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Research Article

Exploration of Diuretic Potential of *Carica papaya* Linn Root Extract Through in Vitro Evaluation

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ABSTRACT

The present study was carried out to evaluate the phytochemical constituents and carbonic anhydrase inhibitory activity of Carica papaya Linn. root extract. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, phytosterols, terpenoids, tannins, and proteins. The extract exhibited concentration-dependent carbonic anhydrase inhibition, with inhibition increasing from 21.41% at 50 µg/mL to 79.71% at 250 µg/mL. The IC_{50} value of the extract was 146.72 µg/mL, whereas acetazolamide showed an IC_{50} of 120.30 µg/mL. The results indicate that C. papaya root extract possesses promising carbonic anhydrase inhibitory potential and may have a possible role in diuretic activity

INTRODUCTION

The word diuretic has a Greek stem, Diu(through), ovpein (to urinate). Diuretics are the drugs that improve the removal of sodium and water from urine (producing a natriuretic effect).(1)

Diuretics have been used for more than 50 years to treat a variety of illnesses and are among the most often prescribed medications. All diuretics work primarily by preventing the renal tubules from reabsorbing sodium, which increases the fractional secretion of electrolytes such as potassium, sodium, and chloride ions and lowers the amount of blood flowing through the cardiovascular system. Diuretics are primarily used for edematous disorders such as hypertension, nephrotic syndrome, hepatic cirrhosis, heart failure, and blood pressure. Certain diuretic medications used to treat more specialized conditions like glaucoma, cerebral edema, hypocalcaemia, hypercalciuria, and diabetes insipidus are effective in controlling volume 000 in patients who are difficult to treat and have several underlying issues. (2)

Herbal drugs have gain important and popularity in recent year because of there safety, efficacy and

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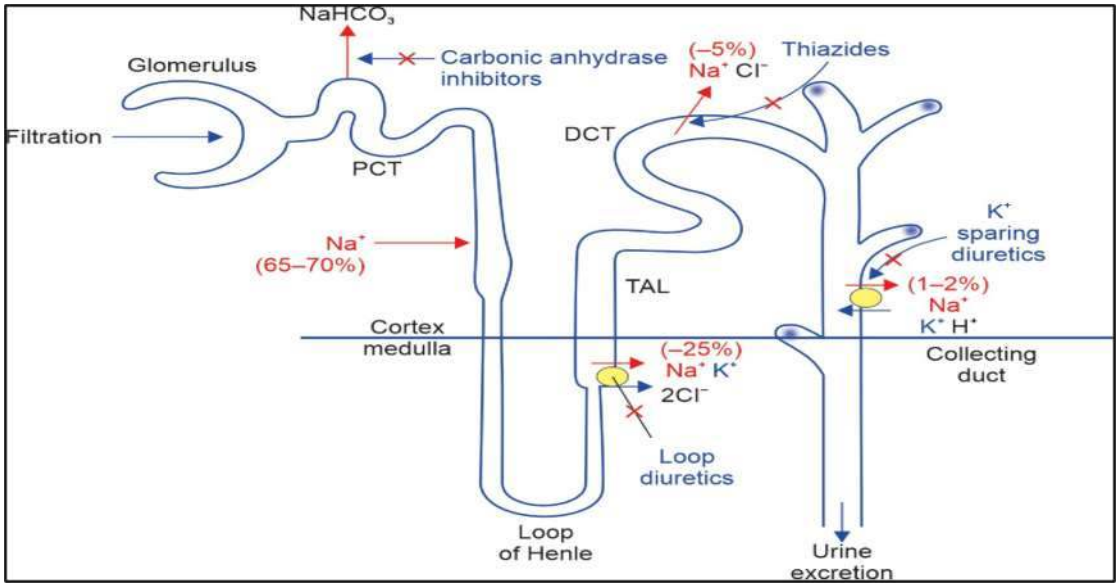
cost effectiveness. The traditional medicine like ayurvedic, siddha and Unani are based on the use of plant material. One of the important and well documented uses of plant product is their uses as diuretic agent.

Diuretics are among the most commonly used drugs and the majority act by reducing sodium chloride reabsorption at different sites in the nephron. Some herbal diuretics produce diuresis by inhibiting the release of ADH (anti-diuretic hormone) or by inhibiting the action of ADH on the uriniferous tubules.[3]

Diuresis in the body is regulated by ADH. ADH is secreted by neurohypophysis and pulmonary veins regulate the rate of ADH release, which depends on body hydration.

Medicinal herbs are the significant source of diuretics. Mono and poly herbal preparation have been used as diuretics. According to one estimate, more than 650 mono and poly herbal preparations in the form of decoction, tincture, tablets and capsules from more than 75 plants are in clinical use. [4]



HERBALS UTILIZED AS DIURETICS IN INDIA [5]

Herbs utilized as a diuretic have been utilized in India for promoted the world over by driving drugs. Plant medication was generally utilized for conventional treatment of some renal sickness, and many plants have been found to show massive diuretic activity. Numerous specialists have shown that investigations of natural plants from India utilized in customary medication as diuretics have expanded in recent years; furthermore, they may be a valuable device in treating hypertension. Hypertension is viewed as one of the principal and hazardous difficulties of diabetes mellitus.

