

**Review Article****Rotula aquatica. Lour-A Review on Medicinal uses Phytochemistry and Pharmacological actions**Bency Baby T<sup>\*1</sup>, Remya K<sup>1</sup>, Smitha Rani<sup>1</sup>, Shebina P Rasheed<sup>2</sup>, Azeem A.K<sup>1</sup><sup>1</sup>Department of Pharmacognosy, Al Shifa College of Pharmacy, Kerala, India<sup>2</sup>Department of Pharmaceutical chemistry, Al Shifa College of Pharmacy, Kerala, India**ARTICLE INFO:****Article history:**

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**ABSTRACT**

*Rotula aquatica* Lour is an important medicinal herb in Ayurvedic system of medicine. The plant occurs as a small branched shrub that grows characteristically among rocks and trailing over pebbles in stream beds, belonging to the family Boraginaceae and widely distributed throughout India. It is commonly called as Pasanabheda in Ayurveda. As only few studies are done on this plant, the comprehensive review presenting here will summarize regarding its Phytochemical and Pharmacological activities with the help of available and update information. Various studies indicated the antiurolithiatic, hypoglycemic, anti-inflammatory, antibacterial and anthelmintic properties of *Rotula aquatica*. Although the information are very encouraging and revealed some of the constituents of the plant like polyphenols and tannins as important therapeutically agents. The herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects. Various traditional claims of the plant are still remain to be validated scientifically. Clinical trials for the reported preclinical studies should be performed urgently to further validate the claims on humans

**Introduction**

The plants with medicinal property were accepted as an alternative to improve the health of the folk, since it helps in reducing the adverse effects and treatment costs of the synthetic medicines. Nearly 80% of the universal populations relying on drugs obtained from plant sources for various ailments. Drugs from plants were widely used by various traditional methods of treatment. Plants with known therapeutic action were used by traditional Indian system of treatment. One among them is *Rotula aquatica*, belonging to family Boraginaceae. The plant is a medicinal shrub with fragrance flowers and lives in rheophytic habitat. This plant is native to India and seen as a part of the lotic aquatic system. This is not a common plant and its presence is limited to certain river banks that are rocky in nature. However, this is widely known in ayurvedic system of medicines. It is represented by about 100 genera and 2000 species. *Rotula aquatica* is one of the most widely used medicinal plants in the ayurvedic system, especially in the Indian states of Kerala and Karnataka for dissolving urinary calculi and kidney stones [1, 2, 3, 4, 5, 6].

The roots possess astringent, sudorific, bitter, and antiurolithiatic properties. Traditionally roots are used in renal

and vesicle calculi, hemorrhoids, venereal diseases and diabetes [1]. The roots also used for the treatment of piles and as a laxative [7]. It exhibits cooling, bitter and astringent properties. Diuretic and cardiogenic properties were exhibited by this plant as per Ayurveda. It is widely used in the treatment of kidney and bladder stones. [8, 9].

**Methodology of the review**

The literature search was performed from January to June 2017 using electronic search engines such as Google, Google scholar, publishing sites such as Elsevier, science Direct, BioMed Central (BMC) and PubMed. The following keywords were used to search literature sources. :“*Rotula*”, “Phytochemistry”, “Urolithiasis”, “Phytotherapy,” “Natural Products,” and “Medicinal Plants.” Other literature sources included papers published in international journals, reports from international, regional and national organizations, conference papers, books, theses, websites and other grey literature.

**Taxonomic classification**

Kingdom - Plantae

Subkingdom - Trachiobionta

Division - Magnoliophyta

Class - Magnoliopsida  
Subclass - Asteridae  
Order - Lamiales  
Family – Boraginaceae  
Genus – *Rotula*  
Species- *Rotula aquatica*

#### Synonyms

Latin: *Rhabdia lyciodes*, *Carmona viminea*, *Rhabdia viminea*,  
*Ethretia viminea*

#### Vernacular names

Sanskrit: Pashanbheda  
Tamil: Seppunerinji  
Malayalam: Kallurvanchi  
Marathi: Machim  
Telugu: Pashanbada  
Kannada: Pashanbada [10-14]

#### Botanical description

It is a small branched aromatic shrub, 60-180cm height.

