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Fingerprint chromatogram analysis and detection of antioxidants markers in various extracts from the leaves of *Hemigraphis colorata* (blume) H.G. Hallier by high performance thin layer chromatography

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ABSTRACT

A simple and reliable high performance thin layer chromatographic (HPTLC) method has been developed and validated for the study of fingerprint chromatograms of the hexane , chloroform, ethyl acetate and aqueous extracts of leaves of *Hemigraphis colorata* (blume) H.G. Hallier .HPTLC separation of the all extracts was performed and scanned at ultraviolet absorbance at 254 nm. A mobile phase composed of toluene: ethyl acetate: formic acid :methanol (3:6:1.6:0.4).The chromatogram obtained and the peak profile of the components collected by scanning could made up the fingerprint of the various extracts from leaves of *Hemigraphis colorata* and compared with antioxidants markers of flavonoids and phenolic acids. The fingerprint chromatograms had a good stability, precision, and reproducibility. The method is suitable for differentiation of extracts from the leaves of *Hemigraphis colorata*, and can be used as a quality control method for this plant.

Keywords: Hemigraphis colorata, ethyl acetate extract, rutin ,quercetin ,Gallic acid

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INTRODUCTION

Plants are gifted with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stannins, flavonoids, , coumarins, alkaloids, amines, and other metabolites, which are rich in antioxidant activity[1] Studies have revealed that many of these antioxidant Compounds own anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities[2]. The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing[3,4]. In recent years, there has been a worldwide inclination towards the use of the natural phytochemical present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables[5]. The therapeutic efficiency of the drugs depends greatly on the use of proper and genuine raw materials. Because of this, the guarantee of safety, quality and subsequent efficacy of the medicinal plants and herbal products have now become a major and explanation area under discussion, so the standardization of plant materials is compulsory[6]. Hence High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs due to its simplicity, high sensitivity, accuracy and less expensive[7]. *Hemigraphis colorata* (Blume), is a versatile tropical, low creeping perennial herb that reaches a height of 15 to 30 cm. It prostrates and spreads with rooting stems when grown on ground, and on hanging baskets it cascades over beautifully is a prostrate growing plant with spreading, rooting stems. The tiny white flowers grow intermittently throughout the year [8,9]. This plant possesses various medicinal properties, only a few are reported like, the whole plant or leaves are used to treat fresh wound, cuts, ulcers, inflammation[10] and in folk medicines it is used internally to cure anaemia, gallstone, diuretic, haemorrhoids and diabetic mellitus [11]. Anti-bacterial activity Benzene extract of *Hemigraphis colorata* leaves has showed its activity against acinetobacter species and *Streptococcus aureus* [12]. Phenolic compounds found in the extract are responsible for the activity [13]. Traditionally, the leaves are consumed to mend gall stones, excessive menstruation and as a contraceptive. In Vanuatu, sap of leaf buds are squeezed in water and drunk at dawn for 4 days as contraceptive and to induce sterility. In Java, leaves are used to treat bloody dysentery and haemorrhoids. It is also credited with diuretic competence. The whole plant of *Hemigraphis colorata* is ground into a paste with water and used for diabetes mellitus [14]. The crude leaf paste promotes excision wound healing [15,16]. In mice, the leaf paste provides faster wound contraction and epithelialisation but oral administration is seen ineffective [17]. The excision and incision wound model studies revealed that methanolic extract is

comparable to standard reference Vokadine [18]. Ethyl acetate extract of *Hemigraphis colorata* leaves shows anti-inflammatory and wound healing properties and inhibit 5 lipoxygenase and cyclooxygenase1 and 2 enzymes[19]. So the endeavour of the present job is to screen by HPTLC finger print analysis of hexane ,chloroform, ethyl acetate and aqueous extracts of *Hemigraphis colorata* leaf.

MATERIALS AND METHOD

Collection and preparation of extract

The entire plant of *Hemigraphis colorata* was collected during flowering season in 2016 from river banks and fallow fields in malapuram area, Kerala, India. Mr.V.Chelladurai Research Officer of Botany, Central Council for Ayurveda and Siddha, Government of India. A voucher specimen was preserved in our laboratory for future reference. Coarse leaf powder of *Hemigraphis colorata* (20g) was subjected for sequential maceration separately with n-hexane , chloroform, and ethyl acetate (each100ml) at room temperature (Cooper & Colin Gunn, 1957).The extracts were then filtered and the solvents were evaporated to dryness under reduced pressure in Eyele Rotary evaporator (Japan) at 40^{o}C. The percentage yield of the extracts was 4.2%,2.8% and 7.8% respectively.