Some plant which exhibits diuretic property:[6]

- *Mangifera indica:*

It is a species of mango in the Anacardiaceae family. It is found in the wild India. They use ethyl acetate, ethanol and water extract of Mangifera indica for evaluation of diuretic activity.

- *Euphorbia thymifolia:*

Euphorbia thymifolia (Euphorbiaceae) is a small branched, pubescent, prostate annual herb, commonly known as laghududhika or choti-dudhi. Thet use crude ethanolic extract of Euphorbia thymifolia for evaluation of diuretic activity.

- *Allium sativum:*

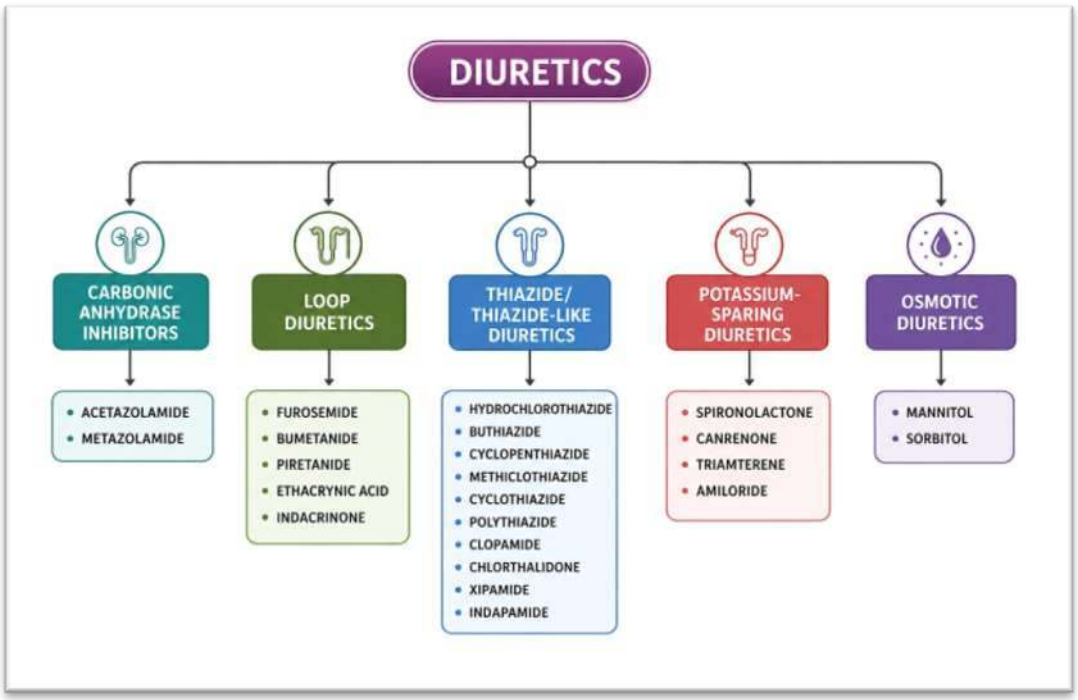
It is also known as garlic, belongs to Liliaceae family and genus Allium. The purified garlic fractions brings a supportive dose dependent effect on Na+-K+-ATPase. Therefore it may cause diuresis by increasing the volume of urine.

- *Mimosa pudica:*

It is also called as sensitive plant; sleepy plant is a creeping annual or perennial herb. Diuretic test of aqueous extract of Mimosa pudica Linn. leaves were evaluated using Lipschitz test.

- *Hibiscus sabdariffa L.*

It belongs to malvaceae family and it is an annual herbaceous plant shrub that can grow up to 2.4 m in tall, with smooth cylindrical red stems. Diuretic activity of aqueous extract was studied from the calyces of Hibiscus sabdariffa linn.



CLASSIFICATION:

- **Carbonic Anhydrase Inhibitors**[7]

Acetazolamide inhibits carbonic anhydrase in the proximal tubule. Causes diuresis and may produce metabolic acidosis. Used to reduce intraocular and intracranial pressure.

- **Loop Diuretics**[8]

Block $Na^+/K^+/2Cl^-$ cotransporter in the ascending loop of Henle. Produce strong natriuresis and diuresis and are effective even at low GFR. Examples: Furosemide, Bumetanide.

- **Thiazide Diuretics**[9]

Two types: Thiazide and thiazide-like diuretics. Block sodium reabsorption in the distal convoluted tubule. Used mainly for hypertension and edema.

- **Potassium-Sparing Diuretics**

Act mainly on the collecting duct and distal tubule. Reduce Na^+ reabsorption and prevent excessive K^+ loss. Example: Amiloride.

