was about 36.2mmHg, representing about 42.35% decrease (%control). A repeat of the dose-effect experiment with a group of atropinized rats, showed that the dose-response curve shifted to the left. This atropine-antagonism appeared to be surmounted by raising the concentration of the extract. In the hypertensive group, the extract (80µg/kg.i.v) produced a maximum decrease in BP of about 24.7% and 63.6% for the systolic and diastolic pressures respectively. Both decreases were significant ($p < 0.05$ in both cases). Also, the extract (80µg/kg.i.v) markedly depressed the heart rate of hypertensive rats. The depression was up to about 73.91% ($P < 0.05$).

Propranolol (0.5µg/kg.iv) failed to prevent the extract-induced reduction of BP. The extract (10µg/kg.iv) reduced noradrenaline-induced increase in BP, and when it (extract) was given in combination with ACh, the action of the extract was enhanced by ACh.

We conclude that the crude ethanolic extract from the leaves of *E. drupifera* probably contains acetylcholine-like agent(s) which interferes with the cholinergic mechanism. The presence of other agents in the extract are however, not ruled out.

INTRODUCTION

Hypertension is a significant health problem because of the percentage of the population affected and the serious consequences of uncontrolled high blood pressure. In general terms, hypertension continues to be a major risk factor for stroke, congestive heart failure and coronary artery disease (Akinkigbe, 2001).

The worldwide increasing demand for medicine from natural sources (Lapa, 1992) has motivated us to search for plants with potential hypotensive activity.

Plants of the family Euphorbiaceae are frequently used in indigenous practice of medicine. Their pharmacological properties include anti-tumor, antibacterial and antihypertensive activities (Schiff, 1970). However, little literature is available on the medicinal uses of the species *Elaeophorbia drupifera* (Thonn). Stapf, ("Akpa Mbiet"); although it is listed among the "plants that heal" (Ampofo, 1977). Ingenol (Kinghorn and Evans, 1974, Abo, 1990) and

lectins (Lynn and Radford, 1986) have been isolated from the latex of *E. drupifera*. The fruit is succulent (Kinghorn and Evans, 1974) but the latex has skin irritant effect (Kinghorn and Evans, 1975), and it is reported to promote inflammatory reactions (Abo, 1994). The leaf extract is said to contain hypoglycemic agent(s) (Eno and Itam, 1996) and stimulates autonomic cholinergic receptors in the rat uterus (Eno and Itam, 1998). Recently, the leaf extract has been found to moderately inhibit HIV-1 and HIV-2 proviral and DNA copying (Ayisi and Nyadedzor, 2003), increases gastric motility (Eno and Azah, 2004) and induces the secretion of pepsin-rich gastric juice (Eno, et al; 2004).

This local herb is used by traditional herbalists for the treatment of hypertension, diabetes and many other ailments. Ground leaves (paste) are dissolved in either water or soft drink and administered orally in doses determined by age.

In cases of over dosage producing any adverse effect, fresh coconut water is administered to the patient as an antidote

(personal comm.).

The present study is aimed at investigating the scientific basis of employing this leaf extract as an antihypertensive agent by our traditional herbalists. This study became necessary following earlier report that the roots of this plant has hypotensive activity (Eno and Owo, 1999).

MATERIALS AND METHODS

(A) PREPARATION OF EXTRACT

Samples of *E. drupifera* (Thonn) Stapf. were collected from a local farm in Calabar municipality and were identified at the herbarium of the Department of Botany, University of Calabar.

Fresh leaf samples (3.5kg) were oven dried (40 °C) and ground, yielding 1.86kg. of powder. The powder was extracted with a mixture of ethanol: water (ratio 70:30) by a conventional reflux method for 1hr. The total volume of the mixture was 21.5L. The extraction was repeated 3 times and the filtered hydro-ethanolic extract was mixed and evaporated under reduced pressure. The concentrated extract was frozen and finally freeze-dried to yield 162 g of powder.

Thus, 1mg of lyophilized extract was obtained from 11.5g of dry leaf powder.

