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EVALUATION OF DIURETIC ACTIVITY OF CENTELLA ASIATICA ROOTS IN WISTAR RATS

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ABSTRACT

Medicinal plants have been important in treating many diseases since ancient times. Herbal medicine still gives important ideas for making new drugs today. It is known for its medicinal properties, which include healing wounds, protecting the nervous system, reducing inflammation, fighting bacteria and viruses, acting as an antioxidant, and helping the body adapt. Synthetic diuretics such as furosemide, hydrochlorothiazide, and spironolactone can be beneficial; however, they may induce electrolyte imbalance, dehydration, metabolic alkalosis, and hypokalemia. To find out the percentage yield of the ethanolic extract, the following formula was used: The weight of the extract obtained is the same as the percentage yield. The weight of the plant powder used times 100 To find the percentage yield, multiply the weight of the plant powder used by 100 and then divide that by the weight of the extract obtained.

KEYWORDS: Wistar rats, hypokalemia, Centella asiatica roots.

1. INTRODUCTION

Medicinal plants have played a vital role in the management of various diseases since ancient times. Herbal medicine continues to provide important therapeutic leads for modern drug development. Among the many medicinal herbs, *Centella asiatica* (family: Apiaceae), commonly known as Gotu Kola or Indian pennywort, is widely used in Ayurveda, Unani, and traditional Chinese medicine.

Centella asiatica is a perennial creeping herb found throughout tropical and subtropical regions. It is known for its pharmacological properties such as wound healing, neuroprotective, anti-inflammatory, antimicrobial, antioxidant, and adaptogenic effects. Traditionally, *Centella asiatica* has also been used as a mild diuretic and blood purifier.

Diuretics are agents that promote the excretion of water and electrolytes through the kidneys. They play a critical role in the management of hypertension, congestive heart failure, renal failure, and edema. However, synthetic diuretics such as furosemide and thiazides can cause side effects including electrolyte imbalance and dehydration. Therefore, there is growing interest in evaluating herbal alternatives with fewer adverse effects.

Diuretics and Their Importance

Diuretics are pharmacological agents that promote the excretion of **water and electrolytes** (mainly sodium and chloride) through the kidneys. They play a central role in the management of:

- **Hypertension**
- **Congestive heart failure**
- **Edematous states** associated with renal or hepatic disease
- **Toxin elimination** through enhanced urine output

Despite their therapeutic value, synthetic diuretics (such as furosemide, hydrochlorothiazide, and spironolactone) are often associated with **electrolyte imbalance, dehydration, metabolic alkalosis**, and **hypokalemia**. Hence, there is a growing interest in herbal diuretics that can provide comparable efficacy with fewer side effects.

Mechanism of Diuretic Action

Diuretics act at different sites of the **nephron**—the functional unit of the kidney—by inhibiting ion transporters and preventing reabsorption of sodium and chloride.

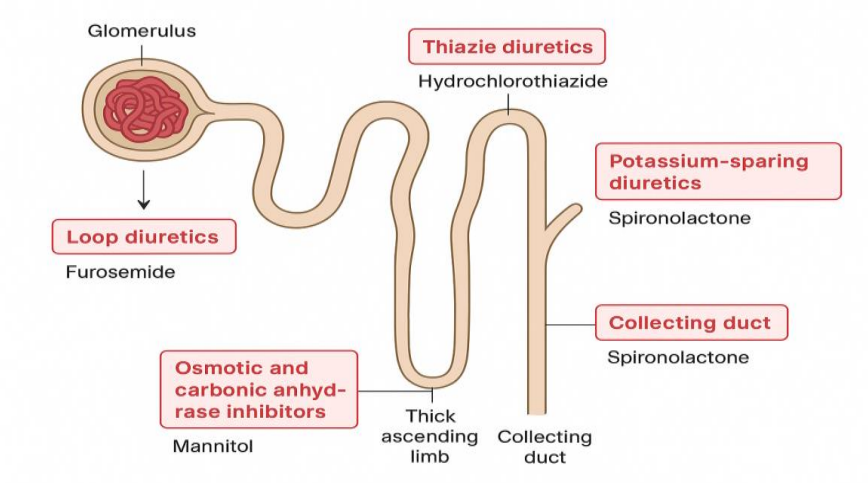


Figure 1: Diagram of the Nephron Showing Sites of Diuretic Action.

