Research Article

Int J. Pharm. Drug. Anal, Vol: 12, Issue: 3, 2024; 11-15

International Journal of Pharmaceutics and Drug Analysis

Content Available at www.ijpda.org ISSN: 2348:8948

INVESTIGATION OF DIURETIC EFFECTS: UTILIZING ALCOHOLIC EXTRACT FROM RIPEN AEGLE MARMELOS FRUIT IN RAT MODEL

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Received: 10 May 2024 Revised: 11 June 2024 Accepted: 19 July 2024

Abstract

The kidneys play a crucial role in maintaining the body's fluid balance through precise regulation of sodium, chloride, and water levels. They expel sodium and chloride, prompting water release and reducing fluid volume. Conversely, retaining these elements leads to water retention and increased fluid volume. This intricate mechanism ensures internal stability. Kidneys filter these substances into tubules, with most being reabsorbed into the blood and exiting as urine. Complex processes in various tubule segments complicate sodium and chloride reabsorption. Diuretics are valuable in treating edema, congestive heart failure, and hypertension. Bael (A. marmelos), a significant herb in Ayurveda, contains compounds like flavonoids and alkaloids. It's used for diarrhea, diabetes, inflammation, and hyperlipidemia. An alcoholic extract from the mature fruit of Aeglemarmelos exhibits diuretic activity, potentially assisting with common kidney ailments. Bael's therapeutic potential arises from its diverse compounds and established use in traditional medicine.

Keywords: Diuretics, Bael (A. marmelos), alcoholic extract, herb.

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DOI: https://doi.org/10.47957/ijpda.v12i3.592

Produced and Published by

South Asian Academic Publications

Introduction

Aeglemarmelos, commonly known as Bael and native to India, possesses remarkable therapeutic potential. This plant belongs to the Rutaceae family and carries different names in various regions, both inside and outside of India. Bael holds significant cultural and religious importance, particularly in Hinduism, where it symbolizes Lord Shiva, and its leaves are in high demand during the Sawan season. In traditional Indian medicine systems, every part of the Bael tree, including the root, bark, leaves, flowers, fruits, seeds, and latex, is highly valued for its effectiveness in treating various diseases. Bael is a medium-sized deciduous tree with thorny branches.Bael, a plant native to India, thrives in dry forests, the outer Himalayas, Shivalik hills, and the southern regions, ranging from 250 to 1200 meters in altitude. It's cultivated for its highly medicinal fruit, which offers a wide range of benefits including anti-dyspepsia, anti-diarrhea, anti-dysentery, and treatments for various ailments. Beyond its medicinal properties, Bael trees act as natural pollution absorbers, neutralizing harmful gases in the environment.46 They belong to a group of plants known as 'Climate Purifiers' that release more oxygen in sunlight than other plants and are also considered 'Fragrant' due to their aromatic nature.Aeglemarmelos leaves have active compounds like Limonene, α-Phellandrene, p-cymene, lupeol, rutin, marmeline, marmesinin, and tannin called skimmianine, which is a 4,7,8-trimethoxyfuroquinoline.Aegle marmelos fruit contains compounds such as Marmelosin, Luvangetin, Aurapten, Psoralen, Marmelide, α-Phellandrene, p-cymene, tannins (highest in January), carotenoids for color, and therapeutically active principles Marmelosin, Skimmianine, and Umbelliferone, along with alkaloids like aegeline and marmeline.Aeglemarmelos root comprises coumarins, including Scoporone, scopoletin, umbelliferone, marmesin, and skimming.*Aeglemarmelos* bark contains Skimmianine, Fagarine, and Marmin.The seeds of Aeglemarmelos contain Anthraquinones, Linoleic acid, Linolenic acid, Palmitic acid, Stearic acid, and Essential oils such as D-limonene, A-D-phellandrene, Cineol, Citronellal, Citral, and P-cymene, along with Cumin aldehydes.In this study, we assess the diuretic activity of Aeglemarmelos [1,2,3,4,5].

