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REVIEWARTICLE

NOVELINVITRO APPRO A CHES FOR SCREENING AND IDENTIFICATION OF ANTI- UROLITHIATIC ACTIVITY: A COMPREHENSIVE REVIEW

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Abstract

Urolithiasis is characterized by the formation of renal calculi, remains a significant global health concern,with high recurrence rates and limitations in current treatment options.The major types of renal stones include calciu m phosphate, uric acid, and.Current treatment strategies such as extracorpo real shock wave lithotripsy, ureteroscopy, and percutaneous nephrolithoto my effectively fragment or remove stones but often fail to prevent recurren ce and carry the risks.Many medicinal plants show anti urolithiasis activiti es caused by chemical constituents which includes flavonoids, saponins, phenols, tannins, and alkaloids with antioxidant, diuretic, and anti inflamm atory properties. In-vitro studies are conducted on membrane of the egg to break down the calcium oxalate crystals by using ethyl acetate extract of B.pinnatum and A.lanata. These extracts show anti urolithiatic activity mai nly because of the presence of phytochemicals such as flavonoids and tannins.

Introduction: -

Urolithiasisis also known as kidney stones, is the one of the most common disorders that affect the urinary tract. The condition involves the formation of hard, crystalline masses within the kidney stones or urinary systems, often composed of calcium oxalate, phosphate, uric acid or other minerals. kidney stones are not only painful but can also leads to severe complication if not diagnosed and managed properly. The mechanism is due to the factors which are, metabolic disorders, dietary habits, genetic predisposition, dehydration, infection and lifestyle factors such as low physical activity.

Crystallization starts by super saturation of the urine with stone-forming constituents, leading to nucleation, growth, aggregation, and retention of crystals within the urinary tract. In light of limitations and side effects of conventional therapies, there has been an increasing interest in the use ofplant remedies and natural supplements to prevent and manage kidney stones

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Traditional systems of medicine such as Ayurveda,have made use of plants for anti-urolithiatic properties. Itworks through multiple mechanisms. such as cessation of stonesformation, crystal aggregationreduction,enhancing diuresis and increasing the expulsion of stones.

phytochemical such as flavonoids, saponins, alkaloids, tannins, glycosides and terpenoids are commonly found in these plants and are responsible for their therapeutic effects. these compounds exhibit antioxidant, ant-inflammatory antispasmodic and antimicrobial properties, all of which contribute to the inhibition of stones formation and improvement of urinary health. Others may act by modulating oxalate metabolism, decreased urinary calcium excretion, or increasing magnesium levels, which help prevent calcium oxalate crystallization [1]

2 Herbal plants play a significant role in ancient methods of medicine, so plants are the primary source for producing cost effective medicines. Aervalanata is the plant that have ability to cure a broad range of disease naturally. It includes in Amaranthaceous family and it is commonly seen in tropical plains as Indian weeds show antimicrobial, ant diabetic, diuretic, expectorant, anti-inflammatory, and anti-urolithiatic properties. Itconsists of wide range of phytochemicals like alkaloids,flavonoids,steroids,amino acids,terpenoids,tannins and proteins [11]Bryophyllumpinnatum, are also called as air plant, life plant etc., belongs to the family Crassulaceae. The leaves and bark of the plant possess a bitter taste which can be used to treat vomiting, diarrhea, burns, gastric ulcer, urolithiasis. Plant extract is used to cure asthma, cough, headache, convulsion and edema of legs. The phyto chemicals present in B. pinnate such as flavonoids, steroids, alkaloids, glycosides, phenols, tannins, saponins, arytenoids and organic acids [12,13].

Types of kidney stones:

Kidney stones are solid masses formed from crystals in urine, and they are classified based on chemicalcomposition.Up to 75% of kidney stones are predominantly composed of calcium oxalate. These stones form when calcium binds with oxalate in urine. Monohydrate type includes extremely hard and dark brown with a smooth surface and Dihydrate type include more fragile, yellow to light brown in color, and often havejagged edges.Calcium phosphate stone comprising about10%ofstones,theseareassociated with high urinary pH and metabolic condition such as hyperparathyroidism or renal tubular acidosis.

