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Summary and Conclusion

Urolithiasis is a common health concern where mineral deposits are accumulated in the urinary tract, causing discomfort and potential complications. The occurrence of kidney stones varies by region and is influenced by factors like climate, age, genetics, diet, and the levels of hydration. Common medical treatment involves pain management medications, drugs to relax the urinary tract. Surgical interventions such as shockwave lithotripsy and ureteroscopy are used for the removal of kidney stones. Drinking plenty of water and making dietary changes can help prevent kidney stones. Traditional medicines offer a promising approach to prevent kidney stones, relieve pain, and support kidney health with fewer side effects than conventional medications. These remedies complement conventional kidney stone treatments, enhancing effectiveness and enabling personalized care. Empirical knowledge in herbal medicine supports urolithiasis management and promotes sustained well-being.

Spermacoce articularis L.f., used in traditional medicine for centuries, is used to treat urinary tract infections and kidney stones, due to its pharmacological properties. The current study of 'Antiurolithiatic Potential of *Spermacoce articularis* L.f. through *In Vivo* and *In Silico* Analysis' aims to demonstrate the effectiveness of *S. articularis* to prevent the formation of calcium oxalate crystals, mitigate oxidative stress, and address urinary risk factors related to kidney stones. This investigation could provide a scientific basis for using *S. articularis* in kidney stone treatment, offering new therapeutic approaches for urolithiasis.

The study comprises four phases; the first phase includes a pharmacognostic study, like organoleptic and fluorescence analysis. The results confirmed the purity and quality of the *S. articularis* powder samples. Qualitative analysis of leaf, stem, and root extracts of *S. articularis* using different solvents exhibited a significantly higher number of phytochemicals in the extracts of stem and leaf, when compared to the

root. The quantification of secondary metabolites exhibited the higher concentrations of alkaloids, terpenoids, and tannins in stem methanol and leaf ethanol extracts of *S. articularis*. Indicating its potential as a natural antioxidant, the enzymatic and non-enzymatic ability was reported to be higher in the stem methanol and leaf ethanol extract, when compared to other solvents. Radical scavenging assay revealed significant activity, with stem methanol extracts exhibiting the highest FRAP and ABTS values (13.84±0.085 µg/ml and 20.08±0.144 % µg/ml) and leaf ethanol extracts exhibiting the highest DPPH activity (23.55±1.270 % µg/ml). The results revealed that stem and leaf extracts of *S. articularis* are rich in bioactive compounds with substantial antioxidant properties.

The results of the second phase indicated that *S. articularis* stem methanol and leaf ethanol extracts demonstrated *in vitro* anti-crystallization properties by effectively inhibiting CaOx crystal formation by reducing the size and number of the crystals, with increasing concentrations (100-500 µg/ml). The growth and aggregation of CaOx crystals were significantly reduced by stem methanol extracts, when compared to leaf ethanol extracts. Thus, *in vivo* antiurolithiatic experiments were carried out using *S. articularis* stem methanol (SASM) extracts at two different dosages, 250 mg/kg and 500 mg/kg, in the Wistar albino rat model. Substantial decreases in the kidney weight and CaOx crystal precipitation were noted in the SASM-treated group than the disease control group. In particular, the SASM extract at 500mg/kg exhibited high antioxidant potential and lowered lipid peroxidation, supporting renal health. Besides, the extract decreased the levels of urinary biomarkers like urea, uric acid, and creatinine. The traces of cellular necrosis and tissue inflammation were absent in the kidney sections of the SASM-treated animal groups. The brine shrimp lethality assay demonstrated that SASM extract is relatively safe, exhibiting low toxicity observed at both lower and higher concentrations, with an LC_{50} value of 180.00 µg/ml. The results indicated that SASM, due to its minimal cytotoxic effects, may be considered for further development as a therapeutic agent.

The Phase III revealed the presence of terpenoids and phenols in the SASM extract in both TLC and HPTLC analysis. The presence of these phytoconstituents, terpenoids (16.96 µg/ml) and phenols (13.46 µg/ml) in SASM at 3mg/ml is detected by

HPTLC analysis. A total of 40 bioactive compounds were detected using GC-MS analysis, of which 25 organic compounds with various functional groups were differentiated based on the retention time and peak area. Catechol, D-mannitol, vanillin, gamma-tocopherol, eugenol, and vitamin E were identified as major phytocompounds present in the SASM extract.

The fourth phase highlights the molecular docking studies of 25 major compounds from GC-MS analysis against three key proteins, like Tamm-Horsfall protein (THP), Calcitonin, and calcium Oxidoreductase. Among them, D-mannitol demonstrated the highest binding affinity against all three proteins. The docked complex of D-mannitol and calcitonin exhibited the highest binding affinity of -8.41 kcal/M, and was further validated for its stability. MD simulations suggest that D-mannitol could potentially inhibit calcitonin hormone, which plays a crucial role in regulating kidney function. The bioactive compounds demonstrated favourable pharmacokinetic properties, closely adhering to Lipinski's rule, indicating their drug-like properties. Based on these findings, *Spermacoce articularis* holds potential natural resource for developing antiurolithiasis treatment, warranting further studies.

Recommendations for further studies

1. Further studies are needed to understand the molecular mechanisms underlying D-mannitol's efficacy against kidney stones.
2. Future studies should focus on optimizing extraction methods and developing pharmaceutical formulations of *S. articularis* to harness its therapeutic value.
3. Development of a formulation that combines *S. articularis* extracts with other herbal extracts to create a effective treatment for kidney stones can be explored.