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Isolation and Biological Activity Assessment of Bioactive Compounds from Hygrophila salicifolia

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Abstract - Hygrophila salicifolia, a medicinal plant with historical therapeutic applications, was investigated for its phytochemical, pharmacognostic, and pharmacological properties. This study isolated and characterized bioactive compounds, particularly flavonoids, from its methanolic extract, using advanced chromatographic and spectroscopic methods. The extract demonstrated potent antioxidant and anti-urolithiatic activity in vitro and in vivo. It significantly reduced urinary calcium, oxalate, and phosphate levels while increasing magnesium excretion in an ethylene glycol-induced urolithiasis model in rats. Histopathological analysis revealed reduced renal damage and crystal deposition in treated groups. The flavonoid glycoside, identified as isoquercitrin, exhibited promising therapeutic potential, substantiating the plant's traditional medicinal use. This research underscores Hygrophila salicifolia's potential as a natural source for developing treatments for urolithiasis and other oxidative stress-related conditions.

Keywords: Hygrophila salicifolia, medicinal plants, isoquercitrin, anti-urolithiatic activity, phytochemical analysis, antioxidants

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INTRODUCTION

The "herbal renaissance" is spreading throughout the world as more people choose natural cures over synthetic ones, which are frequently thought to be bad for the environment and human health (Piglili & Runja, 2014; Schulz et al., 2001). For therapeutic purposes, almost 75% of the world's population uses plants and plant extracts. Up to 25% of all medications in Western nations like the United States are plant-based, however in nations like China and India, the proportion might reach 80%. For the economics of nations like India, where traditional healthcare systems in rural regions mostly rely on indigenous traditions, medicinal plants are very important (Zhou & Wu, 2006).

With more than 45,000 plant species, many of which have therapeutic applications, India is among the most biodiverse nations in the world. Approximately 15,000 to 20,000 of these plants are utilized in modern medicine, with several hundred species being employed for therapeutic purposes in traditional treatments like Ayurveda and Unani. The manufacturing of medicines uses parts including roots, stems, bark, and flowers in addition to excretory secretions like gum and resins. Many medications are still made from plants, even in allopathic medicine, and plant-based chemical intermediates are still essential to the production of contemporary medications. The market for drugs made from plants is still thriving worldwide (Zhou & Wu, 2006).

Modern herbal therapy is still influenced by India's rich history of using therapeutic herbs, which is documented in books like the Charak Samhita (700 BC), the Atharvaveda (4500–2500 BC), and the Rigveda (5000 BC). However, the supply of these plants is threatened by biodiversity loss and deforestation. India loses 1.5 million hectares of forest year, and over half of the world's tropical forests have already been destroyed (Thomas et al., 2001; Singabetal, 2014). With 427 species identified as endangered in the Indian Red Data Book and many more in danger of going extinct, this is endangering a large number of useful medicinal plants (Thomas, 1997).

To detoxify and get rid of reactive oxygen species (ROS), plants have evolved antioxidant systems that include enzymes like peroxidase, superoxide dismutase, and catalase. A plant's capacity to handle stress brought on by ROS toxicity is influenced by

these defense systems (Shalata et al., 1998; Kama et al., 2012; Nariya et al., 2014). A major contributor to yearly death rates, thrombosis is a major cause of myocardial infarction, pulmonary embolism, and stroke. Platelet aggregation-induced arterial thrombosis can be lethal. Cardiovascular disease prevention and treatment depend on inhibiting platelet aggregation (Jin et al., 2007; Kamal et al., 2012). The hunt for novel agents derived from natural sources has been fueled by the shortcomings of existing thrombolytics.

LITERATURE REVIEW

In underdeveloped countries like India and Africa, where they are essential to traditional medical systems like Ayurveda, Unani, and Traditional Chinese Medicine, medicinal plants (MPs) have played a significant role in healthcare since ancient times (WHO, 2002). As contemporary technology has advanced, MPs have taken center stage in the hunt for physiologically active phytochemicals, which are crucial for creating remedies for a range of illnesses, including cancer and drug-resistant ailments (Manjula & Mamidala, 2013; Rao et al., 2012).

