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Characterization studies on medicinal plant of *Andrographis paniculata* (NEES).

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Abstract
Andrographis paniculata (Nees), is a valuable traditional medicinal plant and it has many important bioactive compounds. It cures and prevents a number of diseases in human beings. It is a boon of the nature of human healthy life. It cures cold, fever, colic pain, active against inflammatory, antidiabetic activity, antioxidant, antifertility, cardiovascular and anti-virus including inhibited HIV. In this present study, the bioactive compounds were analyzed using IR, NMR spectrum, and GC-MS. Many of these compounds found were effectively used for human benefits.

Keywords: Andrographis paniculata, antidiabetic, inflammatory, HIV, GC-MS and bioactive compounds.

1. Introduction
Ayurveda, Siddha and Unani medicines occupy the greater place of medicinal world. Ayurveda from north India, Siddha from south India (Tamil Nadu and Kerala) and the Unani from Arabic medicine. In olden periods, medicinal plants are used for human health and cure many diseases and disorders. Worldwide, India is a richest biodiversity country and it has a 45,000 plant species. In India, around 20,000 medicinal plants have been recorded recently. 500 traditional communities cure the different disease [1]. *Andrographis paniculata* is a one of the wonderful medicinal plant. Commonly known as "king of bitters" and it comes under the family of *Acanthaceae*. It is a shrub type tropical traditional medicinal plant. The habitat of *Andrographis paniculata* are forest, along roadside, hills and villages. It comes in all types of soil and easily grows and uptake the minimal amount of water. It is an annual herb. It is branched, erect growing up to 1-2 meter in height. Stems are sharply quadrangular. Leaves are dark green, opposite, lens like structure and upper side having hairy. The fruits are green colour capsule and having the 5-8 seeds. The seeds are green, yellow and brown, ovoid shape, and size is small Seeds are the origin of the plant and ubiquitous in its native areas. The seeds are small and remain dormant of 5-6 months [2]. The roots are cylindrical, curved, tapers, 5 -20 cm long and 1.5-5 cm in diameter. It is grayish brown, the inside is starchy white [3].
The leaves and stems of the plant are used to extract the active phytochemicals. The extract of the plants contains diterpenes, flavonoids and stigma sterols [4]. It grows abundantly in southeastern Asia: Indonesia, India, Sri Lanka and Pakistan. It is cultivated extensively in China and Thailand. It grows best in the tropical and sub-tropical areas of the world. It's well-known for medicinal properties. It grows easily in all types of soil. In fact, it grows in poor soil types where almost no other plant can be cultivated. In recent years, medicinal area is expected the traditional plants, hence scientist visions are turned by the herbs and the traditional meditation plants. The whole plant of the *Andrographis paniculata* having medicinal valuable compounds. Approximately 28 species of *Andrographis paniculata* are known and indigenous to Asia. Screening of active compounds from the plant is used against so many diseases. This present investigation was carried out to know the bioactive compounds of *Andrographis paniculata* and they are analyzed by IR, NMR spectrum and GC-MS.

Medicinal value of *Andrographis paniculata*
The medicinal value of these plants is due to some chemical active substances that produce a definite physiological action on the human body [5]. Many medicinal plants and herbs are active against antimicrobial activity [6, 7]. Methanol extracts of *Andrographis paniculata* have antimicrobial activity against two bacterial pathogen *viz., Pseudomonas aeruginosa* and *S. aureus* [8, 9]. *Andrographis paniculata* found a valuable medicinal plant in many popular systems of medicine including Ayurveda, Siddha and Unani. It cures dysentery, antivenom,

fever, cholera, diabetes, influenza and swelling [10, 11]. The decoction of this plant used against jaundice. The extract of this plant exhibit antifertility, anti-fungal and antinematicidal activities. *Andrograpis paniculata* Snake repellent and locally called or in Tamil Nilavembu, Siriyanangai and Periyanangai in Tamil Nadu [12]. Headaches, nasal and throat symptoms and general malaise showed the most improvement. Ayurveda and other traditional medicinal system for the treatment of diabetes, describe a number of plants used as herbal drugs. It has a lower side-effect and reasonable cost. The active principles present in medicinal plants have been reported to possess the pancreatic beta cells re- generating, insulin releasing and fighting the problem of insulin resistance [13]. *Andrographis paniculata* contains diterpens, locations and flavonoid, flavonoids mainly exist in the root, but also can be isolated from the leaves. The leaves contain two bitter lactone andrographolide, and kalmegh, Active compounds extracted with ethanol or methanol from the whole plant, leaf and stem of *Andrographis paniculata* include over 20 diterpenoids and over 10 flavonoids [14, 15].