Chemicals and Instruments

Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. TLC was carried out using Merck aluminium sheet coated with silica gel GF_{254} (0.2 mm).

Sample application

10µl of test solutions and 5µl of standard solution were loaded as 6mm band length in the 10 x 10 Silica gel 60F_{254}TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (standards) and the plate was developed in the respective mobile phase up to 80mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was Photo documented the images at UV 254nm.

Scanning

The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted. The software used was

winCATS 1.3.4 version A HPTLC profile is done for the sample Hemigraphis colorata. The HPTLC image is shown in Figure1.

RESULTS AND DISCUSSION

The following different solvent compositions were tried for monitoring the elution of components in different extracts. [20].Ethyl acetate: glacial acetic acid formic acid: water (100:3:3:28), Ethyl Acetate: Methanol: Water Toluene (100:13:10:13), Chloroform: ethyl acetate: methanol (6:4:0.3). Ethyl Acetate: Methanol :Water Toluene (100:15.5:13.5:2), Ethyl acetate: methanol: water (100:15.5:13.5), Toluene :ethyl acetate : formic acid :methanol (3:6:1.6:0.4), Ethyl acetate: methanol : water(100:13.5:10),Toluene : ethyl acetate (93:7). Totally 8 mobile phase were trailed for better elution of formulations. Of which Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) were given better elution for all the extracts to screen in one plate. The optimized chamber saturation time for mobile phase was 3.0 min at room temperature (25 ± 1°C). The densitometry analysis was performed at 254nm in reflectance mode. The elution of all the extracts were carried out in mobile phase of toluene: ethyl acetate: formic acid: methanol (3:6:1.6: 0.4) and in this mobile phase elution was good results were tabulated by considering each Rf value for one ingredients of extracts whether it may be pharmacologically active or inert but for screening the number of principle in the extracts can be considered as one of the principle in it. Therefore the obtained Rfvalue were compared with Rf value of the standard and well known free radical scavengers rutin, quercetin and gallic acid in *Hemigraphis colorata* various extracts (Fig:1 and Table :1). For identifying these free radical scavengers rutin, quercetin and gallic acid, we used UV light at 254 nm. Chromatogram of *Hemigraphis colorata* Hexane Extract(Figure: 2) shows rutin in hexane extract. Chromatogram of Chloroform Extract shows (Figure: 4) shows the presence of quercetin in extracts. Chromatogram of *Hemigraphis colorata* Ethyl acetate Extract (Figure: 6).

shows the presence of gallic acid and quercetin and Chromatogram of aqueous extract shows the presence of rutin(Figure :8). Figure:3, 5 and 7 are standard marker rutin, gallic acid and quercetin respectively.

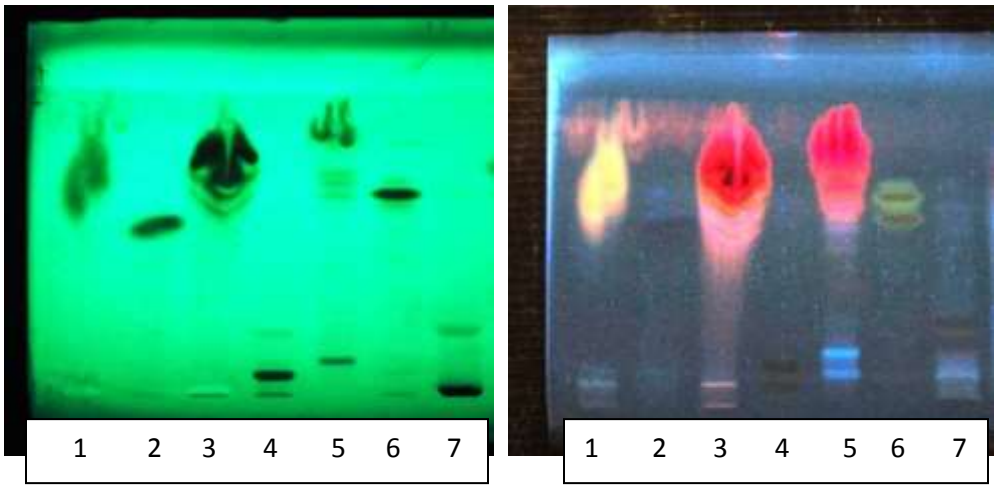

Figure 1: TLC Profile various extracts of *Hemigraphis colorata* at 254 nm

1. *Hemigraphis colorata* Hexane Extract (HCHE) 2.Standard marker Gallic acid

3. *Hemigraphis colorata* Chloroform Extract (HCCE)