Because of their pharmacological qualities, which have been passed down through the generations, plants have long been utilized to treat illnesses. Clinical studies of 156 of the 1,000 plants examined in 2010 demonstrated the growing demand for plant-based medications and dietary supplements (Cravotto et al., 2010; Raja et al., 2012). While some medicinal plants grow naturally, others are grown under cultivation (Vaidya & Devasagayam, 2007).

India has a wide variety of medicinal herbs, and its traditional knowledge is respected all over the world. The use of plants for medicinal reasons is documented in ancient literature such as the Vedas and Samhitas. More than 2,300 species of medicinal plants are described in the Charak and Sushrut Samhitas, which were composed between 700 and 200 BC (Sumner, 2000; Joy et al., 2001). The Red Data Book of India lists 427 species of plants, 28 of which are extinct, indicating that many of these plants are endangered (Thomson et al., 2001).

Traditional medicine is still significant despite the emergence of synthetic drugs because of problems including drug resistance, the high expense of therapy, and the negative consequences of synthetic drugs. The continued use of plant-based therapies is fueled by the pharmacological efficacy and bioselectivity of numerous plant-derived substances (Balandrin & Klocke, 1988; Kama et al., 2008a; Kamal & Naina, 2010).

METHODOLOGY

In August and September, Hygrophila salicifolia was gathered from Jharkhand, and taxonomists from the Bio-science department at YBN, Ranchi, confirmed its identify. For additional phytochemical, pharmacological, and pharmacognostic research, the plant was cleaned, let to dry in the shade, and then milled into a powder. A spray dryer, muffle furnace, hot air oven, and microscope were among the equipment utilized. The analytical-grade chemicals and reagents came from Sigma Aldrich in India. To evaluate the herb's qualities, a number of analytical methods were used, including the fabrication of leaf samples, the computation of the stomatal index, and proximate analysis (such as ash content and extractive values).

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Several analytical methods were used to assess Hygrophila salicifolia's chemical makeup and biological characteristics. Initially, techniques like sulfurated and nitrated ash determination were employed, in which the sample residues were heated to a regulated temperature of no more than 650°C after being treated with diluted sulfuric and nitric acids, respectively (Bartels et al., 1972). After that, the proportion of ash was determined. By dissolving narcotics in alcohol or chloroform, letting them macerate, and then measuring the extractable components by evaporating the solvent, extractive resources were also investigated (Sethi, 2001). Additionally, the sample was examined under a microscope or with the naked eye to identify and weigh any alien living materials (Evans, 2009).

Using female Wistar albino rats given 1000 mg/kg of methanolic extract, the acute toxicological investigation was conducted according to Aebi's (1984) methodology. The rats' weight, caloric intake, and clinical symptoms were tracked for 14 days. To evaluate the impact, the organs were weighed after dissection. Last but not least, the plant's possible anti-urolithiatic effectiveness was examined both in vivo using ethylene glycol-induced urolithiasis in rats and in vitro utilizing double-diffusion gel growth and conductometric measurement (Upadhyay & Malviya, 2013; Bhat et al., 2012).

Reagents such as NaOH and CuSO4 were used to analyze proteins and amino acids, while tests such as the FeCl3 and gelatin tests were used to analyze phenolic compounds and tannins. Tests for saponification and Sudan red staining were used to identify the fats and oils. Female Wistar albino rats were given 1000 mg/kg of methanolic extract as part of the acute toxicological investigation. For 14 days, the rats' weight, caloric intake, and clinical symptoms

were tracked. To evaluate the impact, the organs were weighed after dissection. Last but not least, the plant's possible anti-urolithiatic effectiveness was examined both in vivo using ethylene glycol-induced urolithiasis in rats and in vitro utilizing double-diffusion gel growth and conductometric measurement. By evaluating crystal growth and conductance in solution, these experiments assessed the extract's capacity to prevent kidney stone formation; paired t-tests were used to determine statistical significance.

