

## Hypertensive and Sympathomimetic Effects of the Methanol and Aqueous Extracts of *Scoparia dulcis* in Anaesthetized Male Wistar Rats

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### ABSTRACT

#### Objective

*Scoparia dulcis*, a widely used medicinal plant is believed to have antihypertensive effects among traditional medical healers. This study investigates the efficacy of *Scoparia dulcis* as an antihypertensive agent in Wistar rats.

#### Methods

Plant specimens were collected from local gardens in Portharcourt and identified in Department of plant Science, University of Portharcourt. Methanol and aqueous extracts were made from the leaves of the plant. Phytochemical screening was done using conventional methods and acute toxicity studies carried out to determine the LD<sub>50</sub> using the Arithmetic method of Karber. Rats were anaesthetized using urethane, and endotracheally-intubated. The rats were then cannulated via the jugular vein and carotid artery. The carotid artery was connected to a pre-calibrated Ugo Basile double channel pen recorder. Adrenaline (0.5µg/kg), Acetylcholine (1.5µg/kg), and 20, 40, 80 and 160mg/kg of the aqueous and methanol extracts were administered through the jugular vein and their effects on the blood pressure (BP) and heart rate (HR) recorded. The mean arterial pressure (MAP) was calculated. This was repeated in four series for each extract and the mean of all experiment ± standard error of mean calculated. Results were analyzed statistically using Kruskal-Wallis, Dunn's and Tukey-Kramer multiple comparisons test and Friedman nonparametric test.

#### Results

The phytochemical analysis reveals the presence of Tannins, Phlobatannins, Steroids, Terpenoids, Flavonoids and Cardiac glycosides in the leaves of *Scoparia dulcis*. There were no Saponins. The LD<sub>50</sub> was 450 and 650mg/kg for the methanol and aqueous respectively. There was significant (P<0.05) hypertensive and sympathomimetic effects at 40, 80 and 160mg/kg dose levels for both extracts.

#### Discussion

Though, *Scoparia dulcis* plant is used traditionally in the treatment of hypertension, scientific research has not demonstrated this property but rather has shown this plant to increase the BP, MAP and HR as revealed by the above results. The plant contains catecholamines which accounts for these effects after parenteral administration. In the traditional medicine system, there are no scientific means of diagnosis of hypertension such as measurement of BP, other than perceived symptoms such as headache, insomnia and malaise. Extracts of *Scoparia dulcis* plant are reported to have analgesic, anti-inflammatory and sedative effects and it is likely that the improvement in the above symptoms in hypertensive patients that is erroneously believed to be the BP lowering effects among traditional medical healers.

**Key words:** Hypertensive and Sympathomimetic effects, Ethanol and Aqueous extracts, *Scoparia dulcis*, Wistar rats, LD<sub>50</sub>.

## INTRODUCTION

*Scoparia dulcis* belongs to the family *Scrophulariaceae*, genus *Scoparia* and Species *dulcis*. Its synonyms are *Scoparia grandiflora*, *Scoparia ternata*, *Capraria dulcis*, *Gratiola micrantha*. Other common names by which this plant is known include vassourinha, broomweed, bitterbroom, riceweed, sweetbroom, licorice weed, and Omiemie in Urhobo. *Scoparia dulcis* is an erect annual herb with serrated leaves, producing white flowers and measuring up to a half meter in height when fully grown<sup>1</sup>. This plant is found in abundance in South America and the Amazon rainforest and is widely distributed in Nigeria and many tropical countries in the world.

In every country where *Scoparia dulcis* is found, it holds a long history of use by indigenous peoples and herbalists. Among the Igede people of Benue State, a North-central state in Nigeria, the leaves are used as a tonic, also used in the treatment of fever and toothache<sup>2</sup>. The aerial parts, the leaf and the root of this plant have been traditionally used in herbal medicine for their analgesic, hypoglycaemic, antibacterial, antifungal, anti-herpetic, anti-inflammatory, antiseptic, antispasmodic, antiviral, cytotoxic, emmenagogue, emollient, febrifuge, and hypotensive effects<sup>3,4</sup>. It is sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes<sup>5,6</sup>.

