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ORIGINAL ARTICLE

The Biochemical Analysis and Characterization of *Strobilanthes ciliatus* Nees (Bremek): Leaf Extracts

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The present study was aimed to analysis and characterizes the biochemical elements of *Strobilanthes ciliatus* Nees leaf extract. The healthy leaves of *S. ciliatus* were collected and the plant extracts were prepared as dried powder. Biochemical analysis was conducted using standard procedures for the leaf extracts. The various analysis and charecterization of *S.ciliatus* was determined via total chlorophyll content. The results have envisaged that plant extract have significantly with a presence of bio compounds. The present study shows *S. ciliatus* to be an important medicinal plant, since the leaves showed good biochemical activity. Thus, it may use in the treatment of diseases and may also use in the preparation of natural or herbal drugs due to the presence of biochemical.

Key words: Strobilanthes ciliatus, Biochemical, Diseases, Herbal, Characterization

Natural sources such as plants and herbs have accepted considerable attention for the discovery and development of leads as new drug able molecules, because of its diversity (Anjana *et al.,* 2013). *S. ciliatus Nees* (Bremek) is a traditionally known and medicinally potent plant that belongs to the genus *'Strobilanthes'* (Venu, 2006; Warrier *et al.,* 1994). This plant is original to the evergreen forests of Southwestern Ghats of India (Thomas and Rajeshkumar, 2014) and has of properly been included in the RED DATA list of South Indian medicinal plants by the IUCN (Ravikumar *et al.,* 2000; Ved *et al.,* 2007). *Strobilanthes* pertains to Acanthaceae and the second largest genus of this family. It comprises of approximately 300 species in tropical Asia. The Indian subcontinent has nearly 150 species, out of which 59 are seen in peninsular India. The genus is not greatly explored for economic benefit. *S. ciliatus* is one of the endemic and capable of taking action medicinal plants. It is manageable used in Ayurveda as a source of the drug 'Sahacharya' (Venu *et al.,* 2006). *S. ciliatus* has received considerable attention due to its wide range of secondary metabolites and its traditional usage in Indian system of medicine. It is a common herb, growing aloft to a height of 1.2 M and it is distributed in Western Ghats (Khare, 2008). The roots are bitter, sweet, Thermogenic, emollient, diuretic, febrifuge, diaphoretic, depurative, anti-inflammatory, expectorant and tonic. Conventionally, the plant has been used for the treatment for rheumatalgia, lumbago, sciatica, limping, chest congestion, strangury fever, leucoderma, skin ailment, inflammation, cough, bronchitis, odontalgia and general poor health. The leaves are diaphoretic, expectorant, depurative and febrifuge, and are useful in whooping cough, fever, bronchitis, dropsy, leucoderma, leprosy, pruritus, inflammation. A disease causing glandular swelling (scrofula) and fever (Blois *et al.,* 2014). *S. ciliatus* has been used as traditional medicine for its Anti-inflammatory, Analgesic, Anticancer, Antimicrobial, Antidiabetic and Hepatoprotective. It has a strong aroma and is used medicinally. The plant has got some therapeutic properties. The plant is used for a variety of ailments like rheumatalgia, fever, leukoderma, skin diseases, inflammations, cough, bronchitis, and general debility (Reneela et al., 2010). At the time of gathered in, more than 70% of medicinal plants are facing risk due to the harmful collection of plant parts which makes them endangered (Thiyagarajan and Venkatachalam, 2013). The previous study was phytoconstituents present in the petroleum ether extract by GC – MS analysis in *S. ciliatus* (Gomathi *et al.,* 2021). Therefore, the present study has been experimented for Biochemical analysis and Characterization of *S. ciliates*.

MATERIALS AND METHODS

2.1 Plant Material:

S. ciliatus plants were collected from MS Swaminathan Research Foundation, Wayanad, Kerala and maintained in shade house, Department of Biotechnology, Alagappa University, Tamil Nadu. Leaves dark green, elliptic acuminate at both ends, serrate glabrous, main nerves 6-7 pairs raised above. Flower white to lilac in axillary slender glabrous spikes. Fruits very rarely formed. Adventitious roots arise from a few basal nodes also. The main roots as well as these nodal roots are used as raw materials (Raiby and Sathish 2012). The images of the plants studied in this work are shown in [Fig.1].

