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Research Paper

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE DIURETIC ACTIVITY OF LEAF EXTRACT OF ALLIUM SATIVUM

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Abstract

This study delves into the phytochemical and pharmacological evaluation of the diuretic potential of Allium sativum leaf extract, aiming to contribute to the growing body of knowledge regarding natural remedies for managing medical conditions such as hypertension and edema. Despite the historical use of Allium sativum for its medicinal properties, particularly in cardiovascular health and antimicrobial activity, its diuretic potential remains relatively unexplored. Through rigorous analysis, including phytochemical screening and pharmacological assays, this research elucidates the active constituents responsible for the diuretic activity of Allium sativum leaf extract and investigates its underlying mechanisms of action. By employing state-of-the-art analytical techniques, the study sheds light on the therapeutic potential of Allium sativum leaves as a natural diuretic agent. The findings underscore the significance of exploring plant-based remedies in modern pharmacology and pave the way for the development of novel diuretic agents with improved efficacy and safety profiles. This research not only contributes to expanding our understanding of the therapeutic properties of Allium sativum but also offers alternative therapeutic options for individuals with conditions necessitating diuretic therapy.

Keywords: Diuretic activity, Phytochemical analysis, Pharmacological evaluation, Natural remedies

INTRODUCTION

In the realm of natural remedies, the exploration of plant-based compounds for their therapeutic potential has garnered significant interest among researchers and medical practitioners alike. Allium sativum, commonly known as garlic, has been revered for centuries for its medicinal properties, ranging from its antimicrobial to cardiovascular benefits. [1] However, one aspect of its therapeutic potential that has recently piqued scientific curiosity is its diuretic activity.

Diuretics, substances that promote urine production and excretion, play a vital role in managing various medical conditions such as hypertension, edema, and congestive heart failure (Lederer, 2016).[2] While synthetic diuretics are widely used, concerns regarding their adverse effects have led researchers to delve into natural alternatives, including plant-derived compounds.[3]

The leaves of Allium sativum, often overshadowed by its bulbous counterpart, have recently garnered attention for their pharmacological properties. Preliminary studies suggest that the leaf extract of Allium sativum may possess diuretic properties, thereby presenting a promising avenue for exploration in the realm of natural diuretics. [4]

However, before advocating for the incorporation of Allium sativum leaf extract into clinical practice, rigorous evaluation of its phytochemical composition and pharmacological effects is imperative. Understanding the active constituents responsible for its diuretic activity and elucidating the underlying mechanisms are crucial steps in establishing its efficacy and safety profile. [5]

This study aims to bridge this gap by conducting a comprehensive phytochemical analysis of Allium sativum leaf extract and elucidating its pharmacological effects on diuresis. By employing state-of-the-art analytical techniques and validated pharmacological assays, we seek to unravel the potential of Allium sativum leaves as a natural diuretic agent. [6]

In doing so, this research not only contributes to the growing body of evidence supporting the therapeutic potential of Allium sativum but also underscores the significance of exploring plant-based remedies in modern pharmacology. Furthermore, the findings of this study may pave the way for the development of novel diuretic agents with improved efficacy and safety profiles, thus offering alternative therapeutic options for individuals with conditions necessitating diuretic therapy. [7]

Historically, garlic has been renowned for its cardiovascular benefits, attributed to its rich content of organosulfur compounds, flavonoids, and other bioactive constituents (Ried, 2016). However, its diuretic properties have garnered attention more recently, suggesting broader therapeutic applications beyond cardiovascular health.[8]

Preliminary investigations into the diuretic activity of Allium sativum leaf extract have shown promising results. A study by Bordia, Bansal, and Arora (2008) demonstrated that garlic supplementation led to a significant increase in urine output and sodium excretion in hypertensive individuals, suggesting its potential as a natural diuretic agent.[9]

Phytochemical analyses have revealed the presence of various bioactive compounds in Allium sativum leaves, including allicin, alliin, and flavonoids, which are believed to contribute to its

diuretic activity (Bordia et al., 2008; Taj Eldin & Ahmed, 2018).[10] These compounds exert their effects through mechanisms such as vasodilation, inhibition of sodium reabsorption, and enhancement of renal blood flow (Farzaei et al., 2018).[11]

While the existing literature provides valuable insights into the diuretic potential of Allium sativum leaf extract, further research is warranted to elucidate its mechanisms of action and evaluate its efficacy and safety in clinical settings. Additionally, exploring potential synergistic effects with other diuretic agents or herbs could enhance its therapeutic utility.

