



Modulatory role of alizarin from *Rubia cordifolia* L. against genotoxicity of mutagens

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ABSTRACT

Rubia cordifolia L. (Rubiaceae) is an important medicinal plant used in the Ayurvedic medicinal system. Its use as a traditional therapeutic has been related to the treatment of skin disorders and cancer. Besides its medicinal value, anthraquinones from this plant are used as natural food colourants and as natural hair dyes. Dyes derived from natural sources have emerged as important alternatives to synthetic dyes. Alizarin (1,2-dihydroxyanthraquinone) was isolated and characterized from *R. cordifolia* L. and evaluated for its antigenotoxic potential against a battery of mutagens viz. 4-nitro-*o*-phenylenediamine (NPD) and 2-aminofluorene (2-AF) in Ames assay using TA98 tester strain of *Salmonella typhimurium*; hydrogen peroxide (H₂O₂) and 4-nitroquinoline-1-oxide (4NQO) in SOS chromotest using PQ37 strain of *Escherichia coli* and in Comet assay using human blood lymphocytes. Our results showed that alizarin possessed significant modulatory role against the genotoxicity of mutagens.

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1. Introduction

It is well established that mutations in somatic cells play a key role in cancer initiation and other stages of the carcinogenesis process (De Flora and Ferguson, 2005). A large number of mutagens have been identified and are known to be potentially deleterious to human health. To minimize human exposure to different environmental mutagens, it is pertinent to identify various mutagenic substances as well as to enhance the exposure to antimutagenic agents, such as those naturally occurring in plants as secondary metabolites (Edenharder et al., 2002; Ikuma et al., 2006; Jeong et al., 2006). Plant derived medicines are based on the fact that they contain natural substances whose consumption may provide health benefits and diminish illness. Interactions among bioactive compounds are complicated and ubiquitous, making the detection and identification of natural mutagens and antimutagens important (Greenwald et al., 2001). Knowledge of sources of natural antimutagens will help people to make selections of food or drink containing substantial amounts of active compounds, thereby enhancing their health status. In the last two decades, a wide range of evidence from epidemiological and laboratory studies have demonstrated that some plants eaten whole, or some of their active principles taken in isolation, have substantial protective effects against human carcinogenesis and mutagenesis (Surh and

Ferguson, 2003). Several plant extracts have proved to contain a wide variety of antimutagenic/antigenotoxic substances (Vershaeve et al., 2004; Scassellati-Sforzolini et al., 1999; Kaur et al., 1998, 2000, 2001, 2009) and some can prevent cancer (Nishino, 1998; Nagpal et al., 2000; Saleem et al., 2005). *Rubia cordifolia* L. is a well known medicinal plant and is commonly known as Indian madder, belonging to the family Rubiaceae. It has been used widely in traditional Chinese medicine for its antibacterial, antioxidant and anti-inflammatory activities. This plant contains substantial amounts of anthraquinones, especially in the roots. Literature reports showed that anthraquinone molecules possess antigenotoxic/antimutagenic activities (Huang et al., 1985; Choi et al., 1997; Yen et al., 2000; Jasril et al., 2003; Lee et al., 2005). In the present investigation, we isolated an anthraquinone fraction (alizarin) from *R. cordifolia* and evaluated its antigenotoxic/antimutagenic potential.

2. Material and methods

2.1. Bacterial strains and chemicals

2.1.1. *Escherichia coli*

PQ37 strain was purchased from Institut Pasteur, France. *Salmonella typhimurium* TA98 strain was kindly provided by Professor B.N. Ames, University of California, Berkeley, USA. Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G6P), normal melting point agarose (NMPA), low melting point agarose (LMPA), ethidium bromide and ortho-nitrophenyl-β-D-galactopyranoside (ONPG), were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Para-nitrophenylphosphate (PNPP), were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India; polyethyleneglycol-4-tetraoctylphenolether (Triton

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X-100), hydrogen peroxide, dimethyl sulphoxide from Qualigens Fine Chemicals, Mumbai, India. Histopaque 1077, from Sigma Chemicals (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Plant material and isolation

The roots of *R. cordifolia* were purchased from local market at Amritsar, Punjab, India. Voucher specimen No. 0342-B-03/2006, has been submitted to the Herbarium of Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India. The roots were washed with running water to remove any dust impurities and dried at 40 °C. They were finely powdered and percolated with 80% methanol to obtain the methanol extract. The dried methanol extract was made aqueous with distilled water in a separating funnel and further fractionated with series of organic solvents to obtain the fractions, viz. hexane fraction and chloroform fraction (Flow Chart 1). The chloroform fraction was subjected to aluminium oxide column chromatography which yielded the fraction ('RUC-1') when eluted with ethyl acetate:methanol (10:90).

2.3. Phytochemical analysis

The thin layer chromatography (TLC) of the fraction ('RUC-1') revealed it to be a single compound, which was characterized structurally using ¹H NMR, ¹³C NMR and mass spectroscopy.

2.4. Anti-genotoxicity assays

2.4.1. Ames assay

The plate incorporation assay (Maron and Ames, 1983) with little modification (Grover and Bala, 1993) was used for the present investigation. To check the anti-mutagenic potential, two sets of experiments were designed.

