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RESEARCH ARTICLE

QUANTITATIVE DETERMINATION OF RUBIADIN IN DIFFERENT ACCESSIONS OF *RUBIA CORDIFOLIA* LINN. BY ISOCRATIC RP-HPLC

Mohd Mughees[1], Shipra Rani Jha[2], Shabnam Akhtar[2],Javed Ahmad[2] and Altaf Ahmad[3]*.

1. Department of Biotechnology, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi-110062, India.
2. Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi-110062, India.
3. Department of Botany, Aligarh Muslim University, Aligarh-202001, India.

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Abstract

Rubia cordifolia L. (common name-Indian Madder, Majith, Manjistha) from the family Rubiaceae is commonly known as is widely dispersed throughout the lower hills of India. The plant is famous drug in the Ayurvedic treatments. Extracts of this plant have shown many important medicinal properties.In this study, we quantified the amount of rubiadin in both roots and aerial part of the plant. Theamount of the active principle is affected by the geographical areas. So, we also compared the amount of rubiadin in different accessions of the *R. cordifolia* Linn. that have been collected from different geographical areas of the India.

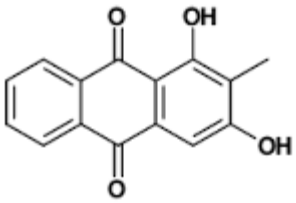
Introduction:-

Rubia cordifolia L. (common name-Indian Madder, Majith, Manjistha) from the family Rubiaceae is commonly known as is widely dispersed throughout the lower hills of Indian Himalayas (Shekhar et al ., 2010; Radha et al., 2011). The plant is important and famous drug in the Ayurvedic treatments. Extracts of this plant have shown hepatoprotective, antineoplastic properties and is also useful in the disintegration and elimination of urinary stones (Gilani and Janbaz,1995; Divakar et al., 2010). Anti-inflammatory, anti-ulcer and anti-dysentricactivities are also found in the roots of *Rubia cordifolia* Linn. (Deoda et al., 2011). It is also used in the treatment of diuretic, liver complaints, joint pains, uterine pains, in rheumatoid arthritis (Shekhar et al., 2010).

The major compounds of this plant are anthraquinones, alizarin, purpurin and their derivatives. It also contains ruberythric acid (alizarin-primeyeroside), pseudopurpurin and lucidinprimeveroside, rubiadin(1,3-Dihydroxy-2-methylanthracene-9,10-dione), munjistin, quinizarin, lucidin and 1,8-dihydroxyanthraquinone (Banyal et al., 2006).

Rubiadin(1,3-Dihydroxy-2-methylanthracene-9,10-dione), a dihydroxy anthraquinone, possesses effective antioxidant property and prevents lipid peroxidation (Tripathi et al., 1997). It also has hepatoprotective and anti-bacterial activity (Rao et al., 2006;Comini et al., 2011). For estimation of specific compounds in a sample HPLC is one of the several chromatographic techniques that are being generously utilized in laboratories all over the world (Mythili et al., 2011).

Corresponding Author:-Altaf Ahmad.
Address:-Department of Botany, Aligarh Muslim University, Aligarh-202001, India.



Rubiadin

Although, HPLC method for determination of rubiadin in the roots of *Rubia cordifolia* Linn. has been reported in literature. But quantitative determination of rubiadin in different accessions of the plant has not been reported yet. Further we also quantified rubiadin in both roots and aerial parts of *Rubia cordifolia* Linn. which has also not been reported yet. Present study deals with the quantitative determination of rubiadin by RP-HPLC in methanolic extracts of root and aerial parts of the different accessions of the plant. Briefly, in this study we verified the RP-HPLC method for the quantification of the rubiadin in different accessions of the plant.

Material and Methods:-

Plant material:-

Six different accessions of *Rubia cordifolia* Linn.were used in this study. Roots and aerial parts of plant were collected from six different geographical areas (Table. 1) of India. These samples were identified by Prof. M. P. Sharma, Head, Dept. of Botany, Jamia Hamdard. Voucher specimen is deposited at the herbarium of Department of Botany, Jamia Hamdard. New Delhi.

