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Mohua Sarker
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Towkir Ahmed
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Md. Nazmul Islam
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Bithi Biswas
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Shrabanti Dev
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Bishwajit Bokshi
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Asish Kumar Das
Pharmacy Discipline, Khulna
University, Khulna-9208,
Bangladesh

Nripendra Nath Biswas
Professor, Pharmacy Discipline,
Khulna University, Khulna-
9208, Bangladesh

Corresponding Author:
Nripendra Nath Biswas
Professor, Pharmacy Discipline,
Khulna University, Khulna-
9208, Bangladesh

Diuretic and laxative activities of *Kandelia candel* and *Brownlowia tersa* in experimental mice

Mohua Sarker, Towkir Ahmed, Md. Nazmul Islam, Bithi Biswas, Shrabanti Dev, Bishwajit Bokshi, Asish Kumar Das and Nripendra Nath Biswas

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Abstract

Different fractionated extracts (in ethanol, *n*-hexane, chloroform, ethyl acetate and water) of two mangrove plants *Kandelia candel* and *Brownlowia tersa* have been investigated here for diuretic and laxative bioactivity. In diuretic test, frusemide (5 mg/kbw) was used as standard and the parameters like volume of urine up to 6th hour, pH, density, conductivity and Na⁺, K⁺, Cl⁻ contents of collected urine were measured. Ethyl acetate fraction of *Kandelia candel* showed significant diuretic activity ($p < 0.001$) at 200 mg/kbw and 400 mg/kbw dose compared with control. For aerial parts of *Brownlowia tersa*, chloroform fraction demonstrated better diuretic activity. Both the active fractions were found to work as loop diuretics. In laxative activity test, bisacodyl (10 mg/kbw) was used as standard and a significant percent increment of fecal output ($p < 0.0001$) was observed for ethyl acetate fraction of *Kandelia candel* at both the doses. For *Brownlowia tersa*, the ethyl acetate and water fractions demonstrated significant laxative effect. Phytochemical analysis for both the plants extracts exhibited the presence of carbohydrate, reducing sugar, steroids, saponins, glycosides, tannins, alkaloids, terpenoids and flavonoids etc. Further studies may be carried to isolate and characterize the active compound(s) responsible for the claimed activity.

Keywords: *Kandelia candel*, *Brownlowia tersa*, diuretic, laxative, fractions, phytochemical

Introduction

The plants which are used in herbalism and have therapeutic potentials are called 'medicinal plants' [1]. These plants are rich in different types of ingredients which can be utilized in drug design and synthesis. Sunder bans is situated in the southwestern part of Bangladesh and is considered as the largest single block of tidal halophytic mangrove forest in the world [2]. A wide variety of mangrove plants are available here having traditional medicinal value. The plants may be important sources of novel drug compounds for mankind. But many of them are still unexplored scientifically especially for unveiling the chemical nature or bioactivity. It provides a safe habitat for many rare medicinal plant species, which can provide. Diuretics are the drugs that increase the rate of urination. It plays important roles in the management of edema, hypertension and weight loss. This function is mainly due to an increase in net negative water and solute balance [3]. Diuretics are used to treat heart failure, liver cirrhosis, hypertension, influenza, water poisoning, and certain kidney diseases [4]. The conventional diuretic drugs sometimes cause electrolyte imbalance and hypotension [5]. Phytoconstituents like steroid, saponins, terpenoids and phenolic compounds show diuretic effects [6]. Laxatives soften the stool consistency, increase bowel movements and thereby facilitate bowel evacuation. They are prescribed for treating or preventing constipation. Constipation is chronic gastro-intestinal disorders which cause discomfort and affect the normal life [7]. To treat this situation laxatives are used. Laxatives either accelerate fecal passage or decrease fecal consistency facilitating the fecal evacuation mechanism. Phytochemicals like terpenoids, sterols, flavonoids and alkaloids can be responsible for the laxative effects.

