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Research Article

Functional Group Analysis of Extracts of Leaf and Stem of *Ipomoea Indica* by using FTIR Spectrum

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A B S T R A C T

The present study was carried out to characterize the bioactive constituents present in extracts of *Ipomoeaindica*. Fourier Transform Infra-Red (FTIR) analysis in various solvents extracts and to establish its antioxidant potential. The plants samples were collected from Islamabad, Pakistan. FTIR analysis confirmed the presence of various functional group i.e. carboxylic acid, amines, phenol, aromatic compounds. Phytochemical analysis confirmed the presence of phytochemical constituents i.e. flavonoids, alkaloids, saponins, volatile oils and reducing sugar which are known to exhibit medicinal as well as physiological activities. Methanolic extracts of anthraquinones and steroids showed negative results.

Keywords: Fourier Transform Infra-Red (FTIR) Analysis, Phytochemical Analysis, *Ipomoea Indica,* Methanolic Extracts, Anthraquinones

Introduction

Medicinal plants are a rich source of phytochemicals and for centuries have been utilized as traditional medicines. Particular phytochemicals and concentrates of their plant sources can decrease the danger for chronic diseases by affectation of catalysts or enzymes included in xenobiotic metabolism, huge numbers of which likewise have anti-inflammatory and antioxidant functions. Medicinal plants shows the vicinity of particular phytochemicals, which can possibly secure against chronic degenerative diseases (Shahat *et al*., 2013).

Ipomoea indica, Blue Morning Glory as it is commonly known is developed in the Mediterranean basin. It is positioned as the seventh most vital nourishment crop in worldwide generation. This product generated in South America yet today it is developed in most tropical, temperate regions and subtropical of the world (O'Brien, 1972).

Thenmozhi *et al*., (2011) used FTIR strategies for compound identification. For structural elucidation of compounds isolated from herbal plants the utilization of Infrared (IR) spectroscopical strategy is restricted. It is likewise discovered helpful for contrasting a natural and synthetic sample in phytochemical studies as "fingerprinting" devices. Undoubtedly, slight contrasts in the spectra inside the same plant species may not be evident and by and large not noticeable to the naked eye. The use of infra-red spectroscopy in herbal analysis is confined only to the areas like microbiology, pharmaceutical industry, food and beverage industry (Seasholtz, 1999). Keeping in view the therapeutic significance of *Ipomoea indica*, the present study was developed with following aims and objectives.

- To evaluate the phyto-chemical parameters of *Ipomoea indica* i.e. alkaloids, tannins, flavonoids, polyphenolics, anthraquinones, saponins, cardiac glycosides, phenol etc.
- Determination of functional group by using FTIR.

Collection and Pre-Treatment of Plant Sample

Ipomoea Indica (Blue Morning Glory) leaves and stem were collected for analysis from Quaid-e-Azam University Islamabad in fine plastic packs appropriately named with

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number and date of gathering of samples. Standard herbal methods were taken after for the accumulation of plant specimens. Specie was affirmed by comparison with herbarium reference materials at Department of Botany, Hazara University, Mansehra, Pakistan.

Figure 1.Ipomoea indica

Preparation of Crude Extract

Different parts of plants were washed and cut into small pieces. Firstly the samples were dried under shade and then by oven drying at 60°C for 24 hours. By electric grinder these dried parts were converted into powder form and was extracted in methanol for further use.

Figure 2.Powderd form of Stem and Leaves of Ipomoea indica

Phytochemical Analysis

Powdered plant material was analysed to test the presence of phytoconstituents. Presence of flavonoids, phenolic acid and tannins was detected by the Harbone's method (Harbone, 2005). Method described by Kaur & Arora (2009) was used for detecting saponins. Standard procedure prescribed by Trease & Evans (1989) was used for testing extracts obtained from the plant materials.