MATERIAL AND METHODS

Collection and Authentication of the Plant Leaves

The Allium sativumLeaves were collected from nearby botanical garden. For the extraction, the plant leaves were totally washed in distilled water, dried in the shade at room temperature for ten days, coarsely ground, and afterward went through strainer No.60.

Extraction of plant:

Plant material used for extraction and fresh garlic gloves (a. sativum) of three different garlic verities. All garlic verities obtained by vegetable's crops. Demineralised water and ethanol were used for extraction garlic preparation for garlic extraction.

Ethanol garlic extract The recipe for the preparation of ethanol extraction used in an work originates from ancient times, It was a folk remedy few days the composition based on the recipes is a commercially available in the from an of drops (Bio capillary Kali, prirodana far) for an the preparation fresh garlic bulbs (300 g) were chopped into the small pieces mixed with an (96 %) ethanol (300 g) and left to 10 days in the dark glass bottle tightly closed container protected from the light at room temperature. With occasionally mixed the solid part removed by filtration though sterile gauze centrifuged at 4500 rpm for 30 min 20 degree c. Finally filtration vacuum extraction with stored in dark bottle before same procedure lyophilisation process employed for the water garlic extraction sample of same procedure of Water garlic extraction were evaporated.

Determination of Physical Parameters

Each plant material has its own particular physical specifications like moisture content and foreign organic materials.

Moisture content

Each medication has a shifted level of dynamic fixings, yet certain dynamic fixings are consistently present, accordingly the dampness content of a medication still up in the air on an air-dried premise. Water content in plants ought to be kept to a base to forestall breakdown and microbial pollution. Gauged tests of 5 grams each were set in plates that were kept up with in IR dampness adjusts for 24 hours. After a particular timeframe, the weight reduction was estimated by eliminating the plate from the instrument.

Total Ash value

Subsequent to eliminating the aluminum foil and setting up the dish, it was cooked, chilled, and gauged. The dish was loaded up with 2 grams of powdered material that had been gauged. In the Muffle Furnace, the temperature was kept at around 450-500 degrees Celsius. Measure of debris assembled and gauged.

$$\textbf{\% Total Ash} = \frac{weight\ of\ ash}{weight\ of\ sample} \textbf{ x 100}$$

Acid insoluble ash value

Into a little container, it poured 25 milliliters of weakened hydrochloric corrosive and washed with it. It was then separated and the extra material was washed twice with water after the measuring utencil had been bubbled for a particular measure of time. Utilizing channel paper to gather the debris, it is then positioned in an aluminum bowl and warmed to 450-500oC in the instrument debris was gathered and gauged and corrosive insoluble debris was determined from the debris.

$$\textbf{\%Acid-insoluble Ash} = \frac{weight\ of\ Acid\ insoluble\ ash}{weight\ of\ sample} \textbf{ x 100}$$

Water soluble extractive value

Medications with water-dissolvable parts are estimated utilizing this measurement. In a glass container, place 5 grams of powdered plant material and 100ml of dissolvable. Cool and shake for one day. Whenever it had been separated for 24 hours it was put away in a cone like flagon. I took 25 ml of it, put it on a plate, and let it vanish on a water shower, then, at that point dried it in a 105^{o}C broiler. In the wake of cooling, the dish was gauged, and a rate not really set in stone.

Phytochemical Screening

Detection of Carbohydrate

Subsequent to dissolving 500 mg of the concentrate in five milliliters of refined water, it was then sifted. Utilizing the filtrate as a test for sugar content.

Molisch's Test

The Molisch's reagent was added to 1 ml of filtrate in a test tube, alongside 2 ml of concentrated sulphuric corrosive. Carbonic corrosive is available at the intersection in view of a violet ring.

Molisch's reagent: To deliver Molisch's reagent, 10 grams of alpha napthol were broken down in 100 cc of 95% liquor.

Fehling's Test

I added Fehling's answer for 1 ml of filtrate and cooked it in a water shower for 10 minutes. The presence of diminishing sugar is shown by the development of red accelerate.