Kandelia candel (L.) is a mangrove species in the family Rhizophoraceae. Locally it is known as Vathkathi. It grows as a shrub or a small tree up to 6 meters tall. It is found locally on the banks of tidal rivers among other mangrove species in India, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, and Indonesia [8]. Traditionally this plant is used as a folk medicine against rheumatoid arthritis and as anthelmintic [9, 10]. It has some therapeutic activities, such as anti-bacterial [11], anti-fungal activity [12], antiulcer effects of stem [13], anthelmintic efficacy of

leaves [14], antidiabetic activity [15], antifouling activity from leaves [16], anti-inflammatory [10] and antioxidant activities [17]. *Brownlowia tersa* (Family: Malvaceae) is a climbing shrub, usually growing up to 1.5 -5 m tall, smaller branches are covered with a dense layer flat scale [18]. This species is found along tidal creeks, canals and shallow channels. This is a brackish water species that grows to two meters, and rarely to five meters. *Brownlowia tersa* has long been used as a traditional folk remedy for dysentery, wounds and boils. Roots have been found to possess significant antibacterial ability. Leaves possess anti-inflammatory, antioxidant, and analgesic activities [18]. It has also antibacterial and antidiarrheal activity [19,20].

Upon literature survey it was found that although some research works have been performed on the *Kandelia candel* and *Brownlowia tersa*, no work has been done on the diuretic and laxative activity of these plants. So, the aim of this project work was to investigate the laxative and diuretic activity and phytochemical screening of fractionated extracts.

Materials and Methods

Collection and Identification of Plants

The targeted parts i.e. leaves of *Kandelia candel* and aerial parts of *Brownlowia tersa* were collected from Koromjol, Sunder bans during month of march and was identified by experts at Bangladesh National Herbarium, Mirpur, Dhaka, and a voucher specimen was submitted (voucher no. 47555 DACB and 43139 DACB respectively) for future reference.

Drying, Grinding and Extraction

The collected samples were separated from undesirable materials or plant parts and dried under shade. Then these were ground into a coarse powder with the help of a grinder and kept macerated in 96% ethanol for 14 days with occasional shaking and stirring [21]. The two mixtures were then underwent a coarse filtration separately by through clean cloth. Then it was filtered through filter paper and the filtrates thus obtained (ethanol extract) were concentrated using a rotary evaporator at temp of 45 °C and stored in two different containers. It rendered concentrate of greenish black color paste types. The concentrates were designated as ethanolic crude extract of *Kandelia candel* and *Brownlowia tersa* respectively. The yields were 4.86 % and 3.44% respectively.

Partitioning in different polar and non-polar solvents

Previously obtained two crude ethanolic extracts were then partitioned individually in four different solvents with varying polarity i.e. water (10.2) > ethyl acetate (4.4) > chloroform (4.1) > n-hexane (0.1). In brief, at first 6 gm of ethanolic crude extract of *Kandelia candel* was dissolved in 200 mL distilled water and shook well to ensure optimum mixing in a separating funnel. 200 mL of n-hexane solvent was added to the funnel and shaken together vigorously. The funnel was inverted and the tap was carefully opened to release excess vapor pressure. The separating funnel was kept on a stand for a while to allow for the complete separation of the phases. The non-polar compounds are more soluble in n-hexane than in water. So, they would be dissolved in n-hexane. The top and the bottom tap were then opened and the two phases were collected in two different glass beakers. Then the water part was again transferred to same separating funnel and ethyl acetate was added and separated the two phases following the procedure mentioned above. The process was repeated using chloroform as well. After that the organic fractions were evaporated individually using rotary evaporator but the water

fraction was dried using freeze dryer. The same procedure was repeated using *Brownlowia tersa* ethanolic extract. The yield was 16-31% for *K. candel* and 23-27% for *B. tersa* (Table 1).

Table 1: Yield of different fractions after partitioning

Fractions	%Yield (<i>Kandelia candel</i>)	%Yield (<i>Brownlowia tersa</i>)
n-hexane	31	23
Chloroform	25	27
Ethyl acetate	28	25
Water	16	25

Phytochemical screening

The preliminary phytochemical tests indicated the presence or absence of different chemical groups in test extracts. Different tests on crude extract as well as four fractions of each plant were performed following the methods developed by Sarkar and co-workers; Trease and co-workers [22, 23].

