RASĀYAN *J. Chem.*
Vol. 19 | No. 1 | 167-173 | January-March | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

UNLOCKING THE PHARMACOLOGICAL POTENTIAL OF *Stenochlaena palustris*: BIOACTIVE COMPOUNDS AND THERAPEUTIC PERSPECTIVES

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ABSTRACT

Stenochlaena palustris, commonly known as kalakai, is a tropical fern widely distributed across Southeast Asia and traditionally valued as both a vegetable and a herbal remedy. Recent studies have highlighted its diverse pharmacological properties, supported by bioactive compounds such as flavonoids, alkaloids, and phenolics. These constituents contribute to antioxidant, anti-inflammatory, antimicrobial, antifungal, and wound-healing activities, while evidence also suggests potential benefits in anaemia, diabetes, cancer, malaria, and maternal health. Additional findings indicate protective roles against heavy metal toxicity, ultraviolet radiation, and metabolic disorders, including obesity. Despite these promising results, current research remains largely limited to in vitro and small-scale in vivo studies, with insufficient clinical validation. Standardised extraction protocols, comprehensive safety assessments, and well-designed clinical trials are essential to confirm efficacy and ensure safe application. Overall, *Stenochlaena palustris* represents a promising natural resource that integrates traditional knowledge with emerging therapeutic potential.

Keywords: *Stenochlaena palustris*; Bioactive Compounds, Pharmacological Activities, Natural Resources, Literature Review.

RASĀYAN *J. Chem.*, Vol. 19, No.1 2026

INTRODUCTION

Stenochlaena palustris, commonly referred to as kalakai, is a fern native to Central Kalimantan, particularly the Palangkaraya region. This species is highly adaptive, able to grow not only in fertile peat soils but also in less favourable environments such as quartz sand and alluvial deposits. Its distribution is not confined to Indonesia; the plant also occurs in other tropical and subtropical countries, including Australia, Malaysia, Thailand, Papua New Guinea, India, and China. As a pteridophyte, *Stenochlaena palustris* grows vigorously during the rainy season. The plant usually reaches about 50 cm in height, with leaf lengths between 7.5 and 10.2 cm. Two main leaf types are commonly observed: young red leaves and mature green leaves. Growth is more rapid during wet months, supported by higher water availability and biomass production, while it slows in drier conditions. Its relatively short harvest cycle of 4–6 days makes it well-suited to humid environments, particularly peatlands. Although widely available in Central Kalimantan, utilisation of *Stenochlaena palustris* remains limited. It is mainly consumed as food or used in traditional medicine by the Dayak community. The leaves and stems are most often prepared, usually cooked as a vegetable side dish with rice, or brewed into herbal drinks, often mixed with ginger to enhance aroma and therapeutic properties. Ethnobotanical accounts indicate its role in treating anaemia within the Dayak community, while broader phytochemical databases also record its traditional use as an aperient and for fever management.[1]

Pharmacological Potentials

Dietary Iron Source

Multiple studies have explored the potential of *Stenochlaena palustris* as a natural anaemia prevention and treatment across different populations and formulations. In a quasi-experimental study among midwifery students aged 17–19, daily consumption of 250 g of fresh *Stenochlaena palustris* for one week resulted in an average of 3.24 g/dL in haemoglobin level, comparable to the effect of ferrous fumarate tablets. Similarly, oral administration of 250 mg *Stenochlaena palustris* extract daily over seven days yielded an average improvement of 1.81 g/dL in adolescent girls with anaemia. Another quasi-experimental study involving 68 participants demonstrated that *Stenochlaena palustris* leaf tea combined with iron tablets resulted in a 19.6% increase in haemoglobin, compared to a 14.67% increase in the control group that received only iron tablets. Daily intake of 100 g *Stenochlaena palustris* leaves over a week in another study also significantly improved haemoglobin levels in anaemic pregnant women, with post-intervention values reaching 11.8–12.7 g/dL from a baseline of 9.2 g/dL ($p < 0.05$). The synergistic effect of *Stenochlaena palustris* and iron supplementation was also further observed in pregnant women, where the combination raised median haemoglobin levels from 11.20 g/dL to 13.10 g/dL over 10 days, with the most notable rise (1.7 g/dL) occurring in women in their third trimester.[2–7] Beyond beverages and raw consumption, functional food innovations have incorporated *Stenochlaena palustris* into seaweed–papaya puddings, with one formulation (150 g seaweed, 50 g *Stenochlaena palustris*, 200 g papaya) showing the most favourable nutritional profile for anaemia prevention among women of reproductive age.[8] The use of nanoparticles from *Stenochlaena palustris* leaves combined with lemon has demonstrated a significant effect in improving haematological parameters among adolescent girls with anaemia. In a randomised controlled trial involving 34 participants, the intervention group received 2 g of *Stenochlaena palustris* leaf nanoparticles and 3 g of lemon daily for 14 days, while the control group was given ferrous fumarate tablets. The intervention led to a notable increase in haemoglobin levels (mean difference: 1.29 g/dL, $p = 0.000$), erythrocyte counts (mean difference: 0.82 million/μL, $p = 0.000$), and hematocrit values (mean difference: 1.64%, $p = 0.000$).[9]

