Review Article

Pharmacological and toxicological effects of *Rubia cordifolia* - A review

A. Sandhya, Anitha Roy*

ABSTRACT

Rubia cordifolia (binomial name-*R. cordifolia* L.,), commonly known as Indian madder, is a traditional Chinese medicinal plant that has been listed at the Chinese Pharmacopoeia in 2015. It belongs to the family *Rubiaceae* and is one of the 70 species that belong to the genus Rubi. It is widely distributed around the world and is cultivated during the ancient days. It is a perennial climbing plant; roots are long with a thin outer red layer while their stem is long with woody base. The presence of dibutyl phthalate in the fruits of *R. cordifolia* has toxicological effects. It is used to treat many diseases such as treating skin disease and cancer; moreover, *R. cordifolia* was proven to have anticancer, anti-inflammatory, antioxidant, and antimicrobial effects. *R. cordifolia* is an old medicinal plant which contains various pharmacological activities such as anti-arthritic, antidiabetic, anticonvulsant, and wound healing property and its fruits have toxicological effects. This study results in an overview of pharmacological and toxicological effects of *R. cordifolia*.

KEY WORDS: *Rubia cordifolia*, Anti-arthritic, Antidiabetic, Anticonvulsant, Wound healing property

INTRODUCTION

Rubia cordifolia is a perennial climbing herbaceous plant. It is also known as Indian madder, which is a flowering plant species in the coffee family, *Rubiaceae*. A red pigment is derived from its root hence it is cultivated. Genus Rubia fell into about 70 species distributed widely around the world, a total of 36 species and two varieties were reported from China. The extracts and phytochemicals of Rubia plants had drawn considerable attention due to their potent bioactivities.[1] Leaves are arranged in four whorls whereas the stem is slender rough and woody at the base. Flowers are in cymes, greenish white. Fruits are smooth, shining, and purplish black when ripe.[2] The root of the plant is commonly known as Manjistha and is sweet, bitter, acrid. These roots, which cluster in the soil, are aubergine or orange-red. The elongating and rough stems slightly lignify at the base. The branches are four-edge shaped.[3,4]

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

The root extract is used against ailments such as arthralgia, arthritis, cephalalgia, cough, diabetes, discoloration of the skin, dysmenorrhea, emmenagogue, general debility, hemorrhoids, hepatopathy, intermittent fevers, jaundice, leukorrhea, neuralgia, pectoral diseases, pharyngitis, and also many pharmacological actions, whereas the roots are used for laxative, analgesic, rheumatism, dropsy, paralysis and intestinal ulcers, etc. In these cases such as in blood, skin and urinogenital disorders, dysentery, piles, ulcers, and inflammations the stem of rubia is used.[2] The major constituent of the root is ruberythric acid and is widely used as a phytotherapeutic drug in the treatment of calcium-containing stones in the urinary tract.[4]

Studies revealed that screening of biologically active compounds from various solvent extracts of root, stem, and leaf in *R. cordifolia* revealed the presence of anthraquinones, glycosides saponins, steroids, phenols, and flavonoids. Biologically active compounds are chemical in nature they have the potential to cure various diseases. *R. cordifolia* also revealed important phytochemical compounds and evidenced that this plant as an important for curing various diseases in traditional medicine.

Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

***Corresponding author:** Dr. Anitha Roy, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Chennai - 600 077, Tamil Nadu, India. E-mail: anitharoy2015@gmail.com

Received on: 14-02-2019; Revised on: 17-03-2019; Accepted on: 21-04-2019

Anthraquinones were mainly found in root, stem, and leaf which have been shown to be antibacterial, antifungal, and laxative and were also used as natural dyes.[5,6]

PHYTOCHEMISTRY

The pharmacological action of the crude drug is largely depends on the metabolites present in it.[6] *R. cordifolia* (Manjistha) basically known for its anthraquinones and naphthohydroquinones phytochemical constituents.[7] The major phytoconstituents of *R. cordifolia* reported include rubiadin, rubicordone A, rubiasins A-C, rubiatriol (triterpenoid), 6-methoxygeniposidic acid an iridoid glycoside and two pentacyclic triterpenoid-rubicoumaric acid, and rubifolic acid. Mollugin, furomollugin, and dehydro-alpha-lapachone are isolated from chloroform fraction.[8-12]