Analysis of Different Constituents

Determination of Tannins

A portion of the extract was dissolved in water and clarified by filtration. Ferric chloride solution (10%) was put in resulting filtrate. Presence of tannins is indicated by appearance of bluish color (Harbone, 2005).

Determination of Alkaloids

Five (5) ml of HCl 1% put in 0.5g of the extract on steam bath and then filtered. Few drops of Wagner's reagent were added to filtrate and few drops of distilled water were also added. Presence of alkaloids is indicated by appearance of reddish brown precipitate (Trease and Evans, 1989).

Determination of Cardiac Glycosides

Glacial acetic acid (2.0ml) having $FeCl_3$ solution's drop was added to 0.5g of extracts. Conc.H_2SO_4. (2ml) was added. Presence of deoxy sugars is indicated by formation of a brown ring at the interphase (Trease and Evans, 1989).

Determination of Flavonoids

Two (2.0) ml of the extracts was dissolved in 2.0ml of diluted NaOH. Presence of flavonoids is indicated by the appearance of a yellow color (Harbone, 2005).

Determination of Saponins

One (1.0) ml extract was mixed with 1.0ml distilled water and shaken forcefully. The presence of saponins is indicated by appearance of stable persistent froth (Kaur and Arora, 2009).

Determination of Phenols

$FeCl_3$ and extractswere mixed in equal concentration. Deep bluish solutions show existence of phenols (Harbone, 2005).

Determination of Anthraquinones

Ten (10) ml of benzene was mixed with extract (0.5g) and filtered then ammonia solution 10% was added to filtrate and the mixture was shaken. Presence of anthraquinones is indicated by the formation of red, violet or pink color on the ammonical phase (Trease and Evans, 1989).

Determination of Reducing Sugars

Distilled water (5ml) was added in 3.0ml of extract and Fehling's A and B solution was added and then boiled. Presence of reducing compounds is indicated by appearance of a red precipitate (Khan *et al.,* 2011).

Determination of Volatile Oils

Ethanol (90%) was added in extract followed by drops of $FeCl_3$. Presence of volatile oils is indicated by formation of green color (Trease and Evans, 1989).

Determination of Steroid

Chloroform (3ml) was added in extract (0.5g) and then filtered. Conc. H_2SO_4 was added to the filtrate. A steroid ring is indicated by appearance of reddish brown color at interphase (Trease and Evans, 1989).

Determination of Amino Acid

In 1.0ml of extract ninhydrin reagents' drops were added. Presence of amino acid is indicated by appearance of purple color (Trease and Evans, 1989).

Phytochemical and FTIR Analysis

Ipoemea Indica extracts were screened for phytochemicals including alkaloids, tannins, flavonoids, saponins, cardiac glycosides, steroids, phenols and reducing sugars using basic tests for phytoconstituents (Prajapati and Patel, 2012). The extracts were also subjected to Fourier Transform Infrared (FTIR) analysis to identify the functional groups present in each extract. Each sample was loaded in FTIR spectrophotometer (Shimadzu, Japan) with a scan range from 400-4000cm^{-1} with a resolution of 4cm^{-1} (Saxena and Saxena, 2012).

Result and Discussion

Phytochemical Estimations

The present study was done on the leaves and stem of *Ipomoea indica* collected from Islamabad. Starting phytochemical screening was performed for the plant material and the study demonstrated that saponins alkaloids, flavonoids, and reducing sugars were available in stem and leaves of the plant while tannins were missing in both leaves and stem showed in Tables 1,2 and 3. Anthraquinones were absent in stem and leaves. Similarly steroids were present in chloroform and distilled water extracts showed in Tables 1 and 3 and absent in methanolic extract showed in Table 2. The presence of saponins showed that the plant has cytotoxic effects as saponins are cytotoxic. The presence of alkaloids showed that plant has analgesic, antispasmodic and bacterial properties. The presence of saponins showed the antibacterial properties of plant (Chung *et al.,* 1998).

