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PHYTOCHEMICAL INVESTIGATION AND SCREENING OF DIURETIC ACTIVITY OF EMBELLIA ROOT

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Abstract:

The present study evaluated the diuretic potential of chloroform, methanol, and aqueous extracts of Embelia root in Wistar albino rats. The roots were authenticated, processed, and subjected to successive extraction followed by phytochemical screening, which revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, and phenolic compounds. Acute oral toxicity studies indicated that all extracts were safe up to 2000 mg/kg body weight. Diuretic activity was assessed using urine volume and urinary electrolyte concentrations (Na^+, K^+, Cl^-) as primary parameters.

Methanol and aqueous extracts significantly increased urinary output compared to the control group. The methanol extract produced a marked increase in sodium and potassium excretion, while the aqueous extract moderately enhanced potassium excretion. In contrast, the chloroform extract showed no significant effect on urine volume or electrolyte excretion. The enhanced diuretic activity of the methanol extract may be attributed to its higher content of flavonoids, tannins, and phenolic compounds, which are known to influence renal function by increasing glomerular filtration rate and modulating tubular reabsorption.

Overall, the findings suggest that Embelia root possesses significant diuretic activity, particularly in its methanol extract, supporting its potential therapeutic application as a natural diuretic agent.

Keywords: *Embelia root, urine volume, Diuretic activity,*

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INTRODUCTION:

KIDNEY

The kidney is a vital organ that removes waste products in the blood and also regulates fluid volume in the body. The basic entities of the kidneys are called nephrons, which filter the blood and remove the waste products through urine [1, 2]. The body's urinary system is made together with the two ureters, the bladder, the single urethra, and the kidneys. Human beings, and all other vertebral species typically have two kidneys. In the body, kidneys are dark red and have a shape of beans in which one side is convex, or rounded, and the other is concave. The adult human kidneys are about 10 to 13 cm (4 to 5 inches) long and about 5 to 7.5 cm (2 to 3 inches) wide with the size of a computer mouse.

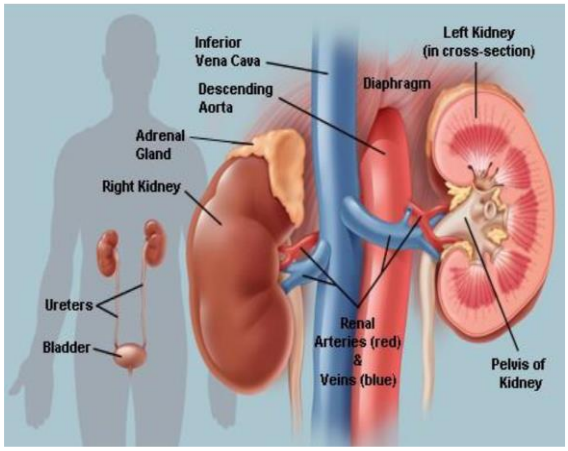


Fig No.1: Structure of Kidney, WebMD, 2009

The kidney is present in a thin fibrous capsule that is adherent at the hilum. The cut surface of the kidney is made up of a well-demarcated peripheral cortex and inner medulla in which the cortex is 1.2 to 1.5 cm in thickness showing striations called medullary rays formed by the collecting tubules, ascending limbs, and straight portions of the proximal convoluted tubules. The medulla is composed of several cone-shaped renal pyramids, whose apex is called papilla and is related to a calyx. 2 to 3 major calyces form a funnel-shaped dilated proximal part of the ureter called the pelvis. Major calyce is subdivided into 3 to 4 minor calyces into which the papillae project [1, 2].