They may be off-white grey, or yellowish. Uric acid stones develop in acid urine and are linked to conditions like diabetes, obesity, and metabolic syndrome. They are often reddish, brown, or yellow color. Struvite stones are usually associated with urinary tract infection caused by urease-producing bacteria. These calculi frequently grow into large staghorn shapes and typically develop in alkaline urine.Cystine stones are rare, accounting for only 1-2 % of cases, and result from a genetic condition called cystinuria. These stones are waxy, yellowish, and resistant to standard treatments like shock wave lithotripsy [9]

Pathophysiology of nephrolithiasis:

Kidney stone formation is a complex biological process influenced by a combination of internal and external factors. A key early step is urinary super saturation, in which the concentration of certain solutes surpasses their solubility limit, creating conditions for crystal precipitation—particularly when urine flow is sluggish. Super saturation is expressed as the ratio of the actual solute concentration to its saturation point; values above 1 indicate a higher likelihood of crystal development.

The body produces several natural inhibitors of crystallization, including citrate, pyrophosphate, magnesium, and specific proteins such as uromodulin and osteopontin. These agents reduce stone risk by binding to ions responsible for crystal formation, thereby limiting their availability. Low urine volume elevates solute concentration, increasing the risk. Urinary pH also plays a major role: alkaline urine promotes calcium phosphate and struvite stones, whereas acidic urine favors uric acid and cystine stones.

Super saturation alone does not guarantee stone development—nucleation sites are often necessary. These may include damaged kidney epithelial cells or Randall's plaques, which are sub epithelial deposits of calcium phosphate found in the renal papillae and often serve as anchors for crystal growth.

These plaques can rupture into the urinary tract, providing a surface for calcium oxalate or phosphate crystals to adhere and grow into larger stones through aggregation. Genetics also influence stone formation. Variants in genes regulating calcium, oxalate, citrate, and uric acid excretion can predispose individuals to nephrolithiasis. Also, monogenic disorders like cystinuria, Dent's disease, or primary hyperoxaluria directly cause stones disease through metabolic abnormalities. Such conditions may warrant genetic testing, particularly in individuals with early-onset or recurrent stones disease and a positive family history.[8].

Phy to constituents:

Many natural compounds show potential by inhibiting crystal formation, reducing oxidative stress and enhancing antioxidant defense mechanism. Berberine: it is an is oquinoline alkaloid found pre dominantly in Berberis vulgaris bark, has demonstrated significant anti-urolithiatic effect. It reduces crystal formation by modulating crystallization process and exhibits strong antioxidant properties, helping to alleviate oxidative stress associated with kidney stones formation. Barbering is a key component in in several plant species, including Berberi saquifolium, Berbereisaristata, Mahoniaaquifolium, cordifolia and coptischinensis. Quercetin:

A well-known flavonoid quercetin has shown promising result in experimental model of urolithiasis.it destroy the stone formation because of diuretic and antioxidant Activity Quercetin-rich plants such as Morus alba, Camellia sinensis, Centellaasiatica, and others exhibit notable anti-urolithiatic potential. Flavonoids, a class of plant-based polyphenols, are well recognized for their ability to prevent or reduce urinary stones formation through multiple protective biological mechanisms. Which play a role in inhibiting stones formation.it can modulate the inflammatory response, reducing production of pro-inflammatory mediators involved in kidney stones formation.

Some flavonoids exhibit diuretic properties, increasing urine production and helping to flush out small stones or precursors of stone formation. Triterpenoids—including oleanolic acid, lupeol, ursolic acid, and betulinic acid—exhibit lipid-lowering effects beneficial in kidney stone prevention by inhibiting calcium oxalate crystallization and minimizing stone promoting factors.These bioactive occur in plants such as Crataevanurvala, Aeglemarmelos, Betul aalba, Calendula officinalis, Cucumissativus, Prunusamygdalus, and Nerium oleander. Saponins, present in species like Tribulusterrestris and Asparagus racemosus, aid prevention by increasing urine output, dispersing mucoproteins that assist stone aggregation, and offering anti-inflammatory benefits. Coumarins contribute by disrupting early stone formation stages, thereby preventing crystal development within the urinary tract.Coumarin also prevent the clumping together of crystals, reducing likelihood of stones forming in the kidney or urinary tract. It contain plan textract suchas those from Aeglemarmelos and Kalanchoelaciniata,can inhibit calcium oxalate crystal formation and growth [4].

