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Phytochemical and FTIR analysis of different plant parts of *Acmella ciliata* (Kunth) Cass.

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Abstract
Acmella ciliata (Kunth) Cass. of Family Asteraceae, is used as a spice and as a traditional medicine for the treatment of toothaches. The leaf extract has several uses in folk medicine, because of its analgesic and anti-inflammatory properties and the plant is the active ingredient in some herbal medicinal products. The present study was undertaken to detect the phytochemical components from selected plant parts. Methanolic extract of flower heads, leaf, stem and root samples of *A. ciliata* subjected to preliminary qualitative phytochemical analysis revealed reducing sugar, flavanoids, terpenoids, steroids, tannins, coumarins, alkaloid, saponins, glycoside, anthraquinone and phlobatannins, whose presence varied invariably in the samples tested. The FTIR spectrum was used to identify the functional groups of the active components present in the powder samples of the flower heads, stem, leaf and root samples of *A. ciliata* that revealed the presence of amide as functional group which is substantiated with the presence of C=O stretch thereby indicating the occurrence of akylamides.

Keywords: *Acmella ciliata*, FTIR, phytochemical analysis, alkylamides

Introduction
Acmella ciliata (Kunth) Cass. belonging to Family Asteraceae is used as a spice and as a traditional medicine for the treatment of toothaches. It is the active ingredient in the herbal medicinal product Spolera® which is used to treat abrasions and distortions [1]. The extract from the leaves of *A. ciliata* has several uses in folk medicine, because of its analgesic and anti-inflammatory properties [2, 3]. Various phytochemicals such as phenolic compounds, flavanoids, alkaloids, fructose, carbohydrate, lactose, saponins, sesquiterpene lactones, sucrose, tannins and essential oil are found in this plant [4].
Spilanthes is also mentioned as *Acmella* in some of the literature. Monographs have been written about both genera *Acmella* and *Spilanthes* [5, 6] and the "toothache plant" was placed in the genus *Acmella* [6]. Comparative morphological, molecular and chromosome studies indicated these two are discrete genera. There are 30 species and 9 intra-specific taxa in *Acmella* [6]. The occurrence of spilanthol, a high value bioactive molecule was reported from the flower heads of *S. acmella* [7]. Later, it was also obtained from the aerial parts of *S. acmella* [8, 9]. Another report showed similar results with major presence of spilanthol in *S. oleracerea* [10, 11]. Thereafter, several studies have been conducted in different *Spilanthes* spp. which revealed vast occurrence of spilanthol in flower heads [12, 13, 14] and there is no information regarding 'spilanthol' in *Acmella ciliata*. Hence as a preliminary step, the present study was undertaken with the objective of screening of phytochemical components by preliminary phytochemical analyses from selected plant parts of *Acmella ciliata* using soxhlet extraction and maceration as well as the FTIR analysis of the powdered samples.

Material and methods
Plant material
Acmella ciliata (Kunth) Cass. Plants collected from Anchal, Kollam District of Kerala was used as the plant material for preparation of extract for the analysis.

Phytochemical analysis
Sample preparation
Soxhlet extraction and maceration techniques were adopted for the sample preparation in the present study.

Soxhlet extraction of flower heads
The flower heads of *Acmella ciliata* was shade dried and powdered using a mortar and pestle. The phytochemicals present in the flower head samples (10 g) were extracted by the distillation method using Soxhlet apparatus and methanol was used as the solvent for the extraction. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hrs at boiling point of methanol. The extract was concentrated

to dryness and the residue were transferred to pre-weighed sample bottles and were stored in dessicator for further studies.

Maceration of plant material
The whole plant was shade dried and powdered using a mortar and pestle. Powdered samples of 10 g each of flower head, stem, leaves and root in 100 ml of methanol was kept overnight in a rotary shaker. The extract was then subjected to centrifugation three times. The supernatant was collected for further analyses.

Qualitative Analysis
Preliminary phytochemical tests were performed for the detection of secondary metabolites like glycosides, coumarins, flavanoids, alkaloids, saponins, tannins, terpenoids, steroids, phlobatannins, iridoids and anthraquinones using standardized procedures [15].

Test for Reducing Sugars (Fehling's Test)
The aqueous methanol extract was added to boiling Fehling solution in a test tube. Formation of a brick red colour indicated the presence of reducing sugars.