Function of the kidney

The kidney's primary function is to eliminate the poisonous wastes from the blood [3]. The nitrogen-containing compounds urea and uric acid are chief among these waste products. Life-threatening diseases occur when large quantities of waste products accumulate in the blood stream. Fortunately, a healthy kidney can easily clear the body of these substances. Kidneys serve multiple functions, including the following:

- Excretion of metabolic waste products and foreign chemicals Regulation of water and electrolyte balance
- Regulation of body fluid osmolarity and electrolyte concentrations Regulation of arterial pressure
- Regulation of acid-base balance
- Secretion, metabolism, and excretion of hormones
- Gluconeogenesis

Excretion of metabolic waste products, foreign chemicals, drugs, and hormone metabolites:

The main function of kidneys is to eliminate waste products of metabolism that are not required by the body, including urea (from the metabolism of amino acids), creatinine (from muscle creatine), uric acid (from nucleic acids), end products of hemoglobin breakdown (such as bilirubin) and metabolites of various hormones. These waste products must be eliminated through the kidneys as rapidly as they are produced. The kidneys also play an important role in eliminating most toxins and other foreign substances that are either produced by the body or ingested, such as pesticides, drugs, and food additives.

Regulation of water and electrolyte balances:

For maintenance of homeostasis, excretion of water and maintenance of electrolytes must accurately match. If intake exceeds excretion, the amount of that substance in the body will increase and vice versa. Kidneys help regulate the blood levels of several ions most essentially sodium, potassium, calcium, chloride, and phosphate ions.

Regulation of Arterial Pressure:

Kidneys play an important role in the long-term regulation of arterial pressure by excreting variable amounts of sodium and water. The kidneys also contribute to secreting vasoactive factors or substances, such as renin, that lead to the formation of vasoactive products (eg., angiotensin-II).

Regulation of Acid-Base balance:

The kidneys are also involved in the regulation of acid- base balance, along with the lungs and body fluid buffers, by excreting acids and regulating the body fluid buffer stores. The kidneys are the only route for the excretion of certain types of acids, such as sulphuric and phosphoric acid, produced by the metabolism of proteins.

Regulation of Erythrocyte Production:

The kidneys secrete erythropoietin, which stimulates the production of RBCs and a significant stimulus for erythropoietin release by the kidneys is hypoxia, which normally accounts for almost all the erythropoietin secreted into the circulation. In people with severe kidney disease or nephrectomy or who have been placed on hemodialysis, severe

anemia develops as a result of reduced erythropoietin production.

Regulation of 1, 25-Dihydroxyvitamin D3 Production:
The kidneys secrete the active form of vitamin D, 1, 25-Dihydroxyvitamin D3 (Calcitriol), which is essential for normal calcium deposition in bone and calcium reabsorption by the GIT. Calcitriol plays a significant role in calcium and phosphate regulation [3-4].

Glucose Synthesis:
The kidneys synthesize glucose, from amino acids and other precursors during prolonged fasting, which is called gluconeogenesis.

MATERIALS AND METHODS:

MATERIALS

Collection and authentication of plant material
In the present study, the roots of embelia roots were collected from the local areas of our college. The plants were authenticated by Dr. Madhavan Chetty, Assistant Professor, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The roots of embelia roots were then washed with water to remove physical impurities like soil and dirt, and dried at room temperature.

Extraction
The shade-dried roots of embelia roots were reduced to course powder and around 200 g of powdered plant material was subjected to successively hot continuous extraction (Soxhlet extractor) with petroleum ether (only for defatting), chloroform, and methanol. Each time before extracting with the next solvent, the powdered material was dried in a hot air oven below 50 ^{0}C. Finally, the marc was macerated with distilled water, and a few drops of chloroform were added as a preservative for more than 24 hours to obtain the aqueous extract. Each extract was then distilled to dryness under reduced pressure using a Buchi Rota evaporator. The extract obtained with each solvent was weighed and its percentage in terms of the air-dried weight of the plant material was calculated. Also, the color and consistency of the extract were noted.

PHARMACOLOGICAL INVESTIGATION

Acute Toxicity Studies
Acute Toxicity Study[5]
The acute toxicity study is used to establish the therapeutic index, i.e. the ratio between the pharmacologically effective dose and lethal dose on the same strain and species (LD50/ED50). The greater the index; the safer the compound and vice versa. The acute toxicity study was done according to OECD (Organization of Economic Co-operation and Development) guidelines 425- Fixed Dose Procedure (FDP), as in annex 2D.

