

Pharmacological Evaluation Of Diuretic Activity Of Ethanolic Extract Of Camellia Sinensis Leaves In Albino Rats

Himani Rana*, Ajeet Pal Singh, Amar Pal Singh

Department of Pharmacology, St. Soldier Institute of Pharmacy, Lidhran Campus, Behind NIT (R.E.C.), Jalandhar –Amritsar by pass, NH-1, Jalandhar -144011, Punjab, India.

ABSTRACT

Objective: The study delves into the diuretic potential of an alcoholic extract derived from the leaves of Camellia Sinensis.

Methods: Male animals were separated into groups for the investigation. Two doses of Camellia Sinensis alcoholic extract (200.0 mg/kg and 400.0 mg/kg) were given to test its diuretic effects. Additionally, a standard diuretic, Frusemide (10.0 mg/kg), was included as a reference for comparative analysis. Urinary output measurements were conducted to gauge the impact of these substances. Moreover, the concentrations of key ions in the urine, including Sodium, Potassium, and Chloride, were quantified to provide a comprehensive view of the diuretic activity.

Results: The study findings are noteworthy. Both doses of the Camellia Sinensis extract, as well as the standard diuretic (Frusemide), produced substantial increases in urinary output. Of particular significance, the 400 mg/kg dosage of the Camellia Sinensis extract exhibited diuretic activity that was comparable to Frusemide, a well-established diuretic medication. Furthermore, through phytochemical analysis, the presence of specific phytoconstituents recognized for their diuretic effects was identified in the extract. These findings serve to corroborate the traditional wisdom associated with Camellia Sinensis as a diuretic agent.

Conclusion: In conclusion, the alcoholic extract derived from Camellia Sinensis leaves, particularly at the 400 mg/kg dosage, demonstrated significant diuretic activity. This suggests developing new diuretics or improving current ones. Further research is needed to isolate and evaluate the therapeutic diuretic constituents present in Camellia Sinensis. These compounds may hold the key to treating conditions associated with edema, offering safer and more effective alternatives to conventional diuretics that may carry adverse side effects.

Keywords: Camellia Sinensis, diuretic activity, phytoconstituents, ethanol extract, traditional herbal medicine, fluid retention, edema, phytochemical analysis.

INTRODUCTION

The human kidney is a pair of reddish bean-shaped organs situated on each side of the spine, under the rib cage. They are around 10 to 12 cm in size, approximately the size of a handful. The human excretory system, which includes the kidneys as its primary organs, filters blood before urine is produced [1]. The kidneys are two organs that resemble beans. The renal cortex, also known as the peripheral cortex, the inner medulla, sometimes known as the renal medulla, and the renal pelvis are the three primary parts of the kidney [2]. Diuretics are medications that change how the kidneys work. They treat renal and

cardiovascular conditions. Drugs and their metabolites are removed from the body in large part by the kidneys. Drugs known as diuretics cause an increase in the excretion of sodium and water ions. The kidney's primary job is to keep the body's internal environment stable by eliminating waste, controlling body temperature, and preserving fluid volume, pH, and electrolyte balance. In order to prevent major consequences like decreased cardiac blood output or renal failure, salt intake and removal must be managed completely [3-4].

The world's most popular non-alcoholic beverage is green tea, which is also the most consumed beverage

