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Thin Layer Chromatography (TLC) and Infra – red (IR) Spectral Analysis of components of *Acalypha indica* Linn

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Abstract

*Acalypha indica*is an important medicinal plant and traditionally used as throat infections, wound healing, arthritis, ulcer and diabetes mellitus. Thin layer chromatography (TLC) the present study showed separation of two deep violet colour spots with R_F values 0.31 and 0.72 which may represent the presence of alkaloids. Infra-Red (IR) spectral analysis of *Acalypha indica* showed C-H, -C=C-, N-H bend, C-C, C-H rock, O-H bend and C-C1 stretchings which may be attributed to the presence of functional groups like alcohol, alkenes, primary amines, alphatic amines and alkylhalides.

Key words: TLC, IR spectral, *Acalypha indica,* medicinal plants, phyto components.

Introduction

The thin layer chromatography (TLC) method is an important analytical tool for the separation, identification and estimation of different classes of bioactive compounds[1]. *Acalypha indica*, in particular have been found contain phenolics, Tannin and Flavonoids[2]. These compounds have various biological properties, such as antioxidant, antimicrobial, antidiabetic, immunomodulatory, antiulcer, antiarthritic and hepatoprotective functions[3]. These phytoconstituents give special characterstics and properties to plants. Therefore, the analysis of these bioactive compounds in plants would help in determining various biological activities of plants[4].

Infrared spectroscopy is now widely used in biology. The IR spectrum analysis allows determining physical-chemical or biological characterstics of a sample, for example, chemical compostion, granulesize, demsity, etc.[5]. At present, there are databases of infrared spectra of food products, technical and food additives, medicines, poly and monomers, plasticizers, toxic chemicals, solvents, petroleum products, toxic substances, steroids, and other compounds having mainly plant-specific single-component compostition[6,7].

Materials and methods:

Preparation of TLC plates

25×10 cm glass plates were washed with distilled water followed by smearing with acetone. After drying the plates were placed on the template in row. The slurry of silica gel G prepared with glass distilled water in the radio 1:2 (w/v) was poured in the applicator. The glass plates were immediately coated with a layer of silica gel i n 500µm thickness. The coated plates were activated at 80^{o} C for 3 h. Then the plates were stored in a plate chmber for further study. In that study, chloroform and methanol were used as solvents in the ratio of 96:4[8].

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Preparation of Dragendprff's reagent

Solution A

0.6 g of Bismuth subnitrate was dissolved in 2ml of concentrated hydrochloric acid and added 10ml of distilled water.

Solution B

6g of Potassium iodide was dissolved in 10ml of water.

The solutions A and B were mixed together with 7ml of concentrated hydrochloric acid and 15ml of water. The whole content was diluted to 400 ml with distilled water.

Solvent

Acetic acid : ethanol (1:3)

Loading of substances

The concentrated plant extract of 2.5mg was loaded on the TLC plates just above 2cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. After the solvent front reacted approximately 18cm height, the plates were removed and allowed at room temperature for 30 min. Then the plates were observed by spraying with Dragendorff's reagent and recorded the R_f value of visualized spots.

Infra-Red (IR) Spectral analysis of components of *A. indica*

IR spectra may be measured on plants substances in an automatic recording IR spectra photometer either in solution (in chloroform) or carbon tetrachloride (1-5%), as a mull with potassium bromide (KBr). In the latter case, a thin disc was prepared under anhydrous condition from a powder containing about 1mg of material and 10 to 100mg KBr, using a mould and presss. The range of measurement is from 2.5 to 15μ and the spectru takes about three minutes to be recorded.

Instrument description

IR spectroscopy is an automated make of Jasco-FTIR-40 using KBr pellet method.

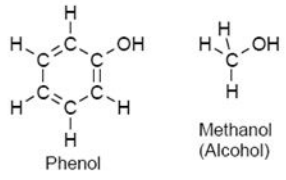
TLC analysis of *A. indica* extract

Separation of phytochemical fractions from the ethanolic extract of *A. indica* was presented in Table 1 & Fig.1. The fractions were separated on the form of two deep violet colour spots with R_f values 0.31 and 0.72 respectively.

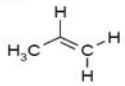
Infra red (IR) spectral analysis of *A. indica* extract

The IR spectrum of ethanolic extract of *A. indica* was analysed and the IR spectral data were reported (Table 2; Fig.2) with position of characteristic bonds, stretches and functional groups.

– Hydrogen bonded OH

Phenols & Alcohols: 	**3600-3100** (Note: Phenols MUST have Aromatic Ring Absorptions too.)	Hydrogen-bonded O-H Stretch (This peak usually appears much broader than the other IR absorptions.

bond : C–H stretch **Functional group :** alkanes

Alkenes: 	**3100-3000**	C=C-H Asymmetric Stretch
	1675-1600	C-C=C Symmetric Stretch

1653.00 – bond –C=C– stretch **Functional group:** alkenes
1620.21 – bond : N–H bend **Functional group:** 1° amines
1402.25 – bond C–C stretch (in–ring) **Functional group:** aromatics
1363.67 – bond : C–H rock **Functional group**:alkanes
1276.88 – bond: C–H wag (–CH2X) **Functional group:** alkyl halides
1240.23 – 1060.84 –Bond: C–N stretch **Functional group :**aliphatic amines
21.97 – bond: O–H bend **Functional group:** Carboxylic Acids
835.81 – bond: C–Cl stretch **Functional group:** alkyl halides

