International Journal of Pharmacy and Herbal Technology (Online)

Home Page: https://www.ijprdjournal.com/

IN VITRO ANTIUROLITHIATIC ACTIVITY OF PIPER BETEL EXTRACT

Shobha K. Gavade, * Ashwini B. Zade, Sanjay K. Bais

Fabtech College of Pharmacy, Sangola

Tal-Sangola, Dist.-Solapur

Maharashtra -413307

ABSTRACT

The most common urinary disorder in the world, urolithiasis, is caused by saturated crystals called kidney stones, which is from elements existing in the urine. Stalactites, another name for the phosphoric crystals, is created from infections of the urinary system. Although there are many synthetic medications available, using conventional drugs or botanicals to treat kidney stones has proven to be a promising approach. To explore an anti-lipidoglycan effectiveness among the conventional, Planting: Bitter melon an extract made from water of Piper betle Linn. leaves have been utilized. In the current experiment, the extract of Piper betel leaves was tested using three different types of assays: nucleation, aggregation, and oxalate depletion assays.

The primary goals of the research are to assess the in vitro impact on kidney stone disintegration and explore the possibility of Piper betel in the prevention or treatment of kidney stones. The capacity of Piper betle's aqueous extract to help dissolve kidney stones has been demonstrated, highlighting the plant's potential use as a natural renal stone cure. According to this research, kidney stones may be treated with piper betle as a non-invasive and perhaps affordable alternative to conventional medical procedures.

Keywords: *Piper betel leaf extract, anti-urolithiatic activity, kidney stone*

*Corresponding Author Email: - shobhagavade01@gmail.com

Received on 10 July, 2024, Accepted 20 July, 2024

Please cite this article as: Gawade Shobha et. al. In Vitro Antiurolithiatic Activity of Piper Betel Extract
International Journal of Pharmacy and Herbal Technology 2024.

INTRODUCTION

The origin and pathophysiology of kidney stones

As a result of food and lifestyle changes, kidney stones are currently the most common and severe urologic illness in the population. Calculi development is a characteristic of lithiasis, or the creation of stones. Nephrolithiasis and urolithiasis are two of its primary forms. Nephrolithiasis is defined by calculi development in the kidney, whereas urolithiasis is known to occur in the bladder, ureter, or any other component of the urinary system. For the purpose of forming bone and teeth, calcification often occurs in carefully regulated biological environments. Kidney stones, or uncontrolled pathological crystallization, are precipitates that develop in the body when a solvent becomes supersaturated.[1]

Potential metabolic process behind the development of kidney stones

In higher plants, ascorbic acid, glycolate, and glyoxylate are biosynthesized to produce oxalic acid. When a person consumes a lot of meals high in oxalate, their body is more likely to experience a major loss of minerals. The body's natural calcium ions can combine with free oxalic acid or oxalate to form insoluble calcium oxalate crystals, which can cause urolithiasis and hypocalcemia. Kidney stones typically consist of an elevated level concentration of calcium oxalate5, followed by trace amounts of calcium carbonate and calcium phosphate. The pathophysiology of calcium oxalate stone development is a multiphase activity comprising the expansion, formation, continuation, including breaking of crystals.[2]

A complex urologic disorder requiring repeated trips to the emergency room as well as prompt urological management is urolithiasis. One Although there has been remarkable technological progress in stone removal, there are still significant limitations due to related difficulties, such as the potential for stone recurrence to be enhanced. Growing interest in a vegetarian one medicine as a substitute for or addition to the traditional medical system has generated a great deal of novel medication discoveries. Piper betel L. was chosen for the current study's limiting factor based on these considerations. A famous herb for healing originating from Asia is betel leaf (Piper betle). The Piperaceae family includes it. To treat a variety of illnesses, traditional medicine is prepared from plant leaves. Due to its enormous abundance and low cost, food firms and the medical device sector may benefit from more research on it. Betel vine is another name for betel leaf. In the majority of nations, including India, it is commonly used as part of chewing exercises to strengthen gums, prevent bad breath, and increase digestive fire. India uses betel leaves as a mouthwash to gargle, whereas Indonesia uses them to treat vaginal douching. In Sri Lanka, skin conditions are treated with betel leaf juice. The astringent taste of betel leaves makes them useful as a cough medication when boiled.[3]

