

UV-VIS AND FTIR SPECTROSCOPIC STUDIES ON *PERISTROPHE BICALYCVLATA* (RETZ.) NEES.JANAKIRAMAN N¹, SAHAYA SATHISH S¹ AND JOHNSON M²¹Department of Botany, St. Joseph's College (Autonomous), Trichy, Tamil Nadu, India. ²Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palaymkottai, Tamil Nadu, India.

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ABSTRACT

Aim: The present study was aimed to produce the UV-VIS and FTIR spectrum profile of *Peristrophe bicalyculata* (Retz.) Nees.

Methods: The extracts were examined under visible and UV light for the proximate analysis. The crude extracts of *P. bicalyculata* were scanned in the wavelength ranging from 200-1100 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR method was performed on a Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peak values and their functional groups. Results: The UV-VIS profile of *P. bicalyculata* aqueous extract showed the peaks at 337, 442, 662 and 774 nm with the absorption 0.013, 0.027, 0.012 and 0.011. The UV-VIS profile of *P. bicalyculata* ethanolic extract showed the peaks at 333, 383, 434, 472, 664, 739 and 783 nm with the absorption 0.119, 0.135, 0.218, 0.186, 0.143, 0.035 and 0.029. The qualitative UV-VIS spectrum profile of *P. bicalyculata* acetone extract showed the peaks at 360, 431, 597 and 661 nm with the absorption 0.072, 0.042, 0.018 and 0.017. The qualitative UV-VIS spectrum profile of *P. bicalyculata* chloroform extract showed the peaks at 335, 433, 458, 664 and 782 nm with the absorption 0.411, 0.969, 0.691, 0.589 and 0.093. The UV-VIS profile of *P. bicalyculata* petroleum ether extract showed the peaks at 333, 445, 661 and 781 nm with the absorption 0.013, 0.028, 0.013 and 0.011 respectively. The FTIR spectrum was used to identify the functional group of the bioactive components based on different peak values in the region of infrared radiation. The results of the present study confirms the presence of amides, ethers, alkanes, deuterated R-OH, organo-phosphorus compounds, deuterated amines, aminoacids, aryl aldehydes, alkenes, ketones, sulfites, aliphatic esters, monosubstituted alkenes, sulfur, aldehydes, carboxylic acids, epoxides, alcohols, ketones, aminoacid hydrochlorides, halogen, aliphatic esters, saturated nitrites, nitroso compounds, benzene ring, silicon, boron, aliphatic nitro compounds, secondary alcohols and bromides in *P. bicalyculata*.

Conclusion: The results of the present study produced the UV-VIS and FTIR spectrum profile for the medicinally important plant *P. bicalyculata* and also used to identify the plant in the pharmaceutical industry.

Keywords: *Peristrophe bicalyculata*, UV-VIS, FTIR, Spectrophotometer, Phytochemistry, Pharmacognosy

INTRODUCTION

Medicinal plant research includes much more than the discovery of new drugs. This field has been expanding to also include diverse subjects as negotiation of power based on medicinal plant knowledge¹. Plants generally contain both primary metabolites as well as secondary metabolites. The different phytoconstituents present in plants include anthraglycosides, arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes and valepotriates. These phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents would help in determining various biological activities of plants. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug².

A variety of techniques can be used to determine and estimate the presence of such phytoconstituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. A survey of analytical methodology developed for estimation of organic compounds in plant samples shows that their preliminary separation such as solvent-extraction is necessary. The determination of phytoconstituents is largely performed by relatively expensive and often laborious techniques such as gas (GC) and liquid (LC) chromatography combined with specific detection schemes or liquid scintillation counting, NMR and ESR^{3,4}. However, simple, cost-effective and rapid tests for detecting phytoconstituents are necessary. Spectroscopic (UV-Vis, fluorescence, FT-IR, Raman) methods together or separate can be used in this sense as well as conventional methods⁵⁻¹⁴.

Molecular absorption spectroscopy has been extensively used for the quantitative determination of compounds in different formulations as well as for the analysis of synthetic mixtures. The use of these techniques have the inherent disadvantage that most active compounds absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination.

