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A Comprehensive Review of *Rubia cordifolia* Linn.

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ABSTRACT

Rubia cordifolia Linn. (*manjishtha*) is popularly known as 'Indian Madder'. Roots are traditionally used as anti-inflammatory, astringent, tonic, antiseptic, deobstruent, antidysenteric, blood purifier. It is an important ingredient of many ayurvedic preparations. The roots are natural red dye and are very effective in purifying blood. Various chemical constituents like anthraquinones, iridoid glycoside, naphthoic acid esters, bicyclic hexapeptides, and triterpenes have been isolated and identified from *Rubia cordifolia* Linn. The present review article is focused on phytochemical, pharmacological and other important aspects of *manjishtha*.

KEYWORDS - *Rubia cordifolia* Linn, *manjishtha*, Rubiaceae, Indian Madder, anthraquinones

INTRODUCTION

Plants play a vital role in maintaining human health and contribute towards improvement of human life. They are important components of medicines, cosmetics, dyes, beverages etc. In the present time focus on plant research has increased all over the globe enormously. There are thousands of plant species having good potential of offering direct therapeutic effect individually or in combinations. Plants are considered as state-of-art chemical laboratories capable of biosynthesizing number of biomolecules of different chemical classes. Many of these are proved to be precursors for development of other drugs. Further more many western drugs have their origin in plant extracts. There are a number of herbal agents which are successfully used for gastrointestinal, cardiovascular, nervous and metabolic disorders. Ethno-botanical and ethno-pharmacological studies on such plants continue to attract investigators throughout the world.

Rubia cordifolia Linn belonging to family Rubiaceae is a well known ayurvedic herb popularly known as Indian Madder (English), *manjeshta* (Marathi), *majit* or *manjit* (Hindi), *manjishtha, aruna, chitra, raktaangi, manjusha* (Sanskrit) *manjeeth iraani* (Unani), *manjitti* (Siddha) (1-2). Synonyms are *Rubia manjista* Roxb. *R. secunda* Moon, *R. mungisth* Desv. The Indian Madder of commerce consists of short rootstocks with numerous cylindrical, smooth and straight roots, about the size of a quill. These are covered with a thin brownish cork, which peels off in flakes, exposing a red-brown bark marked by longitudinal furrows. The root is sweetish followed by acrid and bitter taste (3). Madder has been used in many Asian countries as a dye, for imparting shades of red, scarlet, brown and mauve to cotton and woolen fabrics. In India and neighboring countries, madder also has a long history in skin care and treatment and it has been used internally in disorders of the urinary tract.

Geographical distribution

It is distributed throughout the lower hills of Indian Himalayas in the North and Western Ghats in the South, and Japan, Indonesia, Ceylon, Malay, Peninsula, Java and tropical Africa in moist temperate and tropical forests, up to an altitude of 3500 m. (1), (2). It is a large genus of hardy climbers, with perennial root stocks distributed in the temperate and tropical zones. About 15 species occur in India. Some of these are Indian Madder (*Rubia cordifolia* Linn.), Naga Madder (*Rubia sikkimensis* Kurz), and European Madder (*Rubia tinctorum)*.

Botanical description

Systematic position of the plant (4)
Class: Dicotyledoneae
Subclass: Sympetalae
Order: Rubiales
Family: Rubiaceae
Genus: *Rubia*
Species: *cordifolia*

Rubia comprises about 60 species and is distributed in Europe, Africa and Asia, with three species in tropical Africa. It is closely related to *Galium*. *Rubia cordifolia* an ayurvedic herb is a perennial, climber with very long, cylindric, flexuose roots with a thin red bark (1). Stems are long, rough, grooved and become slightly woody at the base. Bark is white; branches are scandent, quadrangular, glabrous and shining. Leaves are 3.8-9 X 1.6-3.5 cm long arranged in four whorls, ovate, acute lower leaves are larger than the upper, and all are scabrous above, on the nerves beneath and on the margins with minute white prickles. Flowers are in terminal panicled glabrous cymes, branches trichotomous, spreading bracts are ovate, acute and leafy. Calyx is 0.85 mm long, tube globose and glabrous. Corolla greenish and are divided nearly to the base, 5-lobed, ovate, acute, 3 mm long. Styles are 2, stigmas globose. Fruit is 4-6 mm in diameter, didymous or globose, smooth, shining purplish black when ripe (1). *Rubia cordifolia* is an important example of speciation. The process of speciation differs from one plant group to another and each species evolves in its own way. The mode of germination is generally fixed through out a genus or a family. Both epigenous and hypogenous germination have been observed in *Rubia cordifolia* group. The typical *R. cordifolia*

has greenish flowers and fruits becoming yellow brown or orange and then turning purplish black when fully ripe. The cotyledons are hypogenous and the somatic chromosome number is 22. The Himalayan *R. manjith* Roxburgh has dull orange flowers and at first reddish fruits become purplish black at maturity. The somatic chromosome number is 66. The species occurs throughout the Himalayas and Khasia at altitudes generally lower than 2,000 m above the sea level. Another Himalayan race is often found at higher elevations. It has greenish flowers and black berries and somatic chromosome number is 44 or 132 (5).

Ecology: *Rubia cordifolia* has a wide ecological adaptability. It is found in forest edges and clearings, scrub vegetation and dune forest, less commonly in grassland or open, rocky areas, from sea-level up to 2600 m altitude.

Propagation and planting: *Rubia cordifolia* is occasionally cultivated in India (Darjeeling). It can be propagated by seed, cuttings and micro-propagation methods. Seed germinates best when sown immediately after ripening; stored seed takes time to germinate. Plants should be grown in light shade.