Leaves: ovate to oblong, 1.5 cm in length, simple, sessile, rounded at the apex and trichomes on both surfaces, crowded on the branches.

Flower: small, solitary or in clusters, around 6 mm long, corolla pink or reddish, 5 stamens, short pedicel.

Fruits: Subglobose, fleshy, 4mm in diameter, orange- red drupes and with four seeds.

Roots- Cylindrical, often develops from lateral branches [10, 11, 14]

#### Geographical Distribution

The plant is scattered throughout peninsular and Western Ghats of India in the sandy and rocky beds of streams and rivers [15]

#### Phytochemistry

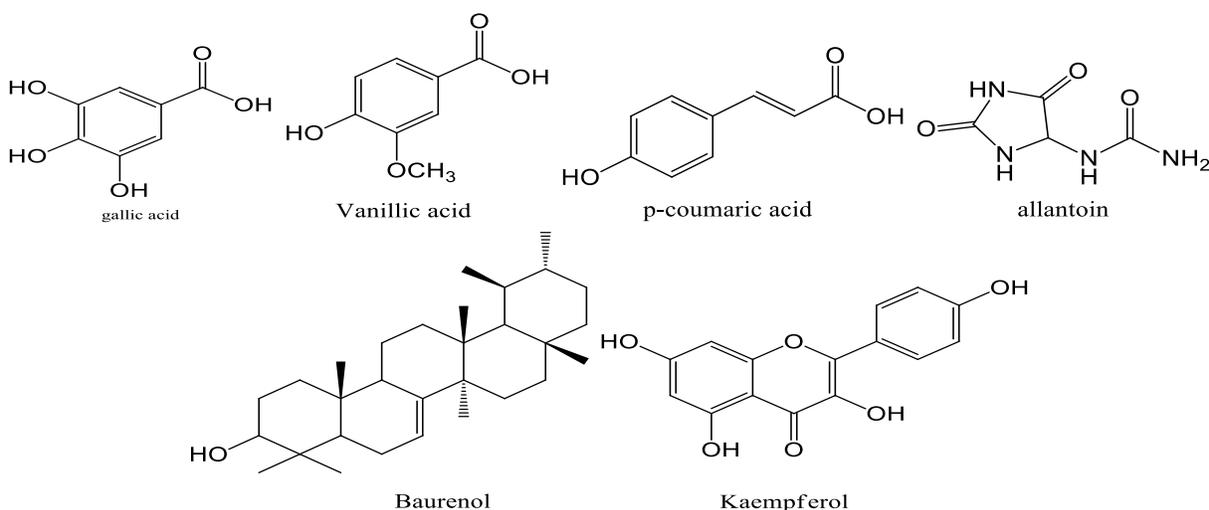
The Phytochemistry of *Rotula aquatica* has not been studied and reported comprehensively. The plant is reported to contain various Phytoconstituents like, alkaloids, Baurenol, flavonoids, phenols saponins, steroids, terpenoids, proteins and amino acids.[3, 7] Pallavi *et al.*, reported the preliminary qualitative phytochemical analysis of *Rotula aquatica*. The methanolic and chloroform extract of roots and leaves showed the presence of carbohydrates, proteins, amino acids and glycosides. Steroids and triterpenoids are present in chloroform extracts whereas absent in methanolic extracts of both. Saponins present only in methanol extracts of root and leaves. Quinones were present in both leaf extracts and in root chloroform extract. Alkaloids, tannins and polyphenols are present in chloroform and methanol extracts of both leaves and roots. Flavonoids showed positive for chloroform extract of root and methanol extracts of both leaves and root. Total phenolic content was estimated by Folin-Ciocalteu method. The methanolic extract of root showed highest concentration of phenolics[3]. The Phytoconstituents responsible for the