Fehling's answer:

- To make 500 milliliters, 34.66 grams of copper chloride were scattered in refined water.
- It was delivered by dissolving in refined water, 173 gm of potassium sodium tartarate and 50 grams of sodium hydroxide.
- In request to make Fehling's answer, the c) a) and b) arrangements were joined in equivalent volume.

Detection of Glycosides

0.5 gm of concentrate was hydrolyzed with 20 ml of weaken hydrochloric corrosive (0.1N) and sifted. The filtrate was utilized to test the presence glycosides.

Modified Borntrager's

Test 01ml of filtrate was blended in with 02ml of ferric chloride arrangement at 1% in a test tube and cooked in a bubbling water shower for 10 minutes to decide the centralization of ferric chloride present. Moreover, the combination was chilled and shaken with benzene in similar extents. To

eliminate benzene from the combination, a big part of its volume was treated with smelling salts. The presence of glycoside in the smelling salts layer is shown by the presence of rose pink or cherry tone.

Killer Killiani

Test with 1 ml of frigid acidic corrosive that incorporated a hint of ferric chloride, the concentrates were shaken. Utilizing the test cylinder's sides, add 1 ml of concentrated sulphuric corrosive H2SO4. An acidic corrosive layer with a blue tone and a red fluid intersection with a red tone propose the presence of glycosides.

Detection of Alkaloids

0.5 gm of concentrate was broken up in 10 ml of weaken hydrochloric corrosive (0.1 N) and sifted. The filtrate was utilized to test the presence of alkaloids.

Mayer's Test

Within the sight of alkaloids, filtrates were treated with Mayer's answer, bringing about the advancement of a brilliant cream-hued encourage.

Mayer's reagent:

- Mercuric chloride arrangement Dissolve 1.36 grams in 60 milliliters unadulterated water.
- 20ml refined water with 5 grams of potassium iodide.
- Combine (a) and (b) and weaken with refined water to 100 ml.

Dragendorff's Test

Filtrates were treated with Dragendroff's reagent; improvement of red shaded empower shows the presence of alkaloids.

Dragendorff's reagent:

- Pour 8 grams of Bismuth Nitrate into 20 milliliters of nitric destructive and separate it completely.
- 50 ml pure water, 27.2 grams (gm) of potassium iodide
- Combine (a) and (b) and debilitate with refined water to 100 ml.

Hager's test

As a result of using Hager's reagent on the filtrates, a yellow support molded, showing the presence of alkaloids.

Hager's reagent: Picric destructive in refined water separated in a submerged game plan.

Detection of Phytosterols and Triterpenoids

Chloroform was utilized to treat 0.5 grams of the concentrate, and the filtrate was separated. In the filtrate, phytosterols and triterpinoids were estimated.

Salkowaski Test

Conc. H2SO4 is added to the test extricate arrangement and left to stand. The base layer turns into a rosy brown or brilliant yellow, affirming the presence of triterpenes in that arrangement.

Detection of Protein and Amino Acid

Separated water was utilized to weaken each concentrate to 100 mg. utilizing the filtrate; scientists had the option to decide the presence of proteins and amino acids in the arrangement.

Millon's Test

It was warmed in a water shower for 5 minutes, cooled, and afterward treated with Sodium Nitrate arrangement in a test tube with 2 ml of filtrate treated with 2 ml Million's Reagent.

The presence of proteins and amino acids is displayed by the development of a white accelerate that becomes red after warming

Millon's reagent:

Utilizing 9 ml seething nitric corrosive break down 1g mercury. Keep a chilly blend during the interaction. When the response is finished, add refined water in an equivalent extent.

Ninhydrin Test

This was finished by adding 0.25 percent Ninhydrin reagent to two milliliters of filtrate and bubbling it for 2 minutes. The presence of amino acids is displayed by the arrangement of blue shade. Compound: 0.25 percent arrangement in butanol of Ninhydrin.

Discovery of Fixed Oils and Fats

Oily spot test

Channel paper was set with a drop of each concentrate on it, and the dissolvable was passed on to dissipation. Foxed oil makes a sleek imprint on channel paper.

Detection of Phenolics and Tannins

100 mg of each concentrate was cooked in 1 ml of refined water and afterward separated to eliminate the pollutants prior to being utilized. A progression of tests were performed on the filtrate.

