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QUANTITATIVE ESTIMATION AND INFRA-RED (IR) SPECTRAL ANALYSIS OF ACALYPHA INDICA LINN

Biochemistry

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ABSTRACT

Acalypha indica present study was to estimate the total phenolic, flavonoids, tannins and alkaloids content of the plant. The total alkaloids content was recorded as 13.6 mg 100-1, total phenolics and tannins content were expressed as tannic acid equivalent and total flavonoids as rutin equivalent. The selected plant sample showed 72.1 mg TAE g-1 of total phenolics, 53.5 mg TAE g-1 of tannins and 24.9 mg RE g-1 of total flavonoids. Acalypha indica showed Infra-Red (IR) spectral analysis of C-H, - C=C, N-H bend, C-C, C-H rock, O-H bend, and C-C1 stretchings which may be attributed for the presence of functional groups like alcohol, alkenes, primary amines, alphatic amines and alkylhalides.

KEYWORDS

Acalypha indica, tannin, phenolic, alkaloid, IR spectral, flavonoids, phytocomponents.

INTRODUCTION

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries the constituents are playing a significant role in the identification of these plants lies in some chemical substances that produces a define physiological action on the human body. Phenolic compounds such as quercetin, rutin, narigin, cattechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents[1]. Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts in free state and glycosides[2]. They are found to have many biological activities including antimicrobial, anti ulcer, anti arthritic, anti cancer, anti anglogenic, anti inflammatory, protein kinase inhibition etc. The flavones and flavonols are the most widely distributed of all the phenolics[3]. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardio vascular disease, certain forms of cancer and age related degeneration of cell components. Their poly phenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals[4]. A variety of dietary plant flavonoids inhibits tumor development in experimental animal models[5].

Infra Red spectroscopy is now widely used in biology. The IR spectrum analysis allows determining physical-chemical or biological characterstics of a sample, for example chemical compotion, granulesize, density etc[6]. 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 composition[7,8].

MATERIALS AND METHODS

Determination Of Total Phenolics And Tannins

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to form a blue coloured complex and the colour intensity was measured at 725 nm.

Procedure

10 ml of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteau reagent and 2.5 ml of 20% sodium carbonate solution were added sequentially in each test tube. Soon after vortexing the reaction mixture the test tubes were placed in dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents[9].

Using the sample extract, the tannins were estimated after the treatment with Poly Vinyl Poly Pyrrolidine (PVPP). 100 mg of PVPP was weighed into a 100×12 mm test tube and to this added 1 ml of distilled water and 1 ml of sample extract. The content was vortexed and kept in the test tube at 4^0C for 4h. Then the sample was centrifuged at 3000 rpm for 10 min. at room temperature and the supernatant was collected. The supernatant has only phenolics whereas the tannins would have been precipitated along with PVPP. The phenolics content of the supernatant was measured and expressed as the content of non-tannin phenolics on a dry matter basis.

From the above results, the tannin content of the sample was calculated as follows

Tannin (%) = Total Phenolics (%) – Non-tannin Phenolics (%)

Determination Of Total Flavonoid Content

0.5 ml of aliquot of appropriately ($10mg^{-1}$ 2ml) diluted sample solution was mixed with 2ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $A1C1_3$ solution was added and allowed to stand for 6 min and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and then the mixture was thoroughly mixed and allowed to stand for 15 min. Absorbance of the mixture was determined at 510nm against water blank. The results were expressed as mg RE (Rutin Equivalent)g^{-1} of extract[10].

Infra-Red (IR) Spectral Analysis Of Components Of Acalypha Indica

IR spectra may be measured on plant 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 1 mg of material and 10 to 100 mg KBr, using a mould and press. The range of measurement is from 2.5 to 15μ and the spectrum takes about three minutes to be recorded[11, 12].

Instrument Description

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

RESULTS AND DISCUSSION

The total alkaloids, total phenolics, total flavonoids and tannin contents of ethanolic extract of Acalypha indica were determined and presented in Table 1. The total alkaloid content was recorded as 13.6 mg 100^{-1}. Total phenolics and tannin contents were expressed as Tannic acid equivalent and total flavonoid as Rutin equivalent. The selected plant sample showed 72.1 mg TAE g^{-1} of total phenolics, 53.5 mg TAE g^{-1} of tannins and 24.9 mg RE g^{-1} of total flavonoids.

Natural products have played an important role in the development of drugs for various diseases. Until 1990's scientists thought that most of the compounds produced by the plants were useless waste products. These waste compounds are called as secondary metabolites. But later it was found that these compounds may perform a huge array of functions. Many of these compounds cannot be synthesized economically on commercial basis. The secondary metabolites have complex stereo structure with many chiral centres which may be essential for various biological activities. The secondary metabolites

from natural sources are good products for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than synthetic drugs. Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines. Plants with natural products exhibit pharmacological and biological activities and play an important role in life-threatening conditions.

Flavonoids are present in all vascular plants and have been reported to exert multiple biological effects including anti-inflammatory, anti-ulcerogenic, anti-allergic, anti-viral and anti-cancerous activities. Tannins have been reported in the leaves of Pomegranate, Tambolan and Guava and medicinally tannins are used in anti-diarrhoeal and anti-haemorrhoidal preparations. Saponins are glycosides of steroids, steroid alkaloids found in plants, especially in plant skins where they form a waxy protective coating. They are useful in lowering cholesterol, as antioxidants and anti-inflammatory agents. Terpenoids are diverse class of naturally occurring organic chemicals found in all classes of living organisms, which may exhibit antibacterial properties. Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia and are found as secondary metabolites in several plants like Digitalis, Convallaris and Euphorbia species.