A number of different principles such as Scoparic acid A, Scoparic acid B, Scopadulcic acid A and B, Scopadulciol, and Scopadulin<sup>7</sup> have been shown to contribute to the observed medicinal effects of the plant<sup>8</sup>. Documented phytochemical constituents of *Scoparia dulcis* are 3'-4'-5'-5'-7-8-hexahydroxy flavone, adrenaline and noradrenaline e.t.c.<sup>9, 10, 11</sup>.

Extract of *Scoparia dulcis* has varying effects on blood pressure. In one study, an entire plant ethanol extract given intravenously (I.V) to dogs and rats at 0.1-0.5mg/kg increased blood pressure. The Chloroform extract, however, had a hypotensive effect.<sup>12</sup> An entire plant water extract in Brazil had hypertensive effect in rats when given intravenously at a dose of 0.1mg/kg<sup>13</sup>.

Freira *et al* in 1996 investigated the sympathomimetic activity of ethanol extract of *Scoparia dulcis* in rats. The result showed that administration of 0.5-2 mg/kg I.V. of the extracts to anaesthetized rats produced dose related hypertension blocked by the alpha-adrenoceptor antagonist prazosin (1mg/kg). Partition of the extract in chloroform–water yielded an aqueous phase 20 times more potent than the extract. This produced hypertension in either reserpine treated or pithed rats. In untreated and reserpine treated rat, the same fraction (1-3x 10<sup>3</sup>µg) produced concentration dependent contraction of the vas deferens musculature parallel to those obtained with noradrenaline (10<sup>-8</sup>-10<sup>-4</sup>). Prazosin, (10<sup>-7</sup>) reduced the maximal contractile effect of the aqueous fraction, and shifted the concentration response curve for adrenaline to the right. The aqueous fraction (25-50µg/ml) increased the inotropism of electrically driven left atria of rat, the effect being blocked by propranolol (0.4µg/ml).<sup>11</sup>

Despite the wide usage of *Scoparia dulcis* as a medicinal plant especially in the treatment of hypertension, scientific research has not been able to substantiate this. This work intends to study the Cardiovascular and sympathomimetic effects of this widely used medicinal plant.

## MATERIAL AND METHODS

### Collection and identification of specimen

The plant was identified in the Department of plant Science University of Port-Harcourt. The leaves of the plants were collected from University of Port-Harcourt and other local gardens in Port-Harcourt. The voucher specimen was deposited in the University of Port-Harcourt Herbarium.

### Extraction process

The leaves were washed with water, sundried to constant weight and blended with Sonic® blender into a fine powder form weighing a total of 600g.

The aqueous extract of *Scoparia dulcis* was prepared by soaking 300g of dried powdered samples in 1000mls of distilled water boiled to about 50°C for 24 hours. The solution was filtered using

Whatman filter paper N0.42 (125mm). The filtrate was evaporated to dryness using a rotary evaporator. The water extract weighed about 8.67gm.

The Methanol extract was prepared by soaking 300g of dried powdered sample in 5 litres of 50% methanol for about 12 hours. The solution containing the active ingredient was filtered using Whatman filter paper N0.42 (125mm). Continuous extraction was done for 24 hours. The filtrate was evaporated to dryness using a rotary evaporator. Methanol extract weighing about 17gm was gotten. Both extracts were stored in sterile containers and preserved in the freezer throughout the course of the study.

#### **Phytochemical screening**

Phytochemical test was carried out on the aqueous and Methanol extracts of the powdered specimens using standard procedures to identify the constituents as described by Sofowara (<sup>14</sup>1993), Trease and Evans (<sup>15</sup>1989) and Harbourne [<sup>16</sup>].

#### **Determination of the LD<sub>50</sub> Of the methanolic and water extracts of *Scoparia dulcis***

In this phase of the study, a pilot study was carried out to determine the LD<sub>100</sub> for the aqueous and the methanol extracts, via the intraperitoneal route, as described by Aliu [<sup>17</sup>]. Then, 30 rats were divided into 6 groups of 5 animals each, and 100mg/kg, 200mg/kg, 300mg/kg, 450mg/kg and 600mg/kg of the water extract was given intraperitoneally to rats in groups 1-5 respectively. The sixth group which was the control group was injected with 0.5ml of normal saline. The acute toxicity test was also carried out using the intraperitoneal route for the methanol extract. Doses of 50mg/kg, 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg were administered to rats in groups 1 – 5 respectively. The sixth group was injected with 0.5ml of normal saline and used as the control. The number of death in each group was noted. The LD<sub>50</sub> was calculated using the Arithmetic method of Karber (as adapted by Aliu) for both extracts.