Anthraquinone

The anthraquinone is more in roots when compared to stem and leaves. Alizarin (1, 3-dihydroxy-2-ethoxymethyl-9, 10-anthraquinone), mollugin (1-hydroxy-2-methyl-9, 10-anthraquinone), 1, 3, 6-trihydroxy-2-methyl-9, 10-anthra-quinone-3-O-(6'-Oacetyl)-α-L-rhamnosyl (1→2)-β-D-glucoside, 1, 3, 6-tri hydroxy-2-methyl-9, 10- anthraqueinone-3-O-β-L-rhamnosyl (1→2)-β-D-glucoside, and 1, 3, 6-trihydrozy-2-methyl-9,10-anthraquinone-3-O-(6'- O-acetyl)-β-D-glucoside are some of the anthraquinone derivatives from the root sof *R. cordifolia*.[2]

Naphthoquinones

The representative of naphthoquinones is mollugin which is proved to have anticancer,[13,14] anti-inflammatory and neuroprotective activities[15-17]. The plant also contains many terpenoids and other phytochemicals.[18]

PHARMACOLOGICAL ACTIVITIES

Anti-acne Property

The anthraquinone rich fraction of *R. cordifolia* is available in a gel formulation which is used against *Propionibacterium acne*, *Staphylococcus epidermidis*, *Malassezia furfur,* etc. It is better when compared with standard clindamycin gel.[19]

Anti-arthritic Property

The ethanolic extract of *R. cordifolia* has imperative anti-arthritic potential and it also showed paw edema inhibition in the induced arthritic model, which is similar to a nonsteroidal anti-inflammatory drug, called aspirin.[20]

Anti-cancer Property

Anticancer activities are experimented through *in vitro* and/or in bioassays based on animal models. The crude aqueous extracts which is demonstrated show growth inhibitory activity on selected cancer cell lines and also on normal human mammary epithelial cells.[21] The quinones and RC-18 exhibited significant anticancer activity against P388 leukemia, L1210, L5178Y, B16 melanoma,[22,23] S-180, and the cyclic hexapeptides against leukemia. The hexapeptides bind to eukaryotic 80S ribosomes, resulting in inhibition of aminoacyl-tRNA binding and peptidyl-tRNA translocation, thus leading to the stoppage of protein synthesis.[24] The cyclic hexapeptide isolated from dried roots showed antitumor activity.[25] Alkyl ether and ester derivatives of RA-V showed significant effects against human nasopharynx carcinoma, P388 lymphocytic leukemia, and MM2 mammary carcinoma cells.[26] In another study, antimutagenicity of purpurin against a number of heterocyclic amines in the Ames mutagenicity test was proven.[27] Mollugin may have potential as a chemotherapeutic agent for human oral squamous cell carcinoma cells through the upregulation of the HO-1 and Nrf2 pathways and the downregulation of NF-κB[28] and an active antiproliferative principle in human colon cancer (Col2) cell line.[29] Methanol fraction of extract exhibited potent inhibition of Hep G32 cell line while found to be less cytotoxic against normal human kidney cells displaying safety for normal cells.[30] The dichloromethane fraction of madder root extract exhibited inhibition of human leukemia cell line and human histolytic lymphoma cell line.[31] The ethanol extract of root showed cytotoxic effect against human larynx carcinoma and human cervical cancer.[32] The methanolic extract of *R. cordifolia* leaf showed nearly 50% MCF-7 cell line (breast cancer) inhibition at 200 µg/ml tested dose.[33]

Antidiabetic Activity

Alcoholic extract of root and leaf extracts was found to have promising antidiabetic activity against animal models. The extract of roots reduced the blood sugar level in alloxan-treated diabetic rats, indicates that the extract has an extrapancreatic effect.[34] The aqueous root extracts were found to normalize hyperglycemia, hypertriglyceridemia, enhanced transaminases of liver and kidney, hypochromic microcytic anemia, and loss of body weight in streptozotocin-induced diabetic rat models.[35] The leaf extract decreased in the blood glucose level compared to the glibenclamide and normal fasted rat and alloxan-induced diabetic rats. In addition, the extract also showed a favorable effect on glucose disposition in glucose-fed hyperglycemic rats.[36,37]