Test for Flavanoids (Shinoda Test)
Take 1 ml of extract and add a few magnesium turnings, followed by the addition of concentrated HCl drop by drop. Development of pink colour indicates the presence of flavanoids.

Liebermann – Burchard Test for Steroids and Terpenoids
A little of the extract was dissolved in dry chloroform and added three drops of acetic anhydride followed by the addition of two to three drops of concentrated sulphuric acid. Appearance of green colour accounts for steroids while pink colour indicated the presence of terpenoids.

Test for Tannins
To 1 ml of extract, add two drops of 5% $FeCl_3$. Presence of dirty green precipitate indicates the presence of tannins.

Test for Coumarins
1 ml of extract was dissolved in methanol followed by the addition of a few drops of alcoholic NaOH. The concentrated HCl was added through the sides of the test tubes. The appearance and disappearance of yellow colour indicates the presence of coumarins.

Test for Alkaloids
5 ml of the extract was warmed with a few drops of Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloid.

Test for Saponins
5 ml of the extract was shaken with 5 ml of distilled water and was heated to the boiling point. Frothing indicates the presence of saponins.

Test for Anthraquinones
To 0.5 g of powdered material add 10 ml of 1% HCl and boiled for five minutes. Filter the sample and allow it to cool. Partition the cool filtrate against equal volume of 10% ammonia solution and allow the layer to separate. Presence of delicate rose pink colour indicates the presence of anthraquinones.

Test for Glycosides (Keller-Killiani Test)
To 0.5 g of extract diluted to 5 ml with distilled water, add 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was treated with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicates the presence of glycosides.

Test for Phlobatannins
Take 1 ml of extract and boil with 5 ml of 1% HCl. Red precipitate indicates the presence of phlobatannins.

FTIR Analysis
The FTIR analysis is done with the powdered samples of flower head, stem, leaf and root of *A. ciliata*. A FTIR spectrometer (FTIR Schimadzu Prestige 21) was used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and a width factor of 30.0 cm^{-1}. De-convolved and second derivative spectra were calculated for Fourier self-deconvoltion, the bands were selected and normalized to unity with Omnic 7 software. Curve fitting of the original spectra was performed with Origin 7 software. The band position of functional group was monitored with knowitall 7.8 software. The spectral region between 3000 and 2800 cm^{-1} was selected to analyze lipids. The

spectral region between 1800 and 1500 cm^{-1} was selected to analyze proteins. The spectral region between 1200 and 1000 cm^{-1} was selected to analyze carbohydrates. Triplicate experiments were conducted and spectra from the first two times of experiments were used for establishment of chemometric models and the spectra from the third time of experiment was used for model validation.

Result and Discussion

Qualitative phytochemical analysis

Methanolic extract of flower heads, leaf, stem and root of *Acmella ciliata* were subjected to preliminary qualitative phytochemical analysis to detect the phytoconstituents such as reducing sugar, flavanoids, terpenoids, steroids, tannins, coumarins, alkaloid, saponins, glycosides, anthraquinone and phlobatannins. The data is given in Table 1. The presence of reducing sugar was confirmed in all the samples except in the root. Test for flavanoids showed positive result in flower head and leaves and it was negative in stem and root samples. Terpenoids were present in leaves and were absent in the other three samples. Test for steroids exhibited positive result only for the stem and leaves and was negative for rest of the samples. Tannins, coumarins and alkaloids were present in flower heads, stem and leaf samples but absent in the root. Glycoside was present in all the samples tested. Test for anthraquinone showed positive result only for leaf samples of *A. ciliata*. Phlobatannins are absent in all samples. A previous study on qualitative phytochemical analysis conducted in *A. ciliata* revealed the presence of steroids, glycosides, alkaloids, tannins, flavanoids, anthraquinones, saponins and cardiac glycosides in methanol leaf extracts [16]. In addition to this, preliminary phytochemical analysis of leaf samples of *A. ciliata* reported the presence of saponins, organic acids, reducing sugars, tannins, alkaloids, steroids and triterpenoids [17] which also support the present finding.