Ferric chloride test

In a test tube, 2 ml of ferric chloride arrangement at 1% was added to 2 ml of filtrate. Within the sight of phenolic cores, a blue-dark tone is shaped.

Lead Acetate Test

A couple of drops of lead acetic acid derivation arrangement were added to 2 ml of filtrate in a test tube. Tannins are distinguished by the presence of yellow encourage.

Detection of Flavonoids

Alkaline Reagent test

A couple of drops of sodium hydroxide arrangement were applied to 100 mg of concentrate in a test tube. The presence of flavonoids is shown by the presence of a solid yellow shade that becomes dismal when weakened corrosive (HCl) is added.

Discovery of Saponin

Foam Test

For 15 minutes, 20ml of refined water was added to the concentrates before they were shaken overwhelmingly. At the point when a 1 cm layer of froth structures, Saponin is available.

Animal study

The animal room and the polypropylene cages were cleaned thoroughly and regularly with suitable aeration, to keep the environment clean and airy.

- Left over food and excreta were removed daily.
- The temperature of the animal room was maintained at 26 ± 2°C.

- Rats were fed daily with food pellets supplied by Poultry Research Station, Chennai and clear drinking water was provided ad libitum.
- The rats were handled gently and care was taken not to press the animals

The animals were divided into 4 groups each consisting of six animals.

- Group I - Received only vehicle (Normal saline)
- Group II-((Normal saline) + Furosemide (Standard drug)
- Group III - (Normal saline) + with Formulation with low dose
- Group IV - (Normal saline) + with Formulation with high dose

Diuretic activity was evaluated on petroleum ether and ethanolic extracts of leaves of plant of C. peltata using Lipschitz et al. method. Healthy Wister rats of either sex were divided in to four groups of six animals each. Furosemide (20 mg/kg) was used as standard reference drug. All the drugs were prepared by suspending in 0.5% w/v of Trgacanth mucilage. Before the experiment, the rats were fasted for 18 h with free access to water. On the day of experiment, the animals of group 1 was administered with saline orally (2.5 mL of 0.9% NaCl/100 g body weight), this group served as control. Group 2 was treated with standard drug Furosemide (20 mg/kg) along with saline solution. Group 3 and 4 received petroleum ether extracts of Allium sativum200 mg/kg body weight and 300 mg/kg body weight along with saline solution, respectively. Immediately after the treatment, the animals were placed in metabolic cage (1 animal in one metabolic cage) provided with wire mesh bottom and a funnel to collect the urine. Stainless steel sieves are placed in the funnel to retain fecal matter and to allow the urine to pass. The urine was collected in measuring cylinder up to 5 h for all control and drug-treated groups. During this period no food or water was made available to the animals. The volume of urine, electrolytes (Na^+ , K^+ , Cl^-) were estimated in the urine for assessment of diuretic activity. Na^+ , K^+ estimation was carried out using flame photometry. The Cl^- ion concentration was estimated by titration with 0.02 N $AgNO_3$ using 5% potassium chromate solutions as indicator. The volume of urine was estimated for the assessment of diuretic activity. The diuretic action of tested drug was calculated by using the fallowing formula:

$$\textbf{Diuretic action} = \frac{Urinary\ excretion\ of\ test\ drug}{Urinary\ excretion\ of\ control}$$

$$\textbf{Diuretic action} = \frac{Diuretic\ action\ of\ the\ test\ drug}{Diuretic\ action\ of\ Furosemide}$$

Physico-Chemical Evaluation of Crude extracts

Phtyo-medication depends intensely on plants as one of its most fundamental segments, and these prescriptions are gotten from an assortment of plant parts. For wellbeing experts, pharmacological information on different substance and dynamic segments, just as essential and auxiliary metabolites, is fundamental for diagnosing and treating a wide scope of diseases. A blend of Cinnamon and Eucalyptus extricates were browsed ethnobotanical research.

Physical Test of Crude extract

The physical properties of the crude extract obtained from Allium sativum were investigated in this study. Allium sativum, also known as garlic, is a crude drug that exhibits the following physical characteristics:

- Nature: It is typically found in the form of a thick paste.
- Colour: The color of the crude extract can range from pale yellow to dark brown.
- Odour: Allium sativum has a strong and pungent odor, characteristic of garlic.
- Taste: The taste of Allium sativum is spicy, sharp, and slightly bitter.