#### **Measurement of blood pressure and heart rate**

Fifteen male rats weighing between 245-375g obtained from the animal house in the Department of Pharmacology, University of Benin

were used for the study on the hypertensive and sympathomimetic effects.

The Ugo Basile double channel pen recorder was calibrated with a clinical sphygmomanometer and set at a recording speed of 10mm per second. Prior to the experiment, the recorder was left to equilibrate for about 1 hour before each series of experiment. Air bubbles in the transducer/sensor were removed to ensure optimal signal transmission. Anesthesia was induced in rats by using 1.75g/kg of Urethane, injected intraperitoneally. Following anaesthesia the rats were placed on the dissecting table and secured with adhesive tapes. The hair around the neck region was removed as much as possible and a horizontal skin incision made and dissected until the trachea, common carotid artery and the jugular vein were identified. The trachea was intubated using a 0.15mm polyethylene tube and secured with thread. The jugular vein and common carotid artery were cannulated using plastic cannulae which were tied securely with thread. When cannulating the jugular vein was difficult, the caudal vein was cannulated. The jugular or caudal vein cannula was connected to a 2-way tap, through which all drugs and extracts were administered, while the common carotid cannula via a 2-way tap, was connected to the transducer/sensor of the Ugo Basile double channel pen recorder. Once the above procedure was completed the 2-way tap connected to the transducer was opened and the blood pressure and heart rate were transmitted directly to the transducer and recorded.

Doses of 20, 40, 80 and 160mg/kg of both extracts were given to the animals. 0.5µg/kg and 1.5µg/kg of Adrenaline and acetylcholine were given as hypertensive and hypotensive agents respectively followed by the 20, 40, 80, and 160mg/kg of the extracts. The blood pressure and heart rate recording were allowed to return to baseline or stabilize before the next dose of a drug or extract was given. Four series of the experiment were conducted for both extracts and the recordings of the blood pressure (BP) and heart rate (HR) were made. The mean arterial pressure (MAP) was calculated from the systolic and diastolic BP from the formula shown below-

**MAP** = (Systolic BP-Diastolic BP/3) + Diastolic.

The BP, HR and MAP were tabulated and the mean  $\pm$  SEM calculated. The statistical analysis was done using INSTAT software and analysis of variation using the Kruskal-Wallis, Dunn's and Tukey-Kramer multiple comparisons test and Friedman nonparametric repeated measures test were applied to the data.

### RESULTS

The phytochemical study carried out on the leaves of *Scoparia dulcis* plant reveals the presence of Tannins, Phlobatannins, Steroids, Terpenoids, Flavonoids and Cardiac glycosides, however it does not contain Saponins.

### LD<sub>50</sub> using arithmetic method of karber

In the pilot study, the LD<sub>100</sub> for the Water and Ethanol extracts was 650 and 450 mg respectively.

### Water extract

Within 24 hours of administration of the water extract of *Scoparia dulcis* at the different dose levels to the five different groups, 2 rats each died in the groups that received 600mg/kg and 450mg/kg (groups 1 and 2 respectively), with one death in the group that had 300mg/kg (group 3). There was no death in the groups that had 100 and 200mg/kg (groups 4 and 5 respectively). No rat died in the control group that had sterile normal saline.

**Table 1: LD<sub>50</sub> of water extract of scoparia dulcis using arithmetic method of karber**

Group	Dose Mg/Kg	Dose Difference	No. Dead	Mean Dead	Dose Difference X Mean Dead
1.	100	0	0	-	-
2.	200	100	0	-	-
3.	300	100	1	0.5	50
4.	450	150	2	1.5	225
5.	600	150	2	2	300
					575

LD<sub>50</sub> = LD<sub>100</sub> - Dose difference x mean dead/total no. of organisms per group

LD<sub>50</sub> = 650 - 575/5 = 535mg/Kg = 0.535g/kg

**Table 2: LD<sub>50</sub> of methanol extract of scoparia dulcis using arithmetic method of karber**

Group	Dose Mg/Kg	Dose Difference	No. Dead	Mean Dead	Dose Difference X Mean Dead
1.	50	0	0	-	-
2.	100	50	0	-	-
3.	200	100	0	-	-
4.	300	100	2	1	100
5.	400	100	2	2	200
					300

