



Review

Rubia cordifolia: a review

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SUMMARY

Medicinal herbs are significant source of synthetic and herbal drugs. In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. Isolated active constituents are used for applied research. For the last few decades, phytochemistry (study of plants) has been making rapid progress and herbal products are becoming popular. According to Ayurveda, the ancient healing system of India, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were written around 1000 B.C. The Ayurvedic Materia Medica includes 600 medicinal plants along with therapeutics. Herbs like turmeric, fenugreek, ginger, garlic and holy basil are integral part of Ayurvedic formulations. The formulations incorporate single herb or more than two herbs (poly-herbal formulations). Medicinal herb contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. *R. cordifolia* is an important medicinal plant commonly used in the traditional and Ayurvedic system of medicine for treatment of different ailments. This review illustrates its major constituents, pharmacological actions substantiating the claims made about this plant in the traditional system of medicine and its clinical applications.

Key words: *Rubia cordifolia*; Ayurveda; Clinical applications

INTRODUCTION

The family Rubiaceae comprises of about 450 genera and 6500 species and includes trees, shrubs and herbs (Williams, 2002). *Rubia* comprises about 60 species. *R. cordifolia* Linn. (Rubiaceae) is a perennial climber with very long, cylindrical, flexuose roots with a thin red bark (Kirtikar and Basu, 1980). In Sanskrit it is known as Manjistha. This plant has

very long, cylindrical, and flexuose roots with a thin red colored bark (Anonymous, 1999). Stems often have a long, rough, grooved, woody base. Plants belonging to this family are known to contain substantial amounts of anthraquinones, especially in the roots (Thomson, 1971; Anonymous, 1999). Flowers are small, white or greenish in terminal panicles or cymes. Fruits are globose, dark purplish or black (Williams, 2002). 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

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and Tropical Africa in moist temperature and tropical forest, upto an altitude of 3500 m. (Kirtikar and Basu, 1980; Khare, 2004).

R. cordifolia is used traditionally in the treatment of liver fluke, dysentery, maggots, wounds and intestinal worms in animals (Jha, 1992). It is used in the treatment of skin disorders and has anticancer activity (Chopra et al., 1957; Adwankar and Chitnis, 1982; Williams, 2002). Roots have tonic, astringent, antidiarrhetic, antiseptic, and deobstruent properties. They are used in rheumatism. Roots are active against *Staphylococcus aureus*. Paste of root is used for application in ulcers, inflammations, and skin conditions. A decoction of leaves and stems is used as a vermifuge (Williams, 2002). Scientific studies show that the anthraquinones of the Rubiaceae family exhibit some interesting *in vivo* biological activities, such as antimicrobial (Sittie et al., 1999), antifungal (Rath et al., 1995), hypotensive, analgesic (Younos et al., 1990), antimalarial (Adwankar and Chitnis, 1982; Koumaglo et al., 1992), antioxidant (Tripathi et al., 1997), antileukemic and mutagenic functions (Chang et al., 1995; Ismail et al., 1997). Apart from its medicinal value, this plant has also been used as natural food colorants and as natural hair dye (Samatha et al., 1996). *R. cordifolia* is often used in formulations to treat uterine and ovarian cancer (Halpern, 2004).

Ayurvedic Properties (Williams EM; CHEMEXIL)

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, Jwarhara, Mutrajanya, Swwdajanana, Twachya

Chemical constituents

Quinones

The plant contains quinines, mainly anthraquinone glycosides and include 1-hydroxy 2-methoxy

anthraquinone, 1, 4- dihydroxy-2- methyl-5-methoxy anthraquinone, 1,3- dimethoxy 2- carboxy anthraquinone and rubiadin (Dosseh et al., 1981).

Iridoids

6-methoxygeniposidic acid is found along with manjistin, garancin and alizarin (Wu et al., 1991).

Oleananes triterpinoid

Rubiprasin A, B, and C along with arborane triterpinoids, like rubiarbonol A, B, C, D, E and F have been isolated (Itokawa et al., 1989; Itokawa et al., 1990).

Bicyclic hexapeptides

The compounds having antitumour activity have been isolated and identified chemically. (Morita et al., 1992; Takeya et al., 1993).