(B) ACUTE TOXICITY TEST

Male white albino mice (20-25g) were assigned to 12 groups of 20 animals per group. Each group was injected (i.p) with one of the following: 10, 20, 40, 80, 120, 200, 250, 300, 350, 400, 450, and 500µg/kg of the crude extract. The maximum volume injected was 0.5ml. The animals were returned to their home cages and given free access to food and water. The mortality in each cage was assessed 24 hours after administration of the extract. The percentage mortalities were converted to probits (a probability unit) and plotted against the \log_{10} of the dose of the extract. Regression lines were fitted by the method of least squares and confidence limits for the LD_{50} value was calculated by the method of Litchfield and Wilcoxon (1949).

(C) INTACT PREPARATION OF NORMO-AND HYPERTENSIVE RATS

Normal white Wistar rats of either sex (200-280g) were anaesthetized with pentobarbital (35mg/kg.i.p). Surgery and cannulations were performed as described by Eno, et al (2000). The blood pressure from the carotid artery was recorded using Washington, F. T. 400 transducer connected to a recorder (Washington Model 400 MD/2C). For

simultaneous heart rate counting, the transducer signal was fed into a biotachometer in one of the polygraph channels. By means of a table heater, the rectal temperature of the animals was maintained at 37 ± 1 °C.

In another group, hypertension was induced using silicone rubber moulds containing deoxycorticosterone acetate (DOCA, 15mg/100g). These were implanted S.C to induce DOCA- salt hypertension. They were given normal saline (0.9% NaCl₂ w/v) in place of drinking water, and fed with rat cubes ad libitum. The animals were considered hypertensive after a period of eight weeks. However, animals with the mean arterial pressure below 160mmHg were not considered hypertensive and were therefore not included in the study. The crude extract (5-80µg/kg.iv) were administered to these hypertensive rats and their BP and heart rate monitored as earlier described.

In another group of normotensive rats, attempts were made to elucidate the possible mechanism of action of the crude extract on BP. Some standard pharmacological agents were employed to influence the action of the extract on blood pressure.

(D) DOSE-EFFECT RELATIONSHIP

Forty normotensive white Wistar rats (180-250g) were obtained. The crude extract was injected (i.v) as a 0.2ml bolus at eight different increasing doses to construct a dose-response curve. The doses (2, 4, 8, 16, 32, 64, 130 and 260µg/kg. were injected at an interval of 5 min, giving cumulative doses of, 2, 6, 14, 30, 62, 126, 250 and 510µg/kg. extract. The effect of a dose was calculated by averaging the last minute of blood pressure and heart rate recording preceding the following dose. In preliminary experiments, a single dose of the extract produced an effect lasting more than 80min. In another group of rats, the dose-response experiment was repeated but the animals received the extract 2min. after atropine (0.2mg/kg.i.v) pretreatment. The mean arterial BP was calculated from the formula, $MAP=DP + 1/3 (SP-DP)$, where DP and SP are the diastolic and systolic pressures respectively.

(E) STATISTICAL ANALYSIS

Regression lines with confidence limits were calculated for the linear portions of log concentration-response curves. The significance of differences in slopes was used as a measure of parallelism of the two lines. Log concentration limits at 50% of the maximum response were used in the analysis of the significance of the concentration differences as described

by (Birmingham et al 1970). Maximum responses were compared by paired Student's t-test.

(F) REFERENCE DRUGS

Noradrenline, mepyramine and propranolol were obtained from Sigma (USA) while Atropine sulphate was obtained from the British Drug House (BDH).

RESULTS

(A) PHYTOCHEMICAL STUDIES

Phytochemical studies (Table 1) revealed that *E. drupifera* leaf extract contained large amount of Saponins and polyphenols. Tannins, flavonoids, phelobatinns and anthraquinones were probably absent. Sodium and particularly potassium were the cations in very high concentration in the extract. Chloride and possibly phosphorus were also in very high levels in the extract as the anions. With a pH of 5.8, the extract must have been very acidic.

2. ACUTE TOXICITY TEST

In lethality studies, a dose-mortality relationship (not show) was apparently sigmoidal. A plot of probit (a probability unit) values (% mortality against the log-dose of extract) produced a straight line. From the straight line graph, the LD₅₀ was extrapolated. This value was about 112.6mg dry wt. of extract per kg, mice (mg/kg mice, i.p). No animal died earlier than 8h. post-injection.