The study conducted here provides the health application at reasonable cost. Medicinal activity is due to presence of secondary metabolites present in plants. Phytochemical screening was done accordingly in the present study. The outcomes displayed here propose that the phytochemical properties for curing different illnesses (Savithramma *et al.,* 2011).

Table 1.Constituents in water Extracts of Ipomoea indica

S. No.	Names	Present (+) Absent (-)
1.	Reducing sugars	+
2.	Alkaloids	+
3.	Tannins	-
4.	Flavonoids	+
5.	Saponins	+
6.	Phenols	+
7.	Anthraquinones	-
8.	Cardiac glycosides	_
9.	Volatile oils	+

Table 2.Constituents in Methanolic Extracts of Ipomoea indica

S. No.	Names	Present (+) Absent (-)
1.	Flavonoids	+
2.	Alkaloids	+
3.	Reducing sugars	+
4.	Cardiac glycosides	-
5.	Saponins	+
6.	Phenols	+
7.	Anthraquinones	-
8.	Tannins	-
9.	Volatile oils	+

Table 3.Constituents in Chloroform Extracts of Ipomoea indica

S. No.	Names	Present (+) Absent (-)
1.	Volatile oils	+
2.	Alkaloids	+
3.	Phenols	+
4.	Flavonoids	+
5.	Saponins	+
6.	Cardiac Glycosides	+
7.	Anthraquinones	-
8.	Reducing sugars	+
9.	Tannins	-

+ = Presence, _ = Absence

FTIR

Extracts of *Ipomoea indica* were screened for functional groups recorded in table 14-21. Extracts of different solvents were used i.e. ethyl acetate, n- hexane, chloroform and aqueous. Each sample was loaded in FTIR spectrophotometer (Shimadzu, Japan) with a scan range from 400-4000cm^{-1} with a resolution of 4cm^{-1} (Saxena and Saxena, 2012). When extract passed into FTIR, on the basis of peak ratio separation of functional groups was done. The results of FTIR affirmed the vicinity different functional groups present in extract based on the peak values in the region of IR radiations i.e. aromatic compounds, amines, carboxylic acid.

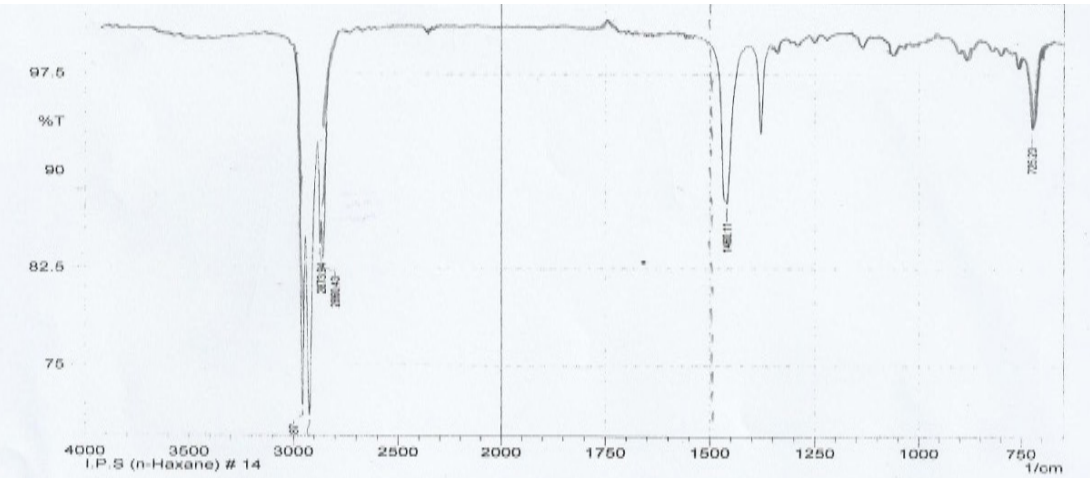
The more intense band happening at 2873.94 cm-1, 3340.71cm-1, 2860.43 cm-1, 1460.11 cm-1, 1416.22 cm-1, 1033.76 cm-1 and 725.23 cm-1 corresponding to N-H/ O-H/ C=O/ C-H stretching, bending, vibrations respectively

show the presence or vicinity of amines, alcohol, amino acids, amides.