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

after water. The flavours of Indian teas are known around the globe. After China, India is the world's second-largest producer of tea. A limited amount of tea is grown in Southern India (Tamil Nādu), whereas the bulk is produced in the Northeast (West Bengal and Assam) and the Northern region (Himachal Pradesh). The tea plant *Camellia sinensis*, a member of the *Camellia sinensis* family, produces tea from the terminal leaves of its shoots. There are two different species of *Sinensis*: *Sinensis* and *Assamica*. *Camellia sinensis* var. *sinensis* is native to Darjeeling, Japan, and South East China. Assam, Thailand, and Sri Lanka are the native habitats of *Camellia sinensis* var. *assamica*. Every year, 700,000 kg of tea are produced in the Kangra area utilising the age-old process of roasting, rolling, and sun-drying tea shoots to render them inactive [5-6]. Kangra The most popular beverage is green tea (*Camellia sinensis*, a member of the *Theaceae* family), due to its unique flavour and scent. Despite having a somewhat bitter flavour, Kangra green tea contains more antioxidants than Darjeeling green tea [7]. Green tea's and its constituent catechins' antioxidant qualities were assessed in relation to a few diseases linked to reactive oxygen species. It has been shown that the antioxidant properties of polyphenols found in teas have both chemo-protective and therapeutic properties. The term polyphenols refer to the catechin that is present in green tea. (-)- epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and (-)-epigallocatechin-3-gallate are the main catechins in green tea. By scavenging reactive oxygen and nitrogen species and retaining metal ions, they function as antioxidants. By scavenging free radicals, a polyphenol extract from *Camellia sinensis* reduced liver transplant damage and increased survival. It has been proposed that the antioxidant properties of polyphenols found in teas have both chemoprotective and therapeutic potential [8].

MATERIAL & METHODS

Drugs and Reagents/ Chemical:

Every chemical and biological reagent utilised in this investigation was of analytical quality and was made just before use. All analytical-grade chemicals were purchased from S. D. Fine Chem. Ltd. in India and Sigma Chemical in the United States.

Collection and identification of plant

The *Camellia Sinensis* plant's leaves were gathered at the St. Soldier Institute of Pharmacy's herbal garden in Jalandhar. Dr. (Mrs.) Sunita Garg, Head of the Raw Materials Herbarium & Museum, Delhi (RHMD), National Institute of Science Communication and Information Resources (CSIR-NISCAIR), New Delhi, discovered and morphologically verified this plant. NISCAIR/RHMD/Consult/2020/3705-06 dated November 27, 2020 is the reference number.

Preparation of plant material

The *Camellia Sinensis* plant's leaves were thoroughly cleaned to get rid of any debris, then ground to a fine powder while preventing moisture contamination. After passing through a screen to achieve a consistent particle size, the powdered crude medication was dissolved in a number of solutions to ascertain its ideal solubility.

Preparation and administration of crude extracts/standard medicines

The most frequent method for extracting active ingredients from crude medicines is extraction. Standard extraction methods use selected solvents to separate medicinally active plant or animal tissues from inactive or inert components. Plants provide impure liquids, semisolids, and powders for oral or external application. Standardized extraction processes for crude medicines (medicinal plant parts) use menstrum to remove undesirable substances and extract therapeutically useful sections. After standardization, the extract may be utilised medicinally. These extracts include a complex combination of alkaloids, glycosides, terpenoids, flavonoids, and lignans [9-11].

Procurement of Animals

An in-vivo research was conducted with the IAEC's required consent. With Protocol No. IAEC/SSIP/2023/PR-034, the Institutional Animal Ethics Committee (IAEC) accepted and approved the experimental study's protocol and process. For the experiment, healthy adult albino rats of either sex weighing between 150 and 200 g will be kept in a conventional laboratory setting with a temperature of



$25 \pm 2^\circ\text{C}$ and a 12-hour light-dark cycle. There will be unlimited access to food and drink.

Experimental Design:

For the research, "Wistar rats" were used. between 150 and 200 grammes in weight. The animal was housed under controlled circumstances in cages made of polypropylene. Normal circumstances in a laboratory: 12-hour cycle of light and dark, with a constant temperature of $25 \pm 2^\circ\text{C}$ $60 \pm 5\%$ relative humidity. The animal is fed a standard pellet diet and unlimited water. The animals were split up into four groups, with six rats in each group. As a normal control, group I will receive vehicle (normal saline 10 ml/kg b.wt.), while group II will receive Furosemide