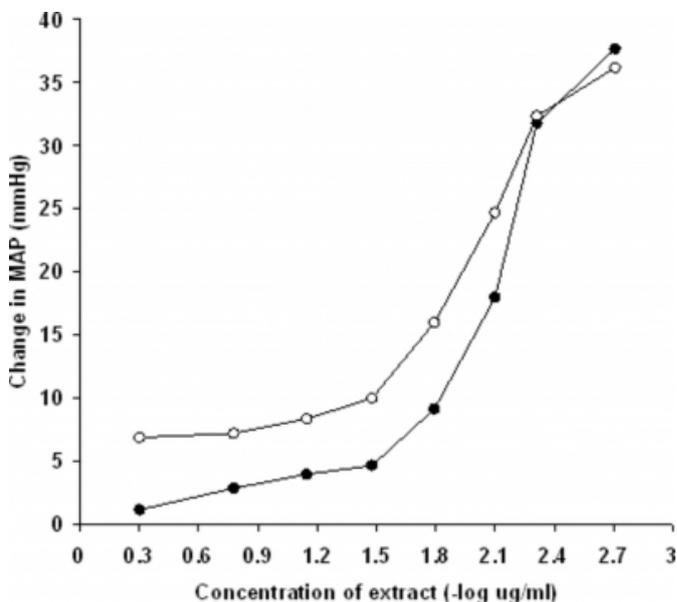
3. EFFECTS OF LEAF EXTRACT ON BLOOD PRESSURE AND HEART RATE

(i) Dose-Effect Relationship

Before the administration of *E. drupifera* leaf extract, the control mean arterial pressure (MAP) in pentobarbital anaesthetized rats was about 85±3.8mmHg (SEM, n=6). Slow intravenous injection of the extract produced a fall in the BP of rats in a dose-dependent manner. Fig 1 shows the extract-induced decrease in BP. (ED₅₀ = 134.90µg/kg). The maximum decrease in BP (36.2mmHg) was achieved with the extract at a cumulative dose of 560µg/kg cumulative dose represents about 42.35 % (%control) depression of BP by the leaf extract. In general, the effect of the extract on the rat BP was quick in onset and the recovery time increased with increasing concentration of the extract.

Figure 1

Figure 1: Dose-effect relationship. The curves show blood pressure changes (mmHg) following cumulative injection of leaf extract (2 - 516µg/kg.i.v.) to a group of normotensive rat (?) and to another group (?) that received atropine (0.2mg/kg.i.v.) pretreatment. Data are the mean values ± SEM (n = 6, p<0.05).



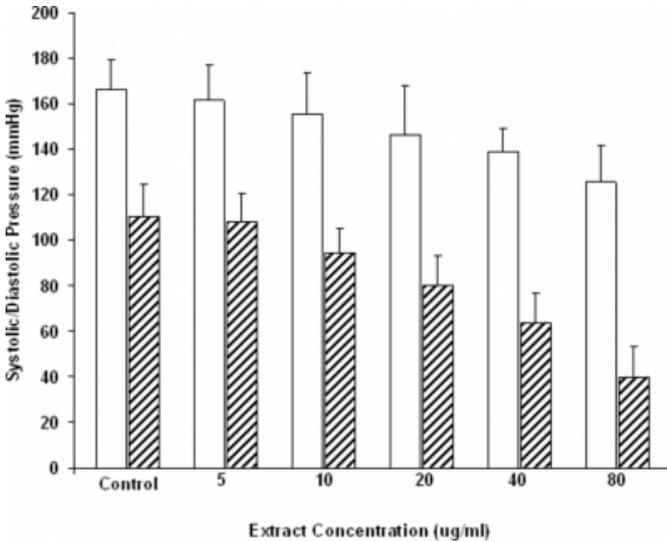
A similar dose-effect experiment was repeated in another group of normal rats (control MAP = 83. 85 ± 9.4 mmHg, n=6). In this group, the animals were pretreated with atropine sulphate (0.2mg/kg.i.v) to block the muscarinic cholinoreceptors, before injecting the extracts (2-516µg/kg.i.v cumulative dose range) as described in the methods section. In atropine-treated animals, the extract-induced decrease in BP was reversed. The concentration response curve (ED₅₀ =83.18µg/kg) was shifted to the left and the atropine-antagonism appeared to be surmounted with higher doses of the extract Fig.1. The maximum decrease in BP (86.24 mmHg) of rats pretreated with atropine before the injection of extract, was not significantly different from the matching controls.