Techniques for using dietary supplements to stop the development of calcium oxalate stones Foods high in oxalate should be consumed in moderation. Oxalate-rich foods include spinach, rhubarb, beets, almonds, the candy, coffee, bread crumbs, and strawberries. Studies have indicated that these foods can elevate oxalate levels and cause a notable rise in excretion of oxalate in the urine. Given that vitamin C is known to convert to oxalate, using larger doses of vitamin C supplements may raise the risk of developing stones and oxaluria. Consuming excessive amounts of fluids, limiting salt and protein intake is recommended. It is recommended to consume calcium during mealtimes to prevent the production of calcium oxalate.[4]

Organic Component

Terpene, Chavicol, P-cymene, as their related compounds, amino acids, oxalic acid, malic acid, estragol, and eugenol is all present in the plant.

Vitamin C, ascorbic acid, and carotin are the three main forms of vitamin C that are present in leaves in good proportions. With the exception of lycine, histidine, and arginine, they also provide sizable levels of all necessary amino acids. While there are good amounts of glycine and proline, there are high quantities of asparagine's. This leaf's fragrant flavor comes from its essential oil. In the root, β-sitosterol is found.[5]

Morphology of Piper betel Linn

Figure No.1: Piper betel leaf

OVERVIEW

Piper betel L. is a member of the Piperaceae family, also referred to as Paan. The land is extensively planted. throughout Southeast Asian region comprises. Taiwan, Thailand, India, and Sri Lanka. The leaves have strong, bitter, somewhat sweet, and caustic flavors. It contains several biomolecules with a variety of pharmacological properties, including laxative, carminative, stomachic, antihelminthic, tonic, and aphrodisiac properties. The leaves of this plant are used to treat a variety of conditions, including sytyptic, bronchitis, ozoena, cough, and foul-smelling mouth.[6]

Kingdom: Plantae
Division: Magnoliophyta
Order: Piperales
Family: Piperaceae
Subfamily: Piperoideae
Genus: Piper
Species: Piper betle Linn [7]

Individuals	Synopsis
Sizes	The leaf is 8–16 cm long. Leaf width: 6 to 12 cm
Hue and state	Dark green to light green, young leaves
The arrangement and venation	straightforward and reticulate
Apex and margin	Whole and shrewd
Base	spherical leaves with a broad cordate base
The Texture and Surface	Thick lamina with a glabrous, smooth surface
The petiole	Extensive petiole Leaf Base length: 1.5 to 4.5 cm Leaves with restrictions
Leaf Base	Stipulate leaves

Table No.1: Piper betle morphological characteristics [8]

Indian languages	Synonym of *Piper betle*
The Ayurvedic	Names for these herbs are Taambula, Naagvallari, Naagini, Saptashiraa, and Bhujangalataa.
Unani healing	food habit and Tambool
Tamil and Sidha	Ventrali The Nagavalli and Kammaaruvetritai
Bangla, Gujraji, Hindi, Urdu	Pashto
Conkani	Phodi paan
Tamil	Veetil
Hindi	Vidyache panan
Tamil	Tamilpaka

Table No.2: Common Names for Piper betle [9]

Examination of plant constituents qualitatively [10]

Picric acid that- An orange colour observed.
Barfoed's test -A red precipitate.
Benedict's test – Green/yellow/red monosaccharides colour.
Ninhydrin test – A purple coloured solution.
Xanthoproetic test – Yellow colour
Keller killani's test – Blue coloured solution.
Ferric chloride test – Green precipitate.
Salkowski test - Golden yellow layer.
Braymer's test – Blue green colour.
Foam test – Persistent foam for 10 min.