- **Osmotic Diuretics[10]**

Increase osmolarity of tubular fluid, causing water excretion. Used in acute renal failure and glaucoma. Examples: Mannitol, Sorbitol.

MEDICAL SIGNIFICANCE OF DIURETICS:[11]

Diuretics used in the treatment of oedema associated with raised venous pressure, reduced plasma colloid osmotic pressure, renal sodium retention.

It used in treatment of

- Cardiac insufficiency.
- Cirrhosis of the liver.
- Hypertension.
- Renal disease.

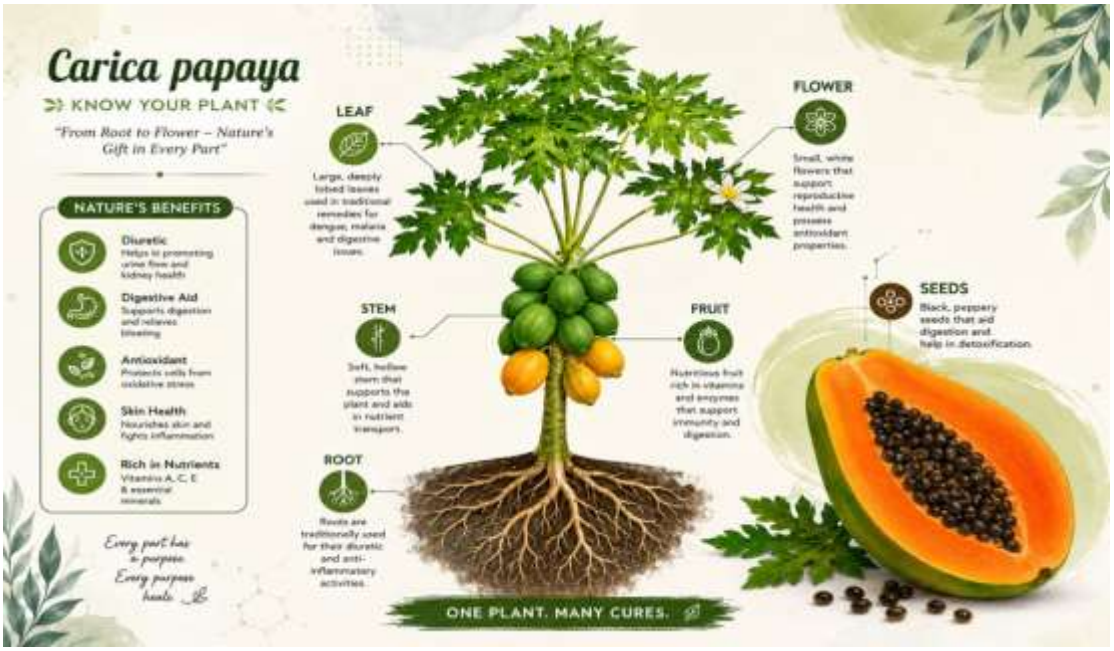
PLANT DESCRIPTION [12]

Papaya is a powerhouse of nutrients and is available throughout the year. It is rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E the minerals, magnesium and potassium, the B vitamin pantothenic acid and folate and Fiber.

In addition to all this, it contains a digestive enzyme –papaintha effectively treats causes of trauma, allergies, and sports injuries.

The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer. It is reported that it helps in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is good source of fibre.

Juices from papaya roots is used in some countries of Asia to ease urinary troubles. Papaya leaf when dried and cured like a cigar, is smoked by asthmatic persons.



TAXONOMICAL DESCRIPTION: [13]

- Domain: Flowering plant
- Kingdom: Plantae
- Sub Kingdom: Tracheobionta
- Class: Magnoliopsida
- Subclass: Dilleniidae
- Super division: Spermatophyta
- Phylum: Steptophyta
- Order: Brassicales
- Family: Caricaceae
- Genus: Carica
- Botanical name: *Carica papaya* linn

GEOGRAPHICAL SOURCE OF CARICA PAPAYA:[14]

- The Carica papaya L. fruits and crops are cultivation in tropical and subtropical world zone. Universal over 6.8 million tones something of papaya fruit was obtain in 2004 on about 389,990 hectares. In 2004 carica papaya was cultivated in 47% in united state America, in 30% Asia and it also cultivated in 20-22% in Africa.
- Brazil is the city of world which is shows largest papaya industry for his continues growth.
- The Carica papaya is originated in Mesoamerica, like in southern Mexico. Carica papaya was introduced plantation selection in India from Malaysia in the 16th century.
- Australia, Hawaii, Philippines, Sri Lanka and south Africa, papaya is a species to not easy to identify weather it is a male and female the height of carica papaya tree is 2 to 10 Meter. Carica papaya is manufacture a pear shaped.