2.2 Preparation of plant extracts:

The plant leaves were washed under running tap water and fresh leaf were shade dried a room temperature and the after drying it was grind at powder form. The powdered plant sample was used for further all biochemical analysis.

2.3 Biochemical screening of plant extracts:

Qualitative biochemical analysis of the crude powder was determined using standard procedures. The extracts were tested qualitatively for the presence of biochemical analysis such as UV, FTIR, HPLC, Chlorophyll a and b, and Antioxidant assay.

2.3.1 UV (Ultra Violet):

UV–Vis spectra was generated by electronic transitions of the molecules that absorb energy in the form of ultraviolet or visible light going from the ground electronic state into excited states, from where the energy is further dissipated by non radioactive processes such as collisions with other molecules.

2.3.2 FTIR (Fourier Transform Infrared Spectrophotometer):

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional group) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried Powder was used for FTIR analysis. 10mg of the dried powder in FTIR spectroscope, with a scan range from 400 to 4000 cm-1 with a resolution of cm^{-1}. The FTIR spectrum of leaf dried powder the data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the leaf dried powder of *S. ciliates.*

2.3.3 HPLC Analysis:

The dried Powder sample (5g/25ml) was extracted successively an organic solvent (chloroform) may be used as the initial extracting and a period of maceration, solid material is then removed by decanting off the extract by filtration (Janovik *et al.,*2014; Sasidharan *et al.,*2011). The filtrate is then concentrated and injected into HPLC for separation.

2.3.4 Estimation of Chlorophyll a and b

1g of the dried leaf powder was weighed in an electronic balance end homogerized using motor and pestle in 10ml of 80% acetone (w/v). The homogenate was centrifuged at 5000 rpm for 10 min and the supernatant was collected. The absorbance was read at 645nm, 663nm against solvent blank. Total Chlorophyll was calculated using the formula William *et al*.,2005; Daniel,1949; Krishnaveni *et al*.,1984).

$$\text{Chlorophyll a content} = \frac{[12.69\,(A663) - 2.69\,(A645)] \times \text{Volume}}{1000 \times \text{Weight of sample}}$$

$$\text{Chlorophyll b content} = \frac{[22.9\,(A645) - 4.68\,(A663)] \times \text{Volume}}{1000 \times \text{Weight of sample}}$$

Where, A645 = Absorbance at 645nm, A663 = Absorbance at 663nm.

2.3.6 Statistical analysis

A descriptive analysis was performed to describe the entire results within each kind of test. Regarding the biochemical composition analysis, antioxidant, activity, and a oneway analysis of variance (ANOVA one-way) followed by DUNCAN test was performed to test possible significant differences among mean values from species. The level of significance was set at $P<0.05$ for all analyses. Statistical analyses were performed using SPSS v.20 software.

RESULTS AND DISCUSSION

Biochemical analysis was conducted for the leaf extracts of *S. ciliatus*. The leaves were positive for the presence of UV and FTIR. Chloroform extracts of leaf showed the presence of GCMS and HPLC.

3.1 UV (Ultra Violet):

The Ultra violet (UV) region scanned is normally from 200 nm, and the visible portion is from 400 to 800 nm. The peak of UV is 277.40 as absorbed (Fig:2). A large matrix is obtained that links the concentrations and absorption for each sample separately. It shows the calibration curve of the absorbance (200–1100) versus the concentration.