Anthraquinones

The coloring matter present in the roots of *R. cordifolia* is a mixture of purpurin (trihydroxy anthraquinone) and manjistin (xanthopurpurin-2-carboxylic acid). The roots contain small amounts of xanthopurpurin or purpuroxanthin and pseudopurpurin (purpurin-3-carboxylic acid) (Anonymous, 1999). The plant also contains dihydromollugin, mollugin, rubilactone (Hoa LK et al., 2001). Purpurin, belonging to the lipocalin family of proteins, is a fast dye for cotton printing and forms complexes with various metal ions. It is a glycosaminoglycan binding protein as well as a retinol binding protein.

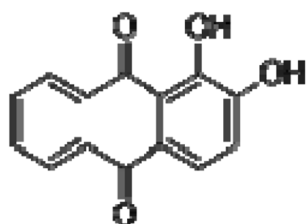
Alizarin, or 1, 2-dihydroxyanthraquinone or mordant red, is the red dye originally derived from the root of the madder plant. In 1869, it became the first natural pigment to be duplicated synthetically. The word alizarin is derived from the Arabic word al-usara, which means juice.

R. cordifolia yielded anthraquinones namely, 1-hydroxy-2 carboxy 3-methoxyanthraquinone, 1-hydroxy-2 methyl 6 or 7-methoxy- anthraquinones. The other compounds were oleanolic acid acetate, β -sitosterol, and scopoletol. Ten fatty acids with

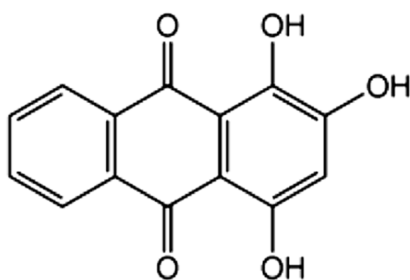
saturated and unsaturated long chains were also identified (Vidal-Tessier *et al.*, 1986).

The methanol extract of *R. cordifolia* roots contains 2 naphthaquinones (Koyama *et al.*, 1992). 2-carbamoyl/2-caurbomyl-3-methoxy-1,4- naphthaquinones and 2-carbamoyl-3-hydroxy-1,4- naphthaquinones.

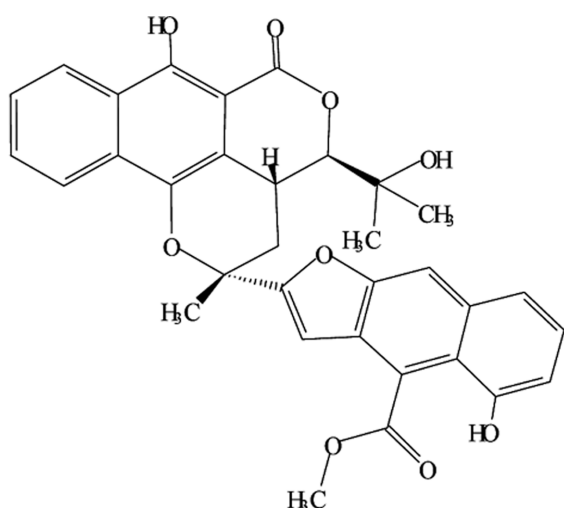
Anthraquinones from *R. cordifolia*:



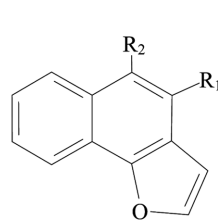
Alizarin (1, 2-dihydroxy-9,10-anthracenedione)



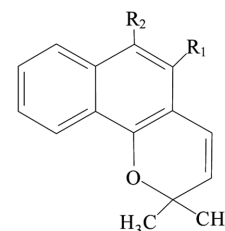
Purpurin (1, 2, 4-trihydroxyanthracene-9, 10-Dione)