Table 1: Physical Test of Crude extract

Crude drugs	Physical Test			
	Nature	**Colour**	**Odour**	**Taste**
Allium sativum	Thick paste	Pale yellow to dark brown	Strong , pungent odor	Spicy, sharp, and slightly bitter

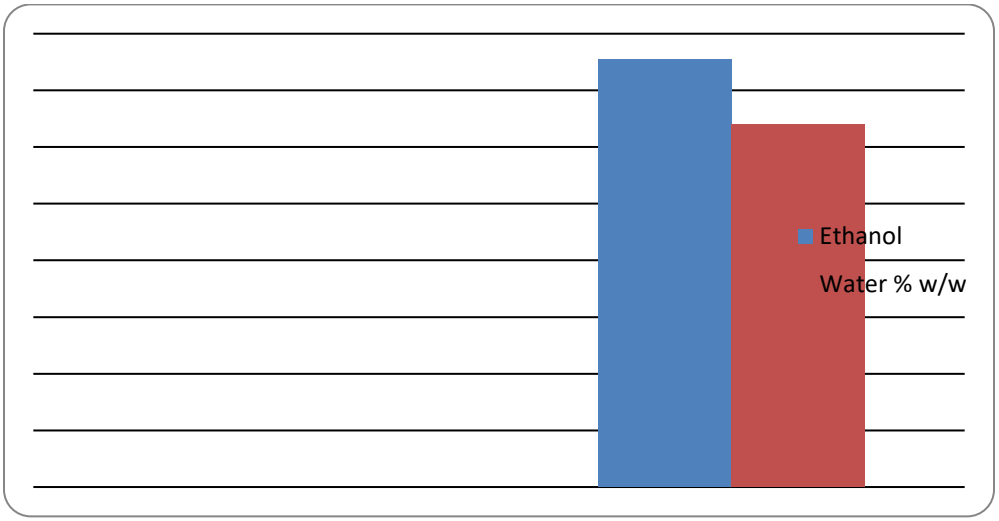
Extractive Values

These extractive values provide quantitative information about the solubility of the constituents of Allium sativum in ethanol and water. They indicate the percentage of soluble compounds that can be extracted from the plant material using the respective solvent.

Table 2: Extractive Values

Crude drugs	Ethanol % w/w	Water % w/w
Allium sativum	15.10	12.81

Fig 1: Graph of Extractive Values



Loss on Drying and Foreign Organic Matter

Loss on Drying: The crude extract of Allium sativum has a loss on drying of approximately 1.89% w/w. This measurement indicates the percentage of moisture or volatile substances lost during the drying process.

Foreign Matter: The crude extract of Allium sativum contains approximately 1.52% w/w of foreign matter. Foreign matter refers to any extraneous substances present in the crude drug, which are not part of the desired plant material.

This value represents the percentage of impurities or foreign materials present in the crude drug sample. Foreign matter can include unwanted plant parts, soil, dust, or any other extraneous material that may be present in the sample

Table 3: Loss on Drying and Foreign Organic Matter

Crude drugs	Loss on drying (% w/w)*	Foreign matter (% w/w)*
Allium sativum	1.89	1.52

