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Original Article

Isolation and Characterization of Renal Protective Compounds from *Orthosiphon stamineus* Leaves: A Traditional Medicinal Plant

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ABSTRACT

Background: *Orthosiphon stamineus*, also known as "cat's whiskers," is traditionally used in several Asian countries, including India, China, Malaysia, and Thailand, for managing conditions like diabetes and chronic renal failure. This plant is rich in bioactive compounds and is believed to possess nephroprotective properties. Its use in traditional medicine, particularly for renal and metabolic health, necessitates empirical validation to support its therapeutic potential. **Materials and Methods:** This study aimed to evaluate the nephroprotective effects of an ethanolic extract and a purified bioactive compound derived from *Orthosiphon stamineus*. Diabetes was induced in rats through a High Fat Diet (HFD) and Streptozotocin (STZ) injection, simulating metabolic and renal complications similar to those in diabetic nephropathy. The effects of the ethanolic extract and the isolated compound were examined by measuring biomarkers related to renal function (urea and creatinine), blood glucose, and cholesterol levels **Results:** The ethanolic extract of *Orthosiphon stamineus* significantly lowered urea, creatinine, blood glucose, and cholesterol levels in diabetic rats. The isolated bioactive compound demonstrated notable hypoglycemic, hypolipidemic, and renal protective effects. These therapeutic benefits are attributed to the presence of phenolic compounds, flavonoids, and anthraquinones within the plant, which contribute to its antioxidative and anti-inflammatory properties. **Conclusion:** The findings of this research support the traditional use of *Orthosiphon stamineus* as a kidney-protecting agent. The observed nephroprotective, hypoglycemic, and hypolipidemic effects validate its historical application in managing diabetes-related complications. Further studies may explore the mechanisms behind these effects, which could support the development of alternative therapeutic options for diabetic nephropathy and related conditions.

Keywords: *Orthosiphon stamineus*, Column chromatography, Spectroscopic techniques, Rhinacanthin-B, Renal protective agent.

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INTRODUCTION

Glomerulonephritis, a serious consequence linked to diabetes and hypertension, impacts almost one-third of people with hyperglycemia and elevated blood pressure. As this condition advances to end-stage renal disease, both mortality and morbidity increases.[1] The pathological alterations induced by nephritis are primarily marked by thickening of the capillary and tubular basement membranes, expansion of the mesangial matrix, loss of podocytes, glomerulosclerosis, and tubulointerstitial fibrosis.[2] No single medication exists for treating renal failure; rather, medications can assist in managing several underlying issues and consequences associated with this condition. The persistent utilisation of medications for healthcare in India is attributed to various factors, including cultural tolerance, ease of access, affordability, and, in certain cases, the unavailability and expensive cost of allopathic medicines.[3] A substantial amount of the global population relies on traditional medicine to fulfil their health needs.[4] Modern pharmaceuticals synthesised in laboratories may exhibit various hazardous effects, but plant-derived medications tend to be less toxic. The isolation, characterisation, and standardisation of bioactive plant chemicals have garnered significant research attention, with the bioactive constituents of medicinal plants accounting for around 25% of pharmaceuticals manufactured in developed nations.[5] *Orthosiphon stamineus* (Lamiaceae) has been utilised in traditional medicine across India, Indochina, Southeast Asia, and tropical Australia, where the plant is commonly located.[6] Several papers analyse preclinical and clinical research concerning the diuretic, antidiabetic, antihypertensive, nephroprotective, hepatoprotective, gastroprotective, antiproliferative, and

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anticancer properties of *Orthosiphon stamineus*. Patients have historically utilised it for the management of diabetes and chronic renal insufficiency.[7] Moreover, advantageous therapeutic results have been documented for specific complications of diabetes, such as diabetic nephropathy. Consequently, the discovery of new antidiabetic agents derived from *Orthosiphon stamineus* is valuable. Nonetheless, examining the preventive impact of *Orthosiphon stamineus* on T2DM nephritis produced by Streptozotocin (STZ) and a High-Fat Diet (HFD) presents significant challenges. This study aimed to assess the impact of *Orthosiphon stamineus* on hyperglycemia-induced nephritis. The antinephritic activity of *Orthosiphon stamineus* and its mechanisms remain unreported. This study aimed to isolate, purify, and characterise an active chemical from *Orthosiphon stamineus* leaves to evaluate its renal protective impact, as well as its hypoglycemic and hypolipidemic effects.