Classification of Diuretics

Table 1: This figure illustrates how different classes of diuretics influence sodium and water excretion by acting on specific regions of the nephron.

Class	Example	Site of Action	Remarks
Carbonic Anhydrase Inhibitors	Acetazolamide	Proximal Tubule	Weak diuretic
Osmotic Diuretics	Mannitol	PCT & Descending Limb	Used to reduce intracranial pressure
Loop Diuretics	Furosemide	Thick Ascending Limb	Potent diuretic
Thiazide Diuretics	Hydrochlorothiazide	DCT	Moderate, long-acting
Potassium-Sparing Diuretics	Spironolactone	Collecting Duct	Prevents K^+ loss

Phytochemistry

The root of *Centella asiatica* contains:

- **Triterpenoids:** Asiatic acid, madecassic acid, asiaticoside, madecassoside
- **Flavonoids:** Quercetin, kaempferol
- **Volatile oils, tannins, and glycosides**

These bioactive compounds are known to exhibit anti-inflammatory, wound-healing, neuroprotective, and nephroprotective activities, which may also contribute to its diuretic properties.

1.7 Rationale for the Study

Traditional medicinal literature describes *Centella asiatica* as a **"mutrala" (diuretic)** herb. However, most pharmacological studies focus on the aerial parts, while the **root extract** remains underexplored despite being rich in triterpenoids.

Therefore, this study aims to **evaluate scientifically the diuretic potential** of the Ethanolic **root extract of** *Centella asiatica* using an experimental rat model. Results may validate traditional claims and provide a basis for developing safer herbal diuretics.

Several reports exist on the aerial parts of *Centella asiatica*, the roots are relatively less explored despite being rich in asiatic acid and related triterpenoids. Traditional healers use the root decoction as a mild diuretic to treat urinary burning and edema, yet there is limited experimental validation.

Thus, evaluating the ethanolic extract of *Centella asiatica* roots for diuretic activity could provide scientific evidence for its ethnopharmacological claims and open possibilities for developing natural diuretic formulations with minimal side effects.

Background

Water and electrolyte balance in the human body is primarily maintained by the kidneys. The kidneys regulate extracellular fluid volume, osmotic balance, and acid–base homeostasis through filtration, reabsorption, and secretion mechanisms. In pathological conditions such as **hypertension**, **congestive heart failure**, **renal failure**, and **hepatic cirrhosis**, these processes are impaired, leading to **edema** and **fluid overload**.

To manage these conditions, **diuretic agents** are prescribed to promote excretion of excess water and electrolytes from the body. However, the long-term use of synthetic diuretics often results in adverse effects such as **electrolyte imbalance, hypokalemia, metabolic disturbances**, and **renal irritation**. Hence, identifying **safer natural alternatives** from medicinal plants has become a focus of pharmacological research.

The Need for Herbal Diuretics

1. Herbal diuretics have gained attention because:
2. They exhibit mild to moderate diuretic action without causing marked electrolyte loss.
3. Many possess antioxidant, anti-inflammatory, and nephroprotective effects, supporting kidney health.
4. They are cost-effective, accessible, and safer for long-term therapy.

5. Several ethnobotanical surveys report the use of medicinal plants such as *Tribulus terrestris*, *Boerhaavia diffusa*, *Asparagus racemosus*, and *Centella asiatica* in urinary disorders.

Among these, *Centella asiatica* is particularly interesting due to its broad pharmacological profile and traditional use in promoting renal and urinary function.

About *Centella asiatica*

Centella asiatica (L.) Urban is a perennial, creeping herb native to India, Sri Lanka, and other parts of Asia. It is commonly known as **Gotu Kola** or **Indian Pennywort**. In Ayurveda, it is described as **"Mandukaparni"**, a herb with **rejuvenating (Rasayana)**, **memory-enhancing**, and **mutrala (diuretic)** properties.

Botanical Description

Family: Apiaceae (Umbelliferae)

Morphology: Slender creeping stems, orbicular-reniform leaves, small pinkish flowers, and thin roots.

Habitat: Moist, shady areas; often found near paddy fields and wetlands.