Estimation of Calcium

The creation of a colored complex with O-cresolphthalein complexone in an alkaline solution serves as the basis for the calcium estimation principle. Using particular reagents such as buffer, color reagent, and calcium standard, test, standard, and blank tubes are prepared. After incubation at 25°C, absorbance at 570 nm is measured. The following formula is used to determine the calcium concentration:

Calcium (mg/dl)=(Abs SAbs T)×10

After adding calcium chloride, oxalate is quantitatively precipitated as calcium oxalate, and the process is titrated with permanganate. Titration with KMnO4 is used to measure the calcium oxalate concentration following sample incubation and precipitation.

Estimating Inorganic Phosphate

The concentration of phosphomolybdate, which is created when inorganic phosphate and ammonium molybdate react, is determined by absorbance at 340 nm. After preparing and adding the phosphate reagent and standards to the sample, the absorbance is measured.

Estimating Magnesium (Calmagite Method)

In an acidic environment, magnesium and calmagite combine to generate a red complex. Results are reported in mEq/L after a standard and test sample are incubated and measured at 510 nm.

Creatinine Estimation (Jaffe's Kinetic Method)

In an alkaline media, creatinine and picric acid combine to generate an orange complex. At 520 nm, the absorbance changes over time. The absorbance change is then used to compute the creatinine concentration.

The Crystalluria

After urine is centrifuged, the supernatant is inspected under a microscope using a hemocytometer to check for crystal formation. The severity of the condition is determined by the number of crystals per high power field.

Isolation by Chromatography

Compounds were separated from Hygrophila salicifolia methanolic preparations using column chromatography. Using solvents such as methanol, ethyl acetate, and chloroform, a gradient elution was used. To isolate a molecule that displayed a single spot under UV light, fractions with comparable Rf values were mixed for additional investigation, such as TLC.

Identification of Flavonoids and Spectroscopic Evaluation

The Shinoda assay verified the presence of flavonoids following chemical isolation. Spectroscopic techniques such as UV-Vis, FT-IR, NMR, and mass spectrometry were used to clarify the structure of the isolated molecule. Determining the melting point was also done.

Verification of the HPTLC Method

High-Performance Thin-Layer Chromatography (HPTLC) was used to evaluate the chemical that was separated from the methanolic extract. With the use of certain solvents and TLC plates for compound analysis, the method's quantification was confirmed. The presence of the isolated chemical was verified by the Rf values and UV analysis conducted at 254 and 366 nm.

RESULTS AND DISCUSSION

Hygrophila salicifolia's pharmacognostical investigations evaluated its anatomical characteristics through in-depth macro- and microscopic analyses. The plant has long, tapering leaves that tip at both ends, giving it a shiny appearance at a macro level. The leaves are subsessile, and the bracteoles are lanceolate. The generally quadrangular stems that grow up to three feet in length give rise to the roots, which start at the base. The flowers are distinguished by a pale-purple corolla with a deeply 2-lipped structure and a calyx that splits along its length, featuring linear, hispid-ciliate lobes. The plant contains an oblong ovary with four viable stamens, each with equal anthers and a long, hairy style. The capsules have a longitudinal or narrowly oblong form and the seeds are hairy, compressed, ovoid, and mucilaginous.

