Strobilanthes callosa Nees, an Alternative for the Vulnerable, High-Market Medicinal Plant, *Strobilanthes ciliata* Nees, Endemic to Western Ghats of India

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ABSTRACT

Strobilanthes Blume (family: Acanthaceae), is popularly known as 'karvi' in the Sahyadri range of Western Ghats, India. The aim of this study was the identification and comparative quantification of phytochemical 'lupeol' in leaf and stem of diverse *Strobilanthes* species by HPTLC method. Five *Strobilanthes* species viz. *S. callosa, S. ciliata, S. integrifolia, S. ixiocephala* and *S. heyneana* were studied. Among these species, *S. callosa* stem showed the highest amount of lupeol in 200 mg crude extract (553 µg), followed by *S. ixiocephala* stem (409.3 µg), *S. heyneana* stem (218.1 µg), *S. ciliata* stem (197.9 µg) and *S. integrifolia* stem (171.7 µg). Similarly, in the leaf *S. integrifolia* showing the lowest amount of lupeol (15.00 µg). The current study confirms the presence of lupeol in all the species of *Strobilanthes* studied for both parts i.e. leaf and stem with the stem showing higher content compared to the leaf. The use in ayurvedic formulations of vulnerable species *S. ciliata* can be minimized by the use of an alternate source, like *S. callosa* (abundantly found in Western Ghats of India) as it contains bioactive lupeol content more than *S. ciliata*.

Key words: Conservation, HPTLC, lupeol

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Introduction

Strobilanthes Blume (Acanthaceae), popularly known as 'karvi', in the Sahyadri range of Western Ghats India, is the second largest genus of the family and consists of approximately 450 species distributed in the tropical regions of Asia (Mabberley, 2017). The phytochemical analysis of *Strobilanthes* species has revealed the presence of terpenoids, phytosterols, flavonoids, polyphenols (Sarpate & Tupkari, 2012; Anvitha & Monnanda, 2015; Subbulekshmi et al., 2015; Fernandes & Krishnan, 2016). The bioactive compound lupeol (Fig. 1) is reported to possess several biological activities such as anti-inflammatory, anti-oxidant, anti-osteoarthritis, anti-diabetic, and anti-microbial (Gallo & Sarachine, 2009). Lupeol has many more important activities like anti-angiogenic (You et al., 2003), anti-oxidative (Nguemfo et al., 2009), wound healing activity (Harish et al., 2008), protective effect in hypercholesterolemia associated with renal damage (Sudhahar et al., 2008), great therapeutic value (Chaturvedi et al., 2008) and reduction of the risk of cancer by dietary supplements rich in lupeol (Saleem et al., 2005).

In Ayurveda, *S. ciliata* is widely used as an important medicine in various ayurvedic formulations (Rameshkumar et al., 2017). Due to prolonged intervals in blooming, the alternative methods like anatomical characters were studied in *Strobilanthes* species since, it is often found difficult to identify the species at vegetative state (Fernandes & Krishnan, 2019). In some states of India, different species of *Strobilanthes* are been used in ayurvedic therapeutics of a well-known drug 'Sahachara' hitherto giving similar results (Ravishankar, 1992). This encouraged us investigating the phytoconstituent 'lupeol' in other selected species and quantify the major compound (Fernandes & Krishnan, 2019a). We studied five medicinally important *Strobilanthes* species (*S. callosa, S. ciliata, S. integrifolia, S. ixiocephala* and *S. heyneana*) for both leaf and stem extracts using GC-MS and found lupeol as the major bioactive compound along with other metabolites. The present contribution is an extension of that study that quantifies lupeol in the leaf and stem of five *Strobilanthes* species using High Performance Thin-Layer Chromatography (HPTLC) system.

