
Review of Literature

Medicinal Plants - A Review

Medicinal plants have been a cornerstone of healthcare in virtually all societies, serving as a rich source of medicine. Ensuring the safety, quality, and efficacy of these plants and their products has become a pressing concern globally. The use of herbal remedies and natural healthcare preparations dates back to ancient texts like the Vedas and the Bible. For thousands of years, medicinal plants have been employed not only to flavor and preserve food but also to treat various health issues and prevent diseases, including epidemics. The knowledge of their therapeutic properties has been passed down through generations within and across communities. The bioactive compounds produced during secondary metabolism in these plants are often responsible for their medicinal properties, making them valuable resources worldwide for treating various ailments, including infectious diseases (Ajlan, 2016). According to Dar *et al.* (2017). Medicinal plants have been a cornerstone of human culture, serving as a primary source of medicine across civilizations. They are a rich source of traditional remedies and have contributed significantly to the development of modern medicines. The biological properties of medicinal plants are often attributed to their secondary metabolites, which also help control microbial growth.

Plant-derived secondary metabolites, or phytochemicals, have various health benefits, including anti-oxidative, anti-allergic, antibiotic, hypoglycemic, and anti-carcinogenic properties. They protect cells from damage caused by free radicals, potentially alleviating allergic reactions, combating infections, regulating blood sugar, and preventing cancer cell growth, ultimately promoting overall well-being (Harini and Nithyalakshmi, 2017). According to Akinyemi (2018), medicinal plants play a crucial role in sustainable human health due to their therapeutic properties and affordability compared to synthetic medications. As interest in

alternative therapies grows, these plants are increasingly used worldwide to maintain and augment human health. However, uncontrolled wild collection and human impact threaten their availability, emphasizing the need for domestication and cultivation to ensure a continuous supply of these valuable plant species.

2.1. Phytochemical Analysis of *Spermacoce* and closely related species

Dahiya and Solanki, (2011) identified phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, amino acids, steroids, and triterpenoids in the methanolic extracts of leaves, stems, and roots of *Spermacoce articularis*. Soosairaj *et al.* (2013) investigated the phytochemical profile of *S. articularis* and screened for the phytochemical constituents using ethanol, acetone, chloroform, and ethyl acetate extracts. They reported the presence of carbohydrates, saponins, phenols, phytosteroids, tannins, flavonoids, and catechins. *S. articularis* did not contain cardiac glycosides and alkaloids. Wong *et al.* (2015) conducted a phytochemical screening of hexane, ethyl acetate, acetone, and methanolic extracts from the whole plant of five species of *Borreria*, viz., and revealed the presence of phenolics, alkaloids, flavonoids, tannins, saponins, and terpenoids. The study found that *B. latifolia* contained phenolics, flavonoids, and tannins, while *B. remotifolia*, *B. exilis* and *R. brasiliensis* contained phenolics, alkaloids, flavonoids, tannins, and terpenoids, and *B. laevicaulis* was found to possess all classes of chemical constituents.

According to Anupriya *et al.* (2016), phytochemical analysis of *Spermacoce hispida* revealed a diverse range of bioactive compounds, including flavonoids, phenols, tannins, alkaloids, and others. The methanol extract showed significantly higher levels of flavonoids (20.6 mg/g), phenols (19.84 mg/g), tannins (11.04 mg/g), and alkaloids (10.42 mg/g) compared to other solvent extracts. These compounds are associated with various pharmacological properties, including antioxidant, anticancer, anti-inflammatory, and anti-ulcer activities, highlighting the plant's potential medicinal value. Anburaj and Manikandan (2016) conducted a phytochemical screening of *Borreria hispida*

leaf extracts, revealing a rich presence of secondary metabolites. The ethanolic extract was found to contain a diverse range of compounds, including tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, phenols, steroids, coumarins, and alkaloids. In contrast, methanol extract predominantly contained alkaloids, tannins, and steroids, while the aqueous extract showed a higher presence of saponins. Recent studies have further elucidated the phytochemical profiles of various *Spermococe* species.

Luo *et al.* (2020) reported that *Spermacoce latifolia* contained various phytochemicals, including phenolics, flavonoids, tannins, steroids, coumarin, iridoid glucosides, triterpenes, naphthoquinones, anthraquinones, alkaloids, terpenes, and fatty acids. Many of these compounds demonstrated significant antibacterial, cytotoxic, and α -glucosidase inhibitory activities. The n-butanol fraction of the whole plant showed the highest total phenolic content (57.97 ± 1.99 mg GAE/g extract), while the ethyl acetate fraction exhibited a TFC value of 168.16 ± 0.65 mg RE/g extract. Sinan *et al.* (2021) documented the pharmacological properties of *S. verticillata*, which include antimicrobial, anthelmintic, anti-inflammatory, analgesic, antinociceptive, and hepatoprotective effects. Their phytochemical analysis of the flowers, roots, aerial parts, and whole plant identified alkaloids such as indole derivatives (Verticillatine A and B) and bis-indole compounds (borrevine and borreverine), terpenoids (including β -sitosterol, caryophyllene, and guanine), flavonoids (like rutin and kaempferol-3-O-rhamnoside), and phytosterols. Among the different extracts, the methanol extract exhibited the highest total phenolic content (TPC: 93.73 mg GAE/g extract) and total flavonoid content (TFC: 85.94 mg RE/g extract), followed by the aqueous extract with TPC of 54.63 mg GAE/g extract and TFC of 39.41 mg RE/g extract.

Sundaram and Vasanthi (2022) conducted both qualitative and quantitative phytochemical screening of *S. hispida* and identified tannins, flavonoids, phenols, terpenoids, alkaloids, saponins, steroids, coumarins, and cardiac glycosides in various parts of the plant. Sharma *et al.* (2022) investigated

the phytochemical components of *Spermacoce hispida* extracts, revealing saponins, tannins, phenolics, steroids, essential oils, flavonoids, and terpenoids. These chemical constituents have contributed to the plant's reported activities, including antidiabetic, antihypertensive, hepatoprotective, anti-inflammatory, antihyperlipidemic, analgesic, antifungal, anticancer, and antioxidant effects.

Combined ethanol extract of *Spermacoce radiata* and *Hypselodelphys poggeana* leaves revealed the phytochemical results indicated substantial amounts of total phenols, alkaloids, and terpenoids, along with moderate amounts of tannins, flavonoids, and steroids (Uroko *et al.*, 2022). Sharma *et al.* (2022) investigated the phytochemical components of *Spermacoce hispida* and reported the presence of saponins, tannins, phenolics, steroids, essential oils, flavonoids, and terpenoids. Puspa *et al.* (2024) showed that the phytochemical analyses conducted on n-hexane, ethyl acetate, and methanol extracts derived from the leaves of *E. sumatrensis* revealed the presence of several bioactive constituents, including flavonoids, phenolics, terpenoids, steroids, and alkaloids. A recent study by Fan *et al.* (2024) reported the presence of glycosides, proteins, and volatile oils in *S. remota*, *S. alata*, and *S. exilis*. Additionally, the extracts of *S. alata* and *S. exilis* revealed the presence of flavonoids and tannins.