Anticonvulsant Activity

In modern medicine, *R. cordifolia* was reported to have anticonvulsant activity. Triterpenes inhibited seizures

induced by maximum electric shock, electrical kindling, and various chemoconvulsants in rats. Brain GABA and serotonin (5-HT) contents were raised by the compound proves its anticonvulsant property.[38]

Anti-inflammatory Activity

Due to the presence of rubimallin from the root of *R. cordifolia* it is used as an anti-inflammatory agent. Studies stated that the aqueous extract showed anti-inflammatory activity in rats with carrageenan paw edema in a dose-dependent manner, which is comparable to that of phenylbutazone.[39] It also inhibited the lipoxygenase enzyme pathway, which catalyzes the production of various inflammatory mediators such as leukotrienes that are involved in asthma, arthritis, and other inflammatory disorders, and the production of cumene hydroperoxide.[40] Notable nitric oxide scavenging activity was exhibited *in vitro* by some extracts of *R. cordifolia*.[41]

Wound Healing Activity

The root extract of *R. cordifolia* was reported as an effective wound healing principle in experimental models as a wound healer.[42] Ethanolic extract and the hydrogel formulation of roots were found to be effective in the functional recovery and healing of wounds and also lead to histopathological alterations.[43]

Antimicrobial Activity

The root extracts of *R. cordifolia* have been studied for their antimicrobial activity against various pathogenic bacteria. Sitosterol and daucosterol possess antibacterial activity. The root extracts constituents such as anthraquinones and flavonoids suppressed the activity phytopathogens of *Gossypium*.[44] Aldehyde acetate, dihydromollugin, and rubimallin reported to have significant antibacterial activity against *Klebsiella pneumonia*.[45] The ethanolic extract inhibited ß-Lactamase producing uropathogenic *Escherichia coli*.[46] The chloroform and the methanol extracts reported to have antibacterial activity on Gram-positive strains, although Gram-negative *Pseudomonas aeruginosa* was also inhibited by the methanol extracts in a dose-dependent manner. According to Basu *et al*., the aqueous extract is active against *Bacillus subtilis* and *Staphylococcus aureus* compared with streptomycin and penicillin G.[47] The ethanolic whole plant extract also showed the same result.[48] Rubiacordone A reported to have considerable antimicrobial activity against Gram-positive bacteria such as *B. subtilis*, *Streptococcus faecalis,* and *B. cereus*.[9]

Antioxidant Activity

R. cordifolia contains a wide variety of antioxidants such as alizarin, hydroxyl anthraquinones[49] and rubiadin[50] which have been using in various medicaments. Hydroxy groups on one benzene ring of the anthraquinone structure were essential for hydroxyl anthraquinones to show the activity; its ortho-dihydroxy structure could greatly enhance their effect, and glycosylation reduced activity.[51] The alcoholic root extract has some effect on body weight due to rubiadin.

Antiperoxidative Activity

Solvent-free alcoholic extract of *R. cordifolia* showed the antiperoxidative property in rat liver homogenate. The cumene hydroperoxide induced malondialdehyde formation accompanied by the reduced glutathione level even in the presence of the above toxin.[40]

Antiplatelet Activating Effect

In the Ayurvedic system, it is stated that the plant is prescribed to cure blood-related ailments. The partially purified fraction of the whole plant itself inhibits the action of platelet-activating factor at its receptor level either by its blocking or by desensitization property.[52]

Antiproliferative Property

Aqueous, ethanolic extract of root reported to have a significant anti-proliferative effect. The antiproliferative property was also tested on A-431 cells (epidermal carcinomoid cells) and 3T3 fibroblast cells and recorded that the inhibition incorporation of [3H]-thymidine, is in a dose-dependent manner. It also inhibited the phorbol 12-myristate 13-acetate induced expression of c-fos genes in A-431 cells due to the inhibition of DNA synthesis.[53] Mollugin found to be an active antiproliferative principle by bioassay-monitored fractionation. It did not exert cytotoxicity to human fibroblast cell line.[54]

Antistress and Nootropic Activity

It is stated that alcoholic extract enhanced brain Y-amino-n-butyric acid levels and decreased the brain dopamine and plasma corticosterone levels.[39] The extract obtained also inhibited the acidity and ulcers caused due to cold restraint stress.