LD<sub>50</sub> = LD<sub>100</sub> - Dose difference X mean dead/total no. of organisms per group

LD<sub>50</sub> = 450 - 300/5 = 390mg/kg = 0.39g/kg

**Methanol extract**

Within 24 hours of administration of the methanol extract of *Scoparia dulcis* at the different dose levels to the five different groups, 2 rats each died in the groups that received 400mg/kg and 300mg/kg (groups 5 and 4 respectively). There was no death in the groups that had 50, 100 and 200mg/kg (groups 1, 2 and 3 respectively) of the extract. The live rats were observed for two weeks. No rat died in the control group that had sterile normal saline.

**Blood pressure and sympathomimetic effects**

When 10, 15 and 20mg/kg of the extracts were given the blood pressure and heart rate remained at baseline values of 105/85mmHg and 215bpm respectively. At a dose of 20mg/kg there was a slight increase in the BP and HR, while at 40, 80, and 160mmHg there was remarkable increase in the BP, MAP and HR above Baseline values and the results are as presented in Tables 3 and 4. The MAP

**Table 3: Effects of the water extract of the leaves of scoparia dulcis on the bp, hr and map of anaesthetized rats**

Parameters	Mean of all experiments± SEM		
	BP (mmHg)	HR(BPM)	MAP(mmHg)
Baseline (control)	101/85±5.4/7.9	289±32	90±7
Adrenaline(0.5µg/Kg)	116/100 <sup>a</sup> ±7.8/9.2	309 <sup>a</sup> ±30	105 <sup>a</sup> ±9
Acetylcholine(1.5 µg/Kg)	87/77 <sup>a</sup> ±8.3/8.4	203*±43	80 <sup>a</sup> ±8
20mg/kg	100/78 <sup>a</sup> ±4.5/7.9	289 <sup>a</sup> ±30	90 <sup>a</sup> ±7
40mg/kg	133/110*±6.4/7.8	329*±19	106*±11
80mg/kg	155/120*±3.2/4.0	346±21	129*±2
160mg/kg	154/124*±3.8/2.2	342*±14	134*±0.6

\*Values are statistically significant compared to control (p<0.05)

<sup>a</sup>Values are not Statistically significant compared to control (p>0.05)

KEY: WT= weight, BP= Blood Pressure, HR= Heart Rate, MAP= Mean Arterial Pressure, MMHG=Millimeter Mercury, SEM= standard error of mean

**Table 4: Effects of the methanol extract of the leaves of scoparia dulcis on the BP, HR and map of anaesthetized rats**

Parameters	Mean of all experiments± SEM		
	BP (mmHg)	HR(BPM)	MAP(mmHg)
Baseline(control)	101/85±5.4/7.9	289±32	90±7
Adrenaline(0.5µg/Kg)	116/100 <sup>a</sup> ±7.8/9.2	309 <sup>a</sup> ±30	105 <sup>a</sup> ±9
Acetylcholine(1.5µg/Kg)	87/77 <sup>a</sup> ±8.3/8.4	203*±43	80 <sup>a</sup> ±8
20mg/kg	100/78 <sup>a</sup> ±4.5/7.9	289 <sup>a</sup> ±30	90 <sup>a</sup> ±7
40mg/kg	133/110*±6.4/7.8	329*±19	106*±11
80mg/kg	155/120*±3.2/4.0	346±21	129*±2
160mg/kg	154/124*±3.8/2.2	342*±14	134*±0.6

\*Values are statistically significant (p>0.05) compared to control

<sup>a</sup> Values are statistically not significant (p>0.05) compared to control