Ayurvedic properties (6-7)

Rasa: Kashaya (astringent), *tikta* (bitter), *madhur* (sweet)

Guna: *Guru* (heavy), *ruksha* (dry)

Veerya: Ushna (hot)

Vipaka: Katu (pungent)

Dosha: Pacifies *kapha* and *pitta*

Karma: Varnya, Jwarahara, Mutrajanana, Swedajanana, Twachya

Safety profile: No adverse effects have been reported at recommended doses and the herb is usually categorised as GRAS (generally recognized as safe) (6).

Dosage: Powdered root: 1-3 g; Decoction: 56-112 ml

TRADITIONAL USAGE

The *Rubia cordifolia* root is sweet, bitter, acrid, heating, alexiteric, antidysenteric, antipyretic, analgesic, anthelmintic; improves the voice and complexion, cures '*kapha*' , inflammations , diseases of the uterus, vagina, eyes, ears, blood; cures leucoderma, erysipelas, ulcers, urinary discharges, jaundice, piles. The roots are very effective in purifying blood. The leaves are sweet and oleaginous; increase appetite; cure '*vata*' and biliousness. The fruit is described to cure diseases of the spleen (1). The root is laxative, analgesic, lactogogue, emmanogogue, diuretic, used in eye sores, paralysis, lethargy, liver complaints, enlargement of the spleen, pains in the joints, rheumatism, uterine pains. In action it is alexipharmic, alternative, antidysenteric, astringent, carminative, deobstruent, febrifuge and tonic. Useful in cough, freckles of skin, hepatic obstruction, indigestion, inflamed parts, ulcers and fractures, mental agony, obstructions in the urinary passage and paralytic affections. In China and Malaya, the root has a certain reputation for its tonic, alternative, and astringent properties. In the Cape Province, natives take a decoction of the leaf or root for pleurisy and other inflammatory conditions of the chest. The decoction of the root is taken to relieve colic, sore throats, chest complaints and to wash the teeth. The stem is described as a cure for snake bite and scorpion sting.

ETHNOVETERINARY USAGE

Rubia cordifolia is used in the treatment of liver fluke, dysentery, maggots, wounds and intestinal worms in animals (8).

Pharmacognosy: Consists mainly in identification of adulterants (9).

Adulterations and substitutes: *Rubia cordifolia* and other *Rubia* species are currently sold together as 'madder'. *Rubia cordifolia* should not be confused with *Oldenlandia umbellata* L., also called Indian madder and found in eastern India, Myanmar and Sri Lanka but not in Africa, which contains alizarin as red dye component.

CHEMICAL CONSTITUENTS

Quinones: Plants belonging to family (Rubiaceae) are known to contain substantial amounts of anthraquinones, especially in the roots. The colouring matter present in the roots of *R. cordifolia* is a mixture of purpurin (trihydroxy anthraquinone) and manjistin (xanthopurpurin-2-carboxylic acid). From roots of *Rubia cordifolia* L. many anthraquinones are isolated like 4-dihydroxy 2-methylanthraquinone and 1, 5-dihydroxy 2-methylanthraquinone and 3-prenyl methoxy 1, 4-naphthoquinone. Mollugin (1-hydroxy-2-methyl-9,10-anthraquinone), alizarin (1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone), lucidin primeveroside, ruberythric acid anthraquinones, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-O-(6'-O-acetyl)-α-rhamnosyl-(1→2)-β-glucoside and 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-O-α-rhamnosyl (1→2)-β-glucoside. The cytotoxic activity of naphthohydroquinones and naphthohydroquinones dimmers had been tested (10-28). Rubiasins, anthracene derivatives have been isolated from the roots and stems of *Rubia cordifolia* (29). In one study, a preparative high-speed countercurrent chromatography (HSCCC) method for isolation and purification of the bioactive component mollugin directly from the ethanol extract of *R. cordifolia* was successfully established (30).

Iridoids: 6-Methoxy geniposidic acid is found along with manjistin, garancin and alizarin (31).

Triterpenoids: Oleananes such as rupiprasin A, B and C along with arborane triterpenoids including rubiarbonol A, B, C, D, E and F have been isolated (32-33).

Pentacyclic triterpenes : Rubicoumaric acid and rubifolic acid isolated from *Rubia cordifolia* have been shown to be 30-hydroxy-3β-*p*-hydroxycoumaryloxy-urs-12-ene-28-oic acid and 3β,30-dihydroxy-urs-12-ene-28-oic acid(30-hydroxyursolic acid) respectively on the basis of ^{1}H NMR, ^{13}C NMR and mass spectral and chemical evidences (34).

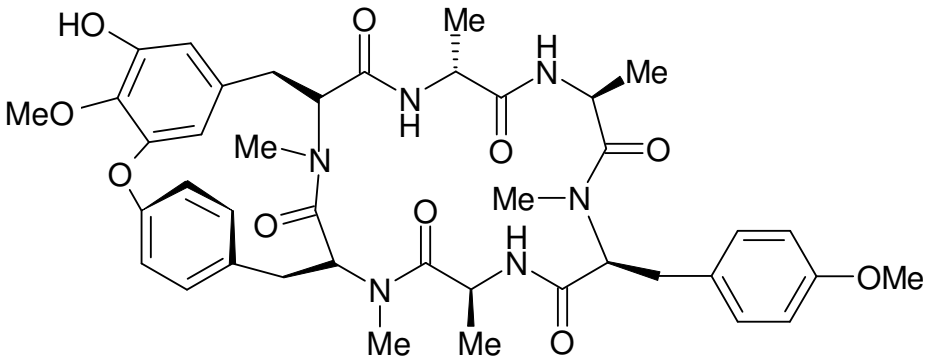
Naphthoic acid esters: These are isolated from the roots of *Rubia cordifolia* (35).