Materials and Methods

Collection of Plant Material

The *Strobilanthes* species was sampled (pooled

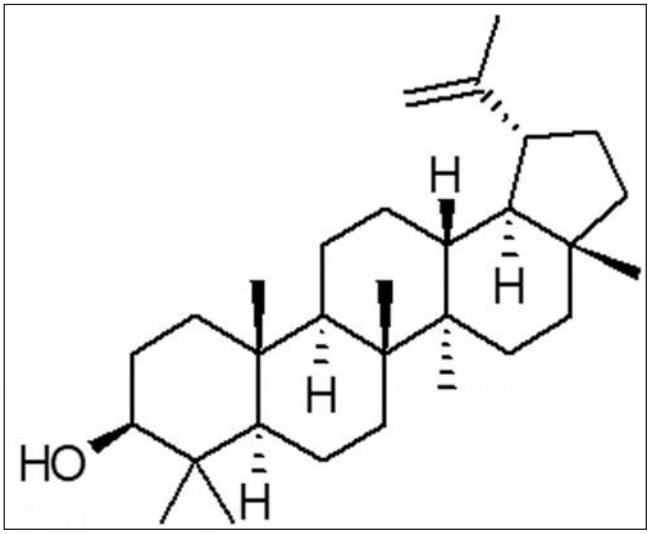

Figure 1. Chemical Structure of lupeol

individuals) approx. 9-10 healthy mature plants of five species i.e. *S. callosa* Nees (Amboli, Maharashtra, India; N15°57'05.2'', E73°59'45.3'', leaf and stem (MS1 & MS6); *S. ciliata* Nees (Bondla, Goa, India; N15°26'485'', E74°05'946'', leaf and stem (MS2 & MS7); *S. integrifolia* Kuntze (Bondla, Goa, India; N15°27'066'', E74°05'755'', leaf and stem (MS3 & MS8); *S. ixiocephala* Benth. (Bondla, Goa, India; N15°25'957'', E74°06'611'', leaf and stem (MS4 & MS9); and *S. heyneana* Nees (Amboli, Maharashtra, India; N15°57'32'8'', E73°59'50.3'', leaf and stem (MS5 & MS10) were collected in the vegetative state for HPTLC analysis. The *Strobilanthes* species are pleitesials i.e. flowers after long periods of vegetative growth, hence all species are not available in flowering state at one time. A voucher specimen of each species was deposited at the Botanical Survey of India (BSI) Pune, India with accession numbers as *S. callosa* (137255), *S. ciliata* (137254), *S. integrifolia* (137251), *S. ixiocephala* (137253) and *S. heyneana* (137256).

Chemicals and Reagents

Lupeol (analytical standard) was procured from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Methanol HPLC grade (Himedia Laboratories Pvt. Ltd. Mumbai, India) was used as solvent for the preparation of samples and standards. Toluene, ethyl acetate, glacial acetic acid and formic acid (Merck Pvt. Ltd. Mumbai, India) were used as mobile phase for HPTLC analysis. *p*-Anisaldehyde-sulphuric acid reagent (ASR) from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) was prepared for derivatisation.

Preparation of Extracts

Matured, healthy and fresh samples of leaf and stem of *Strobilanthes* species (10 g each) were powdered using a grinder and extracted with methanol (70% v/v) using soxhlet extractor (Omega, Mumbai, India) at a temperature of 30°C for 48 hours. The obtained crude extracts were filtered and concentrated at 25°C with rotary evaporator (Heidolph, Germany).

Sample and Standard Solution Preparation

Crude extract (200 mg) was dissolved in 2 ml of HPLC grade methanol, sonicated for 15 min. and filtered through 0.22 µm membrane syringe filter (Merck Pvt. Ltd. Mumbai, India). This solution was used for the HPTLC analysis (Venkatachalapathi & Subban, 2012). The standard lupeol 10 mg/10 mL was dissolved in methanol (stock solution) and stored at 4°C. This solution was diluted 10 times as per the required concentration.