(10 mg/kg, p.o.). Groups III and IV will receive low and high doses of ethanolic extract of *Camellia Sinensis* leaves in vehicle. All rats will be hydrated with saline (10 ml/kg) immediately following the extract treatment, and they will be placed in metabolic cages (two per cage), which are specifically made to separate urine and faeces, and maintained at $21^\circ\text{C} \pm 0.5^\circ\text{C}$. Every five hours, the total volume of urine collected for 24 hours (ml/kg b.wt/5 h) will be measured. Animals will have food accessible to them at this time. Numerous parameters will be measured and estimated, such as the total volume of urine and the concentrations of sodium, potassium, and chloride in the urine. It has been shown that the Lipschitz test is a reliable and effective technique for identifying possible diuretics.

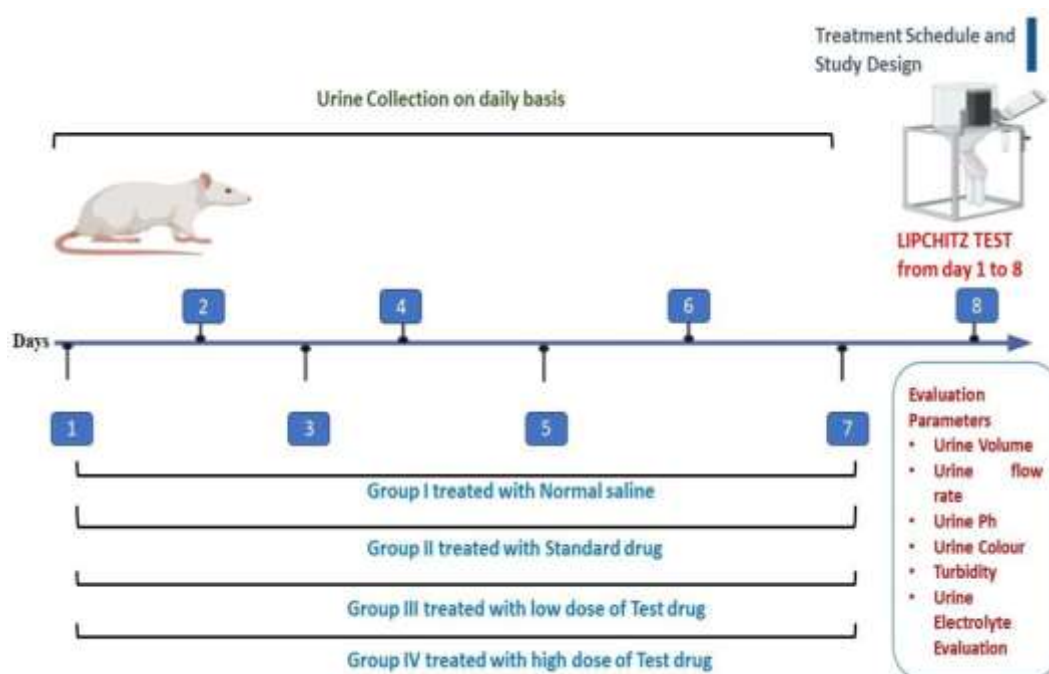


Figure 1: Diagrammatical representation of Treatment schedule and study design in In Vivo studies.

Experimental parameters

Many health conditions associated with oedema, such as congestive heart failure, nephritis, pregnancy-induced toxemia, premenstrual syndrome, and hypertension, can be managed with diuretics that promote water excretion. However, commonly used diuretics like thiazides and loop agents are often linked to adverse effects, including electrolyte disturbances and metabolic alterations. In contrast, several plants recognized in Ayurvedic medicine, such as *Abelmoschus esculentus*, *Bacopa monnieri*,

Barbarea vulgaris, and *Cissampelos pareira*, have been traditionally valued for their natural diuretic properties. [12-14].

Body weight analysis: -

Weekly body weight analysis will be documented concurrently with the research.

Statistical analysis: -



The data obtained will be analyzed using T-test or one way ANOVA (Graph pad prism version 5.00 software) followed by suitable post-test.