Materials and methods

Collection of plant material

The leaves of *A. paniculata* was collected from the trial plots maintain in Faculty of Agriculture, and verified by Dr. Manivannan Professor and Head, Department of Horticulture Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu.

Sample preparation

Fresh and healthy leaves of *Andrographis paniculata* were picked up and washed thoroughly for 3-4 times in running tap water to remove soil particles and finely clean with distilled water. The leaves were dried in shade and ground into fine powder and stored in air tight polyethylene bags. The one gram of shade dried leaf powder was successfully dissolved in 5 ml of methanol. The extract should be kept for 72 h separately. Then the extract was filtered with the help of Whatman No. 1 filter paper and concentrated using vacuum distillation [16].

FT-IR analysis

An experiment was conducted to study the FT-IR spectroscopy analysis of *Andrographis paniculata* leaf extracts. The result was presented in the Table 1 and the fig 1. The infrared spectroscopic analysis gives the information about the possible functional groups of active principles. Solid FT-IR spectroscopic analysis using Kbr disc method was employed for the functional groups analysis of the leaf extract of *Andrographis paniculata.*

NMR- Analysis

In the NMR- spectroscopic analysed and the result was presented in Table 2 and the Fig 2 and 3. ^{1}H AND ^{13} C NMR spectra were recorded on a Bruker Avance AV-500 instrument (Bruker Co., Switzerland) at 500 MHZ. CD_3OD was used as a solvent and TMS was used as an internal standard. Chemical shifts (∂) were expressed in ppm shift. Mass spectra were recorded on an Agilent 1100 LC-MSD- Trap – SL mass spectrometer (Agilent Technologies Co., Ltd, USA). The structure for compound interpretation was determined by ^{1}H NMR and the ^{13} C NMR studies carried out at the Department of Chemistry (Annamalai University).

GC-MS analysis

The GC-MS analysis used Perkin Elmer Clarus 500 & the software used was Turbomass ver 5.2.0. The Clarus 500 GC used in the analysis employed a capillary column with Elite-5MS (5% phenyl 95% dimethyl polysiloxane) with a 30m column length and 250 µm column id. In the initial GC conditions, the oven was maintained @ 50 ºC with a 1 min holding time and @ 8 ºC/ min to 200 ºC (Mass analyzer). There were various parameters involved in the operation of Perkin Elmer Clarus 500 (in case of GC, Carrier gas: Helium @ flow rate 1 ml / min, Split ratio 1: 10, in case of MS, Mass Range: 40-600 amu, Type of ionization: Electron Ionization (EI), Electron energy: 70ev, Transfer line and source temperature: 200 ºC, 180 ºC, Sample injected: 1.0µL).

Identification of compounds

The mass spectrum of GC-MS results were confirmed by using the National Institute of Standards and Technology (NIST), year 2005 library [17]. It has more than 62,000 patterns. The spectrum of separated components was compared with the spectrum of NIST library database for about 95 per cent matching predicting the compound. The name, molecular formula, weight and structure of the component of the test materials were as curtained.