2.4.2. Co-incubation and preincubation

Bacterial culture (0.1 ml), 0.1 ml of direct-acting mutagen NPD and 0.1 ml of non-toxic concentrations of 'RUC-1' fraction were added in the above order into sterile test tubes containing 2 ml of soft agar and poured onto minimal agar plates. In the case of indirect-acting mutagen, 2-aminofluorene (2-AF), 0.1 ml of bacteria, 0.1 ml of 2-AF, 0.5 ml of S9 mix and 0.1 ml of 'RUC-1' fraction were added into

2 ml of soft agar, mixed and poured onto minimal glucose agar plates. In case of pre-incubation mode of treatment the mutagen was incubated with test fraction at 37 °C for 30 min in gyratory incubator. After solidification, the plates were placed in incubator at 37 °C in an inverted position for 48 h.

Non-toxic concentrations were determined to be those where there was no statistically significant difference in the (1) number of spontaneous revertant colonies, (2) size of colonies, and (3) intensity of the background lawn, as compared to the control where no extract/fraction was added. Concurrently, positive control (mutagen but no fraction) was also set. Each concentration was tested in triplicate and the entire experiment was repeated twice.

The antimutagenic activity of each fraction was expressed as percent decrease of reverse mutations as follows:

$$\text{Inhibitory activity(\%)} = a - b/a - c \times 100$$

a = number of histidine revertants induced by mutagen (NPD/2-AF)

b = number of histidine revertants induced by mutagen in the presence of fraction

c = number of histidine revertants induced in the presence of fraction alone and solvent (negative control)

2.4.3. SOS chromotest

For the SOS chromotest, an overnight culture of *E. coli* PQ37 (100 µl) was added to 5 ml of fresh La medium and incubated for 2 h at 37 °C. One ml of this culture was diluted with 9 ml of La medium (Quillardet and Hofnung, 1985). Aliquots of 600 µl of bacterial suspension were distributed to series of glass test tubes, each containing 20 µl of genotoxicant [H₂O₂ (1.0 mM)/4NQO (20 µg/ml)] and 20 µl of 'RUC-1' of different concentrations. Positive control was prepared by exposure of bacteria to either hydrogen peroxide or 4NQO alone. After incubation of 2 h at 37 °C, 300 µl samples each were used for assay of β-galactosidase and alkaline phosphatase activities respectively. The activity of the constitutive enzyme alkaline phosphatase was used as a measure of protein synthesis and toxicity. In order to determine the β-galactosidase activity, 2.7 ml of B-buffer (adjusted to pH 7.5) was added and after 10 min, 600 µl of 0.4% 4-nitrophenyl-β-galactopyranoside (ONPG) solution was added to each of the test tubes of one set. To determine the constitutive alkaline phosphatase activity, P-buffer (adjusted to pH 8.8) was added and after 10 min, 600 µl of 0.4% 4-nitrophenyl phosphate (PNPP) solution was added to another set of tubes. All mixtures were incubated at 37 °C and observed for the colour develop-

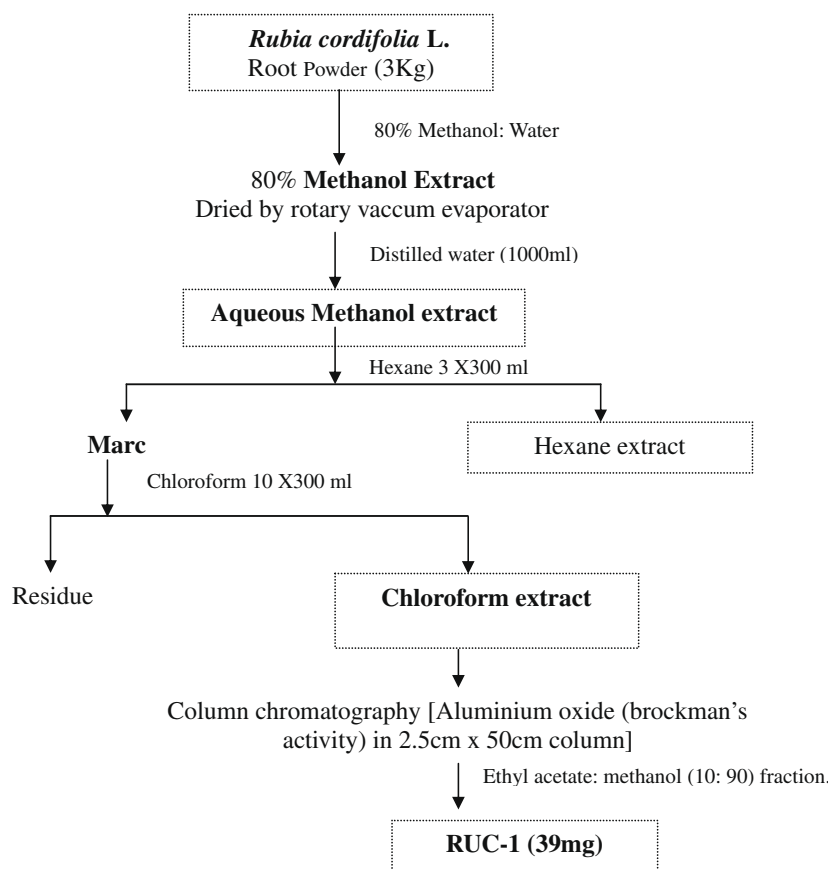


Chart 1. Schematic representation of isolation of 'RUC-1' fraction from chloroform extract of *Rubia cordifolia* L.

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