# EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF *STROBILANTHES SESSILIS* NEES: AN IN-VITRO STUDY"

Kalaiselvi.P[1], T.Venkatachalam, N. Senthil Kumar[1], Jeyaprakash.S[1], Dhayanathan.V[1], Arulprakash.A[1], Mohan.M[1]

1 Department of Pharmaceutical Analysis, JKKMMRF's Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam. Namakkal DT -638183, affiliated The Tamil Nadu Dr MGR medical University, Tamil Nadu

**Address for correspondence**
Dr. P. Kalaiselvi
1 Department of Pharmaceutical Analysis JKKMMRF's Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam. Namakkal DT -638183, Tamil Nadu.

**ABSTRACT:**

The present study was undertaken to evaluate the antioxidant and anti-inflammatory potential of the leaves of *Strobilanthes sessilis* Nees, a member of the family Acanthaceae, traditionally recognized for its medicinal value. Freshly collected leaves were shade-dried, pulverized, and subjected to methanolic extraction. Preliminary phytochemical screening of the extract was carried out using standard qualitative techniques, which revealed the presence of bioactive constituents such as flavonoids, saponins, and glycosides. These phytochemicals are well-documented for their therapeutic role in combating oxidative stress and inflammatory disorders. The antioxidant potential of the methanolic leaf extract was systematically evaluated using three in vitro assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, hydrogen peroxide radical scavenging, and nitric oxide scavenging assays. The extract demonstrated significant free radical scavenging capacity, with inhibitory concentrations ($IC_{50}$) of 16.70 µg/ml for DPPH, 41.02 µg/ml for hydrogen peroxide, and 43.17 µg/ml for nitric oxide, respectively. Anti-inflammatory activity was assessed through the egg albumin denaturation assay, where the extract exhibited notable inhibitory potential with an $IC_{50}$ value of 16.17 µg/ml. The results clearly indicate that *S. sessilis* leaves possess strong antioxidant and anti-inflammatory activities, likely attributable to the flavonoid and glycoside content. These findings support the ethnopharmacological relevance of the plant and suggest its potential application in the management of oxidative stress-related disorders and inflammatory conditions. The study further emphasizes the need for detailed isolation, characterization, and in vivo evaluation of the bioactive constituents to validate the therapeutic efficacy of *S. sessilis* and to explore its possible role as a natural source for novel antioxidant and anti-inflammatory agents.

**KEYWORDS:**

Antioxidant activity, Anti-inflammatory, *Strobilanthes sessilis Nees*, DPPH(1,1-diphenyl-2-picrylhydrazyl),hydrogen peroxide free radical scavenging assay, nitric oxide scavenging assay and egg denaturation assay.

## 1) INTRODUCTION

*Strobilanthes* is a genus of flowering plants comprising approximately 250 species in the family Acanthaceae. The name *Strobilanthes* is derived from the Latin words *"strobilos"* meaning cone and *"anthos"* meaning flower or branch. The genus is predominantly native to tropical Asia and Madagascar, with a few species extending northward into temperate regions of Asia. Many species are cultivated as ornamentals for their characteristic two-lipped, hooded flowers that occur in shades of blue, pink, white, and purple. Most species are frost-sensitive and require protection in frost-prone areas [1]. The genus was first scientifically described in the nineteenth century by Christian Gottfried Daniel Nees von Esenbeck. Species of *Strobilanthes* are remarkable for their unusual flowering behavior, which ranges from annual blooming to mass flowering cycles occurring every 16 years. On a national scale, it is often difficult to identify which species is flowering at a given time due to this variability. *Strobilanthes sessilis* Nees, although its leaves are poisonous and unsuitable for human consumption, has long been used in traditional medicine by local Adivasi communities and rural populations to treat inflammatory disorders. Scientific studies have supported these folk claims, confirming the plant's effectiveness as an anti-inflammatory agent [2,3,4]. Root extracts containing phytoconstituents such as betulin, lupeol, lupenone, and β-sitosterol have demonstrated significant anti-inflammatory properties, while volatile oils derived from the flowering tops have shown antifungal activity [5, 6]. Despite its ethnomedicinal importance, *S. sessilis* has received little scientific attention. Pharmacognostic investigations including macroscopy, anatomical studies, preliminary phytochemical screening, and physicochemical analyses have only recently begun, as the species has largely been overlooked for its phytochemistry and bioactivity. A review of the available literature indicates that limited pharmacological, phytochemical, and pharmacognostical research has been conducted on *S. sessilis* Nees.