RESULTS

Acute oral toxicity study

As per OECD guideline 425, an acute oral toxicity test was conducted on five rats after overnight fasting, with ECS extract administered stepwise at doses up to 1000 mg/kg. The animals were observed for 24 hours and monitored over 14 days for physiological and behavioral changes, but no toxic effects or mortality were noted. The study concluded with the determination of the LD50 value.

The Lipschitz Test and estimation of Urine electrolytes

The diuretic activity of *Camellia sinensis* leaf ethanolic extract was evaluated in albino rats using the Lipschitz method. After acclimatization, animals were divided into four groups and treated with normal saline, frusemide (10 mg/kg), or extract doses of 200 and 400 mg/kg. Urine output was measured at intervals up to 24 hours under controlled conditions in metabolic cages. The extract significantly increased urine volume and electrolyte excretion (Na^+ , K^+ , Cl^-) in a dose-dependent manner, indicating notable diuretic potential. However, elevated potassium loss suggests a possible risk of hypokalaemia, highlighting the need for further investigation into its potassium-sparing effects, likely attributed to flavonoids and glycosides present in the extract.

Estimation of Urinary Electrolytes/Volume/pH

Urine electrolytes, including sodium, potassium, and chloride, were measured using the Ion Selective

Electrode method as outlined in the biochemical kit instructions (Roche Diagnostics Pvt. Ltd., Gurgaon, Haryana). In addition, urine volume, electrolyte concentrations (Na^+ , K^+ , Cl^-), and pH levels (evaluated with standard pH paper) were analyzed to assess diuretic activity.

Statistical analysis: Mean + SEM (n=6) were used to represent experimental findings. The research analyzed unpaired data using Student's t test, with a significance level of ≤ 0.05 and a probability level of ≤ 0.05 . The significance level was assessed.