Procedure:
The animals were divided into two groups and each group consisted of five mice. The defined or fixed dose level of aqueous and ethanolic extracts (2000 mg/kg) was given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological, and autonomic profiles. The toxicity signs were observed after 24 hours to fourteen days for any lethality or death.
The dose administered was 200 mg/kg p.o. for extracts of Biophytum sensitivum and 500 mg/kg p.o. for extracts of Embelia roots.
OECD Guidelines for testing of chemicals, revised draft 420, Documents on acute oral toxicity and acute toxicity class method, Revised Dec. 2001.

Screening of Diuretic Activity in Rats
Diagnostic kits:
Sodium, Potassium (Pariksha Biotech Private Ltd., Hyderabad), Chloride (ERBA diagnostics Mannheim, GmbH).
Instruments:
UV-Spectrophotometer (UV-1800, Shimadzu Corporation, Japan)
Animals Stock:
Wistar albino rats of either sex weighing between 150-200 g were obtained From Jeeva Life Sciences (Reg CCSEA/IAEC/JLS/20/11/23/077),. Animals were housed in groups of 6-8 per cage at a temperature of 25±1 0C and relative humidity of 45-55%. Animals had free access to food and water, however, food and water were withdrawn 18 hours before the experiment. The Institutional Animal Ethics Committee approved the protocol of this study.
Preparation of dosage form:
The emulsion of extracts (chloroform and methanol) was prepared by triturating the accurately weighed quantity of the extract with 1% Tween 80 in a glass mortar, with a gradual addition of distilled water, to make up the required volume. Furosemide Tablets I.P. 40 mg (Lasix 40 mg, Aventis Pharma Ltd.) and aqueous extract of embelia roots were diluted with distilled water.

Diuretic activity:
Animals were divided intoa total of five groups (n = 6 in each group). All animals were deprived of food and water 18 hoursbefore the experiment. On the day of the experiment, the dosing was scheduled as follows:
Group I: Normal saline.
Group II: Furosemide 10 mg/kg p.o. as reference diuretic drug.
Group III: Chloroform extract 200 mg/kg p.o.
Group IV: Methanol extract 200 mg/kg p.o.
Group V: Aqueous extract 200 mg/kg p.o.

Immediately after the dosing, animals were placed in metabolic cages and urine was collected up to 5 h

after dosing. Room temperature was maintained up to 25± 0.5 0C.

During this period no water or food was made available to the animals. Diuretic activity was assessed by measuring the following parameters:

- ✓ Total urine volume.
- ✓ Urine concentration of Na+, K+ and Cl-.[6,7]

The effect of different extracts of embelia roots exhibiting diuretic activity is shown in Table no. 5.4.

Biochemical Estimation:

Sodium Assay

Step-1: Precipitation of Sodium and serum proteins:

Pipette into clean dry test tubes labelled as Standard (S) and Test (T):

	Standard (S)	Test (T)
R1 Precipitating Reagent	1000 µl	1000 µl
R4 Standard	10 µl	-
Serum Sample	-	10 µl

Shake vigorously and incubate at room temperature for 5 minutes. Then Centrifuge at 2000-3000 rpm for 2 minutes to obtain a clear supernatant.

Step- 2: Colour development

Pipette into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T):

	Blank (B)	Standard (S)	Test (T)
R2 Colour Reagent	1000 µl	1000 µl	1000 µl
Supernatant from Step 1		20 µl	20 µl
R1 Precipitating Reagent	20 µl		

Mix well and allow it to stand at room temperature for 5 minutes. Then measure the absorbance of Blank (B), standard (S), and Test (T) on a photo colorimeter with green filter or on a spectrophotometer at 530nm (505 - 530 nm) within 10 minutes.

Calculations:

$$\text{Sodium in mmole/L} = \frac{\text{Abs of B} - \text{Abs of T}}{\text{Abs of B} - \text{Abs of S}} \times 150$$

Potassium:

Principle:

Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed colloidal suspension of potassium tetraphenylboron. The turbidity produced is proportional to the potassium concentration in the sample.

Potassium Assay

Pipette into two clean dry test tubes labeled Standard (S) and Test (T**)**

	Standard (S)	Test (T)
R3 Boron Reagent	1000 µl	1000 µl
R4 Standard	50 µl	-
Serum Sample	-	50 µl

Mix well and Incubate for 5 minutes at RT. Read absorbance of the Standard (Abs.S) and Test Sample (Abs.T) against Distilled water at 620 nm.