## 2) MATERIALS AND METHODS

### Collection of Plant Material

Fresh leaves of *Strobilanthes sessilis* Nees were collected in August 2024 from Parunthumpara, Idukki district, Kerala, India. The leaves were carefully selected based on plant age and overall health to ensure optimal quality. The collected material was shade-dried under ambient conditions and subsequently powdered for further experimental use.

### Taxonomical identification:

The collected plant material was taxonomically identified and authenticated as *Strobilanthes sessilis* Nees (family: Acanthaceae) by Siddha Medicinal Plants Garden, Central Council for Research in Siddha, Ministry of AYUSH, Government of India, Mettur Dam, Tamil Nadu The authentication confirmed the botanical identity of the species selected for the present investigation. A voucher specimen of the plant was prepared and retained in the departmental herbarium for future reference.

## EXTRACTION[23]

Leaf parts of *Strobilanthes sessilis* were collected, thoroughly cleaned, and air-dried in the shade to prevent degradation of heat-sensitive phytoconstituents. The dried leaves were then pulverized into a coarse powder using a mechanical grinder. Each powdered sample was subjected separately to cold maceration in 80% ethanol for 7 days at room temperature (28–30 °C), with intermittent agitation to facilitate efficient extraction of the bioactive compounds. After the extraction period, the mixtures were filtered through Whatman No. 1 filter paper to remove plant residues. The filtrates obtained were concentrated under reduced pressure using a rotary evaporator until complete removal of the solvent, yielding a dry crude extract. The dried extracts were stored in airtight containers under refrigeration at 4 °C in the dark to preserve their stability and prevent phytochemical degradation.

## QUALITATIVE PHYTOCHEMICAL ANALYSIS[24]

Preliminary phytochemical screening of the methanolic leaf extract of *Strobilanthes sessilis* Nees was performed using standard qualitative methods to detect the presence of major classes of secondary metabolites. The extract was subjected to specific chemical tests to identify phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and steroids. The appearance of characteristic color changes or precipitate formation in each test was used as an indicator of the respective compounds. These qualitative assays provided a preliminary profile of the bioactive constituents present in the plant extract.

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control X } 100}$$

## PHYTOCHEMICAL SCREENING OF EXTRACTS: [7, 10, 12, 14].

Preliminary phytochemical analysis of the powdered leaves of *Strobilanthes sessilis* Nees revealed the presence of various bioactive compounds. Comparative evaluation of the powdered sample and its extracts indicated that tannins, flavonoids, alkaloids, and proteins were predominantly present. The abundance of these phytoconstituents is of particular interest, as they are well-recognized for their antioxidant and anti-inflammatory properties, thereby supporting the therapeutic potential of *S. sessilis* as a source of bioactive principles. **Table no: 1** shows the preliminary results of a phytochemical investigation.

| S.No | TEST | PETETHER EXTRACT | ETHYL ACETATE EXTRACT | ALCOHOLIC EXTRACT |
|---|---|---|---|---|
| 1. | Carbohydrate | - | - | - |
| 2. | Test for Gums | + | - | - |
| 3. | Test for Protein | - | - | - |
| 4. | Test for Amino Acid | - | - | - |
| 5. | Test for Steroid | + | - | - |
| 6. | Test for Glycoside | - | - | + |
| 7. | Test for Alkaloids | - | + | - |
| 8. | Test for Flavonoids | - | - | + |
| 9. | Test for Tannis | - | + | - |
| 10. | Test for Saponins | - | - | - |