Cytotoxic Effect

The MTT assay results indicated that fractions obtained from the ethanol and ethyl acetate leaf extracts of *S.palustris* exhibited strong cytotoxic activity against HeLa cancer cells. Phytochemical analysis revealed the presence of flavonoids, alkaloids, phenols, saponins, terpenoids, and tannins, compounds known for their antioxidant and cytotoxic properties. The results suggested that *Stenochlaena palustris* fractions may serve as promising natural sources of antioxidant and anticancer agents.[10] The ethanolic leaf extracts of *Stenochlaena palustris* have shown measurable cytotoxic effects against both breast and liver cancer cell lines. In MCF-7 breast cancer cells, the extract reduced cell viability in a concentration-dependent manner, with an IC_{50} of 493.57 μg/mL, considerably weaker than the positive control, doxorubicin (1 μg/mL), which almost completely inhibited cell growth. The observed activity is likely linked to bioactive constituents such as flavonoids, alkaloids, and steroids, known to influence angiogenesis regulation and apoptosis. Similarly, testing on HepG2 liver cancer cells identified the ethyl acetate fraction of the extract as the most potent (IC_{50} = 224.12 μg/mL), outperforming the n-hexane fraction (568.38 μg/mL) and the crude extract (865.84 μg/mL), while the water fraction exhibited no activity (IC_{50} > 1000 μg/mL). This ethyl acetate fraction also demonstrated selective cytotoxicity (selectivity index = 3.14) and significantly upregulated p53 and caspase-3 protein expression, suggesting apoptosis-mediated anticancer effects. These activities are attributed to secondary metabolites such as flavonoids, alkaloids, and tannins, which have established roles in promoting programmed cell death and suppressing cancer cell proliferation.[11,12] Acylated kaempferol glycosides isolated from *Stenochlaena palustris* demonstrated enhanced biological properties, including strong free radical scavenging activity and notable cytotoxic effects against cancer cell lines. Di-acylated derivatives, particularly kaempferol 3-O-(3′-O-E-p-coumaroyl)-(6′-O-E-feruloyl)-β-D-glucopyranoside (compound A) and kaempferol 3-O-(3′,6′-di-O-E-p-coumaroyl)-β-D-glucopyranoside (compound B), exhibited significantly stronger antioxidant activity compared to their mono- or non-acylated counterparts. Compound A showed the highest radical scavenging activity with an IC50 of 127.6 μM, while compound B displayed potent cytotoxic effects, achieving up to 100% cell death

in MDA-MB-231 breast cancer cells and DU-145 prostate cancer cells at concentrations above 50 μM. Molecular docking studies further revealed that di-acylation enables compound B to bind effectively to multiple active sites on the epidermal growth factor receptor (EGFR), which likely contributes to its anticancer potential. These findings highlight the structural importance of di-acylation in enhancing both antioxidant and anticancer activities of kaempferol glycosides.[13] The ethanol extract of *Stenochlaena palustris* demonstrated significant anticancer activity against HSC-3 tongue cancer cells by decreasing cell viability and inducing cell cycle arrest. The extract demonstrated a concentration- and time-dependent reduction in viable cell numbers, with the most pronounced effect observed at 1000 μg/mL after 48 hours of exposure. Flow cytometry analysis indicated that its primary action was to induce G1-phase cell cycle arrest rather than apoptosis, as evidenced by an increased proportion of cells in the G1 phase and elevated expression of the cyclin-dependent kinase inhibitors p21 and p27. These results suggest that the ethanol extract suppresses cell cycle progression as its main antiproliferative mechanism, highlighting its potential as a natural therapeutic candidate for tongue cancer.[14]