POLLINATION OF CARICA PAPAYA Linn:[12]

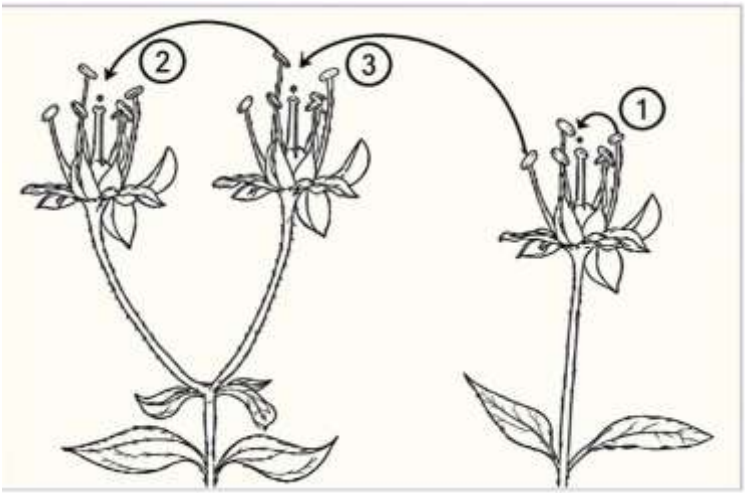
Three methods of pollen transfer,

(1) self-pollination

(2) pollen from same plant but different flower

(3) pollen from different plant

Bisexual flowered plants are self-pollinating, but female plants must be cross pollinated by either bisexual or molar plants.



BOTANICAL DESCRIPTION: [15]

PLANT:

- Large, Single, -stemmed Herbaceous perennial Tree having 20-30 feet height.

FRUIT:

- The fruits are big oval in shape and sometimes called pepo like berries, since they resemble melon by having a central seed cavity.
- Fruits weigh from 0.5 up to 20 lbs, and are green unlike ripe, turning yellow or red orange. Flesh is yellow orange to salmon (pinkish- orange) at maturity.
- Plants begin bearing fruits in 6-12 months

LEAF:

- Papaya leaves range in colour from green to yellow and have a number of advantages, it could be useful in treating dengue, malaria, and different viral illnesses.
- Facilitate digestion- The leaves of the papaya plants contain chemical compounds of carpain, Substance which kills microorganisms that often interfere with the digestive function.

ROOT:

- Papaya root is predominately a non-axial, fibrous system, composed of one or two 0.5-1.0m long tap roots.
- A decoction formed by boiling the outer part of the roots of the papaya tree in the cure of dyspepsia.

FLOWER:

- Papaya plants are dioecious or hermaphroditic, producing only male, female or bisexual flower.
- Prior to opening, bisexual flowers are tubular, while female flowers are pear shaper.

PHARMACOLOGICAL ACTIVITIES OF Carica papaya Linn.[16]

- **Anti-hypertensive activity:** Leaf and stem extracts show ACE inhibitory and vasorelaxant activity, helping to reduce blood pressure.
- **Anti-inflammatory & immunomodulatory activity:** Papaya extracts reduce inflammation and improve immune responses by regulating cytokines.
- **Anti-malarial activity:** Papaya leaves have traditional use in malaria, and antimalarial and antiplasmodial activities have been reported.
- **Nephroprotective activity:** Papaya extracts show dose-dependent protective effects on the kidneys in experimental animals.
- **Anti-ulcer activity:** Leaves and unripe fruits may protect against gastric ulcers and show antibacterial activity against H. pylori.

ETHANOMEDICINAL VALUES OF C.PAPAYA:[12]

- Colon cancer
- Rheumatoid Arthritis
- Anti sickling activity
- Promote Lung health
- Prevent prostate cancer

- Anti-coagulant effect

VERNACULAR NAMES OF C.PAPAYA[17]

- Tamil - Pappali
- Hindi - Papita
- Bengali - Pepe
- Malayalam -Omakai
- Kannada - Papaya

PHYTOCONSTITUENTS OF C.PAPAYA:[18]

- Fruits - Volatile compound, alkaloids, glycosides.
- Juice - Lipid, palmitic, linolenic acid, oleic acid.
- Seeds - Carpine, myrosin, caricin
- Root - Caproside
- Leaves – Vitamin C and E
- Bark – Glucose, fructose, galactose, xylitol
- Latex – Proteolytic enzyme papain, chemopapain.

PHYTOCHEMICAL SCREENING TEST:

Each dry extract was used for screening the following bioactive compounds: alkaloids, Terpenoids, phenol and Tannis, sugar, saponins, flavonoids, quinones, and proteins.

➢ **Alkaloids test**

Mayer's test:

To a few ml of filtrate, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Dragendorff's test:

Take 2 ml of plant extract in a test tube, add 2-3 drops of dilute HCl., and mix. Add 2-3 drops of dragendorff's reagent. Observe for the formation of orange-red precipitate, which indicates the presence of alkaloids.

➢ **Detection of carbohydrates**

Fehling's test;

One ml of filtrate was boiled on water bath with 1 ml each of Fehling solution A and solution B. Red precipitate indicated the presence of sugar.