pharmacological activity may be due to the presence of alkaloids, phenolic compounds, flavonoids, and other nutrients like as amino acid and proteins. The aqueous extracts of *Rotula aquatica* was reported to contain polyphenols[16, 17]. Analysis of methanol extract of the *Rotula aquatica* showed the presence of Phenolic compounds and flavonoids. RP-HPLC study of the extract identified the presence of three major phenolic compounds, vanillic acid gallic acid and p-coumaric acid [18]. The preliminary phytochemical screening aqueous extract of *Rotula aquatica* reported to have alkaloids, flavonoids, phenols, steroids, saponins, terpenoids, tannins, anthraquinones, anthocyanin, quinones, volatile oils, proteins and carbohydrates. An attempt for HPTLC fingerprinting of aqueous extract of *Rotula aquatica* was made by Rameswar *et al.*, HPTLC analysis of aqueous root extract using the mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) showed the presence of seven different types of phenols having the  $R_f$  values ranging from 0.01 to 0.97. One of the  $R_f$  value in sample matches with the marker compound kaempferol indicating the presence of phenolic compound in the extract. The study was further extended to identify the presence of tannins. The mobile phase Toluene-Ethyl acetate-Formic acid-Methanol (3:3:0.8:0.2) used, was able to detect Twelve different types of tannins with better resolution. One of the  $R_f$  value matches with the standard gallic acid indicated the presence of tannin. [4].

Vijayakumari *et al.*, reported the preliminary Phytochemical screening of *Rotula aquatica*. It revealed the presence of alkaloids, flavonoids, phenols saponins, steroids, terpenoids, anthraquinones, proteins and amino acids following the standard procedures.[15].

Allantoin found in root which is responsible for diuretic activity. It also contains sterol, rhabdiol which is found to be active to induce diuresis [10, 15].

Successive solvent extraction of *Rotula aquatica* was reported by Patil *et al.*, various fractions were subjected to phytochemical screening. The phytochemical study indicated the presence of tannins and steroids. They further extended the study by developing HPTLC fingerprints and two compounds identified in HPTLC was isolated were characterized by UV, NMR and Mass spectrum. One of the spot which was having the molecular weight 388 and the molecular formula calculated using elemental analysis was found to be  $C_{26}H_{46}NO$ . The spectral studies reveals that the compound could be a steroidal alcohol with a side chain containing an amide or nitrogen in heterocyclic ring and in such case might be a steroidal alkaloid. They were also successfully isolated a second compound having a molecular weight of 387 and the molecular formula was found to be  $C_{25}H_{39}SO$  and which is reported to be steroidal alcohol[19].



### Pharmacological activities

Various extracts, as well as pure compounds obtained mainly from *Rotula aquatica*, have been shown to express biological activity in both *in vitro* and *in vivo* models.

#### Antiuro lithiatic activity

Urolithiasis is the third most common disease found today throughout the world. In India, a person living in different states utilizes different parts of this plant for curing urolithiasis. Several studies of antiuro lithiatic activity have been done by different researchers. [20]

*Rotula aquatica*, was screened for the anticrystal activity against basic calcium phosphate (BCP), calcium pyrophosphate (CPPD) and monosodium urate monohydrate (MSUM). The effect of plant was assayed on microcrystals in 24-well microplates *in vitro*. The results showed that the aqueous extracts of *R. aquatica* have crystal dissolving activity against MSUM[21]

Prashanthi *et al.*, reported, the antiuro lithiatic potential of *Rotula* roots by a methodical approach, using simple *in-vitro* model. The study involved the preparation of Calcium oxalate and phosphate and its dissolution in presence of *Rotula aquatica*. The successive solvent extracts were screened for *in-vitro* antiuro lithiatic activity using semi-permeable membrane of eggs. The aqueous extract showed high dissolving potential for calcium phosphate. 100% dissolution was found at 38 and 20mg of aqueous extract for Calcium oxalate and calcium phosphate respectively. [9]

Sasikala *et al.*, reported the *in vitro* antiuro lithiatic activity of *Rotula aquatica*. Calcium oxalate crystallization was induced by the addition of 0.01 M sodium oxalate solutions. The effect of extract was studied by the measurement of turbidity in presence or absence of extract at 620 nm by a spectrophotometer. Different extracts of the plant were investigated for the study. The extracts from plants inhibited the crystallization of Calcium oxalate in solution; the extract decreases the particle size with increasing concentration. It also promoted the nucleation of calcium oxalate crystals. The result showed that the aqueous extract of root has a greater

capacity that allows blocking the crystal formation and aggregation as compared to that of petroleum ether, chloroform and methanol extracts of leaf and stem [15]