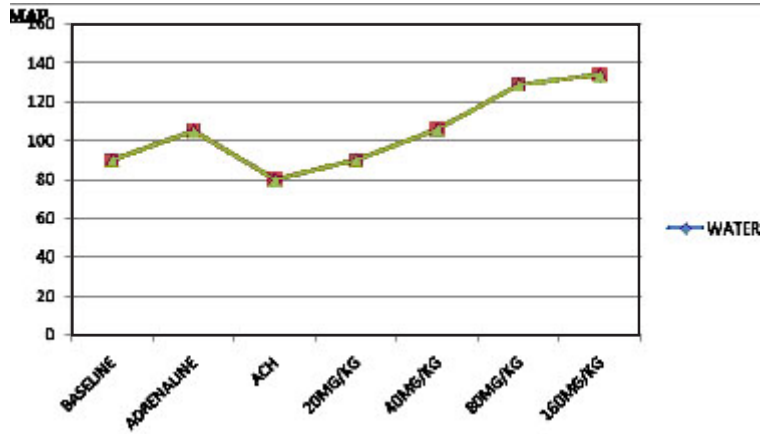


Fig. 1: Dose-response (MAP mmhg) curve of the Water extract

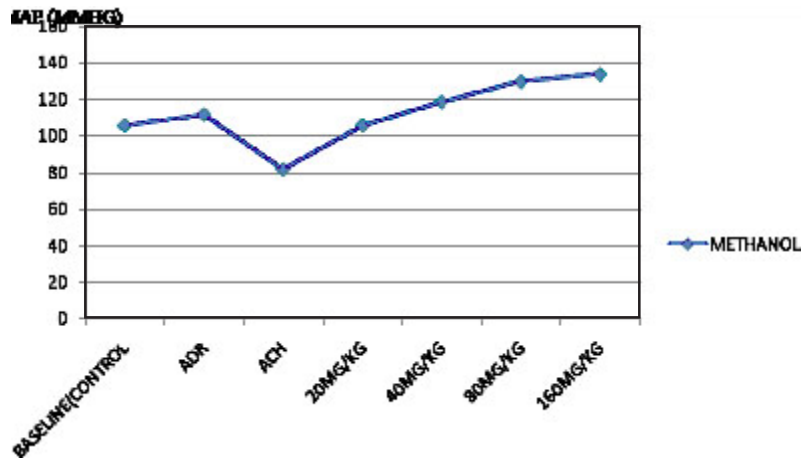


Fig. 2: Dose-response (MAP mmhg) curve of the methanol extract

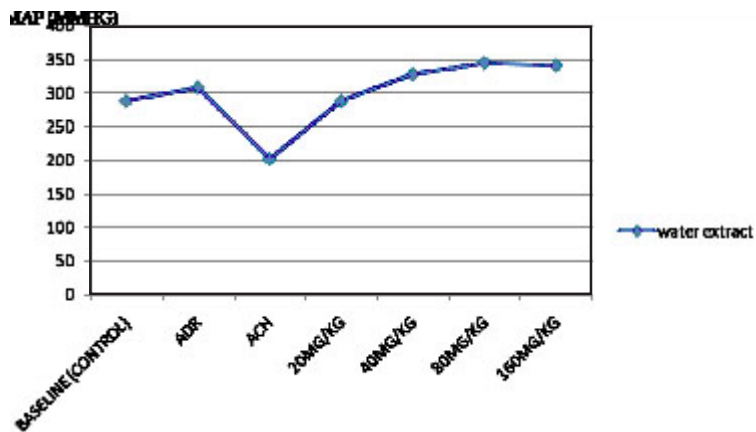


Fig. 3: Dose-response (HR BPM) curve of the Water extract

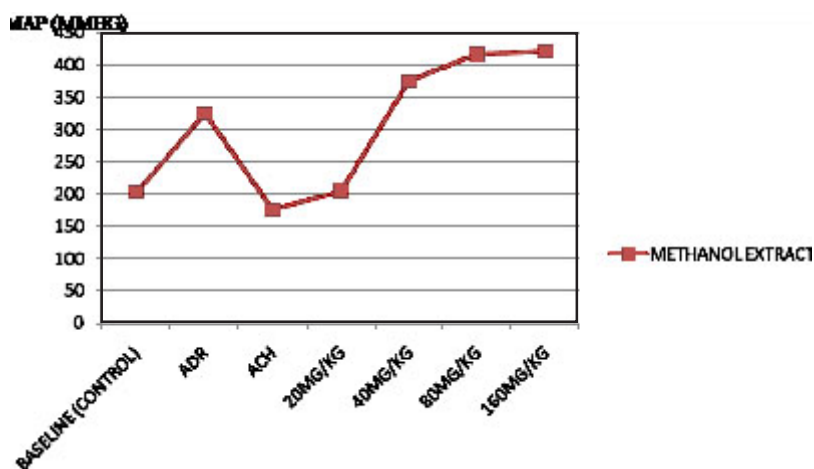


Fig. 4: Dose-response (HR BPM) curve of the Methanol extract

and mean HR dose-response curves are also presented in Figures 1-4.