MATERIALS AND METHOD

Gathering of plants

Piper betel L. leaves were obtained from Sangola's grocery shop and verified and recognized by the department of botany at Sangola College.

Preparing and Extracting

The betel leaf is pounded into a powder after being allowed to dry at room temperature. For a full day at room temperature, 50 grams of betel leaf powder had been immersed in a 200 ml ethanol solution using a soxhlet apparatus. In addition, the solution is evaporated at 40oC in a hot air oven. Twenty milliliters of a viscous, green solution were obtained from the betel leaf the alcohol extraction process.

Aggregation Assay

Calcium oxalate crystals, those are frequently found in kidney stones, can be prevented or lessened from aggregating in an experimental aggregated assay designed to evaluate the anti-urolithiatic effect of betel leaf extract.[11]

Making Crystals of calcium oxalate

Make 10 mM solutions of sodium oxalate (Na2C2O4) and calcium chloride (CaCl2) in distilled water. To create crystals of calcium oxalate, combine equal volumes of Na2C2O4 and CaCl2 solutions. To guarantee total precipitation, let the mixture remain at ambient humidity for one to two hours.

Process

Split the suspension of calcium oxalate crystals among multiple test tubes, e.g., 5 mL each. Fill each test tube with varying amounts of the betel leaf extract. If one is available, provide a positive control and a control tube devoid of extract. To uphold the pH of the tissue while restoring the overall volume to a constant level (e.g., 10 mL), add phosphate buffer (pH 7.4) in every tube. To fully combine the contents, vortex the tubes for a little while. To replicate physiological circumstances, gently shake the tubes or place them in a water bath and incubate them at 37°C for one to two hours.

Measurement

Using a spectrophotometer set to 620 nm, determine the optical density (OD) of the supernatant both before and after incubation. Crystal aggregation and sedimentation are indicated by a drop in OD. As an alternative, count and quantify the aggregate sizes seen under a microscope using image analysis.

Compute and examine:

Examine the variations in calcium oxalate aggregate size and quantity between the test and control samples. Utilizing the following formula, determine the share inhibition of aggregation: Reduction of aggregation (%) = (Aggregate count in control - Aggregate count in test) Aggregates under control: 100Aggregation Inhibition (%) = (Number of Control Aggregates) Number of test aggregates minus number of control aggregates) ×100.[12]

Assay for oxalate depletion

You must gauge the betel leaf extract's capacity to lower the oxalate content in a solution in order to assess the anti-urolithiatic effect of the leaf via an oxalate depletion assay.[13]

Making the Oxalate Solution:

Dissolve the necessary quantity is oxalate salt (Na2C2O4) in distilled Use fluid in create Ten milligram.

Oxalate Depletion Assay:

In many test tubes, combine equal amounts of the sodium oxalate liquid and betel extract of leaves (e.g., 5 mL of each). Place control tubes with distilled water and sodium oxalate solution in place of the betel leaf extract. If needed, use phosphate buffer to bring the mixture's pH down to physiological levels, which are typically around 7.4. Keep the tubes shake-free for a predetermined amount of time (e.g., one to two hours) at 37°C.[14]

Analysis of Oxalate Depletion:

Centrifuge the tubes for 10 minutes at 2000 rpm to get rid of any precipitated materials after incubation. Utilizing the following techniques, determine the amount of oxalate that is still present

Spectrophotometric Method:

As directed by the manufacturer, use an oxalate assay kit that is commercially available. Calculate the supernatant's absorbance at the designated wavelength, which is typically approximately 590 nm. Utilizing a standard curve built with known sodium oxalate concentrations, determine the Examine the differences between the test and control samples' oxalate concentrations. Utilizing the Oxalate depletion (%) is equal to the difference between the initial and final oxalate concentrations in the test. Initial concentration of oxalate) ×100Initial oxalate concentration = Oxalate depletion (%) The difference between the initial and final oxalate concentrations in the test is 100.[15]

By dissolving the necessary amount in distilled water, you can prepare a 10 mM solution of calcium chloride dihydrate (CaCl2·2H2O). Add enough distilled water to dissolve the necessary amount of sodium oxalate (Na2C2O4) to create a 10 mM solution. For best results, preheat both solutions to 37°C.