Result and Discussion

Andrographis paniculata consist several therapeutically important active principle compounds in the aerial parts. [18, 19, 20]. AGPs were further characterized by FTIR for denoting the presence of the different functional groups. The evolution of this studies FT-IR absorbance of characteristic band structures was followed by establishing the ratios between the main observed peaks. The compounds show the leading bands in the regions of 1250- 1020 cm^{-1}, 3000- 2850 cm^{-1}, 1680- 1640 cm^{-1}, 1550- 1475 cm^{-1} and 1550- 1475 cm^{-1}. The appearance of these peaks reveals that the presence of various functional groups like aliphatic amines, alkanes, alkenes, nitro compounds aromatics, alcohols and phenolic groups. The spectrum obtained in this experiment showed a broad and sharp prominent bands *viz*., 1052.34 cm^{-1}, 2920 cm^{-1}, 2845.24 cm^{-1}, 1645.92 cm^{-1}, 1550.70 cm^{-1} and 1504.11 cm^{-1}. According to the peak value of 1052.34 cm^{-1} was present in C-N stretch in between the aliphatic amines. The band at the 2920.20 cm^{-1} and 2845.24 cm^{-1} were revealed that the C-H stretch and it proposed the functional group was alkanes, the frequency value of 1645. 92 cm^{-1} was – C = C stretching and the alkanes groups were present. The bands at the corresponding to the 1550.70 cm-^{1} was condensing the functional group of Nitro aromatic compounds and have the N-O asymmetric stretch, and the bands at 1504.11 cm^{-1} was, according to the N-O asymmetric stretch and the functional group of aromatic were present respectively.

The ^{1}H NMR spectrum of the obtained in this experiment the (figure 2) showed a leading peak *viz.,* the chemical shift was 1.143, 0.962, 1.260, 0.644, 2.050, 4.783, 4.028, 4.860, 6.151, 6.151, 6.128, 7.282, 7.553 and 7.73. The chemical shift 1.143 indicates the proton was tertiary alkyl (R_3CH), and the 0.962 evaluated, namely 14-deoxyandrographisside ($C_{26}H_{40}O_9$) and the type of proton primary alkyl (RCH_3). The ^{1}HNMR of 1.260 and 0.644 exposed the proton was secondary alkyl and the name was 14- Deoxyandrographolide ($C_{20}H_{30}O_4$). The chemical shift of 2.050 indicates the amino group and the name was Andrographiside the molecular formula ($C_{26}H_{40}0_{10}$) and the bond RNH_2. The Vinylic type of proton found in 4.783 and expose the neoandrographolide, $R_2C{=}CH_2$ and the molecular formula ($C_{26}H_{40}O_8$). The chemical shift of 4.028 indicates the vinylic type proton. The namely andrographolide the molecular formula ($C_{20}H_{30}O_5$) the $R_2C{=}CH$ bond. The Aromatic type of proton mentioned 14-Deoxy-12-

hydroxyandrorapholide the molecular formula ($C_{20}H_{30}O_5$) the chemical shift was 6.151. Similar types of functional groups were also observed in *Arabidopsis thaliana* [21]. The phenolic group indicates the 14-O- benzoyl – 3, 19 isopropylidene andrographolide the ArO_4 bond is found. The chemical shift is presented in 7.282, the 7.553 and 7.737 chemical shift mentioned the phenolic group and the ArO_4 bond was present and the name 14-O-benzyl -3, 19 isopropylidene andrographolide was found. The ^{13}C NMR spectrum of the obtained in this experiment the (figure 3) showed a leading peak *viz*., 77.38, 77.06, 76.74, 31.94, 61.67, 27.23, 29.71, 32.20, 29.71, 32.20, 29.71, 135.97, 38. 20, 39.35, 39.35, 27.23 and 167.71.

The chemical shift 77.38 indicate the proton was β glucophranosyl aromatic protein. The ^{13}C NMR peak value 77.06 and 76.74 mentioned D- glucose. The peak value of 31.94 indicates the 14-deoxy-12-hydroxy andrographolide-19-O-β-Dglucuronide. The chemical shift peak value of 61.67 expose the glucose, 27.23 and the 29.71 showed neoandrorapholide ($C_{26}H_{43}0_8$). Following the peak value was 32.20 and 29.71 indicate the neoandrographolide the peak value of 135.97 showed the α, β- unsaturated γ- lactone ring was present, then the following the peak value 39.35, 27.23 was shown neoandrographolide and 167.71 was shown a methoxy group. ^{13}C NMR spectrum six characteristic aromatic carbons at δ 166.1, 165.2, 161.0, 98.5, 96.1 and 95.4 indicated the occurrence of an unsymmetrically substituted phloroglucinol unit [22]. The therapeutic properties include 14-deoxy-11- oxoandrographolide, 14- deoxy -11-12-didehydroandrographolide D, 14- deoxy- andrographolide, non-biter compound neoandrographolide, homoandrographolide, andrographosterol, andrographane, andrographosterine, andrograpanin, α-sitosterol, stidmasterol, apigenin- 7, 4-di-O-methyl ester, 5- hydroxyl 7,8,2,3-tetramethoxy flavones, monohydroxy trimethyl flavones, andrographnin, dihydroxy-di- methoxy flavones, panicolin, andrographoneo, andrographoside, andropaniculosin, isoandrographapholide and skullcaflavone [23].