3.2 FTIR (Fourier Transforms Infrared Spectroscopy)

The FTIR spectrum was used to identify the functional groups of the active compounds in the plant sample based on the peak value in the region of infrared radiation (Fig:3). The dried leaf Powder of *S. ciliatus* showed characteristic absorption peak. The absorption spectra of *S.ciliatus* exhibited a peak at 3421.93 represented the presence of Hydrogen bond (O-H) and Primary and secondary Amine and Amide (N-H). The peak at 2921.13 showed the presence of Alkane (C-H) and Carboxylic Acid (O-H). The peak at 2851.83 showed the presence of Alkane (C-H) and Carboxylic Acid (O-H). The peak at 2025.96 showed the presence of Isothiocyanate (-NCS). The peak at 540.06 showed the presence of Halogen compound [Chloro- compound] (C-CI) and Halogen compound [Indo-compound](C-I). The peak at 604.13 showed the presence of Chloride(C-CI). The peak at 1429.22 showed the presence of Nitro(R-NO_2) (N=O). The peak at 667.83 showed the presence of Chloride (C-CI). The peak at 1633.88 showed the presence of Diketones (POC) and carboxylic acid (C-O). The peak at 1235.39, 1101.07, 1068.72 showed the presence of Amine (C-N) and Alcohol, Ether, Ester, Carboxylic Acid, Anhydride (C-O). The peak at 1318.95 showed the presence of

Alkylketone. The peak at 1543.65 showed the presence of Aromatic (C-C) and Nitro(R-NO_2) (N=O). The peak at 1383.39 showed the presence of -CH_2- (C-H) and Nitro(R-NO_2) (N=O). The peak at 471.77 showed the presence of Polyulfides (S-S) and Aryl disulfides (S-S).

3.3 HPLC fractionation:

HPLC is a versatile, reproducible chromatographic technique for the estimation of secondary metabolites in the plants. It has wide applications in different fields in term of isolation, quantitative and qualitative estimation of active molecules (Fig:4) . The target analytes was achieved enabling the quantification in the sample extract. The peaks of target eluted at retention times are 2.430 min, 5.080 min, 5.776 min, and 11.40 min, respectively, in the sample extract (Table:1).

3.4 Estimation of Chlorophyll a and b:

$$\text{Chlorophyll a content} = \frac{[12.69\,(2.185) - 2.69\,(0.992)] \times 4}{1000 \times 1}$$

$$= 27.7495 - 2.66848 \times \frac{4}{1000}$$

$$= 25.08102 \times 0.004$$

$$= 0.10032408.$$

$$\text{Chlorophyll b content} = \frac{[22.9\,(0.992) - 4.68\,(2.185)] \times 4}{1000 \times 1}$$

$$= 22.7168\text{-}10.2258 \times \frac{4}{1000}$$

$$= 12.491 \times 0.004$$

$$= 0.049964.$$

The leaves were positive for the presence of Chlorophyll a = 0.10032408, Chlorophyll b = 0.049964.