Figure 2: Metabolic cage

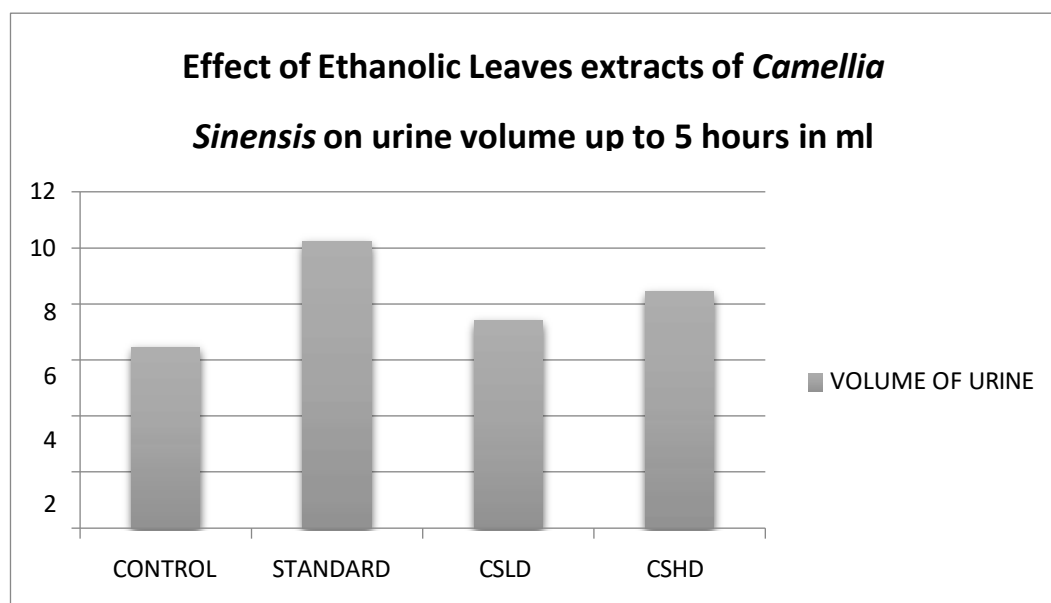
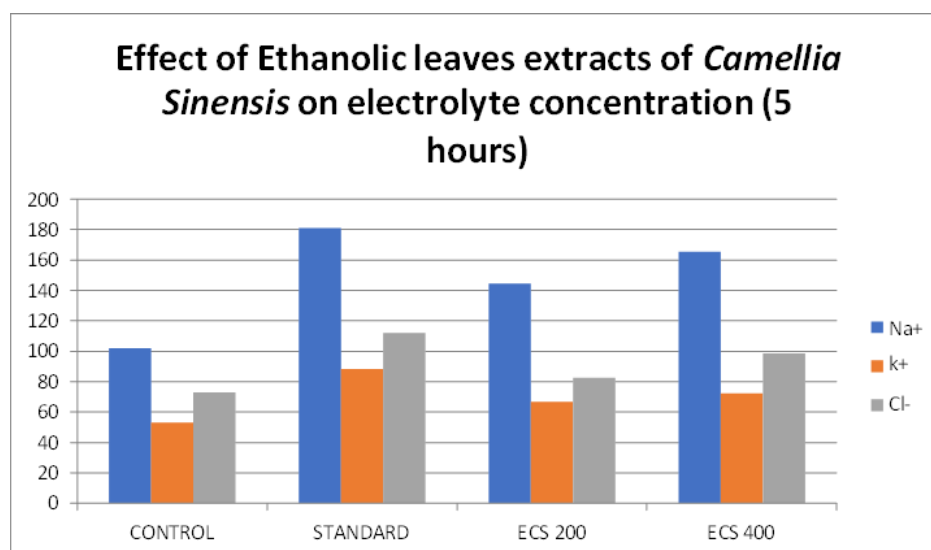
The alcoholic extract of leaves of *Camellia Sinensis* identify the phytoconstituents and it revealed the presence of phenolic compound, Proteins, tannins, glycosides, Carbohydrate, Starch, Vitamins & Minerals etc. In acute toxicity study all the animals were survived even after 14 days. This indicates that the extract was found to be safe up to the maximum dose level tested (1000 mg/kg). No major behavioral changes were observed during this period of study.

Table 1:- Details of Experimental Protocols for Lipschitz Test

Group Name	Number of Animals (n=6 per group)
Control group (received normal saline, 10 ml/kg body weight)	6
Standard group (treated with Furosemide, 10 mg/kg body weight)	6
Test group D1 (ethanolic leaf extract of <i>Camellia sinensis</i> , 200 mg/kg)	6
Test group D2 (ethanolic leaf extract of <i>Camellia sinensis</i> , 400 mg/kg)	6

Table 2:- Percentage yield and Preliminary Phyto-profile of Camellia Sinensis extract

<u>S. No.</u>	<u>Groups</u>	<u>Urine Volume (ml/5 h)</u>	<u>Na⁺ (μmol/L)</u>	<u>K⁺ (μmol/L)</u>	<u>Cl⁻ (μmol/L)</u>
1	Control (10 ml/kg body weight)	6.40 ± 0.10	101.32 ± 2.20	53.46 ± 1.60	73.88 ± 1.52
2	Standard (Furosemide 10 mg/kg body weight)	10.30 ± 0.06 †††	184.12 ± 2.14 †††	89.12 ± 1.70 †††	115.22 ± 1.62 †††
3	Alcoholic leaf extract of Camellia sinensis, low dose (200 mg/kg b.wt)	7.50 ± 0.08 †	145.10 ± 2.12 †	67.90 ± 2.62 †	81.42 ± 1.30 †
4	Alcoholic leaf extract of Camellia sinensis, high dose (400 mg/kg b.wt)	8.56 ± 0.06 ††	167.82 ± 2.28 ††	75.08 ± 1.72 ††	97.64 ± 2.50 ††