Fifty four compounds were identified in a methanol fraction of *Andrographis paniculata* leaves extract by GC-MS analysis. The chromatogram obtained by a methanol fraction of *Andrographis paniculata* Leaf was shown in (Fig 1). The active principle, area of the peak, Concentration (%), Retention time (RT), Molecular formula, Molecular weight and name of the compounds were presented in Table 1. Among the fifty four compounds of *Andrographis paniculata* only the fifteen compounds were found in higher percentage. This shows The following of the compounds are named l - (+) - Ascorbic acid 2,6-dihexadecanoate ($C_{38}H_{68}O_8$), 2- Hexadecen -1-OL, 3, 7, 11, 15 – Tetramethyl-, [R-[($C_{20}H_{40}O$), 1-Phenanthrenecarboxylic acid, 7- Ethenyl ($C2_0H_{30}O_3$), 9, 12, 15 - Octadecatrienoic acid, (Z,Z,Z) - ($C_{18}H_{30}O_2$), 2, 6, 10-Trimethyl, 14 – Ethylene -14- Pentadecne ($C_{20}H_{38}$), (3E,5E,7E) – 6 – Methyl-8 - (2,6,6-Trimethyl-1-Cyclohexenyl) -3, ($C_{18}H_{26}O$), 9 - Octadecenoic acid ($C_{18}H_{34}O_2$), 13,15Octacosadiyne ($C_{28}H_{50}$), Stigmast -5- EN -3 - OL, (3.Beta.) - ($C_{29}H_{50}O$), Octadecanoic acid ($C_{18}H_{36}O_2$), 2-Hexadecen -1- OL, 3,7,11,15-Tetramethyl-, [R-[($C_{20}H_{40}O$), Geranyl-.alpha.-Terpinene ($C_{20}H_{32}$), 9,12,15-Octadecatrienoic acid, Methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$), Methyl 8,10-Octadecadiynoate ($C_{19}H_{30}O_2$), Stigmasterol ($C_{29}H_{48}O$). The highest compound name therapeutic activity is mentioned with reference were shown in (Table 3). The interpretation of mass spectrum GC-MS was confirmed by the database of National Institute Standard and Technique (NIST, 2015). It is having more patterns. Some compounds are unknown, hence the name molecular weight, formula and structure of the compounds of the test material are determined. *Andrographis paniculata*, However, this may not be out of place to mention that the isolation of 1, 1, 3- trietooxypropane, n- hexadecanoic acid, 9, 12-octa decadienolic acid and oleic acid is the first report in *Andrographis paniculata* plant [24]. [25] Similar to our studies also identified as thirteen components by the ethanol extracts. The characterization analysis revealed that the various number of compounds with different chemical structures. The presents of various bioactive compounds confirms application of *Andrograpis paniculata* for various ailments by traditional practitioners.