**Table no: 1** The Preliminary results of a photochemical investigation.

## IN-VITRO STUDIES

### 1. DPPH RADICAL SCAVENGING ASSAY [8,9]

The free radical scavenging activity of different extracts of leaves *Strobilanthes sessilis* Nees. was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, a widely accepted method for determining antioxidant potential [14]. In this assay, a 0.1 mM solution of DPPH in ethanol was freshly prepared and served as the radical source. To assess the activity, 1 ml of this DPPH solution was mixed with 3 ml of plant extracts prepared at varying concentrations were 5, 10, 15, 20, 25, and 30 µg/ml in ethanol. Only extracts that were soluble in ethanol were selected, and their concentrations were adjusted through serial dilution. The reaction mixtures were shaken vigorously to ensure proper mixing and allowed to stand at room temperature for 30 minutes to facilitate interaction between the DPPH radicals and the antioxidant compounds present in the extracts. Following incubation, the decrease in absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Shimadzu) [15]. Ascorbic acid, a well-known natural antioxidant, was used as the reference standard, and all experiments were performed in triplicate to ensure reproducibility and reliability of results [16]. The antioxidant activity was expressed in terms of $IC_{50}$, defined as the concentration of extract required to scavenge 50% of DPPH radicals. The $IC_{50}$ value was calculated using a log-dose inhibition curve, providing a quantitative measure of the potency of the extracts. A lower $IC_{50}$ value indicated stronger radical scavenging activity.

The reduction in absorbance of the reaction mixture correlated with higher free radical scavenging potential of the tested extracts [17]. To quantify the scavenging effect, the following equation was applied:

**DPPH scavenging effect (%) = $(A_0 – A_1) / A_0 \times 100$**

Where $A_0$ is the absorbance of the control reaction (without extract) and $A_1$ is the absorbance in the presence of the test or standard compound [18]. This method provided a clear assessment of the antioxidant capacity of *Strobilanthes sessilis* Nees extracts, highlighting their potential as natural sources of free radical inhibitors and supporting their possible role in mitigating oxidative stress–related disorders. **The DPPH radical scavenging assay result is shown in Table no: 2 and Figure no: 1.**

| S.No | Concentration (µg/ml) | Percentage inhibition (%) of sample | Percentage inhibition (%) of standard (Ascorbic acid ) |
|---|---|---|---|
| **1.** | 10 | 33.19 | 63.58 |
| **2.** | 20 | 57.21 | 80.12 |
| **3.** | 30 | 78.14 | 93.38 |
| **4.** | 40 | 89.02 | 95.41 |
| **5.** | 50 | 92.62 | 98.72 |
| | | **Sample IC 50** | 16.7020 |
| | | **Standard IC 50** | 12.3688 |

**Table no :2 1,1-diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity of Extract**

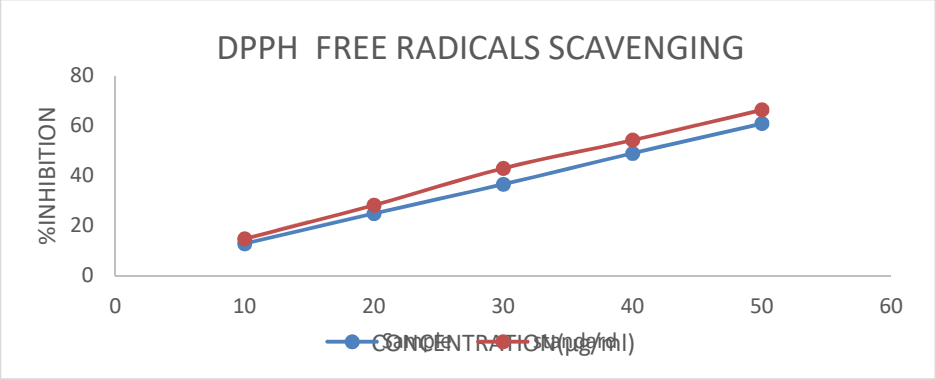


**Figure no: 1. The DPPH radical scavenging assay**

## 2. Hydroxyl Radical Scavenging Assay[11, 13]

The hydroxyl radical scavenging activity of the extract was evaluated using the Fenton reaction, following the method of with slight modifications. Hydroxyl radicals were generated in a reaction system containing ferrous sulfate ($FeSO_4$) and hydrogen peroxide ($H_2O_2$), and subsequently detected by their ability to hydroxylate sodium salicylate. The resulting hydroxylated salicylate complex was measured spectrophotometrically at 562 nm. A reaction mixture with a final volume of 3.0 mL was prepared, consisting of 1.0 mL of 1.5 mM $FeSO_4$ solution, add 0.7 mL of 6 mM $H_2O_2$, add 0.3 mL of 20 mM sodium salicylate, and 1.0 mL of extract at varying concentrations (10, 20, 30, 40, and 50 µg/mL). The mixture was incubated at 37 °C for 60 minutes, after which the absorbance of the hydroxylated salicylate complex was