Table 1: Phytochemical qualitative analysis of different plant parts of *A. ciliate*

Phyto-constituents	Flower head	Stem	Leaf	Root
Reducing sugar	+	+	+	-
Flavanoids	+	-	+	-
Terpenoids	-	-	+	-
Steroids	-	+	+	-
Tannins	+	+	+	-
Coumarins	+	+	+	-
Alkaloid	+	+	+	-
Saponins	-	-	+	-
Glycoside	+	+	+	+
Anthraquinone	-	-	+	-
Phlobatannins	-	-	-	-

+ Present; - Absent

FTIR analysis

The FTIR spectrum was used to identify the functional groups of the active components present in the powder samples based on the peaks values in the region of IR radiation. Here, dried powdered samples of the flower head, stem, leaf and root samples of *A. ciliata* were used for the analysis. A number of previous studies on biochemical analysis revealed that the major secondary metabolites present in *Spilanthes*/ *Acmella* spp. is a group of isobutylamides and its respective isomers [18]. FT-IR spectroscopy of the samples analysed here revealed the presence of amide (secondary metabolite) as functional group which is substantiated by the findings in *S. acmella* flower heads, wherein the spectrum at 1744 cm^{-1} transmittance with C=O stretch indicated the presence of amides [19].

FTIR analysis of flower heads of *A. ciliata*

In the FTIR spectrum of flower heads, 13 major peaks were identified (Fig. 1a) which corresponds to the functional groups like alkyl halides, aromatic amines, aliphatic amines, alcohols carboxylic acid, esters, ethers, alkanes, primary and secondary amines and amides. The highest wave number 3282.30 cm^{-1} contain N-H stretch with primary and secondary amines, whereas the wave number 2920.81 cm^{-1} contains C-H stretch of aromatics. The wave numbers 1630.6 cm^{-1} and 1401.44 cm^{-1} confer C=O Stretch with carboxyls and C-C stretch with aromatics; while 1361.50 cm^{-1} contains C-H rock with alkanes. The spectrum at 1630.66 cm^{-1} transmittance with C=O stretch showed the presence of amides as per previous report [19]. The peaks at 12.35.73 cm^{-1} and 1019.54 cm^{-1} indicated C-N stretch with aliphatic amines. The peak value 508.57 cm^{-1} to 538.56 cm^{-1} contain carbon halogen bonds with alkyl halides (Table 2).