MATERIALS AND METHODS

Drugs and chemicals

STZ was acquired from Sigma Aldrich in Bangalore, India. The antidiabetic medication metformin was obtained from Actavis Pharmaceuticals in Chennai, India. The extraction and phytochemical analyses of the components were performed utilising analytical grade reagents, such as petroleum ether, ethyl acetate, chloroform, ethanol, and methanol, sourced from S.D. Fine Chemicals in India.

Preparation of Plant Extracts

Leaves of *Orthosiphon stamineus* were collected from the Kalakatu forest in the Thirunelveli District of India. The taxonomic identification of the medicinal plants was executed by a botanical survey carried out by the Siddha Unit of the Government of India in Palayamkottai, with the Voucher Specimen Number XCH-40490/2023. The leaves were desiccated in the shade at ambient temperature, ground into a fine powder, and preserved in airtight containers. The sample underwent solvent extraction using solvents of increasing polarity: petroleum ether, ethyl acetate, and ethanol. Each solvent performed a continuous hot extraction process for 72 hr utilising Soxhlet equipment at a temperature of 60°C. The quantity of powdered medication utilised for extraction was 500 g. The extracts were concentrated by applying reduced pressure with a rotating evaporator until a uniform weight was attained. The samples were collected and preserved in a desiccator until utilised for further analysis and the extraction technique was performed thrice.

Experimental animals

Male Wistar rats, weighing 180-220 g, were procured from a CPCSEA-approved vendor in Pondicherry, India, in strict compliance with the parameters set forth by the CPCSEA of the Government of India. We secured previous authorisation from the Institutional Animal Ethics Committee Number 10/IAEC/MG/04/2023-I and granted the rats unrestricted access to rodent laboratory diet and water. Rats were housed in a temperature-regulated environment (20-25°C) with a 12 hr light/dark cycle throughout the experiment.

Induction of Nephritis

The fasting rats received an intravenous injection of 50 mg/kg STZ and were given a high-energy diet comprising 20% sucrose and 10% fat. STZ was solubilised in citrate buffer (0.01 mol/L, pH 4.5) and refrigerated before to application. One week post-STZ administration, animals showing creatinine levels exceeding 5 mg/dL were categorised as having nephritis and utilised in the experiments.[8]

Isolation and Characterization

The ethanol extract of *Orthosiphon stamineus* (10 g) was subjected to chromatography using a column filled with silica gel (60-120 mesh). The column had a diameter of 5 cm and a height of 20 cm, filled with silica gel to a height of roughly 10 cm. Column filling was performed under vacuum to achieve optimal density of the stationary phase. The column was eluted sequentially with different eluents of increasing polarity, specifically n-hexane (100%), a mixture of n-hexane and ethyl acetate, ethyl acetate (100%), a mixture of ethyl acetate and methanol, and methanol (100%). The resultant products were held in an Erlenmeyer flask and designated by their fractions, totalling 21 fractions. Additionally, the solvents in each fraction were evaporated utilising a rotary evaporator, followed by the execution of Thin Layer Chromatography (TLC) on each fraction. The fractions with comparable R_f values, as determined by TLC, were combined and subjected to evaporation at reduced pressure. The principal active fraction (2.5 g) was obtained by elution with a combination of ethyl acetate and methanol (70:30). The mixture was further improved by passing it through a silica gel column (100-200 mesh), yielding a yellow solid (50 mg) following elution with ethyl acetate and methanol (75:25). The melting point of this material was established as 87-89°C. The structure of the isolated compound was elucidated using FTIR, 1H-NMR, 13C-NMR, and MS spectroscopy, and it was assigned the trivial name OS-1.