Current treatment:

Treating kidney stone can be both painful and costly. The intense pain typically results from the sharp presence of, irregularly shaped crystals, which can damage internal tissues. Extracorporeal shock wave Lithotripsy (ESWL): Extracorporeal shock wave Lithotripsy is a method involves directing acoustic shock waves at the kidney stones from

outside the body. High and low frequency waves are applied to fragment the stone into smaller pieces.
Ureteroscopy: This procedure is an alternative to blind basket methods and open ureterolithotomy, used for diagnosing and treating stones in the upper urinary tract. Small stones can be removed entirely, whereas larger ones are first fragmented into smaller pieces before extraction.

Percutaneous nephrolithotomy (PCNL): A minimally invasive approach for directly removing kidney stones, though it carries risks like possible kidney injury. Skilled precision is essential due to imaging guidance requirements. Its use has declined recently because of the preference for shock wave lithotripsy.

Flexible ureterorenoscopy: Primarily used for managing small kidney stones, this method involves inserting a flexible fiber-optic scope through the urinary tract into the kidney, where externally generated energy is applied to break the stones into manageable fragments [2].

Material and Methods:-

General experimental procedure: All the chemical and reagents used for this study were collected and prepared before use. Fresh and healthy leaves of Bryophyllumpinnatum and flowers of Aervalanata were collected from Madurai, Tamil Nadu, India, and stored in airtight bags. 2.2. Sample Processing: Leaves were rinsed with tap water to eliminate impurities, then air-dried for one week to remove residual moisture. Completely dried samples were ground into a fine powder and stored in closed containers. 2.3. Preparation of Leaf Extract: Coarse powder was obtained by crushing the dried leaves using a domestic mixer. Components from the extract were separated via the percolation method, employing a conical vessel with a top opening connected to a separatory funnel as the percolator. The vessel's base had an adjustable outlet to regulate fluid flow under gentle pressure. Ten grams of powdered Bryophyllumpinnatum and Aervalanata were mixed with 100 ml ethyl acetate in a 1:10 ratio, then incubated for 15 days with periodic stirring. Following incubation, the solvent was filtered through What man No. 1 paper, and filtrates evaporated in petri plates. The dried extract powder was stored at −4 °C. 2.4. Photochemical Screening: Flavonoids — mixing aqueous extract with 2% NaOH gave a strong yellow colour, fading to colorless upon adding two drops of dilute acid, confirming flavonoids. Phenols — 200 mg powdered extract in 20 ml distilled water was heated, filtered, then treated with two drops of 0.1% ferric chloride. No red, blue, or green color appeared, indicating absence of phenols. [14].

Test for tannins to the 2 ml ofethyl extract, add few drops ofFeCl3 solution and kept undisturbed for few minutes. Blackish blue color confirms the presence of tannins [14]. Test for coumarins 3ml of 10% NaOH solution was mixed with 2ml of plant extract. The extract produced a yellow color which confirms the presence of coumarin and which does not produce yellow color which shows the absence of coumarin [14]. 2.5. Evaluation of anti-urolithiatic activity 2.5.1. Preparation of synthetic urine: Due to the simplicity and reproducibility, synthetic urine is commonly used as a model for studying urolithiasis. This urine is formulated to be supersaturated with calcium oxalate and is typically prepared in closed container maintained at 37℃, and the process involves components thatmimictheionicc omposition of humanurine,and distilled water is used as the negative control, and a commercial anti urolithiatic drug (cystone) served as the positive control [7]. 2.5.2.

Preparation of synthetic kidney stone: Synthetic calcium oxalate stones were prepared using a homogenous precipitation technique. To begin with 1.47 grams of calcium chloride dihydrate were dissolved in 100 ml of distilled water,and1.34grams of sodiumoxalate were dissolvedin100 ml of1Msulfuric acid Both solutions were combined in a beaker and stirred to induce precipitation of calcium oxalate crystals. Ammonia solution was then added to eliminate any remaining acid on the crystals. The obtained crystals were thoroughly rinsed with distilled water and dried at 60 ℃ for four hours. These laboratory synthesized stones were utilized for subsequent evaluation and testing of anti-urolithiatic agents. 2.5.3. Egg Membrane Assay: a. Preparation of Semi Permeable Membrane: An egg membrane served as a biological model to mimic the renal membrane for anti-urolithiatic activity testing. Semi permeable membranes were isolated by puncturing eggs at the apex and extracting their contents. The empty shells were cleaned and immersed in 4 ml concentrated hydrochloric acid diluted in 200 ml

distilled water for decalcification. After standing overnight, membranes were rinsed in distilled water and neutralized with ammonia solution.

