



Plant Archives

Journal homepage: <http://www.plantarchives.org>DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.2.252>

ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM THE BARK OF THE ENDEMIC TREE *SYZYGium PALGHATENSE* GAMBLE FROM PALAKKAD, KERALA, INDIA

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(Date of Receiving-14-08-2025; Date of Acceptance-21-10-2025)

ABSTRACT

Syzygium is a genus in the Myrtaceae family, which is complex and highly researched. This genus contains essential oils, flavonols, flavonoids, phenolic acids and ellagitannins. The current work aimed to extract, purify, and describe the bioactive compound identified in the bark of *Syzygium palghatense*, an indigenous plant found in the Western Ghats. The methanolic bark extract of *S. palghatense* yielded 4.163 g of crude material, from which 31 fractions were obtained through column chromatography. Fractions 25, 26, and 27, showing identical R_f values (0.95), were pooled and further purified by PTLC, yielding 7 mg of a pure compound (S1). Spectral characterization confirmed its identification. The UV spectrum showed maximum absorbance at 294 nm, FT-IR analysis displayed prominent absorption bands including C=C stretching at 1637 cm⁻¹ and numerous C-H vibrations. The ¹H NMR spectrum exhibited proton signals between δ 2.72–7.73 ppm, while the ¹³C NMR spectrum showed carbon resonances from δ 44.4–191.75 ppm. Mass spectrometry revealed a molecular ion peak at m/z 223.10, consistent with the molecular formula C₁₅H₁₀O₂. GC-MS data and comparison with reported literature confirmed the isolated compound as 2-phenyl-4H-chromen-4-one. Present study provides the first report on isolation of 2-Phenyl-4H-chromen-4-one from the methanolic extract of bark of *S. palghatense*. This study concluded that *S. palghatense* can be a potential source of bioactive compounds. This highlights the importance for future studies of this endemic species.

Key words : Bioactive Compounds, Myrtaceae, *Syzygium palghatense*, Western Ghats.

Introduction

Bioactive compounds are secondary plant metabolites that are known to have pharmacological effects on humans and animals (Bernhoft, 2010). These are not present separately in plants, but rather in combination with other compounds. These are present in many different parts of plants, including leaves, stems, flowers and fruits. Therefore, they can be utilized to determine the existence of these chemicals (Azmir *et al.*, 2013). Phytochemicals in plants can be examined through various methods. These include extraction, separation, purification, identification, structure clarification, physical and chemical property assessment, biosynthesis and quantification (Anulika *et al.*, 2016; Aung *et al.*, 2020).

Syzygium species commonly found in tropical or subtropical habitats, such as limestone forests, swamps, lowland to montane rainforests, savannahs, and ultramafic forests (Soh, 2017). *Syzygium* belongs to family Myrtaceae. This family is known to be the eighth biggest family of angiosperms, which included 150 genera with 3800-5800 species. Many members of this family are valued for their medicinal importance (Ranghoo-Sanmukhiya *et al.*, 2019). *Syzygium palghatense* is a species of this family known only from a single locality (Sreekumar *et al.*, 2020). According to the most recent assessment by the IUCN Red List of Threatened Species, *S. palghatense* has been classified as Critically Endangered (Amitha Bachan and Devika, 2023).

Traditional medicine relies mainly on plants. It serves as the primary source of healthcare for about 80% of the population (Anibogwu *et al.*, 2021; Bora and Sharma, 2011). Natural products that are derived from natural resources like microorganisms, plants, and animals produces a wide range of useful compounds. Studies on phytochemical plays an important role for discovering new substances with promising therapeutic potential (Brusotti *et al.*, 2014). The isolation and characterization of bioactive compounds that are derived from natural sources have recently gained significant attention, especially in food and medicine (El-Maati *et al.*, 2016).

The present study aimed to isolate, purify and characterize the bioactive compounds found in the bark of *Syzygium palghatense*. It is an endemic *Syzygium* species which is native to Western Ghats. Species of the genus *Syzygium* is well known for its medicinal properties. But information on *Syzygium palghatense* is limited. This research focuses on identifying bioactive constituents through a detailed phytochemical investigation of the bark, employing contemporary extraction, chromatographic, and spectroscopic methods.