Bicyclic Hexapeptides: Hexapeptides have been isolated from roots of *Rubia cordifolia* which are found to be cytotoxic with further investigations (36-47).

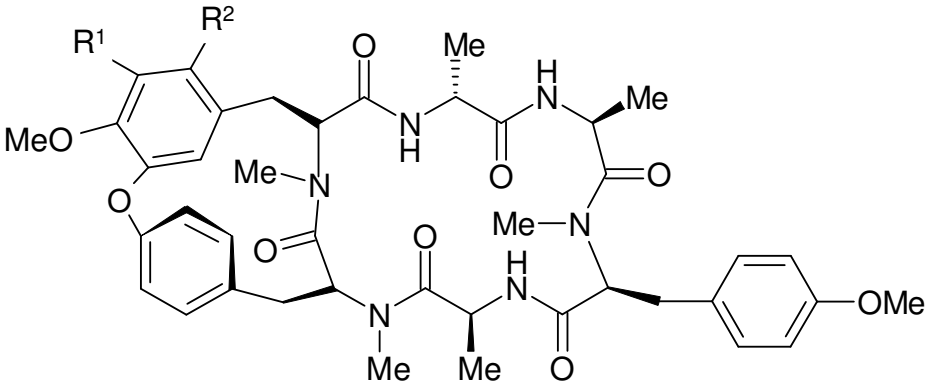
A novel antitumor bicyclic hexapeptide RA-XVII was isolated from the roots of *Rubia cordifolia*. By spectral studies and synthetic approach, its structure was determined to be [2-aminobutyric acid-1] deoxybouvardin. Studies on the effect of side chain at residue 1 on cytotoxic activity and conformation

showed that although it had little effect on the conformation of the molecule, it decreased the activity as it grew longer (48). A new bicyclic peptide of RA-series, RA-XVIII was isolated from the roots of *Rubia cordifolia* L. Its structure was established to be a hydroxylated derivative of RA-VII by the semi-synthesis from deoxybouvardin, and its cytotoxicity against P-388 cells was 0.012 µg/mL (49). Conformational analysis of an antitumor cyclic hexapeptide, RA and its analogues isolated from *Rubia akane* and *R. cordifolia* was conducted by the spectroscopic and computational chemical methods. The biological (antitumour) activity of the N-methyl derivative of RA-VII was found to be less in comparison to RA-VII (50). The structures of antitumor bicyclic hexapeptides, RA-VI and -VIII from *Rubia cordifolia* were elucidated by the spectroscopic and chemical methods (51). Three analogues of RA-VII, an antitumor bicyclic hexapeptide from *Rubia* plants, were synthesized. Three analogues, [Gly-1]RA-VII, [Gly-2]RA-VII , and [Gly-4]RA-VII , in which one of the three alanine residues was replaced by a glycine residue, were prepared by linking of the cycloisodityrosine unit, obtained by degradation of the first compound, to three different glycine-containing tetrapeptides followed by macrocyclization. Out of these analogues, analogue 4 showed the highest cytotoxic activity (52).

Rubia as a dye yielding plant (53-56): Nature has gifted us more than 500 dye-yielding plant species. Dyes derived from natural sources have emerged as important alternatives to synthetic dyes. Natural dyes are colourants having several applications in textiles, inks, cosmetics, etc. Rubia is one of dyes-yielding plants which has been used as a dye in combination with other plants for extraction and preparation of dyes utilizing indigenous processes. *Rubia cordifolia* produces anthraquinone reddish orange dye in roots, stem and leaves, which has been used for dyeing textiles since ancient times. Commercial sonicator dyeing with *Rubia* showed that pretreatment with biomordant, *Eurya acuminata* DC var *euprista* Karth. (Theaceae family) (2 %) shows very good fastness properties for dyed cotton using dry powder as 10 % of the weight of the fabric is optimum. Use of biomordant replaces metal mordants making natural dyeing ecofriendly (57). In West Bengal, the stem is preferred to root for the extraction of the dye, though both are used. A variety, *R. cordifolia* var. *khasiana* Watt, commonly found through out Assam and Manipur and extending westward to Nepal is reported to be richer in the dye than either *R. cordifolia* or *R. sikkimensis*.



Hexapeptide of RA – series isolated from *Rubia cordifolia*



Analogues: R^1, R^2 = H, OH, NH_2, NO_2

MEDICINAL AND PHARMACOLOGICAL ASPECTS

Hepatoprotective activity

Protection against different liver toxins has been established. The hepatoprotective activity of an aqueous-methanol extract of *Rubia cordifolia* (Rubiaceae) was investigated against acetaminophen and CCl_4-induced hepatic damage (58). Pretreatment of rats with plant extract (500 mg/kg) lowered significantly the respective elevated serum GOT and GPT levels in rats. The extract also prevented CCl_4-induced prolongation in pentobarbital sleeping time confirming the hepatoprotective effects of the extract. The hepatoprotective effect of *Rubia* might had been mediated through inhibition of microsomal drug metabolizing enzymes or its calcium channel blocking activity as it was curative against paracetamol as well as mitigated the toxicity due to CCl_4. It has been found to be effective against acute and chronic hepatitis caused by the hepatitis B virus (HBV) in human hepatoma cells (Hep 3B). The quinone derivatives were thought to be the active components (59).

'Rubiadin', a major constituent isolated from *R. cordifolia* was evaluated against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. The substantially elevated serum enzymatic activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and gamma-glutamyltransferase (gamma-GT) due to carbon tetrachloride treatment were dose dependently restored towards normalization. The decreased activities of glutathione S-transferase and glutathione reductase were also restored towards normalization. In addition, *Rubiadin* also significantly prevented the elevation of hepatic malondialdehyde formation and depletion of reduced glutathione content in the liver of CCl_4 intoxicated rats in a dose dependent manner. The biochemical observations were supported with histopathological examination of rat liver sections (60). The results of the study strongly indicated that *rubiadin* may have a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats.