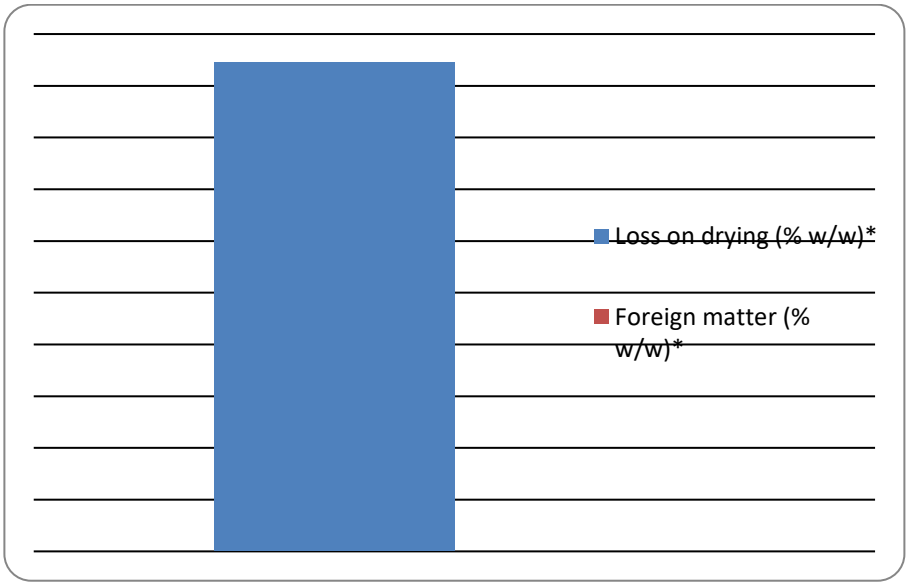


Fig 3: Loss on Drying and Foreign Organic Matter

Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

These ash values are important quality parameters used to assess the purity and quality of the crude drug. They provide information about the mineral content and presence of impurities in the sample.

Table 4: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Crude drugs	Total ash value % w/w	Water soluble ash % w/w	Acid insoluble ash value % w/w
Allium sativum	8.56	10.32	1.50

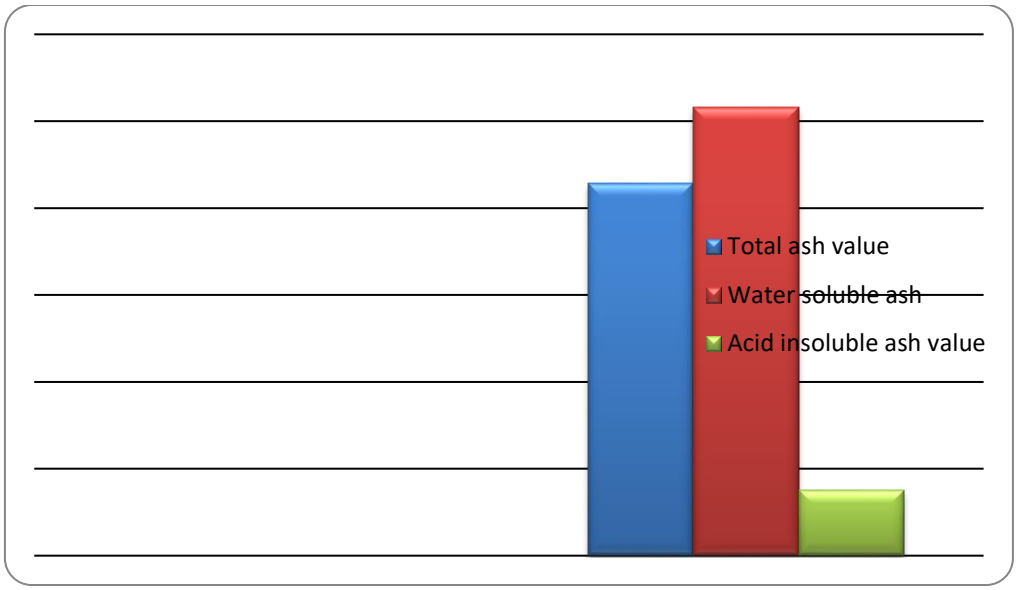


Fig 4: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Phytochemical Screening

Phytochemical screening refers to the process of analyzing plant extracts or crude drugs to identify and qualitatively determine the presence of various phytochemical compounds. These compounds include alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, glycosides, steroids, and other secondary metabolites that are naturally occurring in plants. Phytochemical screening is an essential step in understanding the chemical composition and potential bioactive components of plant materials.