Quantitative Analysis by HPTLC

TLC aluminum-backed plates pre-coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany) were used for sample application. Each sample was applied in triplicate (to minimize sample error**)** on the plate, using a CAMAG (Muttenz, Switzerland) automatic TLC sampler 4 at a speed 150nL/s. The plate layout and application: plate size – 20×10 cm, band type application, start position 8 mm from the lower edge of the plate, first sample position 20 mm, distance between tracks 11.4 mm and band length 8 mm. The plates were developed in toluene:ethyl acetate:glacial acetic acid:formic acid (16:2:1:1 v/v/v/v) mobile phase in CAMAG glass twin-trough chamber 20×10 cm saturated for 20 min at 23°C and humidity 47%. When the solvent front position was at 70 mm, the plate was air dried at room temperature (25°C) for 5 min. The standard lupeol was identified as violet bands on the TLC plates after derivatisation with anisaldehyde sulphuric acid reagent and scanned at 540 nm (white light) using CAMAG visualizer and TLC scanner **(**Fig. 2**)**. The peaks were recorded with CAMAG (Muttenz, Switzerland) computer automated vision CATS 2.3 software and the calibration curve of lupeol was obtained by plotting peak areas against the crude extracts concentration of MS1 to MS10, respectively.

Results

The solvent system (mobile phase) toluene:ethyl

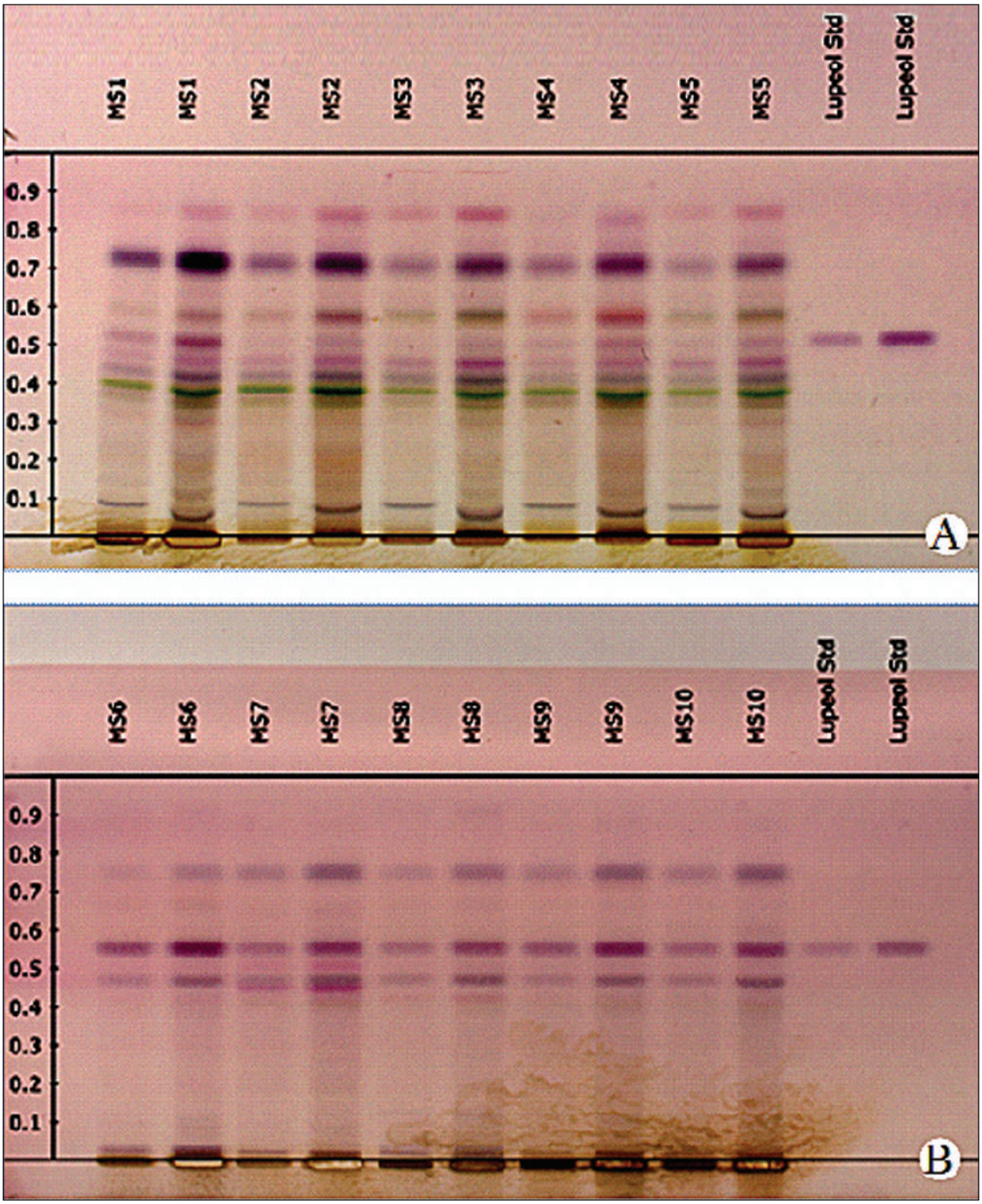

Figure 2. HPTLC profile showing the identification of lupeol in methanolic leaf (A) and stem (B) crude extracts of *Strobilanthes* spp. (A) MS1 to MS5 (*S. callosa, S. ciliata, S. integrifolia, S. ixiocephala, S. heyneana*); (B) MS6 to MS10 (*S. callosa, S. ciliata, S. integrifolia, S. ixiocephala, S. heyneana*) using toluene:ethylacetate:glacial acetic acid:formic acid (16:2:1:1 v/v/v/v) solvent system scanned at 540nm (white light).

acetate:glacial acetic acid:formic acid (16:2:1:1 v/v/v/v) gave good chromatographic separation of MS1–MS10 with well-defined bands at an R_f value of (0.5±0.03). Also, the separation was definite due to saturation of the chamber with CAMAG filter paper with the mobile phase. The highest amount of lupeol in the stem (200 mg of crude extract) was in *S. callosa* (553 µg), and the lowest in *S. integrifolia* stem (171.7 µg). Similarly, the highest amount of lupeol in the leaf (200 mg of crude extract) was in *S. callosa* (115.0 µg) and the lowest in *S. integrifolia* (15.0 µg) (Figs. 3 & 4).