Anti-inflammatory activity

Rubia cordifolia is considered to be traditionally useful as an analgesic, astringent, external application in inflammations, ulcers and skin diseases (61). The plant is also claimed to relieve the symptoms of pruritus, burning and exudation from skin (62). During studies in patients with eczema, the topical application of the plant showed a 50 % reduction in the severity score within 4 days, the oedema, exudation and itching being significantly relieved (63). *Rubia cordifolia* was studied for the anti-inflammatory effect in rats with carrageenan paw oedema. The plant showed significant anti-inflammatory activity at a dose of 10 and 20 ml/kg of the water extracts. The activity was comparable to that of phenylbutazone (100 mg/kg) (64).

Rubia cordifolia inhibited the lipoxygenase enzyme pathway and the production of cumene hydroperoxides. The lipoxygenase pathway catalyses the production of various inflammatory mediators such as the leukotrienes which are involved in asthma, arthritis, and other inflammatory disorders (65).

Anti-platelet activating factor property

Rubia cordifolia is clinically used for the purification of blood by the physicians of the Indian Systems of Medicine. The effect of the partially purified fraction of this whole plant had been studied on rabbit platelets. It inhibited the platelet aggregation induced by PAF (platelet activating factor) but not thrombin. PAF (platelet activating factor) is a phospholipids involved in thrombosis, allergy and nervous disorders. *Rubia cordifolia* extract also inhibited the binding of ^3H labeled-PAF to the platelets in the dose-dependent manner. Thus it appears that *R. cordifolia* inhibits action of PAF at its receptor level either by its blocking or by desensitization (66).

Anti-acne property

Propionibacterium acnes, an anaerobic pathogen, plays an important role in the pathogenesis of acne by inducing certain inflammatory mediators. These mediators include reactive oxygen species (ROS) and pro-inflammatory cytokines. In the study, ROS, interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) were used as the major criteria for the evaluation of anti-inflammatory activity. The polymorphonuclear leukocytes (PMNL) and monocytes were treated with culture supernatant of *P. acnes* in the presence or absence of herb. It was found that *Rubia cordifolia* caused a statistically significant suppression of ROS from PMNL. Thus, *Rubia cordifolia* showed anti-inflammatory activity by suppressing the capacity of *P. acnes*-induced ROS and pro-inflammatory cytokines, the two important inflammatory mediators in acne pathogenesis (67).

Antihyperglycemic activity

The antidiabetic action of *Rubia cordifolia* Linn (Rubiaceae) aqueous root extract (RCAREt) was examined in streptozotocin (STZ)-induced diabetic rat model. Serum glucose, total cholesterol and triglycerides, hematological parameters, and liver and kidney transaminases in normal, STZ diabetic, and RCAREt-treated diabetic rats were measured. The observed hyperglycemia, hypertriglyceridemia, enhanced transaminases of liver and kidney, hypochromic microcytic anemia, and loss of body weight in STZ diabetic rats were normalized by RCAREt treatment, whereas the hypercholesterolemia was not rectified. The beneficial effect of RCAREt treatment might be due to different types of active principles, each with a single or a diverse range of biological activities (68).

The effect of ethyl acetate fraction of roots of *Rubia cordifolia* (RCEAF) was investigated on blood glucose level and glucose utilization to find out the mechanism of action of the extract. Ethanolic extract of roots of *R. cordifolia* L. was reported to be hypoglycemic and it was fractionated by column chromatography. Single dose study of RCEAF (50,100 and 200mg/kg, p.o.) was carried out in i) normal fasted ii) oral glucose tolerance test (OGTT) iii) alloxan (120mg/kg, s.c.)- induced diabetic rats. Repeated dose study of RCEAF (100 and 200 mg/kg, *p.o.*) was carried out for two weeks. It was found that, oral pre-treatment with RCEAF induced a significant ($P<0.05$) decrease in blood glucose levels as compared to diabetic control rats. In the same line an in vitro experiment showed that insulin (0.05 IU/mL) increased

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glucose utilization by an isolated rat diaphragm. Alone RCEAF (25mg/mL) as well as combination of RCEAF (25mg/mL) and insulin (0.05 IU/mL) showed a marked increase ($P<0.05$) of glucose uptake. This exhibited the extra- pancreatic effect of the RCEAF. Further studies with estimation of insulin and insulin receptor may give more insight into the mechanism of the antidiabetic activity of the *R. cordifolia*. (69). Administration of the alcoholic extract of roots of *Rubia cordifolia* showed significant hypoglycemic effect in alloxan-induced diabetic rats (70).

Antistress and nootropic activity

Effect of alcoholic extract of *R. cordifolia* was investigated on cold restraint induced stress and on scopolamine-induced memory impairment. Alcoholic extract enhanced brain gamma-amino-n-butyric acid (GABA) levels and decreased brain dopamine and plasma corticosterone levels. Acidity and ulcers caused due to cold restraint stress were inhibited by alcoholic extract. Animals treated with alcoholic extract spent more time in open arm in elevated plus maze model. It also antagonized scopolamine induced learning and memory impairment. Baclofen induced catatonia was potentiated by alcoholic extract (70).

Calcium channel antagonistic activity

Crude extract of *Rubia cordifolia* (RC) was tested in isolated tissue preparations for its possible calcium channel antagonistic activity. RC suppressed the spontaneous contractions of guinea-pig atria, rabbit jejunum and rat uterus in a concentration dependent manner (0.1-3 mg/ml). The indicated spasmolytic activity suggests the presence of calcium channel blocker like constituent(s) in the plant (71).