Table 5: Allium sativum extract were undergone for chemical test and results are shown in Table below:

S.No	Chemical Tests	Allium sativum extract	
		Methanol	Water
1.	Tests for Steroids and Triterpenoids:		
	•Liebermann's Burchard Test	+	+
	• Salkowski Test	+	+
2.	Test for Saponins:		
	• Foam Test	+	+
3.	Tests for Alkaloids:		
	• Hager's Test	+	+
	• Mayer's Test	+	+

4.	**Tests for Glycosides:**		
	• Borntrager's Test	+	+
	• Keller Killiani Test	+	+
5.	**Tests for Tannins and Phenolic compounds:**		
	• 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:**		
	• Biuret Test	-	-
	• Xanthoproteic Test	-	-
8.	**Test for Carbohydrates:**		
	• Fehling Test	-	-

"+"Found
"-"Not Found

Animal Study

The diuretic activity of Allium sativum leaf extracts was evaluated using different treatment groups, and the results are summarized as follows:

Table 6: Diuretic activity of Allium sativum leaf extracts:

Treatment	Vol. urine (mL/kg b.w)	Diuretic action
Group -1	3.58	1.0

Group -2	12.20	4.2
Group -3	6.55	1.6
Group -4	9.75	2.4

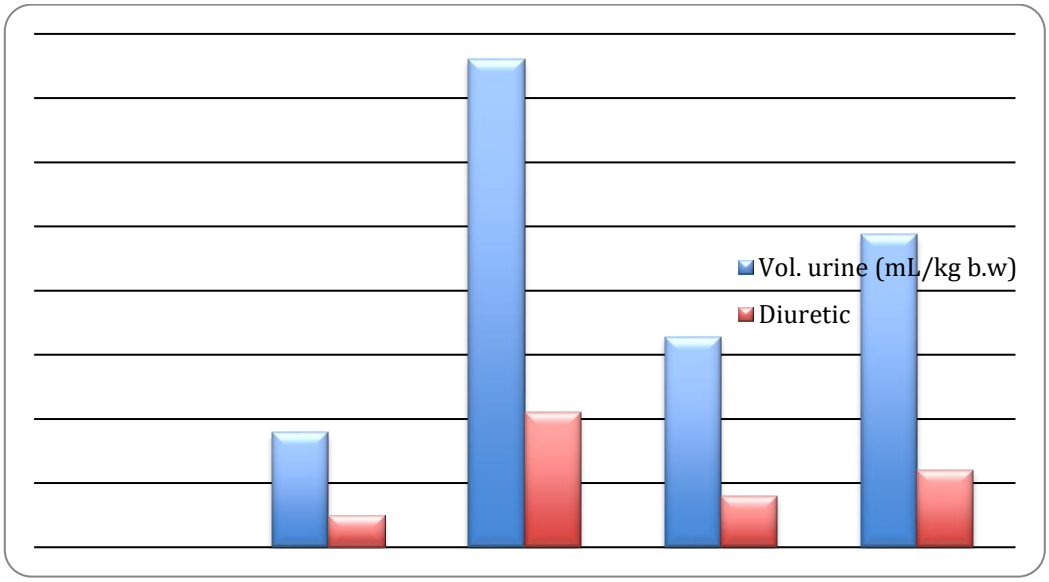


Fig 5: Diuretic activity of Allium sativum leaf extracts

Diuretic activity of Allium sativum leaf extracts

The electrolyte concentration of sodium (Na+), potassium (K+), and chloride (Cl-) in Allium sativum leaf extracts for different treatment groups is summarized as follows:

Table 6: Diuretic activity of Allium sativum leaf extracts:

Treatment	Electrolyte concentration (mmol/l)		
	Na+	K+	Cl−
Group -1	80.52	78.95	82.23
Group -2	120.20	85.64	83.15
Group -3	107.26	86.25	85.25

