

PHYTOCHEMICAL STANDARDIZATION AND DIURETIC ACTIVITY OF ECLIPTA ALBA

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Abstract

Dravyaguna Vigyan, is a branch of Ayurveda that deals with the study of medicinal substances of plant, animal, and mineral origin. It involves the classification, identification, and evaluation of the therapeutic properties of *Eclipta alba*.

Objective:

The aim of this study was to assess phytochemical standardization and diuretic activity of *Eclipta alba*.

Materials and methods:

The coarse powder obtained from the bark apexes of Eclipta alba was subjected to defatted and methanol solvent was used and extracted through the shoxlet. The present investigation aimed to evaluate the diuretic effects of the plant extract derived from Eclipta alba and to standardize the phytochemical composition of the extract. To assess the diuretic activity, Lipschitz procedures were employed on rat models. The findings of our study led to the following conclusions: The Eclipta alba plant extract exhibited distinctive characteristics such as an herbal aroma, a fine and light green powder appearance, a dark green color, a fine and smooth texture, a bitter taste, and a consistently fine texture with slight granularity.

Results and discussion:

The results revealed that The Eclipta alba extract exhibited a total ash content of 5.15% w/w, an acid insoluble ash content of 4.15% w/w, a water insoluble ash content of 7.40% w/w, and a loss on drying of 4.15% w/w. Our study unveiled the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, tannins and phenol compounds, and fixed oils and fats in all extracts of Eclipta alba. The different extracts derived from Eclipta alba demonstrated an

increasing diuretic activity in a dose-dependent manner. This was evidenced by the elevated urine volume and electrolyte levels, including sodium (Na+), potassium (K+), and chloride (Cl-), with higher concentrations of the extract, indicating a potential diuretic effect. The urine output index served as a parameter to gauge the relative urine volume and electrolyte excretion in each treatment group. Higher values indicated augmented urine production and electrolyte excretion in comparison to baseline levels

Conclusions:

In conclusion, this study substantiated the significant diuretic activity associated with the aqueous extract of Eclipta alba, thereby validating its traditional use in Ethiopian folk medicine **Keywords:** *Ash value, phytochemical tests, alkaloids, flavonoids, phenolics, Eclipta alba, rats, saluretic, tannins.*

INTRODUCTION:

Herbal Diuretic Treatment

Many medicines of plant origin had been used since ages without any adverse effects. A treatise published in 1788 by Joseph Plenick (1735–1807) lists several hundred plants, of which 115 have diuretic properties, including garlic, Chinese lantern, saffron, fennel, liquorice, sassafras anddandelion (Taraxacum officinale). Traditional systems of medicine or care by herbals are used throughout the world and from century's herbs have been the original source for most of the drugs. Numerous medicinal plants are available today for having diuretic activityXanthium strumarium; Samanea saman (jacq) Merr; Morinda citrifolia (Linn); Holarrhena antidysentrica; Euphorbia thymifolia, etc ^{1-11.}

Eclipta alba, also known as False Daisy or Bhringraj, is a medicinal herb commonly found in tropical and subtropical regions of the world, including India, China, and Brazil. In Ayurveda, Eclipta alba is considered to be a potent rasayana or rejuvenating herb, and it is believed to have the ability to enhance longevity, improve memory, and strengthen the immune system. The herb is also used as a hair tonic and is believed to promote hair growth and prevent premature greying. In traditional Chinese medicine, Eclipta alba is used for its anti-inflammatory, antitumor, and

hepatoprotective properties. The herb is also believed to have diuretic effects and is used to treat urinary disorders such as nephritis and bladder infections.¹¹⁻¹⁴

Some of the commonly studied plants with reported diuretic activity include:

Boerhavia diffusa; Tribulus terrestris; Taraxacum officinale (Dandelion); Allium sativum (Garlic); Petroselinum crispum (Parsley); Urtica dioica (Stinging Nettle) The leaves, fruits, seeds and roots of the plants contain bioactive compounds that stimulate urine production and increase renal blood flow. And many more plants are there.

Dravyaguna Vigyan, is a branch of Ayurveda that deals with the study of medicinal substances of plant, animal, and mineral origin. It involves the classification, identification, and evaluation of the therapeutic properties of Eclipta alba.