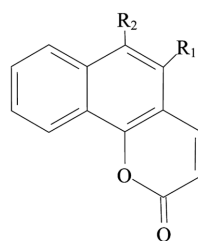
Rubicordifolin



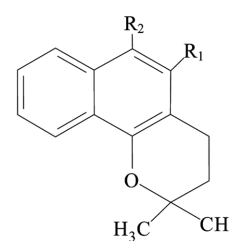
1, 4



2, 5, 7, 8, 9, 12



3, 11



6, 10

No	R ₁	R ₂	
1	COOMe	OH	Mollugin
2	COOMe	OH	Furomollugin
3	COOMe	OH	Rubilactone
4	COOMe	OMe	Methylfuromollugin
5	COOMe	OMe	Mollugin methyl ether
6	COOMe	OH	Dihydromollugin
7	COOMe	OAc	Acetylmollugin
8	COOCH ₂ CH ₂ CH(CH ₃) ₂	OMe	# I
9	COOMe	OCH ₂ Ph	# II
10	COOMe	OMe	Dihydromollugin methyl ether
11	COOMe	OMe	Methylrubilactone
12	COOH	OMe	2,2-Dimethyl-6-methoxy-2H-naphtho-[1,2-b]pyran-5-carboxylic acid

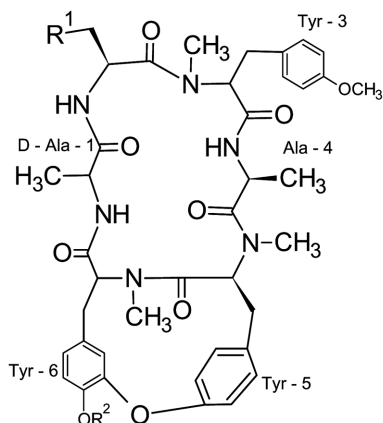
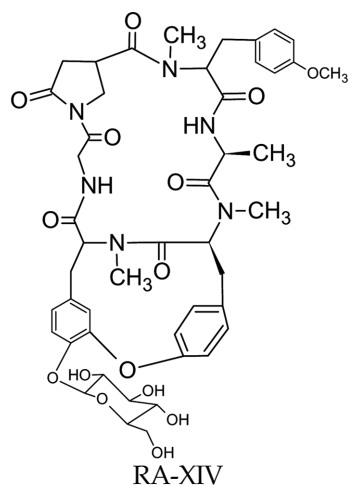
#I - Isopentyl 2, 2-dimethyl-6-methoxy-2H-naphtho-[1,2-b] pyran-5-carboxylate

#II - Methyl 2, 2-dimethyl-6-benzyloxy-2H-naphtho-[1,2-b] pyran-5-carboxylate (Morita *et al.*, 1992).

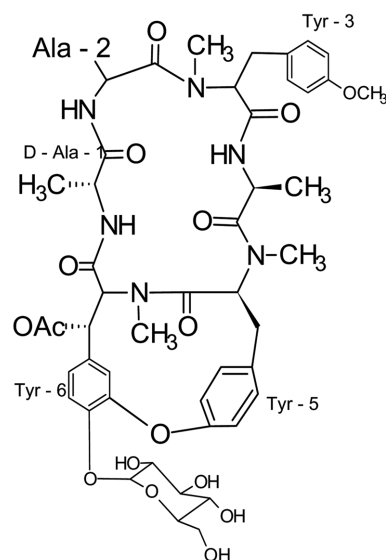
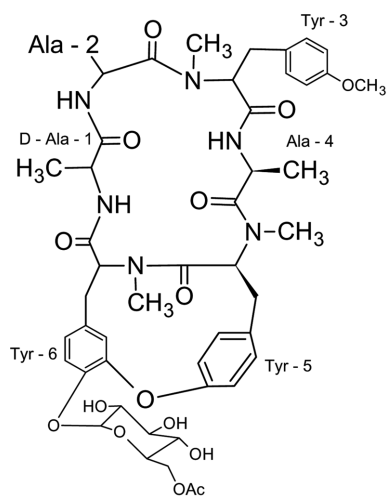
Qiao *et al.* (1990) isolated 12 compounds from roots of *R. cordifolia* of which 9 compounds have been characterized by chemical and spectroscopic methods as -