2.2. Antioxidant Potential of *Spermacoce* species

Purushothaman *et al.* (1979) identified isorhamnetin glycoside in the methanolic extract of *Spermacoce hispida*, although they were unable to isolate the pure flavonoid glycoside. The methanolic extract of *S. hispida* has shown significant antioxidant activity, scavenging both DPPH and nitric oxide radicals. This is notable, as nitric oxide radicals play a key role in inflammation and are implicated in various diseases, including cancer and diabetes. The extract's ability to scavenge nitrous oxide radicals suggests potential anti-inflammatory effects, which could be beneficial in treating inflammatory conditions. Flavonoids have been reported to exert multiple biological properties, including antimicrobial, cytotoxicity, anti-inflammatory, as well as antitumor activities, but the best-described property of almost every group of flavonoids is their capacity

to act as powerful antioxidants, which can protect the human body from free radicals and ROS (Sut *et al.*, 2016).

Farhana and Venkatalakshmi (2017) evaluated the antioxidant activity of the ethanolic extract of *Spermacoce hispida* seeds using various assays. The DPPH scavenging assay showed an IC₅₀ value of 28 µg/ml, indicating significant antioxidant activity. The reducing power assay revealed a dose-dependent increase in reducing power, with an IC₅₀ value of 55 µg/ml. Additionally, the ABTS scavenging assay showed an IC₅₀ value of 140 µg/ml. These results suggest that the ethanolic extract of *S. hispida* seeds possesses notable antioxidant properties. Luo *et al.*, (2020) examined the antioxidant potential of whole plant extract crude extracts of *S. latifolia* by DPPH radical-scavenging assay. The IC₅₀ values were found to be 70.22±0.62, 51.44±0.44, 72.12±0.18, and 2252±2.09 µg/ml for petroleum ether, ethyl acetate, n-butanol, and aqueous. The whole plant extract of *S. latifolia* ethyl acetate fraction exhibited the strongest ferric reducing power of 8.36±0.10 mmol Fe (II)/g extract, followed by the n-butanol fraction 6.11±0.20 mmol Fe (II)/g extract, the petroleum ether fraction 5.89±0.09mmol Fe (II)/g extract and the aqueous fraction 1.49±0.25 mmol Fe (II)/g extract.

Sundaram and Vasanthi, (2022) reported that the antioxidant activity of *S. hispida* establishes its prominent use as a nutraceutical or pharmaceutical agent, both as a prophylactic and therapeutic agent. Sharma *et al.* (2022) reported that the methanolic extract of *S. hispida* exhibited a strong hydrogen-donating ability, with an IC₅₀ value of 60.91 µg/ml. Although this value is higher than that of the standard antioxidant vitamin C (IC₅₀ value: 19.77 µg/ml), it demonstrates the significant antioxidant potential of *S. hispida*. Zhu *et al.* (2024) conducted a comprehensive study to evaluate the antioxidant capacity of *Spermacoce alata* using DPPH, ABTS, and FRAP assays. The study identified 67 compounds in the essential oil, primarily palmitic acid, linoleic acid, and phenylheptatriyne. The essential oil demonstrated moderate to potent biological activities, including antioxidant, anti-acetylcholinesterase, anti-α-glucosidase,

and anti- β -lactamase activities, indicating its potential pharmacological applications in neuroprotection, blood sugar regulation, and combating antibiotic-resistant bacteria.

Adebiyi *et al.* (2017) evaluated the free radical scavenging properties of *Grewia carpinifolia* (EGC) extracts using *in vitro* models, including DPPH, ABTS, and FRAP assays, with ascorbic acid as the standard. EGC showed strong radical scavenging activity in all tests, attributed to its high concentrations of flavonoids and phenolic compounds. The study demonstrated EGC's antioxidant potential, highlighting its ability to reduce lipid oxidation as measured by the TBARS test.

2.3. Antiurolithiatic Activity of Medicinal Plants

Bashir and Gilani's (2009) study demonstrated the antiurolithiatic potential of *Bergenia ligulate* extract, showcasing its ability to inhibit calcium oxalate crystal aggregation, exhibit antioxidant activity, and display diuretic effects in rats. The extract also reduced crystal formation in renal tubules and helped restore kidney function and oxidative stress markers, highlighting its potential as an antiurolithiatic agent with diuretic and antioxidant properties.

Fahad *et al.* (2010) found that *Moringa oleifera* extract (EMO) exhibited antiurolithiatic properties, significantly reducing bladder stone weight in both preventative and curative treatments compared to controls in a zinc disc and ethylene glycol-induced bladder stone model. Bayir *et al.* (2011) found that the ethanolic extract of *Helichrysum plicatum* flower (EFHP) showed potential in treating urolithiasis, significantly reducing elevated biochemical markers, urinary calcium oxalate levels, and preventing kidney damage in an ethylene glycol-induced urolithiasis model, suggesting EFHP's promise as a treatment for kidney stones. The study by Sivaelango *et al.* (2012) found that the ethanolic seed extract of *Spermacoce hispida*, at a dose of 200 mg/kg, significantly reduced cholesterol, phospholipids, triglycerides, and LDL levels, while increasing HDL levels in hyperlipidemic rats. Mi *et al.* (2012) found that aqueous extracts of

Desmodium styracifolium (ADS) and *Pyrosiaepetiolosa* (APP) exhibited antiurolithiasis activity, preventing calcium oxalate stone formation and showing diuretic and antioxidant effects. ADS specifically reduced urine oxalate and increased urine calcium, while APP improved various serum and urine parameters, highlighting their potential in preventing kidney stone formation.

Chauhan and Joshi (2013) found that *Citrus medica* juice and extracts of *Commiphora wightii*, *Boerhaavia diffusa*, and *Rotula aquatica* inhibited struvite crystal growth in an *in vitro* study, showcasing their potential in preventing kidney stone formation. Kishore *et al.* (2013) found that the ethanolic extract of *Portulaca oleracea* aerial parts (EAPO) showed antiurolithiatic activity, improving urine and serum parameters and reducing kidney crystal deposition in an ethylene glycol-induced urolithiasis model, highlighting its potential in preventing kidney stones. Ahmed *et al.* (2013) assessed the antiurolithiatic potential of the ethanolic extract of *Adiantum capillus* (EEAC) in an ethylene glycol-induced urolithiasis model. The study found a significant reduction in crystal formation in the urine of EEAC-treated rats. Additionally, EEAC treatment improved body weight and biochemical markers. Histological analysis of the kidneys showed near-normal renal morphology in the treatment group, confirming the antiurolithiatic potential of EEAC and supporting its traditional use in treating urolithiasis.