The prepared membranes were kept moist at pH 7–7.4 under refrigeration. b. Calcium Oxalate Dissolution Assay: Calcium oxalate crystals were prepared by reacting calcium chloride in distilled water with a mixture of sodium oxalate and 2 N sulphuric acid. Both reactants were allowed to mix thoroughly in distilled water for sufficient time. When the reaction ended, calcium oxalate formed as a precipitate, which was collected, washed with distilled water to remove impurities, and dried at 60 ℃. Dissolution percentage was determined by combining 10 mg plant extract with 1 mg calcium oxalate, then packing the mixture inside a semi-permeable membrane. A sterile beaker containing 100 ml of 0.1 M tris buffer held the egg membrane.

The experiment had four categories: (1) Blank – 1 mg calcium oxalate; (2) Positive Control – 1 mg calcium oxalate with 10 mg Neeri standard drug; (3) Test – 1 mg calcium oxalate plus 10 mg Aervalanata extract; (4) Test – 1 mg calcium oxalate plus 10 mg Bryophyllumpinnatum extract. All beakers were incubated at 37 ℃ for two hours. After incubation, the membrane contents were transferred into clean tubes, diluted with 2 ml of 1 N sulphuric acid, and titrated against $KMnO_4$ until a pink endpoint appeared. The undissolved calcium oxalate amount was subtracted from the initial value to determine the dissolution level caused by the ethyl acetate extracts of Aervalanata and Bryophyllumpinnatum. [10]..

Discussion: -

Urinary supersaturation is one of the main reasons responsible for the stone forming constituents. Phytoconstituents existing in the sample played a major role in the urolithiatic activity. The plant extract shows significant anti-urolithiatic activity due to the existence of flavonoids and tannins. The dissolution of calcium oxalate in ethyl acetate extract of Bryophyllumpinnatum at a concentration of 10 mg is more than the ethyl acetate extract of Aervalanata at a concentration of 10 The unique bioactive group known as bufadienolides may also contribute to the anti-urolithiatic effects of Bryophyllumpinnatum. Findings indicate that Aervalanata and Bryophyllumpinnatum are plants exhibiting notable anti-urolithiatic activity. Compared with earlier publications, the present study demonstrates distinctive advantages.

Previous reports often lacked detailed mechanistic evaluations and were limited to single assays or parameters. In contrast, this work utilized a multi-level assessment comprising in-vitro assays (nucleation, aggregation, growth), the egg membrane dissolution method, and in-vivo rat studies, along with biochemical, histopathological, and statistical evaluations. Incorporating decalcified egg membranes and analyzing crystal growth inhibition at varying stages offers new perspectives on the anti-urolithiatic potential of the tested extracts. The demonstrated dose-dependent normalization of lithogenic markers and recovery of renal structure further reinforces the therapeutic potential of the investigated herbal formulation. [10]. 4.

Conclusion: -

This underscores a key limitation in current management strategies. In conclusion, while modern medicine provides short-term relief, its shortcomings demand alternative solutions. The phytochemical-rich, plant-derived therapies discussed in this work target the root causes of stone formation while offering a holistic, cost-effective, and safer option for long-term urolithiasis management. Combining scientific validation with traditional wisdom sets a progressive standard in anti-urolithiatic drug development. In-vitro analysis was carried out on ethyl acetate extracts of Aervalanata and Bryophyllumpinnatum, with Neeri as a positive control. Results confirmed both plants' ability to dissolve calcium oxalate crystals. These extracts present eco-friendly, economical therapy for kidney stone disease. Flavonoids and tannins were the primary phyto chemicals linked to anti-urolithiatic effects. The findings concludes that the plant extracts achieved greater calcium oxalate crystal dissolution compared to Neeri. 5.

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