To investigate the diuretic activity of Eclipta alba using established experimental models such as the Lipschitz test, and to compare the results with the standard diuretic drug, furosemide. The envisaged research will be carried out in a stepwise manner with each objective being achieved through specific experiments. The research design will involve the use of established experimental models and modern analytical techniques. The findings of this study will contribute to the understanding of the pharmacological properties of this plant and have potential applications in the development of new drugs for the treatment of renal disorders ¹⁴⁻¹⁷.

MATERIALS AND METHODS:

Collection and Authentication of the Plant

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. As per the information collected from local vaidhyas and other traditional anti-inflammatory medicine practitioners, the present study is deals with the evaluatation of activity of Eclipta Alba.

All the plant materials (includes of Leaves, Stem, Bark mainly) on an herbarium sheet had been duly authenticated by the Department of Pharmcognosy & Botanist, *Dr. Vijaya Malik*, Department of Botany, CCS University, Meerut. The plant materials of *Eclipta alba* based on their morphological characteristics.

Eclipta Alba aerial section has been washed, cleaned and dried six days. The plant material was mixed into a coarse powder after the drying and kept for further investigations at room temperature.

Performing the Organoleptic Properties of Plant extract of Eclipta alba¹⁵⁻¹⁷

The physical characteristics of Eclipta Alba extract, in its crude drug form, can provide valuable insights into its nature, color, odor, and taste.

Soxhlet Extraction

Soxhlet extraction is a common method used for the extraction of organic compounds from solid or semi-solid samples. It is widely employed in various industries, including the pharmaceutical, food, and environmental sectors. If you're specifically interested in Soxhlet extraction of Biden pilosa and Rosemary which is the scientific name for the olive leaves and fruit, it suggests you want to extract certain compounds from olive plant material. The freshly collected plant materials were washed, shadow dried and then dried in hot air oven at a temperature not more than 50°C.

The dried powder of aerial parts of plant was extracted in Soxhlet apparatus with different solvent i.e., hexane, petroleum ether, chloroform and methanol using successive extraction method from nonpolar to polar solvent used. The extraction was continued for 72 hours for each solvent at 40-45°C. The extraction was started with hexane then petroleum ether and continued with chloroform and then lastly with methanol. After the completion of extraction, the solvent was evaporated, and residue obtained was then stored in dessicator for further study.

At the beginning of this process, the barks and leaves are removed off of the tree and then dried and fruits afterwards homogenised into fine powder. The measurement of powder was determined in contrast with natural solvents in a conical carafe. It was placed in the rotary shaker at 190-220 rpm for 24 hours. Then the mushroom fabric was sorted and centrifuged. Use of dissolvable refinery gadgets to provide the last volume of a fourth of the first volume had been recovered. It is stored in close-air bottles for future tests at 40°C. The % Yield in different solvents plant extracts were calculated by using the following formula: % *Yield* = (Net weight of powder in gram after extraction /Total weight of powder in gram taken for extraction) $\times 100$

Physicochemical Evaluation

The powdered plant material of Eclipta Alba was evaluated by standard procedure for the determination of following physicochemical parameters.

Evaluation of diuretic activity 8-10

The diuretic study was carried out as per the modified method of Lipschitz. All animals were deprived of food and water 18 h prior to the experiment. Normally, urine output in rats is very low (1-2 ml/rat/day). Hence, to get the measurable quantity of urine, rats of all the groups were administered with distilled water (2 ml/100 g) after 30 min of test drug administration.

Then, the animals were placed individually in metabolic cages with netted floor, and urine was collected in conical flasks placed below the polythene funnel of the metabolic cages. Extreme care was taken to avoid the contamination of urine with fecal matter.

Urine was collected up to 5 h after dosing. Room temperature was maintained up to 25 ± 0.5 °C. During this period, no water or food was made available to the animals. Diuretic activity was assessed by measuring total urine volume and urine electrolyte concentration of Na+, K+, and Cl-. Animals were sacrificed by decapitation at the end of the experiment. Arteriovenous blood was collected in heparinized tubes and centrifuged (3000 rev/min for 10 min). The plasma collected was stored at -20 °C for biochemical analyses.

