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Research Article

"SCREENING OF PRIMARY AND SECONDARY METABOLITES, UV-VIS SPECTRUM AND FTIR" ANALYSIS OF *ACMELLA CALVA (DC.) R.K. JANSEN*. CARMONA RETUSA VAHL. AND *LEPTADENIA RETICULATA W.& A*

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ABSTRACT

In the present study Phytochemical analysis, UV-VIS spectrum and FT-IR analysis of whole plant of *Acmella calva*, *Carmona retusa* and *Leptadenia reticulata* were undertaken. Qualitative phytochemical screening of various solvent extracts of the whole plant of test plants showed the presence of aminoacids, proteins and carbohydrates, alkaloids, flavonoids, tannins, phenolic, quinones, anthroquinones, glycosides, saponins, steroids and sterols, terpenoid, triterpenoids, coumarin, gum and fixed oil. The qualitative UV-VIS spectrum profile of ethanolic extract of *Acmella calva* showed the peaks at 308, 325 and 464 nm with the absorption of 1.938, 2.006 and 0.826, *Carmona retusa* at 212, 261, 270 and 329 nm with the absorption of 3.588, 1.215, 1.257 and 1.134 and *Leptadenia reticulata* at 262, 272, 301 and 328 nm with the absorption of 0.985, 1.058, 0.723 and 0.791 respectively. The results of FTIR peak values indicated the presence of N-H stretching, C-H stretching, O-H bending, C-H Bend out of plane, C-C stretching, phosphates, carbonates, nitrates and silicates. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, aldehydes and amines in ethanolic extract. Further investigations are required in order to test other bioactivities with the aim of increasing the drug arsenal currently used in the treatment and prophylaxis of human diseases.

INTRODUCTION

Plants are the richest source of bioactive organic chemicals on earth. Herbals are effective source of traditional and modern medicines, useful for primary health care. Plants, especially the higher ones have been described as the "sleeping giants" of drug and these medicinal plants have been screened for their chemicals that are potentially active [1].

The importance of medicinal plants, and the contribution of phytomedicine to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines. The active metabolites like phytochemicals from the medicinal plants were under exploration for the development of novel and biodegradable effective drugs as an alternative to the ineffective contemporary medicine. The medicinal plants and their derivatives have long been recognized as an important source of therapeutically effective medicines as they contain secondary metabolites which are potential sources of drugs. Plant based products are healthier, safer and more reliable than synthetic products[2].

The different phytocontituents present in medicinal plants are flavonoids, alkaloids, phenol and tannins, carboxylic acids, terpenes and amino acids and inorganic acids. These phytoconstituents present specific distinctiveness and properties to plants[3]. Therefore, the analysis of these chemical constituents would help in determining various biological activities of plants.

A variety of techniques can be used to determine and estimate the presences of such phytocontituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. The Fourier Transform Infrared (FTIR) spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants. Moreover, FTIR spectroscopy is an established time-saving method to characterize and identify functional groups[4]. Ultraviolet-visible spectrophotometry (UV-Vis) is

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related to the spectroscopy of photons in the UV-visible region. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The colour of the chemicals involved is directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum[5]. Hence, in the present study the phytochemical screening, UV-VIS spectrum and FTIR analysis of three important potential medicinal plants *Acmella calva,* (DC.) R.K. Jansen, *Carmona retusa*, Vahl. and *Leptadenia reticulata*, W. & A. are carried out.

MATERIALS AND METHODS

Collection of plant materials

The test plants were collected from Pillur Beat (Pillur slope RF and Nellithurai RF), Karamadai Range, Western Ghats, Tamil Nadu, India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, Coimbatore by referring the deposited specimen.

Shade drying and powdering of the plant material

Fresh whole plants were collected and washed under tap water to remove adhering dust and then shade dried. The shade dried plant materials were mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and used for Phytochemical, UV-VIS spectrum and FT-IR analysis. Samples were stored in the good grade plastic containers which were maintained at room temperature until analysis [6].

Phytochemical analysis

Qualitative Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in benzene, petroleum ether, ethanol, acetone and aqueous extract of whole plants of test plants.