**Figure 3:- Effect of Ethanolic leaves extracts of Camellia Sinensis on Total Urine Volume (in 5 hours)****Figure 4:- Effect of Ethanolic leaves extracts of Camellia Sinensis on urinary electrolyte concentration (in 5 hours).**

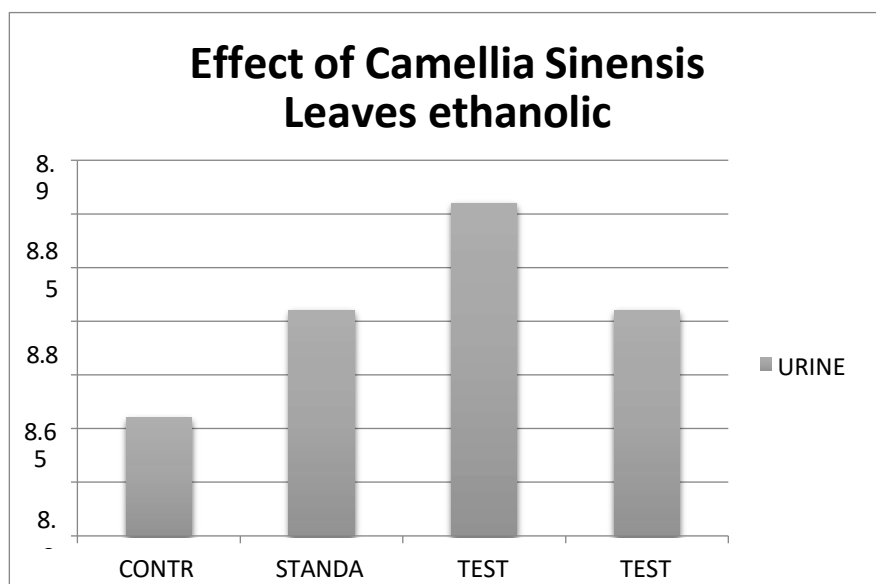


Figure 5:- showing effects of Ethanolic effect of Camellia Sinensis Leaves on pH in hydrated rats

Effects of Ethanolic extract of Camellia Sinensis on pH in hydrated rats

Compared to the control group, the alcoholic extract of *Camellia sinensis* leaves increased urine production and salt, potassium, and chloride ion excretion. Higher doses have stronger diuretic effects. Compared to furosemide, the extract affected urine volume and electrolyte excretion, showing its pharmacological importance. The electrolyte study revealed that the extract enhanced excretion of all main ions tested (Na^+ , K^+ , Cl^-). At the higher dosage, urine volume increased significantly, but urinary pH did not change between treatment groups. These data imply that *Camellia sinensis* leaf alcoholic extract may be an oral diuretic. This action may increase renal blood flow, which increases glomerular filtration rate, or reduce antidiuretic hormone (ADH) release, which increases urine output. Since urinary salt levels increased significantly, endogenous natriuretic peptides, which stimulate sodium and water clearance, may be another contributor.

DISCUSSION

The study demonstrated that the ethanolic extract of *Camellia sinensis* leaves produced a significant increase in urine output and the excretion of sodium, potassium, and chloride in albino rats, with the effects being more pronounced at the higher dose. These findings closely match the performance of frusemide,

a standard diuretic, particularly at 400 mg/kg, which showed diuretic activity comparable to frusemide. This supports traditional claims regarding the plant's diuretic properties and suggests its potential utility in managing conditions associated with fluid retention, such as edema.

Preliminary phytochemical analysis confirmed the presence of various active compounds, including phenolics, tannins, glycosides, and carbohydrates, which may contribute to the observed activity. The dose-dependent response implies that higher doses may yield more pronounced therapeutic effects, though the exact bioactive constituents involved still require precise identification.

CONCLUSION

The findings confirm that an alcoholic extract of *Camellia sinensis* leaves, especially at higher dosages, offers pronounced diuretic effects comparable to conventional drugs like frusemide. These results validate the plant's traditional use as a diuretic and suggest that further research should be devoted to isolating its active compounds and fully characterizing their pharmacological profiles. Plant-based diuretics may provide safer alternatives to synthetic drugs for managing fluid retention, edema, and related health conditions, paving the way for natural, effective, and affordable therapies in modern medicine.