Lilaram and Ahamed (2013) evaluated the effects of an ethanolic extract of *Caesalpinia bonducella* seeds on rats' organs and clinical chemistry. The study involved four groups of Wistar female albino rats treated with different doses (100, 200, 300 mg/kg) of the extract for ten days. Histological analysis showed that tissue structures remained normal. Hematological parameters like RBC count, WBC types, platelet count, hemoglobin, and packed cell volume significantly increased in the treated groups. Serum biochemistry indicated lower cholesterol, triglycerides, and creatinine levels, with significantly higher HDL levels. The findings suggest that *C. bonducella* contains compounds with cytoprotective potential. Selvan *et al.* (2013) tested the efficacy of *Triphala karpa*

Chooranam, a blend of *Terminalia chebula*, *Phyllanthus emblica*, *Terminalia bellerica*, *Karungalisathu*, and *Vengai sathu*, for treating urolithiasis. In a rat model, the formulation showed notable antiurolithiatic activity against ethylene glycol-induced urolithiasis. Histological analysis of the kidneys revealed no damage in the groups treated with the churna formulation and Cystone. Additionally, urine analysis showed a significant reduction in oxalate crystal excretion, highlighting the formulation's potential in treating kidney stones. Jayaramaiah *et al.* (2013) found that Cystone, a polyherbal preparation, inhibited struvite crystal growth *in vitro*, reducing the number, weight, and volume of crystals. It also caused the disintegration of developed crystals at concentrations of 0.5%, 1%, and 2% in a magnesium acetate solution, suggesting its potential in reducing struvite stone formation and aiding fragmentation.

Atodariya *et al.* (2013) studied the antiurolithiatic effects of fractions from *Dolichos biflorus* Linn extract, identifying phenolic, flavonoid, and steroid components. The DBA fraction showed significant dissolution of calcium oxalate stones (around 48%), while cystone was slightly more effective (around 53%). Reddy *et al.* (2014) conducted a long-term study on the effects of Urikind-KM6, a commercial product containing potassium citrate, magnesium citrate, and pyridoxine hydrochloride, on the recurrence of calcium oxalate and phosphate urolithiasis. Over three years, Urikind-KM6 demonstrated a significant increase in 24-hour urine citrate excretion and a rise in urine pH, leading to a reduction in stone recurrence rates from 4 to less than 1 per patient annually. Li *et al.* (2015) investigated catechin's effects on melamine cyanuric acid mixture-induced urolithiasis in both *in vitro* and *in vivo* models. *In vitro*, catechin inhibited crystal formation at various pH levels. *In vivo*, catechin reduced kidney stone formation and nephrotoxicity, decreasing oxidative stress markers, apoptosis, and expression of phospho-p38 and osteopontin. The study highlights catechin's potential as an antiurolithiatic agent due to its ability to suppress stone formation and oxidative damage.

Sridharan *et al.* (2016) investigated the preventive and curative effects of peel extract of *Citrus limon* and formulated tablets of bioflavonoids from *C. limon* against 0.75% EG induced CaOx urolithiasis in male Wistar rats over 50 days. The extract and tablets showed utmost efficacy in reducing urinary calcium, oxalate and phosphate; enhancing urinary citrate levels; reducing serum BUN and creatinine; hampering deterioration of renal tissue integrity; reducing expression of inflammatory mediators viz. nuclear factor- κ B (NF- κ B), monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6); and in downregulating the expression of urinary proteins viz. osteopontin, Tamm-Horsfall protein and transferrin, thus providing substantial evidence for the prophylactic and curing ability of *C. limon* bioflavonoid tablets and *C. limon* peel extract against CaOx urolithiasis Kumar *et al.* (2016) studied the effects of the ethanolic extract of *Peucedanum grande* fruit (EEPG) in an ethylene glycol-induced urolithiasis model. The treatment significantly reduced calcium oxalate crystal formation in urine and lowered serum levels of calcium, phosphorus, creatinine, and urea. EEPG also increased urine output and prevented crystal accumulation in kidney tissue, demonstrating strong antiurolithiatic activity comparable to Cystone.

Ghelani *et al.* (2016) investigated the diuretic and antiurolithiatic properties of *Acorus calamus* rhizome extract (RAC). RAC increased urine volume and enhanced excretion of sodium and potassium ions, exhibiting diuretic effects comparable to furosemide. In an ethylene glycol-induced urolithiasis model, RAC reduced the excretion and deposition of stone-forming substances in urine, blood, and kidney tissues, while improving kidney function. The study suggests that phytochemicals in RAC contribute to its diuretic and nephroprotective effects, supporting its potential as an antiurolithiatic agent. Panigrahi *et al.* (2017) validated the traditional use of *Xanthium strumarium* in an ethylene glycol-induced urolithiasis model. The hydroalcoholic extract of *Xanthium strumarium* (AEXS) improved biochemical abnormalities, reduced stone formation, and decreased oxidative stress. AEXS treatment also lowered

elevated osteopontin levels, suggesting its potential to cure urolithiasis by modulating calcium oxalate production pathways in the kidney.

Prabhu *et al.* (2016) isolated diosmin from *Teucrium gnaphalodes* and evaluated its antiurolithiatic effects in an ethylene glycol-induced urolithiasis model. Diosmin treatment significantly reduced kidney weight, urinary crystals, and biochemical markers, improving kidney function and urine output. At a dose of 20 mg/kg, diosmin exhibited potent antiurolithiatic activity comparable to Cystone, a marketed formulation. Sikarwar *et al.* (2017) studied the effects of methanolic (MECA) and aqueous (AECA) extracts of *Chenopodium album* leaves on ethylene glycol-induced urolithiasis. The extracts also decreased urine volume, pH, and oxalate content, and reduced oxalate levels in the kidneys and the formation of oxalate stones in rats. The effects of MECA and AECA were comparable to the standard treatment, Cystone. Flavonoids and saponins in *Chenopodium album* were identified as responsible for its antiurolithiatic properties, confirming its medicinal use in urolithiasis.

Youn *et al.* (2017) isolated phenolic compounds from *Quercus gilva* Bume (QGB), including (-)-epicatechin, procyanidin B-3, and procyanidin B-4, which showed significant antioxidant and anti-inflammatory properties. These compounds suppressed inflammatory cytokine gene expression, including COX-2 and IL-1, in a dose-dependent manner. QGB extract treatment effectively managed urolithiasis, suggesting its potential role in preventing kidney stone formation. Das *et al.* (2017) evaluated the preventive and therapeutic effects of the aqueous extract of *Phlogacanthus thyriformis* flower (AFPT) and its biofabricated silver nanoparticles (BSN) on struvite and calcium oxalate stone formation. *In vitro* studies showed AFPT and BSN reduced struvite crystal size, while *in vivo* studies demonstrated their effectiveness in removing calcium oxalate stones in rats. Flavonoids were identified as the key active components responsible for the antiurolithiatic effects, highlighting AFPT and BSN's significant therapeutic potential.