Anti-convulsant and behavioral activities of the triterpene from *R. cordifolia*

Effect of a triterpene isolated from the acetone soluble part of petroleum ether extract of *R. cordifolia* was studied on convulsions induced by maximum electro shock (MES), electrical kindling and various chemoconvulsants in rats and mice. The effect of triterpene was also investigated on behavior and gamma-aminobutyric acid (GABA) and serotonin (5-HT) content in mouse brain. Triterpene inhibited seizures induced by MES, electrical kindling, pentylenetetrazol (PTZ), and lithium-pilocarpine. However, seizures induced by strychnine were not inhibited. Triterpene reduced locomotion as well as rearing. Pentobarbitone induced sleep was potentiated and amphetamine induced stereotypy was inhibited. Brain GABA and 5-HT contents were raised by the compound evocative of its anticonvulsant property (72).

Radioprotective effect

The radioprotective potential of alcoholic extract of root of *R. cordifolia* was studied by survival, hemopoietic cell protection and micronucleus assay. The LD_{50} value for the alcoholic root extract was found to be 1200 mg/kg body weight at 72 hrs post irradiation. A significant radiation protection (67 %) as assessed by increased animal survival was observed when *R. cordifolia* (RC) extract was administered intraperitoneally, 90 min. before the radiation exposure. Besides, the extract also inhibited radiation induced lipid peroxidation measured by the inhibition of thiobarbituric acid reactive substance (TBARS). The RC extract at a selected dose of 460 mg/kg body weight was effective in protecting the radiation induced suppression of endogenous colony forming units in spleen. A significant inhibition of radiation-induced micronuclei formation was observed when RC extract was administered 90 minutes prior to irradiation. Thus, it appears that the alcoholic root extract of *R. cordifolia* provides significant protection against radiation induced lipid peroxidation, hemopoietic injury and genotoxicity. The mechanism of action of RC extract appears to be through its anti-oxidant, metal chelation and anti-inflammatory property (73).

Antimicrobial action

Rubia cordifolia is used as a dye from natural sources therefore a study was taken up to test if some natural dyes have inherent antimicrobial activity with a view to develop protective clothing from these. *Rubia cordifolia* was tested against common pathogens *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The textile material impregnated with these natural dyes, however, showed less antimicrobial activity, as uptake of these dyes in textile material is below MIC. The antibacterial activity of the extracts of *Rubia cordifolia* roots prepared with solvents of different successive polarities was evaluated by the agar-well diffusion method. It inhibited both gram positive and gram negative strains (74-75). Many compounds were isolated, established by chemical and spectroscopic methods from the roots of *Rubia cordifolia* L. Some of the compounds showed certain antibacterial activities (76). In one study some antimicrobial agents, emodin and physcion were isolated as the most active constituents (77).

Anti-proliferative property

Psoriasis is a chronic inflammatory skin disorder, which affects approximately 2-3 % of the population worldwide. Traditionally, herbal medicines have been extensively used to treat psoriasis and produced promising clinical results; however, the underlying mechanisms of action have not been systematically investigated. Roots of *Rubia cordifolia* L were extracted with 80 % aqueous ethanol. The dry extract was evaluated for anti-proliferative activity by microplate SRB and MTT assays. It was found to have significant anti-proliferative effect, as measured by MTT assay. *Rubia cordifolia* did not exert cytotoxicity to this human fibroblast cell line (78). The antiproliferative property of *Rubia cordifolia* (Rubiaceae) extract was also tested on A-431 cells (epidermal carcinomoid cells) and 3T3 fibroblast cells. It was observed that a fraction of *Rubia cordifolia* significantly inhibited the incorporation of [^{3}H]-thymidine, induced by fetal bovine serum, in a dose-dependent manner. It also inhibited the PMA (phorbol 12-myristate 13-acetate) induced expression of c-fos genes in A-431 cells. It appears that inhibition of DNA synthesis underlies the mechanism for its antiproliferative properties (79).

Inhibitory action on nitric oxide production

Rubia cordifolia is used for prevention and treatment of inflammatory diseases, therefore water and methanol extracts of *Rubia cordifolia* were screened for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated J774.1 macrophages and in LPS/interferon

(IFN)-γ-stimulated mouse peritoneal exudate macrophages. The methanol extract *Rubia cordifolia*, showed significant inhibition in J774.1 macrophages, while in mouse peritoneal exudate macrophages, water extract of *R. cordifolia* inhibited the NO production. Water extract of *R. cordifolia* showed inhibition on iNOS mRNA expression (80). The inhibition of nitric oxide (NO) is found to be a key mediator in the phenomenon of inflammation. Thus *Rubia cordifolia* was evaluated for inhibitory activity on NO produced *in-vitro* from sodium nitroprusside, and in LPS-activated murine peritoneal macrophages, *ex vivo* (81). The inhibition of NO synthesis was correlated with the reduction of iNOS protein expression through Western blot. Notable NO scavenging activity was exhibited *in-vitro* by extracts of *R. cordifolia* (IC_{50} < 0.2 mg/mL). It showed marked inhibition (60%-80%), *ex vivo*, at a dose of 80 microg/mL without appreciable cytotoxic effect on the cultured macrophages. Immunoblot analysis confirmed that the modulatory effect of the samples had occurred through suppression of iNOS protein suggestive of potential inhibition of NO production.

Wound healing activity

Several drugs of plant, mineral and animal origin are described in the Ayurveda for their wound healing properties under the term '*vranaropaka*'. *Rubia cordifolia* was also found to be effective in experimental models (82).

