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Phytochemical Screening and Evaluation of Diuretic Activity of Methanolic Extract of Leaves of Neolamarckia Cadamba

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ABSTRACT

The phytochemical components and diuretic efficacy of the methanolic extract of Neolamarckia cadamba leaves are investigated in this work. Rich phytoconstituent profile was indicated by preliminary phytochemical screening, revealing alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds. Using adult Wistar albino rats, the diuretic action was evaluated under a standard medication furosemide. Urinary volume and electrolyte excretion (Na^+, K^+, and Cl^-) showed a notable rise in the extract at two dosage levels—200 mg/kg and 400 mg/kg—versed against the control group. The promising diuretic action of the methanolic extract of Neolamarckia cadamba leaves supports its traditional usage in the treatment of fluid retention problems. More investigation is advised to separate and define the bioactive substances causing the reported pharmacological effects

Keywords: *Neolamarckia cadamba, diuretic action, phytochemical screening, methanolic extract, electrolyte excretion, Wistar albino rats, traditional medicine.*

1. INTRODUCTION

Through the practice of Ayurveda, which emphasizes treating the whole person—body, mind, and spirit—by addressing the fundamental causes of disease and promoting balance and longevity, India has a rich and distinguished history of utilizing medicinal plants. Contemporary studies have demonstrated that herbal therapies in Ayurveda, which contain bioactive compounds, are effective in treating a wide range of health issues, including those that require diuretic properties. Diuretics, which enhance urine production, play a crucial role in addressing health conditions such as hypertension, kidney disorders, and heart failure. There are three categories of medications classified by their efficacy: high, medium, and low. These include loop diuretics like furosemide, thiazide diuretics such as hydrochlorothiazide, and potassium-sparing diuretics like spironolactone. Each of these medications operates through specific mechanisms within the nephron structure. Although herbal diuretics in Ayurveda may offer safer and more natural alternatives, the ongoing advancement in renal physiology, cellular transport, and organic compound release underlines the significance of these medications in medical treatment. Furthermore, urinary tract infections remain a considerable public health issue; therefore, it is essential to develop effective treatment strategies and to continue research on both herbal and pharmaceutical options.[1-5]

2. MATERIALS AND METHODS

Materials:

Chemical and reagent:

Table 1. Chemicals and their manufacture

Chemicals	manufacture
Diethyl ether	Merck Ltd. India
Heparin	Chemist Shop
Nacl	Merck Ltd. India
Methanol	Central Drug House (P) Ltd., Daryaganj, New Delhi
H2SO4	Central Drug House (P) Ltd., Daryaganj, New Delhi
Chloroform	Merck Ltd. India
Acetone	Merck Ltd. India

Instruments and equipment:

Table 2: Instruments, equipment's their manufacturers

Equipment	Manufacture
Digital Weighing Balance	Anamed Instruments Pvt. Ltd., India
Refrigerator	Godrej
High Speed Centrifuge	Remi Laboratory Instruments, India
Micropipettes	MicroSidd, India
Dissection Box	Jyoti scientific laboratories
Soxhlet apparatus	Borosil
Heating mantle	Bells, India Ltd
Funnel/ measuring cylinder	Borosil, India Ltd

Collection of Plant Material:

The leaves of Neolamarckia cadamba were collected from the garden of Shri Ram Group of College, Banmore, Morena.

Authentication:

The plant from which the substance is obtained must also be accurately established. A botanist or a plant taxonomist may be interested in the plant's thorough authentication (i.e., classification into its class, order, family, genus, and species). The plant material that will be studied may be chosen based on certain basic common uses (ethnobotanical bioprospecting approach).[6-8]

Preparation of Extraction:

Because of its ease perceived usefulness, the Soxhlet extraction is commonly used throughout the extraction of plant metabolites. Almost all initial and bulk extraction can be done with this process. During an extraction chamber, the plant material is stored in a cellulose thimble, which would be stacked on upper side of receiving flask under a reflux condenser. The flask is filled with an appropriate solution, as well as the rig is heated through reflux. Whenever the thimble has collected some certain amount of concentrated solvent, it is syphoned into the flask underneath. Soxhlet extraction has the benefit of being a continuous operation. New solvent is recondensed when the solvent (saturated with solubilized metabolites) is replenished into the flask, extracting the substance in the thimble regularly. Soxhlet extraction takes less time and uses fewer solvents than percolation or maceration.