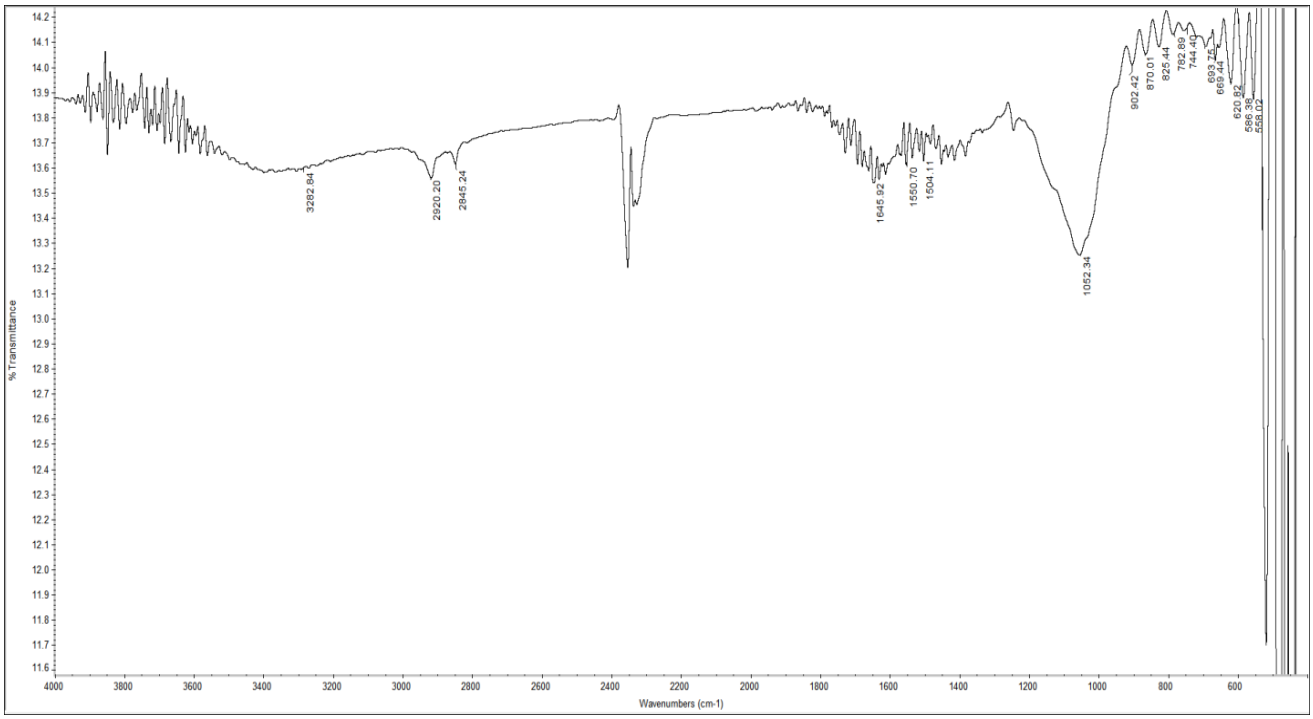

Fig 1: FT-IR leaf extract of *Andrographis paniculata.*

Table 1: Phytoconstituents present in leaf extract of *Andrographis paniculata* (FT-IR)

S.NO	Frequency	Bond	Functional group
1.	1052.34	C-N Stretch	Aliphatic amines
2.	2920.20	C-H Stretch	Alkanes
3.	2845.24	C-H Stretch	Alkanes
4.	1645.92	-C=C Stretch	Alkanes
5.	1550.70	N-O asymmetric Stretch	Nitro compounds aromatics
6.	1504.11	N-O asymmetric Stretch	Aliphatic Amines
7.	3282.84	N-H Stretch	Alcohols, Phenols
8.	902.42	N-H Wag	Primary, Secondary amines
9.	870.01	N-H Wag	Primary, Secondary amines
10.	825.44	N-H Wag	Primary, Secondary amines
11.	782.89	C-C_1 Stretch	Alkyl halides
12.	744.40	C-C_1 Stretch	Alkyl halides
13.	693.75	- C (Triple bond)	Alkynes
14.	669.44	C-H: C-H bond	Alkynes
15.	620.82	C-Br Stretch	Alkyl halides
16.	586.38	-	Unknown
17.	558.02	-	Unknown