Antidiabetic

Bioassay-guided fractionation of WF further led to the isolation of a bioactive compound with pronounced α-glucosidase inhibitory activity. The 0% methanol fraction, obtained via solid-phase extraction, showed the strongest inhibition (EC_{50} = 42.71 μg/mL), outperforming both quercetin and acarbose, and exhibited a non-competitive inhibition mechanism. While its antioxidant capacity, determined by DPPH and superoxide anion scavenging assays, was moderate compared to quercetin, NMR analysis indicated the presence of a pyridine alkaloid as the probable active constituent.[15] Optimisation studies have highlighted that post-harvest handling and extraction conditions greatly influence α-glucosidase inhibitory (AGI) activity. Methanol extraction using fine particle size (<250 μm), a 1:20 (w/v) solvent-to-sample ratio, and a 24-hour extraction period yielded the highest activity, while moisture preservation and delayed senescence maintained bioactivity. Subsequent fractionation and LC-MS/NMR analysis identified kaempferol 3-O-β-glucopyranoside (astragalin) as the primary AGI-active compound.[16] Phytochemical investigations have also led to the isolation of five flavonols they were stenopalustroside A, tiliroside, kaempferol, quercetin, and rutin, from the ethyl acetate extract. Quercetin showed the highest α-glucosidase and α-amylase inhibitory activities (IC_{50} = 43.6 μg/mL and 118.4 μg/mL, respectively), surpassing acarbose in α-glucosidase inhibition. Molecular docking confirmed strong binding affinities of quercetin and rutin to the enzyme active sites.[17] The ethanol extract of *Stenochlaena palustris* leaves has demonstrated antidiabetic potential by effectively reducing blood glucose levels in glucose-induced male white rats. In a controlled experimental study, 15 rats were divided into five groups, including a negative control (CMC suspension), a positive control (glibenclamide), and three treatment groups receiving 50, 100, and 150 mg/kg body weight of *Stenochlaena palustris* ethanol extract. The results revealed that the extract significantly lowered blood glucose levels in a dose-dependent manner, with the 150 mg/kg dose showing the most pronounced hypoglycemic effect compared to lower doses. This activity is attributed to the flavonoid content of *Stenochlaena palustris*, which enhances insulin sensitivity and protects pancreatic β-cells from oxidative damage by neutralising reactive oxygen species. These findings suggest that *Stenochlaena palustris* extract could serve as a natural therapeutic agent for managing hyperglycemia.[18] The ethanol extract of *Stenochlaena palustris* has also demonstrated significant anti-diabetic potential by reducing fasting blood glucose levels through the upregulation of glucose transporter 4 (GLUT 4) expression without altering insulin secretion. In an in vivo study using streptozotocin-nicotinamide-induced type 2 diabetic rats, doses of 400 and 800 mg/kg body weight of *Stenochlaena palustris* extract were administered for 21 days. The 400 mg/kg dose showed the most pronounced effect, lowering fasting glucose levels to near-normal values while restoring GLUT 4 expression in the soleus muscle, comparable to healthy controls and glimepiride treatment. This mechanism is attributed to the flavonoids, alkaloids, and phenolic compounds in *Stenochlaena palustris*, which enhance insulin signalling pathways (IRS-PI3K-Akt), facilitating GLUT 4 translocation and improved glucose uptake. These findings suggest that *Stenochlaena palustris* extract could serve as a promising natural therapeutic agent for managing type 2 diabetes mellitus.[19]

Antibacterial and Antifungal

The antibacterial activity of *Stenochlaena palustris* has been investigated using different plant parts, extraction methods, and target microorganisms. An ethanolic root extract containing alkaloids, saponins, and tannins showed no inhibitory effect against *Escherichia coli* at concentrations ranging from 1–15% in the Kirby–Bauer disc diffusion assay, indicating that the tested doses may have been below the minimum inhibitory concentration (MIC) and that higher concentrations or alternative extraction approaches might be necessary.