The liver and kidneys were dissected out, cleaned of fat material, weighed and stored at -20 °C for biochemical analyses. Urine was collected and the volume determined each hour from the treatment for 6 h (i.e. 1, 2, 3, 4, 5 and 6 h after treatment) and 24 h after in all experimental groups. Electrolyte concentrations (Na+, K+ and Cl–) were measured in 24 h urine and in blood plasma obtained from animals sacrificed 24 h after treatment.

Table: Grouping of Animals.

S.N.	Groups	Dose received		
1	Group-1: Saline control group	10	mL/kg	body

		weight
2	Group - 2: (Saline + Disease Induced	5 mg/kg
	group)	
3	Group-3 :(Treated group low dose)	Received 200 mg/kg
		drug extract
4	Group -4 :(Treated Group high dose)	Received 400 mg/kg
		drug extract
5	Group-5: (Standard drug group)	Received 5 mg/kg
	Furosemide	standard

RESULTS AND DISCUSSIONS:

Report of Physical Test of Crude Drugs

The physical characteristics of Eclipta Alba extract, in its crude drug form, can provide valuable insights into its nature, color, odor, and taste. The physical test results of Eclipta Alba extract are as follows:

Table: The Organoleptic properties of the plant extract were evaluated for Eclipta Alba

S.N	Parameters	Results
1	Odour	Characteristic herbal scent
2	Powder as such	Fine, light green powder
3	Colour	Dark green
4	Texture	Fine and smooth
5	Taste	Bitter
6	Consistency	Fine and slightly granular

Extractive Values

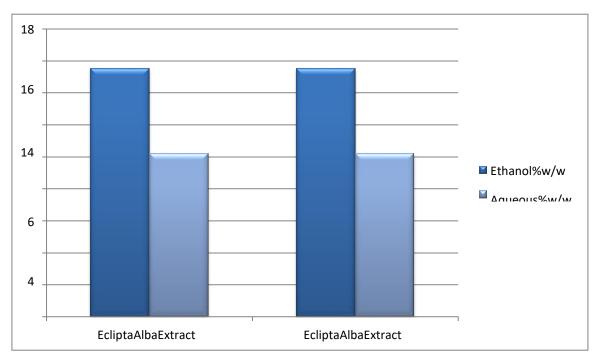
The ethanol extract of eclipta alba fruit was found to contain approximately 9.52% (w/w) of the plant's constituents, while the aqueous extract contained approximately 12.21% (w/w). These values indicate the percentage by weight of the extractable components present in the respective

solvent extracts. The Extractive Values of the plant extract were evaluated for alcoholic and aqueous solutions Eclipta Alba.

Crudedrugs	Ethanol	Aqueous
	%w/w	%w/w
EcliptaAlbaExtract	15.52	10.21

Table3.Extractivevalues

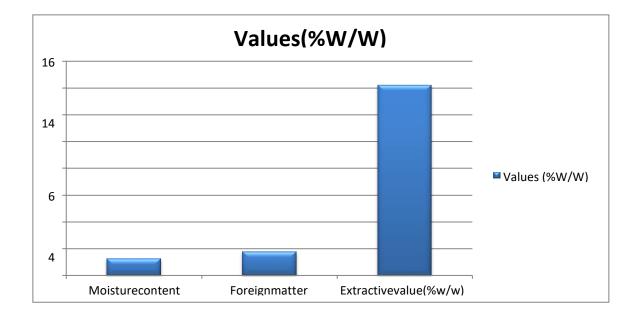
Graph of the Extractive Values:



Graph1.ExtractiveValuesofextractinEthanol&Aqueousmedium

Table 4. Losson Drying and Foreign Organic Matter Eclipta Alba leaves extract

S.No	PhysicalConstant	Values(% W/W)
1.	Moisturecontent	1.25
2.	Foreignmatter	1.76
3.	Extractivevalue(%w/w)	14.23