1. Alizarin
2. 1-hydroxy-2-methyl-9,10-anthraquinone
3. 1,3,6-trihydroxy-2-methyl-9,10-anthraquinone-3-O-(6'-o-acetyl)-alpha-L-rhamnosyl (1 to 2) - beta- D-glucoside
4. 1,3,6-trihydroxy-2-methyl-9,10-anthraquinone-3-O-alpha-L-rhamnosyl (1 to 2)-beta- D-glucoside
5. 1,3,6-trihydroxy-2-methyl-9,10-anthraquinone-3-O-(6'-o-acetyl)-beta-D-glucoside
6. 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone di-beta-D-glucoside
7. Rubimallin
8. beta-sitosterol
9. Damosterol

Bicyclic hexapeptides from *R. cordifolia*:



RA-XI: $R_1 = \text{CH}_2\text{COOH}$; $R_2 = \text{H}$
 RA-XII: $R_1 = \text{H}$; $R_2 = \beta\text{-D-Glucose}$
 RA-XIII: $R_1 = \text{CH}_2\text{COOH}$; $R_2 = \beta\text{-D-Glucose}$ (Morita *et al.*, 1992).



RA-XVI (Takeya *et al.*, 1993)

Traditional uses in different systems of herbal medicine

Unani system of medicine

R. cordifolia has been prescribed for paralysis, dropsy, jaundice, amenorrhoea, urinary tract obstructions, skin disorders of many varieties, menstrual disorders (excessive or painful bleeding), renal stone, urinary disorders and blood detoxification (www.raynaudshern.com).

Chinese system of medicine: Roots help menstrual flow, promote blood circulation, promote urination, stop coughing blood or vomiting blood, nose bleeding. The plant is also useful in treatment of missing menses due to blood stasis, cold damp heat bi (pain and inflammation caused by bleeding and blood circulation stasis), injuries from impacts and in jaundice and edema (www.alternativehealing.org).

Ethnoveterinary usage

R. cordifolia is used in the treatment of liver fluke, dysentery, maggots, wounds and intestinal worms in animals (Jha, 1992).

Pre-Clinical Studies

In vitro studies

Calcium channel blocker(s) in *R. cordifolia*

The calcium channel antagonistic activity of a crude root extract of *R. cordifolia* was tested in isolated tissue preparations. The extract (0.1 - 3 mg/ml) augmented spontaneous contractions of guinea pig atria, rabbit jejunum and rat uterus in a concentration-dependent manner. In rabbit aorta, the extract inhibited norepinephrine-(10 fM) and KCl (80 mM) induced contractions. Spontaneous movements of rabbit jejunum were abolished in a calcium-free solution. Addition of calcium (25 fg/ml) restored the spontaneous movements. When tissues were pretreated with plant extract (1 mg/ml) or verapamil (0.5 fg/ml), addition of calcium failed to restore spontaneous contractions. These results indicate that the extract exhibits spasmolytic activity similar to that of verapamil suggestive of presence

of calcium channel blocker- like constituent(s) (Gilani *et al.*, 1994).

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- γ (TNF- γ) 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 *R. cordifolia* caused a statistically significant suppression of ROS from PMNL. Thus, *R. cordifolia*

Table 1. Some of the ayurvedic preparations containing *R. 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, leucorrhoea etc. Improves body strength and digestive system. Keeps the body cool and also maintain good general health.	Yogaratanakaram
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	Yogaratanakaram
5	Gulguluthikthkarishtam	Recommended in acute rheumatic conditions, chronic skin diseases, sinusitis, lymphadenitis, 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	Manjishthasavam	For all types of skin diseases and vata, pitta, kapha, especially for vitiated pitta and kapha vikaras.	Ashtangahridayam
8	Nimbamrithasavam	Recommended in acute rheumatic conditions, skin diseases, and sinusitis, obesity, and lymphadenitis 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.	Yogaratanakaram
10	Jaatyaadi ghrita	Applied externally for chronic and septic ulcers	Ashtanga Hridaya
11	Phal kalyaan ghrita	Used for amenorrhoea and uterine affections	Bhaishajya Ratnaavali
12	Majishthaadi taila	Used for headache	Sahasrayoga

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 (Jain and Basal, 2003).