In contrast, ethanolic extracts derived from the shoots, rich in flavonoids, alkaloids, saponins, and phenolic compounds, exhibited moderate antibacterial effects against *Bacillus subtilis* (6.00 mm inhibition zone) and *E. coli* (5.28 mm), while showing weaker activity against *Staphylococcus aureus* (4.38 mm) at 50 mg/mL. These differences are likely attributable to the synergistic actions of phenolic and flavonoid constituents present in the shoot extract.[20] Leaf extracts have been more widely studied and often display stronger antibacterial effects, particularly against Gram-positive bacteria. Ethanol extracts selectively inhibited *Streptococcus sobrinus* (13.7 mm at 2%) but had no activity against *Ralstonia solanacearum*, a difference attributed to the structural complexity of Gram-negative bacterial cell walls.[21] Dose-dependent effects were observed against *S. aureus* and *Salmonella typhi*, with maximum inhibition zones of 18.53 mm and 15.20 mm, respectively, at 125,000 µg/mL.

The MIC values were 10.6% (w/v) for *S. aureus* and 9% (w/v) for *S. typhi*, while MBC values were 11% and 10.8% (w/v), with antibacterial efficacy comparable to tetracycline standards.[22] When formulated into hand sanitizer gels, 20–30% extracts inhibited *S. aureus* with zones of 9.00–9.50 mm, exceeding control formulations.[23] Comparative phytochemical analyses revealed that *Stenochlaena palustris* has a high phenolic content (938.68 µg GAE/g DW), including caffeic and ferulic acids, which correlate with notable antibacterial activity, especially against *Pseudomonas aeruginosa*.[24] Applications in dental and oral health have also been reported. Ethanolic leaf extracts inhibited *Enterococcus faecalis*, a root canal pathogen, with inhibition zones increasing from 9.47 mm at 25% concentration to 21.24 mm at 100%.[25] Activity against *Streptococcus mutans* was significant, with MIC and MBC values of 12.5% and 50%, respectively; at higher concentrations, efficacy was comparable to 0.2% chlorhexidine gluconate.[26]

The extract also showed dose-dependent inhibition of *P. aeruginosa* (9.45–29.37 mm), indicating potential for wound-healing applications.[27] Ethyl acetate leaf extracts inhibited *S. typhi* (10.18 mm) and *E. coli* (10.86 mm) at 12.5%, linked to the actions of saponins and flavonoids.[28] Methanol extracts demonstrated strong activity against *Cutibacterium acnes*, producing a 14.47 mm inhibition zone at 100% concentration, and suggesting dermatological applications.[29] Further formulation studies confirmed the potential of *Stenochlaena palustris* in personal care products. A 15% ethanolic gel preparation inhibited *E. coli* (11.68 mm) and *S. aureus* (8.57 mm) while meeting pH, homogeneity, and non-irritancy standards.[30] In contrast, methanolic and fractionated extracts (n-hexane, dichloromethane, ethyl acetate, water) exhibited stronger antioxidant and antiplasmodial activities than antibacterial effects, with only weak inhibition against *Bacillus cereus*, *Listeria monocytogenes*, and *Salmonella typhimurium*.[31]

The ethanolic leaf extract of *Stenochlaena palustris* exhibited notable antifungal activity against *Candida albicans*, with inhibition increasing in a concentration-dependent manner. In the Kirby–Bauer disc diffusion assay, inhibition zones measured 2.9 mm, 5.4 mm, 7.1 mm, 7.7 mm, and 9.3 mm for extract concentrations of 5%, 15%, 25%, 35%, and 45%, respectively. One-way ANOVA revealed that these differences were statistically significant ($p = 0.000$). The antifungal activity is linked to the extract's diverse bioactive constituents, including flavonoids, tannins, steroids, alkaloids, and saponins, which can impair fungal cell membrane integrity, denature proteins, and inhibit chitin synthesis. The 45% extract concentration showed the strongest effect, with activity comparable to the fluconazole positive control. These results suggest that *Stenochlaena palustris* has promising potential as a natural antifungal agent for controlling *C. albicans* infections.[19]

Analgesic and Anti-Inflammatory

Stenochlaena palustris is a notable candidate for anti-inflammatory development owing to its abundance of bioactive molecules, including flavonoids, phenolics, alkaloids, terpenoids, saponins, and steroids.