(B) EFFECT OF CRUDE EXTRACT ON DOCA-INDUCED HYPERTENSIVE RATS

The control mean systolic and diastolic arterial pressures were 166.3 ±12.8 and 110. 4 ± 14.2 mmHg respectively, for the hypertensive group of rats. Following the administration of the crude extract (5-80µg/kg.i.v), both systolic and diastolic pressures were significantly decreased (p<0.05) in a dose-dependent fashion (Fig. 2).

Figure 2

Figure 2: Histograms showing the effects of graded doses (5 - 80 µg/kg.i.v) of leaf extract on the systolic (?) and diastolic (?) blood pressures of DOCA-induced hypertensive rats. Each bar represents the mean value $\hat{A} \pm$ SEM, $p < 0.05$; $n = 6$.

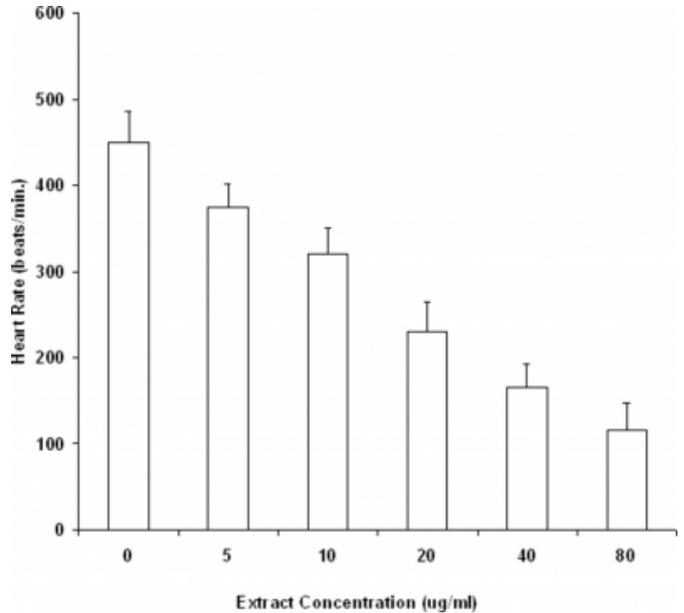


However, the decreases appeared to be more marked in the diastolic than the systolic pressures. The maximum dose of extract tested (80 µg/kg.i.v) produced about 24.7% and 63.6% (% controls) depression of the systolic and diastolic pressures respectively. In general, the extract appeared to have very mild reducing effect on the systolic pressures when compared to its effect on diastolic pressures.

The mean control heart rate (HR) of the hypertensive rats, measured simultaneously with the BP was about 460 beats/min before administering the extract (Fig. 3). Following the injection of the crude extract (5-80 µg/kg. body wt), the HR showed a dose-dependent decrease. The highest dose of extract injected (80 µg/kg) also produced the maximum decrease in HR (120 ± 14 beats/min) which represents about 73.91% (% control) decrease produced by the crude extract.

Figure 3

Figure 3: Histograms showing the effects of graded doses (5 - 80 µg/kg.i.v) of leaf extract on Heart rate measured simultaneously with the blood pressure of DOCA-induced hypertensive rats. Each bar represents the mean value $\hat{A} \pm$ SEM. $P < 0.05$, $n = 6$.