recorded at 562 nm using a UV–Vis spectrophotometer. The hydroxyl radical scavenging activity (%) was calculated according to the following formula:

**% SCAVENGING ACTIVITY = [1-($A_1$-$A_2$)/$A_0$] x 100**

Where $A_0$ is the absorbance of the control, $A_1$ is absorbance in the presence of the extract and $A_2$ is the absorbance in the absence of sodium salicylate. This ensured accurate estimation of radical scavenging ability by eliminating interference from the natural color of the extract. **The hydroxyl radical scavenging assay result is shown in Table no: 3 and Fig no: 2.**

| S.No | Concentration (µg/ml) | Percentage inhibition (%) of sample | Percentage inhibition (%) of standard (Ascorbic acid ) |
|---|---|---|---|
| **1.** | 10 | 12.78 | 14.75 |
| **2.** | 20 | 24.83 | 28.20 |
| **3.** | 30 | 36.57 | 42.95 |
| **4.** | 40 | 48.95 | 54.20 |
| **5.** | 50 | 60.81 | 66.30 |
| | | **Sample IC 50** | 41.0208 |
| | | **Standard IC 50** | 36.7544 |

**Table no: 3 Hydroxyl Radical Scavenging Activity.**

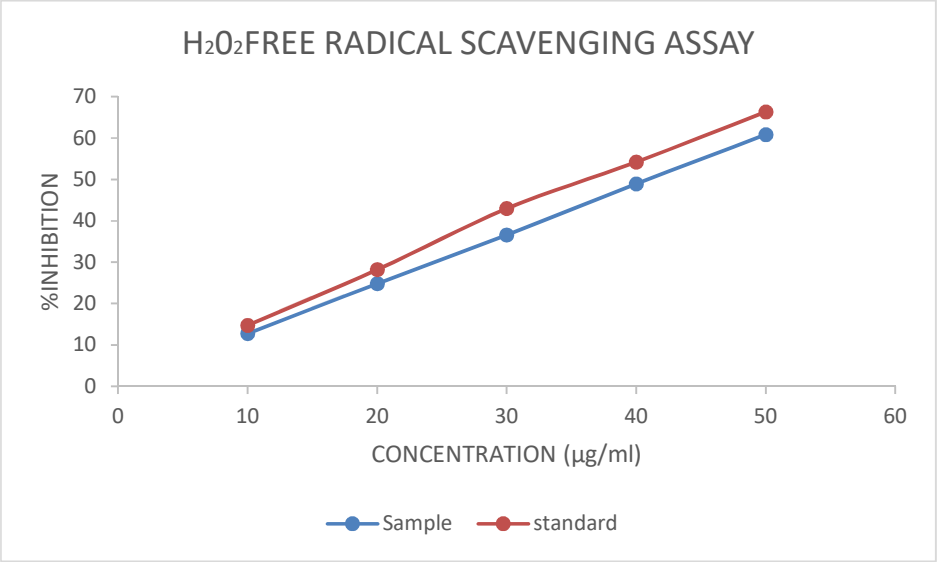


**Figure no: 2.Hydroxyl Radical Scavenging Activity**

### 3. Nitric Oxide Radical Scavenging Assay [14, 24]

The nitric oxide (NO) scavenging activity of the extract was determined according to the sodium nitroprusside method with slight modifications. The reaction mixture (6.0 mL) contained 4.0 mL of sodium nitroprusside (10 mM), 1.0 mL of phosphate-buffered saline (PBS, pH 7.4), and 1.0 mL of the extract dissolved in DMSO at different concentrations, or standard ascorbic acid. The mixtures were incubated at 25 °C for 150 minutes**.** After incubation, 0.5 mL of the reaction mixture was withdrawn and treated with 1.0 mL of sulphanillic acid reagent, mixed thoroughly, and allowed to stand for 5 minutes to complete diazotization. Subsequently, 1.0 mL of N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) was added, and the mixture was incubated for 30 minutes under diffused light.