Compounds such as quercetin and 20-hydroxyecdysone have been reported to attenuate inflammation by blocking phospholipase activity, inhibiting prostaglandin formation, and reducing cytokine release. Its total flavonoid content, measured at 14.5 µg/mL, exceeds that found in other medicinal plants traditionally used in South Kalimantan, such as *Cratoxylum arborescens* and *Eurycoma longifolia*. The use of advanced, non-thermal extraction methods like Pulsed Electric Field (PEF) can further preserve these labile compounds, maintaining their biological activity.[32] The analgesic potential of *S.palustris* extract was assessed using the acetic acid-induced writhing assay. At oral doses of 250 mg/kg and 500 mg/kg, the extract reduced writhing responses by 26% and 60%, respectively, whereas the reference drug diclofenac sodium (25 mg/kg) achieved 78.52% inhibition. In addition to its analgesic effect, the extract also suppressed formaldehyde-induced paw edema in mice at both tested doses. Given that pain and inflammation share many mediators, it is plausible that compounds responsible for the analgesic response may also contribute to anti-inflammatory activity.

Phytoconstituents such as stenopalustrosides, stigmasterol, ethyl linoleate, kaempferol, and their derivatives present in the extract are likely to play a role in these pharmacological effects.[33] In an in vivo study using *Plasmodium berghei*-infected BALB/c mice, four days of oral *Stenochlaena palustris* extract at 10 or 100 mg/kg did not significantly raise IL-10 levels, although the highest average concentration occurred in the 100 mg/kg group on day five post-infection. This modest effect was attributed to the relatively low doses tested, despite the extract's documented anti-inflammatory constituents. Together, its phytochemical richness, compatibility with modern extraction technologies, and preliminary biological findings support further exploration of *Stenochlaena palustris* as a natural anti-inflammatory resource.[34] In another in vivo testing on Wistar rats using carrageenan-induced paw edema showed that a 500 mg/kg BW dose of the extract achieved complete inhibition of inflammation within the observation period. The anti-inflammatory activity is attributed to bioactive secondary metabolites such as flavonoids, saponins, and tannins, which act by neutralising reactive oxygen species (ROS), stabilising lysosomal membranes, and suppressing pro-inflammatory mediators like histamine, prostaglandins, and leukotrienes. Flavonoids function as antioxidants by inhibiting cyclooxygenase and lipoxygenase pathways, while tannins assist by reducing oxidative damage and modulating neutrophil activity. These findings highlight the potential of *Stenochlaena palustris* leaf extract as a natural anti-inflammatory agent with efficacy comparable to, or even exceeding, conventional NSAIDs.[35]

CONCLUSION

Stenochlaena palustris exhibits diverse pharmacological activities supported by a rich phytochemical profile, including flavonoids, alkaloids, phenolics, and related secondary metabolites. Experimental studies have demonstrated its potential as a natural dietary source of iron for anaemia prevention and treatment, with effects comparable to standard iron supplementation. In addition, extracts and isolated compounds have shown cytotoxic activity against several cancer cell lines through mechanisms involving apoptosis induction, cell cycle arrest, and regulation of angiogenesis-related pathways. Its antidiabetic potential is supported by both in vitro and in vivo studies, highlighting α-glucosidase inhibition, enhancement of insulin sensitivity, and upregulation of glucose transporter expression.

The plant also displays antimicrobial and antifungal activities against a range of pathogens, alongside promising wound-healing, analgesic, and anti-inflammatory effects mediated by antioxidant and immunomodulatory mechanisms. Despite these encouraging findings, most evidence remains confined to preclinical or small-scale studies, with limited clinical validation. To advance its application in functional foods, nutraceuticals, and pharmacotherapy, further work is required to establish standardised extraction protocols, elucidate active constituents, evaluate long-term safety, and confirm efficacy through well-designed clinical trials. Overall, *Stenochlaena palustris* represents a valuable link between traditional use and modern therapeutic innovation.

ACKNOWLEDGMENTS

The authors wish to acknowledge with deep appreciation the academic resources, infrastructure, and professional assistance provided throughout the preparation of this study.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: N. H. Akbar *et al.*, *Rasayan J. Chem.*, 19(1), 167-173(2026), https://doi.org/10.31788/RJC.2026.1919551.

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[RJC-9551/2025]