Hygrophila salicifolia was examined under a microscope using free-hand transverse slices (T.S.) of fresh stem, root, and leaf. The dorsiventral leaf has reticulated venation and distinct mesophyll tissues that create spongy parenchyma and palisade. The upper epidermis is made up of a single layer of cells with visible trichomes encased in a thin layer of cuticle, whereas the lower epidermis is primarily covered in stomata. Calcium oxalate crystals are found in the mesophyll cells, and the central vascular bundle is of the collateral, conjoint type, with a parenchymatous bundle sheath around

each vascular bundle and phloem encircling the xylem. The hypodermis of the stem is made up of three to four layers of collenchyma, whereas the epidermis is made up of long, hairy cells that are coated in a thin layer of cuticle. The parenchyma and endodermis make up the cortex, which is located adjacent to the hypodermis. Phloem and xylem make up the stem's vascular bundles, and the xylem vessels exhibit visible fibers and parenchyma. The core pith is packed with parenchymatous tissue, and the medullary rays between the arterial bundles are composed of many layers of polygonal or radially elongated cells.

The epiblema is made up of a single layer of cells with thin walls, and the transverse section of the root is round with a thin outer cork layer. A little cortex of parenchymatous cells follows the cork layer, which is composed of three to four rows of dark brown, tangentially elongated cells. The vascular bundle is made up of phloem and xylem, and the endodermis divides the cortical and vascular regions. The root's parenchymatous tissue contains calcium oxalate crystals in both cluster and prismatic forms. Diacytic stomata were revealed by peeling the leaf surface, and the intricate arrangement of the cortical, vascular, and epidermal tissues was revealed by the stem and root structures. The herb's powder form was also investigated; it was found to have a green color, a distinctive odor, and a strong, bitter flavor. It had a rough, fibrous feel. Under a microscope, the powder included calcium oxalate crystals, trichomes, stomata, and xylem arteries, demonstrating the existence of important anatomical elements seen throughout the entire plant.

Microscopy in statistics

The features of the leaf's quantitative microscopy are shown below. They were essential for leaf identification.

Table 1: Varying Leaf Values

Sr. No	Leaf constant	Value
1	Number of stomata Surface skin	10.0–23.0per sq.mm
	The dermal layer	12–25 per sq.mm
2	Index of stomata Upper dermis	18.40–30.23 per sq.mm
	The dermal layer	32.34–41.65 per sq.mm
3	Spinal vein count	17–35 per sq.mm
4	Endpoint of the vein	19–31 per sq.mm
5	A ratio of palisade	7–12.5

Proximate Analysis

Physico-chemical characteristics, also known as quality parameters, of *Hygrophila Salicifolia* whole herb powder were:

Table 2: Nearby Evaluation

Sr. No.	Parameters	% w/w
1	Overall ash	27.43
2	Silica gel-based powder	14.5
3	Soluble in acid ash	10.12
4	Anhydrous carbonate	15.45
5	Phosphorylated ash	11.23
6	Decomposed ash	12.43
7	Eluted with water-soluble	6.5
8	Extractive solvents that dissolve in alcohol	8.3
9	The Amount of Moisture	7.5
10	Outside substance	1.15

Alkaloids, tannins, sugars, steroids, mucilage, amino acids, and flavonoids were among the bioactive substances found in the entire herb Hygrophila salicifolia after phytochemical screening. Flavonoids were proven to be present using the Shinoda assay. Molisch, Fehling, Barfoed, and Benedict's tests for carbohydrates; Lovermann Burchard and Salkowaski reactions for phytosterols; ferric chloride test for tannins; Mayer, Dragendroff, Hayger, and Wagner tests for alkaloids; Ninhydrin test for amino acids; and Molisch, Fehling, and Salkowaski tests for mucilage were among the tests used to identify the different compounds.

The test did not significantly alter the animals' body weight or feed intake in toxicity trials. For as long as four hours following injection, the Open Field and Actophotometer tests revealed a modest decrease in locomotor activity. Itching, swelling, tremors, convulsions, salivation, diarrhea, insomnia, or coma were not reported side effects. Body weight and feed intake stabilized after 14 days, and there were no recorded deaths. There were no indications of toxicity in the major organs (liver, kidneys, heart, and lungs) examined by histology or gross necropsy.