The key drawback of Soxhlet extraction is that the process of extraction is continuously heated to the boiling point of the solvent, which can weaken thermolabile materials and/or cause artifact production.[7-12]

The plants are collected, washed, and dried in the shade. Approximately 500 grams of coarse-ground powder or the aerial part of the plant is loaded into a continuous Soxhlet extraction device to extract the ethanol. The filtrate was concentrated under pressure using rotary vacuum only and dried in a desiccator until ready for use.

PHYTOCHEMICAL STUDIES

Preliminary phytochemical research can be aqueous solutions or special solvents such as chloroform, methanol, or petroleum ether, depending on the type of natural drug being studied.

Most of the extracted data was subjected to phytochemical analysis to identify different plant species. Using quantitative and qualitative methods to identify phytoconstituents from plant

extracts, including alkaloids, carbohydrates, fixed fats, glycosides, phytosterols and fats, phenolic compounds, tannins, proteins and free amino acids, mucilages and gums, Lignin, Flavonoids, and Alkaloid Testing, the extract was dissolved in dilute hydrochloric acid and analyzed.[13-16]

EVALUATION OF DIURETIC ACTIVITY:

Animal

This research will be using male Wistar rats ranging between 160 and 175 grams. The animals were acquired from the ShriRam College Pharmacy's central animal facility in Banmore, M. P., India, and will be held in a polypropylene cage with rodent pellets at a stable temperature (22 0C) and acclimatized to a 12/12 h light/dark period. Food and water will be available 2 hours before the case. The animals will be cared for and maintained according to the committee's approval for the motive of monitoring and oversight of animal experiments, which will follow the rules of the establishment's ethical committee on animal experimentation.

GROUPING OF ANIMALS

Animals are divided into 4 groups, and each group has 4 animals

Group 1 – in the control group, given normal saline (n=4)

Group 2 – standard group received furosemide (n=4)

Group 3 – Test group received Methanolic extract of *Neolamarckia cadamba leaves* 100/kg (n=4)

Group 4 - Test group received Methanolic extract of *Neolamarckia cadamba leaves* 200 mg/kg (n =4)

Experimental model Lipchitz Method:

A male rat (Wister albino) with a body weight of 160 to 175 grams (filtered) was kept at normal temperature and humidity. Three groups of six mice each did not eat breakfast and were fasted for eighteen hours before the experiment.

The first group of animals was kept under control and administered saline (16 ml/kg orally). The second animal was administered furosemide (10mg/ml, intraperitoneal injection) dissolved in saline.

Three groups were administered 100 mg/kg of extract orally.

The fourth group was administered 200 mg/kg of the extract orally

This study used Wistar rats weighing 160–175 g and placed them in a metabolic chamber with

a metal mesh bottom and a funnel for urine collection. This area is secured by a stainless steel mesh on the funnel, allowing only water to enter the storage and calculations. Do not eat or drink for 15 hours before the test. We live in metabolic cells. Drugs were administered to each group of mice according to the information above. Additionally, 1 ml of saline solution per 100 g was administered to these two rats, diuresis was measured 5 hours later, and the sodium and potassium contents of urine were calculated using an electronic meter.[17-22]

HPLC Analysis of Collected Blood Samples

Sample Collection and Preparation: After the experimental period, blood samples were collected from the retro-orbital plexus using mild anesthesia and placed into EDTA-coated tubes. Plasma was isolated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until analysis.

