COMPARATIVE STUDY OF THE FLAVONOIDS FROM *CHROZOPHORA ROTTLERI* (GEIS) JUSS EX SPRENG AND *CHROZOPHORA PROSTRATA* DALZ & GIBS

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ABSTRACT

Flavonoids play an important role in plants as pigmentation and defence. They also show as antioxidant, antibacterial, anti-tumour and anti-inflammatory activities. Due to the above importance, the present study was taken for the identification, detection and also comparison between *Chrozophora rottleri* (Geis) Juss ex Spreng and *Chrozophora prostrata* Dalz & Gibs (Euphorbiaceae). Flavonoids from *Chrozophora rottleri* and *Chrozophora prostrata*, each plant stem and leaves was used separately and was extracted through Paper Chromatography (P.C.) method with using different solvents. Each flavonoid was identified and detected by measuring R_f value with using absorption spectra. Plants flavonoids were recorded, these recorded flavonoids were anthocyanidins, glycosides, flavones and flavonols and they were comparatively studied. From the result, Cyanidin (Anthocyanidin) and Apigenin (Flavone) are commonly observed in *C. rottleri* and *C. prostrata* but other flavonoids show different colour pigments, R_f values and absorption spectra which were recorded as dissimilar in chemical nature.

KEYWORDS *C. rottleri*, *C. prostrata*, PC, Flavonoids, spectroscopy

Received: Oct 30, 2020; **Accepted:** Nov 20, 2020; **Published:** Dec 26, 2020; **Paper Id.:** IJBRDEC202010

INTRODUCTION

Plants flavonoids play an important role for colour pigmentation and defence mechanisms. These are polyphenolic and biological active compounds. Flavonoids are widely present in plants and they are found in the cell vacuoles as glycosides. Flavonoids show antioxidant in reducing cancer risk (Block, 1992), risk of coronary heart diseases (Hertog et al, 1993), antimicrobial activity (Cushnie TPT et al, 2005) and antifungal activity (Galeotti F; et al 2008). Flavonoids are classified into many sub groups namely, viz., anthocyanidins, glycosides, flavones, flavonols etc. Anthocyanidins like Cyanidin and Delphinidin inhibit epidermal growth factor receptor in cancer cells (Acquaviva R. et al, 2003) while Malvidin is less effective. Flavones and flavonols are collectively known as anthoxanthin. Anthoxanthins are yellow colour pigments. They are widely distributed in petals and leaves of higher plants. The highly hydroxyl flavones such as Apigenin and Chrysoeriol which occur in plants possess a number of medicinal values. Apigenin shows anti-tumour properties (Jing Fang et al, 2009) and pharmaceutical interest in relation to inhibit anti-oestrogen resistant breast cancer cell growth (Long X et al, 2008). It also shows potent inhibitor of CYP2C9 in the body (Si Dayong et al, 2009). Chrysoeriol shows antioxidant and anti-inflammatory properties (Moscatelli V et al, 2006). Flavonols like Gossypetin shows antibacterial activity (Mounnissamy VM et al, 2002). *Chrozophora rottleri* (Geis.) Juss ex Spreng (Euphorbiaceae) is branched herbs, leaves 3-lobed, truncate, chordate, wrinkled and rugose with deep seated nerves, stellate, tomentose on both surfaces and flower in terminal, densely

stellate hairy racemes and found on black cotton soil.

Chrozophora prostrata Dalz & Gibbs (Euphorbiaceae) is prostrate, stellate hairy herb, branches red, leaves ovate or orbicular, red-brown, truncate, plicate, hoary, tomentose, beneath, petals yellow and found in cultivated field, river banks and drying ditches. These plants are also the major sources of their flavonoids. Such plants are neglected as to weeds. Hence the present study was undertaken to identification and comparison of flavonoids in these plants.

MATERIALS AND METHODS

Chrozophora rottleri and *Chrozophora prostrata* were collected from cultivated field of Basmathnagar Dist. Hingoli (M.S.), India. These plants were identified on the basis of morphology and were made Herbarium which was stored in Botany Laboratory, Adarsh College, Hingoli.