Materials and Methods

A mechanical pulverizer was used to coarsely grind the *S. palghatense* bark after it had been well cleaned with running tap water and dried out in the shade. A Soxhlet extraction was performed on 20 g of the powdered material using methanol at room temperature in a 1:4 (w/v) proportion and the extraction was permitted to proceed for 24 hours (Sukhdev *et al.*, 2008).

Separation of Bioactive compounds

Chromatography was used for compound separation. It helps in separation according to the partition between a stationary and mobile phase. This technique was used to separate substances from fractionated extracts.

On TLC plates, a drug solution containing 1 mg/mL was placed as spots and allowed to air dry. Different solvent ratios were then used for making the plates. Spots were seen in an iodine chamber, under UV light, and under visible light. The ratio between the compound's and solvent's travel distances was used to compute the retention factor (Rf). The chemicals that had been isolated were scraped from the plate for further examination (Deinstrop, 2000).

A slurry was made by dissolving the methanolic extract in methanol and combining it with silica gel (100-200 mesh, 60 Å). The mixture was then let to dry overnight at 50°C. The slurry was placed within a glass column that measured 2.5 cm in diameter and 75 cm in length. It

was packed with activated silica gel and allowed to dry for three hours at 80°C. The column was first cleaned using 100 mL of hexane. After loading the extract-silica slurry, elution was carried out, progressively increasing polarity to 100% methanol from 100% hexane. TLC was used to evaluate the 100 mL fractions that were collected. Similar Rf fractions were mixed together, concentrated at 50°C under decreased pressure, and then further purified using preparative thin layer chromatography (PTLC). In order to determine the Rf values for isolation, the separated compounds were analyzed under UV light (254 and 365 nm). Characterization of the isolated compounds was done using spectrum techniques such ESI-MS, UV, IR and NMR (JóŹwiak and Hajnos, 2007).

Glass plates (50 mm x 100 mm) precoated with silica gel layers of 0.25, 0.5, or 2 mm thickness were used for PTLC. Capillary tubes were used to apply the samples. Reactive double bonds and sensitive groups like aldehydes may oxidize during separation because silica gel has a slightly acidic pH. Due to its expense, this approach works best for small-scale separations. These patches were examined under a UV lamp, scraped off, and gathered for additional examination when they had developed in an appropriate solvent (JóŹwiak and Hajnos, 2007).

Characterization of Bioactive compounds

The ultraviolet (UV) spectrum of the isolated compound was recorded using a SHIMADZU (UV-160A) spectrophotometer.

FT-IR Spectrum was obtained using a Perkin Elmer spectrophotometer and the KBr disc technique was used to analyse the functional groups based on vibrational frequencies.

Proton Nuclear Magnetic Resonance (¹H NMR) spectroscopy was performed in CDCl₃ solvent on a 500 MHz Bruker instrument to study the proton environments present in the compound. Carbon-13 Nuclear Magnetic Resonance (¹³C NMR) spectroscopy was carried out using a Bruker 400 MHz instrument, with the carbon spectra recorded at 100.4 MHz in CDCl₃ solvent.

Mass spectrometric analysis was performed using electron spray ionization (ESI) to determine the molecular weight and confirm the molecular formula of the compound.

Results

The yield of *S. palghatense* bark methanol extract was 4.163 g. Airtight containers were used to store the extracts for later use. Several phytochemicals have been found from the methanolic bark extract by TLC analysis. Fractionation using column chromatography produced 31

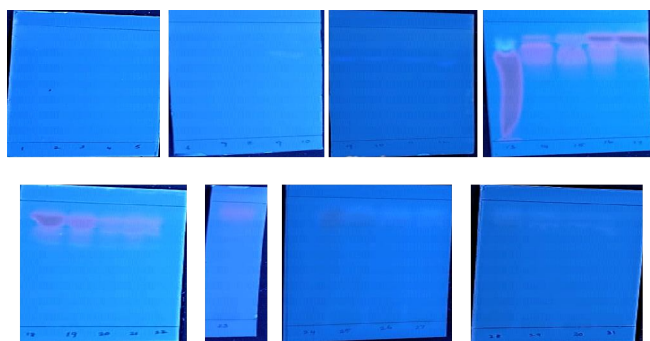


Fig. 1 : Fractions collected viewed under UV light at 365 nm by TLC method.