The intense bands occurring at 3315.63 cm-1, 3300.20 cm-1, 3263.56 cm-1, 1633.71 cm-1, 1373.32 cm-1, 1043.49 cm-1, and 667.37 cm-1 corresponding to O-H / C-O str/ N-H / O-H str/ C-H/ C=O bending, stretching, vibrations show the presence of alcohol, phenol, amines, amides, carboxylic group, ester,ether, amino acids group.

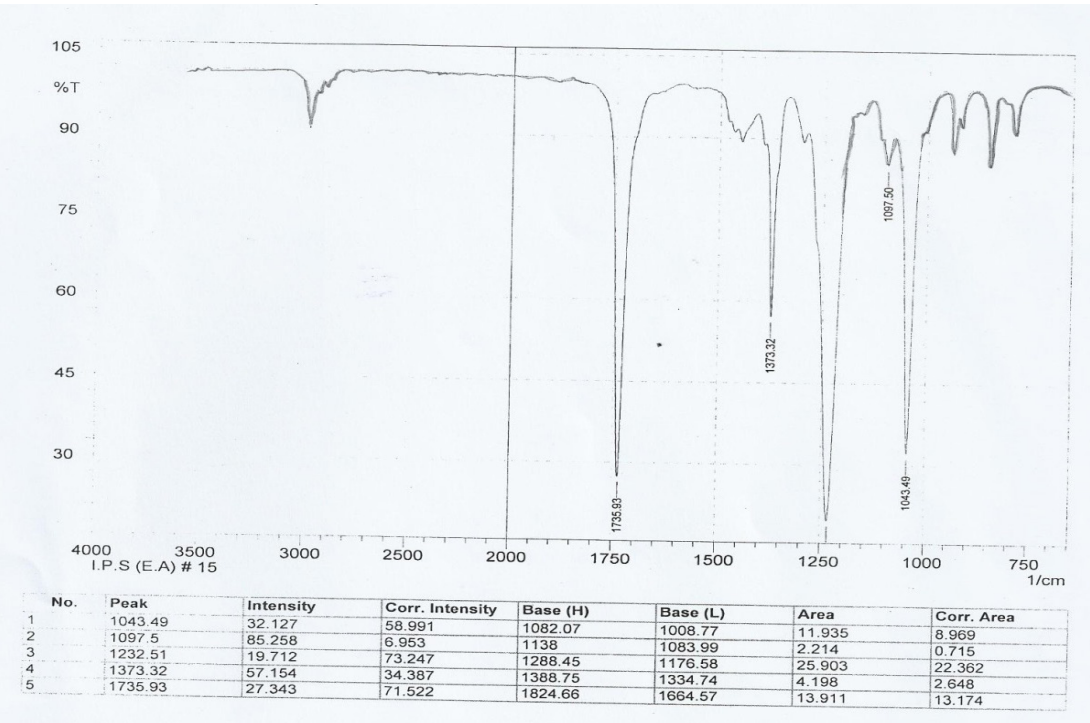
The results of FTIR analysis confirmed the presences of polar groups and phenols in *IPS.*(n-hexane) and alkynes, aromatic compounds, carboxylic acids, amines and phenols in *IPS* (E.A) where as *IPL* (DW) contained alkanes, amines, sulfate esters and phenols. The leaves extract *IPL* (Chl) constituted aromatic compounds, amines, phenols. On the other hand, *IPS* (DW) contained no polar group while contained aromatics and phenol. Presence of C-H, C=C, C=O and C-C and C-O bonding structures indicates the presence of methyl groups, alkyl groups, alcohols, esters, ethers, carboxylic acids, deoxyribose and anhydrides.