Extraction Procedure: To 500 µL of plasma, 1 mL of acetonitrile was added to precipitate proteins. The mixture was vortexed for 2 minutes, followed by centrifugation at 10,000 rpm for 15 minutes. The supernatant was filtered through a 0.22 µm syringe filter before being injected into the HPLC system.[23-29]

HPLC Conditions:

• Instrument: Shimadzu LC-20AT or equivalent

• Column: C18 reverse-phase column (250 mm × 4.6 mm, 5 µm)

• Mobile Phase: Methanol: Water (70:30 v/v), adjusted to pH 3.5 with orthophosphoric acid

• Flow Rate: 1.0 mL/min

• Injection Volume: 20 µL

• Detection Wavelength: 254 nm

• Run Time: 10 minutes

3. RESULTS AND DISCUSSION

Phytochemical Profile

Methanolic extract revealed the presence of key phytochemicals such as flavonoids, alkaloids, glycosides, and tannins, which are known to exhibit diuretic properties.

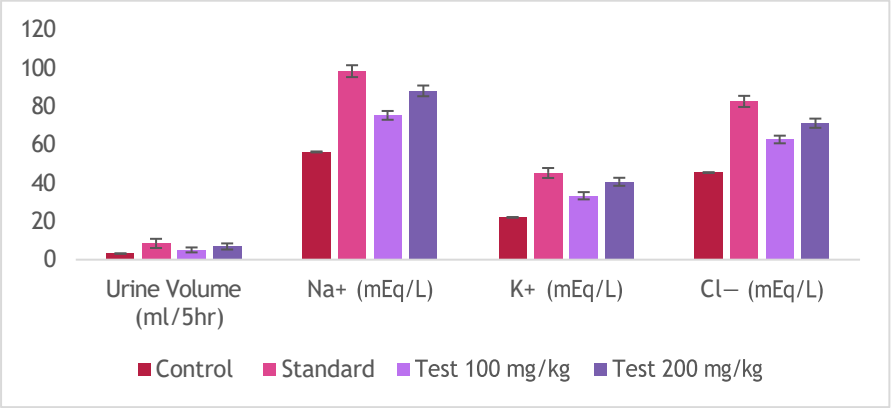
Table 3: Phytochemical Screening of Methanolic Extract of N. cadamba Leaves

S.No	Phytoconstituents	Test Method Used	Result
1	Alkaloids	Mayer's, Wagner's, Dragendorff's	+
2	Flavonoids	Alkaline Reagent Test	+
3	Glycosides	Borntrager's Test	+
4	Tannins and Phenolics	Ferric Chloride, Gelatin Test	+
5	Saponins	Foam Test	+
6	Carbohydrates	Molisch's, Fehling's Test	+
7	Proteins and Amino Acids	Ninhydrin, Xanthoproteic Test	+
8	Sterols and Triterpenoids	Liebermann-Burchard Test	+

2. Diuretic Response

Table 4: Diuretic Response

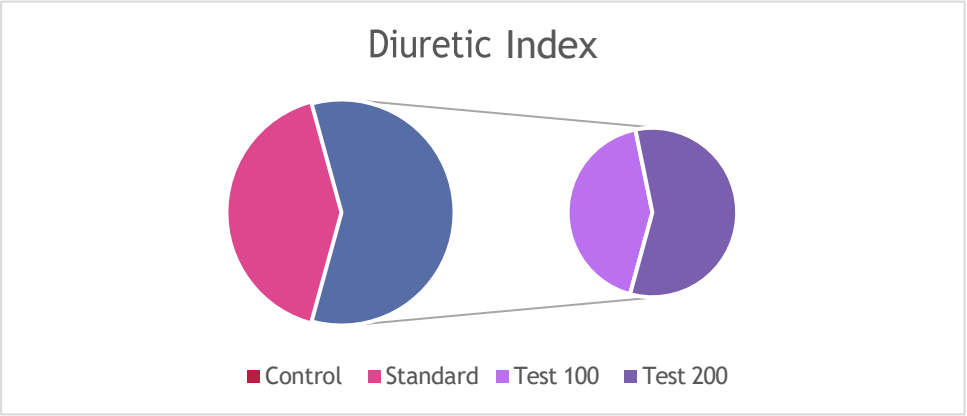
Group	Urine Volume (ml/5hr)	Na+ (mEq/L)	K+ (mEq/L)	Cl− (mEq/L)
Control	3.2 ± 0.12	56.3 ± 2.4	22.1 ± 1.3	45.5 ± 1.6
Standard	8.5 ± 0.25	98.5 ± 3.1	45.3 ± 2.2	82.7 ± 2.8
Test 100 mg/kg	5.1 ± 0.18	75.4 ± 2.6	33.4 ± 1.9	62.8 ± 2.1
Test 200 mg/kg	6.9 ± 0.22	88.2 ± 2.9	40.7 ± 2.0	71.3 ± 2.4