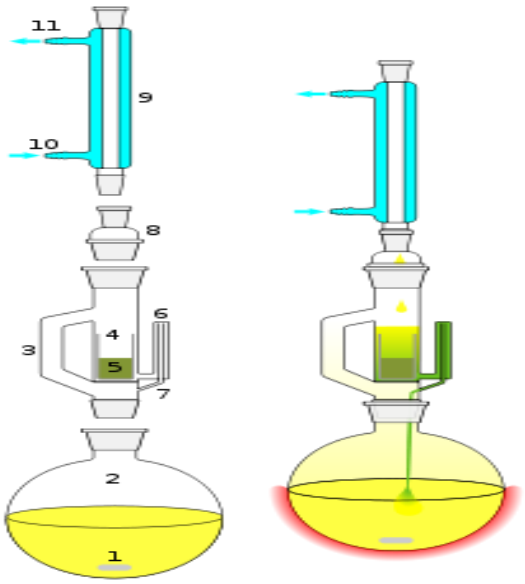
2. Material & methods:

(a) Preparation of plant material: The ripe fruit of the Aeglemarmelos plant is carefully washed to remove dirt, powdered, ensuring moisture-free grinding, and sieved to achieve uniform particle size; subsequently, it is dissolved in various solutions to ascertain its optimal solubility.

(b) Preparation & administration of crude extracts/standard drugs: The common method for isolating active constituents from crude drugs is extraction, where selective solvents separate active components from inert ones. These resulting products from plants are typically impure liquids, semisolids, or powders for oral or external use.The general techniques of medicinal plant extraction include [6]:

- Maceration.
- Infusion.
- Digestion.
- Decoction.
- Cold percolation.
- Hot continuous extraction.

Hot continuous extraction (Soxhlation): TheSoxhlet extractor, created by Franz von Soxhlet in 1879, was initially designed for lipid extraction from solid materials. It's employed when the desired compound has low solubility in a solvent, and it efficiently recycles a small solvent quantity to dissolve a larger material amount, offering unmonitored operation.In the Soxhlet extraction process, a heated solvent undergoes reflux, vaporizes, and condenses in a chamber with solid material. As the chamber fills with warm solvent, the siphon empties it back into the distillation flask, allowing for multiple cycles over hours or days. This method concentrates the desired compound in the distillation flask using a single batch of recycled solvent, which is later removed to yield the extracted compound, often with a rotary evaporator.The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded [7].



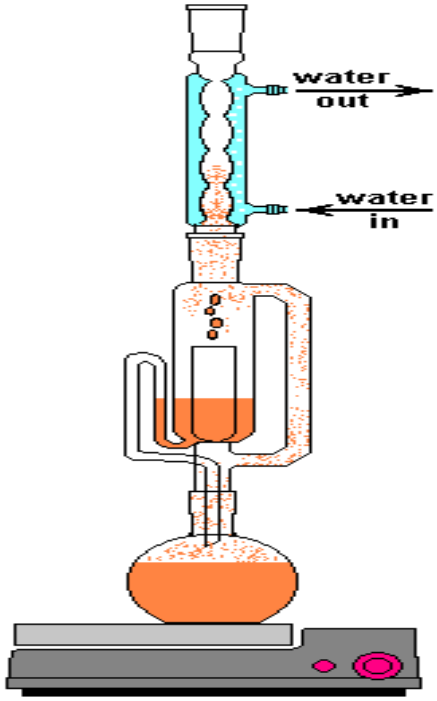


Figure no.1: A schematic representation of a Soxhlet extractor

1: Stirrer bar,**2:** Still pot (the still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the Soxhlet chamber),**3:** Distillation path ,**4:** Thimble ,**5:** Solid ,**6:** Siphon top ,**7:** Siphon exit ,**8:** Expansion adapter ,**9:** Condenser ,**10:** Cooling water in ,**11:** Cooling water out

Ethanolic Extract:To obtain the ethanolic extract, the ripe fruit of the AegleMarmelos plant was first shade-dried and powdered (100 g). This powder was then placed in a Soxhlet apparatus and subjected to continuous hot percolation using 350 ml of ethanol as the solvent for approximately 8 hours. The resulting extract was concentrated into a semi-solid mass under vacuum and thoroughly dried within desiccators.

(c) The percentage yield of the Aeglemarmelosextract:The percentage yield of the AegleMarmelos extract was determined using the formula:

Percentage yield = (weight of extract in grams / weight of drug powder in grams) x 100.