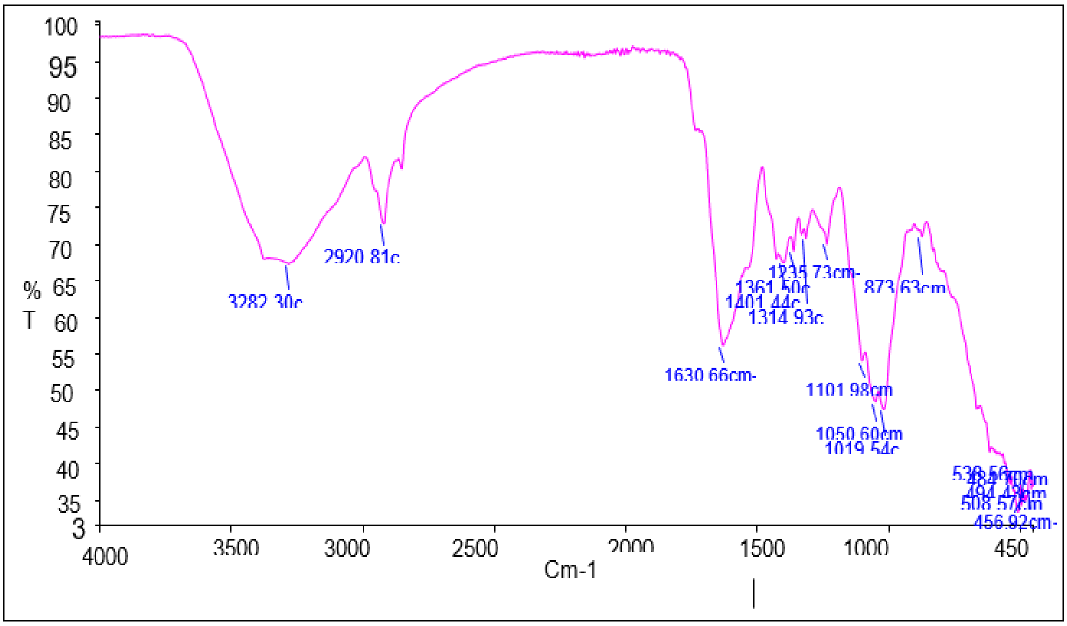

Fig 1a: FTIR spectral diagram of flower heads of *A. ciliata*

Table 2: FTIR frequency table for flower heads of *A. ciliate*

Sl. No	Frequency (cm^{-1})	Bond	Functional group
1.	508.57	C-Cl stretch	Alkyl halides
2.	538.56	C-Br stretch	Alkyl halides
3.	873.63	C-H ‘oop’	Aromatics
4.	1019.54	C-N stretch	Aliphatic amines
5.	1050.60	C-N stretch	Aliphatic amines
6.	1101.98	C-O stretch	Alcohols, Carboxylic acid, ester, ether
7.	1235.73	C-N stretch	Aliphatic amines
8.	1314.93	C-N stretch	Aromatic amines
9.	1361.50	C-H rock	Alkanes
10.	1401.44	C-C stretch	Aromatics
11.	1630.66	C=O stretch	Carboxyls; amides*
12.	2920.81	C-H stretch	Aromatics
13.	3282.30	N-H stretch	Primary, secondary amines, amides

FTIR analysis of stem of *A. ciliata*

The FTIR analysis of the stem showed 7 major peaks between 526.94 cm^{-1} to 3292.10 cm^{-1} depicting carboxylic acids, aldehydes, primary amines, alkanes, aliphatic amines, alkyl halides as the functional groups (Fig. 1b). The highest frequency was 3292.10 cm^{-1} that indicated O-H stretch with carboxylic acids, while 1736.49 cm^{-1} contains C=O stretch of aldehydes. The spectrum at 1736.49 cm^{-1} transmittance with C=O stretch showed the presence of amides here as reported in *S. acmella* [19]. The peaks 1024.81 cm^{-1} and 1216.92 cm^{-1} contains C-N stretch with aliphatic amines and those at 1366.39 cm^{-1} and 1612.72 cm^{-1} revealed C-H rock and N-H bend with alkanes and primary amines respectively (Table 3).

Table 3: FTIR frequency for stem of *A. ciliate*

Sl. No	Frequency (cm-1)	Bond	Functional group
1.	526.94	C-Br stretch	Alkyl halides
2.	1024.81	C- N stretch	Aliphatic amines
3.	1216.92	C-N stretch	Aliphatic amines
4.	1366.39	C-H rock	Alkanes
5.	1612.72	N-H bend	Primary amines
6.	1736.49	C=O stretch	Aldehydes, saturated aliphaticAmides*
7.	3292.10	O-H stretch	Carboxylic acids