Table 4.FTIR analysis of Ipomoea indicastem extract in n-hexane



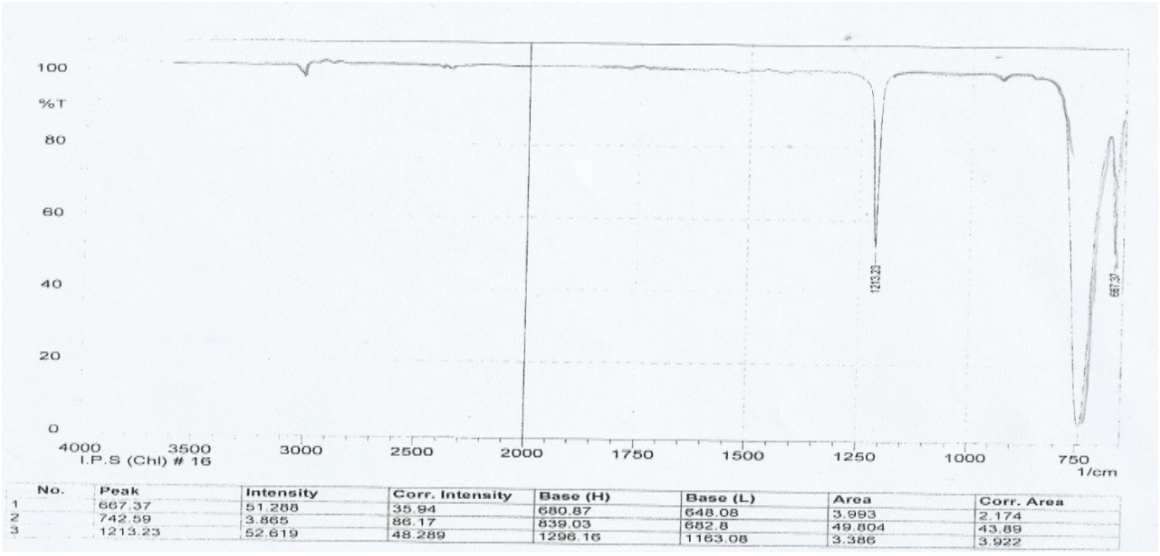
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	725.23	93.435	5.904	748.38	698.23	0.559	0.424
2	1460.11	87.585	0.643	1462.04	1409.96	0.899	0.021
3	2860.43	83.274	3.552	2866.22	2769.78	1.517	0.203
4	2873.94	84.317	3.826	2885.51	2868.15	1.03	0.134
5	2922.16	71.109	16.569	2943.37	2887.44	5.052	2.113
6	2956.87	71.881	14.896	3030.17	2946.3	3.238	1.136