Calculations:

Chloride:

Principle: When chloride is mixed with a solution of undissociated mercuric thiocyanate, the chloride preferentially combines with mercury forming mercuric chloride.

The thiocyanate that is released then combines with ferric ions present in the solution forming strongly colored ferric thiocyanates with an absorption maxima at 480 nm.

Assay procedure
Wavelength 492 (470 - 630) nm
Cuvette 1 cm

	Reagent blank	Standard (Cal.)	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard (Cal.)	-	10 µl	-
Distilled water	10 µl	-	-

Mix and incubate for 5 min. at 37 ^{0}C, measure absorbance at the sample Asam and standard Ast against reagent blank.

Calculation

$$\text{Chloride mmol/L} = \frac{\Delta A_{sam}}{\Delta A_{st}} C_{st}$$

Cst = standard (calibrator) concentration

Diuretic activity

Diuretic activities of normal and treated animals were estimated using an estimation of the difference between the output of urine and consumption of liquids. The percentage of variance between saline administered and test animals was calculated[41].

$$\text{Diureticaction} = \frac{\text{Urinary excretion volume of the test group}}{\text{Urinary excretion volume of the control group}}$$

$$\text{Diureticactivity} = \frac{\text{diureticactionoftest}}{\text{diureticactionofstandard}}$$

Saluretic and natriuretic activity and carbonic anhydrase inhibitor[42]

Saluretic index, natriuretic index, and ion quotient were determined using the following formulae.

$$\text{Salureticindex} = \frac{\text{Urinary excretion of electrolytes of the test group}}{\text{Urinary excretion of electrolytes in the control group}}$$

$$\text{Natriuretic index} = \frac{\text{Urinary excretion of } Na^+}{\text{Urinary excretion of } K^+}$$

$$\text{Carbonic anhydrase inhibition} = \frac{\text{Urinary excretion of } Cl^-}{\text{Urinary excretion of } Na^+ \text{ and } K^+}$$

RESULT & DISCUSSION:

Pharmacological Investigation

Chloroform, methanolic, and aqueous extracts of Biophytum sensitivum and Embelia were subjected to assessment of –

- ✓ Acute oral toxicity study
- ✓ Diuretic activity

Acuteoral toxicity study:

All extracts were found to be safe in the dose used and there was no mortality up to a dose of 2000 mg/kg. The dose administered was 200 mg/kg p.o.

Diuretic Activity of Extracts:

The present study indicates the pharmacological evaluation of the diuretic activity of different extracts of embelia roots and in case the dose of the extract administered was 200 mg/kg b.w. The parameters like urine volume, and the concentration of electrolytes in the urine such as sodium, potassium, and chloride were measured to assess the diuretic potential of all the groups. Significantly ($p<0.001$) there was an increase in urine volume in methanol and aqueous extract compared to control. Methanol extract significantly ($p<0.001$) increased in sodium concentration compared to control. Methanol extract significantly ($p<0.001$) and aqueous extract significantly ($p<0.01$) increased in potassium level compared to control. There was a non-significantly increase in chloride level compared to control. Furosemide showed a highly significant level ($p<0.001$) in urine volume, sodium, potassium, and chloride ions.

Table No. 1 :Effect of different extracts of embelia roots on urinary electrolyte excretion (n = 6 in each group)

Parameters	Control	Standard	Methanol extract (100)	Methanol extract (200)	Methanol extract (300)
Urine Volume (ml)	1.304±0.02626	3.912±0.09458***	1.143±0.05627	2.849±0.1232***	1.901±0.0730***
Na^+ (Meq/L)	11.29±0.4539	22.31±1.435***	10.85±0.2400^{ns}	18.86±1.022***	12.95±0.8823ns
K^+ (Meq/L)	59.61±2.742	87.29±3.336***	67.37±2.184ns	103.49±2.042***	73.92±4.181**
Cl^- (Meq/L)	12.05±0.4745	21.85±1.721***	9.696±0.4168^{ns}	9.173±0.7111^{ns}	11.35±0.5906^{ns}

All values are expressed as mean ± SEM; **P<0.01, ***P<0.001 Vs control, ns= non-significant.