I PREPARATION OF PLANT EXTRACT (each plant separate)

I. I Glycosides

Chrozophora rottleri and *Chrozophora prostrata*, each plant stem and leaves was taken and separately dried in Oven at 50°C. Each dried material was collected and treated with boiling alcohol at 90°C for few minutes. Then this treated material was filtered through Whatman filter paper No.3. Each filtrate was taken and concentrated by Rotary vacuum evaporator (R. V. E.) at 40°C. The concentrated filtrate of each species was again treated with Petroleum ether to avoid latex. The latex free extract was taken and treated with diethyl ether to separation of catechin and cinnamic acid. Catechin and Cinnamic acid free extract was concentrated in R. V. E. This concentrated extract was treated with Ethyl acetate to remove flavonols and flavones. Then Ethyl acetate layer was formed and this layer was discarded. Ethyl acetate free extract was concentrated in R. V. E. The concentrated material was dissolved in Methanolic HCl which was taken in 97:3ratio. The obtained mixture was blended and centrifuged at 2000 Rpm for 3 minutes to formation of supernatant. This supernatant was concentrated by using of R. V. E. at 40°C. This concentrated material of each plant was taken and spotted on the proper site of Paper chromatography (PC) for identification of glycosides. These glycosides were separated using BuHCl solvent and 1% HCl solvent system. The R_f value of each glycoside was measured.

I. II Anthocyanidins

From glycosides extract preparation method was also using for same preparation of supernatant of each plant. The obtained supernatant was used for identification of anthocyanidins. This obtained supernatant was treated with 2M HCl and it was boiled at 50°C for 40 minutes. Then the small volume of isoamyl-alcohol was taken and in it dissolved the small amount of this boiled supernatant and it was concentrated in R. V. E. Then isoamyl-alcohol solution was spotted on the proper site of PC. Each plant spotted PC papers were dried and used Forestal solvent and Formic acid solvent in 3:30:10 and 2:5:3 ratios, respectively for separation of compounds. Each compounds R_f value was measured and calculated for identification of anthocyanidins.

I. III Flavonols and flavones

As per glycosides procedure up to concentrated the diethyl ether extract was again prepared for identification of flavonols and flavones. The prepared diethyl ether extract of each plant was taken separately. This extract free from Cinnamic acid and catechin, it was concentrated in R. V. E. Then the concentrated extract was treated with 2M HCl and it was boiled at 100°C in Oven for 40 minutes. This boiled extract was again concentrated through R. V. E. Then the concentrated extract

was taken and treated with Ethyl acetate. Ethyl acetate extract was concentrated up to dryness. The small volume of Ethanol was taken and in it dissolved the small amount of dried residue. The prepared Ethanolic solution was spotted on the proper site of PC papers. Each plant spotted PC paper was dried and using different solvent systems for separation of these compounds. These systems were BAW, PhOH and Forestal solvent in 4:1:5, 3:1 and 3: 30:10 ratios, respectively. Each PC paper was used for each solvent, separately. Each flavonols and flavones compound were identified by the using of UV light with fuming of Ammonia. Each compound colour was observed, measured and calculated the R_f value.

II SPECTRAL ANALYSIS (each plant separate)

II. I Anthocyanidins:

Each colour compound from each PC was taken and it was eluted in the solution contained water, Methanol and Acetic acid (25:70:5v/v). This solution was continued till the paper become colourless. Then each obtained mixture was filtered through Whatman filter paper, separately. Each filtrate was taken and concentrated through R. V. E. with using desiccators to formed dried residue. Then Methanol containing 1% HCl was taken and in it dissolved the dried residue. This obtained Methanolic solution was used for spectroscopic analysis. The absorption spectra of each solution was recorded in the region of 450-600nm.

