

GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF ETHANOLIC EXTRACT OF ROOTS OF *RUBIA CARDIFOLIA* (MANJISTHA)SHAIKH GAZI^{1,4*}, SADATH ALI², SHAIKH LUQMAN³, SYED HUSSAIN RIZWAN¹

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ABSTRACT

Objective: The present research investigation was carried out with prime objective of studying the phytoconstituents of roots of *Rubia Cardifolia* after isolation with flash chromatography technique.

Methods: The ethanolic root extract of *Rubia Cardifolia* was employed for GC-MS study.

Results: The study on active principles of ethanolic root extract of *Rubia Cardifolia* by GC-MS, showed the presence of two important compounds. Rubiadin (30.81% area) and Purpurin (11.45 % area). More compounds would have appeared, but trying GC-MS with the isolated pure fraction by flash chromatography of root extract lead to isolation and discovery of two principal compounds.

Conclusion: GC-MS analysis after carrying flash chromatography of ethanolic root extract led to identification of 02 pure compounds. The compounds were found to be Rubiadin (1, 3 dihydroxy 2 methyl anthraquinone) and Purpurin (1, 2, 4 trihydroxy anthraquinone). The presence of Rubiadin and Purpurin justifies the usage of the root extract of plant for treatment of skin ailments.

Key words: GC-MS, *Rubia Cardifolia*, Psoriasis, Rubiadin, Purpurin

INTRODUCTION

Since the dawn of history in India, medicines based on herbal origin have been the basis of treatment and cure for various diseases. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, scabies, venereal disease, ulcers, and snake bite. *Rubia cardifolia* is an important medicinal plant which is used for the treatment of various ailments in Ayurvedic system of medicine since ancient times (Fig. 1). *R. cardifolia* is commonly known as "Indian Madder" and sold under the trade name "Manjistha." In the ancient world, Manjistha is reputed as an efficient blood purifier immunomodulator [1] and hence is extensively used against blood, skin, and urinary diseases [2,3]. The root is sweet, bitter, acrid, astringent, thermogenic, antidysenteric, anti-inflammatory [4], antipyretic, analgesic, anodyne, anthelmintic, antiseptic, constipating, diuretic, galacto-purifier, febrifuge, rejuvenating and antioxidant [5], and tonic. It is useful in vitiated conditions of *kapha*; the body fluid principles relate to mucus and *pitta*, an energy principle which uses bile to direct digestion. In modern pharmacopoeia, the plant has been used to treat a variety of ailments [6-9]. Rubiadin is a major component of *R. cardifolia* Linn. and found to possess hepatoprotective and antioxidant property [5,10]. The prime objective of the present research was to explore and establish the possible medicinal value of the ethanolic extract of the root of *R. cardifolia*.

METHODS**Materials**

The roots of *R. cardifolia* (Fig. 2) were procured from local market and authenticated from Govt. Agriculture College, Osmanabad. The root specimen was shade dried, and a herbarium sheet is preserved in our college department for further future references. The roots were shade dried to avoid degradation of phytoconstituents. After drying about 500 g roots were coarsely powdered (Fig. 3) in a lab mixer, subjected to extraction with ethanol (95%) using Soxhlet apparatus for continuous

extraction for 12 hrs, later the extract was cooled at room temperature and evaporation of alcohol afforded a semi-solid mass.

Flash chromatography

The prepared extract was subjected to initial column chromatography using ethyl acetate/N-hexane solvent system, later the bulk of components were subjected to flash chromatography at a wavelength of 2258, EZ Purifier Lisure flash chromatography machine was employed for the study (Fig. 4). It was conducted at Y B Chavan College of Pharmacy, Aurangabad, Maharashtra.

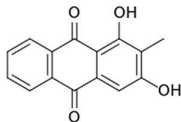
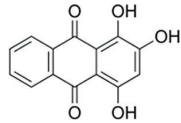
Gas chromatography-mass spectrometry (GC-MS) analysis

The model of the GC-MS employed for mass spectral identification of the methanol extract was an SHIMADZU GC-MS-QP2010 SE interfaced



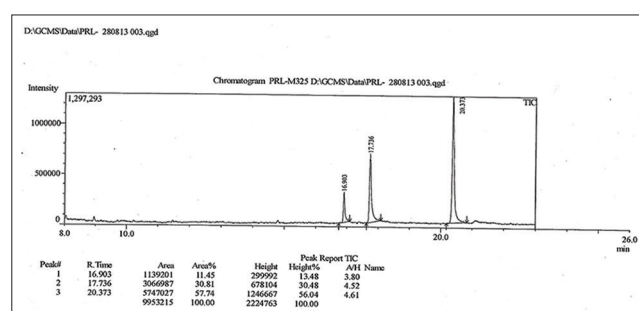
Fig. 1: *Rubia cardifolia* plant

Table 1: Phytochemicals identified in root extract of *R. cardifolia*

S. No	Compound	Structure	RT (min)	Molecular mass	Molecular formula	% area
1	Rubiadin (1,3, dihydroxy 2 methyl anthraquinone)		17.736	256	C ₁₅ H ₁₀ O ₄	30.81
2	Purpurin (1,2,4 Trihydroxy anthraquinone)		16.903	254	C ₁₄ H ₈ O ₅	11.45
3	Standard peak	-	20.373	-	-	57.74

R. cardifolia: *Rubia cardifolia*Fig. 2: Roots of *Rubia cardifolia*

Fig. 4: EZ purifier lisure flash chromatography machine

Fig. 3: Coarse powder of roots of *Rubia cardifolia*Fig. 5: Gas chromatography mass spectrometry spectrum of isolated components of ethanolic root extract of *Rubia cardifolia*

Identification of compounds

Interpretation of the mass spectrum of GC-MS was conducted using a database of IICT, Hyderabad, Telangana, which consists of enormous numbers of patterns. The spectrum of the unknown component was compared with the spectrum of known component inherent to IICT library. The name, molecular weight and structure of the components of the test material were ascertained.

RESULTS AND DISCUSSION

Medicinal plants are being used as the most valuable sources of food and medicine, for the prophylactic treatment of series of diseases and for proper maintenance of human health. Especially in India, since ancient times, many indigenous plants are widely used for the treatment or management of common diseases. The study on active

to a mass-selective detector, at Indian Institute of Chemical Technology (IICT), Hyderabad, Telangana. The capillary column (30 m, 0.25 mm × 0.25 μm film thickness) was ZB-5. The oven temperature was initially maintained at 50°C for 5 minutes and then programmed to 250°C at 10°C minutes⁻¹. The carrier gas used was helium (99.999%), at a flow rate of 1 ml/minutes, and an injection volume of 1 μl was employed (split ratio of 5:1). The electron-impact ionization of the MS was operated at an electron energy of 70 eV. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time was 25 minutes.

principles of ethanolic root extract of *R. cardifolia* by GC-MS showed the presence of two important compounds. More compounds would have appeared, but trying GC-MS with the isolated pure fraction by flash chromatography of root extract lead to isolation and discovery of two principal compounds. The compounds with their retention times, molecular formulae, molecular weights, and concentrations (%) are presented in Table 1 and Fig. 5. Rubiadin had should hepatoprotective and antioxidant activity [5,10], while purpurin showed antioxidant activity [11]. The results, therefore, point that the efficiency of these two compounds can be tested for other skin disease such as psoriasis and leucoderma.

CONCLUSION

In the present research study, two principal constituents from ethanolic root extract of *R. cardifolia* by GC-MS method were isolated and identified. The presence of rubiadin and purpurin justifies the usage of the root extract of the plant for treatment of skin ailments.

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