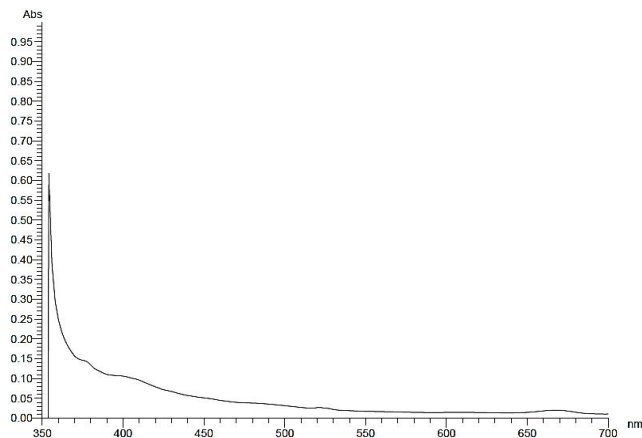


Fig. 2 : UV characterization of S1 isolated from *S. palghatense*.

fractions; fractions 25, 26 and 27 had the same R_f values (0.95) and were combined for PTLC purification. In a solvent solution of ethyl acetate and methanol (3:7), the pure chemical S1 produced 7 mg and fluoresced blue when exposed to UV light at 365 nm (Fig. 1).

Characterization of the isolated compounds was done using spectrum techniques. The molecule had the highest absorbance at 294 nm (UV spectrum) (Fig. 2). The FT-IR spectrum displayed characteristic absorption bands at 1637 cm^{-1} (C=C stretching), along with other prominent peaks at 1566, 1493, 1462, 1371, 1224, 1127, 1027, 904, 766 and 685 cm^{-1} , which correspond to various C–H stretches (Fig. 3).

The ^1H NMR spectrum showed multiple signals ranging between δ 2.72 to 7.73 ppm (Fig. 4) and the ^{13}C NMR spectrum revealed peaks in the range of δ 44.4 to 191.75 ppm (Fig. 5). The mass spectrum displayed a molecular ion peak at m/z 223.10, which corresponded well with the calculated molecular formula of $\text{C}_{15}\text{H}_{10}\text{O}_2$. Fig. 6 illustrates the GCMS characterization of the compound S1 isolated from *S. palghatense*. Spectral analysis and comparison with published literature revealed that molecule S1 was 2-Phenyl-4H-chromen-4-one (Figs. 7-8).

Discussion

Natural antioxidants such flavonoids, curcuminoids, tannins, terpenoids, and lignans are abundant in medicinal plants, herbs, spices, and oilseeds (El-Maati *et al.*, 2016). Some *Syzygium* species, particularly *S. cuminni* and *S. aromaticum*, have drawn a lot of interest due to their phytochemical and health-promoting components. The chemical and biological characteristics of other *Syzygium* species, however, are yet unknown and are not being fully exploited (Rocchetti *et al.*, 2019).

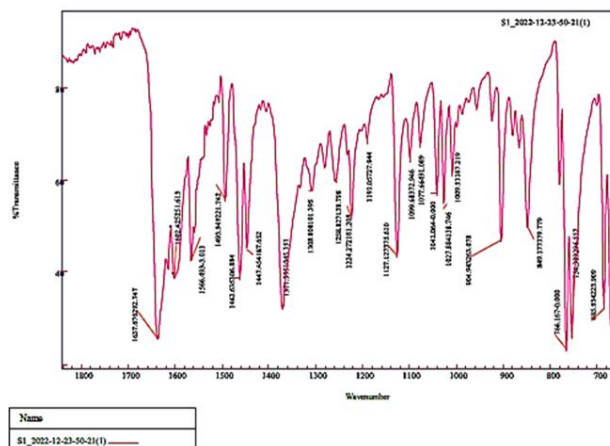


Fig. 3 : FT-IR spectrum of compound S1 isolated from *S. palghatense*.

According to studies, different extracts (methanol, ethanol and aqueous) from various parts (leaves, barks, stem barks, seeds, fruits and flower buds) of *Syzygium* sp. exhibit antioxidant, toxicity, antidiabetic, anticancer, antibacterial, anti-inflammatory, and anthelmintic properties (Aung *et al.*, 2020). It was earlier identified that the methanol extracts of *S. palghatense* leaves comprised carbohydrates, alkaloids, tannins, phenolics, glycosides, reducing sugars, saponins, and essential oils, these secondary metabolites may be the source of its biological characteristics (Snehalatha and Rasmi, 2022).