Diuretic Test Procedure

Diuretic test was carried out following an established protocol adopted by Golla and coworkers Golla, 2014 with minor modification [24]. Twenty-four mice of both sexes (27-30 g) were randomly divided into four groups of six each. Each group was fasted and deprived of water for 18 h prior to the experiment. The first group was provided with normal saline (6x2 mL) as control. The second group received standard drug frusemide (5 mg/kbw) in normal saline as positive control. The third and fourth group was provided with test plant extracts at a dose of 200 mg/kbw and 400 mg/kbw. Each time the total injected volume was 6x2 mL (Vi) for each group. Immediately after dosing, the animals were placed in metabolic cages. Food and water were withheld, and the cages were maintained at (25.0±0.5) °C throughout the experiment (6 hours). All the experiments were conducted on an isolated and noiseless condition and maintain the ethical guidelines of animal handling set out by the Animal ethics committee of Life Science School, Khulna University (Reference: KUAEC-2018/03/04). The urinary output (Vo) was collected every hour and the urine was then stored in freeze (0-4 °C) for further electrolytes analysis. The urinary excretion was calculated as ratio of total urinary output (Vo) by total liquid administered (Vi). The diuretic action was calculated as the ratio of urinary excretion in test group (U_{ET}) and that of control group (U_{EC}). Diuretic activity was calculated as the ratio of diuretic action in test group (D_{AT}) and that of standard group (A_{AF}). The electrolyte content (Na⁺, K⁺) of collected urine sample was measured using a flame photometer and Cl⁻ was measured titrimetrically. pH, conductivity and density were also determined using appropriate equipment and methods. The experiments were duplicated every time.

Analysis of Urine Sample for different cations and anions

The collected urine sample was analyzed for different electrolytes, pH, conductivity density using appropriate apparatus and methods. Na⁺ and K⁺ concentrations were measured using a JENWAY Crop Model PFP7 flame photometer whereas the Cl⁻ concentration was estimated by titration with silver nitrate solution. Conductivity was determined using D-50 Series Handheld Water Quality Meters, HORIBA scientific®. Urine pH was determined directly by using pH meter, Model No. HI 2211, Henna instrument®, China. Density estimation was made by weighing with a four-digit analytical balance on urine volume measured with a micropipette [25,26].

Calculation of Diuretic Indices

The calculated diuretic indices are used to predict the mechanistic behavior of any diuretic agent. It is done by counting the sodium, potassium and chloride ion excretion and derivation of further equations.

$$\text{Diuretic index} = \frac{\text{Urine volume of test group}}{\text{Urine volume of control group}}$$

Calculation of different Saluretic Index

As there was an increase in urine volume in the extract-treated mice in a dose dependent manner, we became interested in investigating the effect of the extract on the concentration of electrolytes in the urine with a view to identifying the mechanism of its diuretic activity. In addition, Saluretic index, Na^+ / K^+ index (Natriuretic Index), K^+ / Na^+ index (Kaliuretic Index) and Carbonic Anhydrase Inhibition (CAI) index are important in describing the mechanism of action of diuretic drugs. These parameters were calculated by the following equations

Here,

$$\text{Saluretic index} = \frac{\text{Urinary excretion of electrolytes of test group}}{\text{Urinary excretion of electrolytes of control group}}$$

$$\text{Natriuretic index} = \frac{\text{Urinary excretion of Sodium ion}}{\text{Urinary excretion of Potassium ion}}$$

$$\text{Kaliuretic index} = \frac{\text{Urinary excretion of Potassium ion}}{\text{Urinary excretion of Sodium ion}}$$

$$\text{CAI index} = \frac{\text{Urinary excretion of Chloride ion}}{\text{Sum of Urinary excretion of Sodium + Potassium ion}}$$

Laxative Test Procedure

At first 24 mice of 27 to 33 gm (age 6-7 weeks) weight were taken and kept fasting for 12 hours period before starting the experiment Golla, 2014 [24]. Water was provided at that time. After 12 hours the mice were weighted and were divided in four groups. The first group was administered with normal saline (6x2 mL) as control. The second group was administered with bisacodyl in saline (10 mg/kg), the third and fourth groups received test plant extracts at different doses (200 and 400 mg/kbw). Then they were housed in a cage lined with clean and white filter paper and observed for 16 hours. No food and water were given at that time. After 16 hours the feces were collected and quantified by measuring the feces weight and consistency was felt. The experiments were duplicated every time.