➢ **Detection of Glycosides**

Borntrager's test;

To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it pink colour indicated the presence of glycosides.

➢ **Detection of saponins by form test**

Take 2 ml of extract add a 1 ml of Distilled water is added. Two cm layer of foam indicated the presence of saponins.

➢ **Detection of phenol**

Ferric chloride test;

The 1ml of extract add a 2 ml of 2% ferric chloride is added. Dark green or black colour indicated the presence of phenolic compounds presence.

➢ Detection of phytosterols

Liebermann-Burchard test;

1ml of extract is mixed with 2 ml of acetic acid. To these one or two drops of concentrated sulphuric acid were added slowly along the side of the test tube. An array of colour changes showed the presence of phytosterols.

➢ Detection of terpenoids

To 2 ml of test solution added 2 ml of chloroform and add 2 ml of sulphuric acid is added. Greyish colour changes showed the presence terpenoids.

➢ Detection of Tannins

To 1 ml of the extract add a few drops of lead acetate is added. Formation of white precipitate indicates tannin.

➢ Detection of Quinines

Take 1 ml of extract add a 1 ml of sodium hydroxide is added. Appearance of Blue colour indicate Quinines presence.

➢ Detection of Flavonoid

Take 1 ml of test solution in ethanol, put a bit of magnesium and add a few drops of HCl. Appearance of deep blue colour indicate flavonoids.

➢ Detection of Protein

Take 1 ml of extract add a few drops of Con.Nitric acid is added. Appearance of yellow colour indicate protein presence.

METHOD OF PREPARATION:

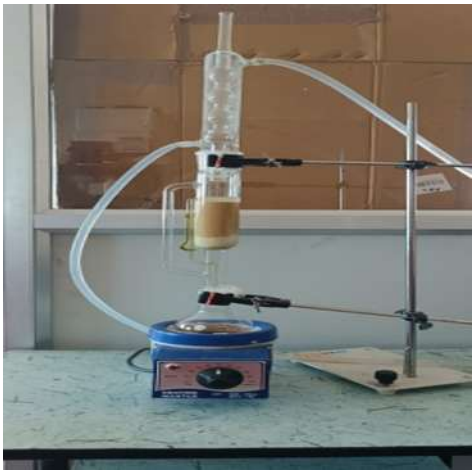
PREPARATION OF PLANT EXTRACT:

Plant extract was prepared by Soxhlet extraction method. It is a continuous extraction technique used to extract bioactive and soluble phytoconstituents from powdered plant materials using a suitable solvent. Solvent extraction of solid samples, which is commonly known as solid-liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest Method for solid sample pretreatment.

PROCEDURE:

- About 40 g of dried, powdered plant material was accurately weighed.
- The powdered material was uniformly packed into a Soxhlet extraction thimble.
- Ethanol was selected as the extraction solvent.
- The thimble was placed inside the Soxhlet apparatus, and the apparatus was properly assembled.
- The solvent was heated gently, allowing it to evaporate, condense and repeatedly pass through the plant material.
- This continuous extraction process was carried out for approximately 24 hours.
- Extraction was continued until the solvent in the siphon tube became almost colourless, indicating completion of extraction.
- The obtained ethanolic extract was collected in a clean container.
- The extract was concentrated by heating on a hot plate at 30–40°C to remove the solvent.
- Heating was continued until a concentrated/dried extract was obtained.
- The dried extract was collected, weighed and transferred into a clean, dry, airtight container.
- The extract was stored at 4°C in a refrigerator until further use.
- The stored extract was used for preliminary phytochemical screening and further analysis.

Soxhlet assisted extraction



Concentration of carica papaya

PERCENTAGE YIELD OF THE EXTRACT:

After extraction and evaporation of the solvent, the obtained dried extract was weighed, and the percentage yield was calculated using the formula;

Percentage yield (%) = (weight of dried extract/ weight of powdered plant material) *100

= (2.34/25) *100

= 9.36%

So, the percentage yield of the ethanolic extract was found to be **9.36%**

METHODOLOGY:

CARBONIC ANHYDRASE INHIBITION[19,20]

Principle:

The carbonic anhydrase (CA) inhibitory activity of the plant extract was evaluated using the p-nitrophenyl acetate (p-NPA) esterase assay, with minor modifications of previously reported spectrophotometric methods. The assay is based on the hydrolysis of p-nitrophenyl acetate by carbonic anhydrase to form p-nitrophenol, which was monitored spectrophotometrically at 348 nm.

Chemicals and reagents:

Bovine carbonic anhydrase, p-nitrophenyl acetate (p-NPA), Tris base, zinc chloride ($ZnCl_2$), acetazolamide, dimethyl sulfoxide (DMSO), and acetonitrile were used. All reagents were of analytical grade.