3. Diuretic and Saluretic Indices

Table 5; Diuretic and Saluretic Indices

Group	Diuretic Index	Saluretic Index (Na+ + Cl−)	Na+/K+ Ratio
Control	–	–	2.55
Standard	2.65	1.78	2.17
Test 100	1.59	1.36	2.26
Test 200	2.15	1.57	2.17



4. Statistical Significance (Dunnett's test vs. Control)

Table 6: Statistical Significance (Dunnett's test vs. Control)

Group	Urine Volume	Na+	K+	Cl−
Standard	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Test 100	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Test 200	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

5. Interpretation

The data indicate that the methanolic extract of Neolamarckia cadamba leaves exhibits a significant, dose-dependent diuretic effect, with the 200 mg/kg dosage nearly matching the efficacy of standard furosemide. The rise in urinary Na+ and Cl− suggests effective natriuretic activity, and the balanced Na+/K+ ratio indicates a lower risk of hypokalemia. The saluretic

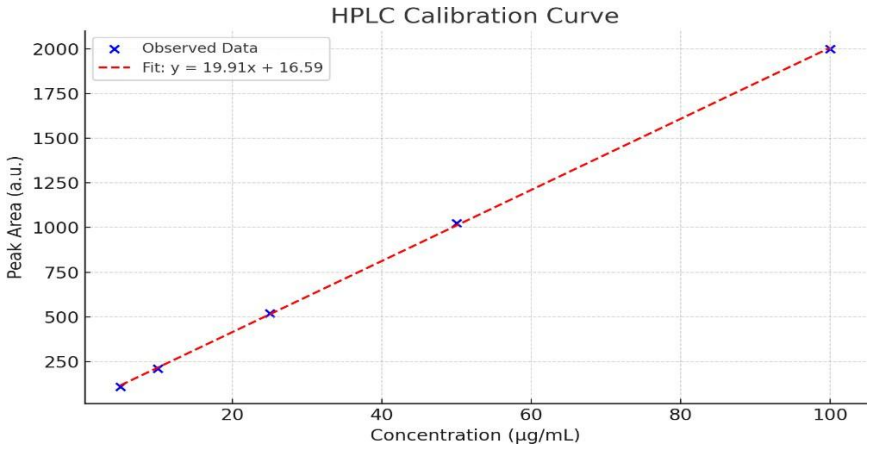
indices reflect a strong potential to enhance overall salt excretion.

Standard Preparation and Calibration:

A standard solution of the marker compound (if known, e.g., flavonoid or alkaloid suspected to be active) was prepared in methanol. A calibration curve was constructed using serial dilutions (e.g., 5, 10, 25, 50, 100 µg/mL). The retention time and area under the curve (AUC) were recorded for both standards and test samples.

Table 7: Standard Preparation with peaks

Concentration (µg/mL)	Peak Area (a.u.)
5	110
10	210
25	520
50	1025
100	2000



Results Interpretation: Retention time (Rt) and peak area for each chromatogram were compared with the standard. The presence of phytoconstituents in plasma confirms systemic absorption of active components from the *Neolamarckia cadamba* extract. Quantitative estimation was performed using the linear regression equation derived from the calibration curve.