The percent of increment of faeces weight for every sample was calculated using the following equation,

% of increment of faeces

$$= \frac{\text{Faeces output in test group} - \text{Faeces output in control group}}{\text{Faeces output in control group}} \times 100$$

Results and Discussion

The phytochemical tests shown the presence of several bioactive constituents such as tannins, flavonoids, steroids etc. and the results are summarized in Table 2.

Table 2: Summary results of phytochemical tests of *Kandelia candel* and *Brownlowia tersa* fractionated extracts

Phytochemical groups	Results (<i>Kandelia candel</i>)					Results (<i>Brownlowia tersa</i>)				
	EtOH Extract	Fr. n-hex	Fr. CHCl ₃	Fr. EtOAc	Fr. H ₂ O	EtOH Extract	Fr. n-hex	Fr. CHCl ₃	Fr. EtOAc	Fr. H ₂ O
Reducing sugar	+	+	+	+	-	+	+	+	+	+
Tannins	+	-	+	+	-	+	+	+	+	+
Flavonoids	+	+	+	+	-	+	+	+	+	+
Saponins	-	-	-	-	-	+	+	+	+	+
Alkaloids	+	+	+	+	-	+	+	+	+	+
Terpenoids	+	+	+	+	-	+	+	+	+	-
Carbohydrates	+	-	+	+	+	+	+	+	+	+
Glycosides	+	-	-	-	+	+	-	-	+	+
Steroids	+	+	+	+	+	+	+	+	+	+

[‘+’ indicates presence and ‘-’ indicates absence]

These types of constituents might be responsible for different medicinal activities. So, further studies were carried out for assessing their diuretic & laxative potentials.

Among four fractions of each plant, the ethyl acetate (*Kandelia candel*) and chloroform fractions (*Brownlowia tersa*) showed better diuretic effect on experimental mice. It may be caused by the presence of phytoconstituents like terpenoids, flavonoids, saponins, phenolics, carbohydrates, steroids etc. and their existence was evident by preliminary phytochemical tests on respective fractionated extracts. This finding may be correlated with the results of some literally available earlier reports. For example, plant constituents like flavonoids [27], glycoside [28], tannins [4], steroids [29] etc. are claimed to be responsible for diuretic activity. And these constituents might cause diuretic effect by stimulating regional blood flow or initial vasodilation by producing inhibition of tubular reabsorption of water and electrolytes, or by increasing renal circulation [30]. Diuresis occurs by two

phenomena including net increase in urine volume and elevated excretion of electrolytes in the urine [31]. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The thiazide diuretics inhibit Na^+/Cl^- symporter (co-transporter system) in the distal convoluted tubule by competing for the Cl^- binding site and increasing the excretion of Na^+ and Cl^- while the loop diuretic reference drug frusemide, increases the urinary excretion of Na^+ by inhibiting $\text{Na}^+/\text{K}^+/\text{Cl}^-$ symporter in the thick ascending limb of loop of Henle [31]. Therefore, in this study both urine volume and electrolyte concentrations were measured to investigate the diuretic mechanism of *Brownlowia tersa*. In the present study, 5 mg/kg dose of frusemide showed significant diuresis in mice over a period of 6h. Our data suggest that the most polar water fraction and the lowest polar n-hexane fraction decrease urine excretion, where the medium polar chloroform and ethyl acetate produced a very high urinary excretion (Table 3).