A pink chromophore was developed due to the diazotization of nitrite ions with sulphanillic acid and subsequent coupling with NEDD. The absorbance of the resulting solutions was measured at **540 nm** against the corresponding blank. Ascorbic acid was used as the reference standard.

**% SCAVENGING ACTIVITY = $[1-(A_0-A_1)/A_0]$ X100**

Where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the sample. The result is shown the nitric oxide radical scavenging assay shown in Table no: 4 and Fig no: 3.

| S.No | Concentration (µg/ml) | Percentage inhibition (%) of sample | Percentage inhibition (%) of standard (Ascorbic acid ) |
|---|---|---|---|
| **1.** | 10 | 11.78 | 15.83 |
| **2.** | 20 | 28.83 | 31.94 |
| **3.** | 30 | 35.57 | 43.50 |
| **4.** | 40 | 45.95 | 57.83 |
| **5.** | 50 | 57.81 | 69.10 |
| | | **Sample IC 50** | 43.1779 |
| | | **Standard IC 50** | 34.8104 |

**Table no: 4 The Nitric Oxide Radical Scavenging Assay**

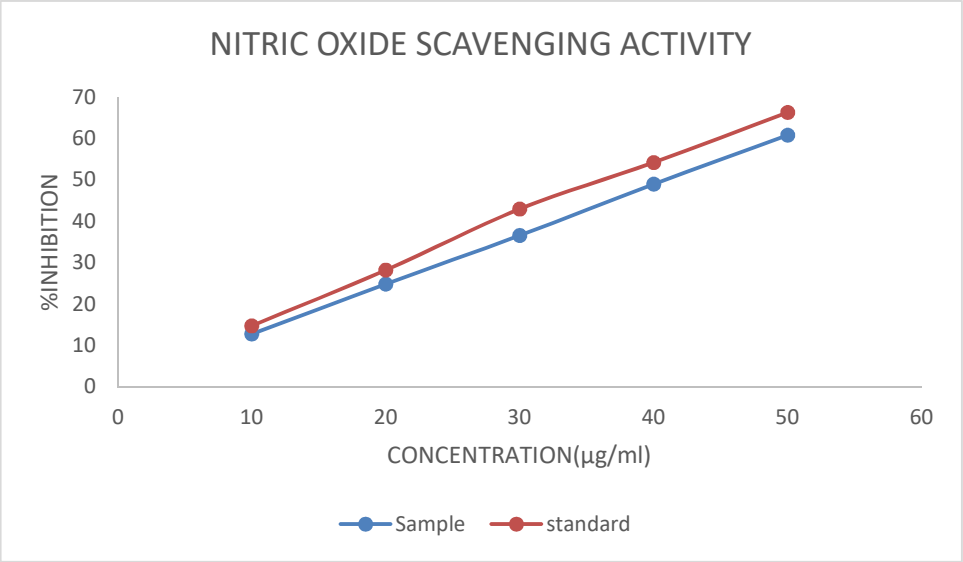


**Figure no: 3. The Nitric Oxide Radical Scavenging Assay**

## 4) Anti-inflammatory study [20, 21]

### Egg Albumin Denaturation Assay

A 1% egg albumin solution was prepared either from fresh hen's eggs or commercially available albumin powder. For the preparation from fresh eggs, the transparent portion (albumin) was carefully separated, and 1 mL was diluted with 100 mL of distilled water under continuous stirring. Cold distilled water was preferred to prevent coagulation of albumin.

The in vitro anti-inflammatory potential of the extract was evaluated by its ability to inhibit heat-induced denaturation of egg albumin. The reaction mixture (5.0 mL) contained 0.2 mL of egg albumin solution (1–2%), 2.0 mL of the extract or standard drug (Diclofenac sodium) at different concentrations, and 2.8 mL of phosphate-buffered saline (PBS, pH 7.4). For the control, 0.2 mL of egg albumin solution was mixed with 2.8 mL PBS and 2.0 mL triple-distilled water. The mixtures were incubated at **37 ± 2 °C for 30 minutes**, followed by heating in a water bath at **70 ± 2 °C for 15 minutes**. After cooling, the absorbance was measured at **280 nm** using a UV–Visible spectrophotometer, with triple-distilled water as blank.