## DISCUSSION

The phytochemical screening of the chemical constituents of *Scoparia dulcis* revealed that the leaves of the plant contains Tannins, Phlobatannins, Steroids, Terpenoids, Flavonoids and Cardiac glycosides, however it does not contain Saponins. The absence of saponins in *S. dulcis* is in consonance with the result reported by Edeoga *et al*<sup>[11]</sup> but contrasts with that of Gill who noted that saponin is one of the active phytochemical constituents of this plant.<sup>[18]</sup> The difference in the phytochemistry could be explained by differences in the geographical location and hence climatic conditions in the areas they were collected as the phytochemical constituents of same plant varies depending on the location.

The water and methanol extracts of *Scoparia dulcis* produced significant ( $p < 0.05$ , using Kruskal-Wallis ordinary ANOVA) dose dependent increase in the blood pressure (BP), mean arterial pressure (MAP), and heart rate (BPM) of the experimental rats from doses between 40- 160mg/kg. At 20mg/kg there was no increase in the BP, MAP and HR above baseline values. Applying the Tukey-Kramer multiple comparisons test reveals that there are no significant difference ( $p > 0.05$ ) between the BP of the baseline versus adrenaline,

acetylcholine and 20mg/kg groups; the adrenaline versus the 20, 40mg/kg groups; the 40mg/kg versus the 80, 160mg/kg and 80 versus 160mg/kg. However there were significant differences ( $p < 0.05$ ) between baseline values versus 40, 80, 160mg/kg; the adrenaline versus acetylcholine group; adrenaline versus 80, 160mg/kg group; acetylcholine versus 40, 80, 160mg/kg groups and the 20mg/kg versus 40, 80, 160mg/kg groups. This implies that at 40mg/kg and higher doses, there is a significant increase in the BP from baseline values while 40mg/kg did not produce significant difference in the BP compared to 0.5µg/kg of adrenaline (Tables 3 and 4).

This dose dependent hypertension produced by the water and methanol extracts of *Scoparia dulcis* is in consonance with the report of Freire *et al*.<sup>[11]</sup> Also there were extremely significant ( $p < 0.05$ ) increases in the MAP of anaesthetized rats both with the Kruskal -Wallis and the Friedman nonparametric repeated measures test. Using the Dunn's multiple comparison test there was extremely significant difference ( $p < 0.01$ ) between the baseline MAP values and that of acetylcholine, 80 and 160mg/kg groups while the adrenaline, 20 and 40mg/kg groups showed no significant difference ( $p > 0.05$ ). This are illustrated graphically in figures 27 and 28.

There were extremely significant increases in the HR ( $p < 0.05$ ) of rats given 40, 80 and 160mg/

kg of both extracts using repeated measures ANOVA, ordinary ANOVA, Friedman nonparametric and Dunn's test. Comparing the MAP of the rats given the methanol and water extracts shows that the mean MAP of the rats given the methanol extracts was higher (though not statistically significant  $p > 0.05$ ) than that of those that received the water extract. This suggests that the methanol extract is more potent than the water extract as observed in the acute toxicity study where the LD50 of the methanol extract was lower than that of the water extract.

Though *Scoparia dulcis* plant is used traditionally in the treatment of hypertension, scientific research has not demonstrated this property but rather has shown this plant to cause increase in the BP, MAP and HR as shown by the above results and that of the sympathomimetic effect reported by Freire *et al* [11]. The plant has been shown to contain catecholamines as one of its phytochemicals and it's the adrenaline and

noradrenaline that accounts for the hypertensive and inotropic effects after parenteral administration [11]. Catecholamines are subject to inactivation by Catechol - O - Methyl transferase enzymes found in the gut and liver, hence oral administration of the extract, as it is usually given by traditional medical healers may not be associated with hypertensive effects.

Paradoxically, this plant is used in various traditional medicine systems in the treatment of hypertension [11, 19]. In the traditional medicine system there are no scientific means of diagnosis of hypertension such as measurement of BP, other than perceived symptoms such as headache, insomnia and malaise. Extracts of *Scoparia dulcis* plant are reported to have analgesic, anti-inflammatory and sedative effects [20], and hence it is very likely that it is improvement in the above symptoms in hypertensive patients that is erroneously believed to be the BP lowering effects among traditional medical healers.

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