Effect of OS-1 on the renal profile in nephritic rats

Acute toxicity assessments were conducted according with OECD-423 guidelines. The rats, both healthy and nephritic, were divided into four groups of 6 rats each. Group I comprised normal rats, while Group II included nephritic rats that received only 1 mL of regular water. Group III rats received a dosage of 100 mg/kg of OS-1. Group IV rats received a dosage of 200 mg/kg of OS-1. The rats in each group underwent oral therapy for a period of 21 days. Upon the conclusion of the trial session, the animals underwent an overnight fast lasting eight hr. Blood samples were subsequently obtained from the retroorbital plexus while the

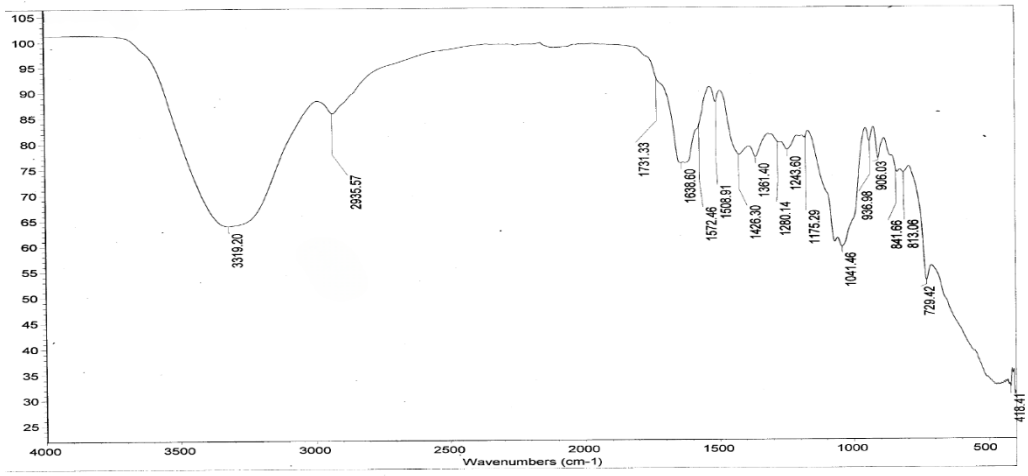

Figure 1: IR Spectrum of OS-1.

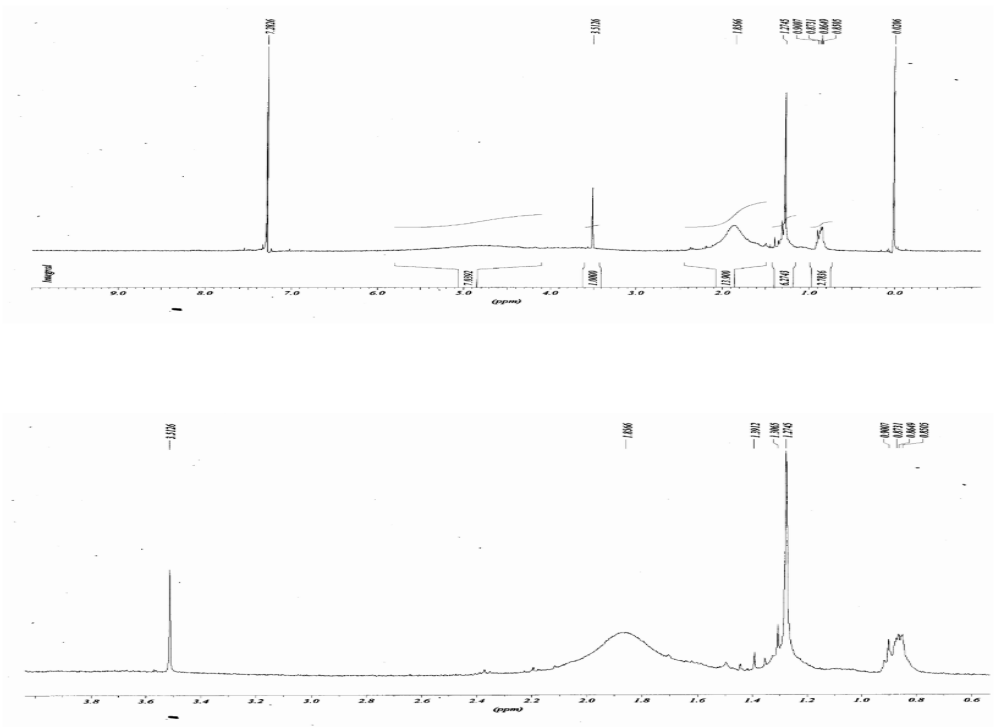

Figure 2: ^{1}H-NMR Spectrum of OS-1.

