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Detection of Flavonoids from *Acalypha indica* L.

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Abstract
Flavonoids from *Acalypha indica* L were extracted by using different solvent and they were identified and detected by Paper Chromatography (PC) and spectroscopic method. Flavonoids like anthocyanidins, glycosides, flavonols and flavones were detected from *A. indica* L.

Keywords: Flavonoids, *A. indica* L., PC, spectroscopy.

INTRODUCTION

Flavonoids comprise a large group of bioactive polyphenolic plant secondary metabolites. They played an important role in plants as defense and pigmentation. Flavonoids are water soluble compounds found in the form of glycosides and located in the vacuoles. Flavonoids show antioxidant properties [1 and 2]. It protects from heart diseases [3 and 4] and also shows antibacterial activity [5] and antifungal activity [6]. Flavones and flavonols show inhibitory activity of CYP2C9 mechanism [7]. The main interest of the present study is that all plants contain flavonoids which are not similar in proportion as well as chemotaxonomically different.

Acalypha indica L. is erect, sparsely pubescent herb, stems angular, leaves ovate-elliptic, serrate, acute, petiole longer than blade, flowers androgynous, axillary, male minute, female several at the base, ovary, globose, fruit greenish, hispid, seed brown and found on waste places, on old wall of houses and along banks of water courses. *Acalypha indica* L. is also source of flavonoids. Hence, the present study was undertaken to detection and identification of flavonoids in the same plant.

MATERIALS AND METHODS

Acalypha indica L. was collected from Basmath, Dist. Hingoli (M.S.), India. It was identified on the basis of the morphological characters up to the species level.

Preparation of plant extract for
a. Glycosides

The plant materials i.e. stem, leaves and flowers were dried at 50^{0}C in oven. The dried material was treated with boiling alcohol 90^{0}C for 10 minutes and it was filtered through Whatman filter paper no.3. The filtrate was concentrated in Rotary vacuum evaporator (R.V.E.) at 40^{0}C. The concentrated filtrate was treated with light petroleum ether. Latex free extract was treated with diethyl ether (to separate cinnamic acid and catechin) and concentrated in R.V.E. The concentrated extract was treated with ethyl acetate (to remove flavones and flavonols). Then ethyl acetate layer was discarded and concentrated in R.V.E. and it was dissolved in methanolic HCl (97:3). The mixture was blended and centrifuged (2000 Rpm) for 3 minutes and obtained supernatant. This supernatant was concentrated in R.V.E. at 40^{0}C and it was used for Paper Chromatography to identification of glycosides. Two chromatograms (Whatman no. 1) were prepared and concentrated supernatant was spotted on the proper site of each chromatogram. Spotted chromatograms were dried by Hair dryer. These dried chromatograms were developed in BAW (n-Butanol-Acetic acid-Water; 4:1:5) and PhOH (Phenol-Water; 3:1) solvent system, respectively. These chromatograms were dried and identified the colors under UV light. The colors were identified and calculated R_f value of each glycoside.

b. Anthocyanidins

Above same preparation of supernatant was prepared and it was taken and boiled with 2M HCl for 40 minutes at 100^{0}C. The small amount of this supernatant was dissolved in the small volume of isoamyl-alcohol and concentrated in R.V.E. at 40^{0}C. Then, this extract was prepared for PC. Two chromatograms (Whatman no. 3) were prepared and concentrated extract was spotted on the proper site of each chromatogram. Spotted chromatograms were dried by Hair dryer. These compounds were separated using Forestal solvent (conc. HCl-acetic acid – water, 3:30:10) and Formic acid solvent system (conc.HCl-Formic acid–water, 2:5:3), respectively. These chromatograms were dried and identified the visible colors. R_f value of each anthocyanidin was measured and calculated.

c. Flavonols and Flavones

Catechin and cinnamic acids free extract was taken and concentrated in R.V.E. at 40^{0}C and it was boiled with 2M HCl for 40 minutes at 100^{0}C in oven. The boiled extract was concentrated in R.V.E. and treated with ethyl acetate. Again, the extract was concentrated under vacuum up to dryness. The small amount of the dried residue was dissolved in the small volume of Ethanol. This

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ethanolic solution was prepared and banded on chromatographic papers (Whatman no. 3). These chromatograms were developed in Forestal (conc. HCl- acetic-acid- water, 3:30:10) and BAW (n-Butanol-Acetic acid-Water; 4:1:5) solvent system, respectively. These papers were dried and identified the color under UV light with the fuming of ammonia. The colors were observed and calculated R_f values of each flavonol and flavone, separately.