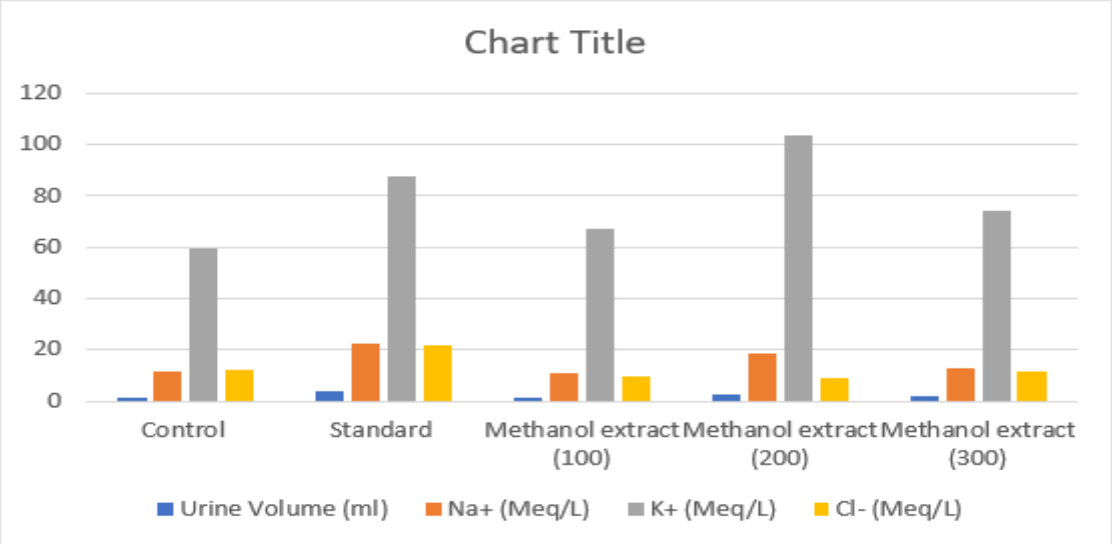
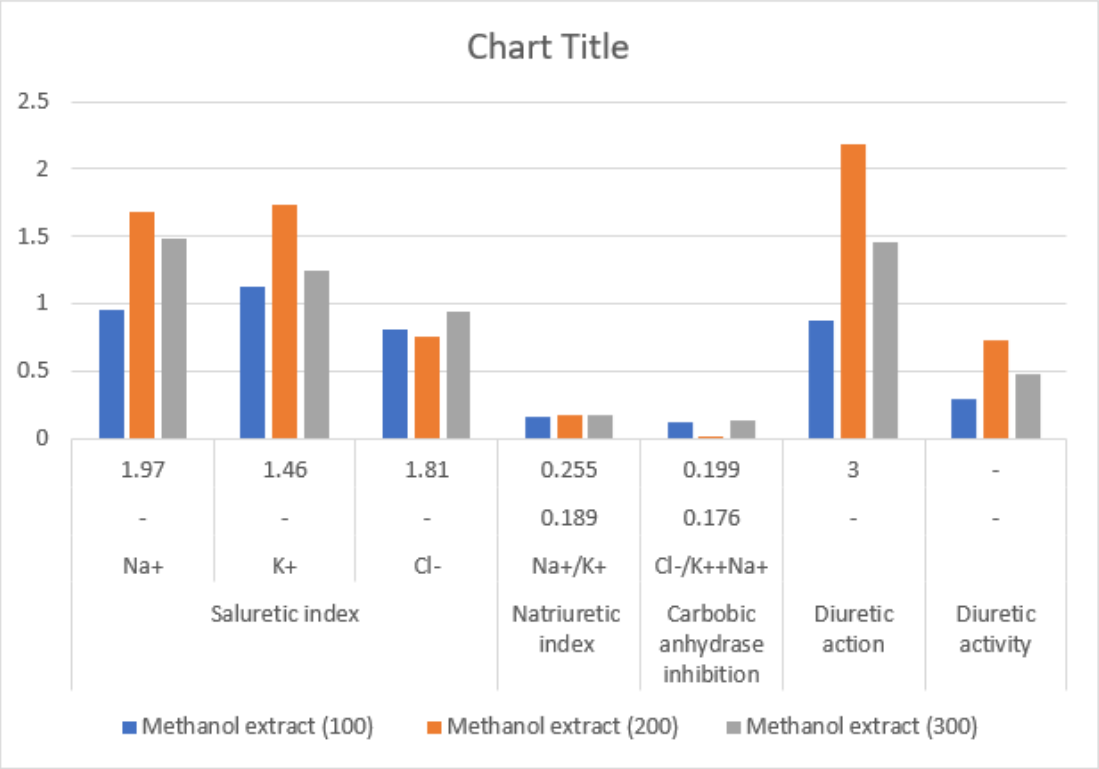