The effects of Hygrophila salicifolia on urine calcium, oxalate, inorganic phosphate, and magnesium levels were evaluated in a study of ethylene glycol (EG)-induced urolithiasis in rats. The calcium concentration in the EG-induced urolithiatic model group was substantially greater (18.0 ± 0.22 mg/dl) than in the control group (2.71 ± 0.29 mg/dl). Calcium levels were successfully lowered by Cystone, MEHS 300 mg/kg, and MEHS 500 mg/kg; however, there were no appreciable variations between the two dosages (13.56 ± 0.31 mg/dl, 16.38 ± 0.19 mg/dl, and 13.75 ± 0.67 mg/dl, respectively).

Additionally, oxalate concentrations were higher in the model group (12.41 ± 0.39 mg/dl) than in the normal control (6.84 ± 0.15 mg/dl). Treatments with Cystone and MEHS at 500 mg/kg resulted in significant decreases in oxalate levels (4.23 ± 0.09 mg/dl, 7.31 ± 0.37 mg/dl, and 5.91 ± 0.44 mg/dl, respectively).

The model group had much higher levels of inorganic phosphate (6.94 ± 0.19 mg/dl), while Cystone, MEHS 300 mg/kg, and MEHS 500 mg/kg

treatments decreased the levels to 3.41 ± 0.17 mg/dl, 6.19 ± 0.20 mg/dl, and 2.82 ± 0.26 mg/dl, respectively. There were statistically significant variations in the reduction of inorganic phosphate levels between the 300 mg/kg and 500 mg/kg dosages.

Lastly, the model group's urine magnesium excretion was considerably lower. Magnesium concentrations were considerably higher in the Cystone and MEHS 500 mg/kg treatments (5.89 ± 0.22 mg/dl and 6.24 ± 0.69 mg/dl, respectively) than in the control group. According to the findings, Hygrophila salicifolia may be used therapeutically to treat urolithiasis by adjusting the amounts of calcium, oxalate, phosphate, and magnesium in the urine.

We evaluated the effect of Hygrophila salicifolia (MEHS) on urine creatinine levels in rats with ethylene glycol (EG)-induced urolithiasis. Urinary creatinine levels were significantly ($p<0.05$) lower in the rats that were given drinking water containing 0.75% ethylene glycol than in the usual control group, as the figure illustrates. However, the groups treated with Cystone, MEHS 300 mg/kg, and MEHS 500 mg/kg had significantly higher urine creatinine levels ($p<0.05$).

Likewise, the lithiatic group's serum creatinine levels were considerably higher ($p<0.05$) than those of the control group. When compared to the model group, pretreatment with Cystone and MEHS (both 300 mg/kg and 500 mg/kg) resulted in a substantial decrease in blood creatinine levels ($p<0.05$), suggesting that Hygrophila salicifolia may be able to reduce creatinine buildup in both urine and serum during urolithiasis.

Potential Risks of Crystalluria

Microscopic investigation revealed that the concentration of crystals in the model group was higher than in the control group. Treatment with 500 mg/kg of cystone and MEHS decreased crystalluria.

Table 3: Methylethylene glycol's Impact on EG Model Crystalluria

Parameter	Normal	Model	Standard	MESH 300 mg/kg	MESH 500 mg/kg
No. of crystals per high power field	----	+++	+	++	+

Crystalluria of rattanine. Crystals in urine (model group; EG): noticeable growth and aggregation; (C) crystals in urine (EG+STD): identical to control group; (A) untreated urine (control group): no crystals identified; (D) EG+MEHS 300 mg/kg crystals in urine: less aggregated crystals (over 8); (E) EG+MEHS 500 mg/kg crystals in urine: identical to control group (original magnification X100).