Table 3: Urinary output and derived calculations after 6 hours of treatment of mice (* $p < 0.005$, ** $p < 0.001$, *** $p < 0.0001$, compared with control)

Treatment Group/Dose (mg/kbw)		Cumulative urine volume (Vo mL) after 6h, Avg \pm SD	Urinary excretion {(Vo/Vi) X 100}	Diuretic action, D _{AT} =(U _{ET} / U _{EC})	Diuretic activity (D _{AT} / D _{AF})
Control (Normal Saline)		2.60 \pm 0.15	21.67	-	-
Standard (Frusemide)		10.15 \pm 0.49***	84.58	3.90	-
<i>Kandelia candel</i>	EtOH (200)	4.57 \pm 0.11*	38.08	1.76	0.45
	EtOH (400)	7.50 \pm 0.05**	62.50	2.88	0.74
	Fr. n-hex (200)	3.70 \pm 0.23*	30.83	1.42	0.36
	Fr. n-hex (400)	4.90 \pm 0.19*	40.83	1.88	0.48
	Fr. CHCl ₃ (200)	4.30 \pm 1.20*	35.83	1.65	0.42
	Fr. CHCl ₃ (400)	5.70 \pm 2.30**	47.50	2.19	0.56
	Fr. EtOAc (200)	4.60 \pm 1.40*	38.33	1.77	0.45
	Fr. EtOAc (400)	7.80 \pm 1.90***	65.00	2.30	0.59
	Fr. H ₂ O (200)	3.80 \pm 0.50	31.67	1.46	0.37
Fr. H ₂ O (400)	4.40 \pm 1.20*	36.67	1.69	0.43	
<i>Brownlowia tersa</i>	EtOH (200)	7.95 \pm 0.21***	66.25	3.06	0.78
	EtOH (400)	8.35 \pm 0.21***	69.58	3.21	0.82
	Fr. n-hex (200)	4.50 \pm 0.14*	37.50	1.73	0.44
	Fr. n-hex (400)	5.65 \pm 0.21*	47.08	2.17	0.56
	Fr. CHCl ₃ (200)	7.20 \pm 0.28**	60.00	2.77	0.71
	Fr. CHCl ₃ (400)	7.95 \pm 0.07***	66.25	3.06	0.78
	Fr. EtOAc (200)	5.70 \pm 0.14*	47.50	2.19	0.56
	Fr. EtOAc (400)	6.35 \pm 0.07*	52.92	2.44	0.63
	Fr. H ₂ O (200)	4.55 \pm 0.35*	37.92	1.75	0.45
Fr. H ₂ O (400)	4.60 \pm 0.57*	38.33	1.77	0.45	

The control of plasma Na⁺ is important in the regulation of blood volume and pressure; the control of plasma K⁺ is required to maintain proper function of cardiac and skeletal muscles [25]. The regulation of Na⁺, K⁺ balance is also intimately related to renal control of acid-base balance. There are two factors on which urine volume depends. One is the rate of glomerular filtration and other is the degree of tubular reabsorption. The observed effect may be attributed to the mechanism like increasing the renal blood flow and the attendant increase in glomerular filtration rate. It is also possible that it has inhibitory effect on antidiuretic hormone (ADH) secretion as inhibition of ADH causes polyuria. Another possible mechanism involved may be stimulation of

release of endogenous natriuretic peptides, which promotes sodium and water secretion [32].

The magnitude of Na⁺, K⁺ and Cl⁻ was determined as a parameter for saluretic activity. The ratio of Na⁺ / K⁺ was calculated as an indicator for natriuretic activity. Values greater than 2.0 indicate a favorable natriuretic effect, while values greater than 10.0 indicate potassium sparing effect [33]. Na⁺/K⁺ ratio (Table 4) for both the active fractions were found to be close to the acceptable value 2 and therefore, it may be possible to claim that the extracts have a good natriuretic activity. With regard to K⁺ excretion almost all doses of the extracts indicate that the extracts are not acting as a potassium sparing agents (None of the value was found greater than 10).