Antiulcer Activity

The effect of alcoholic extracts of roots of *R. cordifolia* and its antiulcer potential on alcohol, ibuprofen, cold-restraint stress, and pyloric ligation-induced gastric lesions was studied along with ranitidine, a standard drug.[55] The extract showed substantial and significant protection against gastric ulcers in all the models compared to ranitidine. In polyherbal formulations, the ulcerogenicity effect in rats showed significantly lesser ulcer effect even at a very high dosage as compared to that of aspirin.[56]

Antiviral Activity

The naphthohydroquinones are reported to have antiviral activity. 6-hydroxy group and a pyran or furan ring of furomollugin and mollugin strongly suppressed

the secretion of hepatitis B surface antigen, in human hepatoma Hep3B cells.[57] The methanolic extracts of leaves have a minimum inhibitory concentration of different virus using HEL cell cultures and Vero cell cultures.[58]

Diuretic Activity

To substantiate the traditional claim, the hydroalcoholic root extract of *R. cordifolia* was evaluated for its diuretic property and got positive results.[59,60] The hydroalcoholic extract as well as the ethanol extract showed a significant increase in urine volume and electrolyte excretion in a dose-dependent manner compared with the reference drugs.[61]

Gastroprotective Activity

R. cordifolia has both gastroprotective and ulcer healing properties.[27] Triterpenoids present in root extracts are the potent antiulcer and antioxidant compound which can be clinically explored.[62] The methanolic extract and the chloroform fraction showed a reduction in ulcer index, lipid peroxidation, and increase in the mucin content, catalase and reduced glutathione in stomach tissue.[63]

Hepatoprotective Activity

The quinone derivatives from *R. cordifolia* reported to have a hepatoprotective effect on animal systems. Animal model studies proved that the methanolic extract protects the liver thioacetamide-induced hepatotoxicity.[64] The aqueous-methanol extract is active against acetaminophen and CCl4-induced hepatic damage in rats.[65]

Immuno-modulating Activity

The alkaloids, cardiac glycosides, tannins, flavonoids, and phenols present in *R. cordifolia* are responsible for enhanced immuno-modulation. Ethanolic extracts of the whole plant were administrated to rats to test immunosuppressive activity and showed enhanced cell-mediated and humoral immuno-potentiating activity.[66] Due to the immuno-modulating activity, it is utilized as a source of immunity-enhancing drug.

Neuroprotection

R. cordifolia contains a wide variety of antioxidants and also exhibited strong free radical scavenging properties against reactive oxygen and nitrogen species. The herb attenuates oxidative stress-mediated cell injury during oxygen-glucose deprivation and exerts the above effects at both the cytosolic and at gene expression level and may be an effective therapeutic tool against ischemic brain damage.[67,68]

Radiation Protection

Oxidative stress induced by oxygen-derived reactive oxygen species produces several adverse effects which are highly implicated in several degenerative diseases such as cancer. The therapeutic applications of *R. cordifolia* extract provide significant protection against radiation-induced lipid peroxidation, hemopoietic injury, and genotoxicity when administered intraperitoneally before the radiation exposure.[69] Single strand breaks induced in plasmid pBR322 DNA following ionizing radiations was effectively prevented by the aqueous extract.[70]

TOXICOLOGICAL EFFECTS

Although some extracts or compounds from RRR have shown antitumor effects, rubiadin was reported to display carcinogenic potential.[4] The results indicated that rubiadin may be a potent carcinogenic ingredient that targeted the proximal tubule cells in the outer medulla.[71] Rubiadin was also considered as both initiator and promoter of carcinogenicity targeting kidney, liver, and large intestine.[72] In madder pigment, alizarin, purpurin, and 1-hydroxyanthraquinone were found to have similar effects as ethidium bromide, a typical DNA intercalator. They exhibited potential genotoxicity in *E. coli*, by blocking gene expression and inducing cell death.[73]

CONCLUSION

R. cordifolia has a long history of use in the treatment of various human diseases. It is proven to have anticancer, anti-inflammatory, antioxidant, antiarthritic, antidiabetic, anticonvulsant, wound healing property, and toxicological effect. This plant has promising effects on various diseases.