Namburu *et al.* (2017) investigated the antiurolithiatic effects of the ethanolic extract of *Phaseolus vulgaris* seeds (ESPV) in an ethylene glycol-induced urolithiasis model. ESPV treatment restored biochemical parameters, including calcium, oxalate, and phosphate levels, to near-normal values and reduced kidney stone accumulation. ESPV also exhibited antioxidant properties, decreasing lipid peroxidation and recovering antioxidant enzyme activity, similar to the standard treatment, Cystone.

Sikarwar *et al.* (2017) carried out the screening of the preventive efficacy of methanolic and aqueous extracts of the leaves of *Chenopodium album* for 28 days against 0.75% v/v EG induced CaOx urolithiasis in male and female Wistar rats. Both the extracts showed significant amelioration in EG-induced alterations in urine, serum, and renal parameters. *C. album* treatment produced a significant reduction in calcium, phosphorus, urea, creatinine, and uric concentration in the urine and serum of rats. It also produced a significant decline in renal oxalate and CaOx crystal deposition, thus exhibiting its potential as a preventive antiurolithiatic.

Bano *et al.* (2018) studied the antilithiatic effect of *Kabab chini* fruit extract (EFKC) in an ethylene glycol-induced urolithiasis model. EFKC treatment reduced urinary crystals, lowered serum creatinine and urea levels, and improved kidney histology. It also altered urine composition, increasing Mg^{2+} and decreasing Ca^{2+} , Na^+ , Cl^- , and phosphorus levels. These findings suggest EFKC's potential in inhibiting calcium oxalate urolithiasis.

Several studies have investigated the inhibitory effects of various plant extracts on calcium oxalate (CaOx) nucleation. Bawari *et al.* (2018) evaluated the effect of *Daucus carota* root ethanol extract on CaOx nucleation and found a significant reduction of $56.1 \pm 1.55\%$ at a concentration of 1000 $\mu\text{g/ml}$ than that produced by cystone of $41.67 \pm 1.03\%$. Kaushik *et al.* (2019) evaluated the toxicity and antiurolithiatic potential of *Tribulus terrestris* fruit extract (AETT). In an ethylene glycol-induced urolithiasis model, AETT improved kidney function, renal morphology, and body weight. It facilitated the excretion of medium-sized

urinary stones and reduced oxidative stress by restoring the p38MAPK pathway. The study provides scientific evidence for AETT's prophylactic and curative properties in urolithiasis. Kaleeswaran *et al.* (2019) evaluated the ethyl acetate extract of *Pedalium murex* (EAPM) against struvite urolithiasis. EAPM showed antibacterial activity against urease-producing bacteria, including *E. coli* and *S. aureus*. It significantly reduced struvite stone size and promoted dissolution *in vitro*. *In vivo* studies demonstrated EAPM's efficacy in reversing renal damage, normalizing biochemical parameters, and improving histopathological outcomes, with effects comparable to or exceeding Cystone.

Yasir *et al.* (2018) evaluated the preventive and curative efficacy of caffeic acid on 0.75% v/v ethylene glycol-induced urolithiasis in Wistar rats in an 8-week study protocol. Preventive and curative treatment with caffeic acid resulted in the elevation of urinary citrate levels. As determined from the real time polymerase chain reaction (PCR) analysis of gene expression, caffeic acid treatment resulted in the upregulation of prothrombin fragment 1, Tamm-Horsfall protein and bikunin gene expression and downregulation of osteopontin gene expression that are supposed to have mediated the preventive and curative efficacy of caffeic acid against urolithiasis in rats. A study conducted by Solomon *et al.* (2019) investigated the inhibitory effect of the ethanol leaf extract of *Maerua angolensis* on calcium oxalate (CaOx) nucleation and evaluated the percentage inhibition of CaOx nucleation at various concentrations of the extract. The results showed that the highest concentration of the extract (30 mg/ml) exhibited a significant inhibitory effect, with a percentage inhibition equivalent to 22 mg of cystone per milliliter. Bawari *et al.* (2020) studied the antiurolithiatic effects of *Daucus carota* root extract (HEDC) in an ethylene glycol and ammonium chloride-induced urolithiasis model. HEDC treatment restored biochemical parameters, reduced kidney damage, and alleviated oxidative stress, showing both preventive and curative effects against urolithiasis. Cystone is a standard inhibitor used as a reference for comparison. This finding suggests that the ethanol leaf extract of *Maerua angolensis* has potential as a natural inhibitor of CaOx nucleation.

A study conducted by Gonzalez (2020) investigated the potential antilithiatic properties of aqueous and hydroalcoholic extracts of the stems of *Caesalpinia bahamensis*. The effectiveness of these extracts was evaluated in preventing the formation of renal stones, a common and painful condition. The results showed that the extracts significantly reduced the concentrations of calcium and oxalate in the urine of lithiasis patients, which are key factors in the formation of renal stones. This finding suggests that the extracts may have a protective effect against renal stone formation. The optimal dosage for antilithiatic activity was found to be 200 mg/kg, with a significant effect also observed at 100 mg/kg. However, the study also revealed that the extracts did not have a significant impact on renal damage caused by renal stones. The lithiasis and treated groups showed higher plasmatic creatinine levels compared to the control group, indicating renal damage. Furthermore, histological examination of kidney sections stained with hematoxylin/eosin revealed signs of renal damage, including loss of the morphology of the Bowman Capsule and renal tubules.

Mousa *et al.* (2020) found that the ethyl acetate fraction of *Coffea arabica* has a high concentration of calcium ions, which enables it to effectively dissolve calcium oxalate monohydrate crystals, even outperforming the standard anti-urolithic drug Cystone. They reported that *Coffea arabica* extract has anti-urolithic properties, which work by reducing the size of calcium oxalate crystals and promoting the formation of less harmful calcium oxalate dehydrate crystals. This reduction in crystal size, combined with the extract's diuretic effect, helps to prevent the formation of kidney stones and facilitates their elimination. Furthermore, the study showed that different fractions of *Coffea arabica* inhibit the crystallization of calcium oxalate monohydrate and promote the formation of calcium oxalate dihydrate, resulting in fewer and smaller particles. This is significant because calcium oxalate monohydrate has a stronger affinity for cell membranes, making it a higher risk factor for stone formation.