4. Standard marker Rutin 5. *Hemigraphis colorata* Ethyl acetate Extract (HCEAE)

6. Standard marker Quercetin 7. *Hemigraphis colorata* Aqueous Extract (HCAE)

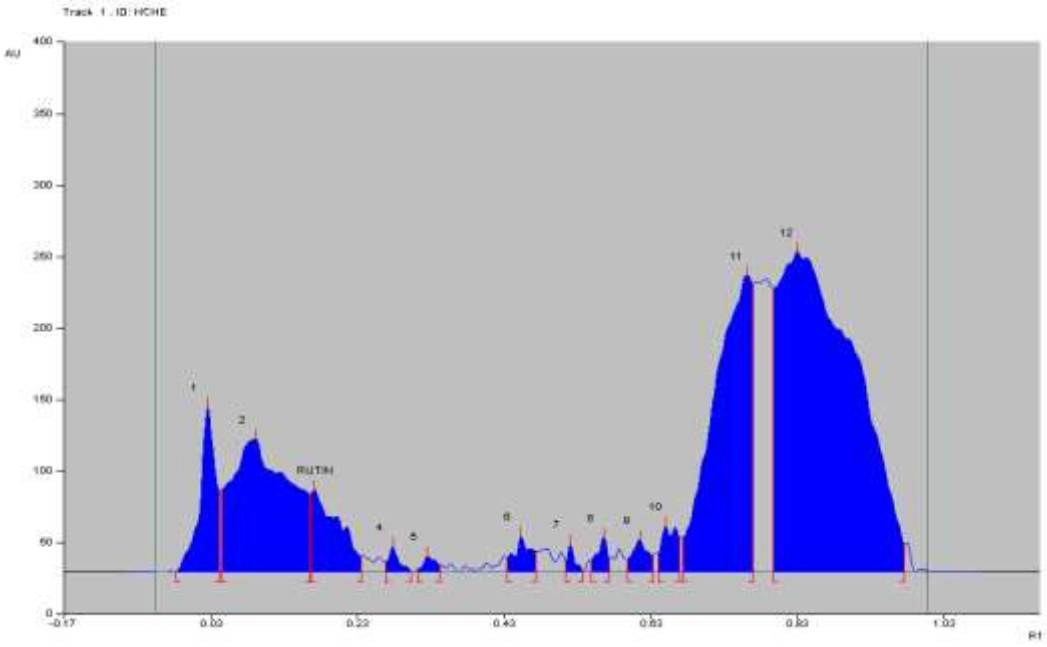

Figure 2: Chromatogram of *Hemigraphis colorata* Hexane Extract

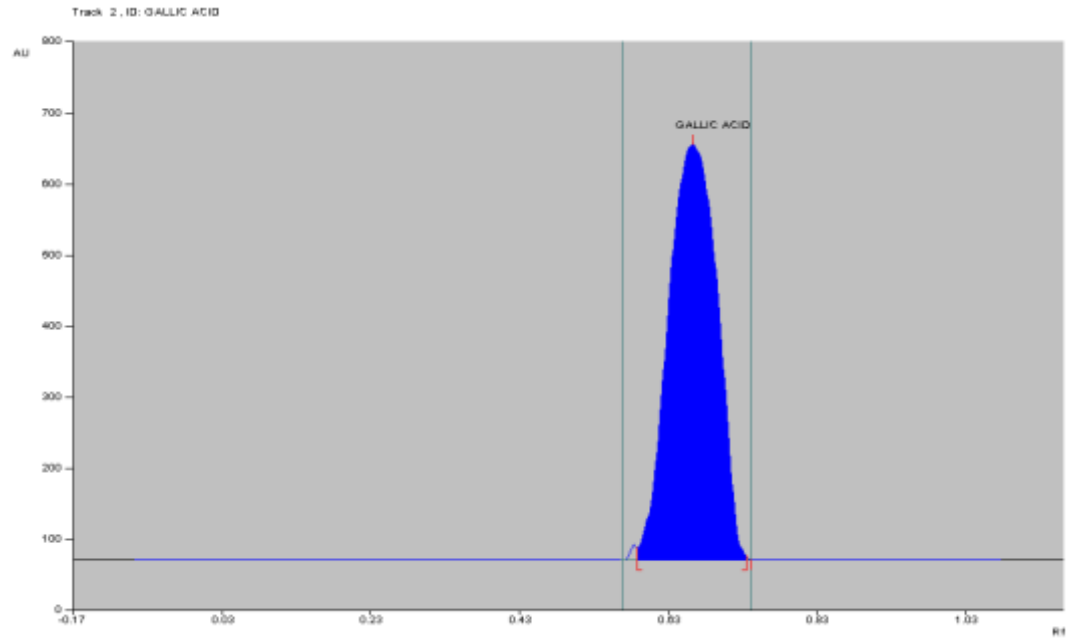

Figure 3: Chromatogram of standard marker Gallic acid

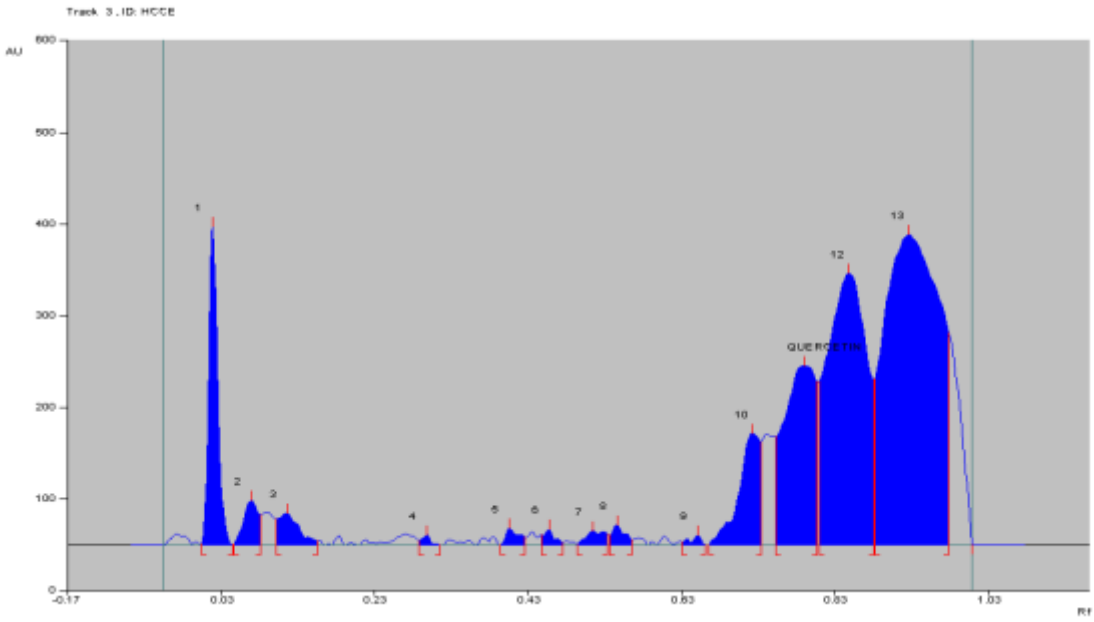

Figure 4: Chromatogram of *Hemigraphis colorata* Chloroform Extract

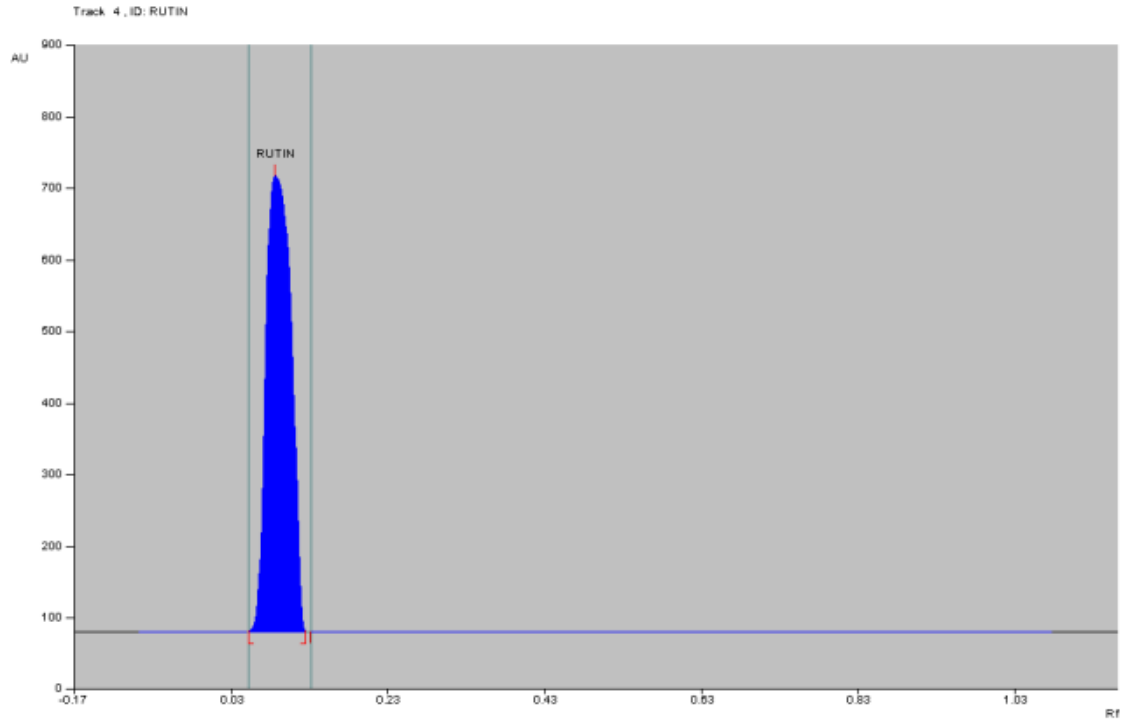

Figure 5: Chromatogram of standard marker rutin

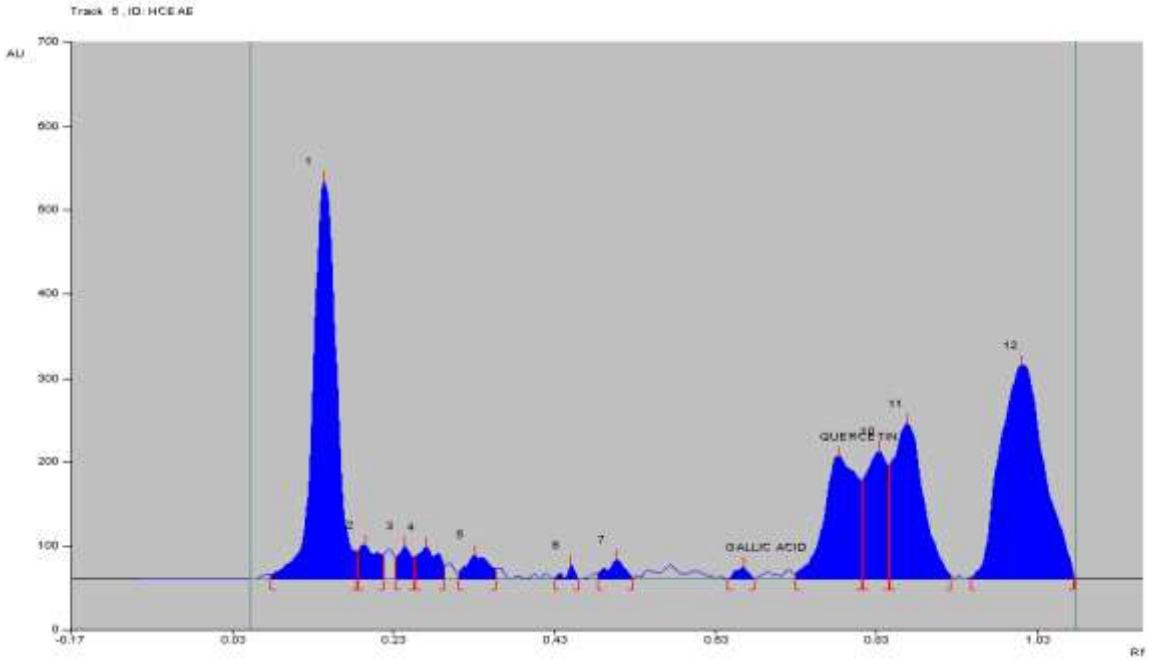

Figure 6: Chromatogram of *Hemigraphis colorata* Ethyl acetate Extract

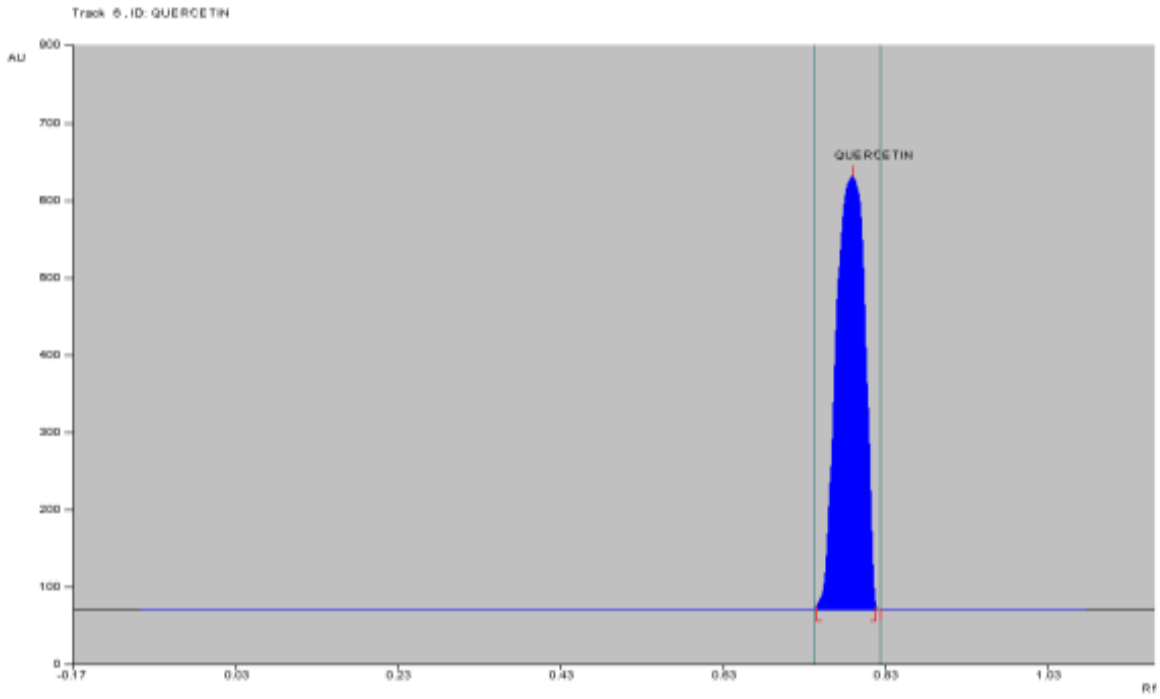

Figure 7: Chromatogram of standard marker Quercetin

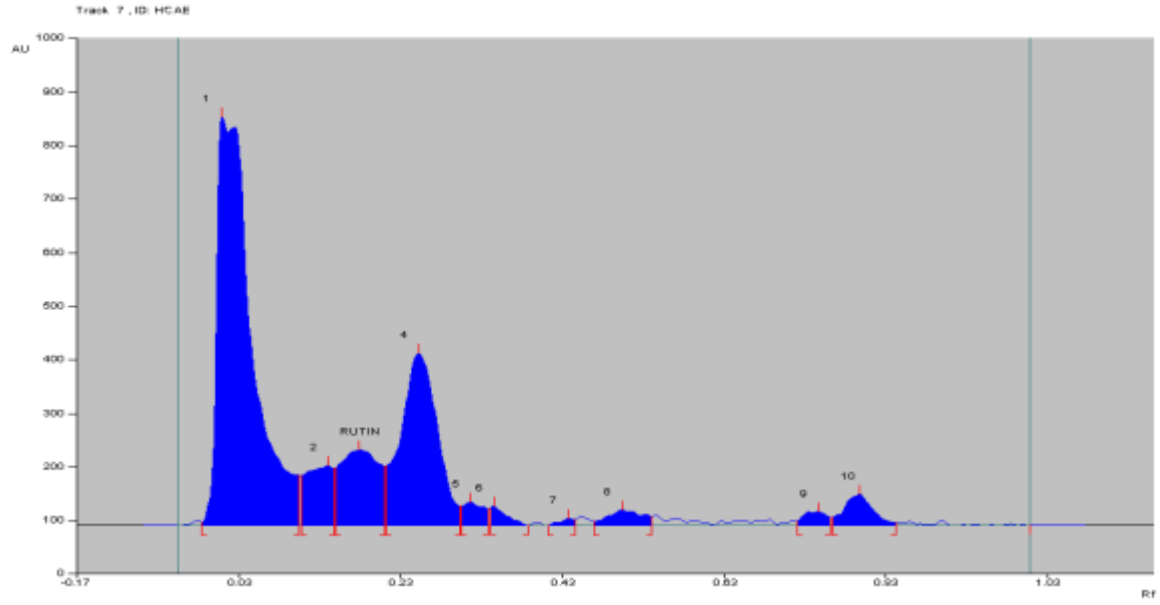