The Fourier Transform Infrared (FTIR) spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants¹⁵. The analysis can be performed both on pure compounds and complex mixtures, without separation into individual components. IR spectrometry is more sensitive and selective than colorimetric methods. Moreover, FT-IR spectroscopy is an established time-saving method to characterize and analyze microorganisms and monitor biotechnological processes¹⁶.

The family Acanthaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents. *Peristrophe bicalyculata* (Retz.) Nees. is a medicinally important plant which is erect, hispid herb or under shrub, 60-120 cm height found in forest undergrowth, hedges and waste band almost throughout India. The leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhoea. This plant is used by the traditional healers for curing many skin related problems; it is also used as an antidote for snake poison when macerated in an infusion of rice, and as an insect repellent. This plant is also used for horse feed and ploughed into the soil as green manure¹⁷. Although undocumented, the plant is used in South West Nigeria in the treatment of hypertension and other cardiovascular diseases. It was recently discovered to have hypolipidemic effects¹⁸ and such effects are known to protect against cardiovascular diseases, including hypertension. With this knowledge, the present research work was aimed to produce the UV-VIS and FTIR spectrum profile of *P. bicalyculata*.

MATERIALS AND METHODS

Collection and preparation of plant material

Peristrophe bicalyculata (Retz.) Nees. was collected by handpicking from the natural habitats of Tiruchirappalli district, Tamil Nadu, India. The whole plant samples were washed thoroughly in running tap water to remove soil particles and adhered debris followed by sterile distilled water. The washed plants were blotted on the

blotting paper and spread out at room temperature in shade. Shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in a refrigerator for further use.

Plant sample extraction

2 g of air dried powder of sample was extracted with 50 ml of solvents such as ethanol, acetone, chloroform, petroleum ether and aqueous with gentle stirring for 72 h. The sample was kept in dark for 72 h with intermittent shaking. The complete extraction was carried out with the following solvents in the increasing order of polarity. After incubation, the solution was filtered through Whatmann No. 1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4°C before use.

Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV-VIS and FTIR spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. To detect the UV-VIS spectrum profile of the crude extracts of *P. bicalyculata*, the extracts were scanned in the wavelength ranging from 200-1100 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were

recorded. Each and every analysis was repeated twice and confirmed the spectrum.

RESULTS AND DISCUSSION

The qualitative UV-VIS profile of aqueous extract of *P. bicalyculata* was taken at the wavelength of 300 nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 337, 442, 662 and 774 nm with the absorption 0.013, 0.027, 0.012 and 0.011 respectively (Fig-1A; Table-1). The qualitative UV-VIS profile of *P. bicalyculata* ethanolic extract was selected at wavelength from 300 nm to 800 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 333, 383, 434, 472, 664, 739 and 783 nm with the absorption of 0.119, 0.135, 0.218, 0.186, 0.143, 0.035 and 0.029 respectively (Fig-1B; Table-1). The qualitative UV-VIS spectrum profile of acetone extract of *P. bicalyculata* was chosen at a wavelength of 300 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 360, 431, 597 and 661 nm with the absorption 0.072, 0.042, 0.018 and 0.017 respectively (Fig-1C; Table-1). The qualitative UV-VIS spectrum profile of *P. bicalyculata* chloroform extract was taken at the wavelength of 300 nm to 800 nm due to the sharpness of the peak and proper baseline. The profile showed the peaks at 335, 433, 458, 664 and 782 nm with the absorption 0.411, 0.969, 0.691, 0.589 and 0.093 respectively (Fig-1D; Table-1). The UV-VIS profile of *P. bicalyculata* petroleum ether extract taken at the wavelength of 300 nm to 800 nm due to the sharpness of the peak and proper baseline. The spectrum profile showed the peaks at 333, 445, 661 and 781 nm with the absorption 0.013, 0.028, 0.013 and 0.011 respectively (Fig-1E; Table-1).

Table 1: UV-VIS Peak Values of Different Extracts of *Peristrophe bicalyculata* (Retz.) Nees.