A number of secondary metabolites were identified by the phytochemical analysis of the plant components. Numerous studies have previously identified different flavanones from *Syzygium* species. MeOH extract of *S. grande* leaves yielded one flavonoid glycoside, myricetin 42 -methyl ether 3-O- β -d-xylopyranoside, along with thirteen other recognized substances (Samy *et al.*, 2014). Memon *et al.* (2015) isolated two flavanones were namely (2S)-7-Hydroxy-5-methoxy-6,8-dimethyl flavanone and (S)-5,7-dihydroxy-6,8-dimethyl-flavanone from *S. campanulatum* leaves. The methanol extract of *S. aqueum* yielded one flavonoid compound called 5,7-dihydroxy 6,8-dimethyl flavanone

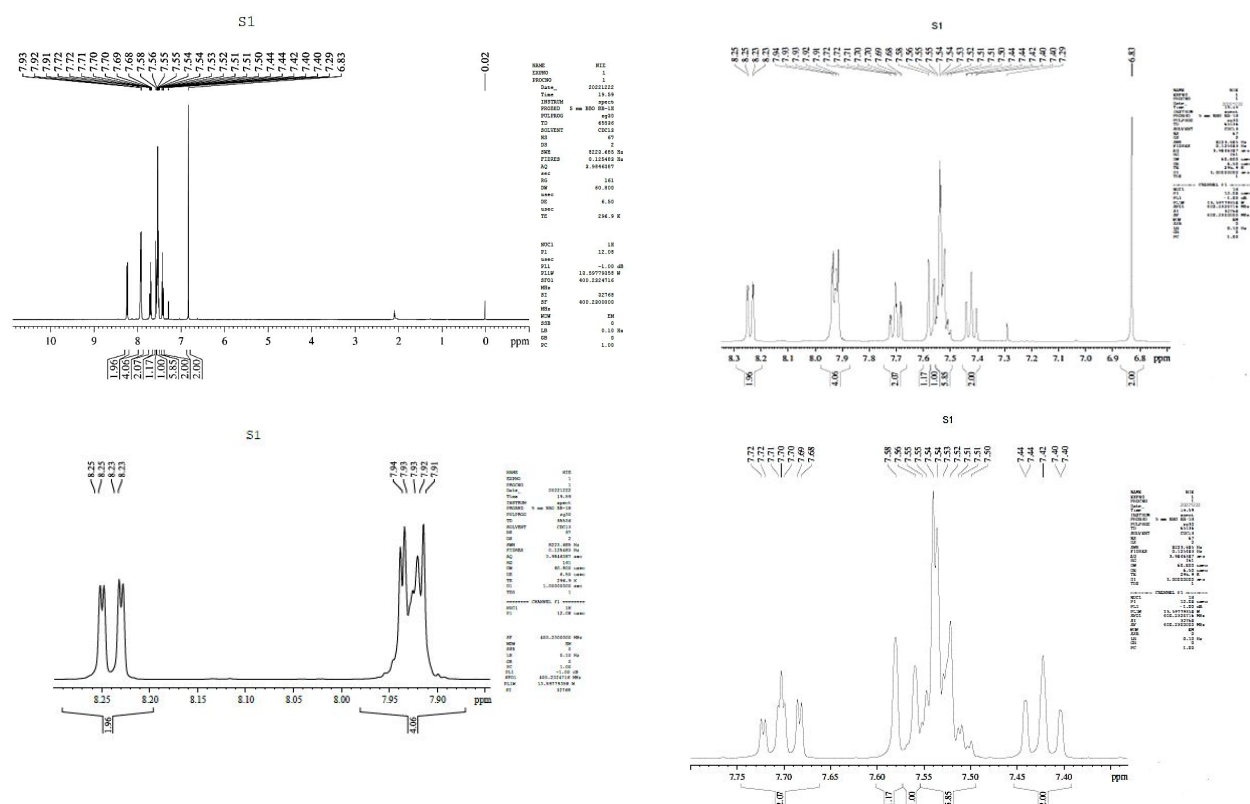


Fig. 4 : ^1H characterization of the compound S1 isolated from *S.palghatase*.