Anticancer activity

The cyclic hexapeptides and quinones of *Rubia* exhibited a significant anticancer activity against various proliferating cells. The hexapeptides showed potent antitumour activity by binding to eukaryotic 80S ribosomes resulting in inhibition of aminoacyl -tRNA binding and peptidyl – tRNA translocation, thus leading to the stoppage of protein synthesis (83-85). The antitumor activity of RA-700, a cyclic hexapeptide isolated from *Rubia cordifolia*, was evaluated in comparison with deoxy-bouvardin and vincristine (VCR). The antitumor activity of RA-700 was similar to that of deoxy-bouvardin and VCR against P388 leukemia. As with deoxy-bouvardin and VCR, the therapeutic efficacy of RA-700 depends on the time schedule. RA-700 showed marginal activity against L1210 leukemia (50 % ILS), similar to that of deoxy-bouvardin but inferior to that of VCR. RA-700 inhibited Lewis tumor growth in the early stage after tumor implantation, whereas deoxy-bouvardin and VCR did not. A slight reduction of peripheral WBC counts was observed with the drug, but no reduction of RBC and platelet counts. Bilirubin, creatinine, GPT and GOT levels in plasma did not change with the administration of the drug (86). The anticancer as well as antiviral property had been reviewed (87). From the chloroform fraction of *Rubia cordifolia* roots three constituents were isolated namely mollugin, furomollugin and dehydro-a-lapchone. Mollugin has shown inhibition of passive cutaneous anaphylaxis (PCA) and protection of mast cell degranulation in rats. It also exhibited considerable activity against lymphoid leukemia (P338) in mice (88).

The *Rubia cordifolia* extract had shown a mitodepressive effect on the rate of cell division in bone marrow cells of Swiss male mice. This exposure-time-dependent reduction was attributed to the effect of inhibiting protein synthesis, suggesting probable effect of *Rubia* extract on the biosynthesis of certain amino acids as well as RNA synthesis (89). *Rubia cordifolia* had shown an increase in leukocyte count in leucopenia (90).

The cytotoxic action of *Rubia cordifolia* had been evaluated with DNA Topoisomerases I and II inhibition and cytotoxicity of constituents isolated from the roots was tested. Topoisomerases I and II inhibitory activities were measured by assessing the relaxation of supercoiled pBR 322 plasmid DNA. The tetrazolium-based colorimetric assay (MTT assay) was used for the cytotoxicity towards human colon carcinoma (HT-29), human breast carcinoma (MCF-7) and human liver carcinoma (HepG2) cell lines. Seven compounds were isolated possessing cytotoxic activity (91).

Anti-tumor activity of RC-18, a pure isolate from *Rubia cordifolia*, was repeatedly tested in different sets of experiments on a spectrum of experimental murine tumors, *viz* P388, L1210, L5178Y, B16 melanoma, Lewis lung carcinoma and sarcoma-180. RC-18 exhibited significant increase in life span of ascites leukemia P388, L1210, L5178Y and a solid tumor B16 melanoma. However, it failed to show any inhibitory effect on solid tumors, Lewis lung carcinoma and sarcoma 180. Promising results against a spectrum of experimental tumors suggested that RC-18 may lead to the development of a potential anti-cancer agent (92). The anti-cancer activity of extracts of *Rubia cordifolia*, tested against the P388 tumor system in BDF1 mice, compared well with that of the positive control, 5-fluorouracil (93).

Antioxidant activity

The antioxidant properties have been well established. The herb significantly inhibits $FeSO_4$ induced lipid peroxidation and glutathione depletion (94). The generation of free radicals has been predominantly involved in pathophysiology of many disease conditions. The alcoholic extract of *Rubia cordifolia* was tested for antioxidant potential in ethanol-treated rats. The parameters like phagocytosis, total leukocyte count (TLC), humoral and cell-mediated immune responses, lipid peroxidation (LPO), reduced glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activities were assessed. Chronic administration of ethanol decreased the humoral and cell-mediated immune response, phagocytosis, phagocytosis index, TLC, GSH, CAT and SOD activities and increased the LPO. These influences of ethanol were prevented by concurrent daily administration of extract of *Rubia cordifolia* and the effect was comparable with that of the combination of vitamins E and C. The ethanol-induced immunosuppression was due to oxidative stress and *Rubia cordifolia* could have prevented the same by virtue of its *in vivo* antioxidant property (95-97).

The inhibition of $FeSO_4$ induced lipid peroxidation in rat liver by alcoholic extract of *Rubia cordifolia* and by one of its constituent 'rubiadin' (1, 3-dihydroxy-2-methyl anthraquinone) (pure form) had been compared. Both have been found to inhibit lipid peroxidation in a dose dependent manner. Whereas the former showed both oxidizing and reducing properties with Fe^{2+} and Fe^{3+}, the latter showed oxidizing property only by converting Fe^{2+} to Fe^{3+}. The former

inhibited the oxidation of reduced glutathione while the latter did not (98).

The antioxidant activity (Trolox equivalent antioxidant capacity) of the phenolic constituents from the roots of *Rubia cordifolia* was evaluated using the improved 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt method. Hydroxyanthraquinones were found to be the predominant antioxidant constituents. The structure-radical scavenging activity relationships showed that the hydroxy groups on one benzene ring of the anthraquinone structure were essential for hydroxyanthraquinones to show activity, the ortho-dihydroxy structure in the hydroxyanthraquinone molecules could greatly enhance their radical scavenging effect, and glycosylation of the hydroxyanthraquinones reduced the activity (99).