Figure 8 Chromatogram of *Hemigraphis colorata* Aqueous Extract

Table 1: Rf values of anti oxidant markers and various extracts of *Hemigraphis colorata*

Track No	**Amount of Sample in µl**	**Number of compounds**	**Rf value of the compounds in extracts**	**Presence of marker in extracts**	**Area of Standard Marker**	**Area of marker in sample**	**Amount of marker present µg/ 5 µl**	**% of marker in Extracts**
1	Hexane extract 5 µl	12	0.17	Rutin	--------	1445.8	0.38	0.076%
			0.66	Gallic acid	---------	479.2	0.09	0.018%
			0.77,	Quercetin	---------	207.0	0.03	0.06%
2	Gallic acid 5 µl	01	0.67	Standard	27522.2		5 µg	**100%**
3	Chloroform extract 5 µl	11	0.66,	Gallic acid	---------	98.5	0.02	0.04%
			0.79	Quercetin	---------	2373.1	0.41	0.82%
4	Rutin 5µl	1	0.17	Standard	19052.5	-------	5 µg	**100%**
5	Ethyl acetate extract5 µl	13	0.67	Gallic acid	--------	150.8	0.03	0.06%
			0.77	Quercetin	------	4192.8	0.71	1.42%
6	Quercetin 5 µl	01	0.79	Standard	29137.6	-----	5 µg	**100%**
7	Aqueous Extract 5 µl extract	10	0.18	Rutin	------	4786.3	1.26	2.52%
			0.75	Quercetin	------	517.5	0.09	0.18%

DISCUSSSION

By comparing with the Rf value of standard antioxidants markers in various extracts like rutin, quercetin and gallic acid (Table 1). Quercetin was present in all extracts but ethyl acetate extract contains nearly (1.42%) when comparing with other three extracts. Aqueous extract contain comparatively more rutin (2.52%) than other extracts. This plant was reported to response for anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities by Sahaa et al 2008[2] .By fingerprint analysis and screening for antioxidants markers in various extracts of *Hemigraphis