(d) LD50 Determination of the *EAM* extracts as per OECD guideline: The determination of the lethal dose (LD50), which represents the dose causing mortality in 50% of a test animal population, is a crucial parameter for evaluating acute toxicity. It also serves as an initial step in screening chemical and pharmacological agents for potential toxicity.An acute toxicity study of the ethanolic extract from the ripe fruit of the AegleMarmelos plant will adhere to OECD guidelines No: 423. Three different doses, categorized as low, medium, and high, will be chosen for the treatment.

Method: 5rats, which have fasted overnight, will receive the ethanolic extract of AegleMarmelos (EAM) orally at

different dose levels (5, 50, 300, and 1000) using a gavages. The rats will be continuously observed for the first 2 hours and again at 24 hours to detect any changes in behavior, tremors, convulsions, salivation, diarrhea, lethargy, sleepiness, or coma. Monitoring will continue for up to 14 days to assess toxic symptoms and mortality. Rats that survive the 14-day period will be rehabilitated and used for further experiments.

(e)The diuretic effects of the ethanolic extract derived from ripe Aeglemarmelos fruit, by Lipschitzmethod: The Lipschitz method for testing diuretic activity in rats quantifies water and sodium excretion in test animals relative to a urea control, expressed as the "Lipschitz value." In this study, male Wistar rats (150–200 g) are used. Three rats per group are placed in metabolic cages for urine collection after a 15-hour fasting period. They receive either the test compound (50 mg/kg), urea (1 g/kg), or a saline solution. Urine output is measured at 5 and 24 hours, and sodium content is determined. Active compounds are further tested at lower doses. The Lipschitz test assesses diuretic effects by calculating the "Lipschitz-value," which is the ratio of a test compound's response (T) to that of urea treatment (U). Values of 1.0 or higher indicate a positive effect, with potent diuretics reaching values of 2.0 or more. This index helps determine the duration of the diuretic effect. Similar calculations can be made for sodium excretion. Dose-response curves can be created, with loop diuretics having steep curves and uretic drugs like hydrochlorothiazide showing values around 1.8, while loop diuretics like Frusemide can reach values of 4.0 or higher. The Lipschitz test is a standard and valuable tool for screening potential diuretics. The cage underwent improvements, including a built-in sieve cone for separating urine and feces. It was equipped with a urine measurement device that collected urine in a vessel connected to a pressure sensor. The sensor's initial pressure reading was recorded on a chart recorder, which had been calibrated with distilled water to measure urine volume and voiding timing accurately [8,9,10.11,12,13].

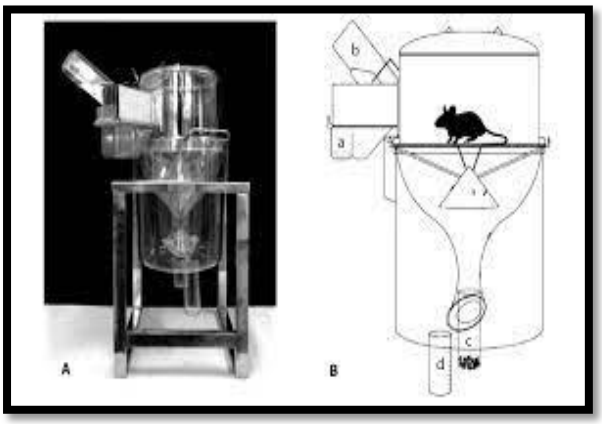


Figure no. 2: The Lipschitz Method

(f) Urine Electrolytes Determination by Ion Selective Electrode Method: Urine electrolyte concentrations, specifically sodium (Na+ mmol/L), potassium (K+ mmol/L), and chloride (Cl- mmol/L), are assessed using the Ion Selective Electrode technique, in accordance with the instructions outlined in the user manual for the biochemical kits.