Attempts were made in order to elucidate the possible mechanism of action employed by the crude extract (Figs. 4 and 5) using normotensive rats propranolol (0.5 µg/kg) caused a reduction in BP and this condition was aggravated by the extract (10 µg/kg) administered about 10 min after the injection of the drug (Fig. 4a). Noradrenaline-induced increase in BP was as well depressed by the extract (10 µg/kg.i.v) (Fig. 4b). This reduction was by about 23.5% ($p < 0.05$). However, in atropine-treated (1.0 µg/kg) animals, the extract (10 µg/kg.i.v) failed to depress the BP and even tended to raise it (Fig. 4c). Figure 5 shows the effects of administering high doses (above the ED_{50} value) of the extract on blood pressure. In all animals tested with this dose of extract (0.2 mg/kg.i.v), results showed decreased pulse rate and increased pulse pressure (Fig. 5). This was the lowest dose of extract that could produce this effect. Propranolol (0.5 µg/kg.i.v) and mepyramine (2 mg/kg.i.v) failed to ameliorate this effect (Fig. 5a and b) while ACh in combination with the extract, aggravated it (Fig. 5c), resulting to the death of these animals. However, these responses (decreased pulse rate and increased pulse pressure) were readily reversed by the administration of atropine sulphate (1.5 µg/kg.i.v) (Fig. 5b).

Figure 4

Figure 4: Representative tracing on arterial blood pressure (BP) of normotensive rats. Record shows the influence of propranolol (Prop), noradrenaline (NA) and atropine sulphate (Atr) on extract (Ext.)-induced decrease in BP.

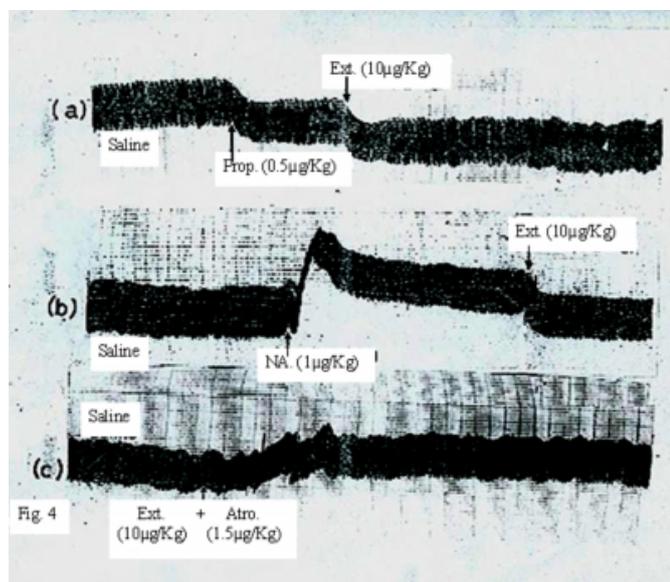


Figure 5

Figure 5: Representative tracing on arterial BP. of normotensive rats. Record shows the effects of a high dose (0.2mg/kg.i.v) of the extract (above LD value) on the blood pressure and the influence of propranolol (Prop.), mepyramine (mepy.), atropine (Atr.) and acetylcholine (Ach) on the BP.

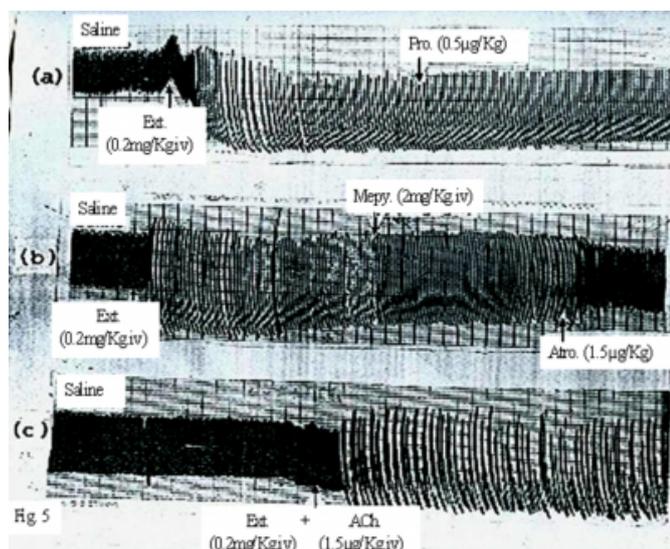


Figure 6

Table 1: Chemical characteristics of leaf extract.