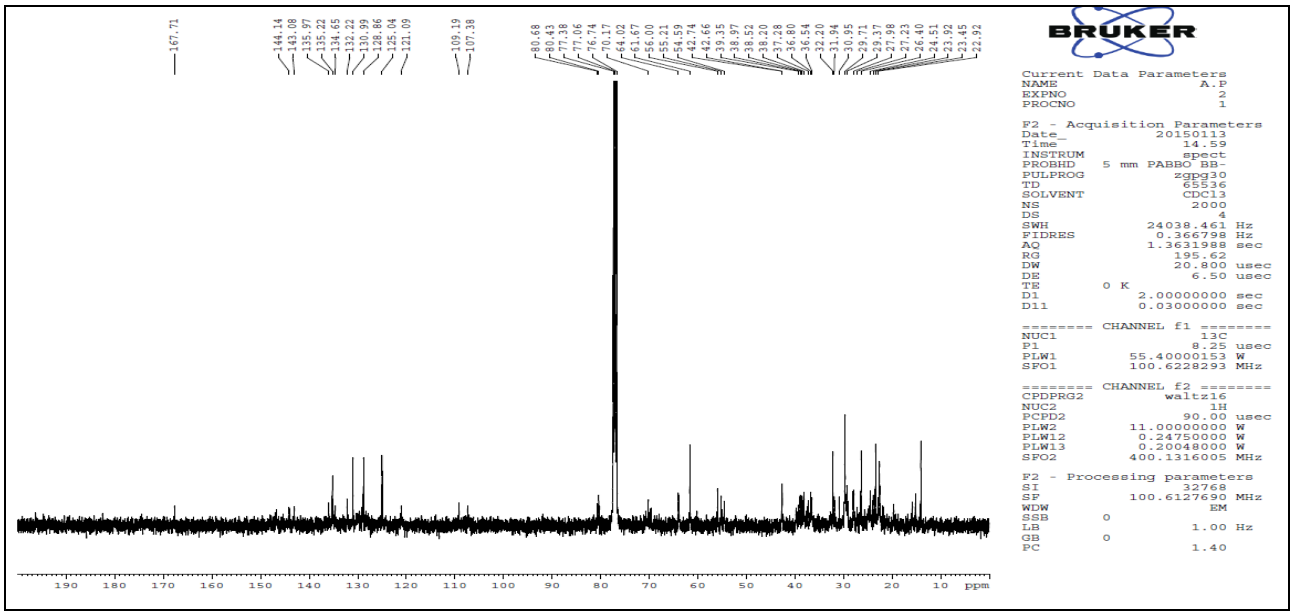

Fig 2: H NMR chemical shifts of flavonoids in methanol

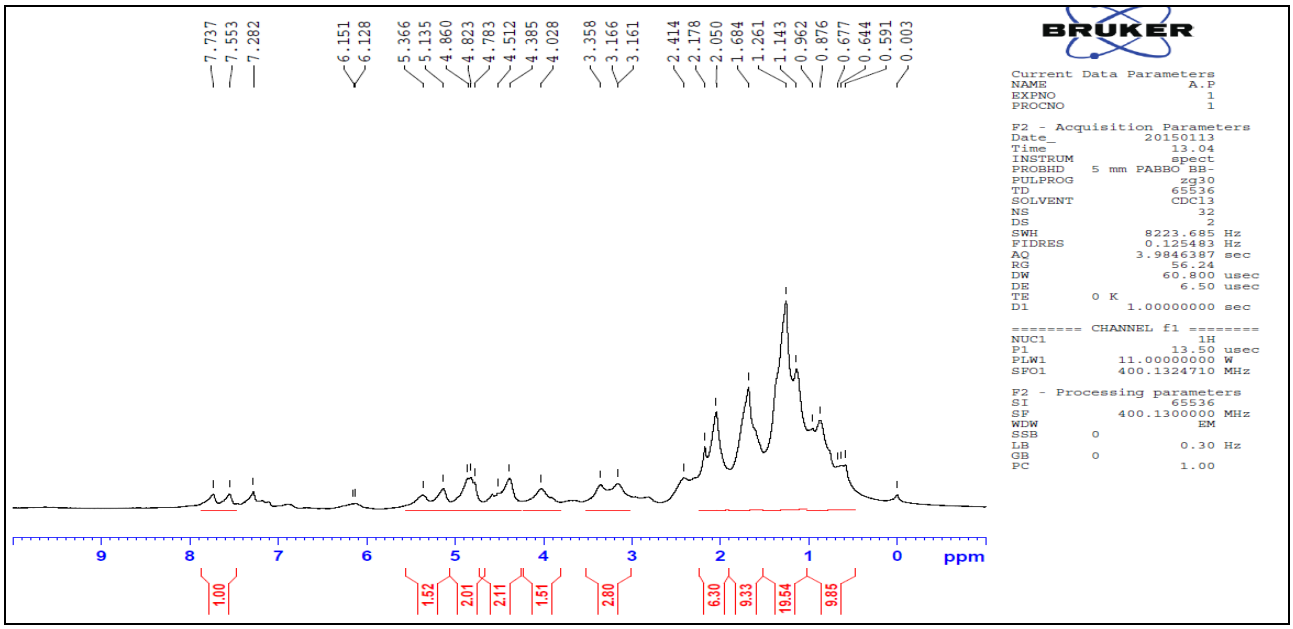

Fig 3: H NMR chemical shifts of flavonoids in methanol

Table 2: Chemical Shift and proton position of *Andrographis paniculata* (^{13} CNMR).

S.NO	Chemical shift	Compounds
1.	77.38	β glucophranosyl aromeric proton
2.	77.06	D-glucose
3.	76.04	Glucuronic acid
4.	31.94	14- deoxy-12-hydroxy-andrographolide-19-O-β- D-glucose
5.	61.67	Glucose
6.	27.23	Unknown
7.	29.71	Neoandrographolide
8.	32.20	Unknown
9.	29.71	Neoandrographolide
10.	32.20	Unknown
11.	29.71	Neoandrographolide
12.	135.97	α,β- unsaturated γ- lactone ring
13.	38.20	Unknown
14.	39.35	Neoandrographolide
15.	27.23	Unknown
16.	167.71	Methoxy group

Table 3: Chemical Shift and proton position of *Andrographis paniculata* (^{1}H NMR).