Preparation of 50 mM Tris-sulfate buffer containing 0.1 mM $ZnCl_2$:

Tris base (0.605 g) was dissolved in approximately 80 mL of distilled water to prepare 100 mL of 50 mM Tris buffer. The pH was adjusted to 7.6 using dilute sulphuric acid. Zinc chloride was added to obtain a final concentration of 0.1 mM (approximately 1.36 mg anhydrous $ZnCl_2$/100 mL). The final volume was adjusted to 100 mL with distilled water. The buffer was freshly prepared or stored under appropriate laboratory conditions.

Preparation of Stock solution:

The dried plant extract was dissolved in a suitable solvent, preferably DMSO, to prepare a concentrated stock solution. The stock was subsequently diluted with assay buffer to obtain a concentration range suitable for inhibition studies, from 50, 100, 150, 200 and 250 µg/mL. The final concentration of DMSO in the assay should be kept constant and low enough that it does not affect enzyme activity.

Preparation of p-nitrophenyl acetate substrate:

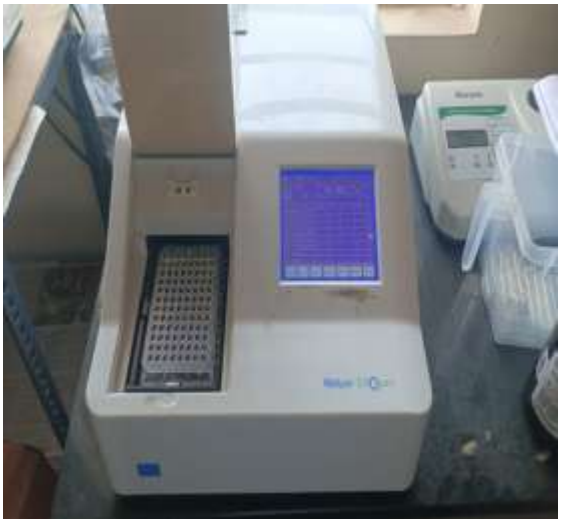
A fresh 6 mM p-nitrophenyl acetate stock solution was prepared immediately before use by dissolving 10.89 mg p-nitrophenyl acetate in 10 mL of a suitable solvent system, followed by dilution with assay buffer. The organic solvent content should be kept below approximately 5% in the substrate preparation. The substrate was prepared fresh for each experiment because p-NPA undergoes spontaneous hydrolysis. Published CA esterase assays commonly use a fresh 6 mM p-NPA stock and a final substrate concentration of approximately 0.6 mM.

PROCEDURE:

- The assay was performed in a 96-well microplate.
- For each reaction, 120 µL of 50 mM Tris-sulfate buffer containing 0.1 mM $ZnCl_2$ (pH 7.6), 20 µL of plant extract at different

concentrations, and 20 µL of bovine carbonic anhydrase enzyme were mixed.

- The enzyme–extract mixture was incubated for 10 min at room temperature (approximately 25°C) to allow enzyme–inhibitor interaction.
- The assay composition follows published p-NPA-based CA inhibition procedures.
- Following pre-incubation, 40 µL of freshly prepared 6 mM p-NPA was added to each well.
- This provided a final p-NPA concentration of approximately 0.6 mM. The total reaction volume was 200 µL.
- The reaction mixture was mixed thoroughly and incubated for 30 min at room temperature.
- Absorbance was measured at 348 nm using a microplate reader. The wavelength of 348 nm is used because it allows spectrophotometric monitoring of the p-nitrophenol/p-nitrophenolate product formed during p-NPA hydrolysis.



Experimental controls:

The following controls were included:

1. **Enzyme control:** enzyme + buffer + p-NPA, without plant extract; considered 100% enzyme activity.
2. **Sample blank:** plant extract + buffer + p-NPA, without enzyme; used to correct for the intrinsic absorbance or color of the extract.
3. **Reagent blank:** buffer + p-NPA without enzyme or plant extract.
4. **Positive control:** acetazolamide + carbonic anhydrase + p-NPA.
5. **Solvent control:** corresponding solvent at the same final concentration used for the plant extract.

Calculation of carbonic anhydrase inhibition:

For assays where higher absorbance/change in absorbance represents higher enzyme activity:

$$\% \text{ CA inhibition} = \left[1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{control}}}\right] \times 100$$

where:

- ΔA_{sample} = change in absorbance with plant extract
- $\Delta A_{control}$ = change in absorbance without extract

If extract color/turbidity interferes with the reading, correct using the corresponding sample blank before calculation.

Determination of IC_{50}:

The plant extract was tested at a minimum of five different concentrations spanning low to high inhibition. The percentage inhibition values were plotted against the logarithm of extract concentration.

The IC_{50} value, defined as the concentration of plant extract required to inhibit 50% of carbonic anhydrase activity, was calculated by nonlinear regression using a four-parameter logistic dose–response model in Graph Pad Prism or equivalent statistical software.

The same procedure was applied to acetazolamide as the reference inhibitor.

The results were expressed as mean ± standard deviation (SD) of three independent determinations (n = 3). Where appropriate, statistical comparisons were performed using one-way ANOVA followed by an appropriate post-hoc test, with $p < 0.05$ considered statistically significant.