Preparation of plant extract for UV-VIS spectrum and FTIR analysis

The shade dried leaves of *Acmella calva, Carmona retusa*, and *Leptadenia reticulata* (at 25°C) were powdered in mechanical grinder. 20 gms of whole plant powder was weighed; 150 ml of ethanol was added and kept for 3 days. The extract was filtered using Whatman No.1 filter paper and the supernatant was collected. The residue was again extracted two times (with 3 days of interval for each extraction) and supernatants were collected. The supernatants were pooled and evaporated (at room temperature, 28 ± 1°C) until the volume was reduced to 150 ml. The prepared extract (whole plant powder with ethanol) was stored in air tight bottles for subsequent analysis.

UV-VIS Spectrum analysis

The extract was centrifuged at 3000rpm for 10min and filtered through Whatmann No.1 filter paper. The sample was diluted to 1:10 with the same solvent. The extract was scanned at wave length ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

Table 1 Preliminary phytochemical analysis of test plants

S. No.	Metabolites	*Acmella calva*					*Carmona retusa*					*Leptadenia reticulata*				
		B	E	PE	A	W	B	E	PE	A	W	B	E	PE	A	W
1.	Carbohydrate Test Barfored's Test	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-
2.	Protein & Amino acid 1.Biuret Test	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+
	2.Ninhydrin Test	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+
3.	Alkaloid 1.Mayer's Test	+	+	+	+	+	+	+	-	+	-	+	+	-	-	+
	2.Wagner's Test	+	+	+	+	-	+	+	-	+	-	+	+	-	-	-
4.	Tannin Ferric Chloride test	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+
5.	Phenols	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+
6.	Flavonoids Ferric chloride Test	+	+	-	-	+	+	+	+	-	+	+	+	-	-	+
7.	Terpenoid Test	+	+	-	+	+	-	-	-	+	+	+	+	-	-	+
8.	Triterpenoids Libermann-Burchard Test	+	+	-	-	-	+	+	+	+	+	+	-	-	+	+
9.	Saponin Test Froth formation Test	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
10.	Steriod Test	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
11.	Glycoside Test Borntrager's Test	-	+	-	+	+	+	+	-	-	+	+	+	-	+	+
12.	Anthraquinone Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	Quinone Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14.	Coumarin Test	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+
15.	Gum Test	+	+	-	-	+	+	-	-	-	-	+	+	+	+	+
16.	Starch Test	+	+	-	-	+	+	+	+	-	-	+	-	-	-	+
17.	Fixed oil Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: '+'= Present; '-' = Absent; B= Benzene; E=Ethanol; PE= Petroleum ether; A= Acetone; W= Water

Extraction Procedure

Coarsely powdered plant material were extracted using water, ethanol, benzene, acetone and Petroleum ether through Soxhlet apparatus. The collected extracts were then used for testing the Preliminary phytochemicals.

The peak values of the UV-VIS were recorded.

FTIR analysis

Dried powder of test plant was used for FTIR analysis. 1 mg of the dried powder was encapsulated in 10 mg of KBr pellet, in order to prepare translucent sample discs. The powdered

sample of the pellet was loaded in FTIR spectroscope (Shimadzu, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Successful evaluation of botanical phytocompounds from plant material was largely dependent on the type of solvent used in the extraction procedure. The present phytochemical study on the whole plant of *Acmella calva*, *Carmona retusa*, and *Leptadenia reticulata* using different solvent extracts revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, amino acids, tannins, terpenoids, quinones, anthraquinones and coumarin (Table - 1).

Next to benzene, ethanol and water extracts showed the presence of rich variety of secondary metabolites. Our results are in agreement with the report of Edeoga [7] in several plant samples. Carbohydrates, proteins and amino acids were present in all the extracts of all the test plants. This is in accordance with findings of Leon Stephan Raj [8].

The phytochemical constituents such as alkaloids, glycosides, flavonoids, reducing sugar, tannins, anthraquinone, saponins, polyphenols and cardiac glycosides were present in various extracts of plant parts of *Acmella calva*[9], (Shanthi and Amudha, 2010), *Carmona retusa* [10] (Chandrappa *et al.,* 2012) and in *Leptadenia reticulata*[11] (Girish, *et al*., 2013).

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. UV-VIS spectroscopy is simple, cost-effective and rapid tests for detecting phytocomponents. Fourier Transform Infrared Spectrophotometer (FTIR) was perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed was the salient feature of chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Dried powders of ethanolic extract of *Acmella calva*, *Carmona retusa*, and *Leptadenia reticulata* were subjected to UV-VIS spectrum and FTIR analysis.