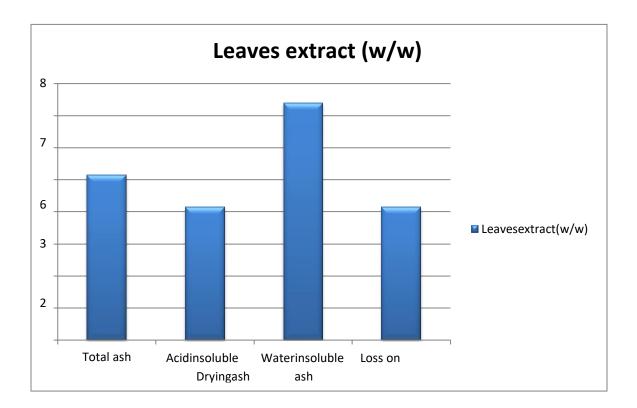


Graph 2.Losson Drying and Foreign Organic Matter Eclipta alba leaves extract



Table	1.	Totalash	5.15
5.Tota	2.	Acidinsolubleash	4.15
l Ash,	3.	Waterinsolubleash	7.40
Acid	4.	LossonDrying	4.15
Insolu			

ble Ash And Water Soluble Ash Values Eclipta Alba extrcat



Graph3.Total Ash, Acid Insoluble Ash And Water Soluble Ash Values Eclipta Alba extract.

Phytochemical Screening

The present study revealed that the carbohydrates, proteins, amino acids, glycosides, flavonoids, tannin and phenol compound, and fixed oils and fats present in all extracts. However, the steroids were present only in petroleum ether, ethyl acetate, and aqueous extracts. Alkaloids were present in chloroform, methanol, and aqueous extracts. Saponins were present in chloroform, ethanol, and aqueous extracts. Quinones were present in petroleum ether, ethyl acetate, methanol, and aqueous extracts. Terpenes were determined to be absent in all the extracts. Compared to all

other solvent extracts, aqueous extracts were shown high values of secondary metabolites. Phytochemical screening using different solvents has been reported on this plant. The phytoconstituents such as flavonoids, tannins, and phenolic compound extracts were liable for its antioxidant property.

S.No	ChemicalTests	Eclipta AlbaExtrac t
1.	TestsforSteroidsandT riterpenoids:	
	Liebermann'sBurchardTest	-
	• SalkowskiTest	-
2.	TestforSaponins:	
	• FoamTest	-

Table 6.Phytochemical screening for extract of Eclipta Alba Extract.

3.	Tests for Alkaloids:	
	• Hager'sTest	+
	• Mayer'sTest	+
4.	TestsforGlycosides:	
	• Borntrager'sTest	+
		I.
	• KellerKillianiTest	+
5.	Tests for Tannins	
	andPhenoliccompou nds:	
	Gelatin Test	+
	Ferric Chloride Test	+
	Lead Acetate Test	+
	• Dilute Nitric acid Test	+
6.	Tests for Flavonoids:	
	• Ferric chloride Test	+
	• Alkaline reagent Test	-
	• Lead acetate Test	+
7.	Tests for Proteins:	
	• BiuretTest	+
	Xanthoproteic Test	+
8.	Test for Carbohydrates:	
	• Fehling Test	+

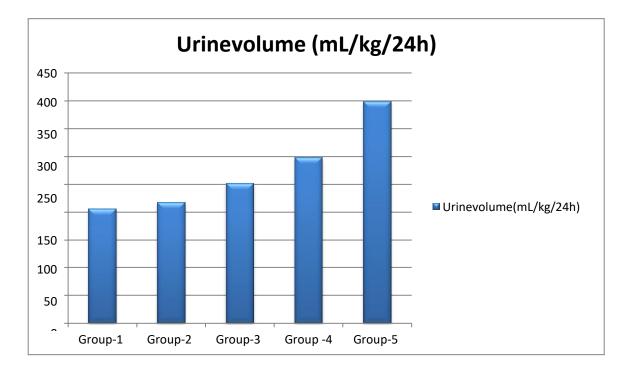
Diuretic activity

The administration of extracts derived from Eclipta Alba exhibited a notable enhancement in urinary output and the excretion of electrolytes, specifically sodium and potassium, when compared to the control group. Conversely, the chloroform extract of Eclipta Alba did not produce a significant effect on urine output or the concentrations of sodium, potassium, and chloride ions in the urine. The diuretic potential of all groups was evaluated by measuring urine volume and the concentrations of sodium, chloride, and potassium ions in the urine.