individuals were under light ether anaesthesia. The plasma was extracted, and the concentrations of urea and creatinine were analysed via Jaffe's alkaline picrate-kinetic method.[9]

Statistical analysis

The data are expressed as the mean±standard error of the mean. The statistical analysis applied one-way ANOVA. The least significant difference test was employed to compare means, with a significance threshold of $p<0.05$ to ascertain statistical significance.

RESULTS

Characterization of OS-1

Spectral analysis of the IR spectrum of the OS-1 compound

The IR spectra exhibited a broad absorption band at 3319 cm^{-1} for the OH group, distinct bands at 1731 cm^{-1} for the C=O group, and absorption bands at 1638 cm^{-1} for the C=C group, as illustrated in Figure 1.

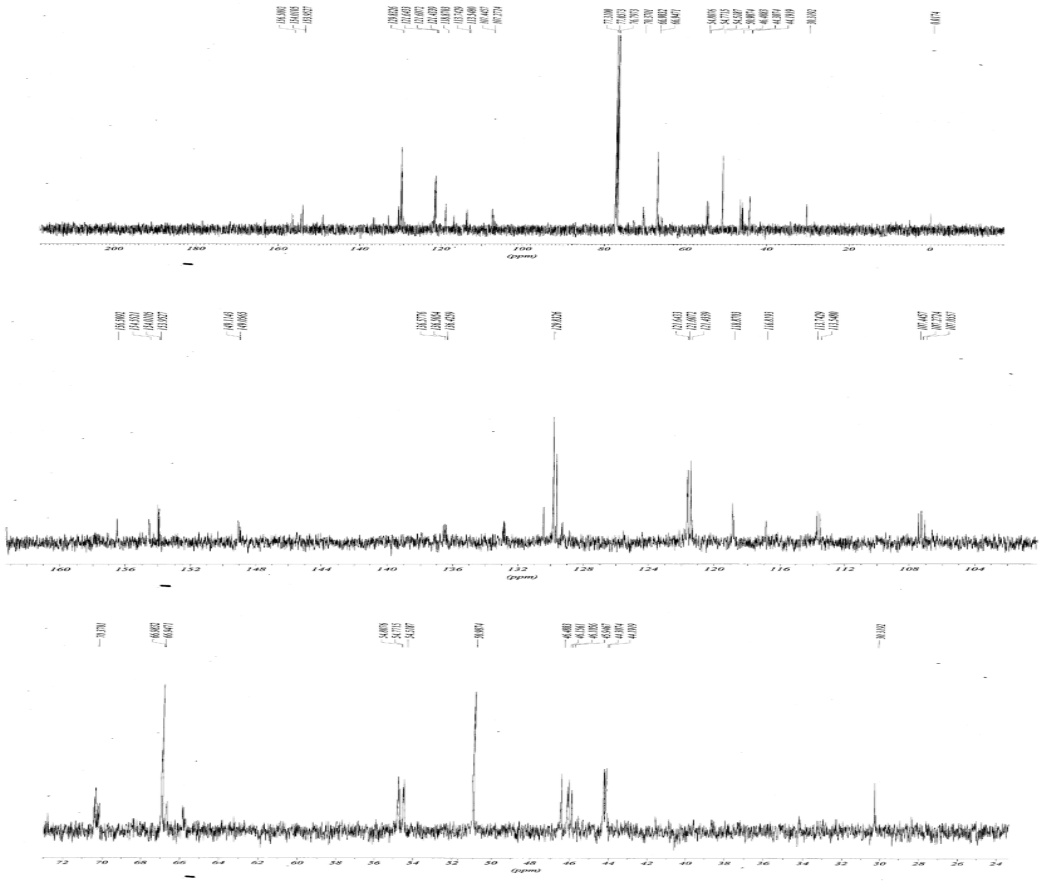

Figure 3: ^{13}C-NMR Spectrum of OS-1.