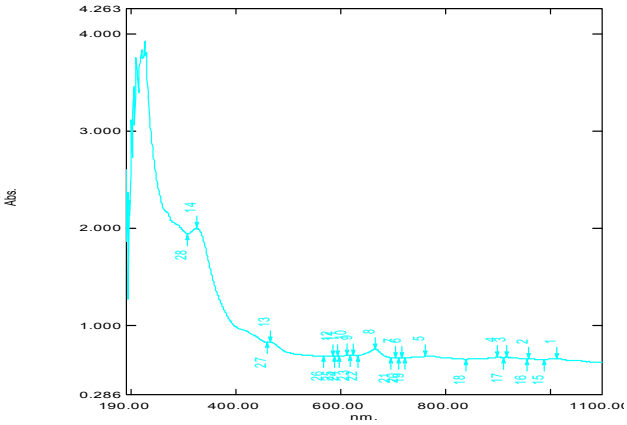

Fig 1 Ultraviolet-Visible Spectroscopy analysis of ethanolic extract of *Acmella calva*

The qualitative UV-VIS spectrum profile of ethanolic extract of *Acmella calva*, *Carmona retusa*, and *Leptadenia reticulata* was selected at wavelength from 260 to 1078 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks for *Acmella calva* at 308, 325 and 464 nm with the absorption of 1.938, 2.006 and 0.826, *Carmona retusa* at 212, 261, 270 and 329 nm with the absorption of 3.588, 1.215, 1.257 and 1.134 and *Leptadenia reticulata* at 262, 272, 301 and 328 nm with the absorption of 0.985, 1.058, 0.723 and 0.791 respectively (Fig. 1, 3 & 5; Table 2, 4 & 6). The results exhibited the presence of flavonoids in all the test plants. Sahaya [12] studied the UV-VIS profile of *Vitex altissima* which showed peaks ranging from 400-700 nm with different absorption respectively, *Acorus calamus* exhibited the peaks at 304 and 237 for flavonoid [13], *Bougainvillea glabra* showed the peaks at 324and 290 for flavonoid [14].

Table 2 UV-VIS Spectrum Peak values of ethanolic extract of *Acmella calva*

S. No.	Wavelength nm.	Abs.
1.	1013.00	0.660
2.	666.00	0.752
3.	624.00	0.690
4.	613.00	0.690
5.	593.00	0.686
6.	584.00	0.684
7.	464.00	0.826
8.	325.00	2.006
9.	632.00	0.686
10.	618.00	0.688
11.	598.00	0.685
12.	589.00	0.684
13.	460.00	0.825
14.	308.00	1.938

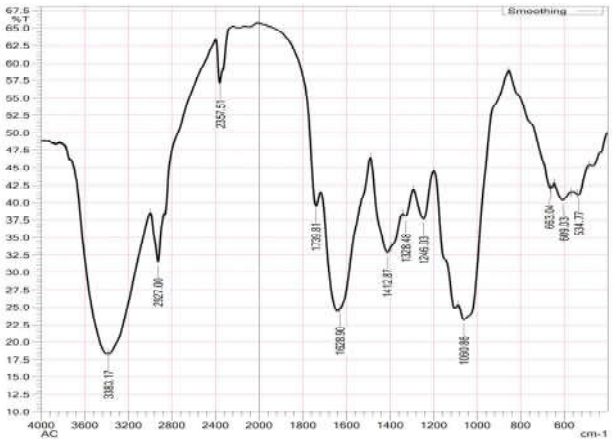

Fig 2 Fourier-Transform Infrared Spectroscopy analysis of ethanolic extract of *Acmella calva*

Table 3 FTIR peak values and functional groups of ethanolic extract of *Acmella calva*

Extracts	Peak value	Functional group	Name of functional group	Vibrations
Ethanolic extract of *Acmella calva*	534.77	C-Br	Bromoalkane	-
	609.03	C-H	Alkane	Rocking
	663.04	C-H	Alkane	Rocking
	1060.86	C-F	Haloalkane	-
	1246.03	C-F	Haloalkane	-
	1328.48	C-F	Haloalkane	-
	1412.87	Carbonates	-	-
	1628.9	C=O	Aldehyde	Stretch
	1739.81	C=O	Aldehyde	Stretch
	2357.51	C≡C	Alkyne	Stretch
	2927	C-H	Alkane	Stretch
	3383.17	O-H	Hydroxyl	Stretch