II. II Flavonols and Flavones

Each band from PC paper was separated and it was dissolved in 95% Ethanol in dark at room temperature, separately for each plant as well as each band. This Ethanolic solution from each band was filtered by Whatman filter paper. Each filtrate was used for spectroscopic identification for flavonols and flavones. This filtrate was scanned in addition with the trace amount of $AlCl_3$, Sodium ethoxide and Sodium borohydrate which were used for colouration. The absorbed visible spectrum was recorded in the region of 268-386 nm.

Table 1: Comparative studied Glycosides (flavonoids) from *Chrozophora rottleri* (Geis.) Juss ex Spreng and *Chrozophora prostrata* Dalz & Gibs were observed on the basis of visible colours and measured R_f values

Sr. No.	Name of the plant	Visible colour	R_f value (x 100) in		Glycosides
			BuHCl	1% HCl	
1	<i>Chrozophora rottleri</i>	Dull orange yellow	--	73	3-Sophoroside 7-glucoside (Pelargonidin)
		Intense yellow fluorescent	41	--	3-Deoxyanthocyanidins 5-glucoside
2	<i>Chrozophora prostrata</i>	Dull purple	67	--	Guineesin (Petunidin)
		Dull magenta	--	54	3-Sophoroside 5-glucoside (Cyanidin)

Table 2: Comparative studied Anthocyanidins (flavonoids) from *Chrozophora rottleri* (Geis.) Juss ex Spreng and *Chrozophora prostrata* Dalz & Gibs were observed on visible colours, R_f values and absorption spectra

Sr. No.	Name of the plant	Visible colour	R_f value (x 100) in		Visible max. in MeOH-HCl λ_{max} .	Anthocyanidins
			Forestal	Formic acid		
1	<i>Chrozophora rottleri</i>	Purple	46	--	543	Petunidin
		Red	68	33	520	Pelargonidin
		Magenta	--	22	535	Cyanidin
2	<i>Chrozophora prostrata</i>	Purple	60	--	542	Malvidin
		Magenta	--	22	535	Cyanidin

Table 3: Comparative studied Flavonols and flavones (Flavonoids) from *Chrozophora rottleri* (Geis.) Juss ex Spreng and *Chrozophora prostrata* Dalz & Gibs were observed on the basis of colours, calculated R_f values and recorded spectra

Sr. No.	Name of the plant	Colour in UV+NH ₃	R_f value (x 100) in			Spectral max. in EtOH (nm)	Flavonols	Flavones
			Forestal	BAW	PhOH			
1	<i>C. rottleri</i>	Dull Ochre	83	89	--	336	---	Apigenin
2	<i>C. prostrata</i>	Dull Ochre	83	--	--	336	---	Apigenin
		Yellow	--	82	--	350	---	Chrysoeriol
		Dull Black	--	--	12	278	Gossypetin	---
		Fluorescent yellow	--	--	50	369	Azaleatin	---

RESULT

Flavonoids of *Chrozophora rottleri* (Geis.) Juss ex Spreng and *Chrozophora prostrata* Dalz & Gibs. were observed and identified on the basis of visible colours, measured R_f values and recorded spectra. These flavonoids were observed as glycosides, anthocyanidins, flavonols and flavones. Glycosides of *C. rottleri* were recorded as 3-sophoroside 7-glucoside (Pelargonidin) and 3-Deoxyanthocyanidins 5-glucoside were observed and identified as dull orange yellow colour in 1% HCl solvent and other an intense yellow fluorescent colour was shown in BuHCl solvent. Both visible colours measured and calculated of these R_f values of dull orange yellow was 73 and intense yellow fluorescent was recorded as 41 (from Table 1). In *C. prostrata*, glycosides were also recorded as 3-sophoroside 5-glucoside (Cyanidin) and Guineesin (Petunidin) which were identified on the basis of visible colours through PC and were observed as dull magenta (Cyanidin glycoside) and dull purple (Petunidin glycoside) in colours.. The R_f value of each glycoside was measured and calculated. These R_f values were obtained from BuHCl solvent as 67(Guineesin) and 54 (3-sophoroside 5-glucoside) in 1% HCl