Antimicrobial action

R. 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. *R. 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 *R. 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 (Singh et al., 2005; Vlietinck, 1995). Many compounds were isolated, established by chemical and spectroscopic methods from the roots of *R. cordifolia* L. Some of the compounds showed certain antibacterial activities (Qiao et al., 1990). In one study some antimicrobial agents, emodin and physcion were isolated as the most active constituents (Basu et al., 2005).

In vivo studies

Antidiabetic Action

The antidiabetic action of *R. 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 (Baskar et al., 2006). The effect of ethyl acetate fraction of roots of *R. 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 200 mg/kg, p.o.) was carried out in i) normal fasted ii) oral glucose tolerance test (OGTT) iii) alloxan (120 mg/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 glucose utilization by an isolated rat diaphragm. Alone RCEAF (25 mg/mL) as well as combination of RCEAF (25 mg/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* (Somani et al., 2006). Administration of the alcoholic extract of roots of *R. cordifolia* showed significant hypoglycemic effect in alloxan induced diabetic rats (Patil et al., 2006).

Anti-oxidant activity

Prolonged oral treatment of rats with ethanol (2 g/kg of 20% w/v) significantly increased the levels of lipid peroxidase (LPO), decreased the activities of superoxide dismutase (SOD) and catalase (CAT) and reduced the content of glutathione (GSH). The concurrent treatment of ethanol-administered rats with *R. cordifolia* prevented these above mentioned ethanol-induced changes in the markers of oxidative

stress. Influences of *R. cordifolia* on ethanol-induced changes were comparable with those produced by vitamin E and C treatment (Joharanpurkar *et al.*, 2003).

Rubiadin, a dihydroxy anthraquinone, isolated from alcoholic extract of *R. cordifolia*, possesses potent antioxidant property. It prevents lipid peroxidation induced by FeSO₄ and t-butylhydroperoxide (t-BHP) in a dose-dependent manner. The percent inhibition was more in the case of Fe²⁺ induced lipid peroxidation. The antioxidant property of the preparation was better than the EDTA, Tris, mannitol, Vitamin E and p-benzoquinone (Tripathi *et al.*, 1997).

Antiproliferative activity

The antiproliferative property of *R. cordifolia* extract has been studied on two different cell types, A-431 cells (epidermal carcinomoid cells) and 3T3 fibroblast cells. A fraction of *R. cordifolia* significantly inhibited the incorporation of 3H-thymidine induced by fetal bovine serum, in a dose-dependent manner. It also inhibited the phorbol 12-myristate 13-acetate (PMA)-induced expression of c-fos genes in A-431 cells. Inhibition of DNA synthesis underlies the mechanism for its antiproliferative properties (Tripathi *et al.*, 1998).

Hepatoprotective Activity

The hepatoprotective activity of an aqueous-methanol extract of *R. cordifolia* was investigated against acetaminophen and CCl₄-induced hepatic damage. Acetaminophen produced 100% mortality at a dose of 1 g/kg in mice while pretreatment of animals with *R. cordifolia* extract reduced mortality to 30%. Acetaminophen at a dose of 640 mg/kg produced liver damage in rats as manifested by the rise in serum levels of glutamic oxaloacetic transaminase (SGOT) and glutamate pyruvate transaminase (SGPT). Pretreatment of rats with *R. cordifolia* extract lowered significantly the SGOT and SGPT levels. Similarly, hepatotoxic dose of CCl₄ raised the SGOT and SGPT levels respectively compared with respective control. The same dose of *R. cordifolia* was able to

prevent significantly the CCl₄-induced rise in serum enzymes and the estimated values of SGOT and SGPT. Moreover, it prevented CCl₄-induced prolongation in pentobarbital-induced sleep confirming the hepatoprotective effects of the extract (Gilani *et al.*, 1995).

The hepatoprotective activity of rubiadin was evaluated against carbon tetrachloride (CCl₄)-induced hepatic damage in rats. Rubiadin at a dose of 50, 100 and 200 mg/kg was administered orally once daily for 14 days. The substantially elevated serum enzymatic activities of SGOT, SGPT, serum alkaline phosphatase (SALP) and γ -glutamyltransferase (γ -GT) due to carbon tetrachloride treatment were dose dependently restored towards normalization. Meanwhile, the decreased activities of glutathione S-transferase and glutathione reductase were also restored towards normal. In addition, rubiadin also significantly prevented the elevation of hepatic melondialdehyde formation and depletion of reduced glutathione content in the liver of CCl₄ intoxicated rats in a dose dependent manner. Silymarin used as reference standard also exhibited significant hepatoprotective activity on post treatment against carbon tetrachloride induced hepatotoxicity in rats. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate that rubiadin has a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats (Mohana *et al.*, 2006).