Table 4: Effect of extracts on urinary electrolytes excretion in mice

Treatment Group/Dose (mg/kbw)	Cumulative Concentrations of ions (mEq/L/6h)			Saluretic Index			Na ⁺ /K ⁺	K ⁺ /Na ⁺	CAI {Cl ⁻ /(Na ⁺ +K ⁺)}	
	Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻				
Control (Normal saline)	49.06 \pm 2.69	20.93 \pm 7.72	73.75 \pm 1.25	1.00	1.00	1.00	2.34	0.43	1.05	
Standard Furosemide (5)	154.11 \pm 0.00**	28.65 \pm 0.00	100.00 \pm 2.50	3.14	1.37	1.36	5.38	0.19	0.54	
<i>Kandelia candel</i>	EtOH (200)	119.09 \pm 8.08*	59.51 \pm 6.50**	137.50 \pm 12.50*	2.57	3.48	3.95	2.00	0.50	0.77
	EtOH (400)	148.73 \pm 5.38**	67.22 \pm 7.72**	212.50 \pm 13.50*	3.21	3.94	3.94	2.21	0.45	0.98
	Fr. n-hex (200)	79.66 \pm 2.64	36.37 \pm 7.45	190.00 \pm 12.50**	1.72	2.13	2.13	2.19	0.46	1.64
	Fr. n-hex (400)	58.57 \pm 2.64	59.50 \pm 0.00**	208.50 \pm 21.25***	1.26	3.47	3.88	0.98	1.02	1.77
	Fr. CHCl ₃ (200)	71.74 \pm 0.00	13.23 \pm 0.00	135.00 \pm 32.51**	1.55	0.77	2.51	5.42	0.18	1.59
	Fr. CHCl ₃ (400)	84.93 \pm 2.64**	20.93 \pm 0.00	152.50 \pm 22.50**	1.83	1.23	2.84	4.06	0.25	1.44
	Fr. EtOAc (200)	179.83 \pm 2.62***	51.79 \pm 7.71*	201.25 \pm 31.25***	3.88	3.03	3.74	3.47	0.29	0.85
	Fr. EtOAc (400)	200.92 \pm 2.64***	59.50 \pm 0.00**	228.75 \pm 21.23***	4.33	3.46	4.26	3.38	0.30	0.87
	Fr. H ₂ O (200)	105.98 \pm 2.68**	44.08 \pm 0.00*	176.25 \pm 27.24**	2.29	2.58	3.28	2.40	0.42	1.17
Fr. H ₂ O (400)	121.83 \pm 2.64**	51.79 \pm 7.71**	193.75 \pm 15.25**	2.63	3.04	3.61	2.35	0.43	1.12	
<i>Brownlowia tersa</i>	EtOH (200)	116.40 \pm 5.39*	36.36 \pm 7.71	105.00 \pm 5.00*	2.37	1.74	1.42	1.36	0.73	0.35
	EtOH (400)	226.83 \pm 8.08***	74.93 \pm 0.00*	201.25 \pm 1.25***	4.62	3.58	2.73	1.29	0.77	0.33
	Fr. n-hex (200)	59.84 \pm 2.70	51.79 \pm 7.71	70.00 \pm 2.5	1.21	2.47	0.95	1.16	0.87	0.63
	Fr. n-hex (400)	100.24 \pm 0.00*	59.50 \pm 0.00*	91.25 \pm 1.25*	2.04	2.84	1.24	1.68	0.59	0.57
	Fr. CHCl ₃ (200)	199.90 \pm 8.08**	74.93 \pm 0.00*	176.25 \pm 1.24***	4.07	3.58	2.39	2.67	0.37	0.64
	Fr. CHCl ₃ (400)	207.98 \pm 0.00***	82.65 \pm 7.71*	205.00 \pm 2.5***	4.24	3.95	2.78	2.52	0.39	0.71
	Fr. EtOAc (200)	51.76 \pm 5.39	51.78 \pm 7.72	115.00 \pm 2.3**	1.06	2.47	1.56	0.99	1.00	1.11
	Fr. EtOAc (400)	84.08 \pm 0.00*	90.36 \pm 0.00*	166.25 \pm 3.75**	1.71	4.32	2.25	0.93	1.07	0.95
	Fr. H ₂ O (200)	54.45 \pm 2.70	36.36 \pm 7.71	58.75 \pm 3.75	1.11	1.74	0.80	1.49	0.67	0.65
Fr. H ₂ O (400)	92.16 \pm 8.08*	28.65 \pm 0.00	79.10 \pm 4.10	1.88	1.34	1.07	3.22	0.31	0.65	