The nucleation of calcium oxalate crystals is accomplished by mixing equal amounts (e.g., 5 mL each) of both pre-warmed calcium chloride and sodium oxalate solutions in a series of test tubes. Put various amounts of betel leaf extract into the test tubes right away. Don't forget to add an extract-free control tube. To keep the pH of each tube physiological and to ensure that the volume remains constant, add phosphate buffer (pH 7.4) for every unit (e.g., 10 mL). To fully mix the ingredients, vortex the tubes for a brief moment. For a predetermined amount of time (such as one to two hours), gently shake the tubes while they are incubated at 37°C to replicate physiological conditions.[16]

Measurement of Nucleation:

Using a spectrophotometer set to 620 nm, determine the visibility of the solutions following incubation. Higher calcium oxalate crystal nucleation is indicated by increased turbidity. An alternative method for settling the crystals would be to centrifuge the tubes for 10 minutes at 2000 rpm. To resuspend the crystals, toss the supernatant and use a tiny amount of distilled water (one milliliter, for example). A small amount of the suspension should be placed on a little slide, then covered with a coverslip. Examine the slide at a magnification of 40x or greater using a light microscope. Keep track of the size and quantity of calcium oxalate crystals by observation. [17]

Use the following formula to determine the quantity inhibition of nucleation

Nucleation in control − Nucleation in test = Inhibition of nucleation (%) Controlled nucleation) ×100Nucleation in control = Inhibition of nucleation (%) Nucleation in test) ×100 − Nucleation in control. [18]

RESULT

The present investigation was performed to test the antiurolithiatic potential of the traditional plant Piper betle, for which a series of concentrations of aqueous extract of Piper betle leaves (0.5%, 1%, 1.5% 2%, 2.5%) were prepared and were compared with the standard drug (Cystone). Sections of Colocasia esculenta leaf and petiole showing raphides (17) along with struvite crystals prepared from the crystallization method were utilized for the study. It was observed that with a 0.5% concentration of aqueous leaf extract, there was no considerable decrease in the number of raphides (calcium oxalate) and struvite crystals. The concentrations of 1% and 2% showed a slight change in the quantity of both crystals. A moderate change in the size and number was seen in raphides and struvite crystals treated with 4% and 6% aqueous leaf extract of Piper betle. The highest degree of change was seen with concentrations of 8% and 10%, by dissolving and/or minimizing the size of each crystal. Similar effects were seen using the standard drug (Cystone).

DISCUSSION

Renal stones are reportedly affecting humankind for a long time and have been one of the causes of renal failure. The reappearance of kidney stones in the human body is of crucial concern in the therapeutic management of urolithiasis. Advancement in drug therapy has met the needs of the population's healthcare. There are many vital areas in medical sciences such as infectious diseases, arthritis, liver diseases and cancer where the use of ordinary and synthetic medications are devoid of satisfactory treatments.

CONCLUSION

A serious medical condition known as urolithiasis is brought on by the supersaturation of urine, which leads to kidney stone recurrence and subsequent pain and inflammation. An herbal method is a complex corrective measure as happens because of alterations Way of being alive by means of nutrition; inside medications Along with Chamomile treatment particularly successful within administration for kidney blocks. Right now, investigation indicated watery leaf extract from Pipe betle had a favorable inhibiting impact Regarding laboratory-based the formation crystals of the mineral calcium oxide in addition to grout rocks. Piper betle leaves were characterized and phytochemicals were screened, and the results showed that the leaves had an intricate structure with active substances that can dissolve, minimize, or neutralize the crystals of calcium oxalate as Struvite that have basic qualities. The results this examine support the traditional notion The Piper's Bettle leaf is a viable pharmaceutical product with a variety of therapeutic properties, as supported by prior research studies.

CONFLICTS OF INTEREST

Nil.