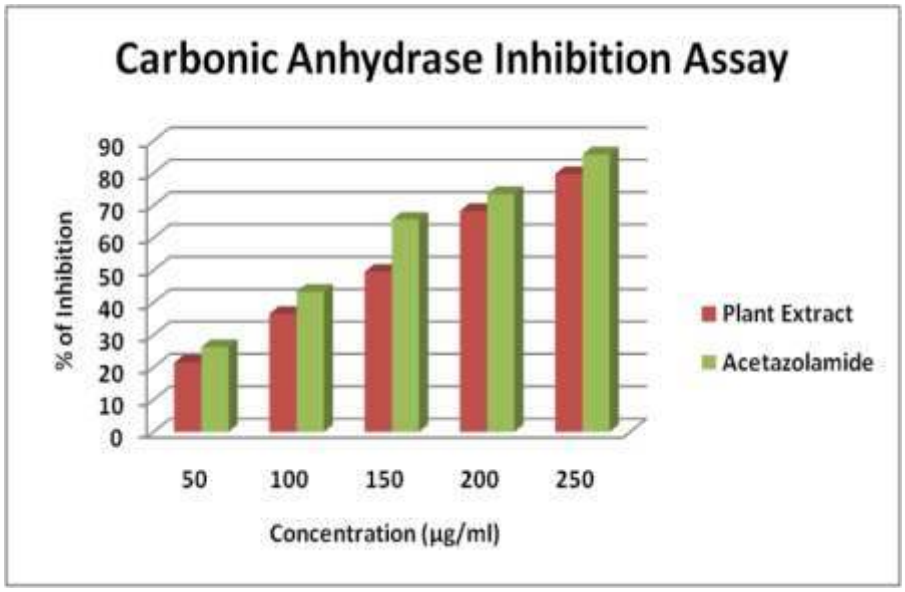
RESULTS AND DISCUSSION:

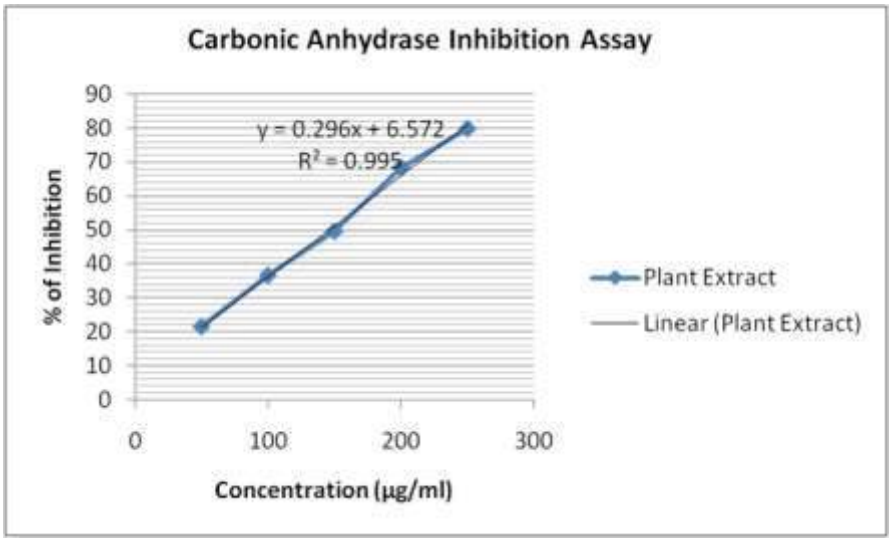
➢ **PHYTOCHEMICAL ANALYSIS;**

Phytochemicals	Method	Plant extract
Alkaloid	Mayers Test	+
	Dragendorff's	+
Carbohydrate	Fehling's Test	+
Glycosides	General Test	-
Saponins	Water Test	-
Phenol	Ferric Chloride Test	-
Phytosterols	Libermann Burchard Test	+
Terpenoids	General Test	+
Tannins	General Test	+
Quininines	Alkaline Reagent test	-
Flavonoid	Shinoda test	-
Protein	Biuret test	+