Values are expressed as Mean \pm SD (n =2); * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; compared with the control group (Student's unpaired t –test)

Observing the experiment results, it can be explained that the extracts exert their diuretic effect by inhibiting tubular reabsorption of water with excretion of electrolytes. The $Cl^- / (Na^+ + K)$ ratio also called Carbonic anhydrase (CA) index was calculated. The CA index is an indicator of CA enzyme inhibition [34]. The lower CA index indicates higher carbonic anhydrase inhibition [35]. The CA index and pH value (slightly

alkaline) of urine for active fractions indicates that one possible mechanism of diuretic activity may be carbonic anhydrase inhibition (low CA value than that of control group). The conductivity and density were found to be more due to higher electrolyte excretion through urine presented in the Table 5.

Table 5: Effect of extracts on urinary volume, diuretic index, conductivity, pH and density of excreted urine

Treatment Group/Dose (mg/kbw)	Urine Volume (mL)	Diuretic Index	pH	Conductivity (dS/m)	Density (g/mL)	
Control (Normal Saline)	2.7 ± 0.10	1.00	7.18 ± 0.02	51.5 ± 1.5	0.027	
Standard (Frusemide)	10.5 ± 0.35***	3.89	8.33 ± 0.03*	63.5 ± 0.5*	0.031	
<i>Kandelia candel</i>	EtOH (200)	6.65 ± 0.40**	2.95	8.54	62.50	0.028
	EtOH (400)	6.90 ± 0.17**	3.07	8.60	63.00	0.027
	Fr. n-hex (200)	2.80 ± 0.15	1.24	7.22	56.00	0.025
	Fr. n-hex (400)	3.80 ± 0.55*	1.69	7.23	58.00	0.027
	Fr. CHCl ₃ (200)	2.40 ± 0.15	1.07	7.94	50.50	0.025
	Fr. CHCl ₃ (400)	4.20 ± 0.45*	1.87	7.24	55.50	0.032
	Fr. EtOAc (200)	3.40 ± 0.45*	1.51	7.99	63.50	0.027
	Fr. EtOAc (400)	4.60 ± 0.40*	2.04	8.02	66.00	0.029
	Fr. H ₂ O (200)	2.80 ± 0.50	1.24	7.08	46.50	0.027
Fr. H ₂ O (400)	3.30 ± 0.05*	1.47	7.56	47.50	0.026	
<i>Brownlowia tersa</i>	EtOH (200)	8.1±0.15**	3.00	7.60	53.0	0.026
	EtOH (400)	8.5±0.15**	3.15	8.35	62.0	0.032
	Fr. n-hex (200)	4.6±0.1	1.70	7.84	42.5	0.029
	Fr. n-hex (400)	5.8±0.05*	2.15	8.00	48.0	0.027
	Fr. CHCl ₃ (200)	7.4±0.2**	2.74	7.38	49.0	0.028
	Fr. CHCl ₃ (400)	8.0±0.05**	2.96	7.39	54.5	0.027
	Fr. EtOAc (200)	5.8±0.10	2.15	6.78	37.0	0.026
	Fr. EtOAc (400)	6.4±0.10*	2.37	8.65	63.5	0.028
	Fr. H ₂ O (200)	4.8±0.25	1.78	7.31	28.5	0.025
Fr. H ₂ O (400)	5.0±0.40	1.85	6.97	36.0	0.026	

Expressed as Mean ± SEM., * $p < 0.005$. ** $p < 0.001$, *** $p < 0.0001$, compared with the control group

A significant laxative activity (more stool with soft consistency) was seen for the chloroform (*Kandelia candel*) and ethyl acetate (*Brownlowia tersa*) fractions in experimental animal comparing to the control group and presented in the Table 6.