TreatmentGrou	Urine	Na+((mEq/kg/2	K+(mEq/kg/24h	Cl-
ps	volume(mL/	4h))	(mEq/kg/24h)
	kg/24h)			
	• • • • · ·			
Group-1	205.61	22.12	19.47	21.23
Group-2	217.24	58.12	51.25	43.65
Group-3	251.22	112.13	105.62	65.21
Group-4	298.32	165.20	155.20	58.23
Group-5	398.75	101.62	95.11	43.27

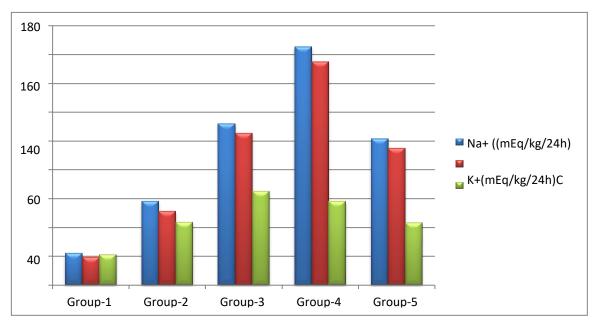
 Table7.Diuretic activityofdifferentextractsofEcliptaAlbaExtract

(n=5ineachgroup)



$Graph 4. Diuretic activity of different extracts of {\it EcliptaAlba Extract}$

(n=5ineachgroup)



Graph5.Concentration of Electrolytes in Urine.

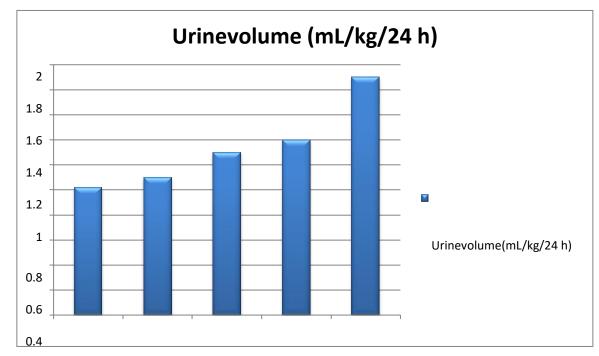
Urine out put index

The Urine index in negative control group was slightly decreased in groups treated with furosemide. Slightly increased in groups receiving the extract with the doses. However, these changes were not statistically significant compared to the control. Diuretic index of the plant extract is lower than that of furosemide.

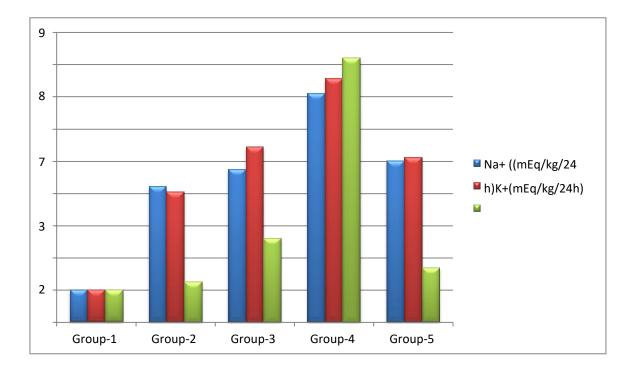
TreatmentGrou	Urine	Na+((mEq/kg/2	K+	Cl-
ps	volume(mL/	4h)	(mEq/kg/24h)	(mEq/kg/24h)
	kg/24h)			
Group-1	1.02	1	1	1
Group-2	1.1	4.21	4.05	1.27
Group-3	1.3	4.75	5.45	2.60
Group-4	1.4	7.10	7.56	8.20

Group-5	1.9	5.02	5.12	1.70	

Table8.Urineoutputindex



Graph6. Diuretic index: urine volume of test group/urine volume of control group.



Graph7.Na+ &K- index, sodium & potassium excretion in test group/sodium excretion in control group.

DISCUSSION:

Diuretic medications enhance urine production and facilitate the elimination of excess water from both extracellular and intracellular spaces within edematous tissues. They achieve this by either augmenting the glomerular filtration rate (GFR) or reducing fluid reabsorption from the tubules. These pharmacological agents, commonly referred to as diuretics, exhibit beneficial effects in various life-threatening medical conditions, including congestive heart failure, nephritic syndrome, cirrhosis, renal failure, and hypertension. Naturally occurring diuretics, such as caffeine found in coffee, tea, and cola, inhibit the reabsorption of sodium ions, while alcohol present in beer, wine, and mixed drinks inhibits the secretion of anti-diuretic hormones.