Extraction of Plant material:-

The plant samples were air dried and ground into a fine powder using a grinder. Then, the plant material was extracted using different solvent system in a soxhlet apparatus and by other methods as well. 20g dried plant material was extracted using 200 ml of each solvent system of increasing polarity like petroleum ether, chloroform, acetone, ethanol (80%) , methanol ethyl acetate, n-butanol and water respectively in a soxhlet assembly for 12 h. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on water bath or using Rotary evaporator. Extracts were also used directly for various tests. Extracts were collected and used for different phytochemical tests (Results in Table. 2)

HPLC chemicals and reagents:-

HPLC grade standard compound rubiadin was purchased from Natural Remedies (India). All the solvents and reagents used in the experiments were of HPLC grade. HPLC grade methanol and water were purchased from Merck, India. HPLC analysis was carried out on a Waters HPLC system (Binary Pump 600 controller), Waters PDA detector (996) and an auto sampler (2707). Empower 2 software was used to control the system and for monitoring and analysis of results. For chromatographic separation R_pC_{18} column (250×4.6 mm, particle size 5 µm) was used. Further, a sonicator, rotary evaporator (R-200/205/V (Buchi), a pH meter and hot air oven were also used.

Preparation of sample solution for HPLC analysis:-

Powdered samples of *R. cordifolia* (roots and aerial part)**,** plant sample (1g, accurately weighed) were extracted with methanol (2×25mL) for 24h at room temperature in sonicator. The combined extracts were filtered through Whatman Filter paper No 42. Extracts obtained were concentrated on rotary evaporator (R-200/205/V (Buchi) in vacuum to 10mL.

Preparation of Standard Solutions:-

A stock solution of rubiadin (1000 ug/mL) was prepared by dissolving 2.0 mg of standard rubiadin accurately weighed in 2 mL methanol in an eppendorf tube. Standard solution of 200 µg /mL was prepared from the stock solution by transferring 200 uL of stock solution, and diluting to volume with methanol (800 uL). Appropriate quantities of this standard solution was injected to obtain rubiadin in the range of 200-1000 ng.

HPLC Conditions:-

Chromatography was performed using Waters system (Miford, MA, USA),616 pump and 996 PDA detector. The volume injected was 20µL. Quantitative determination of rubiadin was performed at 295nm on a R_PC_{18} column (250×4.6 mm, 5 µm). The mobile phase consisted of methanol: water (80:20, v/v) (Khodke et al, 2010) . The flow-

rate was 1 mL/min. The solvent was filtered through a nylon membrane (0.45µm) and degassed by sonication before use. UV spectra was recorded from 210-400nm at a rate of 1.00 spectrum/sec and a resolution of 1.2 nm.

Calibration:-
Standard solutions of 10-200 µg/mL of rubiadin were prepared in methanol from the stock solution of 1 mg/mL and were used for the preparation of calibration graph. 20 µL of each of the standard solution was injected by the auto sampler with concentrations mentioned above and the linearity of response for rubiadin was determined. Calibration curve was drawn by plotting the peak areas rubiadin against the corresponding concentration.

Results & Discussion:-

The separation of rubiadin by RP-HPLC was carried out under optimized conditions. Optimization of mobile phase was carried out using various concentrations of methanol and water. Three different compositions of methanol and water were used, 75:25, 85:15 and 80:20. The optimum mobile phase was found to be Methanol: Water (HPLC grade) in the ratio of 80:20 it is the same as reported in the Khodke et al, 2010 method. Retention time of rubiadin was found to be 7.920 min as shown in Fig.1.A linear relationship between peak areas and concentrations was obtained in the range of 10-50µg/ml. This shows that method is linear. Repeatability studies show %RSD to be less than 2%.This shows that method is precise. %RSD for inter-day precision was higher than that of intra-day precision. Excellent recoveries were obtained at each level of added concentration as the mean recovery found to be within 98% to 102% for rubiadin. The limit of detection and limit of Quantitation of method was found to be 55.75ng/ml and 200ng/ml (Table.3). As it was found that rubiadin peak gets well resolved from peaks of other chemical constituents, hence we conclude that method is selective. Further, the quantification of rubiadin in different samples of *R. cordifolia* was also successfully done (Fig. 2; Table. 4)
Quantification of rubiadin in roots of *R. cordifolia* has been reported in the previous study (Khodke et al., 2010). This analysis showed that rubiadin is present in roots only but in our study we quantified the rubiadin in both roots and aerial parts of this plant. This study not only supports the previous study that roots are good source of rubiadin but also revealed that significant amount of rubiadin is also present in the aerial part of this plant. Thus, the whole plant can be utilized to extract rubiadin. Further, we also quantified the rubiadin in aerial and roots of the *R.cordifolia*collected from different geographical areas which has not been reported earlier. Among all the six accession that has been used in the study the accession no.5 has the higher amount of rubiadin in its roots.