**Inhibition of denaturation (%) =A control−A sample/ A control X100**

Where, A control is the absorbance of the control and A ample is the absorbance in the presence of the extract or standard. The egg albumin denaturation assay result is shown in **Table no: 5 and Figure no:4.**

| S.No | Concentration (µg/ml) | Percentage inhibition (%) of sample | Percentage inhibition (%) of standard (Ascorbic acid ) |
|---|---|---|---|
| **1.** | 10 | 48.54 | 66.97 |
| **2.** | 20 | 52.97 | 75.56 |
| **3.** | 30 | 55.41 | 91.84 |
| **4.** | 40 | 62.02 | 93.64 |
| **5.** | 50 | 77.96 | 98.04 |
| | | **Sample IC 50** | 16.1761 |
| | | **Standard IC 50** | 13.8918 |

**Table no: 5 The egg albumin denaturation assay**

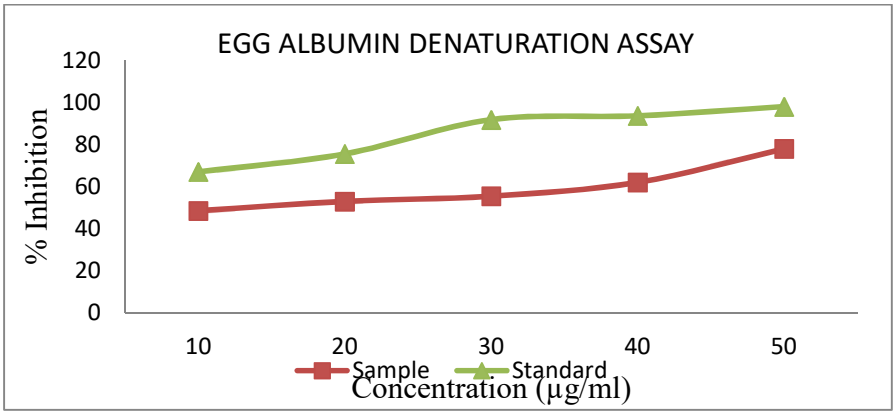


**Figure no: 4. The egg albumin denaturation assay**

## RESULTS

Medicinal plants exert diverse pharmacological effects primarily through their secondary metabolites, such as flavonoids, glycosides, saponins, and steroids. These bioactive compounds, studied under phytochemistry, are widely recognized for their beneficial roles in human health. Hence, phytochemical identification is a critical step in evaluating the therapeutic potential of medicinal plants. Various extraction techniques are employed for isolating phytochemicals, including maceration, Soxhlet extraction, supercritical fluid extraction, and microwave-assisted extraction. In the present study, Soxhlet extraction with methanol was selected, as methanol is highly efficient in extracting a broad spectrum of phytochemicals, particularly polyphenolic compounds. Qualitative phytochemical screening of *Strobilanthes sessilis* Nees extracts was performed using standard procedures. The analysis confirmed the presence of key bioactive constituents—flavonoids, glycosides, saponins, and steroids—which are likely to contribute to the plant's pharmacological and therapeutic potential.

## DISCUSSION

The plant *Strobilanthes sessilis* Nees, belonging to the family Acanthaceae, was investigated to evaluate its antioxidant potential. Phytochemical screening confirmed the presence of glycosides, flavonoids, saponins, and steroids. The methanolic extract of *S. sessilis* exhibited a dose-dependent increase in antioxidant activity and anti-inflammatory effects, which were comparable to those of standard reference compounds. These findings suggest that the bioactive constituents present in the extract may contribute significantly to its pharmacological properties, indicating its potential use as a natural antioxidant and anti-inflammatory agent. Further studies, including chemical characterization, isolation of active principles, and identification of specific antioxidant and anti-inflammatory markers, are warranted to establish the therapeutic value of this species.

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