The major damaging factor during and after the ischemic / hypoxic insult is the generation of free radicals, which leads to apoptosis, necrosis and ultimately cell death. Therefore *Rubia cordifolia* was tested for its neuroprotective ability and the associated mechanism in rats. Hippocampal slices were subjected to OGD (oxygen glucose deprivation) and cytosolic Cu-Zn superoxide dismutase (Cu-Zn SOD), reduced glutathione (GSH), glutathione peroxidase (GPx), nitric oxide (NO) was measured as nitrite (NO_2) in the supernatant and protein assays were performed. *Rubia cordifolia* was found to be effective in elevating the GSH levels, expression of the gamma-glutamylcysteine ligase and Cu-Zn SOD genes. It also exhibited strong free radical scavenging property against reactive oxygen and nitrogen species. In addition it significantly diminished the expression of iNOS gene after 48 hours which plays a major role in neuronal injury during hypoxia/ischemia. *Rubia cordifolia* therefore attenuates oxidative stress mediated cell injury during OGD and exerts the effects at both the cytosolic as well as at gene expression level and may be an effective therapeutic tool against ischemic brain damage (100).

Rubia cordifolia Linn. (Rubiaceae) is an important component of the ayurvedic system of medicine. It has a variety of uses such as blood purifier, immunomodulant, anti-inflammatory and anti-PAF. The anti-peroxidative property of the solvent free alcoholic extract of *R. cordifolia* had been studied in rat liver homogenate. It prevented the cumene hydroperoxide induced malondialdehyde formation in the dose and time dependent manner. This effect was accompanied by the maintained reduced glutathione level even in the presence of above toxin (101). Rubiadin, a dihydroxyanthraquinone, isolated from alcoholic extract of *Rubia cordifolia* prevented the lipid peroxidation induced by $FeSO_4$ and t-butylhydroperoxide (t-BHP) in a dose dependent manner. The percent inhibition was more in the case of Fe^{2+} induced lipid peroxidation. The antioxidant property of the preparation had been found to be better than that of EDTA, Tris, mannitol, Vitamin E and *p*-benzoquinone (102).

Some of the ayurvedic preparations containing *Rubia cordifolia* Linn. (Rubiaceae)

Sr.no.	Name of preparation	Indications	Citations
1	Aswagandharistam	Epilepsy, faintness, fatigue, psychic problems, piles, indigestion, rheumatic complaints etc. Improves memory power.	Baishajyaratnavali
2	Chandanasavam	Burning micturition, leucorrhocat etc. Improves body strength and digestive system. Keeps the body cool and also maintain good general health.	Yogaratnakaram
3	Devadarvarishtam	Diabetes, rheumatic complaints, sprue syndrome, piles, difficult urination, skin diseases etc.	Baishajyaratnavali
4	Eladyarishtam	Chickenpox, skin diseases like urticaria, dermatitis and allergic itching.	Yogaratnakaram
5	Gulguluthikthkarishtam	Recommended in acute rheumatic conditions, chronic skin diseases, sinusitis, lymph adunosis, diseases related with head, neck and throat, abscess and obesity.	Ashtangahridayam
6	Madhookasavam	Diabetes, skin diseases, sprue syndrome and oedema. Also as a tonic for general well-being.	Ashtangahridayam
7	Manjishtasavam	For all types of skin diseases and vatha sonitha, especially for vitiated pitha and kapha vikaras.	Ashtangahridayam
8	Nimbamrithasavam	Recommended in acute rheumatic conditions, skin diseases, and sinusitis, obesity, and lymph adenoids around neck, diseases related with head, neck and throat abscess.	Ashtangahridayam
9	Useerasavam	For burning sensation of head and body, vertigo, disturbed sleep, tachycardia etc.	Yogaratnakaram
10	Jaatyaadi ghrita	Applied externally for chronic and septic ulcers	Ashtaanga Hridaya
11	Phal kalyaan ghrita	Used for amenorrhea and uterine affections	Bhaishajya Ratnaavali
12	Majishthaadi taila	Used for headache	Sahasrayoga

Other activities:

The plant has activity against allergies (103), excessive bleeding (104), and diabetic ulcer (105).

Clinical trails conducted of indigenous drug combinations containing *Rubia cordifolia*

A therapeutic trial was conducted of 'Mahayograj guggul' in cases of rheumatoid and osteoarthritis at Sadar Hospital, Purnea, India. Mahayograj guggul is used for these conditions from time since immortal. It contains *Rubia cordifolia* (13 mg) as one of many ingredients. The results corroborated that it was as effective as aspirin in pain relief although there had been some initial delay in response (106).

Rumalaya tablets and Rumalaya cream (marketed preparations) have proved to be beneficial in relieving the pain in degenerative cases of osteoarthritis of the cervical spine, lumbar spine, knee joints, shoulder joints etc. as well as in other collagen disorders like rheumatoid arthritis, ankylosing spondylosis (107). Rumalaya tablets and Rumalaya cream have anti-inflammatory, analgesic, anti-arthritic and antiphlogistic properties. Clinical trial was conducted at Central Institute of Orthopaedics, Safdarjang Hospital, New Delhi on eighty patients suffering from classical types of cervical spondylosis, lumbar spondylosis, periarthritis of the shoulder joints, and osteoarthritis of the shoulder joints, osteoarthritis of the knee joint, rheumatoid arthritis, ankylosing spondylitis, fibrofascitis and gout. The data of the trial showed that this ayurvedic formulation is very effective in the management of osteoarthritis, spondylysis and related disorders on short and long term basis. Many clinical trials are accomplished on Rumalaya tablets at various clinics and hospitals. The results have proved it a beneficial remedy for the osteoarthritis, spondylysis and related disorders (108-112).

An open clinical trial of an herbal antiseptic cream was conducted at Bangalore Medical College, Bangalore, India on patients with minor lacerated wounds sustained by mild trauma injuries. The findings suggested that the Antiseptic Cream is an excellent additive to the total therapeutic management of minor lacerated wounds, minor cuts and burns. *Rubia cordifolia* (manjistha) is credited with astringent, antibacterial, antiallergic and antiseptic properties (113). However, recent investigations have clarified the efficacy of herbal topical formulations used in the treatment of these conditions.