(g) Urine pH determination with the help of standard pH paper: Urine volume, along with the levels of electrolytes (Na+ and K+ cations, and Cl- anions), and urine pH, was determined using standard pH paper. Estimating urine volume was crucial for assessing diuretic activity. The diuretic effect of the tested drug was quantified using the following formula:[11]

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

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

3. Results (Table& Graphs)

Phytochemical Screening

Preliminary tests for *Aeglemarmelos*

Phytochemicals	Prresent (+) / Absent (-)
Alkaloids	+
Steroids	+
Terpenoids	+
Flavonoids	+
Saponins	+
Phenolic compounds	+
Tannin	+
Carbohydrates	+
Protein	+
Cumarins	+

Table No. 1: Percentage yield and Preliminary Phyto-profile of *AegleMarmelos* extract

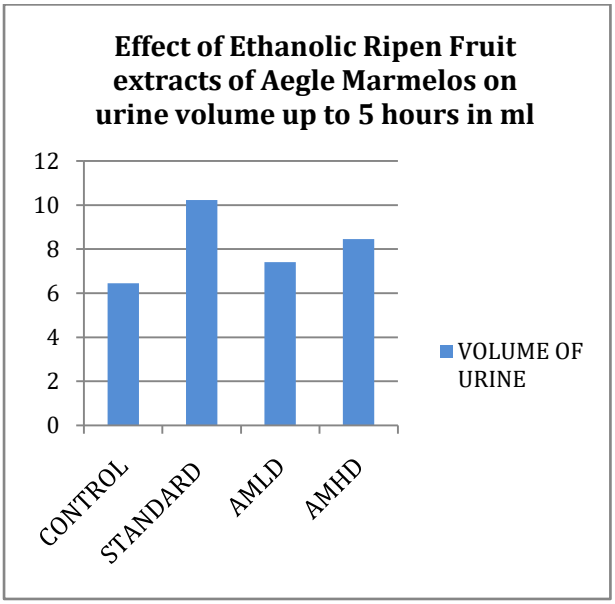
SNo.	Name of Extract	Wt. of Powder \ Color	Wt. of extract/state	Percentage yield (w\w)
1	Ethanolic Extract	100 gm / Brown	7.98 gm/ Powder	7.98%

Table No. 2: Effect of alcoholic extract of ripen fruit of *AegleMarmelos* on urine volume up to 5 hours and electrolyte concentration in hydrated rat model in albino rats

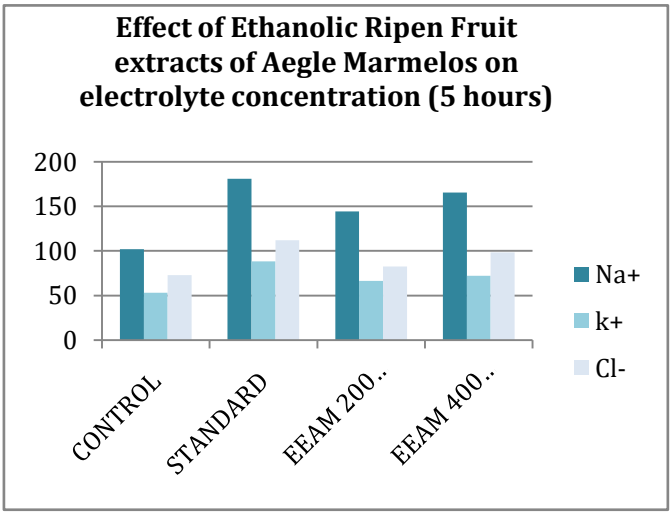
Groups	Total UrineVol. (ml/5 hours)	Na+µmol/L	K+µmol/L	Cl– µmol/L
Control (10 ml/Kg	6.56±0.06	104.04 ± 2.18	56.08 ± 1.52	72.96 ± 1.42

Standard (Frusemide 10mg/kg	10.43±0.04***	182.06±2.08***	88.22±1.64***	114.06±1.68***
Alcoholic extract of The Ripen fruit part of plant *AegleMarmelos* Low dose (200 mg/kg)	7.82±0.06*	146.44±2.06*	68.44±2.68*	84.66±1.26*
Alcoholic extract of The Ripen fruit part of plant *AegleMarmelos* High dose (400 mg/kg)	8.48±0.08**	166.54±2.38**	72.22±1.76**	98.38±2.46**

The data was presented as mean ± SEM (n=6), and statistical analysis was conducted using one-way ANOVA followed by Dunnett's' test via Graph Pad Prism software. Significance levels were denoted as * for p<0.05, ** for p<0.01, and *** for p<0.001 when compared to the control group.