The results of FTIR peak values and functional groups were represented in Table 3, 5 & 7. The FTIR spectrum profile was illustrated in Fig. 2, 4 & 6. The FTIR gave broad peak at 3383.17 cm^{-1} which indicated the presence of O-H stretching. It showed strong peaks at 2357.51, 1739.81 and 1.628.9 cm^{-1} which indicated the presence of alkyne and aldehyde in *Acmella calva*, 3414.51 cm^{-1} attributed to O-H stretching vibrations, the peak around 2923.14, 2857.08 and 2358.96 cm^{-1} are due to alkane and alkyne in *Carmona retusa* and peak at 3425.61 cm^{-1} attributed to O-H stretching, the peak around 2923.14, 2856.12 and 2360.41 cm^{-1} are due to alkyne and alkane in *Leptadenia reticulata*. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, aldehydes and amines in ethanolic extract of all the test plants.

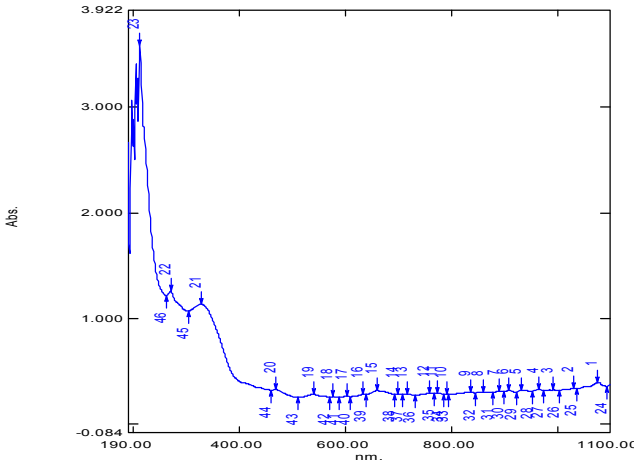

Fig 3 Ultraviolet-Visible Spectroscopy analysis of ethanolic extract of *Carmona retusa*

Table 4 UV-VIS Spectrum Peak values of ethanolic extract of *Carmona retusa*

S. No.	Wavelength nm.	Abs.
1.	1077.00	0.392
2.	1032.00	0.338
3.	993.00	0.322
4.	964.00	0.324
5.	329.00	1.134
6.	270.00	1.257
7.	212.00	3.588
8.	1094.00	0.351
9.	1037.00	0.330
10.	1004.00	0.317
11.	973.00	0.313
12.	953.00	0.305
13.	899.00	0.304
14.	303.00	1.067
15.	261.00	1.215

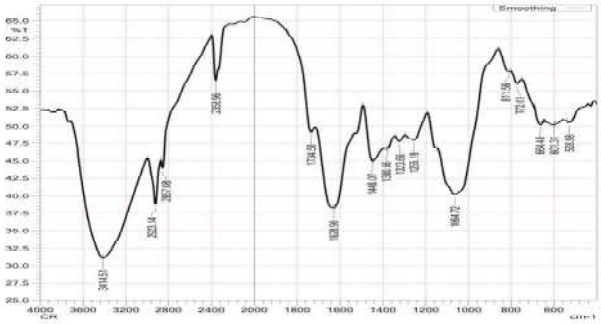

Fig 4 Fourier-Transform Infrared Spectroscopy analysis of ethanolic extract of *Carmona retusa*

All these compounds belong to secondary plant metabolites as per researcher explanations [16, 17]. The presence of above said secondary metabolites could be responsible for the various medicinal properties of *Acmella calva*, *Carmona retusa* and *Leptadenia reticulata* [15]. The FTIR signal at 900, 1500, 1714, 3000, 3100cm-1 in the study plants were considered as an indicator of polyphenols[18]. (Geethu *et al.*, 2014).