S.NO	Chemical shift	Type of proton	Bond and Compounds
1.	1.143	Tertiary alkyl	R_3CH
2.	0.962	Primary alkyl	14-deoxyandrographiside ($C_{26}H_{40}O_9$), RCH_3
3.	1.26	Secondary alkyl	14-deoxyandrographolide ($C_{20}H_{30}O_4$), R_2CH_2
4.	0.644	Alcohol hydroxyl	ROH
5.	2.050	Amino	14-deoxyandrographiside ($C_{26}H_{40}O_{10}$)
6.	4.783	Vinylic	Neoandrographolide ($C_{26}H_{40}O_8$)
7.	4.028	Alkyl fluoride	3,14 Dideoxyandrographolide ($C_{20}H_{30}O_3$), RCH_2F
8.	4.860	Vinylic	andrographolide ($C_{20}H_{30}O_5$) $R_2C=CH$
9.	6.151	Aromatic	14-Deoxy-12-hydroxyandrographolide ($C_{20}H_{30}O_5$) ArH
10.	7.82	Phenolic	14-O-benzoyl-3,19 isopropylidene andrographolide (ArO_4)
11.	7.553	Phenolic	ArO_4
12.	7.737	phenolic	14-O-BENZOYL-3,19 isopropylidene andrographolide.(ArO_4)