Figure 1: Morphology of *Strobilanthes ciliatus*

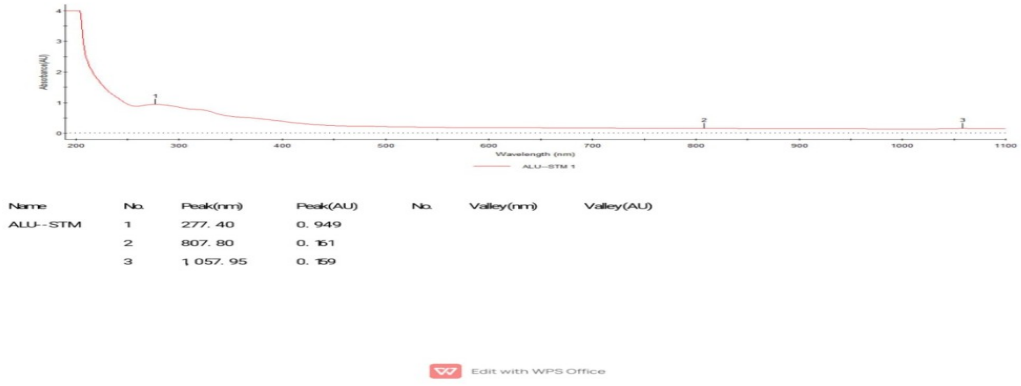

Figure 2: UV analysis of *S. ciliatus Nees (Bremek)*

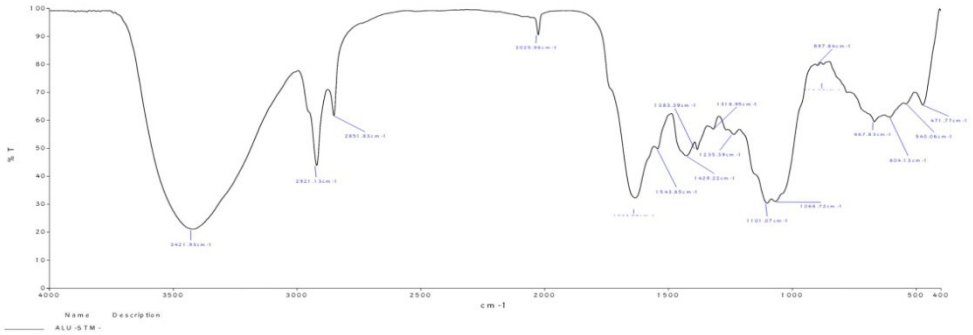

Figure 3: FTIR analysis of *S. ciliatus* Nees (Bremek)

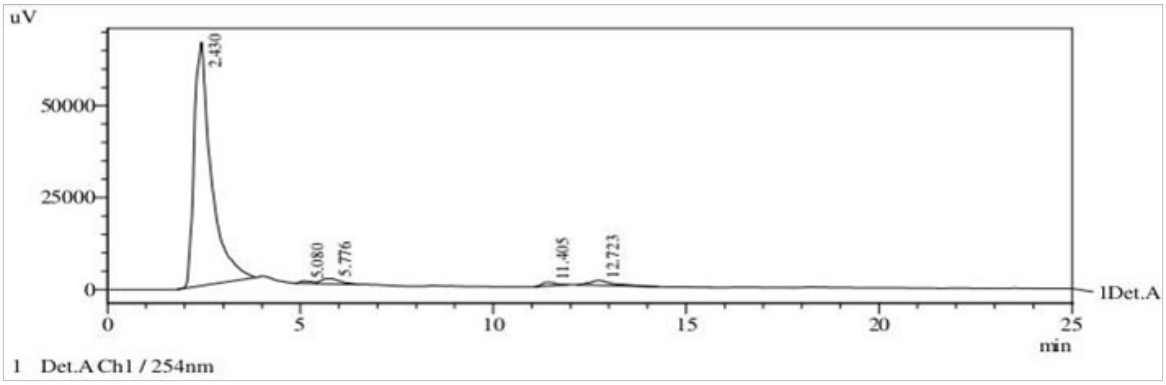

Figure 4: Plant extracts for HPLC analysis

Table.1: Chromatogram of HPLC for Chloroform extract for Steroids

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.430	2060322	66247	93.429	93.764
2	5.080	14853	658	0.674	0.932
3	5.776	49300	1424	2.236	2.016
4	11.405	23438	997	1.063	1.411
5	12.723	57323	1326	2.599	1.877
Total		2205237	70653	100.000	100.000

CONCLUSION

The present research was done for the biochemical characterization of the chloroform extract of *S. ciliates.* The various phytochemicals including flavonoids, alkaloids, were found to be present during qualitative analysis of the extract. The presence of these phytochemicals was also supported by the spectroscopic studies showing the characteristic peaks obtained in Ultraviolet and Visible region. The presence of phenolic, alcohols and aromatic compounds was also indicated by FTIR spectrum. The value of absorption obtained at these wavelengths indicates the presence of flavonoids and its derivatives. The stem of *S. ciliatus* was found to have analgesic activity. The study indicates that the data obtained on basis for further studies and application of this plant. The promising pharmacological potential of the various parts of *S.ciliatus* and may provide useful lead for the development of drug able moieties from the plant. The study showed that chloroform extract of aerial parts of *S. ciliatus* can be effectively used as an anti-inflammatory

agent. Phytochemical analysis of *S. ciliatus* has led to the isolation of new compounds. Some of these fractions contain terpenoids and lignans that may have new structures. The present study was *S. ciliatus* and may provide useful lead for the development of Drug Analysis and Clinical trial. The leaves of *S. ciliatus* were found to have biochemical activity.

CONFLICT OF INTERESTS

The author declare that there is no conflict of interest.

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