FUNDING

No financial interest

REFERENCE

1. Niharika M, Himabindu J, Ramanjaneyulu K. Evaluation of In Vitro Antiurolithiatic Activity Scf Tridax procumbens. Int J Sci Res.2018,7(1):38–39.
2. Sharma D, Dey YN, Sikarwar I, Sijoria R, Wanjari MM, Jadhav AD. In vitro study of aqueous leaf extract of Chenopodium album for inhibition of calcium oxalate and brushite crystallization. Egyptian Journal of Basic and Applied Sciences.2016,3(2):164 – 171.
3. Nagarajan Y, Boopathi R, Yahoob SAM, Venkatraman A. In Vitro Evaluation of Anti Urolithiatic Activity of Bryophyllum pinnatum Lam. International Journal of Scientific Research in Multidisciplinary Studies. 2019,5(8): 97–102.
4. Banu AS, Siddiqua S, Maliha S, Fatima S, Farooq A, Altaf N, et al. Ayurvedic Remedies for Kidney Stones. International Journal of Pharmaceutical Research and Applications. 2022,7(4):1338–1342.
5. Baby B, Swetha AS, Veluraja K. Studies on the effect of Phyllanthus emblica extract on the growth of urinary type struvite crystals invitro. Clinical Phytoscience, 2015; 1.
6. Smrethe R, et al. In Vitro Study of Inhibitory Effect of Alpinia purpurata Leaves Extract on Struvite Crystals. International Journal of Recent Scientific Research. 2018,9(12):30021–30025.
7. Gudulkar S, Rajbhar K, Dawda H, Mukundan U.Screening of selected plants for their effectiveness in the treatment of kidney stone. Annals of Phytomedicine: An International Journal.2020,9(1):213–217.

8. Jaiswal S, Patel M, Saxena D, Naik S. Antioxidant Properties of Piper Betel (L) Leaf Extracts from Six Different Geographical Domain of India. Journal of Bioresource Engineering and Technology.2014,2(2):12–20.
9. Patel N, Jain D, Suryawanshi H, Pawar S.Phytopharmacological Study of Piper Betle Leaf. Saudi Journal of Medical and Pharmaceutical Sciences.2019,(11):964–971.
10. Pandit A, Joshi A. A Short Overview on Significance of Betel Leaf (Piper betle) And Its Applications. Int J Health Sci Res.2022,12(11):251–254.
11. Dinnimath BM, Jalalpure SS, Patil UK. Antiurolithiatic activity of natural constituents isolated from Aerva lanata. J Ayurveda Integr Med. 2017,8(4):226–232.
12. Nandiyanto A, Oktiani R, Ragadhita R. How to Read and Interpret FTIR Spectroscope of Organic Material. Indonesian Journal of Science and Technology.2019,4(1):97– 118.
13. Ashwini B. Zade, Sanjay K. Bais, Vinayak G. Kamble Role of Ayurveda in kidney stone International journal of Advanced Research in science communication and technology 2024,2(1)1323-1329.
14. SK Bais, P V Ghatage, Herbal Excipient and Novel Drug Delivery System Used in Liposome and Ethio some, International Journal of Creative Research Thoughts, 2023,11(2):720- 724.
15. J P Lavande, S K Bais, Balika Ashok Nagane, Novel Drug Delivery System International Journal of Advanced Research in Science Communication and Technology 2023,3(2):2581-2585
16. SD Sonawane, S K Bais, SA More, Novel Drug Design International Journal of Advanced Research in Science Communication and Technology, 2023,3(1):528-536
17. Amol Pore, Sanjay Bais, Roshan Navanath Galave, Novel Herbal Drug Delivery System Its Analytical Aspects and Applications International Journal of Creative Research Thoughts, 2023,11(1):76-80
18. Praveen V Patil, Sanjay K Bais, Ganesh V Gudge., Review on Novel Herbal Drug Delivery System International Journal of Advanced Research in Science Communication and Technology, 2023,3(1):92-96