Table 5.FTIR analysis of Ipomoea indica stem extract in Ethyl acetate



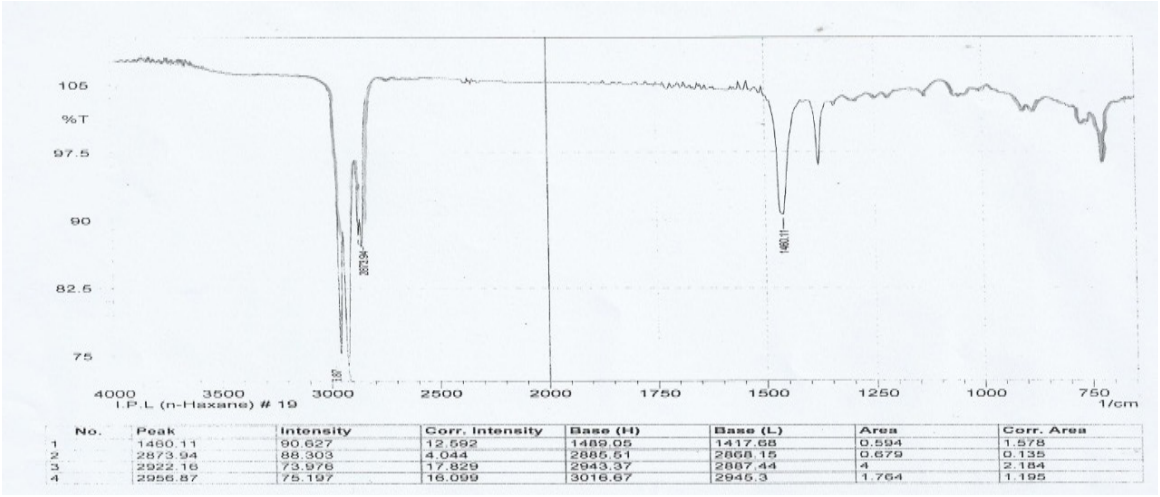
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1043.49	32.127	58.991	1082.07	1008.77	11.935	8.969
2	1097.5	85.258	6.953	1138	1083.99	2.214	0.715
3	1232.51	19.712	73.247	1288.45	1176.58	25.903	22.362
4	1373.32	57.154	34.387	1388.75	1334.74	4.198	2.648
5	1735.93	27.343	71.522	1824.66	1664.57	13.911	13.174

Table 6.FTIR analysis of Ipomoea indicastem extract in chloroform



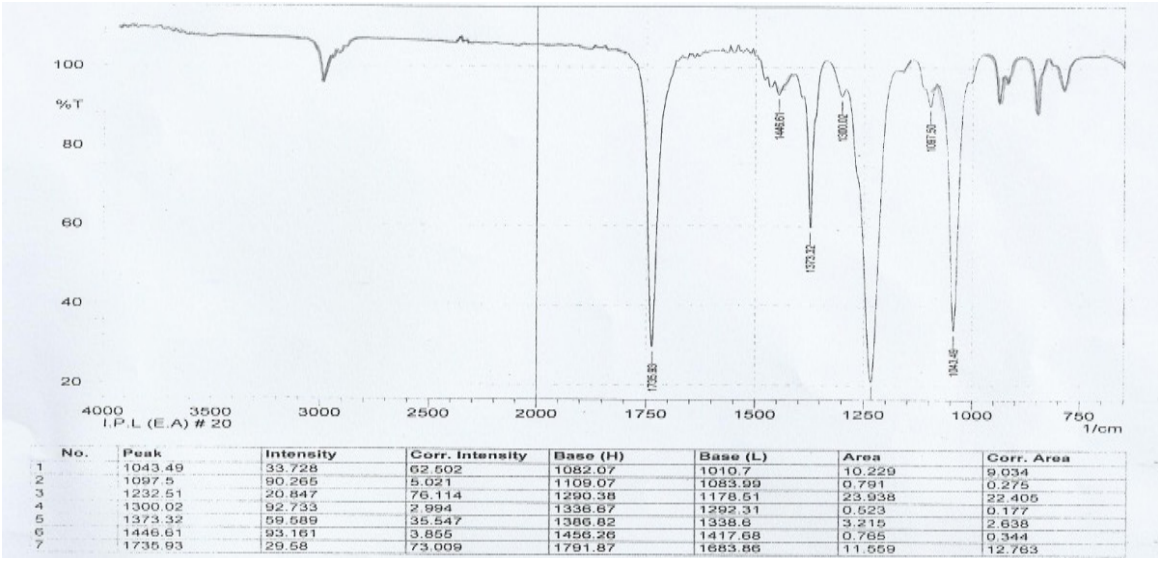
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	667.37	51.288	35.94	680.87	648.08	3.993	2.174
2	742.59	3.865	86.17	839.03	682.8	49.804	43.89
3	1213.23	52.619	48.289	1296.16	1163.08	3.386	3.922