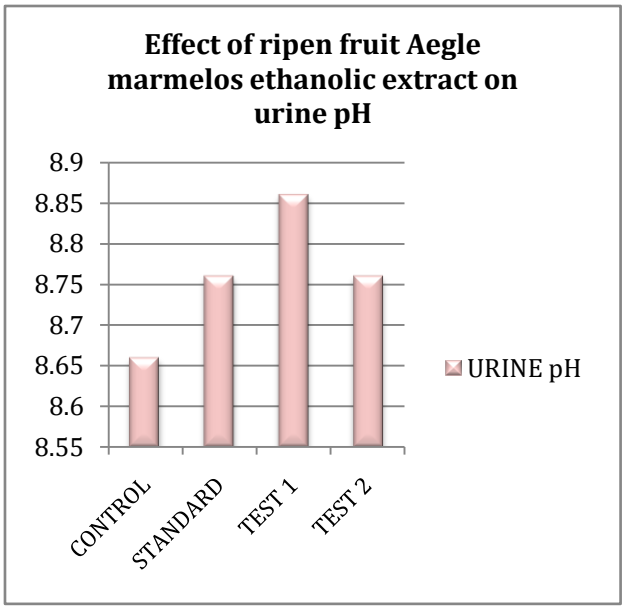
Graph 1: Effect of Ethanolic leaves extracts of *AegleMarmelos* on Total Urine Volume (in 5 hours)



Graph 2: Effect of Ethanolic Ripen Fruit extracts of *AegleMarmelos* on urinary electrolyte concentration (in 5 hours)

Urine pH Analysis:

Group	pH of Urine
Control (Normal Saline 10 ml/Kg)	8.64±0.14
Standard (Frusemide 10mg/kg)	8.78±0.28
EAMLD (200 mg/kg)	8.66±0.24
EAMHD (400 mg/kg)	8.74±0.24



Graph 3: Effect of Ethanolic Ripen Fruit extracts of *AegleMarmelos* on urinary on pH in hydrated rats

Discussion

In Acute oral toxicity study, The LD50 of EAM, determined following OECD 425 guidelines using five rats, revealed no acute oral toxicity at the tested doses ranging from 5 mg/kg to 1000 mg/kg, as no observable signs of toxicity or

mortality were observed during the 14-day observation period.

Statistical analysis

The experimental results, presented as mean ± SEM (n=6), were analyzed using Student's t-test for unpaired data, with statistical significance set at a significance level (α) of ≤0.05. The resulting p-values were assessed and interpreted accordingly.The alcoholic extract of ripe fruit from the AegleMarmelos plant contains various phytoconstituents, including phenolic compounds, proteins, tannins, glycosides, carbohydrates, starch, vitamins, and minerals. In acute toxicity testing, all animals survived for 14 days, indicating the extract's safety up to the highest tested dose of 1000 mg/kg, with no significant behavioral changes observed.

The alcoholic extract of ripe AegleMarmelos fruit demonstrated significant dose-dependent diuretic activity compared to the control, with increased urinary output and elevated excretion of sodium, potassium, and chloride ions. When compared to the standard diuretic drug Frusemide, the extract showed notable diuretic effects, indicating its potential as a diuretic agent.

Conclusion

Scientists from various fields are exploring plants for potential diuretic properties due to species extinction concerns. Thousands of Phytochemicals with diuretic inhibition effects have been identified in vitro, warranting further animal and human studies, including toxicity assessments. Standardized extraction and testing methods are needed for systematic research, and alternative anti-infection mechanisms, like adhesion disruption, should be considered. The study found that a single dose of the alcoholic extract of AegleMarmelos fruit significantly increased urine output and the excretion of sodium, potassium, and chloride ions, comparable to the standard drug Frusemide. This supports the traditional use of AegleMarmelos for diuretic purposes and highlights its potential for future therapeutic applications in human medicine.

Acknowledgment

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

Conflicts of Interests

There are no conflicts of interest.

Funding

Nil

Authors Contributions

All the authors have contributed equally.

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