Table :2 Effect extracts of embelia roots Saluretic index, Natriuretic index, Carbonic anhydrase inhibition, diuretic action, and diuretic activity

Treatment	Saluretic index			Natriuretic index	Carbobic anhydrase inhibition	Diuretic action	Diuretic activity
	Na^+	K^+	Cl^-	Na^+/K^+	$Cl^-/K^+ + Na^+$		
Control	-	-	-	0.189	0.176	-	-
Standard	1.97	1.46	1.81	0.255	0.199	3	-
Methanol extract (100)	0.96	1.13	0.81	0.161	0.123	0.876	0.292
Methanol extract (200)	1.68	1.74	0.76	0.182	0.023	2.18	0.726
Methanol extract (300)	1.48	1.24	0.94	0.175	0.130	1.46	0.486



DISCUSSION:

The methanol and aqueous extracts of embelia roots significantly increased the urinary output and excretion of electrolyte concentrations of sodium and potassium comparatively better than the control. The chloroform extract of embelia roots had an insignificant effect on urine output and electrolyte concentrations of sodium, potassium, and chloride ions (As shown in table no. 5.4). Urine volume, the concentration of electrolytes in the urine such as sodium, chloride, and potassium were the parameters measured while assessing the diuretic potential of all the groups. There are two factors on which urine volume depends. One is the glomerular filtration rate (GFR) and the other is the degree of tubular re-absorption. The observed effect may be attributed to mechanisms like increasing the renal blood flow and the attendant increase in GFR.[68] The present study demonstrates that methanol and aqueous extracts of embelia roots significantly increased urinary output. Methanol extract increased sodium and potassium ions significantly, and aqueous extract moderately increased only potassium ions. The chloroform extract was the least potent compared to other extracts that showed the absence of diuretic activity. Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, and phenolic compounds. Therefore, some of these components may have played a role in the observed diuretic activity profile.

SUMMARY AND CONCLUSION:

In the present study, the roots of embelia roots were collected and authenticated. The roots were then subjected to size reduction to get coarse powder (40#) and subjected to extractive values and successive extraction with chloroform, methanol, and water, then identification of major chemical constituents and their estimation was carried out as per pharmacopeia/literature. As per the phytochemical investigation: alkaloids, carbohydrates, flavonoids, tannins, and phenolic compounds were found to be present in the various extracts of the root of embelia roots. The various extracts were studied for acute oral toxicity study as per OECD/OCDE guidelines. According to the literature, all extracts were found to be safe in the dose used and there was no mortality up to a dose of 2000 mg/kg, b.w. embelia roots. After the acute oral toxicity study, the various extracts were screened for diuretic activity in Wistar albino rats.\ From the extracts of embelia roots, methanol showed prominent diuretic activity aqueous extract showed moderate diuretic activity and chloroform extract showed absence of diuretic activity. The diuretic activity of methanol extract fromthe root of embelia roots may be due to the single or combined effects of flavonoids, carbohydrates, tannins, and phenolic compounds. But there is the probability that flavonoids or/with tannins may be the most responsible constituents for diuretic activity because as per literature flavonoids or/with tannins do possess the action on the urinary system.[69,70]Thus we

can conclude that embelia roots does possess diuretic activity.

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