The effectiveness of alcoholic extract of *Rotula aquatica* for the treatment of urolithiasis was reported by Gilhotra *et al.*, Urolithiasis was induced in albino rats by administering 1% ethylene glycol in drinking water for 28 days. The induced condition was manifested by high urinary calcium, phosphate, oxalate, protein, uric acid, creatinine and low urinary magnesium content. The extract was then administered at a dose of 200 mg/kg body weight orally for 28 days. Serum analysis and histopathological studies of the experimental animals reveals that the group treated with plant extract significantly reduced the level of urinary calcium, phosphate, oxalate, protein, uric acid, and creatinine. These groups of animals were also observed to have an increased urine output thereby minimizing the chance for crystallization. The results of histopathological studies showed reduction in the microcrystal deposition in the section of kidney reaffirms the potential of the extract for the treatment of urolithiasis. It was observed that *Rotula aquatica* lowered the excretion of uric acid and reduces the risk of stone formation. The study proposed a possible mechanism by which the extract effectively control the levels of calcium and oxalate by inhibiting the synthesis of oxalate and by increasing the bioavailability of nitric oxide to sequester calcium through the cGMP pathway.[16]

In addition Gilhotra *et al.*, performed a study on Antiuro lithiatic activity of poly-herbal formulation containing hydroalcoholic extracts of *Rotula aquatica* by *in-vitro* method. In their study they developed a herbal tablet formulation of *Kalancho epinnata* and *Rotula aquatica*. The antiuro lithiatic activity was compared with the aqueous extract of cystone (a marketed herbal formulation for urolithiasis). The homogenous precipitation method was used to study the antiuro lithiatic activity. The results showed inhibitory effect on calcium oxalate crystallization which is beneficial in the treatment of renal lithiasis. All these results in the study revealed the antiuro lithiatic activity of *Rotula aquatica*. [22]

### Antioxidant Effect

Many researchers studied about the antioxidant and free radical scavenging activity of *Rotula aquatica*. *In vitro* antioxidant activity of *Rotula aquatica* roots and leaves were assessed using various radical scavenging activities such as DPPH, superoxide anion, nitric oxide, hydroxyl and iron chelating activity at different concentrations. The methanolic extract of roots and leaves exhibited significant antioxidant activity. The study concluded that the antioxidant activity could be attributed to the, polyphenols, tannins, flavonoids, glycosides etc.[3]

The aqueous extracts of *Rotula aquatica* showed a marked antioxidant activity when compared to ascorbic acid. The above studies were also carried out with fractionated extracts of the two drugs using solvents of increasing polarity. This strategic approach further helped to target the phytoconstituents responsible for the antioxidant activity. The study revealed that the polyphenols present in the polar extracts have a stronger antioxidant activity compared to the non-polar phytoconstituents. The study concluded that herbal drugs by their antioxidant activity might be useful in the treatment of cancer. Further, the above herbs can be used as a protective measure in cancer patients undergoing radiation therapy, since it has been demonstrated that radiation therapy causes mutations and cancer due to increased production of free radicals. In such cases, the above herbs can be used as a protective measure in cancer patients undergoing radiation therapy [17].

The antioxidant effects of methanolic extract of *Rotula aquatica* have been implicated in another study using *in vitro* assays. Antioxidant activity of the plant extracts were determined by total antioxidant capacity, ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] and DPPH [2,2-diphenyl-1-picrylhydrazyl] free radical scavenging activity, inhibitory activity toward  $\beta$ -carotene bleaching and lipid peroxidation, and DNA protection activity. It is obvious from the study, that the investigated extracts have the ability to quench free radicals. This indicates that the screened plant extracts are a potential source of natural antioxidants [18].