A study conducted by Marhoume *et al.* (2021) investigated the potential of *Rubia tinctorum* L. extracts to prevent kidney stone formation in an experimental

rat model using ethanolic (E-RT) and ethyl acetate (EA-RT) extracts. The results showed that both extracts prevented the formation of calcium oxalate crystals in the urine and reduced the associated biochemical alterations and kidney tissue damage. However, the ethanolic extract (E-RT) had a more pronounced preventive effect due to its higher antioxidant activity and polyphenol content. The study suggests that *Rubia tinctorum* L. extracts, particularly the ethanolic extract, may be a useful natural remedy for preventing kidney stone formation. The antioxidant and polyphenol-rich properties of these extracts may help to mitigate the oxidative stress and inflammation associated with kidney stone formation, providing a potential therapeutic strategy for the prevention and treatment of urolithiasis.

According to Hewagama and Hewawasam (2022), research on plant extracts' ability to inhibit calcium oxalate nucleation revealed promising results. *Kalanchoe laciniata* hexane extract demonstrated notable inhibition, while *Aegle marmelos* extracts, particularly the hexane extract, showed significant dose-dependent inhibition, reaching 83.56% at 1000 µg/ml. Similarly, *Drymoglossum piloselloides* ethanol extract consistently inhibited nucleation by 71.13% across different concentrations. These findings suggest that these plant extracts possess potential as natural inhibitors of calcium oxalate crystallization, which could have implications for preventing kidney stone formation. The study highlights the therapeutic potential of these plants in managing calcium oxalate-related disorders. A study conducted by Swarnalatha, (2022) investigated the anti-urolithiatic activity of the ethanolic extract of *Sterculia urens*. The results showed that the extract exhibited a significant inhibitory effect on calcium oxalate crystallization, with a percentage inhibition of 35% at a concentration of 600 µg/ml. Notably, this inhibitory effect was higher than that of Cystone, a standard anti-urolithiatic drug, which showed a percentage inhibition of 27% at the same concentration. This finding suggests that the ethanolic extract of *Sterculia urens* has potential as a natural anti-urolithiatic agent, with a more pronounced inhibitory effect than cystone at the tested concentration.

Vishnupriya and Tamilselvi (2022) conducted a study to evaluate the anti-urolithiatic activity of the ethanolic extract of *Aerva lanata* leaves. The results showed that the extract exhibited a remarkable inhibitory effect, with a percentage inhibition of 87.93% at a concentration of 500 µg/ml. This inhibitory effect was comparable to that of Cystone, a standard anti-urolithiatic drug, which showed a percentage inhibition of 90.64% at the same concentration. The findings suggest that the ethanolic extract of *Aerva lanata* leaves has significant anti-urolithiatic potential, with an inhibitory effect similar to that of cystone. According to Deka *et al.* (2024), the study evaluated the antiurolithiatic potential of *Calamus floribundus* Griff.'s methanolic extract using *in vitro* and *in vivo* methods. The extract demonstrated promising results, inhibiting calcium oxalate nucleation and aggregation with IC₅₀ values of 767.394±0.22 µg/ml and 759.435±0.29 µg/ml, respectively. *In vivo* studies showed normalization of biochemical parameters in a dose-dependent manner. However, the extract exhibited cytotoxicity on NRK-52E cell lines with an IC₅₀ value of 11.1 µg/ml. Overall, the findings suggest that *Calamus floribundus* Griff. possesses antiurolithiatic potential, warranting further investigation.

2.4 *In vitro* Cytotoxicity of Antiurolithiatic Plants

The study by Gullien-Melendez *et al.* (2022) investigated the effect of the methanolic extract of *Mimosa malacophylla*. A Gray on Vero and HEK-293 cell lines, as well as the morphology of the kidneys and bladder in rats with induced urolithiasis. The results showed that concentrations of the methanolic extract below 300 µg/ml did not cause cytotoxicity in renal Vero and HEK-293 cells. In the *in vivo* analysis, a 15-day treatment with extract concentrations ranging from 50 mg/kg to 150 mg/kg proved effective as an antiurolithic treatment, with no morphological changes observed in the kidneys or bladder of the murine urolithiasis model. According to Nafiu *et al.* (2018), the study examining the cytotoxic effects of saponin-rich extract of *Dianthus basuticus* (SEDB) treatment on HEK293 cell lines revealed a concentration-dependent impact on cell viability and cytotoxicity. Notably, cells treated with the highest dose exhibited a mere

15.17% survival rate, whereas those receiving the lowest dose showed a significantly higher survival rate of 89.72%. Furthermore, the calculated CC₅₀ value for the extract in HEK293 cells was 301.56 µg/ml after 72 hours.

2.5. Chromatographic Profiling of Phytocompounds

Flavonoids, a group of polyphenolic compounds, are known for their ability to scavenge free radicals, inhibit hydrolytic and oxidative enzymes, and exhibit anti-inflammatory effects (Atanassova *et al.*, 2011). The aerial part of the plant showed terpenoids, campesterol, β-sitosterol, stigmasterol, and mixtures of aliphatic acids, tri-O-acylglycerols, sucrose phytol, 1,8-cineole, α-α-pinene, and p-cymene were identified by GC-MS analysis. The first report of the occurrence of four triterpenes, two flavonoids, one anthraquinone, and two phytosteroids was investigated in *S. verticillata* (Ferreira *et al.*, 2012). Recent research has highlighted that phenolic compounds in *Spermacoce hispida* possess a wide range of biologically active constituents, making them a major focus of study. One of the key properties of phenolics is their exceptional radical scavenging activity (Upadhyaya *et al.*, 2013). GC-MS analysis of *S. articularis* L.f. extracts has identified 25 biochemical constituents, with major compounds including 2-Benzylidene-3-oxo-4-(octylsulfanyl)-2,3-dihydrothiophene-1-dioxide (27.71%), Tridecanoic acid (17.46%), 3,7-Dimethyl-3-hydroxy-4-isopropenyl-6-octadiene (6.73%), Octadecanoic acid (5.60%), and Methyl-threo-9,10-dichlorooctadecanoate (4.05%). These findings highlight the potential therapeutic value of *Spermacoce* species and warrant further research into their phytochemical constituents.

Garba *et al.* (2019) isolated two compounds, labeled A and B, from the crude extract of *Spermacoce verticillata* through chromatographic separations (TLC and Column). Spectroscopic analyses using IR, NMR, and GC-MS revealed that Compound A is Hexacosanoic acid, while Compound B is 3-Hydroxyurs-12-en-28-oic acid. Compound A's GC-MS spectrum showed a molecular ion at m/z 392, while its IR spectrum exhibited a broad hydroxyl (OH) group at 3417.24 cm⁻¹ and a strong carbonyl (C=O) absorption at 1701.06 cm⁻¹.