Alkaloids are the largest class of secondary plant substances present in higher plants of some families viz., Papaveraceae, Liliaceae, Solanaceae, Rubiaceae, Rutaceae, Boraginaceae and Asclepiadaceae and are reported to possess defensive effects. Alkaloids are stored predominantly in actively growing yound tissues, roots, stem barks, flowers and seedlings. In the present study, the ethanolic extract of A. indica was reported to contain 13.6 mg 100 g^{-1} of alkaloids (Table 1). Presence of alkaloids was reported in Cephaelis species, Anthocephalus cadamba and Cinchona sps.

Phenolics are the most important secondary metabolites present in plant kingdom. These diverse group of compounds serve as potential natural antioxidant in terms of the ability to act as both efficient radical scavenger and as metal chelator. It has been reported that the antioxidant activity of phenols is mainly due to their ability to act as hydrogen donor, singlet oxygen quencher and their redox property. In the present study, the total phenolics content of ethanolic extract of A. indica was estimated as 72.1 mg TAE g^{-1} extract (Table 1).

Tannins are water soluble plant polyphenolics which cause protein precipitation from aqueous solutions, located in vacuoles. This group of compound has received a great deal of attention in recent years. Since it was suggested that the consumption of tannin containing beverages especially green teas and red wines can cure or prevent a variety of illness. Tannins are complex moieties produced by most of the plants as protective agent with wide pharmacological activities. They have been shown to possess astringent, anti-inflammatory, antidiarrhoel, antioxidant and antimicrobial properties.

In the present study, the tannin content of ethanolic extract of A. indica was recorded as 53.5 mg TAE g^{-1} (Table 1). This is in line with the report of Kannan et.al., in Rubia cordifolia.

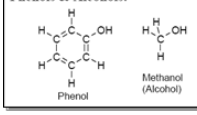
Flavonoids are well known phytochemicals having the biological effects such as free radical scavenging, modulation of enzymatic activity, antibiotic and anti-inflammatory activities. It is reported that flavonoids are natural products which are shown to exhibit biological properties related to antioxidant activity.

The flavonoid content of the present study plant was found to be 24.9 mg RE g^{-1} (Table 1). This correlates with the report of Lopes et.al.,in Chiococca braquiata.

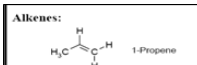
The findings of the present study indicated that the selected plant sample was reported to contain alkaloids, phenolic compounds, tannins and flavonoids which may contribute to the antioxidant activity of the ethanolic extract of A. indica. This result correlates well with the findings of Ashafa et.al., who reported that the leaf extract of Felicia muricata had shown significant antioxidant activity due to the presence of secondary metabolites such as alkaloids, flavonoids and phenols.

The IR spectrum of ethanolic extract of Acalypha indica was analysed and the IR spectral data were reported (Table 2; Fig 1) with position of characteristic bands, stretches and functional groups.

3383.14 – 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.

2931.80 – 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

The identification of an organic compound by the infrared technique is usually carried out by 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 found at 700 – 800 cm^{-1}. 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-C1 stretchings which may be attributed to the presence of functional groups like alcohol, alkenes, primary amines, aliphatic amines and alkyl halides.

Phytochemicals naturally occur in medicinal plants leaves, vegetables and roots that have defense mechanisms and protect from various diseases. Phytochemicals are primary and secondary compounds, chlorophyll, protein and common sugars are included in primary and secondary compounds have terpenoid, alkaloid and phenolic compounds. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas.

Acalypha indica used in traditional system of Indian medicine for the treatment of anti cancer, anti inflammatory, hepatoprotective, antiulcer, anti diabetic, anti viral, wound healing, asthma, pneumonia, bronchitis and rheumatism, skin disease, snake bite, anti microbial, anti oxidant, analgesic, cough, acne and Digestive disorders.

Table 1 Quantitative analysis of phytochemicals in ethanolic extract of Acalypha indica

Constituents	Concentration	Unit
Total Alkaloids	13.6±3.4	mg $100g^{-1}$
Total Phenolics	72.1±2.8	mg $TAEg^{-1}$ extract
Tannins	53.5±4.2	mg $TAEg^{-1}$ extract
Total Flavonoids	24.9±9.1	mg REg^{-1} extract

Values are means of three independent analyses of the extract ; ± standard deviation (n=3)
TAE - Tannic acid equivalent
RE - Rutin equivalent

Table 2 IR Spectral data of Acalypha 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
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

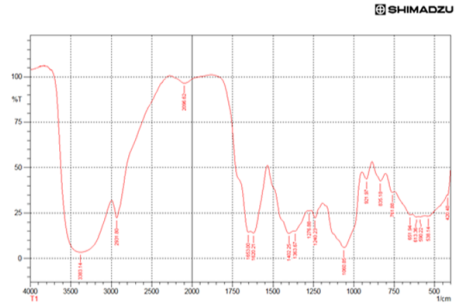


Fig 2 IR Spectrum of ethanolic extract of Acalypha indica extract

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