Traditional Uses

Used as a **tonic for the brain**, **wound healing**, **anti-inflammatory**, **antioxidant**, and **diuretic agent** in Indian and Chinese traditional medicine systems.

Phytochemical Profile

Phytochemical studies of *Centella asiatica* have identified several **bioactive triterpenoids**, including:

- **Asiatic acid**
- **Madecassic acid**
- **Asiaticoside**
- **Madecassoside**

1. In addition, it contains **flavonoids (quercetin, kaempferol)**, **tannins**, **steroids**, and **volatile oils.**
2. These compounds are known for **anti-inflammatory, wound-healing, neuroprotective, hepatoprotective**, and **renal-protective** properties.
3. Triterpenoids are believed to play a central role in enhancing renal function and may inhibit tubular reabsorption of electrolytes, thereby increasing urine flow.

Significance

- Provides scientific validation of a traditionally used diuretic plant.
- May contribute to the development of safe, plant-based diuretics.
- Encourages phytochemical standardization and pharmacological profiling of *Centella asiatica* roots.
- Strengthens the interface between traditional knowledge and modern pharmacology.

Preparation

1. Prepare the sample: Grind or crush the sample to increase surface area.
2. Choose a solvent: Select a suitable solvent for the extraction (e.g., hexane for fat extraction).
3. Assemble the apparatus: Set up the Soxhlet apparatus, ensuring all connections are secure.

Procedure

1. Add sample to thimble: Place the sample in the thimble, usually made of filter paper or cellulose.
2. Add solvent to flask: Add the solvent to the round-bottom flask.
3. Heat the flask: Heat the flask using a heating mantle or water bath.
4. Start extraction: The solvent vaporizes, condenses, and drips into the thimble, extracting the sample.
5. Allow extraction to run: Let the process run for several hours (typically 4-24 hours).
6. Stop heating: Stop heating and let the apparatus cool.
7. Collect extract: Collect the extract in the flask and evaporate the solvent if needed.

Post-extraction

1. Clean the apparatus: Clean the apparatus thoroughly to prevent contamination.
2. Analyze the extract: Analyze the extract using techniques like chromatography or spectroscopy.

AIM AND OBJECTIVE

Aim

To **evaluate the diuretic activity** of the **ethanolic extract of** *Centella asiatica* **roots** in Wistar albino rats and compare its effects with the standard drug **furosemide**.

Objectives

- ✓ To collect, authenticate, and extract the roots of *Centella asiatica* using ethanol.
- ✓ To perform preliminary phytochemical screening of the ethanolic extract.
- ✓ To evaluate the diuretic effect of the extract in Wistar rats.
- ✓ To compare the urine volume and electrolyte excretion with that of the standard drug, furosemide.

MATERIALS AND METHODS

Sl.No	Chemical name	Manufactured by
1.	Ethanol	China Changshu Yangyuan Chemica
2.	Furosemide	Nice chemicals pvt.lid., kochi
3.	Normal saline	Burgoyne urbidgess& co. (India). Mumbai.
4.	Corboxymethyl cellulose	Hetal
5.	C.asiatica roots	S.D.Fine-Chem.Limited.,Mumbai
6.	Potassium (K^+)	Pharma.chem,hyderabad.
7.	Chloride (Cl^-)	Pharma.chem, hyderabad
8.	Sodium	Pharma.chem, hyderabad

METHODS

Preparation of ethanolic Extract

1. The roots of Centella asiatica that were gathered were washed with water to get rid of dirt and other things. Then, they were dried in the shade at room temperature for 10 to 15 days.
2. A mechanical grinder was used to roughly grind the dried roots.
3. About 250 g of the powdered root material was extracted by using 95% ethanol in a Soxhlet apparatus for 18 to 20 hours.
4. A rotary evaporator was used to concentrate the extract at low pressure, which left behind a thick, dark brown residue.
5. The residue was dried in a desiccator, weighed, and stored in an airtight container at 4°C until it was needed.

To find out the percentage yield of the ethanolic extract, the following formula was used: The weight of the extract obtained is the same as the percentage yield. The weight of the plant powder used is 100 times more than the weight of the plant.