Table 7.FTIR analysis of Ipomoea indica leaves extract in n-hexane



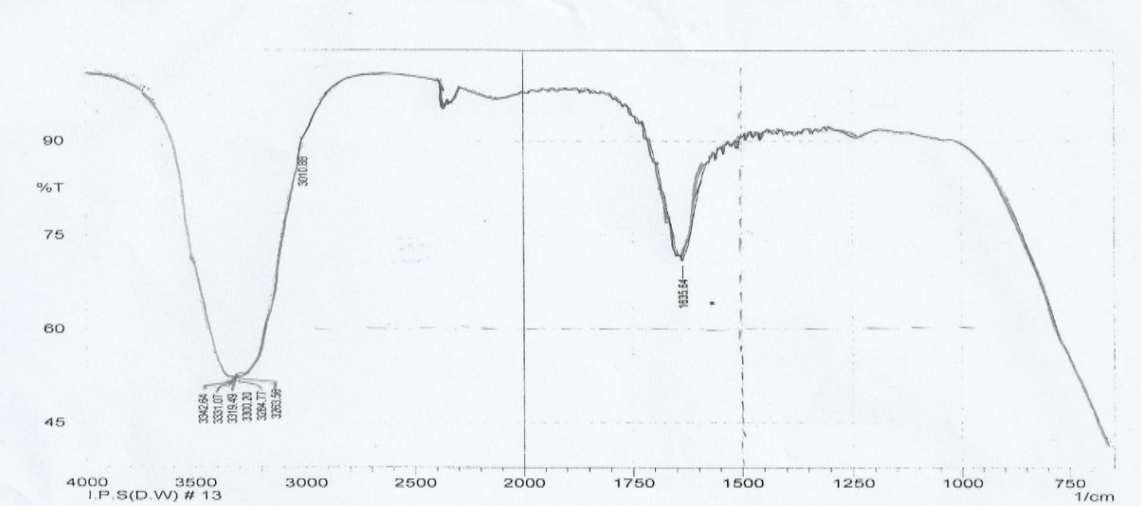
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1460.11	90.627	12.592	1489.05	1417.68	0.594	1.578
2	2873.94	88.303	4.044	2885.51	2868.15	0.679	0.135
3	2922.16	73.976	17.829	2943.37	2867.44	4	2.184
4	2956.87	75.197	16.099	3016.67	2945.3	1.764	1.195

Table 8.FTIR analysis of Ipomoea indica leaves extract in Ethyl acetate



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1043.49	33.728	62.502	1082.07	1010.7	10.229	9.034
2	1097.5	90.265	5.021	1109.07	1083.99	0.791	0.275
3	1232.51	20.847	76.114	1290.38	1178.51	23.938	22.405
4	1300.02	92.733	2.994	1336.67	1292.31	0.523	0.177
5	1373.32	59.589	35.547	1386.82	1338.6	3.215	2.638
6	1446.61	93.161	3.865	1456.26	1417.68	0.765	0.344
7	1735.93	29.58	73.009	1791.87	1683.86	11.559	12.763

Table 9.FTIR analysis of Ipomoea indicastem extract in Aqueous



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	650.01	40.888	0.279	663.51	648.08	5.879	0.015
2	1635.64	70.937	2.349	1641.42	1579.7	6.599	0.356
3	3010.88	90.382	0.396	3014.74	2781.35	3.421	0.017
4	3263.56	53.248	0.609	3267.41	3016.67	41.496	2.528
5	3284.77	52.921	0.126	3290.56	3269.34	5.843	0.015
6	3300.2	52.791	0.103	3304.06	3292.49	3.203	0.005
7	3319.49	52.484	0.108	3323.35	3305.99	4.842	0.013
8	3331.07	52.407	0.093	3336.85	3325.28	3.244	0.006
9	3342.64	52.454	0.067	3352.28	3338.78	3.778	0.003

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