REFERENCES

1. Verma A, Kumar B, Alam P, Singh V. *Rubia cordifolia* a review on pharmaconosy and phytochemistry. Int J Pharm Sci Res 2016;7:2720-31.
2. Priya MD, Siril EA. Traditional and modern use of Indian madder (*Rubia Cordifolia* L.): An overview. Int J Pharm Sci Rev Res 2014;25:154-64.
3. Pathania S, Daman R, Bhandari S, Singh B, Lal B. Comparative studies of *Rubia cordifolia* L. And its commercial samples. Ethnobotanical Leaf 2006;11:179-88.
4. Shan M, Yu S, Yan H, Chen P, Zhang L, Ding A, *et al.* A review of the botany, phytochemistry, pharmacology and toxicology of *Rubiae* radix et rhizoma. Molecules 2016;21:E1747.
5. Pendli S, Talari S, Nemali G, Azmeera SN. Phytochemical analysis of root, stem and leaf extracts in *Rubia cordifolia* L. An important medicinal plant. World J Pharm Pharm Sci 2014;3:826-38.
6. Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S. Herbs as an antioxidant arsenal for periodontal diseases. J Intercult Ethnopharmacol 2016;5:92-6.
7. Itokawa H, Qiao Y, Takeya K. Anthraquinones and naphthohydroquinone from *Rubia cordifolia*. Phytochemistry 1989;28:3465-8.
8. Rao GM, Rao CV, Pushpangadan P, Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. J Ethnopharmacol 2006;103:484-90.
9. Li X, Liu Z, Chen Y, Wang LJ, Zheng YN, Sun GZ, *et al.* Rubiacordone A: A new anthraquinone glycoside from the