Table 1:- List of Plant materials (*Rubia cordifolia* Linn.) used in this study.

Plant name	Explant	Sample	Locality
Rubia cordifolia Linn.	Aerial part	Rb-1	IHBT(Palampur)
	Aerial part	Rb-2	Indus nursery (Bangalore)
	Root	Rb-3	Chopta forest (Himalyas)
	Root	Rb-4	University of Kashmir (Srinagar)
	Root	Rb-5	Chamba (Himachal Pradesh)
	Root	Rb-6	Hamdard laboratory (Ghaziabad)

Table 2:- Phytochemical screening of *Rubia cordifolia* Linn.

Constituent	ME	PF	CF	EAF	WF
Anthraquinones	+	+	+	+	+
Glycosides	+	-	+	+	+
Tannins	+	-	-	-	-
Saponins	+	+	+	+	+
Triterpinoids	+	+	+	-	-
Alkaloids	-	-	-	-	-

ME: Methanol Extracts, PF: Petroleum ether fraction, CF: chloroform fraction
EAF: Ethyl acetate fraction and WF: Water fraction: + = present, - = absent

Anthraquinone and glycosides are present in almost all fractions of*Rubia cordifolia*Linn. and roots are rich in anthraquinones butalkaloids were not present.

Table 3:- Calibration data

Parameter (Units)	**Rubiadin**
Regression equation	Y=8580X + 26481
Linearity range (µg/mL)	10-200
r^2±SD	0.993781 ± 0.0005

Table 4:- Quantitative estimation of rubiadin in methanolic extracts of *Rubia cordifolia* Linn.

Accession no.	**Plant part**	**Content of rubiadin (% w/w) of sample Mean ± SD**
1	Aerial part	0.12 ± 0.091
2	Aerial part	0.15 ± 0.091
3	Root	0.48 ± 0.070
4	Root	0.27 ± 0.073
5	Root	0.54 ± 0.071
6	Root	0.38 ± 0.070