4. CONCLUSION

The results of this study indicate that the methanolic extract of Neolamarckia cadamba leaves exhibits significant diuretic activity that is both dose-dependent and statistically supported. Specifically, the 200 mg/kg dose led to a notable rise in urinary output and electrolyte excretion (Na^+, K^+, and Cl^-), achieving efficacy levels similar to the standard diuretic drug, furosemide. This finding suggests the presence of potent bioactive compounds within the plant that may affect renal physiology, likely through mechanisms related to the inhibition of sodium and chloride reabsorption in the renal tubules.

The diuretic effect observed is consistent with the plant's traditional use in treating conditions such as edema, urinary tract disorders, and fluid retention. Preliminary phytochemical screening confirmed the presence of phytoconstituents like flavonoids, saponins, alkaloids, and glycosides, supporting the theory that these compounds may work synergistically to enhance the diuretic effect. [29-32]

This study not only validates the traditional applications of Neolamarckia cadamba but also sets the stage for future pharmacological investigations. It is crucial to conduct further research to isolate, identify, and characterize the specific active compounds responsible for the diuretic effect. Moreover, mechanistic studies at the molecular and cellular levels will provide further insights into the underlying pathways, facilitating the development of novel plant-based diuretic agents that could potentially have fewer side effects than synthetic options.

REFERENCES

[1] Mukherjee PK, Harwansh RK, Bahadur S, Banerjee S, Kar A, Chanda J, Biswas S, Ahmmed SM, Katiyar CK. Development of Ayurveda–tradition to trend. Journal of ethnopharmacology. 2017 Feb 2;197:10-24.

[2] Chauhan A, Semwal DK, Mishra SP, Semwal RB. Ayurvedic research and methodology: Present status and future strategies. Ayu. 2015 Oct;36(4):364.

[3] Firenzuoli F, Gori L. Herbal medicine today: clinical and research issues. Evidence-based complementary and alternative medicine. 2007 Sep 1;4:37-40.

[4] Builders PF. Introductory chapter: Introduction to herbal medicine. InHerbal medicine 2018 Nov 5. IntechOpen.

[5] Dutta KN, Chetia P, Lahkar S, Das S. Herbal plants used as diuretics: a comprehensive review. J Pharm Chem Biol Sci. 2014 May;2(1):27-32.

[6] Agbodjogbe WK, Aïkpe JF, Ayedoun MA, Assogba FM, Dansou PH, Gbenou JD. Diuretic and natriuretic activities from ten medicinal plants used in south Benin. Journal of Chemical and Pharmaceutical Research. 2015;7(12):1145-52.

[7] Wile D. Diuretics: a review. Annals of clinical biochemistry. 2012 Sep;49(5):419-31.

[8] Roush GC, Kaur R, Ernst ME. Diuretics: a review and update. Journal of cardiovascular pharmacology and therapeutics. 2014 Jan;19(1):5-13.

[9] Klabunde RE. Cardiovascular Pharmacology Concepts, Beta-Adrenoceptor Antagonists (Beta-Blockers).

[10] Ioannidis K, Papachristos A, Athanassa Z, Katsouda E, Skarlatinis I, Paskalis H. Safety and effectiveness of the combination acetazolamide and bicarbonates to induce alkaline diuresis in patients with rhabdomyolysis. European Journal of Hospital Pharmacy. 2015 Apr 27.

[11] Kossmann D, Ailamaki A, Balazinska M, Candan K, Diao Y, Dyreson C, Loannidis Y, Jensen C, Milo T, Spinola F. Letter from the sigmod executive committee. SIGMOD Record. 2015 Sep;44(3):5-6.

[12] Tripathi KD (2013). Essential Pharmacology. New Delhi • Ahmedabad • Bengaluru • Chennai • Hyderabad • Kochi • Kolkata: JAYPEE BROTHERS MEDICAL PUBLISHERS (P) LTD 557 - 570.

[13] Barash P, Cullen BF, Stoelting RK, Cahalan M, Stock MC, Ortega R. Handbook of clinical anesthesia. Lippincott Williams & Wilkins; 2013 May 8.