Tubules of normal size were observed in the control group's renal tissue according to histopathological investigation. The EG model group, on the other hand, showed tubular crystal accumulation, which resulted in dilatation, inflammation, tubular necrosis, and cell flattening. Cystone and MEHS (500 mg/kg) treatment decreased these problems, and the kidney structure of the EG+MEHS 500 mg/kg group was similar to that of the control group. Additionally, compared to the control group, mice treated with MEHS 500 mg/kg had considerably increased levels of calcium, oxalate, and inorganic phosphate and significantly lower levels of magnesium, a stone inhibitor. In a different investigation, column chromatography was used to remove a flavonoid glycoside from the methanolic extract. The Shinoda test and thin-layer chromatography (TLC) using the solvent system of ethyl acetate, glacial acetic acid, and formic acid revealed UV-active components, confirming the chemical identity.

Table 4: The substance that was isolated using column chromatography and its Rf value were measured on a TLC plate.

Sample No.	Fraction No	Rf Value	UV 254nm	Shinoda test
Isolated compound	(89-92) Ethyl acetate: methanol (9:1, v/v)	0.76	Dark black	Positive

The isolated chemical, which showed up as an amorphous yellowish substance, included a flavonoid glycoside, as validated by the Shinoda test. In the methanolic solution of the isolated chemical, spectroscopic investigations employing UV spectroscopy revealed distinctive absorption peaks at 359.24 nm and 256.87 nm. A bathochromic shift was noted with λmax values moving to 404.7 nm and 276.78 nm following the addition of 0.5M NaOH.

The methanolic extract of Hygrophila salicifolia was validated and quantified using high-performance thin-layer chromatography (HPTLC). The wavelengths used for the detection were 254 nm and 366 nm. The concentration of the isolated chemical was 460 ng/band. With intra- and inter-day percentage RSD values less than 2, precision studies demonstrated outstanding repeatability and reproducibility, demonstrating great technique accuracy.

Table 5:Accuracy within a single day

Conc. (ng/band)	Amount Found			% Recovery			Ave	RSD
600	587.39	606.352	602.474	97.8984	101.059	100.412	99.789	1.363
800	799.966	817.687	827.116	99.9957	102.211	103.39	101.865	1.406
1000	980.986	1005.24	991.148	98.0986	100.524	99.1148	99.245	0.994

Table 6: Typical accuracy level

Conc. (ng/band)	Amount Found			% Recovery			Ave	RSD
600	583.598	571.703	555.792	97.2663	95.2839	92.632	95.0607	1.898
800	799.966	809.343	835.735	99.9957	101.168	104.467	101.877	1.892
1000	1032.7	1014.46	1001.78	103.27	101.446	100.178	101.632	1.268

With limits of detection (LOD) of 75.676 ng/band and 229.321 ng/band, the approach showed good

sensitivity and the capacity to detect low concentrations. As demonstrated by % RSD values being less than 2%, robustness testing revealed that small changes to protocol parameters, such as mobile phase ratio, saturation time, and wavelength, had no discernible impact on the outcomes. Rf values were significantly affected by changes in the mobile phase's ethyl acetate content, indicating that exact control over ethyl acetate concentration is essential for preserving Rf consistency and ideal outcomes.

DISCUSSION

Numerous investigations were conducted to examine Hygrophila salicifolia's pharmacognostical, phytochemical, and anti-urolithiatic qualities. This plant, which belongs to the Acanthaceae family, has been used traditionally for therapeutic purposes, including as treating inflammatory sores and as a poultice for fevers and headaches. The study offers insightful information on its potential as a treatment. Hygrophila salicifolia's leaf, stem, and root were examined under a microscope to reveal specific details about its structural makeup. The leaf's epidermis is thick-walled, single-layered, and has glandular trichomes on both the top and lower surfaces. In addition to calcium oxalate crystals, the leaf's central vein included a collateral vascular bundle with phloem and xylem surrounding it. The root has layers of cork and endodermis, but the stem has a hypodermis with a cortex, phloem, and xylem arrangement. The herb's powder showed stomata, calcium oxalate crystals, and cystolith trichomes.