Study of cardiac function of a formulation (114): There are many studies on the subject of anti-neoplastic drugs for acute and chronic cardiac toxic function. It is important to check previously the cardiac toxicity of the new anti-neoplastic drugs. The first stage examination of RA-700, isolated from *Rubia cordifolia* was done and several kinds of parameters assessed were ECG, ultrasonic cardiograms and arterio-grams on cardiac toxicities of RA-700. The finding regarding the cardiac function, blood pressure, sigma QRS, ejection fraction suggested that the cardiac function must be checked while giving anti-neoplastic drugs to neoplastic patients.

Evaluation of the efficacy and safety of a diaper rash cream (115): Infantile irritant diaper dermatitis (IIDD) is an inflammation of the infantile skin covering the groin, lower stomach, upper thighs and buttocks. The study was conducted to evaluate the efficacy and safety of "Diaper Rash Cream" in the management of IIDD at Department of Pediatrics, Medical College and Hospital, Kolkata, India. The study was a prospective, phase III clinical trial, conducted as per the good clinical practice guidelines. A total of 15 infants, who were suffering from IIDD, and whose parents were willing to give informed written consent were included in the study. The positive benefits observed in this study might be due to the synergistic action of the active ingredients of the formulation. viz., anti-inflammatory activities (of *Aloe vera, Vitex negundo* and *Rubia cordifolia*), antibacterial activities (of Zinc calx, *Aloe vera*, *Vitex negundo* and *Rubia cordifolia*), wound healing activities (of *Aloe vera*), and antioxidant activities (of *Vitex negundo, Prunus amygdalus* and *Rubia cordifolia*). *Rubia cordifolia* was among the major ingredients which have proved to posses antibacterial, antioxidant, anti-inflammatory, wound healing activities. Therefore, it can be concluded that "Diaper Rash Cream" is effective and safe in the management of IIDD.

Tissue culture studies on *Rubia cordifolia*

The transformation of *Rubia cordifolia* L. cells by the 35S-rolB and 35S- rolC genes of *Agrobacterium rhizogenes* caused a growth inhibition of the resulting cultures and an induction of the biosynthesis of anthraquinone-type phytoalexins (116). The results indicated a lack of calcium homeostasis in both transgenic cultures and that induction of anthraquinone production in non-transgenic and transgenic cultures do not proceed through the activation of the common Ca (2+)-dependent NADPH oxidase pathway that mediates signal transduction between an elicitor-receptor complexes via transcriptional activation of defense genes. This was further supported by the studies on anthraquinone production in non-transformed and transformed cultures by Ca (2+) channel blockers, as well as by diphenylene iodonium, an inhibitor of NADPH oxidase, and by the protein kinase inhibitor staurosporine. Anthraquinone production was not inhibited thus indicated that the induction of anthraquinone production in transgenic cultures does not involve the activation of Ca (2+)-dependent NADPH oxidase pathway (117). It has been suggested that the *rol* genes of *Agrobacterium rhizogenes* could play an essential role in the activation of secondary metabolites production in plant transformed cultures. The anthraquinone content was shown to be significantly increased in transgenic (35S-*rolB* and 35S-*rolC* genes) cultures, thus providing evidence that the *rol*-gene transformation can be used for the activation of secondary metabolism in plant cells (118). An attempt was made to study increase in anthraquinone production in calli. Manjistin and purpurin were identified as the major components of anthraquinone pigments produced by callus cultures of *R. cordifolia*. Anthraquinone content in calluses was 0.62–1.22% (by dry wt.) depending on the source of explants (119).

CONCLUSION

The extensive survey of the literature revealed that *Rubia cordifolia* is an important medicinal plant from the time immemorial and the roots are used mainly as anticancer, antiinflammatory agent, antioxidant, as a dye etc. It has been

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used in many ayurvedic preparations. The *Rubia cordifolia* growing in the Himalaya is known as '*manjishta*'. It contains many useful phytoconstituents like quinones, hexapeptides, naphthoic acids, triterpenoids etc. From the roots of *Rubia cordifolia* subspecies *pratensis* (Maxim.) Kitam, collected in China, 11 anthraquinones and their glycosides and 4 naphthohydroquinones (among which dihydromollugin) and their glycosides were isolated. The derivatives of 1, 3, 6-trihydroxy-2-methylanthraquinone so far isolated are only found in some forms of *Rubia cordifolia* and in *Rubia akane* Nakai from Asia. Differences in the composition of roots and stems noticed in the literature have pointed to the need for a systematic chemotaxonomic research on the various forms of the plant, both in Asia and Africa. In concern with the therapeutic potential, bicyclic hexapeptides are shown to be most active anticancer agents.

Genetic resources: *Rubia cordifolia* is widely distributed and is liable to genetic erosion. Its extreme variability requires more attention.

Speciation: *Rubia cordifolia* is an important example of speciation. Therefore considering therapeutic prospective of the genus *Rubia*, speciation should be studied in detail along with evolutionary, cytogenetical, ecological, chemical aspects and responsible environmental factors and interrelationships between the different species. It is also important to investigate the role of polyploidy.

Prospects: *Rubia cordifolia* has at present lost much of its former importance as a dye producing plant, but since it produces a great range of beautiful, fast colors and is easy to propagate, the current growing interest in natural dyes as renewable resources may make it a useful crop. Its effectiveness in disease treatments deserves more research and is being investigated in India where the plant was used in the Ayurvedic and Unani systems of medicine, besides being part of an anticancer compound in Tibetan medicine. Clinical trials of preparations and formulations should be conducted scientifically. Extensive phytochemical and pharmacological study on *manjishta* may give an important 'lead' having potent anticancer activity and better efficacy. Standardization and quality control aspects also are to be studied in detail.

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