^{1}H-NMR spectrum of the OS-1 compound

In the ^{1}H-NMR spectra, signals were detected at 0.85 ppm for a methyl group, a broad signal at 1.27 ppm, and a signal at 1.85 ppm for a lengthy chain of methylene groups, with protons beneath oxygen functional groups observed at 3.50 ppm. The signal for the unsaturated protons is at 5.0 ppm, as illustrated in Figure 2.

Spectral analysis of the ^{13}C-NMR spectrum of compound OS-1

The ^{13}C-NMR spectra displayed signals at 107.27, 113.54, 118.87, 121.43, 121.60, 121.60, 121.64, 129.00, 153.95, 154.01, and 156.50 cm-1, suggesting the existence of a minimum of five C=C groups. The signals at 66.94, 66.98, and 70.37 cm-1 indicate the existence of three carbon atoms associated with oxygen functional groups. -CH-O or -CH2OH. The signals at 44.19, 44.30, 46.38, 50.98, 54.51, 54.27, and 54.80 ppm signify the existence of carbons in oxygen functional circumstances, as illustrated in Figure 3.

Spectral analysis of the OS-1 compound of mass spectrum

The mass spectrum of the molecule showed a peak at m/z 595.20 (Electrospray Ionization-Mass Spectrometry positive mode) and a peak at m/z 563.05 (Electrospray Ionization-Mass Spectrometry negative mode), indicating a molecular weight of 594.0, as illustrated in Figure 4. Based on the aforementioned analytical and spectral data, the hypothesised structures for the newly isolated compound rhinacanthin-B are illustrated in Figure 5.

Effect of OS-1 on the renal profile of nephritic rats

The acute toxicity test indicated that oral treatment of OS-1 to rats did not elicit any adverse effects. The LD_{50}, indicating the fatal dose, was established at 2000 mg/kg of body weight. The two doses of OS-1 (low dose 100 mg/kg and high dose 200 mg/kg) were allocated in accordance with the OECD-423 criteria for subsequent investigations. OS-1 markedly ($p< 0.05$) diminished urea and creatinine concentrations in diabetic rats, as detailed in Table 1. Furthermore, OS-1 induced a substantial ($p<0.05$) decrease in serum glucose and cholesterol concentrations.

DISCUSSION

Various sections of the plant material function as reservoirs for potent biochemical substances. The field of generating novel active pharmaceuticals for diabetes and hyperlipidaemia primarily concentrates on identifying herbal treatments derived from traditional folk medicine.[10] The rising importance of the probable application of secondary metabolites for human and plant disease management has led to the direct research of new sources of biologically active natural products.[11] *Orthosiphon*