Phytochemical content		Electrolyte content	
Chemical constituent	Results	Ionic Species (mg/L)	Conc. in Extract (mg/L)
Saponins	(++)	Chloride as Cl ⁻	808.5
Cardiac glycosides	(+)	Phosphorus as PO ₄ ⁻	189.8
Tannins	(-)	Potassium as K ⁺	2.998.5
Alkaloids	(+)	Sodium as Na ⁺	485.0
Polyphenols	(++)	Calcium as Ca ²⁺	39.5
Flavonoids	(-)	Iron as Fe ²⁺	51.7
Reducing compounds	(+)	Conductivity (mhos/cm)	160
Phelobatins	(-)	pH	5.8
Hydroxymethyl-antraquinones	(+)		
Antraquinones	(-)		

Phytochemical and ionic composition of crude aqueous extract from *E. drupifera* leaves. Values are determined in the laboratories of the National Fertilizer Company of Nigeria “NAFCON”. NIGERIA. (+ = present; ++ = greater presence, - = not present)

DISCUSSION

The crude ethanolic extract from *E. drupifera* leaves seems to be relatively non-toxic in view of the observed low LD₅₀ value in mice. Another important observation about the general activity of the extract was the fast onset of action. This may point to a low molecular weight compound present in the extract, which may penetrate rapidly to its site of action. Alternatively, it could merely reflect the high concentration of the active principle present in the extract (Bowman and Rand, 1980).

The present studies show clearly that *E. drupifera* leaf extract markedly depressed the blood pressure (BP) and heart rate (HR) of DOCA-salt-induced hypertensive rats when compared with that in the normotensive controls. Although the cause of death in some extract-treated animals is not clear, cardiotoxicity may not be ruled out. This is because, severe hypotension could reach the level at which blood flow to vital organs (heart, brain and kidney) is impaired, this effect resulting in death (Tarazi and Gifford, 1979). Secondly, the observation that the extract (dose above the LD₅₀ value) produced decreased pulse rate and increased pulse pressure may also point to cardiotoxic effect.

Blood pressure measurements reflect the status of the cardiovascular system (Milnor, 1980), and the maintenance of an adequate BP in the aorta depends on the product of two

factors, the cardiac output and the total peripheral resistance of the vessels (Guyton, 2005). Therefore, the present studies were focused on the effect of the crude extract from *E. drupifera* leaves on blood pressure and heart rate. From our experimental results, there are some convincing evidences that this crude extract could possess hypotensive property which is probably due to some interference with the cholinergic transmission, although some sympathetic-like effect may not be completely ruled out. This is suggested because blocking the sympathetic innervation to the heart with propranolol (a beta-adrenoceptor antagonist) failed to prevent the extract-induced depression of BP, suggesting that the extract was acting at a different site. The observation that noradrenaline-induced increase in BP was blocked by the extract is in line with this contention. Also, this leaf extract was found to induce the contraction of an isolated guinea pig ileum (Eno, et al, 1999) just like ACh and histamine, suggesting a cholinergic pathway for the extract. We cannot suspect that the extract influenced the histaminergic transmission since mepyramine also failed to block the action of the extract. The overwhelming evidence may therefore point to interference with cholinergic transmission. Possibly, the extract contains ACh-like agent(s) that could stimulate the autonomic cholinergic receptors. This is suggested because atropine sulphate (a muscarinic cholinergic receptor blocker), abolished or prevented the actions of both the low and high doses of the extract. Secondly, ACh, administered in combination with the extract, was found to enhance the action of the extract. Such enhancement caused by ACh, aggravated the hypotensive condition. Finally, studies in atropinized- animals revealed that the extract-induced decrease in BP was reversed and the dose-response curve shifted to the left, thus suggesting atropine-antagonism of a competitive type, since it was surmounted with higher doses of the extract.

Although, we are unable to explain why *E. drupifera* leaf extract markedly depressed the diastolic pressure but mildly depressed the systolic pressure. However, this seemingly selective action could be the underlying property of the extract that enhances its efficacy in blood pressure depression. This is suggested since the mean arterial pressure (MAP) is determined more by the diastolic than the systolic pressure (Bowman and Rand, 1980).

In conclusion, it appears, therefore, that the crude ethanolic extract from the leaves of *E. drupifera* contains several pharmacologically active constituents. One such group may be acetylcholine-like agent(s) showing predominantly

cholinergic activity and this reduces arterial blood pressure. However, given that the active agent, let alone its chemical structure, is yet unknown, further progress must await refinements in the separation techniques.

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