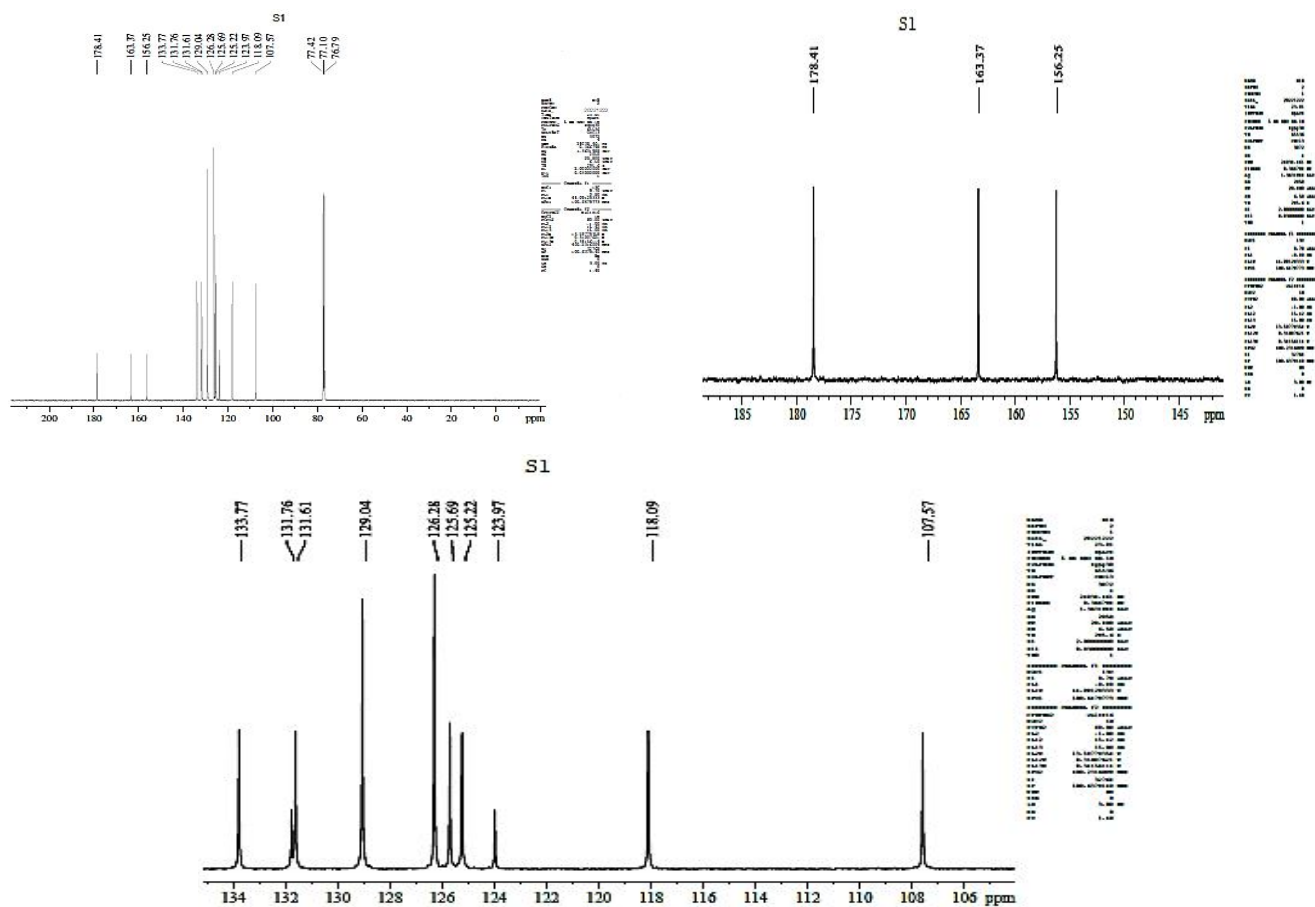


Fig. 5 : ^{13}C characterization of the compound S1 isolated from *S.palghatase*.

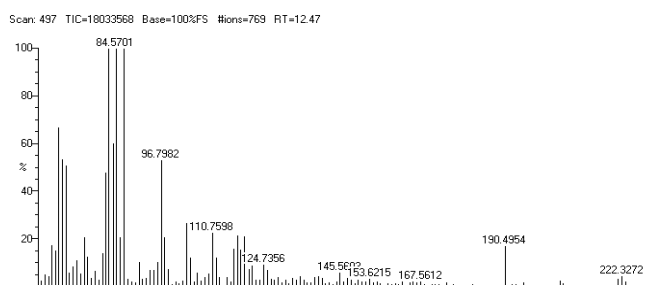


Fig. 6 : GCMS characterization of the compound S1 isolated from *S. palghatense*.