Table 1. Separation of phytocomponents of *A. indica* by TLC

Fractions/spot	Colour of the spot	R_f value of the spot
Spot-I	Deep violet	0.31
Spot-II	Deep violet	0.72

Fig.1 TLC analysis of Phytocomponents of *A.indica*

Table 2. IR Spectral data of *A. indica* extract

S.No	Peak	Intensity	Corr. intensity	Base (H)	Base (L)	Area	Corr. area
1	420.48	34.84	1.33	422.41	399.26	9.04	0.48
2	538.14	23.28	1.74	555.5	424.34	73.93	3.01
3	590.22	22.91	0.27	599.86	565.14	22.07	0.1
4	613.36	22.79	0.72	636.51	601.79	22.01	0.29
5	651.94	24	1.96	746.45	638.44	57.89	1.29
6	761.88	36.41	2.93	802.39	748.38	21.61	0.93
7	835.18	42.61	6.57	885.33	804.32	27.37	2.82
8	921.97	43.73	6.34	948.96	887.26	20.1	1.76
9	1060.85	6.06	33.71	1192.01	950.91	195.55	94.58
10	1240.23	22.3	5.34	1261.45	1193.94	39.44	2.53
11	1276.88	26.13	0.07	1280.73	1263.37	10.1	0.02
12	1363.67	15.06	1.03	1371.39	1282.66	62.32	0.92

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13	1402.25	13.8	7.88	1533.41	1373.32	98.26	9.24
14	1602.21	13.94	7.71	1639.49	1535.34	62.26	5.14
15	1653	14.46	4.65	1878.67	1641.42	57.05	0.83
16	2096.62	96.47	4.34	2279.86	1890.24	2.05	3.45
17	2931.8	22.23	15.93	2995.45	2281.79	145.74	14.71
18	3383.14	3.41	66.23	3732.26	2997.38	653.33	477.03

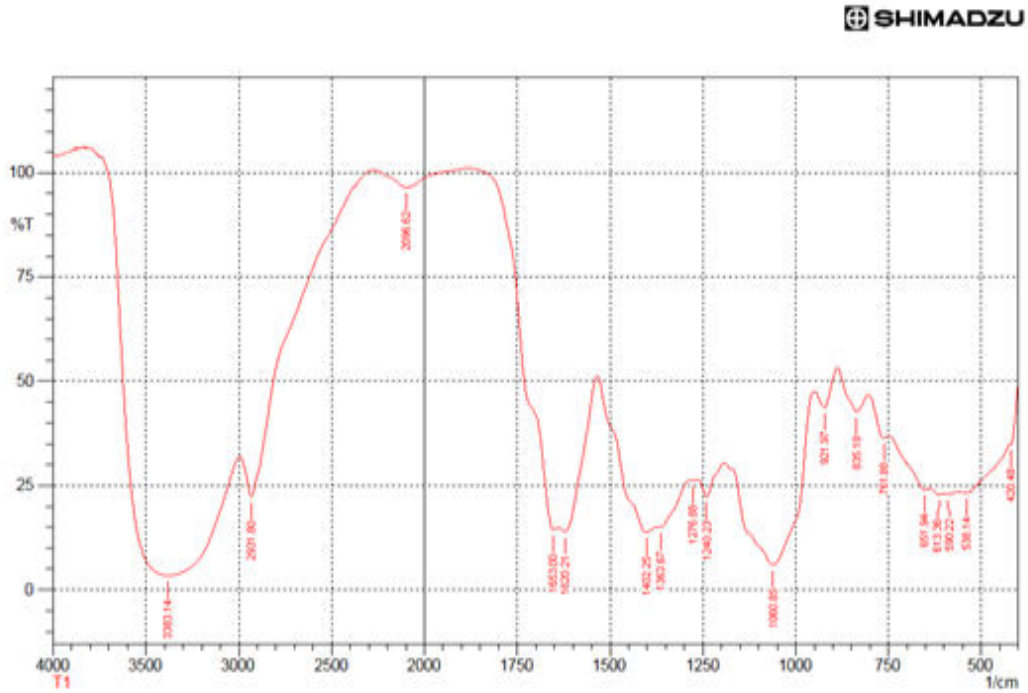

Fig. 2. IR Spectrum of ethanolic extract of *Acalypha indica* extract

Conclusion

TLC serves as one of the many methods in providing a chromatographic plant extract finger print[11]. Gabriela[12] suggested that the colours of the spotsin TLC and their position relative to standard substances are the two important characteristics for plant extract identification. The present study showed separation of two deep violet colour spots with $R_f$values 0.31 and 0.72 which may represent the presence of alkaloids in the selected plant extract(Table 1; Fig.1) similar phytochemical analysis was carried out in plant drug[13].

The identification of an organic compound by the infrared technique is usually carried out be examining certain regions of the spectrum in a systematic way. The absorption peaks obtained in the region of 3000-2850 cm^{-1} are due to the presence of aliphatic CH vibration, the carbonyl stretching vibration at 1700 cm^{-1} due to the presence of ketones, aldehydes, acids, amides and carbonates and C-O-C stretching vibration in esters and ethers are sound at 700-800 cm^{-1}[10]. In the present study, the IR spectral data given in Table 2 & Fig.2 showed C-H, -C=C-, N-H bend, C-C, C-H rock, O-H bend and C-Cl stretchings which may be attributed to the presence of functional groups like alcohol, alkenes, primary amines, aliphatic amines and alkyl halides.

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