Hygrophila salicifolia's chemical and physical characteristics were assessed using the Indian Ayurvedic Pharmacopoeia. We measured the moisture content, alcohol and water-soluble extractive values, and ash content. According to the study, the percentage of acid-insoluble ash was 11.12%, while the total ash was 27.43%. It was discovered that the water-soluble extractives were 6.5% and the methanol-soluble extractives were 8.3%. The low levels of moisture and foreign debris verified the purity of the plant. The presence of alkaloids, carbohydrates, phytosterols, fixed oils and fats, tannins, gums, and mucilage was discovered during preliminary phytochemical screening. Additionally, flavonoids and tannins were found, which may help explain the plant's therapeutic qualities.

An OECD guideline 423 acute toxicity research was used to evaluate the methanolic extract's diuretic qualities in the pharmacological testing of Hygrophila salicifolia. At dosages of 2000 mg/kg, the extract exhibited no toxicity, suggesting its safety. In vitro tests revealed that the methanolic extract prevented calcium oxalate crystals from growing, indicating a possible anti-urolithiatic activity.

The extract dramatically decreased kidney stone formation, according to additional in vivo tests conducted on male Wistar albino rats. The extract decreased urinary calcium, oxalate, and inorganic phosphate levels while increasing urine volume. Histopathological investigations confirmed the plant extract's anti-urolithiatic effect by demonstrating a decrease in kidney damage and crystal deposition after therapy. Using UV-Vis, IR, NMR, mass spectrometry, and TLC, the flavonoid component that was extracted from the methanolic extract of Hygrophila salicifolia was described. The substance was determined to be isoquercitrin, a flavonoid with anti-inflammatory and antioxidant qualities. The known structure of isoquercitrin was supported by the spectroscopic data, which included the UV absorption spectra, IR peaks, and NMR shifts. The compound's identification was further validated by the Rf value derived from TLC.

CONCLUSION

The methanolic extract of Hygrophila salicifolia showed the highest activity among the other extracts. After being tested for acute toxicity, this methanolic extract was shown to be entirely harmless. The methanolic extract showed the most anti-urolithiatic properties when tested in vitro. The ethylene glycol model was used in an in vivo investigation, and the findings demonstrated a potent anti-urolithiatic effect. Column chromatography was used to separate a flavonoid component from the methanolic extract, and methods like thin-layer chromatography (TLC) and the Shinoda test were used to further validate the product's identity. The substance was identified as isoquercitrin by means of mass spectrometry, ultraviolet (UV) light, infrared (IR) spectroscopy, thermal analysis, and TLC.

REFERENCES

1. Piglili, M., & Runja, M. (2014). "Herbal Renaissance: A Global Phenomenon." Journal of Herbal Medicine, 7(3), 200-208.
2. Schulz, V., Hänsel, R., & Blumenthal, M. (2001). Herbal Medicine: From the Traditional Use to Modern Application. 2nd ed. New York: Springer-Verlag.
3. Zhou, S. F., & Wu, X. L. (2006). "Pharmacokinetic Studies on Medicinal Plants in Traditional Chinese Medicine." Journal of Ethnopharmacology, 106(3), 282-293.
4. Thomas, R. E., Vaidyanathan, R., & Singh, S. (2001). "Deforestation and Its Impact on Medicinal Plants in India." Environmental Biology, 23(4), 317-324.
5. Singabetal, P., Kapoor, P., & Khan, S. (2014). "Biodiversity Loss and Threatened Species in India." Conservation Biology, 28(6), 1185-1197.
6. Thomas, J. T. (1997). The Red Data Book of India: Endangered Plant Species. New Delhi: Ministry of Environment and Forests, Government of India.
7. Shalata, A., M. Volokita, & M. Ron, (1998). "Defense mechanisms in plants under oxidative stress." Environmental and Experimental Botany, 40(2), 137-148.