In recent years, there has been an increased interest in exploring herbal remedies as therapeutic options due to their minimal side effects. Extensive animal studies have indicated that most of these herbal products are non-toxic. However, there have been a limited number of isolated reports focusing on understanding the mechanisms of diuretic activity in certain Indian medicines.

Eclipta alba, also known as False Daisy or Bhringraj, is a medicinal plant traditionally used in Ayurvedic medicine to address various health conditions. While it is commonly associated with potential benefits for hair health, its diuretic properties have also been investigated.

A diuretic refers to a substance that stimulates urine production and enhances the elimination of water and electrolytes from the body. Diuretics are frequently employed to manage conditions such as hypertension, edema (fluid retention), and specific kidney disorders.

Studies have suggested that Eclipta alba may possess diuretic effects. The plant contains bioactive compounds such as wedelolactone, eclalbasaponins, and flavonoids, which are believed to contribute to its diuretic activity.

One study, published in the Journal of Ethnopharmacology, examined the diuretic activity of Eclipta alba in rats. The findings demonstrated that the ethanolic extract of Eclipta alba significantly increased urine output and sodium excretion, thereby exhibiting notable diuretic effects. Another study, published in the Indian Journal of Pharmacology, evaluated the diuretic activity of Eclipta alba in human volunteers. The researchers observed an increase in urine output and sodium excretion among the participants who received the Eclipta alba extract.

Nevertheless, it is important to note that further research is required to comprehensively comprehend the mechanisms of action of Eclipta alba, determine its efficacy and safety in humans, and assess its overall potential as a diuretic agent. Moreover, it is always advisable to consult with a healthcare professional before using any herbal remedies or supplements, including Eclipta alba, especially if one has underlying medical conditions or is taking medications.

The present investigation aimed to evaluate the diuretic effects of the plant extract derived from Eclipta alba and to standardize the phytochemical composition of the extract. To assess the diuretic activity, Lipschitz procedures were employed on rat models. The findings of our study led to the following conclusions:

The Eclipta alba plant extract exhibited distinctive characteristics such as an herbal aroma, a fine and light green powder appearance, a dark green color, a fine and smooth texture, a bitter taste, and a consistently fine texture with slight granularity.

The extractive values of the Eclipta alba plant extract were determined to be 15.52% w/w in ethanol and 10.21% w/w in the aqueous solution.

The moisture content of the Eclipta alba plant extract was found to be 1.25% w/w, while foreign matter constituted 1.76% w/w. Additionally, the extractive value was measured to be 14.23% w/w.

The Eclipta alba extract exhibited a total ash content of 5.15% w/w, an acid insoluble ash content of 4.15% w/w, a water insoluble ash content of 7.40% w/w, and a loss on drying of 4.15% w/w. Our study unveiled the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, tannins and phenol compounds, and fixed oils and fats in all extracts of Eclipta alba. However, steroids were exclusively detected in the petroleum ether, ethyl acetate, and aqueous extracts. Alkaloids were present in the chloroform, methanol, and aqueous extracts. Saponins were present in the chloroform, ethanol, and aqueous extracts. Terpenes, on the other hand, were absent in all the extracts. Compared to other solvent extracts, the aqueous extracts exhibited higher concentrations of secondary metabolites. The literature also reports the phytochemical screening of this plant using various solvents.

The different extracts derived from Eclipta alba demonstrated an increasing diuretic activity in a dose-dependent manner. This was evidenced by the elevated urine volume and electrolyte levels, including sodium (Na+), potassium (K+), and chloride (Cl-), with higher concentrations of the extract, indicating a potential diuretic effect. The urine output index served as a parameter to gauge the relative urine volume and electrolyte excretion in each treatment group. Higher values indicated augmented urine production and electrolyte excretion in comparison to baseline levels

CONCLUSION:

The findings of the present study indicate that Eclipta alba exhibits potential as a diuretic agent, offering a promising alternative to currently available diuretics with commonly observed adverse effects. Nonetheless, further investigations are warranted to determine the specific active phytochemical constituent and elucidate the precise mechanism underlying its diuretic effects.

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