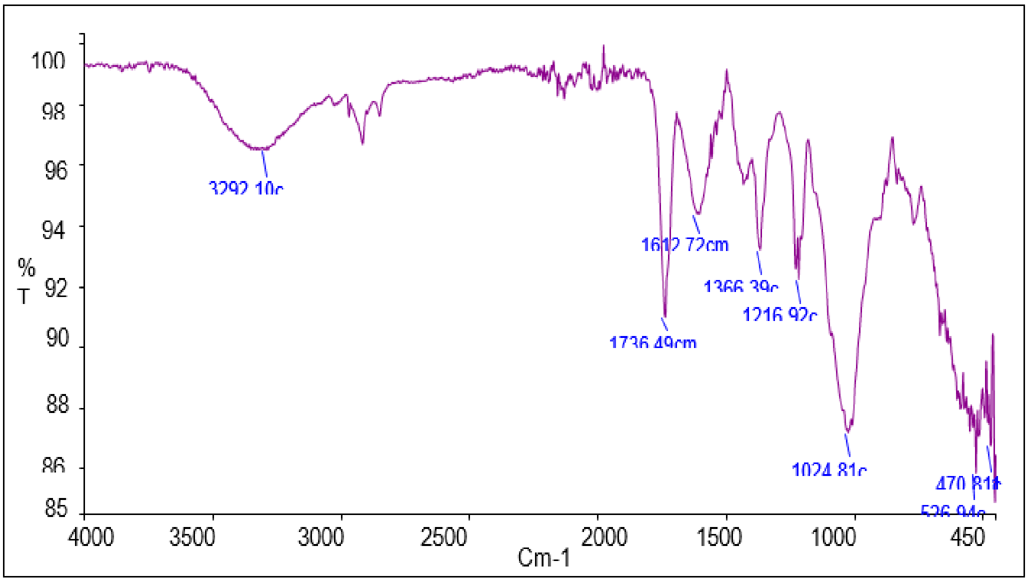

Fig 1b: FTIR spectral diagram of stem of *A. ciliata*

FTIR analysis of leaves of *A. ciliata*

The FTIR analysis of the leaf exhibited 9 major peaks between 527.42 cm^{-1} and 3289.97 cm^{-1} with carboxylic acids, alkanes, aldehydes, primary amines, alkyl halides, aliphatic amines as functional groups (Fig. 1c). The highest frequency 3289.97 cm^{-1} contains O-H stretch with carboxylic acid, while that at 2917.70 cm^{-1} and 1371.60 cm^{-1} corresponds to C-H stretch with alkanes. Primary amines has N-H bend at frequency 1601.89 cm^{-1} while the frequency at 1735.36 cm^{-1} is that due to C=O stretch revealing the presence of amides that was substantiated by previous report [19]. Alkyl halides has C-Br stretch with frequencies 527.42 cm^{-1} and 517.86 cm^{-1} (Table 4).

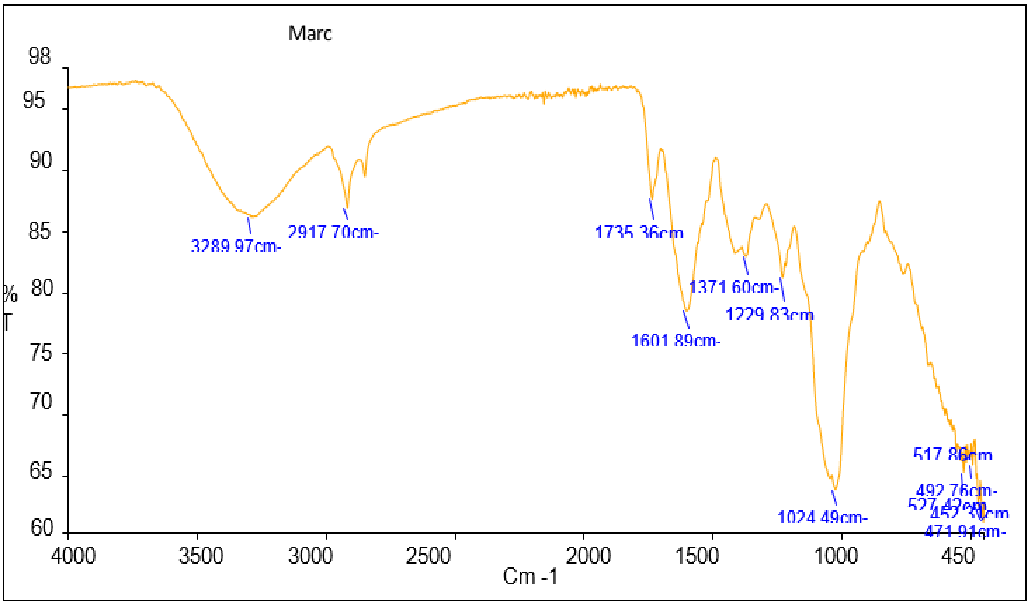

Fig 1c: FTIR spectral diagram of leaf of *A. ciliata*

Table 4: FTIR frequency table of leaf of *A. ciliate*

Sl. No	Frequency (cm^{-1})	Bond	Functional group
1.	527.42	C-Br stretch	Alkyl halides
2.	517.86	C-Br stretch	Alkyl halides
3.	1024 49	C-N stretch	Aliphatic amines
4.	1229.83	C-H wag (CH_2X)	Alkyl halides
5.	1371.60	C-H rock	Alkanes
6.	1601.89	N-H bend	Primary amines
7.	1735.36	C=O stretch	Aldehydes, saturated aliphatic
8.	2917.70	C-H stretch	Alkanes
9.	3289.97	O-H stretch	Carboxylic acids