Additionally, absorption peaks at 2915.42 and 2848.17 cm^{-1} indicated C-H stretching of methyl and methylene groups. Compound B, a white amorphous substance with a melting point of 240.5-242.4°C, showed a molecular ion ratio (m/z) of 456, consistent with the molecular formula $\text{C}_{30}\text{H}_{48}\text{O}_3$. Its IR spectrum revealed a hydroxyl group at 3395.46 cm^{-1} , a carbonyl (C=O) at 1737.25 cm^{-1} , and an olefinic group (C=C) at 1686 cm^{-1} . The characteristic C-H stretching for unsaturated and saturated functional groups was observed between 3000 cm^{-1} and 2922.06 cm^{-1} .

Sekandi *et al.* (2023) identified eleven compounds in *S. princeae* extracts using *n*-hexane, ethyl acetate, methanol, and distilled water. The compounds included quercetin kaempferol-3-O-rutinoside rutin, *myo*-inositol, asperulosidic acid hexadecanoic acid, β -sitosterol stigmasterol, campesterol ursolic acid and β -sitosterol glucoside. The study found that kaempferol-3-O-rutinoside and rutin exhibited good radical scavenging activity, with an IC_{50} value of 64.81 $\mu\text{g}/\text{ml}$ for kaempferol-3-O-rutinoside and 58.58% inhibition for rutin. In comparison, ascorbic acid, used as a positive control, showed a significantly lower IC_{50} value of 2.59×10^{-16} $\mu\text{g}/\text{ml}$, highlighting its potent antioxidant activity. *Spermacoce hispida* is a nutrient-rich plant containing significant amounts of calcium and phosphorus. It also boasts an array of notable compounds, including beta-sitosterol, ursolic acid, iso-rhamnetin, and D-mannitol.

The ethanolic extract of *S. articularis* subjected to GC-MS analysis by Soosairaj *et al.* (2013) revealed twenty-five chemical constituents with major compounds including 2-Benzylidene-3-oxo-4-(octylsulfanyl)-2,3-dihydrothiophene-1 dioxide, Tridecanoic acid 3,7-Dimethyl-3-hydroxyl-4-isopropenyl-6-octadiene, Octadecanoic acid (CAS), and Methyl-threo-9,10-dichloro-octadecanoate. Sukari *et al.* (2013) isolated ursolic acid and stigmasterol from *Spermacoce articularis* and *Spermacoce exilis*. Besides, benzo[*g*]isoquinoline-5,10-dione, hexadecanoic acid, sitosterol and pentatriacontane from *Spermacoce exilis*.

Gnandeebam and Vishwanathan (2014) conducted a GC-MS analysis of the methanolic extract of *Spermacoce articularis* L.f. leaves, identifying 30 compounds. The chromatogram showed six prominent peaks within the retention time range of 4.88-40.12 minutes. The major phytochemicals identified in the methanolic extract of *in vivo* leaves included 3-[4'-(2"-Chlorophenyl)-2'-thiazolyl]-2,4-dioxo-1,2,3,4-tetrahydroquinazoline, 2-N-Propyl-1-d1-Aziridine, and 6-Imino-2,3,4,6-tetrahydro-1,3-thiazini[3,2b]isoquinoline-11-carbonitrile. Other compounds identified included Phytol, Octadecanoic acid, 9,10-dichloro-methyl ester, and 9,12,15-Octadecatrienoic acid, methyl ester. In contrast, the major phytochemicals in the *in vitro* leaf extract included Ethyl 3-amino-4-{4'-[(5-chloro-2-methoxybenzoyl)amino]phenyl}-6-(p-fluorophenyl)thieno[2,3-b]pyridine (13.75% peak area), Spiro[Adamantylidene-1,1'-2',2'-diphenyloxirane] (13.09% peak area), and Guanosine (7.74% peak area). Other notable compounds identified in the *in vitro* leaf extract included Decanoic acid, Cholesta-7,14-dien-3-ol, and Phytol.

Lima *et al.* (2014) conducted a phytochemical analysis of *Spermacoce verticillata* leaves, utilizing HPLC-DAD and NMR techniques. The study revealed the presence of chlorogenic acid and three flavonoids: quercetin-3-O-rutinoside (rutin), kaempferol-3-O-rutinoside, and an unidentified flavonoid derivative (retention time: 7.934 min). The HPLC conditions enabled effective separation of the phenolic acids and flavonoids. Dhevi and Elango (2015) analysed the GC-MS chromatogram of the methanolic extract of *Spermacoce hispida* seeds, identifying several bioactive compounds. The major compounds included 2-Furancarboxaldehyde, 5- (hydroxymethyl)- (7.21%); Hexadecanoic acid, ethyl ester (8.52%), and 9,12-Octadecadienoic acid, ethyl ester (35.58%). Notably; 9,12-Octadecadienoic acid was prominent fatty acid, present in various forms, including methyl ethyl ester, 2- hydroxy-1- (hydroxymethyl) ethyl esters. *Spermacoce latifolia* has yielded novel ursane and oleanane triterpene acids, specifically 3- β , β -23-trihydroxyurs-12,20(30)-dien-28-oic acid and 3 β , β -29-trihydroxy-olean-12-en-28-oic acid, alongside seven known triterpenic acids. Notably, these compounds exhibit anticancer properties against MCF-7, NCI-

H460, and HepG-2 human cell lines, with IC₅₀ values between 9.53 and 80.20 µM (Luo *et al.*, 2015).

Anupriya *et al.* (2016) performed a comprehensive GC-MS analysis of *Spermacoce hispida* extracts, revealing a diverse array of phytoconstituents. The methanolic extract contained 30 compounds, with seven major compounds, including Triacetin (15.0%), Hexatriacontane (7.6%), Linoleic acid, and methyl ester (4.0%). The remaining 23 compounds were present in minor amounts. The ethyl acetate extract contained six compounds, with three major compounds, including Cis-Vaccenic Acid (9.77%) and Alphamonoacetin (8.82%). The chloroform extract showed eight compounds, with five major compounds, including Phenol, 2,4-bis(1,1-dimethylethyl) (17.7%) and Tetradecyl trifluoroacetate (12.9%). The hexane extract contained 22 compounds, with 10 major compounds, including 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (10.4%). The aqueous extract revealed four major compounds, including Eicosane (16.6%), Tetratetracontane (16.1%), and Hexatriacontane (12.8%). The study identified a range of aliphatic and aromatic compounds, which were matched with the NIST/NBS spectral database. Saha *et al.* (2016) studied the chemical composition of the non-polar fractions of *Spermacoce latifolia* using GC-MS spectrophotometry. The study revealed the presence of 35 non-polar compounds, including aliphatic and aromatic hydrocarbons, fatty acids, esters, ethers, carbonyl compounds, alcohol, and terpenoids.