In another study, Mengi *et al.*, reported the DPPH scavenging activity of aqueous plant extracts by spectrophotometry. The extracts dissolved in distilled water were added to the methanol solution of DPPH (200 $\mu$ M) at different concentrations (4 to 25  $\mu$ g / mL). After 30 minutes, the decrease in absorbance of the test mixture caused by DPPH free radical quenching was read at 517 nm and the percent inhibition was calculated. Ascorbic acid was used as a reference free radical scavenger. Aqueous extracts showed significant free radical scavenging in DPPH assays with an IC<sub>50</sub> of 11.07  $\mu$ g / mL. Ascorbic acid as a known antioxidant had an IC<sub>50</sub> of 1.07  $\mu$ g / mL [6].

### Anti-inflammatory activity

Many studies have been reported the anti-inflammatory activity of *Rotula aquatica*. The aqueous extract was screened for anti-inflammatory activity using chronic and acute studies. Animal models like Carrageenan-induced rat paw edema,

cotton pellet induced granuloma as well as crystal-induced inflammation was performed to evaluate its potential. The result indicates that the aqueous extract at a dose of 200 mg/kg effectively inhibited the IL-6 levels. And the percentage reduction of edema was significant when compared with its standard. In addition to these the extract demonstrated marked reduction in total white blood cell count in crystal-induced inflammation. All these results indicate the anti-inflammatory potential of the aqueous extract [6].

Kamurthy *et al.*, reported the anti-inflammatory activity of petroleum ether and ethyl acetate extracts of whole plant of *Rotula aquatica* using acute (carrageenan-induced paw edema) and sub-acute (cotton pellet granuloma) models of inflammation. This study revealed that petroleum ether and ethyl acetate extracts of whole plant at a dose of 200 mg/kg possessed significant anti-inflammatory activity in experimental animals [7].

### Anthelmintic activity

The aqueous extract of *Rotula aquatica* roots have been evaluated for anthelmintic activity and compared to standard drug piperazine citrate. The assay was performed on adult Indian earth worm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Anthelmintic activity of *Rotula aquatica* was confirmed by examining the time taken for paralysis (P) and death (D) for *Pheretima posthuma* worms. Time taken was measured in minutes. The result of the study reveals that aqueous extract of *Rotula aquatica* at concentration of 75 mg/ml have more effective anthelmintic activity as compared with the standard drug. This support to use of this plant for eradication of worms[23].

Another study reported *in-vitro* anthelmintic effect of aqueous extract of *Rotula aquatica* and *Aerva Lanata* on adult earth worm's *Pheritima posthuma*, using piperazine citrate 15 mg/ml as standard drug. The study reported that the aqueous extract of *Aerva lanata* is more effective than *Rotula aquatica* when compared to standard drug piperazine citrate. [24]

### Antibacterial effect

Vittal *et al.*, reported Antibacterial activity of *Rotula aquatica* using wide range of bacterial strains which include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Klebsiella pneumonia* and *Shigella flexneri*. Different plant extracts were used in the study. Agar disc diffusion method was used to study the antibacterial activity of the plant extract The methanolic extract showed effective antibacterial activity against the bacterial strains *Escherichia coli* and *Salmonella typhi*. [18]

Another study reported the antibacterial activities of aqueous extract of *Rotula aquatica*. The dried roots of *Rotula aquatica* were extracted by successive solvent extraction using solvents in the increasing order of polarity. Among the organisms-Gram +ve - *Bacillus cereus* and Gram-ve *Salmonella abony*, *Klebsella pneumoniae*, *Pseudomonas aeruginosa* were found

to be susceptible to aqueous extract. The antibacterial activity increased in a dose dependent manner at 250, 500, 750µg.[25]

#### Antidiabetic activity

Methanolic extracts of aerial parts of *Rotula aquatica* significantly reduces blood glucose levels at streptozotocin induced diabetes in rats. The activity showed by aqueous extract was comparable to that of standard oral hypoglycemic agent glibenclamide. The experimental results indicated that Oral management of 400 mg/kg of methanolic extract of aerial parts of *Rotula aquatica* produced highly significant (P<0.001) percent reduction in blood glucose levels at 4,8,12 and 18<sup>th</sup>hrs compared to the control group [26].