colorata*, the pharmacological action may be due to presence of above detected flavonoids and phenolic acids and many scientific literature also support these markers as therapeutic component for various diseases. This was supplementary confirmed by Kandaswami et al 1994 and Duthie et al 1999 reported that rutin scavenges free radicals,[20,21]; Middleton reveled that rutin suppresses cellular immunity [22] as well as anti-inflammatory [23] . Quercetin has been reported to inhibit the allergic and inflammatory responses of the immune system [24] by modulating several aspects of cell function relevant to inflammatory arthritis. At the molecular level, quercetin is known to inhibit nuclear factor kappa B (NF-kB), a central transcription factor in inflammatory and proliferative diseases [25].Based on the fingerprint chromatogram analysis and detection of antioxidants markers in various extracts from the leaves of *Hemigraphis colorata* (blume) *H.G.* Hallier by high performance thin layer chromatography we conclude that the pharmacological action may be due to presence of rutin,quercetin and gallic acids.

CONCLUSION

It can be concluded that rutin, quercetin and gallic acid were simultaneously detected in various extracts from the leaves of *Hemigraphis colorata* (blume) *H.G.* Hallier by high performance thin layer chromatography we conclude that the pharmacological action may be due to presence of rutin, quercetin and gallic acids. The developed HPTLC method may be adopted for routine detection of rutin, quercetin, and gallic acid in various plant extracts by simultaneous detection..

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