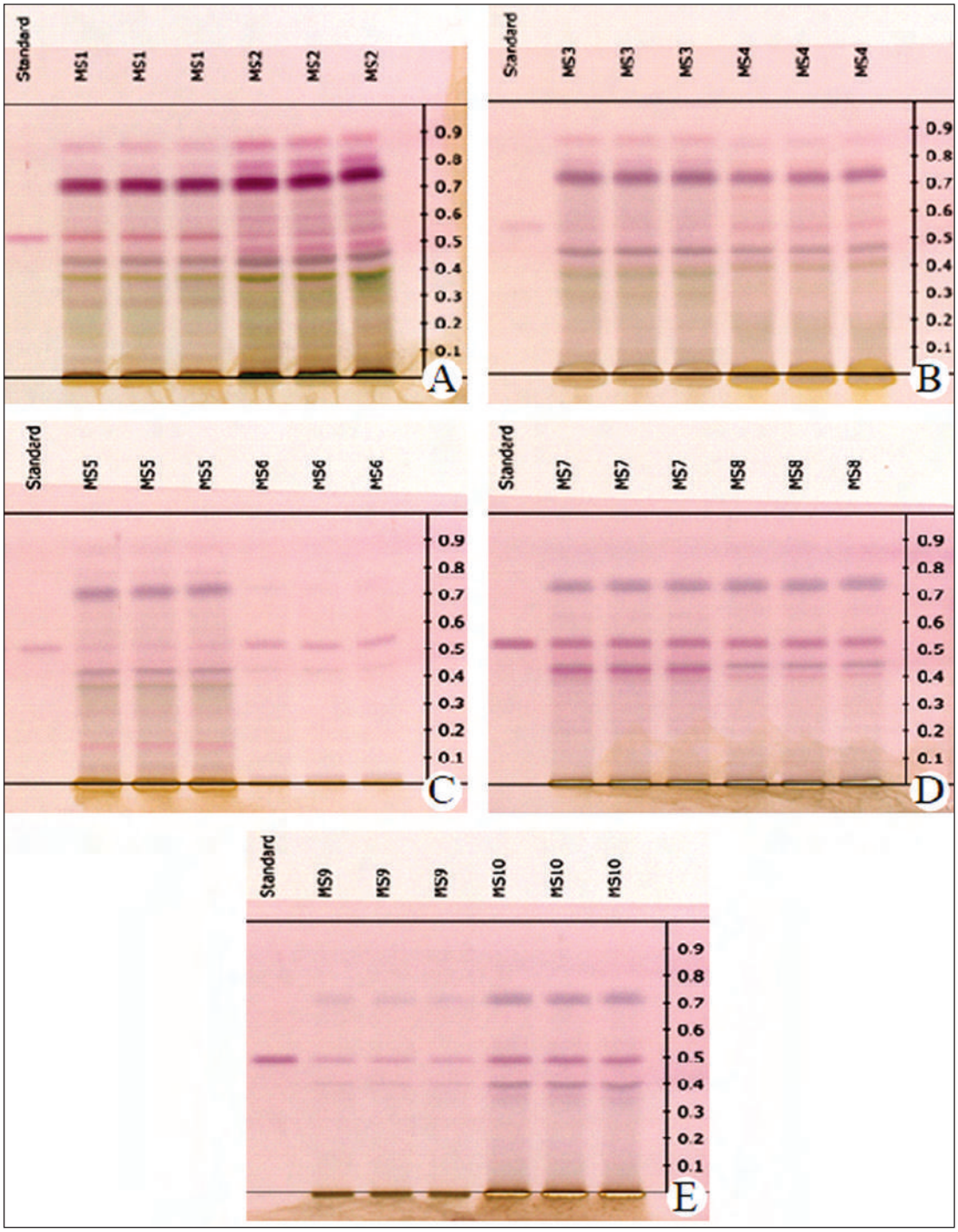

Figure 3. HPTLC profile during the comparative quantification of lupeol in methanolic leaf and stem crude extracts of *Strobilanthes* spp. (A) MS1 to MS2; (B) MS3 to MS4; (C) MS5 to MS6; (D) MS7 to MS8; (E) MS9 to MS10 scanned at 540nm (white light).

Discussion

The current study confirms the presence of lupeol in all the species of *Strobilanthes* in two parts, leaf and stem; the stem shows higher content of lupeol as compared to the leaf. However, the level in stems of *S. callosa* differed from that in a previous study (Sarpate & Tupkari, 2012). These authors found only 0.013% of lupeol in the dried stem powder of *S. callosa* using HPTLC method, whereas in the present study (Table 1; Fig. 4), lupeol was much higher (0.276%). Unexpectedly, *S. ciliata* stem (0.098%) showed lower levels of lupeol in our study compared to *S. callosa*