Percentage yield is the amount of extract you get. The weight of the plant powder used times 100 To find the percentage yield, multiply the weight of the plant powder used by 100 and then divide that by the weight of the extract obtained.

Phytochemical Testing

We used standard methods to do preliminary phytochemical tests on the ethanolic extract to see if it had important parts like Alkaloids (Dragendorff's and Mayer's tests), Flavonoids (Shinoda test), Saponins (Foam test), Tannins and phenolic compounds (Ferric chloride test), Triterpenoids and steroids (Liebermann–Burchard test), and Glycosides (Keller–Killiani test).

Experimental Animals: Wistar albino rats Sex: Any sex Source: [insert institution name] Weight range: 150–200 g Central Animal House Facility.

Housing conditions: Polypropylene cages with bedding made of paddy husk, a 12-hour light/dark cycle, a temperature of 25 ± 2°C, and a relative humidity of 55 ± 10%. Food: Regular pellets and unlimited water access.

Acute Toxicity Study

No mortality or behavioral abnormalities were observed up to **2000 mg/kg** (p.o.), suggesting that the extract is safe for pharmacological use. Therefore, **100 mg/kg** (1/20th of maximum safe dose) was selected for the study.

5.3. Diuretic Activity

Table 5.2: Effect of ethanolic extract of Centella asiatica roots on urine volume and electrolyte excretion.

Group	Dose	Urine vol ml	Na+	K+	Cl-
Control	25 ml/kg	2.5 ml	334.4	391.2	50
Standard	15 mg/kg	13.5 ml	358.8	165.8	79
Low Dose	250 mg/kg	5.9 ml	188.4	323.3	66
High Dose	500 mg/kg	8 ml	226.2	337.7	77

Values are Mean ± SEM, n = 6.

Significance compared to control:

p < 0.05, ** p < 0.01, *** p < 0.001 (ANOVA followed by Dunnett's test).

Diuretic Index and Lipschitz Value

Parameter	Furosemide(15mg/kg)	C. asiatica(250mg/kg)	C. asiatica(500mg/kg)
DiureticIndex(DI)	2.83	1.80	3.30
LipschitzValue(LV)	1.00	0.64	1.5

Interpretation

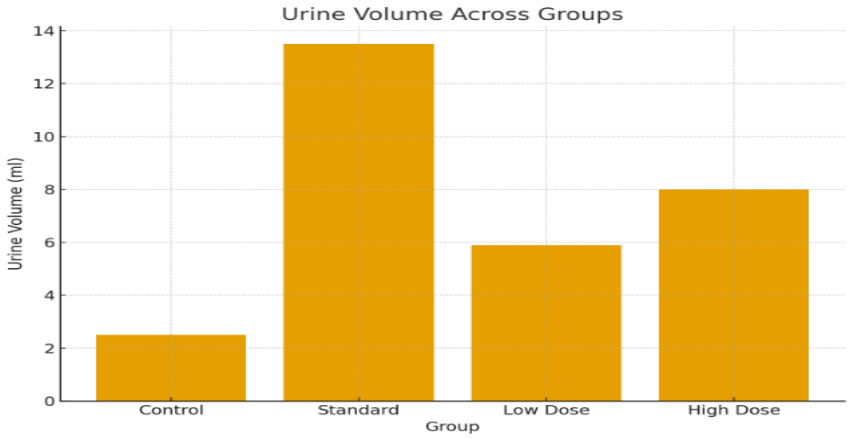
- The extract increased urine output by ~80% compared to control.

The Lipschitz value (0.64) suggests the extract exhibits approximately 64% of the diuretic potency of furosemide at the tested dose.