Igwe *et al.* (2016) studied the GC-MS chromatogram of the ethanolic extract of *Acalypha wilkesiana* leaves, revealing the presence of 12 phytochemicals. The identified compounds included 3-methylene-1-vinyl-1-cyclopentene, 2-Vinylbicyclo [2.1.1] hex-2-ene, Acetophenone or Methyl phenyl ketone, Butane-1,4-diol, 3-Methyl-6-hepten-1-ol, Acrylic acid butyl ester, n-Hexadecanoic acid or Palmitic acid, 1,4-Dimethylbenzene or 1,4-Xylene, Styryl alcohol, phenyl ethyl alcohol, 2-Ethyl-1-hexene and 2-Butenyl propionate. A recent study by Singh *et al.* (2023) employed GC-MS analysis to identify 43 compounds in the chromatogram of *Apple Cider* Vinegar (ACV) fruit. The

chromatogram exhibited high resolution and detected a substantial number of phytoconstituents within the 10.0- 28.0-minute retention time range. Notably, the dominant compounds included Five-Hydroxymethylfurfural, 1,5-Anhydro-6-Deoxyhexo-2,3-Diulose, and 3-Deoxy-d-mannonic lactone, among others. These findings suggest that the identified compounds may possess therapeutic potential for the treatment of bladder stones.

Garba *et al.* (2019) conducted Chromatographic and Spectroscopic analyses of *Spermacoce verticillate* using GC-MS, leading to the Isolation of two compounds, namely Hexacosanoic acid (fatty acid) and 3-hydroxyurs-12-en-28-oic acid (Pentacyclic triterpenoid). Perumal *et al.* (2021) analysed the ethyl acetate extract of the aerial parts of *Spermacoce hispida* Linn using GC-MS, identifying various bioactive molecules. These compounds included n-hexadecanoic acid, 2-((Octan-2-yloxy) carbonyl) benzoic acid, Squalene, gamma-Tocopherol, dl-lpha-Tocopherol, Campesterol, Stigmasterol, beta-sitosterol, E,E,Z-1,3,12-Nonadecatriene-5,14-diol. The presence of these molecules may contribute to the medicinal roles of the plant, supporting its traditional use in ethnomedicinal practices

According to Pinipay *et al.* (2023), GC-MS analysis of the ethyl acetate crude seed extract of *Ficus religiosa* revealed a complex phytochemical profile, comprising 62 peaks with multiple compounds exhibiting retention time variability. Notably, hexadecan-1-ol displayed dual peaks at distinct retention times. The identified compounds spanned various classes, with fatty acid methyl esters, hydrocarbons, fatty alcohols, phenols, and fatty acids being the predominant constituents. This comprehensive phytochemical analysis provides valuable insights into the potential bioactive compounds responsible for the extract's anti-urolithiatic activity.

Sowmya *et al.* (2024) conducted a phytochemical analysis of the ethanolic extract of *Tinospora cordifolia*, identifying key components with high probability percentages. The major compounds detected, including Capsazepine, Khellin, and Phytol, exhibited significant retention times. Notably, the compounds with

the highest peak area percentages demonstrated substantial pharmacological activity, suggesting that *T. cordifolia*'s phytochemicals play a crucial role in modulating the urolithiasis pathway. Zhu *et al.* (2024) conducted a GC-MS analysis of *Spermacoce alata* Aubl, revealing its main components. The predominant compounds included palmitic acid (30.74%), linolenic acid (16.13%), phenylheptatriyne (8.07%), and hexahydrofarnesyl acetone (4.44%). The composition was characterized by a high proportion of fatty acids (55.03%), followed by sesquiterpenoids (9.70%), and monoterpenoids (9.49%). These indicate that *S. alata* can be classified as a fatty acid chemotype. Despite these extensive phytochemical characterizations, the direct correlation between specific compounds and Antiurolithiatic mechanisms remains to be elucidated.

2.6. *In silico* Analysis

2.6.1 Molecular Docking

Sasikala *et al.* (2015) *in silico* study investigated the antiurolithiatic potential of *Rotula aquatica* Lour. The research identified 3-O-Acetyl-11-Keto-P-Boswellic acid, a triterpenoid compound extracted from the plant's aqueous root extract, as a promising candidate. Notably, this compound demonstrated significant interaction with Tamm-Horsfall protein, yielding the lowest glide score. This finding suggests that 3-O-Acetyl-11-Keto-P-Boswellic acid may possess antiurolithiatic properties, warranting further investigation. Radha *et al.* (2015) study employed *in silico* screening to explore the antiurolithiatic potential of *Crateva magna* (Lour.) DC. The investigation revealed that 14-hydroxy-12-abietene-7-one, a diterpenoid compound isolated from the aqueous bark extract, exhibited significant interaction with Tamm-Horsfall protein, characterized by the lowest glide score. This finding suggests that 14-hydroxy-12-abietene-7-one possesses antiurolithiatic properties, meriting further research.

An *in silico* study by Bala *et al.* (2022) investigated the therapeutic potential of *Hybanthus enneaspermus* against urolithiasis. The research revealed that compounds from the plant's ethanolic extract exhibited strong

binding affinity towards key enzymes, including Adenine phosphoribosyltransferase, Oxalate oxidase, and Glycolate oxidase. Notably, the docking of 2 ETE with rutin showed a high binding energy of -11.28 kJ/mol, characterized by hydrogen bonding interactions with specific amino acid residues (Asn 167, Gly 87, Trp 83, Asn 152, Ala 164, and Leu 149). These findings suggest that the ethanolic extract of *Hybanthus enneaspermus* may possess significant anti-urolithiatic activity, warranting further investigation.

According to Swetha *et al.* (2022) molecular docking study revealed that compounds from *Acalypha indica*, *Aerva lanata*, and *Kalanchoe pinnata* exhibit potent interactions with enzymes (2ETE, 1JRP, 4WRN, 2RDU, 5FBH) implicated in urolithiasis. Notably, quercetin, acalyphin, and kaempferol demonstrated exceptional binding affinities, with docking scores of -8.27, -8.19, and -9.32 Kcal/mol, respectively, when interacting with proteins 1JRP and 2RDU. These findings suggest that these compounds may exhibit effective anti-urolithiatic activity, as lower docking scores indicate more stable interactions between the compounds and target proteins.

Molecular docking, a computational technique predicting the binding affinity of ligands to receptor proteins, has emerged as a powerful tool in drug development. Nutraceuticals, bioactive substances found in food sources, can be utilized for disease management by targeting specific molecular pathways (Agu *et al.*, 2023). When a plant's crude extract exhibits significant biological activity, isolating and characterizing its active compounds enables researchers to use *in-silico* studies, such as molecular docking, to investigate potential drug targets. This approach involves leveraging known structures from databases to identify new targets, including docking phytochemicals from a specific plant onto drug targets for emerging diseases. Correlating the biological activity of medicinal plants with their molecular targets enables researchers to discover novel, disease-specific therapies, revealing the potential of medicinal plants to combat a range of diseases (Asiamah *et al.* 2023).