Spectral analysis

a. Anthocyanidins

The proper band of each chromatogram was marked by pencil. Each band was cut out and taken in clean test-tube and eluted with water, methanol and acetic acid in the proportion of 25:70:5 v/v mixture, separately. Each mixture was filtered through Whatman filter paper no. 3 and filtrate was concentrated in R.V.E. at 40^0C and finally evaporated up to dryness in desiccators. Each dried residue was dissolved in methanol containing 1% HCl, separately. The solution was used for spectrophotometric identification. The anthocyanidins were measured by scanning the sample in the region between 450-600 nm.

b. Flavonols and Flavones

The proper band of each chromatogram was marked by pencil. Each band was cut out and taken in clean test-tube and eluted with 95% of Ethanol, separately. Each elution was continued till the paper become colorless. The ethanolic solution was filtered through Whatman filter paper no. 3, separately. Each filtrate was used for spectral analysis. Spectral maxima was recorded by using the trace amount of $AlCl_3$, sodium ethoxide and sodium borohydrate in ethanolic solution for coloration reaction. The absorption spectra of each solution of pure flavonol and flavones were determined in the region between 268-386 nm.

Table 1. Detection of flavonoids (anthocyanidins) from *Acalypha indica* L. on the basis of the colors, R_f values and absorption maxima.

Sr. No.	Visible colors	R_f (x100) in		Visible *max.* in MeOH-HCl λ*max.* (nm)	Anthocyanidins
		Forestal	Formic Acid		
1	Red	68	---	520	Pelargonidin
2	Magenta	---	76	524	Rosinidin
3	Orange-red	---	54	511	Columnidin

Table 2. Detection of glycosides from *Acalypha indica* L. on the basis of the colors and R_f values.

Sr.No.	Visible colors	R_f (x100) in		Glycosides
		HOAc-HCl	BAW	
1	Dull orange	---	71	3-rhamnoside (Pelargonidin)
2	Dull	72	---	3,5-diglycoside (Rosinidin)

Table 3. Detection of flavonoids (Flavonols and Flavones) from *Acalypha indica* L. on thebasis of the colors, R_f values and absorption maxima.

Sr. No.	Flavonoids	Color in UV Plus NH_3	R_f (x100) in		Spectral *max.* in EtOH (nm)
			Forestal	BAW	
1	**Flavonols**				
	Kaempferol	Bright yellow	---	83	368
2	**Flavones**				
	Apigenin	Dull ochre	83	---	336
	Luteolin	Yellow	66	---	350

RESULTS AND DISCUSSION

Flavonoids like glycosides, anthocyanidins, flavonols and flavones were detected from *Acalypha indica* L. The anthocyanidins like Pelargonidin, Rosinidin and Columnidin were detected by PC. The visible colors of Pelargonidin, Rosinidin and Columnidin were observed on chromatograms as red, magenta and orange-red, respectively. The R_f values of these anthocyanidins were measured as 68 (Pelargonidin) in Forestal and 76 (Rosinidin) and 54 (Columnidin) in Formic acid solvent system. The spectral values of these anthocyanidins were recorded as 524, 524 and 511 nm, respectively. Rosinidin and Columnidin were not observed in Forestal solvent and Pelargonidin was not recorded in Formic acid solvent system (Table 1).

Glycosides like 3-rhamnoside (Pelargonidin) and 3, 5-diglycoside (Rosinidin) were identified as a dull and dull orange in colors, respectively. The R_f values of these glycosides were measured as 72 (3-rhamnoside) in acetic acid-HCl and 71 (3, 5-diglycoside) in BAW solvent system. Pelargonidin (3-rhamnoside) was not observed in BAW solvent and Rosinidin (3, 5-diglycoside) was not recorded in acetic acid-HCl (Table 2).

Some flavonols and flavones are also recorded from *Acalypha indica* L. Kaempferol (Flavonol) was appeared as bright yellow in color in presence of ammonia fuming under UV light. The R_f value of this flavonol was measured as 83 in BAW solvent system. The spectral value of kaempferol was observed in 95% Ethanol as 368 nm. Flavones were observed as dull ochre (Apigenin) and yellow (Luteolin) in coloration. The R_f values of these flavones are measured as 83 and 66 in Forestal solvent system and the spectral values were recorded as 336 and 350 nm in 95% of Ethanol, respectively (Table 2).

Acalypha indica L. is distributed as weed but it contains a various phytochemical compounds like flavonoids. These flavonoids widely used in medicine, because it shows the antioxidant properties,

antimicrobial activity, antifungal activity and it protects from heart diseases. So this weed plant is also source of flavonoids.

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