roots of *Rubia cordifolia*. Molecules 2009;14:566-72.
10. Chang LC, Chavez D, Gills JJ, Fong HH, Pezzuto JM, Kinghorn AD. Rubiasins A-C, new anthracene derivatives from the roots and stems of *Rubia cordifolia*. Tetrahedron Lett 2000;41:7157-62.
11. Arisawa M, Ueno H, Nimura M, Hayashi T, Morita N. Rubiatriol, a new triterpenoid from the Chinese drug "Qian Cao Gen," *Rubia cordifolia*. J Nat Prod 1986;49:1114-6.
12. Wu LJ, Wang SX, Hua HM, Li X, Zhu TR, Miyase T. Ueno A. 6-methoxygeniposidic acid, an iridoid glycoside from *Rubia cordifolia*. Phytochemistry 1991;30:1710-1.
13. Do MT, Hwang YP, Kim HG, Na M, Jeong HG. Mollugin inhibits proliferation and induces apoptosis by suppressing fatty acid synthase in HER2-overexpressing cancer cells. J Cell Physiol 2013;228:1087-97.
14. Zhang L, Wang H, Zhu J, Xu J, Ding K. Mollugin induces tumor cell apoptosis and autophagy via the PI3K/AKT/mTOR/p70S6K and ERK signaling pathways. Biochem Biophys Res Commun 2014;450:247-54.
15. Zhu ZG, Jin H, Yu PJ, Tian YX, Zhang JJ, Wu SG, *et al.* Mollugin inhibits the inflammatory response in lipopolysaccharide-stimulated RAW264.7 macrophages by blocking the janus kinase-signal transducers and activators of transcription signaling pathway. Biol Pharm Bull 2013;36:399-406.
16. Jeong GS, Lee DS, Kim DC, Jahng Y, Son JK, Lee SH, *et al.* Neuroprotective and anti-inflammatory effects of mollugin via up-regulation of heme oxygenase-1 in mouse hippocampal and microglial cells. Eur J Pharmacol 2011;654:226-34.
17. Kim KJ, Lee JS, Kwak MK, Choi HG, Yong CS, Kim JA, *et al.* Anti-inflammatory action of mollugin and its synthetic derivatives in HT-29 human colonic epithelial cells is mediated through inhibition of NF-kappaB activation. Eur J Pharmacol 2009;622:52-7.
18. Jacob JJ, Kumar BP. Dual COX/LOX inhibition: Screening and evaluation of effect of medicinal plants of Kerala as antiinflammatory agents. J Pharmacogn Phytochem 2015;3:62-6.
19. Khan N, Karodi R, Siddiqui A, Thube S, Rub R. Development of anti-acne gel formulation of anthraquinones rich fraction from *Rubia cordifolia* (*Rubiaceae*). Int J Appl Res Natl Prod 2012;4:28-36.
20. Jaijesh P, Srinivasan KK, Kumar PB, Sreejith G, Ciraj AM. Anti-arthritic property of the plant *Rubia cordifolia* Linn. Pharmacologyonline 2008;1:107-13.
21. Bokadia GS, Priya J, Ariga P. A systematic review on cancer therapy in ayurveda. J Pharm Sci Res 2018;10:211-3.
22. Adwankar MK, Chitnis MP. *In vivo* anti-cancer activity of RC-18: A plant isolate from *Rubia cordifolia*, Linn. Against a spectrum of experimental tumour models. Chemotherapy 1982;28:291-3.
23. Kintzios SE. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Crit Rev Plant Sci 2006;25:79-113.
24. Morita H, Yamamiya T, Takeya K, Itokawa H, Sakuma C, Yamada J, *et al.* Conformational recognition of RA-XII by 80S ribosomes: A differential line broadening study in 1H NMR spectroscopy. Chem Pharm Bull (Tokyo) 1993;41:781-3.
25. Itokawa H, Takeya K, Mori N, Hamanaka T, Sonobe T, Mihara K, *et al.* Isolation and antitumor activity of cyclic hexapeptides isolated from rubiae radix. Chem Pharm Bull (Tokyo) 1984;32:284-90.
26. Itokawa H, Takeya K, Mori N, Takanashi M, Yamamoto H, Sonobe T, *et al.* Cell growth-inhibitory effects of derivatives of antitumor cyclic hexapeptide RA-V obtained from *Rubiae* radix (V). Gan 1984;75:929-36.
27. Marczylo T, Arimoto-Kobayashi S, Hayatsu H. Protection against Trp-P-2 mutagenicity by purpurin: Mechanism of *in vitro* antimutagenesis. Mutagenesis 2000;15:223-8.
28. Lee YM, Auh QS, Lee DW, Kim JY, Jung HJ, Lee SH, *et al.* Involvement of nrf2-mediated upregulation of heme oxygenase-1 in mollugin-induced growth inhibition and apoptosis in human oral cancer cells. Biomed Res Int 2013;2013:210604.