Anti-platelet activating factor activity

R. 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. *R. 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 (Tripathi *et al.*, 1993).

Antitumour 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 (Morita, 1992, Morita, 1993, Itokawa, 1993). The antitumor activity of RA-700, a cyclic hexapeptide isolated from *R. 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 (Kato, 1987). The anticancer as well as antiviral property had been reviewed (Rastogi and Dhawan, 1990). From the chloroform fraction of *R. cordifolia* roots three constituents were isolated namely mollugin, furomollugin and dehydro-alapchone. 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 (Gupta, 1999). The *R. 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 (Abderrahman, 2004). *R. cordifolia* had shown an increase in leukocyte count in leucopenia (Zhang, 1983).

The cytotoxic action of *R. 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 (Son JK, 2006). Antitumor activity of RC-18, a pure isolate from *R. 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 (Adwankar and Chitnis, 1982). The anticancer activity of extracts of *R. cordifolia*, tested against the P388 tumor system in BDF1 mice, compared well with that of the positive control, 5-fluorouracil (Adwankar *et al.*, 1980).

Anti-inflammatory activity

R. cordifolia is considered to be traditionally useful as an analgesic, astringent, external application in inflammations, ulcers and skin diseases (Khalid, 1995). The plant is also claimed to relieve the symptoms of pruritus, burning and exudation

from skin (Nadkarni, 1976). 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 (Bapalal, 1965). *R. 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) (Antarkar *et al*, 1983). *R. 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 (Tripathi *et al*, 1995).

Radioprotective effect

The radioprotective potential of alcoholic extract of root of *R. cordifolia* was studied by survival, hemopoietic cell protection and micronucleus assay. The LD₅₀ value for the alcoholic root extract was found to be 1200 mg/kg body weight at 72 h post irradiation. A significant radiation protection (67%) as assessed by increased animal survival was observed when *R. cordifolia* 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 *R. cordifolia* 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 *R. cordifolia* extract was administered 90 min 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 *R. cordifolia* extract

appears to be through its anti-oxidant, metal chelation and anti-inflammatory property (Tripathi and Singh, 2007).

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 *R. 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. *R. cordifolia* did not exert cytotoxicity to this human fibroblast cell line (Tse *et al.*, 2006). The antiproliferative property of *R. cordifolia* (Rubiaceae) extract was also tested on A-431 cells (epidermal carcinomoid cells) and 3T3 fibroblast cells. It was observed that a fraction of *R. cordifolia* significantly inhibited the incorporation of [3H]-thymidine, induced by fetal bovine serum, in a dosedependent 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 (Tripathi and Shukla, 1998).

Inhibitory action on nitric oxide production

R. cordifolia is used for prevention and treatment of inflammatory diseases, therefore water and methanol extracts of *R. cordifolia* were screened for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated J774.1 macrophages and in LPS/interferon (IFN)-B-stimulated mouse peritoneal exudate macrophages. The methanol extract *R. cordifolia*, showed significant inhibition in J774.1 macrophages, while in mouse peritoneal exudate macrophages, water extract of *R. cordifolia* inhibited the nitric oxide (NO)

production. Water extract of *R. cordifolia* showed inhibition on iNOS mRNA expression (Tezuka *et al.*, 2001). The inhibition of NO is found to be a key mediator in the phenomenon of inflammation. Thus *R. cordifolia* was evaluated for inhibitory activity on NO produced in-vitro from sodium nitroprusside, and in LPS-activated murine peritoneal macrophages, *ex vivo* (Basu and Hazra, 2006). 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 ug/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'. *R. cordifolia* was also found to be effective in experimental models (Biswas and Mukherjee, 2003).

Anti-allergic activity

Alcoholic extract of *R. cordifolia* inhibited passive cutaneous anaphylaxis (PCA) in the mouse and rat (Gupta *et al.*, 1993).