S. No	Aqueous		Ethanol		Acetone		Chloroform		Petrol. ether	
	nm	Abs	nm	Abs	nm	Abs	nm	Abs	nm	Abs
1	337	0.013	333	0.119	360	0.072	335	0.411	333	0.013
2	442	0.027	383	0.135	431	0.042	433	0.969	445	0.028
3	662	0.012	434	0.218	597	0.018	458	0.691	661	0.013
4	774	0.011	472	0.186	661	0.017	664	0.589	781	0.011
5			664	0.143			782	0.093		
6			739	0.035						
7			783	0.029						

Table 2: FTIR Peak Values of Aqueous Extracts of *Peristrophe bicalyculata* (Retz.) Nees.

S. No	Peak values	Functional Groups
1	3430.12	Unknown
2	2949.24	Alkanes
3	2844.12	Aldehydes
4	2117.39	Alkynes
5	1638.30	Amides
6	1403.73	Carboxylic acids
7	1243.53	Epoxides
8	1022.77	Alcohols
9	675.20	Sulfur compounds

Table 3: FTIR Peak Values of Ethanol Extracts of *Peristrophe bicalyculata* (Retz.) Nees.

S. No	Peak values	Functional Groups
1	3935.08	Unknown
2	3823.22	Unknown
3	3419.75	Ketones
4	2980.71	Aminoacid hydrochlorides
5	2092.12	Aminoacids
6	1638.59	Amides
7	1396.77	Halogen compounds
8	1253.49	Aliphatic esters
9	1053.96	Sulfur compounds
10	661.92	Halogen compounds

Table 4: FTIR Peak Values of Acetone Extracts of *Peristrophe bicalyculata* (Retz.) Nees.

S. No	Peak values	Functional Groups
1	3928.28	Unknown
2	3875.75	Unknown
3	3774.57	Unknown
4	3728.54	Unknown
5	3439.60	Amides
6	3004.30	Ethers
7	2923.36	Alkanes
8	2779.58	Deuterated R-OH
9	2679.17	Aminoacids
10	2575.95	Organo-phosphorus compounds
11	2459.72	Deuterated amines
12	2088.39	Aminoacids
13	1713.20	Aryl aldehydes
14	1643.55	Alkenes
15	1427.19	Ketones
16	1365.08	Alkane
17	1225.08	Sulfites
18	1094.69	Aliphatic esters
19	906.28	Monosubstituted alkenes
20	533.84	Sulfur compounds

Table 5: FTIR Peak Values of Chloroform Extracts of *Peristrophe bicalyculata* (Retz.) Nees.

S. No	Peak values	Functional Groups
1	3943.50	Unknown
2	3783.19	Unknown
3	3708.39	Unknown
4	3420.50	Unknown
5	2923.73	Alkanes
6	2241.98	Saturated nitrites
7	1632.40	Nitroso compounds
8	1384.91	Alkane
9	1221.37	Sulfites
10	1108.91	Secondary alcohols
11	770.33	Benzene ring
12	594.47	Bromides

Table 6: FTIR Peak Values of Petroleum Ether Extracts of *Peristrophe bicalyculata* (Retz.) Nees.

S. No	Peak values	Functional Groups
1	3929.33	Unknown
2	3787.63	Unknown
3	3446.44	Unknown
4	2957.38	Alkanes
5	2666.29	Organo-phosphorus compounds
6	2047.30	Silicon and Boron compounds
7	1631.48	Ketones
8	1457.69	Alkanes
9	1375.37	Aliphatic nitro compounds
10	1106.43	Secondary alcohols
11	596.93	Bromides

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The aqueous extract of *P. bicalyculata* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis confirmed the presence of alkanes, aldehydes, alkynes, amides, carboxylic acids, epoxides, alcohols and sulfur compounds which shows major peaks at 2949.24, 2844.12, 2117.39, 1638.30, 1403.73, 1243.53, 1022.77 and 675.20 respectively (Fig-1F; Table-2). The FTIR analysis results of ethanolic extract of *P. bicalyculata* proved the presence of ketones, aminoacid hydrochlorides, aminoacids, amides, halogen compounds, aliphatic esters and sulfur compounds (Fig-1G; Table-3). The FTIR analysis results of acetone extract of *P. bicalyculata* revealed the presence of amides, ethers, alkanes, deuterated R-OH, aminoacids, organo-phosphorus compounds, deuterated amines, aminoacids, aryl aldehydes, alkenes, ketones, sulfites, aliphatic esters, monosubstituted alkenes and sulfur compounds (Fig-1H; Table-4). The FTIR analysis results of chloroform extract of *P. bicalyculata* validated the presence of alkanes, saturated nitrites,

nitroso compounds, sulfites, secondary alcohols, benzene ring and bromides (Fig-1I; Table-5). The FTIR analysis results of petroleum ether extract of *P. bicalyculata* authenticated the presence of alkanes, organo-phosphorus compounds, silicon and boron compounds, ketones, aliphatic nitro compounds, secondary alcohols and bromides (Fig-1J; Table-6).