FTIR analysis of roots of *A. ciliata*

The FTIR analysis of roots exhibited 8 major peaks between 530.66 cm^{-1} and 3320.11 cm^{-1} with alkynes, alkanes, esters, primary amines, aromatics, aliphatic amines and alkyl halides as functional group (Fig. 1d). The highest frequency 3320.11 cm^{-1} contains –C (triple bond) C-H:C-H stretch with alkynes; while that at 2917.70 cm^{-1} contains C-H stretch with alkanes. Esters with frequency 1736.77 cm^{-1} contain C=O stretch and 1602.88 cm^{-1} contains N-H bend with primary amines. The spectrum at 1736.77 cm^{-1} transmittance due to C=O stretch denoted the presence of amides as in *S. acmella* [19], while the same at 1420.28 cm^{-1} revealed C-C stretch of aromatics. The peak at 1229.68 cm^{-1} contains C-H wag – (CH2X) with alkyl halides, whereas that at 1029.45 cm^{-1} indicated the presence of aliphatic amines because of C-N stretch. The lowest frequency 530.66 cm^{-1} indicated the presence of alkyl halides with C-Br stretch (Table 5).

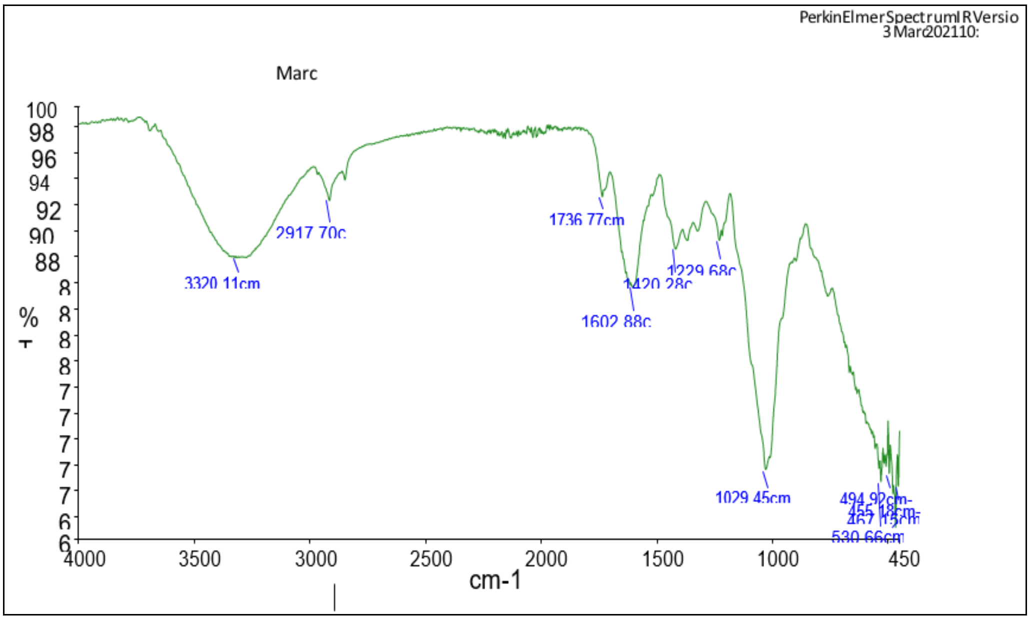

Fig 3d: FTIR spectral diagram of roots of *A. ciliata*

Table 5: FTIR frequency table of roots of *A. ciliate*

Sl. No	Frequency (cm^{-1})	Bond	Functional group
1.	530.66	C-Br stretch	Alkyl halides
2.	1029.45	C-N stretch	Aliphatic amines
3.	1229.68	C-H wag (CH_2X)	Alkyl halides
4.	1420.28	C-C stretch	Aromatics
5.	1602.88	N-H bend	Primary amines
6.	1736.77	C=O stretch	Esters, saturated aliphatic amides*
7.	3320.11	-C(triple bond)C-H:C-H stretch	Alkynes

Conclusion

Here, the preliminary qualitative phytochemical analysis revealed reducing sugar, flavanoids, terpenoids, steroids, tannins, coumarins, alkaloid, saponins, glycoside, anthraquinone and phlobatannins in *A. ciliata* whose presence varied invariably in different samples. The FTIR spectrum used to identify the functional groups of the active components present in the powder samples of the flower heads, stem, leaf and root samples of *A. ciliata* revealed the presence of amide functional group which is substantiated with the presence of C=O stretch thereby indicating the occurrence of alkylamides. The alkylamide 'spilanthol' has been previously identified as the key bioactive compound in *Acmella* spp. and there are no reports yet regarding the determination of this N-alkylamide in different plant parts of *A. ciliata*; the findings established here would be a stepping stone for exploration of *A. ciliata* as a source of spilanthol.

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