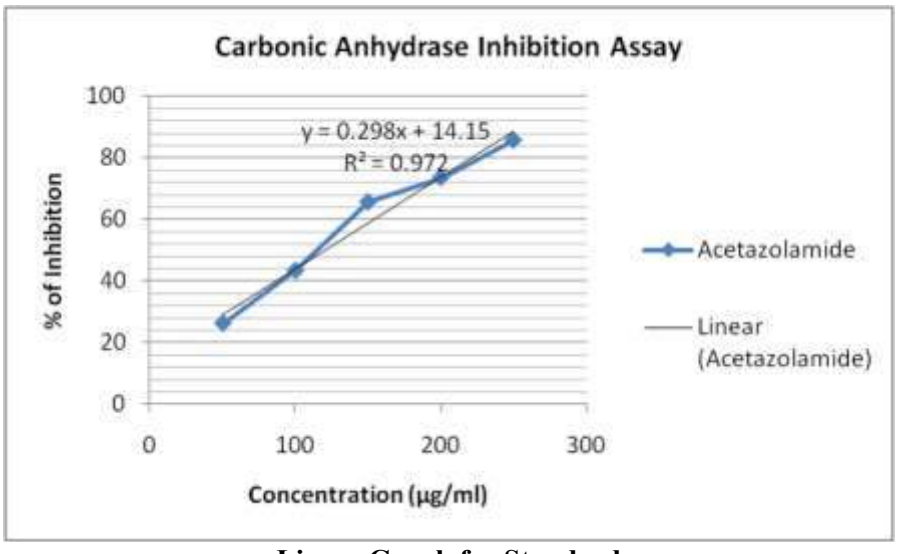
➢ **COMPARATIVE INHIBITORY ACTIVITY OF PLANT EXTRACT AND ACETAZOLAMIDE BASED ON IC_{50} VALUES;**

Concentration (µg/ml)	Plant Extract	Acetazolamide
50	21.41 ± 0.05^{a}	26.26 ± 0.12^{a}
100	36.52 ± 0.12^{b}	43.36 ± 0.15^{b}
150	49.49 ± 0.09^{c}	65.63 ± 0.42^{c}
200	68.30 ± 1.02^{d}	73.55 ± 1.15^{d}
250	79.71 ± 0.09^{e}	85.76 ± 0.5^{e}
IC50 value	146.72 µg/mL	120.30 µg/mL





Linear Graph for Plant Extract



Linear Graph for Standard

- The plant extract demonstrated concentration-dependent carbonic anhydrase inhibitory activity, increasing from 21.41 ± 0.05% at 50 µg/mL to 79.71 ± 0.09% at 250 µg/mL.
- Acetazolamide exhibited comparatively higher inhibition, ranging from 26.26 ± 0.12% to 85.76 ± 0.50%. One-way ANOVA revealed a statistically significant difference among the treatment groups ($F = 5345.27$, $p < 0.0001$).
- The IC_{50} values obtained from four-parameter logistic regression were 146.72 µg/mL for the plant extract and 120.30 µg/mL for acetazolamide, indicating comparatively lower CA inhibitory potency of the plant extract.

DISCUSSION:

The present study was undertaken to evaluate the phytochemical constituents and carbonic anhydrase inhibitory potential of Carica papaya Linn. root extract. The phytochemical screening revealed the presence of alkaloids, carbohydrates, phytosterols, terpenoids, tannins, and proteins, while glycosides, saponins, phenols, quinones, and flavonoids were not detected. The presence of these bioactive phytoconstituents may contribute to the pharmacological properties of the plant. Previous studies have also reported the presence of various bioactive compounds in Carica papaya, supporting its traditional medicinal importance.

The carbonic anhydrase inhibition assay demonstrated that the plant extract produced a concentration-dependent inhibitory effect. The percentage inhibition increased from 21.41 ± 0.05% at 50 µg/mL to 79.71 ± 0.09% at 250 µg/mL. Acetazolamide, used as the standard drug, showed comparatively higher inhibition, increasing from 26.26 ± 0.12% to 85.76 ± 0.50% over the same concentration range.

The IC_{50} value of the Carica papaya root extract was found to be 146.72 µg/mL, whereas acetazolamide showed an IC_{50} of 120.30 µg/mL. The lower IC_{50} value of acetazolamide indicates greater carbonic anhydrase inhibitory potency compared with the plant extract. However, the extract demonstrated appreciable inhibition, suggesting that its phytochemical constituents may possess carbonic anhydrase inhibitory potential.

Statistical analysis showed a significant difference among the treatment groups, with $F = 5345.27$ and $p < 0.0001$, indicating that the observed concentration-dependent changes in inhibitory activity were statistically significant.

Overall, the findings provide preliminary scientific support for the potential of Carica papaya root extract as a natural source of compounds with carbonic anhydrase inhibitory activity. Further studies are required to identify the individual active constituents and to establish their exact mechanism of action and therapeutic potential.

CONCLUSION:

The present study concluded that Carica papaya Linn. root extract contains various phytoconstituents such as alkaloids, carbohydrates, phytosterols, terpenoids, tannins and proteins. The extract showed concentration-dependent carbonic anhydrase inhibitory activity, which inhibits the carbonic anhydrase enzyme thereby reducing the bicarbonate resorption and promoting the urinary excretion of bicarbonate, sodium and water leads to the diuretic effect. Thus, Inhibition increasing from 21.41% at 50 µg/mL to 79.71% at 250 µg/mL.

The IC_{50} value of the plant extract was 146.72 µg/mL, while acetazolamide showed 120.30 µg/mL, indicating that the standard was more potent.

Overall, the findings suggest that Carica papaya root extract possesses promising carbonic anhydrase inhibitory potential.

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