The crude extracts subjected to UV-VIS and FTIR analysis is used for the identification of chemical constituents present in *P. bicalyculata*. The alkanes are found in the plant cuticle and epicuticular wax of many species. They protect the plant against water loss, prevent the leaching of important minerals by the rain, and protect against bacteria, fungi, and harmful insects¹⁹. In the present study we observed the presence of alkanes in *P. bicalyculata* and Giwa et al²⁰ observed the anti-microbial activity of *P. bicalyculata* against the selected pathogens. The results of the present study supported and supplemented the Giwa et al²⁰ observations. Alkynes occur in certain plants (Ichthyothere, Chrysanthemum, and other members of the Asteraceae family). Alkynes are highly bioactive

as nematocides²¹. The result of the present study confirms the alkynes presence in *P. bicalyculata* suggests that this plant can be used as nematocides agent in the near future. Carboxylic acids are biologically very important in the formation of fat in the body. The drug aspirin is a carboxylic acid, and some people are sensitive to its acidity. The non-aspirin pain reliever ibuprofen is also a carboxylic acid²². The Sulfur compounds are present in the plant in three forms: in the amino acids of proteins, i.e., cystine, methionine, and others; volatile compounds; and sulfates²³. Halogen compounds function within the context of the plant cell to generate chlorinated tryptophan, which is then shuttled into monoterpene indole alkaloid metabolism to yield chlorinated alkaloids. A new functional group halide is introduced into the complex metabolism of *C. roseus*, and is incorporated in a predictable and regioselective manner onto the plant alkaloid products²⁴. In the present study we observed the halogens presence in *P. bicalyculata*. Giwa et al²⁰ confirmed the presence of alkaloids in *P. bicalyculata*, we also confirmed the alkaloids presence in the *P. bicalyculata* by the qualitative analysis. Bromide is needed by eosinophils (white blood cells of the granulocyte class, specialized for dealing with multi-cellular parasites), which use it to generate anti-parasitic brominating compounds by the action of eosinophil peroxidase, a haloperoxidase enzyme which is able to use chloride, but preferentially uses bromide when available²⁵. Esters are widespread in nature. They occur both in plants and in animals.

Small esters, in combination with other volatile compounds, produce the pleasant aroma of fruits. Many chemicals in combination are responsible for specific fruity fragrances; however, often a single compound plays the leading role²⁶. Aldehydes are mainly used in the production of resins when combined with urea, melamine, and phenol (e.g., Bakelite)²⁷. The results of the present study confirms the presence of amides, ethers, alkanes, deuterated R-OH, organo-phosphorus compounds, deuterated amines, aminoacids, aryl aldehydes, alkenes, ketones, sulfites, aliphatic esters, monosubstituted alkenes, sulfur, aldehydes, carboxylic acids, epoxides, alcohols, ketones, aminoacid hydrochlorides, halogen, aliphatic esters, saturated nitrites, nitroso compounds, benzene ring, silicon, boron, aliphatic nitro compounds, secondary alcohols and bromides in *P. bicalyculata*. The results of the present study suggest that *P. bicalyculata* can be used as anti-parasitic and antimicrobial agents in the near future. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition²⁸. In the present study also we produced the UV-VIS and FTIR spectrum for the medicinally important plant. Thus the present studies on *P. bicalyculata* exhibited novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also the presence of adulterants in pharmaceutical industry. Further advanced spectroscopic studies are required for the structural elucidation and identification of compounds.