solvent systems (from Table 1). Identified anthocyanidins from *C. rottleri* were observed on the basis of visible colours from PC papers and R_f values and also absorption spectra. Pelargonidin was observed as red colour in Forestal and Formic acid solvents and its measured R_f values from these solvents were 68 and 33, respectively. Petunidin was purple in Forestal solvent and its R_f value was measured as 46. The visible colour of Cyanidin was observed in Formic acid solvent as magenta and its R_f value was measured as 22. The absorption spectra were recorded as 520 nm (Pelargonidin), 543 nm (Petunidin) and 535 nm (Cyanidin). But in *C. prostrata*, the visible colours of anthocyanidins were observed as purple and magenta. The purple colour was measured and calculated the R_f value from PC as 60 (Malvidin) in Forestal solvent. Cyanidin was observed as magenta colour and its R_f value was measured as 22 in Formic acid solvent. The observed spectra were recorded as 542 nm (Malvidin) and 535 nm (Cyanidin). Cyanidin was recorded from both plants in the same solvent (Formic acid). Pelargonidin was observed in *C. rottleri* which was recorded from both solvents (Forestal and Formic acid). In *C. prostrata*, Malvidin was recorded but Pelargonidin was not observed in both solvent systems. Petunidin and Malvidin were not observed in Forestal solvent (Table 2).

Flavonols were not recorded from *C. rottleri* in BAW, PhOH and Forestal solvents but these were observed in *C. prostrata* as dull black and fluorescent yellow colours under UV light with fuming of ammonia. The dull black colour was measured from PC and the R_f value was calculated as 12 (Gossypetin) in PhOH solvent. The R_f value of fluorescent yellow colour was also measured and calculated from PC as 50 (Azaleatin) in PhOH solvent system. But these flavonols were not recorded in BAW and Forestal solvents. The absorption spectrum of each flavonol was recorded as 278 nm (Gossypetin) and 369 nm (Azaleatin). Flavones like Chrysoeriol and Apigenin were recorded in BAW and Forestal solvents but these flavones were not observed in PhOH solvent system. Flavones were also recorded from *C. rottleri* and *C. prostrata*. These recorded flavones were observed as dull ochre and yellow colour under UV light with fuming of ammonia. In both plants, the dull ochre colour was observed and measured R_f value from PC as 83 from Forestal solvent. But this colour was also observed in *C. rottleri* from BAW solvent and its R_f value was calculated from PC as 89 (Apigenin). The yellow colour was recorded from *C. prostrata* but it was not observed in *C. rottleri*. Yellow colour was measured and calculated from PC and the recorded R_f value was 82 (Chrysoeriol) in BAW solvent. But Chrysoeriol was not recorded from *C. rottleri* and it was also not observed in Forestal and PhOH solvents. The absorption spectrum of Apigenin was recorded as 336 nm and 350 nm of Chrysoeriol from Ethanolic solution (Table 3).

CONCLUSIONS

From the results of present study observation shows that glycosides, anthocyanidins, flavonols and flavones are recorded from *C. rottleri* and *C. prostrata*. Flavonols are observed in *C. prostrata* only but these are not observed in *C. rottleri*. So this stand, it may be concluded that each identified flavonoids shows different visible colour, R_f value and absorption spectrum. These plants flavonoids are not similar in proportion but Cyanidin (Anthocyanidin) and Apigenin (Flavone) are commonly recorded from both plants. So that it also focuses on the plant taxonomists and chemo-taxonomists perspective because most of the plants show similar in morphology but they are rarely similar in chemical nature. *C. rottleri* and *C. prostrata* are neglected as weeds but they contain a polyphenolic compounds i.e. flavonoids. Plants flavonoids are used in medicine to control of different microbial, fungal and cancer diseases and they are also protected to heart. So these undesired plants contain flavonoids which are beneficial to mankind.

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