8. Kama, M., M. Nisha, & P. Prasad, (2012). "Antioxidant defense system in plants." International Journal of Plant Biology, 34(4), 354-368.
9. Nariya, H., P. Jain, & D. Kumar, (2014). "Role of antioxidants in plant stress responses." Journal of Agricultural Science, 12(5), 213-222.
10. Mackman, N. (2008). "Role of thrombosis in cardiovascular diseases." Nature Reviews Cardiology, 5(1), 18-26.
11. Jin, X., L. Zhang, & F. Wang, (2007). "Inhibition of platelet aggregation as a therapeutic strategy for cardiovascular diseases." Journal of Cardiovascular Pharmacology, 50(3), 263-272.
12. Kamal, R., M. Gupta, & R. Singh, (2012). "Search for novel thrombolytic agents from natural sources." Phytomedicine, 19(6), 1-6.
13. WHO. (2002). Traditional Medicine Strategy 2002–2005.
14. Manjula, S., & Mamidala, P. (2013). "The Role of Medicinal Plants in Modern Medicine." Indian Journal of Traditional Knowledge, 12(3), 297-308.
15. Rao, M., et al. (2012). "Biomining of Medicinal Plants for Therapeutic Compounds." Phytochemical Analysis, 23(5), 123-132.
16. Cravotto, G., et al. (2010). "Pharmacological Investigations on Medicinal Plants." Phytomedicine, 17(7), 610-615.
17. Raja, R., et al. (2012). "Plant-based Medicines and Nutritional Supplements." Journal of Herbal Pharmacology, 24(6), 303-310.
18. Vaidya, A., & Devasagayam, T. (2007). "Medicinal Plants and their Role in Traditional Medicine." Indian Journal of Clinical Medicine, 15(2), 135-142.
19. Sumner, L. (2000). "Medicinal Plant Usage in Ancient India." Indian Journal of History of Science, 35(4), 253-258.
20. Joy, J., et al. (2001). "Endangered Medicinal Plants in India." Journal of Ethnopharmacology, 47(1), 45-57.
21. Thomson, A., et al. (2001). "Red Data Book of Endangered Medicinal Species." Indian Journal of Biodiversity, 22(2), 134-141.
22. Balandrin, M., & Klocke, J. (1988). "Pharmacologically Active Plant Compounds." Phytotherapy Research, 6(5), 293-301.
23. Kama, M., et al. (2008a). "Medicinal Plants: Active Ingredients and Pharmacological Effects." Journal of Pharmacology and Therapeutics, 13(3), 101-114.
24. Kamal, R., & Naina, B. (2010). "Plant-based Therapeutics and Their Medicinal Value." Herbal Medicine Journal, 9(6), 119-124.
25. Raskin, I., et al. (2002). "Bioactive Compounds in Medicinal Plants." Phytochemical Reviews, 1(1), 93-102.
26. Aebi, H. (1984). "Catalase in vitro." Methods in Enzymology, 105, 121-126.
27. Bartels, R., et al. (1972). "Calcium Measurement by O-Cresolphthalein Complexone Method." Clinica Chimica Acta, 37(2), 227-229.
28. Bhat, S. R., et al. (2012). "Antiurolithic Potential of Herbal Medicines." Journal of Medicinal Plants Research, 6(21), 4167-4172.
29. Evans, W.C. (2009). Trease and Evans' Pharmacognosy. 16th Edition. Saunders Elsevier.
30. Harborne, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edition. Springer.
31. Kokate, C.K., Purohit, A.P., & Gokhale, S.B. (2001). Pharmacognosy. 12th Edition. Nirali Prakashan.
32. Sethi, P.D. (2001). High-Performance Thin-Layer Chromatography. 1st Edition. CBS Publishers & Distributors.
33. Upadhyay, A., & Malviya, R. (2013). "An Overview on Urolithiasis." Indian Journal of Pharmaceutical Sciences, 75(3), 235-243.

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