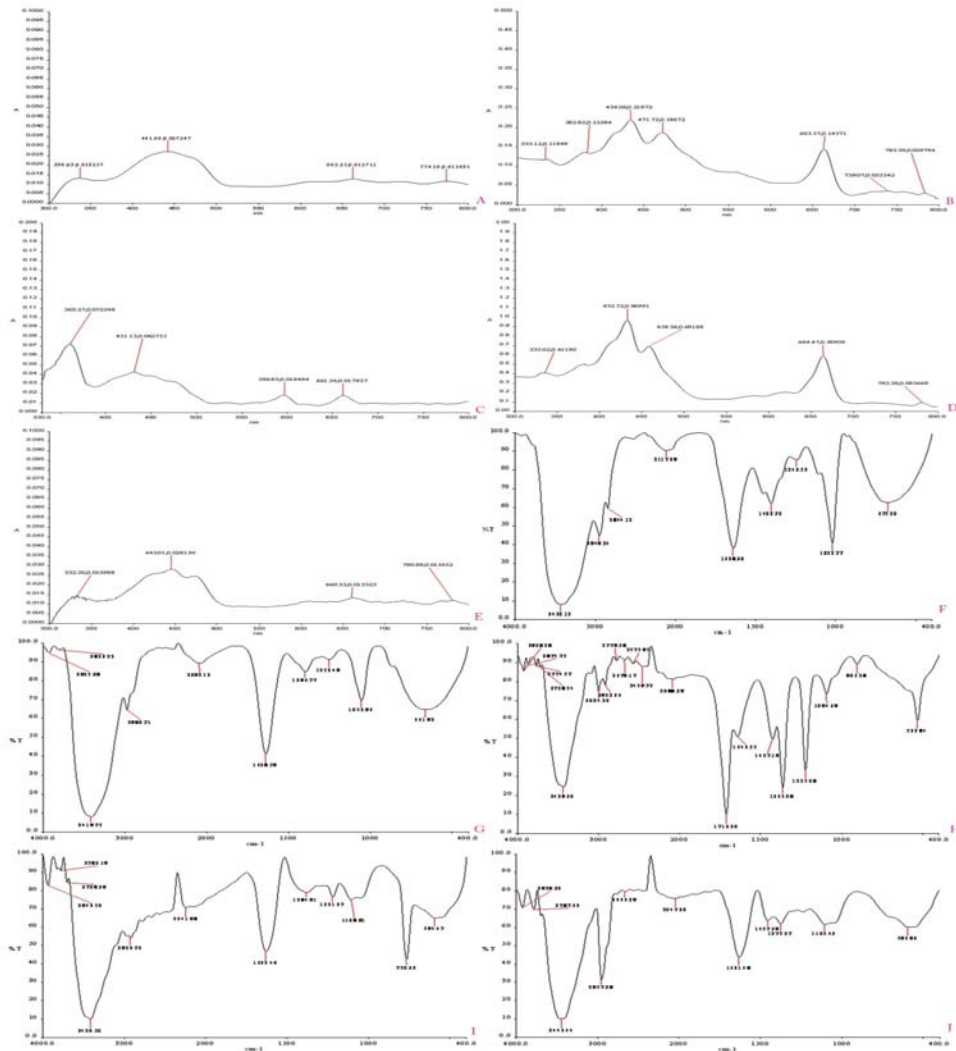


Fig. 1: UV-VIS and FTIR spectrum of *Peristrophe bicalyculata* (Retz.) Nees.

- A. UV-VIS Spectrum of Aqueous Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- B. UV-VIS Spectrum of Ethanol Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- C. UV-VIS Spectrum of Acetone Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- D. UV-VIS Spectrum of Chloroform Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- E. UV-VIS Spectrum of Petroleum Ether Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- F. FTIR Spectrum of Aqueous Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- G. FTIR Spectrum of Ethanol Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- H. FTIR Spectrum of Acetone Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- I. FTIR Spectrum of Chloroform Extract of *Peristrophe bicalyculata* (Retz.) Nees.
- J. FTIR Spectrum of Petroleum Ether Extract of *Peristrophe bicalyculata* (Retz.) Nees.

CONCLUSION

The results of the present study showed that *P. bicalyculata* may be rich sources of phytoconstituents which can be isolated and further screened for different kinds of biological activities, depending on their reported therapeutic uses. Further research will be needed to found out the bioactive class of compounds which may be subjected to subsequent target isolation.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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