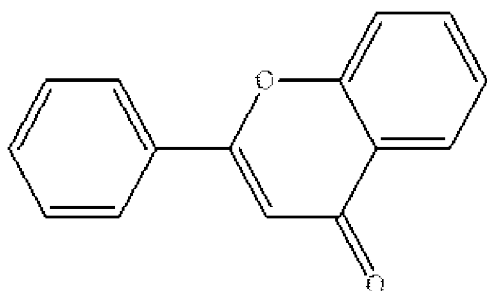


Fig. 7 : Compound S1 (2-phenyl-4H-chromen-4-one) isolated from *S. palghatense*.

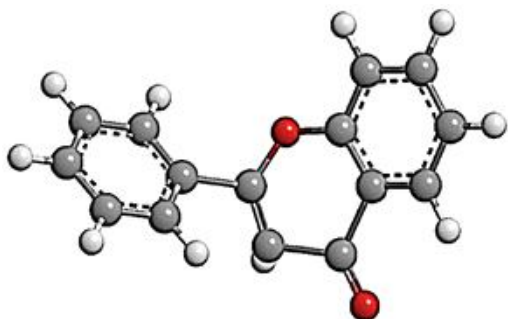


Fig. 8 : 3-Dimensional structure of 2-phenyl-4H-chromen-4-one.

(demethoxymatteucinol). It exhibited modest xanthine oxidase inhibitory and antioxidant properties (Insanu *et al.*, 2018). HPLC-PDA-MS/MS examination of *S. jambos* leaf extract revealed the presence of 17 secondary metabolites, including flavones, flavonol glycosides, flavonol di-glycosides, phenolic acids, and ellagitannins (Sobeh *et al.*, 2018). 5,7-dihydroxyflavanone 7-O- α -D-(62 2 -O-galloylglucopyranoside), a flavanone glucoside had been identified from the leaves of *S. cerasiforme* (Hai Ninh *et al.*, 2023). The presence of 2-Phenyl-4H-chromen-4-one, a flavonoid derivative known for its pharmacological relevance in the methanol extract of *S. palghatense* bark has never been documented before.

The leaves and bark of *S. palghatense* exhibit substantial pharmacognostic qualities as well as possible antioxidant and antidiabetic substances (Snehalatha and

Rasmi, 2021). It was demonstrated by Kiruthiga *et al.* (2018) docking result of phenyl-4H-chromen-one, that these compounds were highly connected with the predicted anti-diabetic effect. Xiao *et al.* (2022) and Singh *et al.* (2022) synthesized a number of 2-phenyl-4H-chromen-4-one compounds. These molecules have a variety of pharmacological properties, which may pave the way for the creation of powerful medications to treat Alzheimer's disease (Singh *et al.*, 2022). The results of the antifungal investigation line up with molecular docking, and drugs bind to the proteins most active region. In silico research suggests that substances containing lipophilic electron-withdrawing groups may function as chitinase inhibitors that break down chitin which aid in the formation of fungal membranes (Devi *et al.*, 2022). It was also found that one of these compounds of phenyl-4H-chromen-one suppresses inflammation via controlling the TLR4/MAPK pathway and can be evaluated further for therapy development (Xiao *et al.*, 2022).

Conclusion

Present study provides the first report on isolation of 2-Phenyl-4H-chromen-4-one from the methanolic extract of bark of *S. palghatense*. Spectral characterization of this isolated compound revealed consistent findings across multiple analytical techniques. The UV analysis showed its highest absorbance at 294 nm, FT-IR spectrum exhibited characteristic functional groups (C=C, C-H stretches). The ^1H and ^{13}C NMR profiles provided the information about the hydrogen and carbon environments, which supports the presence of aromatic rings and carbonyl functionalities. Mass spectrometric analysis confirmed the molecular weight of 223.10 g/mol, corresponding to the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_2$. These results provide the structure of the isolated flavonoid derivative, 2-phenyl-4H-chromen-4-one. This outcome highlights the potential of *S. palghatense* as a valuable source of bioactive compounds with possible pharmacological significance. It is concluded from this study that *S. palghatense* is a good source of bioactive flavonoids and highlight its potential for future application in drug discovery and pharmacological research and also underscoring the importance of conserving this threatened species.

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