[14] Cechinel-Zanchett CC, Bolda Mariano LN, Boeing T, da Costa JD, Da Silva LM, Bastos JK, Cechinel-Filho V, de Souza P. Diuretic and renal protective effect of kaempferol 3-O-alpha-l-rhamnoside (afzelin) in normotensive and hypertensive rats. Journal of Natural Products. 2020 May 26;83(6):1980-9.

[15] Sisodia, L., Tiwari, R., Bhalerao, H., Mishra, R., & Akram, W. INTRODUCTION TO PHARMACODYNAMICS: THE BASIS OF DRUG THERAPY.

[16] Mathew GE, Mathew B, Shaneeb MM, Nyanthara B. Diuretic activity of leaves of Garcinia cambogia in rats. Indian journal of pharmaceutical sciences. 2011 Mar;73(2):228.

[17] Fatima, S., Mishra, R., Jain, V., & Jain, S. (2024). Evaluation of sun protection factor (SPF) of common fruit and vegetable extracts using UV-visible spectroscopy: An herbal approach. Nanotechnology Perceptions, 20(7), 2363-2378. https://doi.org/10.62441/nano-ntp.v20i7.4377

[18] Ntchapda F, Abakar D, Kom B, Nana P, Bonabe C, Kakesse M, Talla E, Dimo T. Diuretic activity of the aqueous extract leaves of Ficus glumosa Del.(Moraceae) in rats. The Scientific World Journal. 2014 Oct 14;2014.

[19] Santhanam R, Karunakaran T, Sowndhararajan K, Zulkifli MF, Govindan Kothandaraman M, Aravindhan V, Wan Ismail WI. Photoprotective Potential, Cytotoxicity, and UPLC-QTOF/MS Analysis on Bioactive Solvent Fractions of Moringa concanensis Nimmo Bark. Evidence-Based Complementary and Alternative Medicine. 2022 Apr 23;2022.

[20] Satyajit D, Sarker Z, Latif A, Gray I. Natural products isolation. Methods in Molecular Biology. 2006;864.

[21] Kontagora GF, Lawal N, Adebote DA, Kamba B, Nafiu MI, Jufare AI. Some preliminary phytochemical screening and assessment of four solvents extracts of button weed (Borreria verticillata). Journal of Applied Sciences and Environmental Management. 2020;24(12):2085-8.

[22] Sengupta A, Sengupta C, Das PK. The triglyceride composition ofMoringa concanensis seed fat. Lipids. 1971 Sep;6(9):666-9.

[23] Manzoor M, Anwar F, Iqbal T, Bhanger MI. Physico-chemical characterization of Moringa concanensis seeds and seed oil. Journal of the American Oil Chemists' Society. 2007 May;84:413-9.

[24] Balakrishnan BB, Krishnasamy KA. Evaluation of free radical screening and antioxidant potential of Moringa concanensis nimmo-a medicinal plant used in Indian traditional medication system. Int J Pharm Pharm Sci. 2018;10(7):91-7.

[25] Balamurugan V, Balakrishnan V. Evaluation of phytochemical, Pharmacognostical and antimicrobial activity from the bark of Moringa concanensis Nimmo. Int J Curr Microbiol App Sci. 2013a. 2013;2:117-25.

[26] Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020 Mar;8(2):603-8.

[27] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002 Jul 1;10(3).

[28] Brater DC. Pharmacology of diuretics. The American journal of the medical sciences. 2000 Jan 1;319(1):38-50.

[29] Mishra, M. R., PHARM, M., Rathore, M. H., Tiwari, M. R., & Jain, V. RADIOPHARMACEUTICALS.

[30] Greger R, Wangemann P. Loop diuretics. Kidney and Blood Pressure Research. 1987 Nov 7;10(3-4):174-83.

[31] Oh SW, Han SY. Loop diuretics in clinical practice. Electrolytes & Blood Pressure: E & BP. 2015 Jun;13(1):17.

[32] Tiwari, R., Rathore, H., Mishra, R., & Jain, V. (2023). Andrographolide and its analogues in colon cancer (anti-tumor activity). J. Coast. Life Med, 11, 616-631