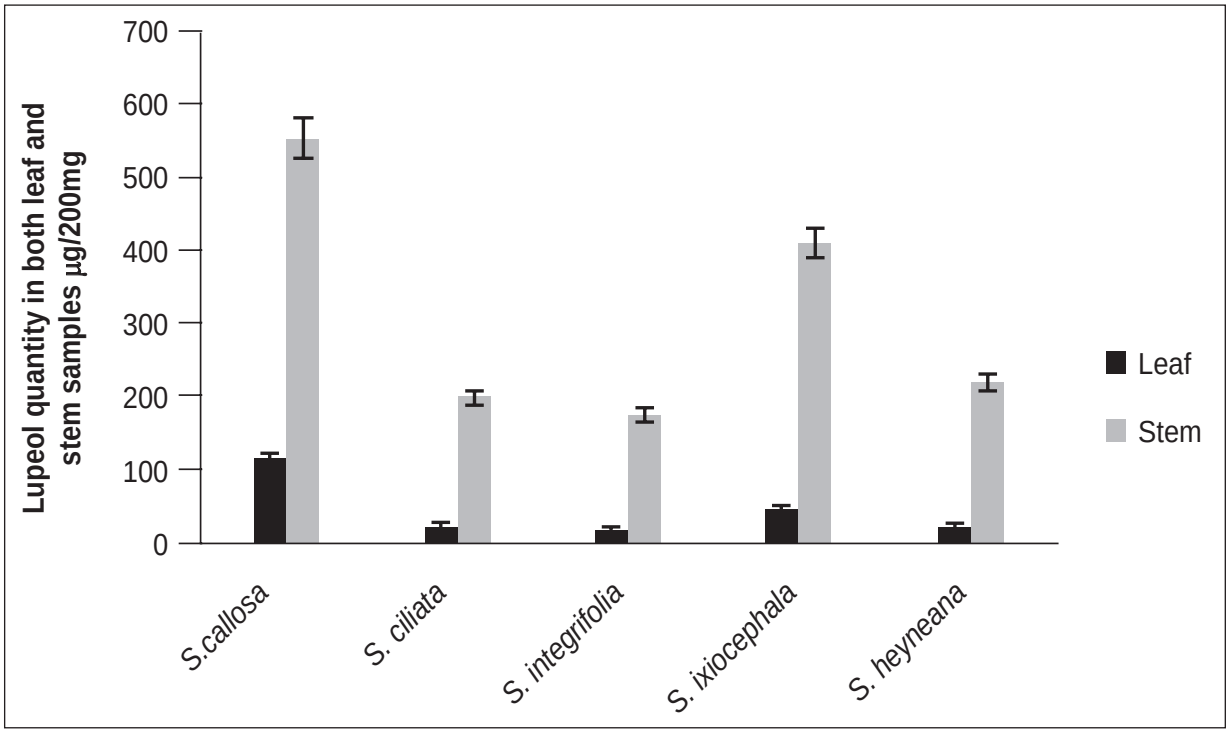

Figure 4. Quantification results. Lupeol comparison in methanolic leaf and stem crude extracts (µg/200 mg) of *Strobilanthes* spp. Results presented as mean ± SD of three samples: *P<0.05.

Table 1. Quantity (mean percentages) of the phytochemical lupeol in leaves and stems of *Strobilanthes* species.

Strobilanthes species	Lupeol quantity in leaf (200 mg crude extract)		Lupeol quantity in stem (200 mg crude extract)	
	Mean		Mean	
	(µg)	(%)	(µg)	(%)
S. callosa	MS1 = 115.0±0.005	0.057	MS6 = 553.0±0.03	0.276
S. ixiocephala	MS4 = 45.16±0.006	0.022	MS9 = 409.3±0.02	0.204
S. heyneana	MS5 = 17.81±0.0005	0.008	MS10 = 218.1±0.01	0.109
S. ciliata	MS2 = 17.82±0.0005	0.008	MS7 = 197.9±0.003	0.098
S. integrifolia	MS3 = 15.00±0.0003	0.007	MS8 = 171.7±0.002	0.085

*Each value expressed as mean ± SD of 3 samples.

stem and *S. ixiocephala* stem, and slightly lower than the level found (0.16±0.02% w/w) in the same species by Venkatachalapathi and Subban (2012) using HPTLC from air dried aerial parts of *Strobilanthes ciliata* extracted with petroleum ether using cold percolation technique. These differences may be due to differences in solvents used for extraction, sampling of plant during vegetative/flowering state, parts of plant used and geographical locations. It can be noted that the presence of the active compounds can also vary due to season of collection of species, climatic conditions and extraction techniques (Sridhar et al., 2018). Traditional healers even when uses different species of *Strobilanthes* which gives similar therapeutic value because lupeol is the active constituent which have shown its effects in biological activity such as anti-inflammatory, anti-arthritic, anti-tumor, anti-microbial (Gallo & Sarachine, 2009). Because stems contain high levels of the compound, commercial healers and traders need not uproot the plant and destroy the species from its natural habitats.

Conclusion

The present study claims that an alternative to *S. ciliata* [IUCN Red List status: Vulnerable (VU)], *S. callosa* (abundantly found in Western Ghats, India) could be exploited as it contains bioactive lupeol content more than *S. ciliata*. This result will help to reduce the

pressure of overexploitation of vulnerable species *S. ciliata* and appropriate strategies could be evolved for conservation of the species biodiversity.

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