RESULTS

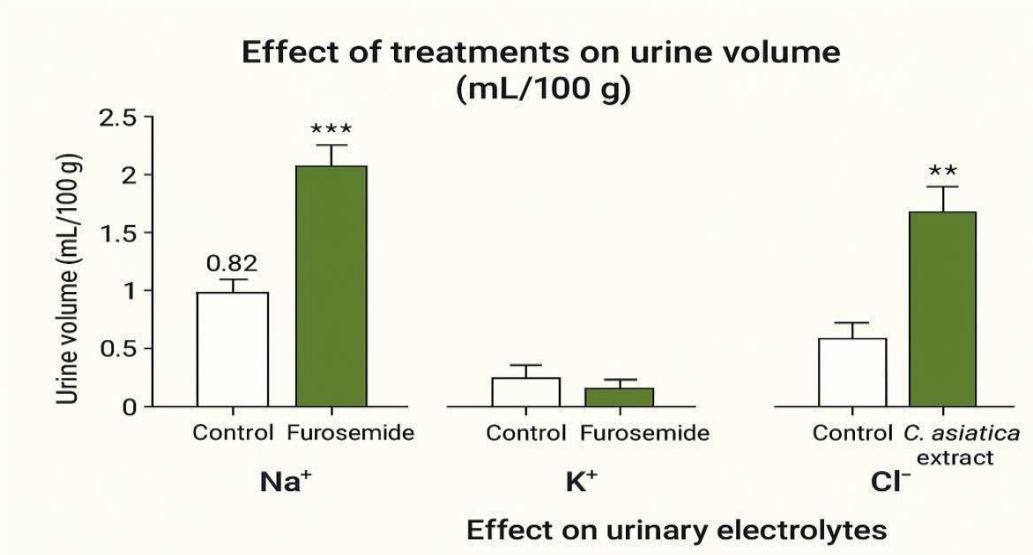
Graphical Representation

Effect of treatments on urine volume (mL/100 g)



Treatment	Urine Volume (Mean±SEM)
Control	0.82±0.04
Furosemide	2.32±0.12
C. asiatica Extract	1.48±0.08

Graph: Y-axis – Urine volume



DISCUSSION

1. The current study illustrated that the ethanolic extract of Centella asiatica roots markedly enhanced urine production and electrolyte elimination in Wistar rats, thereby validating its diuretic efficacy.
2. The rise in urine volume and Na^+ and Cl^- excretion indicates that the extract enhances saluretic activity (NaCl excretion), akin to loop diuretics.
3. The moderate K^+ excretion and Na^+/K^+ ratio >2 suggest a balanced diuretic effect with a low risk of hypokalemia when compared to synthetic agents.
4. Phytochemical analysis showed that triterpenoids and flavonoids were present. These compounds are known to affect how the kidneys work and may stop sodium from being reabsorbed in the nephron.

SUMMARY AND CONCLUSION

The current study was conducted to assess the diuretic efficacy of the ethanolic extract of Centella asiatica roots in Wistar albino rats. The study sought to substantiate the conventional application of this plant in the treatment of urinary and renal ailments.

Parameter	Control	Furosemide (20 mg/kg)	C. asiatica (100 mg/kg)
Urine Volume (mL/100 g)	0.82 ± 0.04	2.32 ± 0.12	1.48 ± 0.08
Na^+ (mEq/L)	38.5 ± 1.6	78.6 ± 2.3	59.2 ± 2.1
K^+ (mEq/L)	22.1 ± 1.1	34.5 ± 1.8	28.9 ± 1.3
Cl^- (mEq/L)	30.8 ± 1.3	63.9 ± 2.4	49.6 ± 1.8
Na^+/K^+ Ratio	1.74	2.28	2.05
Diuretic Index	1.00	2.83	1.80
Lipschitz Value	-	1.00	0.64

Summary finding table-7

CONCLUSION

- ✓ The ethanolic extract of Centella asiatica roots at 100 mg/kg demonstrated a significant enhancement in urine production and electrolyte elimination, confirming its moderate diuretic efficacy relative to the standard medication furosemide.
- ✓ The study's main steps were: Gathering and extracting: Fresh roots of Centella asiatica were gathered, dried in the shade, ground into a powder, and then extracted with ethanol using a Soxhlet apparatus. The yield was concentrated, kept, and used for drug testing.
- ✓ Phytochemical screening: The ethanolic extract contained triterpenoids, flavonoids, saponins, tannins, and glycosides, which are known to affect kidney function and help with diuretic activity.
- ✓ Acute toxicity study: There were no deaths or signs of toxicity up to 2000 mg/kg, which means the extract is safe. Thus, 100 mg/kg (p.o.) was chosen for the pharmacological assessment.

Experimental design: Wistar rats were categorized into three groups: control, standard (furosemide 20 mg/kg), and test (extract 100 mg/kg). They collected urine for six hours after giving the drug.

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