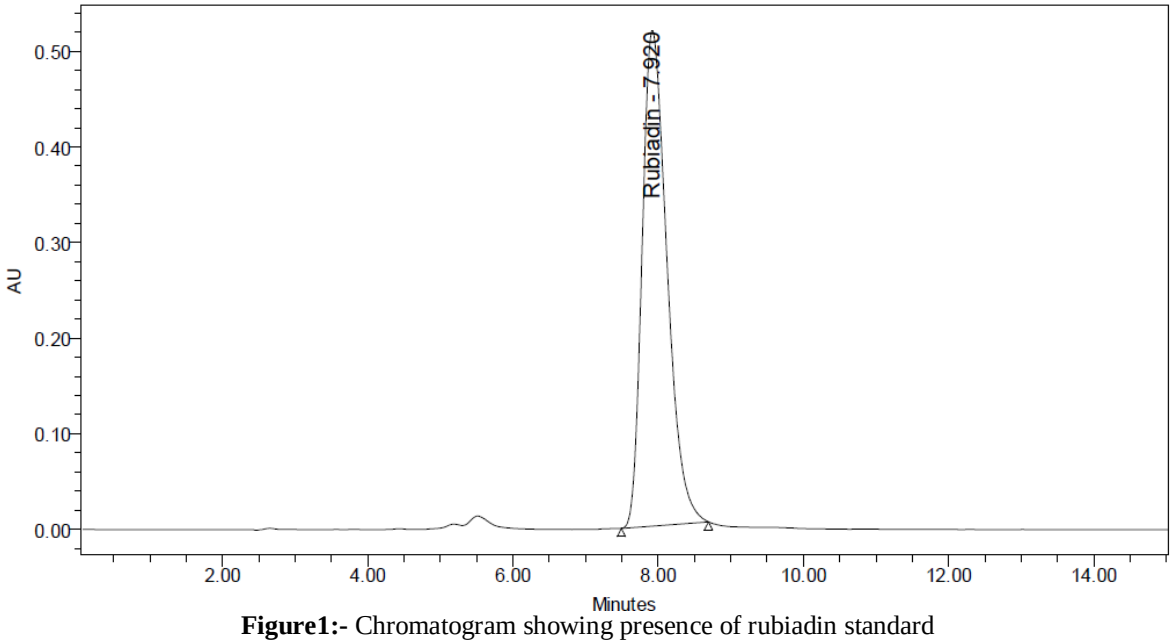


Figure1:- Chromatogram showing presence of rubiadin standard

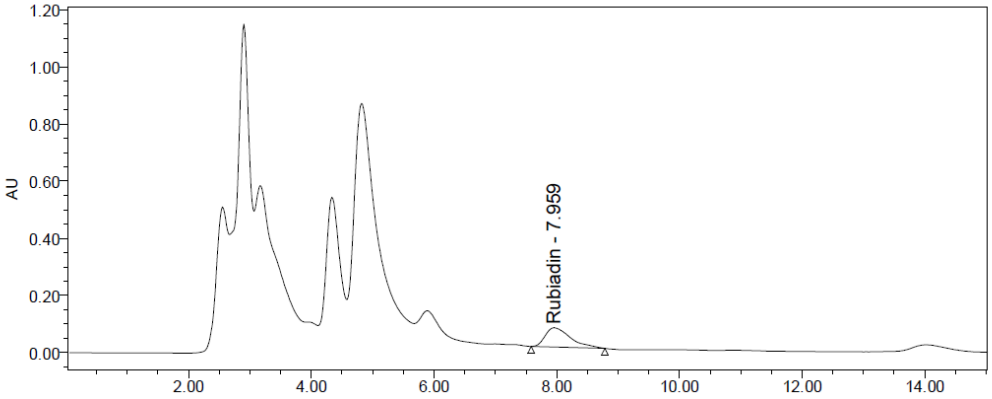


Figure 2:- Chromatogram showsthe presence of rubiadin in mixture of compounds in root extract of *R. cordifolia*Linn.

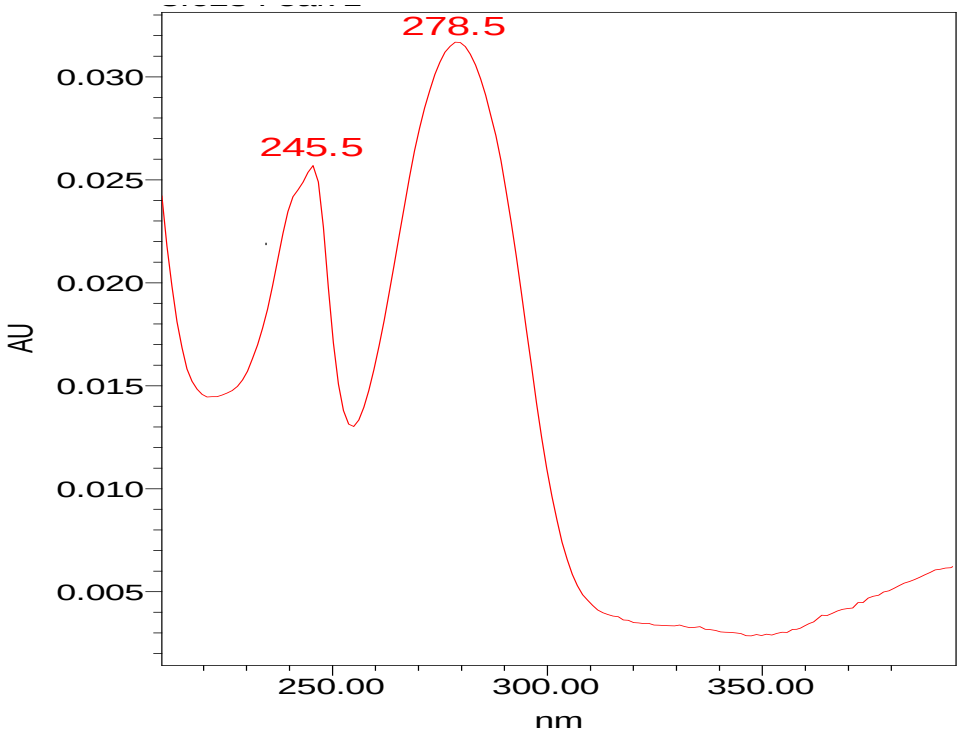


Figure3:- Spectra of peak of rubiadin in standard

Conclusion:-

In our study, we compared the amount of rubiadin in different samples of the *R. cordifolia* plant that have been collected from the different geographical areas.

Conflict of interest:-

The authors have no conflict of interest.

Acknowledgment:-

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