AUTHORS CONTRIBUTIONS

All authors have contributed equally

Funding: Nill

Conflict of Interest: No conflict of interest

Acknowledgment

It's our privilege to express the profound sense of gratitude and cordial thanks to our respected chairman Mr. Anil Chopra and Vice Chairperson Ms. Sangeeta Chopra, St. Soldier Educational Society, Jalandhar for providing the necessary facilities to complete this research work.

Data Availability All data generated and analyzed are included within this research article..

REFERENCES

1. Dehkordi FR, Kamkhah AF. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. *Fundam Clin Pharmacol*. 2008;22(4):447-52.
2. Crowson MG, Patki A, Tucci DL. A systematic review of diuretics in the medical management of Ménière's disease. *Otolaryngology--Head and Neck Surgery*. 2016;154(5):824-34.
3. Agarwal R, Verma A, Georgianos PI. Diuretics in patients with chronic kidney disease. *Nature Reviews Nephrology*. 2025;21(4):264-78.
4. Kaur A, Kaur M, Kaur P, Kaur H, Kaur S, Kaur K. Estimation and comparison of total phenolic and total antioxidants in green tea and black tea. *Glob J Biosci Biotechnol*. 2015;4(1):116-20.
5. Gulati A, Rawat R, Singh B, Ravindranath SD. Application of microwave energy in the manufacture of enhanced-quality green tea. *J Agric Food Chem*. 2003;51(16):4764-8.
6. Kaur A, Jain Y, Mittal K, Mittal N. Synergistic effect of pineapple peels and green tea on antioxidant levels of pineapple peels infused Kangra green tea. *Int J Sci Res*. 2018;7(3):392-5.
7. Solanki D, Choudhary S, Vora A, Ghose T, Mantri RR, Modi N, Sawhney JP, Singhal A, Kumar A, Edakutty R, Pande A. Loop Diuretics Unique Mechanism of Action. *J Assoc Physicians India*. 2024;72:14-5.
8. Handa SS. An overview of extraction techniques for medicinal and aromatic plants. In: *Extraction Technologies for Medicinal and Aromatic Plants*. UNIDO & ICS-UNIDO, Italy. 2008;11,22-7.
9. Jarald EE, Jarald SE. *Textbook of Pharmacognosy and Phytochemistry*. 1st ed. New Delhi: CBS Publishers and Distributors Pvt. Ltd.; 2007:104-5.
10. Harwood LM, Moody CJ. *Experimental Organic Chemistry: Principles and Practice*. Illustrated ed. Oxford: Wiley-Blackwell; 1989:122-5. ISBN: 0-632-02017-2.
11. Ayoti R, Khan Z, Mlaw G, Gupta A. Assessing the Effectiveness and Safety of Combination Diuretic Therapy in Heart Failure: A Systematic Review and Meta-Analysis. *Cureus*. 2024;22;16(10).
12. Lipschitz WL, Hadidian Z, Kerpcsar A. Bioassay of diuretics. *J Pharmacol Exp Ther*. 1943;79:97-110.
13. Vogel HG, ed. Diuretic activity in rats (Lipschitz test). In: *Drug Discovery and Evaluation: Pharmacological Assays*. 2nd ed. Springer; 2002:522.
14. Tietz NW. *Clinical Guide to Laboratory Tests*. Philadelphia: WB Saunders Co; 1983:110,398,446.

HOW TO CITE: Himani Rana, Ajeet Pal Singh, Amar Pal Singh, Pharmacological Evaluation Of Diuretic Activity Of Ethanolic Extract Of *Camellia Sinensis* Leaves In Albino Rats, *J. Pharm. Sci.*, 2026, 2 (4), 1770-1776. <https://doi.org/10.5281/zenodo.19642674>