29. Chang LC, Chávez D, Gills JJ, Fong HH, Pezzuto JM, Kinghorn AD. Rubiasins A-C, new anthracene derivatives from the roots and stems of *Rubia cordifolia*. Tetrahedron Lett 2000;41:7157-62.
30. Patel PR, Patel NN, Suthar MP, Rajesh KS, Patel LD. *In vitro* anticancer activity of *Rubia cordifolia* against HepG 32 cell line. Pharmagene 2013;1:1-3.
31. Patel PR, Raval BP, Karanth HA, Patel VR. Potent antitumor activity of *Rubia cordifolia*. Int J Phytomed 2010;2:44-6.
32. Patel PR, Nagar AA, Patel RC, Rathod DK, Patel VR. *In vitro* anticancer activity of *Rubia cordifolia* against HELA and HEP-2 cell lines. Int J Pharm Pharm Sci 2011;3:70-1.
33. Aditya JV, Kumar LN, Mokkapati A. *In vitro* anticancer activities of few plant extracts against MCF-7 and HT-29 cell lines. Int J Pharm Sci 2013;3:185-8.
34. Patil RA, Jagdale SC, Kasture SB. Antihyperglycemic, antistress and nootropic activity of roots of *Rubia cordifolia* Linn. Indian J Exp Biol 2006;44:987-92.
35. Baskar R, Bhakshu LM, Bharathi GV, Reddy SS, Karuna R, Reddy GK, *et al*. Antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats. Pharm Biol 2006;44:478-9.
36. Viswanathaswamy AH, Koti BC, Singh AK, Thippeswamy AH. Antihyperglycemic and antihyperlipidemic effect of *Rubia cordifolia* leaf extract on Alloxan-induced Diabetes. RJPS 2011;1:49-52.
37. Rani S, Mandave P, Khadke S, Jagtap S, Patil S, Kuvalekar A. Antiglycation, antioxidant and antidiabetic activity of traditional medicinal plant: *Rubia cordifolia* Linn. For management of hyperglycemia. Int J Plant Anim Environ Sci 2013;3:42-9.
38. Kasture VS, Deshmukh VK, Chopde CT. Anticonvulsant and behavioral actions of triterpene isolated from *Rubia cordifolia* Linn. Indian J Exp Biol 2000;38:675-80.
39. Antarkar SS, Chinwalla T, Bhatt N. Anti-inflammatory activity of *Rubia cordifolia* Linn. In rats. Indian J Pharmacol 1983;15:185-8.
40. Xu K, Wang P, Yuan B, Cheng Y, Li Q, Lei H. Structural and bioactive studies of terpenes and cyclopeptides from the Genus Rubia. Chem Cent J 2013;7:81.
41. Basu S, Hazra B. Evaluation of nitric oxide scavenging activity, *in vitro* and *ex vivo*, of selected medicinal plants traditionally used in inflammatory diseases. Phytother Res 2006;20:896-900.
42. Biswas TK, Mukherjee B. Plant medicines of Indian origin for wound healing activity: A review. Int J Low Extrem Wounds 2003;2:25-39.
43. Karodi R, Jadhav M, Rub R, Bafna A. Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. Int J Appl Res Natl Prod 2009;2:12-8.
44. Naidu KC, Lalam R, Bobbarala V. Antimicrobial agents from *Rubia cordifolia* and *Glycyrrhiza glabra* against phytopathogens of *Gossypium*. Int J Pharm Technol Res 2009;1:1512-8.
45. Singh R, Jain A, Panwar S, Gupta D, Khare SK. Antimicrobial activity of some naturaldyes. Dyes Pigm 2005;66:99-102.
46. Sawhney R, Berry V, Kumar A. Inhibitory activity of *Rubia cordifolia* plant extract against ESBL producing urinary *E. coli* isolates. J Pharm Res 2012;5:1328-30.
47. Basu S, Ghosh A, Hazra B. Evaluation of the antibacterial activity of ventilago madraspatana gaertn. *Rubia cordifolia* Linn. And *Lantana camara* Linn.: Isolation of emodin and physcion as active antibacterial agents. Phytother Res 2005;19:888-94.
48. Sharma BC. *In vitro* antibacterial activity of certain folk medicinal plants from Darjeeling Himalayas used to treat microbial infections. J Pharamcogn Phytochem 2013;2:1-5.
49. Xu K, Wang P, Yuan B, Cheng Y, Li Q, Lei H .Structural and bioactive studies of terpenes and cyclopeptides from the Genus *Rubia*. Chem Cent J. 2013; 7: 81.
50. Lodia S, Kansala L. Antioxidant activity of *Rubia cordifolia* against lead toxicity. Int J Pharm Sci Res 2012;3:2224-32.
51. Cai Y, Sun M, Xing J, Corke H. Antioxidant phenolic constituents in roots of rheum officinale and *Rubia cordifolia*:

Structure-radical scavenging activity relationships. J Agric Food Chem 2004;52:7884-90.
52. Tripathi YB, Pandey S, Shukla SD. Anti-platelet activating factor property of *Rubia cordifolia* Linn. Indian J Exp Biol 1993;31:533-5.
53. Tripathi YB, Shukla SD. *Rubia cordifolia* extract inhibits cell proliferation in A-431 cells. Phytother Res1998;12:454-6.
54. Tse WP, Che CT, Liu K, Lin ZX. Evaluation of the anti-proliferative properties of selected psoriasis-treating Chinese medicines on cultured haCaT cells. J Ethnopharmacol 2006;108:133-41.
55. Kalra P, Datusalia AK, Sharma S. Antiulcer potential of *Rubia cordifolia* Linn. In experimental animals. Int J Green Pharm 2011;5:149-54.
56. Gupta M, Shaw BP, Mukerjee A. Studies on antipyretic-analgesic and ulcerogenic activity of polyherbal preparation in rats and mice. Int J Pharmacol 2008;4:88-94.
57. Ho LK, Don MJ, Chen HC, Yeh SF, Chen JM. Inhibition of hepatitis B surface antigen secretion on human hepatoma cells. Components from *Rubia cordifolia*. J Nat Prod 1996;59:330-3.
58. Prajapati SN, Parmar KA. Anti-viral and *in vitro* free radical scavenging activity of leaves of *Rubia cordifolia*. Int J Phytomed 2011;3:98-107.
59. Pawar AT, Divakar K, Chandrasekar SB, Goli D. Diuretic activity of root extract of *Rubia cordifolia* Linn. Pharmacologyonline 2009;1:597-603.
60. Pawar AT, Anap RM, Ghodasara JV, Kuchekar BS. Protective effect of hydroalcoholic root extract of *Rubia cordifolia* in indomethacin-induced enterocolitis in rats. Indian J Pharm Sci 2011;73:250-3.
61. Tripathi YB, Sharma M, Manickam M. Rubiadin, a new antioxidant from *Rubia cordifolia*. Indian J Biochem Biophys 1997;34:302-6.
62. Deoda RS, Kumar D, Kadam PV, Yadav KN, Bhujbal SS, Patil MJ. Pharmacognostic and Biological studies of the roots of *Rubia cordifolia* Linn. (*Rubiaceae*). Int J Drug Dev Res 2011;3:148-58.
63. Deoda RS, Kumar D, Bhujbal SS. Gastroprotective effect of rubia cordifolia linn. On aspirin plus pylorus-ligated ulcer. Evid Based Complement Alternat Med 2011;2011:541624.
64. Babita MH, Chhaya G, Goldee P. Hepatoprotective activity of *Rubia cordifolia*. Pharmacologyonline 2007;3:73-9.
65. Gilani AH, Janbaz KH. Effect of *Rubia cordifolia* extract on acetaminophen and CCl4 induced hepatotoxicity. Phytother Res 1995;9:372-5.
66. Kannan M, Singh R, Narayanan M. Phytochemistry and immunopharmacological investigation of *Rubia cordifolia* Linn. (*Rubiaceae*). Pharmacologyonline 2009;3:653-62.
67. Rawal A, Muddeshwar M, Biswas S. Effect of *Rubia cordifolia*, *Fagonia cretica* Linn, and *Tinospora cordifolia* on free radical generation and lipid peroxidation during oxygen-glucose deprivation in rat hippocampal slices. Biochem Biophys Res Commun 2004;324:588-96.
68. Rawal AK, Muddeshwar MG, Biswas SK. *Rubia cordifolia*, *Fagonia cretica* Linn and *Tinospora cordifolia* exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation. BMC Complement Altern Med 2004;4:11.
69. Tripathi YB, Singh AV. Role of *Rubia cordifolia* Linn. In radiation protection. Indian J Exp Biol 2007;45:620-5.
70. Shah V, Kumar S, Maurya DK, Nair CK. Protection of plasmid pBR322 DNA and membranes of rat liver microsome and mitochondria by *Rubia cordifolia* extract. Indian J Radiat Res 2004;1:46.
71. Inoue K, Yoshida M, Takahashi M, Fujimoto H, Ohnishi K, Nakashima K, *et al.* Possible contribution of rubiadin, a metabolite of madder color, to renal carcinogenesis in rats. Food Chem Toxicol 2009;47:752-9.
72. Inoue K, Yoshida M, Takahashi M, Fujimoto H, Shibutani M, Hirose M, *et al.* Carcinogenic potential of alizarin and rubiadin, components of madder color, in a rat medium-term multi-organ bioassay. Cancer Sci 2009;100:2261-7.
73. Li CC, Li JS, Huang GX, Yan LJ, Wang QQ. Study on potential toxicity of madder pigment. Guangdong Agric Sci 2013;40:35-9.

Source of support: Nil; Conflict of interest: None Declared