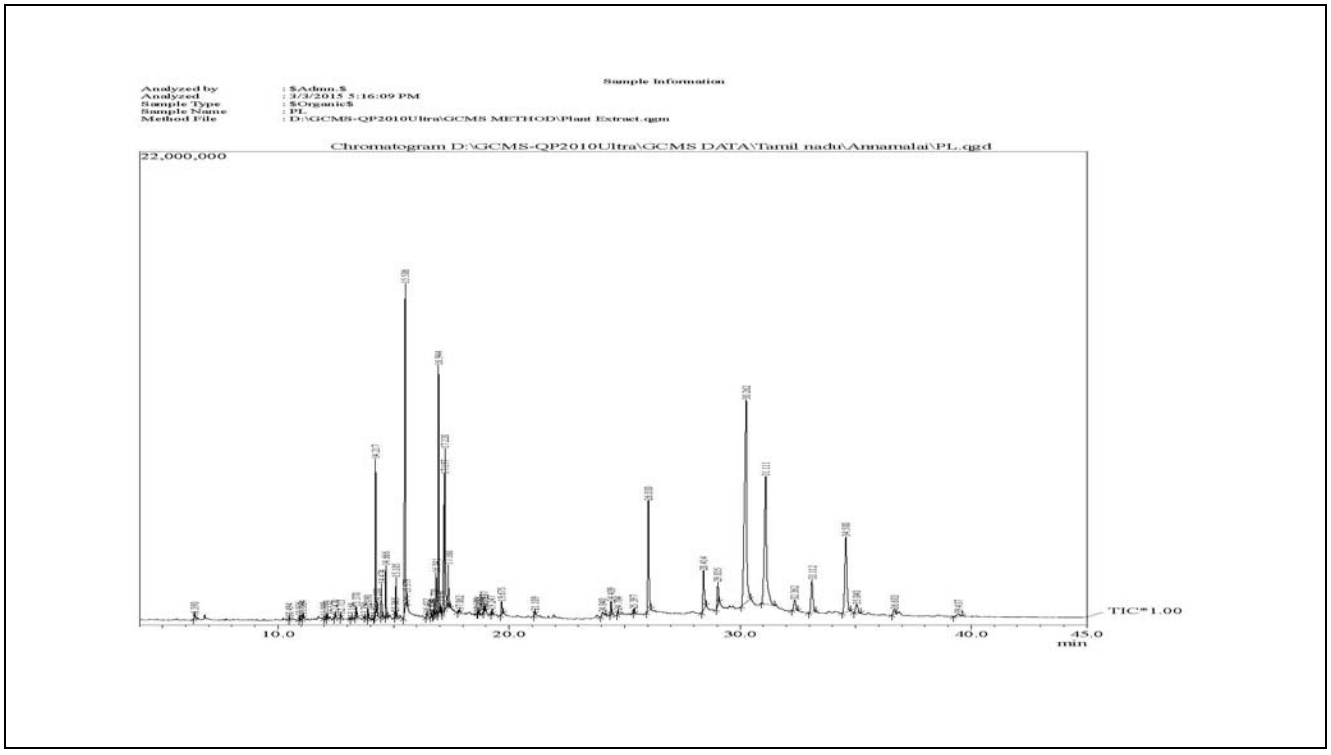

Fig 4: GCMS Chromatogram of methanolic leaf extract of *Andrographis paniculata.*

Table 4: Phytoconsitituents present in methanolic leaf extract of *Andrographis paniculata* and its medicinal properties.

Peak No	R. Time	Area %	Name of the compounds	Molecular weight	Molecular formula
1	15.506	12.74	l-(+)-Ascorbic acid 2,6-dihexadecanoate	652	$C_{38}H_{68}O_8$
2	16.944	6.54	2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl-, [R-[	296	$C_{20}H_{40}O$
3	30.262	20.06	1Phenanthrenecarboxylic acid, 7- Ethenyl	318	$C2_0H_{30}O_3$
4	17.228	4.71	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$
5	14.217	4.36	2,6,10-Trimethyl,14 -Ethylene-14-Pentadecne	278	$C_{20}H_{38}$
6	31.111	12.88	(3E,5E,7E)-6-Methyl-8-(2,6,6-Trimethyl-1-Cyclohexenyl)-3,	258	$C_{18}H_{26}O$
7	17.197	6.14	9-Octadecenoic acid	282	$C_{18}H_{34}O_2$
8	26.030	5.50	13,15-Octacosadiyne	386	$C_{28}H_{50}$
9	34.580	7.20	Stigmast-5-EN-3-OL, (3.Beta.)-	414	$C_{29}H_{50}O$
10	17.368	1.09	Octadecanoic acid	284	$C_{18}H_{36}O_2$
11	14.666	1.47	2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl-, [R-[	296	$C_{20}H_{40}O$
12	28.414	2.49	Geranyl-.alpha.-Terpinene	272	$C_{20}H_{32}$
13	16.841	1.28	9,12,15-Octadecatrienoic acid, Methyl ester, (Z,Z,Z)-	292	$C_{19}H_{32}O_2$
14	29.035	1.18	Methyl 8,10-Octadecadiynoate	290	$C_{19}H_{30}O_2$
15	33.112	2.84	Stigmasterol	412	$C_{29}H_{48}O$

Conclusion

The results of the present study reported that *Andrograpis paniculata* leaves have numerous bioactive compounds. The mass spectra are used for analysis of different chemical compounds. It can be used for various medicinal applications, and it very improve the healthy human life.

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