Aswini *et al.*, reported the hypoglycemic and antidiabetic potential of *Rotula aquatica*. The aqueous extracts of roots effectively normalized the blood glucose levels in streptozotocin-induced diabetes at dose dependent manner. The effect was compared with that of standard drug glibenclamide. Parameters such as oral glucose tolerance test, fasting blood glucose level, serum lipid levels were assessed. The oral glucose tolerance test showed decrease in blood glucose level at a dose of 200 mg/ kg. The serum lipid levels were reduced which was comparable to normal rats. The study concluded that the antidiabetic effect could be attributed to the phytoconstituents and their synergetic effect [8].

#### Antidiarrhoeal activity

Sing *et al.*, evaluated the antidiarrhoeal effect of alcoholic extract of *Rotula aquatica* using different experimental models. Alcoholic extract was selected for antidiarrhoeal activity. Antidiarrhoeal effect was evaluated by castor oil induced diarrhoea, charcoal meal test and PGE2 induced diarrhea. Both 100 mg/kg and 200 mg/kg of alcoholic extract showed protection against PGE2 induced enteropooling which might be due to the inhibition of synthesis of prostaglandins. The underlying mechanism appears to be spasmolytic and an anti-enteropooling property by which the extract produced relief in diarrhea [27].

#### Anticancer activity

The aqueous extract of *Rotula aquatica* was evaluated for antimetastatic activity. Preliminary antimetastatic activity was done using *Allium cepa* root tip assay. The mitotic index of the root tips markedly decreased with increasing concentration of the aqueous extract. The different fractions obtained by successive extraction of the plant were evaluated for antibiotic activity. The fractions which contain tannin showed better activity. Experiments were also carried out with the incorporation of folic acid in the aqueous extract. Folic acid inhibited the antimetastatic activity of aqueous extract of *Rotula aquatica* in a dose dependant manner. The results obtained were compared with standard drug methotrexate. The studies were extended to human cells using 3 pancreatic cell lines, viz; HPAF-II, BxPC-3 and CAPAN-2. extract of *Rotula aquatica* was found to be extremely effective in the prevention of cell proliferation of the pancreatic cell lines[19].

#### In silico studies

Vijayakumari *et al.*, reported a molecular docking study using the compound 3-O-acetyl-11-keto-β-boswellic acid which was isolated from aqueous extract of *Rotula aquatica* roots. In the study they have prepared the target, Tamm–Horsfall protein structure through homology modelling and upon docking, the compound showed good interaction with the active site amino acid residues with a docking score of -5.465. The reported *insilico* study describes the effectiveness of the compound to inhibit calcium oxalate crystallization in urolithiatic condition[28].

#### In vitro micropropagation studies

Attempts have been made for the *invitro* micropropagation of *Rotula aquatica*. Axillary bud multiplication and indirect organogenesis were successfully performed using Murashige and Skoog (MS) medium. The media fortified with 0.5 mg l-1 indole-3-butyric acid (IBA) and 1.0 mg l-1 N6-benzylaminopurine (BAP) were found to have enhanced axillary bud proliferation. In vitro multiplication was done through subculture of the shoots using same concentration BAP and IBA. The root development found to be better MS medium supplemented with 0.5 mg l-1 Naphthalene acetic acid (NAA). Rooted shoots, after acclimation in the greenhouse, were successfully transferred to field conditions, and 80% of the plants were survived [29].

#### Conclusion

The use of natural remedies dates back thousands of years. [30] Use of traditional medicines for improving immunity and treating various diseases has been approved by WHO. This review has presented a comprehensive view about the Phytochemistry and Pharmacology of *Rotula aquatica*. However the research is very limited in some areas and further study on phytochemicals and their mode of actions revealing pharmacological effects are required to be fully understand in concern with the traditional uses. Pharmacological and chemical studies have demonstrated that the extracts of the plant possess various pharmacological actions viz. antiurolithiatic, anti-inflammatory, antioxidant, antidiabetic, anthelmintic and anticancer. In addition majority of pharmacological studies were conducted using crude and poorly other solvent extracts. In such case more bioactive compounds should be identified through bioassay guided isolation. Molecular mechanisms should be established for therapeutic applications. More clinical studies on the toxicity of extracts from different parts and the isolated compounds from this plant need to be assessed for ensuring the safe application as modern medicines.

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