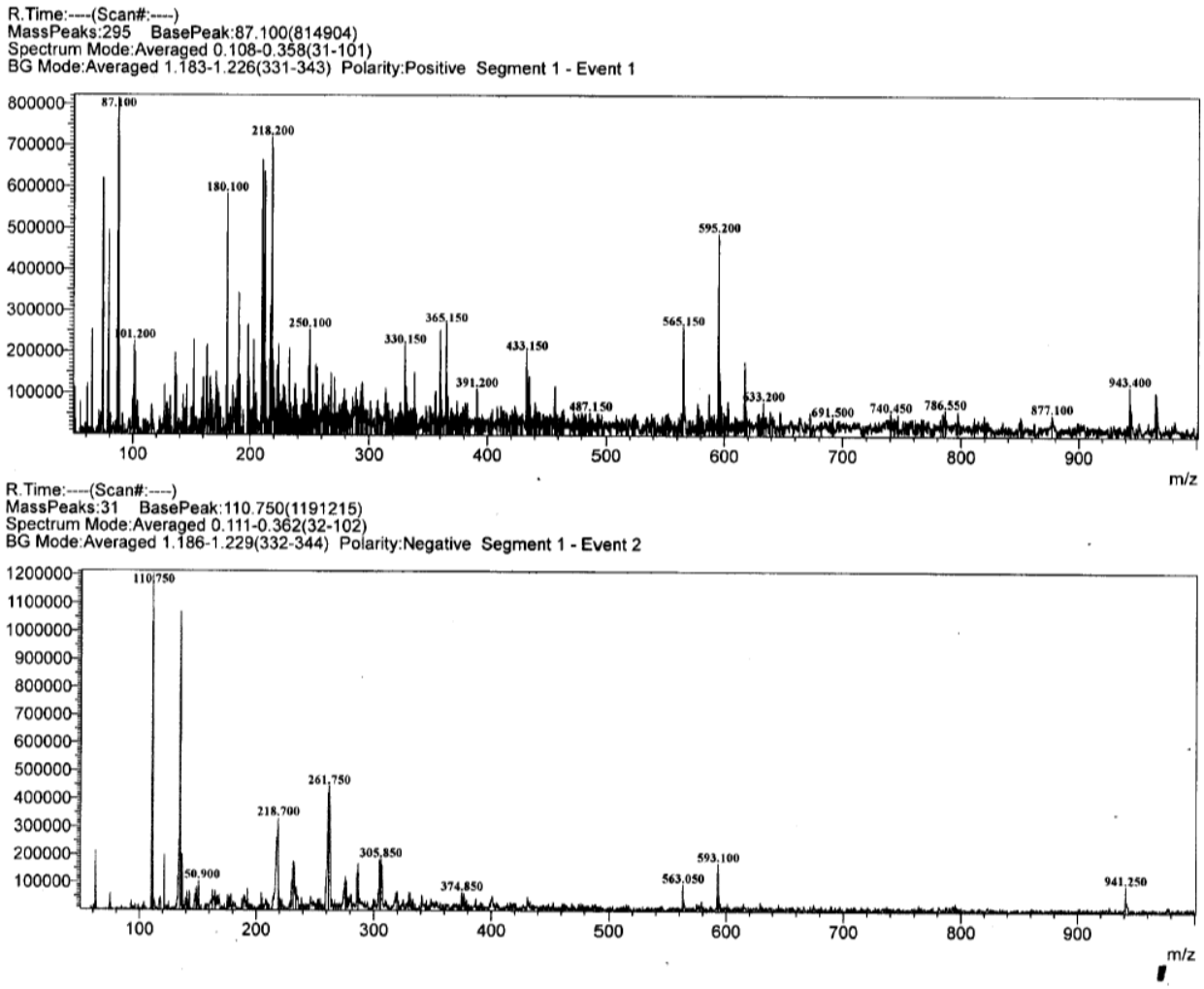

Figure 4: Mass Spectrum of OS-1.

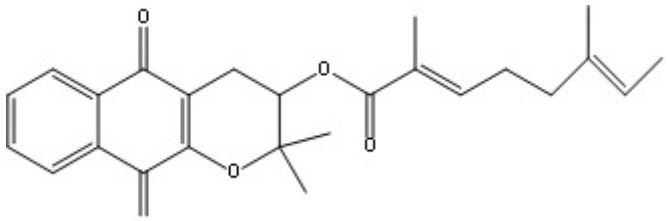

Figure 5: Structure of Rhinacanthin-B.

Table 1: Effect of OS -1 on the Serum Urea, Creatinine, Glucose and Cholesterol Level.

Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Blood Glucose (mg/dL)	Cholesterol (mg/dL)
Normal Control	09.36±0.32	0.98±0.13	85.98 ±4.10	167.6±4.3
Nephritic Control	30.13±1.51	5.98±1.50	248.86±5.56	204.5±5.4
OS-1 (100 mg/kg)	18.87±0.38	2.57±0.19	176.98±3.09	190.6±4.6
OS-1 (200 mg/kg)	11.80± 0.33*	1.32±0.14*	149.84±4.72*	181.3±2.9*

n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests. *p<0.05, compared to normal control group.

stamineus yielded over 20 flavonoids. The majority of these chemicals are flavones, particularly polymethoxy-substituted flavones.[12] Furthermore, over 60 diterpenoids have been identified thus far, exhibiting diverse skeletal structures, including isopimarane,[13] staminane,[14] secoisopimarane,[15] norstaminane, and secostaminane.[16] Furthermore, about 20 triterpenoids have been extracted from *Orthosiphon stamineus*.[17] A significant number of the chemicals examined serve as the primary pharmacodynamic constituents of *Orthosiphon stamineus* in the management of diabetes and associated consequences. In this study, we employed STZ and HFD to generate diabetic nephritis. Through direct alkylation, STZ induces cell death specifically targeting beta cells, leading to elevated blood glucose levels. This effect manifested at a dosage of 45 mg per kilogramme of body weight. STZ promotes hyperglycemia and hypoinsulinemia, subsequently disrupting many metabolic and enzymatic processes in the kidney, resulting in renal damage. Kidney-related issues in diabetic patients are associated with alterations in enzyme levels.[18] Serum urea and creatinine levels serve as key indicators for assessing renal function in individuals with nephritic disorders.[19] The present investigation shown that OS-1 (100 mg/kg) significantly decreased high urea and creatinine levels in rats with diabetic nephritis. The resultant yellow, amorphous substance performed IR, mass spectrometry, NMR, 13C-NMR, and 1H-NMR analyses. The findings validated that the OS-1 structure resembled that of Rhinacanthin-B, as illustrated in Figure 5; OS-1 exhibited antifungal, antiallergic, anti-inflammatory, anti-Alzheimer, antitumor, antiparkinsonian, hypoglycemic, and hypolipidemic properties.[20] The isolated chemical OS-1 shown substantial efficacy in reducing blood glucose and cholesterol levels. The improvements in the lipid profile noted in diabetic rats after the treatment of OS-1 may provide beneficial benefits in mitigating diabetes complications and optimising lipid metabolism in diabetic patients. The precise mechanism of action of isolated OS-1 remains unidentified and will be the subject of next investigations.

CONCLUSION

The objective of this study was to evaluate the efficacy of an ethanol extract from the leaves of *Orthosiphon stamineus* in safeguarding renal function. The findings indicated that the anthraquinones, flavonoids, and phenols in the ethanolic extract of *Orthosiphon stamineus* exert significant protective effects on the kidneys. A reduction in blood glucose, cholesterol, creatinine, and urea following administration of an isolated molecule indicates that OS-1 may possess hypoglycemic and hypolipidemic effects, in addition to exhibiting renoprotective qualities. This establishes a solid foundation for employing OS-1 as a nephroprotective agent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

OECD: Organization for Economic Cooperation Development; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **^{1}H-NMR:** Proton nuclear magnetic resonance; **^{13}C-NMR:** Carbon nuclear magnetic resonance; **MS:** Mass spectra; **FTIR:** Fourier Transform Infra-Red; **T2DM:** Type 2 diabetes mellitus; **ANOVA:** Analysis of variance.