Kumar *et al.* (2024) conducted a multi-target screening of phytochemicals from *Heliotropium indicum* L. and found that pestalamide-B exhibited the strongest binding affinity across most protein targets associated with anti-urolithiasic activity. In contrast, rapanone and supinine showed good binding affinity with only a subset of targets, with lower binding energies compared to pestalamide-B. An *in silico* study by Sujatha and Rajini, (2024) investigated the potential of compounds from *Musa paradisiaca* (L.) exudate against urolithiasis. The results revealed that the olean-12-ene-3 beta, 28-diol demonstrated the highest docking score (-7.2 kcal/mol) on the glycolate oxidase receptor, indicating its potential as a promising lead molecule for treating urolithiasis.

2.6.2 Molecular Dynamics Simulations

The integration of molecular docking with molecular dynamics simulations offers a powerful approach to investigating the dynamic behavior of protein-ligand complexes. This combined methodology enables researchers to elucidate the conformational changes that occur upon ligand binding and assess the stability of the complex (Dhanik *et al.*, 2013). A key advantage of this approach is its ability to explicitly consider structural flexibility and entropic effects, thereby providing a more accurate estimation of the thermodynamic and kinetic parameters governing drug-target recognition and binding (De Vivo *et al.*, 2016). A study by Kumar *et al.* (2024) employed a multi-target-based screening approach to identify potential anti-urolithiatic agents from the aerial parts of *Heliotropium indicum* L. The simulation-based study revealed that Pestalamide B, Rapanone, and Supinine exhibited significant potential against multiple anti-urolithiatic targets, outperforming other compounds. Although experimental validation is still required to confirm these findings, the study provides valuable insights that could pave the way for the development of novel anti-urolithiasis agents for the treatment of kidney stones.

2.6.3 Pharmacokinetic properties

According to Ram *et al.* (2021), ADMET analysis, diosmetin and hispidulin demonstrated overall drug scores of 0.83 and 0.77, respectively, with a molecular weight of 300 g/mol. Notably, luteolin exhibited the most favorable pharmacokinetic profile, with a molecular weight of 286 g/mol, cLogP of 1.99, solubility of -2.56, and a drug likeness score of 1.9, resulting in the highest overall drug score of 0.84. The analysis suggests that these compounds have a high likelihood of qualifying as drug candidates, given their optimal balance of drug likeness, lipophilicity, solubility, molecular weight, and toxicity risks. Furthermore, the study confirmed that the compounds comply with Lipinski's rule and are non-toxic.

Pinipay *et al.* (2023) evaluated the medicinal chemistry of 53 compounds identified in *Ficus religiosa* seeds through GC-MS analysis. The compounds were assessed for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties based on Lipinski's rule of 5 and their quantitative estimate of drug-likeness (QED) score. The results showed that 16 compounds exhibited low gastrointestinal absorption, while 37 compounds showed high absorption. Furthermore, distribution studies revealed volume distribution values ranging from 0.221 L/Kg to 8.866 L/Kg. The study further investigated the anti-urolithiasis potential of the compounds based on their *in vitro* antioxidant properties, *in silico* ADMET, and molecular docking studies. Roy *et al.* (2023) found that natural compounds like atisine, kutkin, and phylloquinone, derived from renal protective herbs, act as P-glycoprotein inhibitors, enhancing drug bioavailability and potentially improving renal function. These compounds' strong binding affinity could help overcome drug efflux, making them valuable for therapeutic applications.

Adams *et al.* (2023) identified four bioactive compounds—dimethylmatairesinol, flavodic acid, sakuranetin, and sesartermin—as potential antifolates against *Plasmodium falciparum* dihydrofolate reductase (PfDHFR) through molecular docking. ADMET analysis showed they comply with key

pharmacological rules, exhibiting high gastrointestinal absorption, low toxicity, and strong binding affinities. Further *in vitro* and *in vivo* testing is warranted to develop these compounds as novel malaria treatments. Kurnia *et al.* (2023) found that apigenin-7-glucuronide and dihydrokaempferol-3-glucoside showed strong binding to the main protease enzyme, with affinities of -8.77 kcal/mol and -8.96 kcal/mol, respectively. Both compounds interacted with key residues CYS145 and HIS41. ADMET analysis indicated good pharmacokinetic profiles, although aesculetin raised some concerns. The study highlights the potential of these compounds as protease inhibitors. James and Sindhu (2024) conducted *in silico* ADMET screening of 18 phytoconstituents from plants like Amrita Balli, Neem, Tulasi, and Indian Borage. The compounds showed favorable physicochemical properties and drug likeness, with most having good bioavailability, oral absorption, and safety profiles. However, Xanosporic acid had potential oral bioavailability issues due to violating two of Lipinski's rules. Overall, the study suggests these phytoconstituents have potential as therapeutic agents.

2.7 Biological activities of *Spermacoce articularis*

The plant has been traditionally used to treat various ailments, with its seeds serving as a remedy for nerve and kidney injuries. It's also believed to have anti-aging properties, enhance vitality, and have been used by tribes in Kerala's Western Ghats for centuries, showcasing their potential therapeutic value (Sekar and Francis, 1999). *Spermacoce articularis* has been traditionally used to treat various health issues, including ophthalmia, eye and gum inflammation, blindness, fever, spleen disorders, sores, and conjunctivitis (Parthasarathy *et al.*, 2010). The extracts serve as an astringent for hemorrhoids and gallstones, while the seeds are used for diarrhea and dysentery. A decoction of the herb is used to treat headaches. Additionally, traditional preparations like choornam and lehyam have been found beneficial in reducing obesity, controlling bloody diarrhea, alleviating internal heat, and treating venereal diseases.

Spermacoce hispida is a valuable plant in both allopathic and Siddha medicine, with all its parts possessing significant ethnomedicinal properties. It's

used to treat various ailments, including hypertension, and has shown hepatoprotective, anti-inflammatory, and antioxidant effects. Additionally, the seeds have demonstrated antidiabetic and anti-hyperlipidemic activity in rats, highlighting the plant's potential as a natural remedy (Meti *et al.*, 2013; Dhevi *et al.*, 2014). Arasi and Krishnaveni (2018), investigated the antipyretic activity of aqueous leaf extracts of *Annona muricata* L. and *Smallanthus articulatus* L.f. in albino rats with Brewer's yeast-induced pyrexia.

A study by Sankaran *et al.* (2007) found that various extracts of *Spermacoce articulatis* exhibited significant anti-inflammatory effects in rats with carrageenan-induced paw edema. The extracts, including petroleum ether, chloroform, ethyl acetate, water, and benzene, showed 27-74% inhibition of edema, with the water extract being the most effective. Although less potent than the control drug Celecoxib, all extracts demonstrated notable anti-inflammatory activity, potentially due to the presence of ursolic acid.