Table 5 FTIR peak values and functional groups of ethanolic extract of *Carmona retusa*

Extracts	Peak value	Functional Group	Name of functional group	Vibrations
Ethanolic extract of *Carmona retusa*	528.98	C-Br	Bromoalkane	-
	601.31	C-H	Alkyl	Rocking
	664.48	C-H	Alkyl	Rocking
	772.01	C-Cl	Haloalkane	-
	1064.72811.56	C-H	Alkyl	Bend-out-of plane
	1064.72	C-F	Haloalkane	-
	1255.19	Phosphates	-	-
	1323.66	C-H	Alkyl	Bend-in-plane
	1380.56	Nitrates	-	-
	1448.07	Carbonates	-	-
	1628.9	N-H	Amine	Bending
	1734.5	C-O	Aldehyde	Stretch
	2358.96	C≡C	Alkyne	Stretch
	2857.08	C-H	Alkane	Stretch
	2923.14	C-H	Alkane	Stretch
	3414.51	O-H	Hydroxyl	Stretch

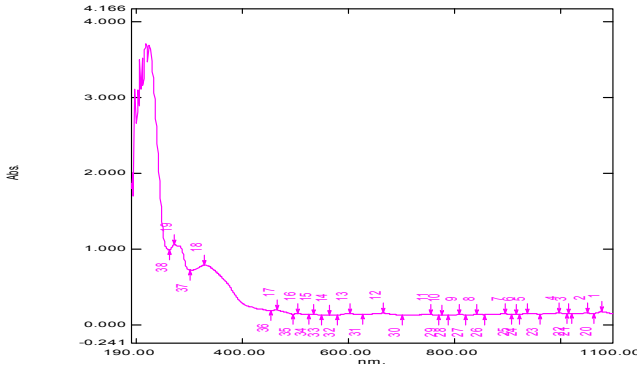

Fig 5 Ultraviolet-Visible Spectroscopy Analysis of ethanolic Extract of *Leptadenia reticulate*

Table 6 UV-VIS Spectrum Peak values of ethanolic extract of *Leptadenia reticulata*

S. o.	Wavelength nm.	Abs.
1.	1078.00	0.174
2.	1051.00	0.156
3.	1017.00	0.150
4.	998.00	0.158
5.	938.00	0.152
6.	503.00	0.145
7.	272.00	1.058
8.	1064.00	0.151
9.	1023.00	0.147
10.	1012.00	0.146
11.	962.00	0.140
12.	922.00	0.141
13.	907.00	0.141
14.	301.00	0.723
15.	262.00	0.985

The FTIR spectroscopic anaylsis of *Acmella calva*, *Carmona retusa*, and *Leptadenia reticulata* revealed the presence of alkaloids due to N-H stretching. Polyphenols and flavonoids

due to O-H stretching, terpens due to C-H group [15]. The functional groups represent the presence of aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters, lactones, ethers, quinones and organic halogen compounds in the test plants.

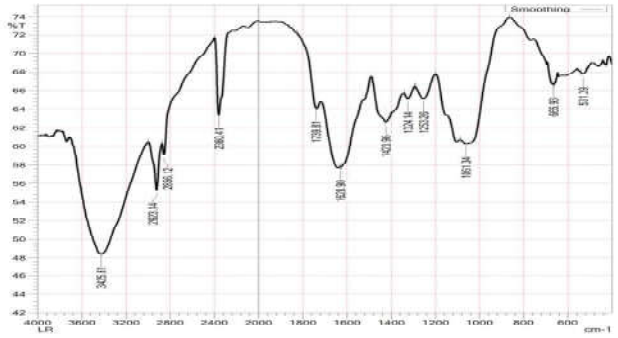

Fig 6 Fourier-Transform Infrared Spectroscopy analysis of ethanolic extract of *Leptadenia reticulate*

Table 7 FTIR peak values and functional groups of ethanolic extract of *Leptadenia reticulata*

Extracts	Peak value	Functional Group	Name of functional group	Vibrations
Ethanolic extract of *Leptadenia reticulata*	531.39	C-Br	Bromoalkane	-
	665.93	C-H	Alkane	Rocking
	1061.34	Phosphates	-	-
	1253.26	O-H	Hydroxyl	Bending
	1324.14	O-H	Hydroxyl	Bending
	1423.96	C-H	Alkane	Bend-in-plane
	1628.9	N-H	Amine	Bending
	1739.81	C-O	Aldehyde	Stretch
	2360.41	C≡C	Alkyne	Stretch
	2856.12	C-H	Alkane	Stretch
	2923.14	C-H	Alkane	Stretch
	3425.61	O-H	Hydroxyl	Stretch

CONCLUSION

The study plants *Acmella calva*, *Carmona retusa* and *Leptadenia reticulata* can be further subjected to isolation of some other new therapeutically valuable substances to carry out further pharmacological evaluation. This investigation has opened up the possibility of the use of these plants in drug development for human consumption possible for the treatment of many ailments and the plants also had protective metabolites against oxidative stress.

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