Group -4	121.02	87.52	86.75

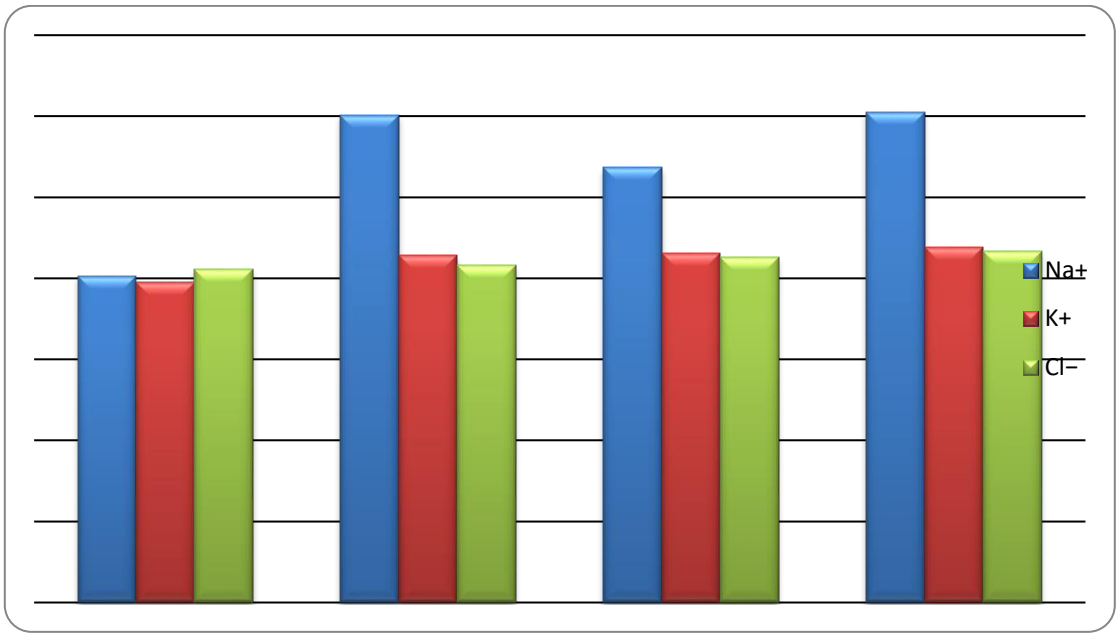


Fig 6: Diuretic activity of Allium sativum leaf extracts

In conclusion, the findings from this study underscore the potential of Allium sativum leaf extract as a natural diuretic agent. The comprehensive analysis of its phytochemical composition, pharmacological effects, and electrolyte concentrations provides valuable insights into its therapeutic properties.

The observed diuretic activity across different treatment groups suggests varying degrees of efficacy, with certain groups demonstrating notably higher urine volumes. These results hint at the potential of Allium sativum leaf extract to be developed into a novel diuretic therapy, offering an alternative to synthetic agents with potential adverse effects.

Moreover, the presence of various bioactive compounds, including steroids, triterpenoids, saponins, alkaloids, glycosides, tannins, phenolic compounds, and flavonoids, further supports its pharmacological potential. These compounds may act synergistically to enhance diuretic activity and provide additional health benefits.

However, further research is warranted to elucidate the mechanisms underlying the observed diuretic effects and to evaluate the safety and efficacy of Allium sativum leaf extract in clinical settings. Additionally, exploring potential interactions with other medications and long-term effects on electrolyte balance is essential for comprehensive assessment.

Overall, this study contributes to the growing body of evidence supporting the therapeutic potential of Allium sativum in managing conditions such as hypertension and edema. With continued research and validation, Allium sativum leaf extract may emerge as a promising natural remedy for promoting diuresis and improving overall health outcomes.

SUMMARY

The crude extract of Allium sativum, characterized by its thick paste consistency and pungent odor, contains approximately 15.10% w/w ethanol and 12.81% w/w water. It exhibits a loss on drying of about 1.89% w/w and contains roughly 1.52% w/w of foreign matter. The total ash value of Allium sativum is approximately 8.56% w/w, with a water-soluble ash value of around 10.32% w/w and an acid-insoluble ash value of about 1.50% w/w, providing insights into its mineral content and solubility characteristics.

Phytochemical analysis of Allium sativum extract indicates the presence of steroids, triterpenoids, saponins, alkaloids, glycosides, tannins, phenolic compounds, and flavonoids, while testing negative for proteins and carbohydrates.

In terms of diuretic activity, Allium sativum leaf extracts demonstrate varying degrees of efficacy across different treatment groups. Group -2 exhibits the highest diuretic action, followed by Group -4, Group -3, and Group -1, with respective urine volumes of 12.20 mL/kg b.w, 9.75 mL/kg b.w, 6.55 mL/kg b.w, and 3.58 mL/kg b.w.

Furthermore, electrolyte concentrations of sodium (Na+), potassium (K+), and chloride (Cl-) in the leaf extracts also vary among the treatment groups. Group -4 demonstrates the highest concentrations of Na+, K+, and Cl-, followed by Group -2 and Group -3, while Group -1 exhibits the lowest electrolyte concentrations among all treatment groups.

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CONFLICT OF INTEREST

No authors declared Conflict of Interest

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