Table 6: Results of Laxative test (* $p < 0.05$, ** $p < 0.001$, compared with the control group)

Treatment Groups (Dose: mg/kg)	Weight of Faeces (gm)		Mean weight of faeces (gm)	% increment of Faeces weight		% increment in weight of Faeces ± S.E.M.	
	Rep 1	Rep 2		Rep 1	Rep 2		
Control (Normal Saline)	0.53	0.56	0.55	-	-	-	
Standard Bisacodyl (10)	0.78	0.70	0.74	47.17	41.07	44.12 ± 3.05**	
<i>Kandelia candel</i>	EtOH (200)	0.89	0.82	0.91	53.45	54.72	54.09 ± 2.54
	EtOH (400)	0.65	0.63	0.54	12.06	18.87	15.46 ± 1.23
	Fr. n-hex (200)	0.530	0.320	0.330	0	0	0
	Fr. n-hex (400)	0.870	0.760	0.815	50.000	46.420	48.21±2.53*
	Fr. CHCl ₃ (200)	1.537	1.380	1.459	165.000	160.377	103.13 ± 1.98**
	Fr. CHCl ₃ (400)	1.377	1.265	1.321	137.413	138.679	162.69 ± 3.27**
	Fr. EtOAc (200)	0.770	0.725	0.748	32.762	36.793	34.76 ± 2.85*
	Fr. EtOAc (400)	0.733	0.683	0.708	26.379	28.868	27.62±1.76*
	Fr. H ₂ O (200)	0.950	0.897	0.924	63.793	69.245	66.52 ± 3.86*
Fr. H ₂ O (400)	1.170	1.084	1.127	101.724	104.528	138.05 ± 1.32**	
<i>Brownlowia tersa</i>	EtOH (200)	0.69	0.68	0.69	30.19	21.43	25.81± 4.38
	EtOH (400)	0.90	0.96	0.93	69.81	71.43	70.62 ± 0.81**
	Fr. n-hex (200)	0.56	0.59	0.58	5.66	5.36	5.51±0.15
	Fr. n-hex (400)	0.84	0.81	0.83	58.49	44.64	51.57±6.93*
	Fr. CHCl ₃ (200)	0.77	0.80	0.79	45.28	42.86	44.07± 1.21
	Fr. CHCl ₃ (400)	0.55	0.59	0.57	3.77	5.36	4.57± 0.80
	Fr. EtOAc (200)	0.76	0.79	0.78	43.39	41.07	42.23 ± 1.16
	Fr. EtOAc (400)	1.07	1.13	1.10	101.88	101.79	101.84±0.05***
	Fr. H ₂ O (200)	0.49	0.47	0.48	0	0	0
Fr. H ₂ O (400)	0.95	0.90	0.93	79.23	66.07	72.65 ± 6.58**	

The method described by Golla, 2014, is an effective method to evaluate the laxative activity of the crude extract of the plant. In this method, bisacodyl tablet was used as standard for comparison [24]. The administration of the test extracts to the mice was effective in increasing defecation frequency,

fecal volume, and consistency. These are indications of the laxative property of the plant extract. The laxative effect of the extract could also be due to changes in the intestinal motility, which produced acceleration in the intestinal transit and colonic movement [36].

From phytochemical screening of ethanolic extract and different fractions of both the plants revealed the presence Phenolic compounds which produce laxative activity of different fractions in a dose dependent manner. Laxative activity is also studied based on the fecal consistency. Fecal consistency is correlated to the ratio of the water holding capacity of the insoluble solids, such as those derived from dietary fiber and to the total water in the lumen^[37]. The active fractions produced soft faeces with retention of water and the softness of was observed as the dose increased.

Conclusion

Ethyl acetate fraction for *Kandelia candel* and chloroform fraction for *Brownlowia tersa* were found to be more active both in diuretic and laxative tests among their respective four fractions. Among these two plants *Brownlowia tersa* showed better diuretic effect than *Kandelia candel* but as laxative *Kandelia candel* was found to be more active. The extracts demonstrated the diuretic activity as loop diuretics or carbonic anhydrase inhibitors. In phytochemical tests, the active fractions showed the presence of multiple compounds such as steroids, terpenoids, flavonoids, glycosides, phenolics, etc. Further chromatographic and spectroscopic techniques (NMR and Mass) may be applied to isolate and characterize the pure compound responsible for the claimed diuretic and laxative activity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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