REFERENCES

1. Marks JB, Raskin P. Nephropathy and hypertension in diabetes. Med Clin North Am. 1998;82(4):877-907. doi: 10.1016/s0025-7125(05)70028-6, PMID 9706125.
2. Weiss JW, Woodell TB. Chronic kidney disease, dialysis, and transplantation. 4th ed; 2019.
3. Xie X, Liu Y, Perkovic V, Li X, Ninomiya T, Hou W, *et al.* Renin-angiotensin system inhibitors and kidney and cardiovascular outcomes in patients with CKD: A bayesian network meta-analysis of randomized clinical trials. Am J Kidney Dis. 2016;67(5):728-41. doi: 10.1053/j.ajkd.2015.10.011, PMID 26597926.
4. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, *et al.* Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv. 2015;33(8):1582-614. doi: 10.1016/j.biotechadv .2015.08.001, PMID 26281720.
5. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta). 2022;65(3):399-411. doi: 10.1 007/s13237-022-00405-3, PMID 36276225.
6. Hui Gan S, Chai Tham T, Xiang Ng M, Suan Chua L, Aziz R, Redza Baba M, *et al.* Study on retention of metabolites composition in misai kucing (*Orthosiphon stamineus*) by heat pump assisted solar drying. J Food Process Preserv. 2017;41(6):13262. doi: 10.1 111/jfpp.13262.
7. Yuliana ND, Khatib A, Link-Struensee AM. Adenosine A(1) receptor binding activity of methoxy flavonoids from *Orthosiphon stamineus*. Planta Med. 2009;75:132-6.
8. Natarajan V, Devarasu P, Velmurugan A, Sendhamaraikannan T. Active fraction isolated from *Orthosiphon stamineus* leaf extract has an antinephritic effect on STZ-induced diabetic rats. Yugato. 2024;76:1-10.
9. Helger R, Rindfrey H, Hilgenfeldt J. Direct estimation of creatinine in serum and in urine without deproteinization using a modified Jaffé method. Z Klin Chem Klin Biochem. 1974;12(7):344-9. PMID 4428848.
10. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: from herbal medicine to modern drug discovery. Biology (Basel). 2020;9(9):252. doi: 10.3390/bi ology9090252, PMID 32872226.
11. Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ, Saeed M. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci. 2012;4(1):10-20. doi: 10.4103/0975-7406.92725, PMID 22368394.
12. Hossain MA, Ismail Z. Isolation and characterization of triterpenes from the leaves of *Orthosiphon stamineus*. Arab J Chem. 2013;6(3):295-98. doi: 10.1016/j.arabjc.201 0.10.009.
13. Adam Y, Somchit MN, Sulaiman MR, Nasaruddin AA, Zuraini A, Bustamam AA, *et al.* Diuretic properties of *Orthosiphon stamineus* Benth. J Ethnopharmacol. 2009;124(1):154-8. doi: 10.1016/j.jep.2009.04.014, PMID 19375494.
14. Mohamed EA, Yam MF, Ang LF, Mohamed AJ, Asmawi MZ. Antidiabetic properties and mechanism of action of *Orthosiphon stamineus* Benth. bioactive subfraction in streptozotocin-induced diabetic rats. J Acupunct Meridian Stud. 2013;6(1):31-40. doi: 10.1016/j.jams.2013.01.005, PMID 23433053.
15. Ameer OZ, Salman IM, Asmawi MZ, Ibraheem ZO, Yam MF. *Orthosiphon stamineus*: traditional uses, phytochemistry, pharmacology, and toxicology. J Med Food. 2012;15(8):678-90. doi: 10.1089/jmf.2011.1973, PMID 22846075.
16. Ashraf K, Sultan S, Adam A. *Orthosiphon stamineus* Benth. is an outstanding food medicine: review of phytochemical and pharmacological activities. J Pharm Bioallied Sci. 2018;10(3):109-18. doi: 10.4103/jpbs.JPBS_253_17, PMID 30237681.

17. Seyedan A, Alshawsh MA, Alshagga MA, Mohamed Z. Antiobesity and lipid-lowering effects of *Orthosiphon stamineus* in high-fat diet-induced obese mice. Planta Med. 2017;83(8):684-92. doi: 10.1055/s-0042-121754, PMID 27992939.
18. Zafar M, Naqvi SN, Ahmed M. Renal morphology and enzymes altered in rats with diabetes induced by streptozotocin. Int J Morphol. 2009;27(3):783-90.
19. Zhao LL, Makinde EA, Shah MA, Olatunji OJ, Panichayupakaranant PJ. Rhinacanthins-rich extract and rhinacanthin C ameliorate oxidative stress and inflammation in streptozotocin-nicotinamide-induced diabetic nephropathy. J Food Biochem. 2019;43(4):e12812. doi: 10.1111/jfbc.12812, PMID 31353582.
20. Suksawat T, Panichayupakaranant P. Variation of rhinacanthin content in *Rhinacanthus nasutus* and its health products. J Pharm Biomed Anal. 2023;224:115177. doi: 10.1016/j.jpba.2022.115177, PMID 36436487.

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