Anticonvulsant activity

Triterpenes isolated from the acetone soluble part of petroleum ether extract of *R. cordifolia* inhibited seizures induced by maximum electroshock (MES), electrical kindling, pentylenetetrazol (PTZ), and lithium-pilocarpine. However seizures induced by strychnine were not inhibited (Kasture *et al.*, 2000).

Pentobarbitone-induced sleep

Mice treated orally with triterpenes isolated from the petroleum ether extract of *R. cordifolia* significantly

prolonged the pentobarbitone-induced sleeping time (Kasture *et al.*, 2000).

Antinociceptive activity

Mice treated with triterpenes isolated from the petroleum ether extract of *R. cordifolia* exhibited significant antinociceptive activity as indicated by increase in the reaction time in the hot plate analgesiometer (Kasture *et al.*, 2000).

Anxiolytic activity

Mice treated with triterpenes isolated from the petroleum ether extract of *R. cordifolia* exhibited anxiogenic activity by remaining for most of the time in the closed arm 38. Whereas, the ethanolic extract exhibited anxiolytic activity as indicated by a significant increase in open arm occupancy (Kasture *et al.*, 2000).

Nootropic activity

Alcoholic extract of roots of *R. cordifolia* decreased transfer latency in the elevated plus maze paradigm suggesting nootropic activity. Alcoholic extract of *R. cordifolia* antagonized amnesic effect of scopolamine (Patil *et al.*, 2006).

Effect on motor co-ordination

Alcoholic extract of roots of *R. cordifolia* significantly decreased the fall off time when placed on rotarod rotating at the speed of 20 rev/min (Patil *et al.*, 2006).

Antistress activity

Alcoholic extract of roots of *R. cordifolia* significantly decreased ulcer index, acidity, plasma corticosterone level, brain content of dopamine in a dose-dependent manner in animals under cold restraint stress (CRS) when compared with diazepam. Alcoholic extract of *R. cordifolia* increased brain content of GABA in dose-dependent manner in CRS treated animals (Patil *et al.*, 2006).

Effect on baclofen induced catatonia

Alcoholic extract of *R. cordifolia* potentiated baclofen-

induced catatonia (Patil *et al.*, 2006).

Clinical studies

Eczema

During recent 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 (Antarkar *et al.*, 1978).

Phase I Study of RA-700

Phase I clinical study was conducted on an antitumour substance RA-700 isolated from *Rubia akane* or *R. cordifolia*. This clinical study was carried out in 6 institutions. The effects of a single dose administration and 5-day administration were evaluated in 14 patients. RA-700 was given from 0.2 to 1.4 mg/M2 in single i.v. dose and from 0.4 to 2.0 mg/M2 in 5-day i.v. dose schedule. Nausea, vomiting, fever, stomachache, mild hypotension and slight abnormality of electrocardiogram were observed. In pharmacokinetic study, the elimination half-lives ($t_{1/2}$) of RA-700 in plasma were: 55 min of alpha-phase and 3.9 h of beta-phase by single dose study, and 23 - 25 min of alpha-phase and 6 - 14 h of beta-phase by 5-day schedule study. Accumulation was not found in 5-day schedule administration and metabolites were not observed in plasma and urine. It seems that RA-700 was metabolized by the liver and excreted in the feces. In conclusion, the maximum tolerated dose was 1.4 mg/M2 for 5-day schedule administration (Majima *et al.*, 1993).

Inhibition of Hepatitis B Surface Antigen Secretion on Human Hepatoma Cells by Components of *R. cordifolia*

The antiviral activity in the roots of *R. cordifolia* was examined, and three naphthohydroquinones, furomollugin, mollugin, and rubilactone were isolated from it. Furomollugin and mollugin strongly suppressed the secretion of hepatitis B surface antigen (HBsAg), furomollugin and mollugin showed

IC₅₀ = 2.0 µg/mL, in human hepatoma Hep3B cells while having little effect on the viability of the cells. A 6-hydroxy group and a pyran or furan ring contribute to suppressive effect of furomollugin and mollugin (Ho *et al.*, 1996).

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