

Pharmacognostic and Biological Studies of the Roots of *Rubia cordifolia* Linn. (Rubiaceae)

RAMESH S. DEODA*¹, DINESH KUMAR², PRASAD V.KADAM¹, KAVITA N YADAV¹,
SANTOSH S. BHUJBAL³, MANOHAR J.PATIL¹

1. Marathwada Mitra mandal's college of Pharmacy, Thergaon, Kalewadi, 2.Department of Pharmaceutical sciences, University of Kashmir, Srinagar 3.Padam Shree Dr. D. Y. Patil Institute of Pharmaceutical sciences and research, Pimpri, Pune,

Abstract

Rubia cordifolia (Rubiaceae) is also known as, Manjishtha, Indian madder known to contain substantial amounts of anthraquinones, especially in the roots which is responsible for anti-tumor, anti-inflammatory, urinary disorders, antistress antimicrobial, hepatoprotective, radio protective, and anticancer, antimicrobial, antifungal, hypotensive, analgesic, antimalarial, antioxidant, antileukemic and mutagenic functions, immunomodulatory, anti-inflammatory and antioxidant activity. The plant contains substantial amounts of anthraquinones, triterpenoids especially in the roots, which is responsible for most of its pharmacological activity. In present study the chloroform fraction from methanolic extract of roots of *R. cordifolia* were evaluated for its biological effect and compared with the parent extract and remaining fraction, where chloroform fraction showed potent protective action for stress induced complications in mice.

Key words:

Rubia cordifolia, stress induced ulcer, triterpenoids;

How to Cite this Paper:

Ramesh S. Deoda, Dinesh Kumar, Prasad V.Kadam, Kavita N. Yadav, Santosh S. Bhujbal, Manohar J. Patil. "Pharmacognostic And Biological Studies of The Roots of *Rubia Cordifolia* Linn. (Rubiaceae)" Int. J. Drug Dev. & Res., July-Sep 2011, 3(3): 148-158

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Article History:-----

Date of Submission: 24-04-2010

Date of Acceptance: 15-07-2011

Conflict of Interest: NIL

Source of Support: NONE

*Corresponding author, Mailing address:

Mr. Ramesh S. Deoda
M. Pharm (Pharmacognosy)
Marathwada Mitra mandal's college of Pharmacy,
Thergaon, Kalewadi, Pune
Email: ramesh.deoda@gmail.com
Mobile: 09923899299

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). 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. 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[1]. *Rubia cordifolia* is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine[2].

MATERIALS

Plant material and Extraction:

Roots of plant *Rubia cordifolia* were collected from Bhimashankar Hills (Ghat regions of the sahyandri hills), Taluka Khed, District Pune, Maharashtra and authenticated by Dr. (Mr.) Rajesh Dabur, research officer, from Regional Research Institute (AY) Kothrud, Pune, As Specimen Voucher no.- 64. It was shade dried, powdered and extracted with methanol. The Extract was vacuum dried and successively fractionate with chloroform using continuous soxhlet apparatus. Parent extract along with chloroform fraction and its residual fraction was used for the further experimental models [3].

Chemicals:

Alcian Blue, Absolute alcohol, Carboxy Methyl Cellulose Sodium Salt Disodium hydrogen phosphate, 5,5 – Dithio Bis(2- nitro Benzoic acid), Tris buffer, EDTA, Magnesium Chloride hexahydrate crystals, Phenolphthalein (Research Lab Fine Chem. Industries, Mumbai), Bovine serum albumin (Himedia pvt.Ltd,Mumbai) Folin- Ciocalteu's Phenol reagent (Loba chemicals, Mumbai), Solution of Hydrogen Peroxide (Oswal Pharmaceuticals, Pune), Potassium dihydrogen orthophosphate (Pure chem. laboratories,Pune), Thiobarbituric acid(Spectrochem Pvt. Ltd,Bombay) Tyrosine (Central drug house P Ltd, Bombay) Ranitidine (GlaxoSmithKline Pharmaceuticals ltd., Mumbai).

Instruments:

Cooling Centrifuge, Homogeniser, Incubator (Remi), P^H meter (Toshniwal inst.Mfg. Pvt.Ltd. Ajmer), Rotatory vacuum evaporator (JSGW), UV visible Spectrophotometer (Schimadzu), Verniar callipers (Mitutoyo Corp.), Weighing balance (Schimadzu), Weighing balance, Mice (Docbel Braun), Digital Microscope B-1 Advance series (Motic).

HPTLC Analysis:

The sample was spotted in a form of a band by means of Hamilton microsyringe on precoated silica gel F 254 (sigma) plates with the help of Linomat IV spotter (CAMAG) in Camag HPTLC(Switzerland) instrument and

photodocumented by Digi store-2 Documentation System with win CATS & Video Scan Software.

Animals

Mice of either sex weighing between 25-50 gm were used for the study. Animals were maintained under controlled conditions of temperature $26 \pm 2^{\circ}\text{C}$, relative humidity 44-56%, and photo-schedule (12 h light and 12 h dark). Animals were provided with standard pellet diet (Amrut feeds, Mumbai, India) and water *ad libitum*. Institutional Animal Ethics Committee approved the experimental protocol (198/99/CPCSEA). The pharmacological work was carried out as per norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute Toxicity Study: (OECD Guidelines-423, 2004)

Nine mice in a group of three were fasted overnight and maintained with water *ad libitum*. All three animal of either group received single dose of methanolic extract, its chloroform fraction and residual fraction of *Rubia cordifolia* (2,000 mg/kg, p.o.) respectively. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then monitored for any mortality for the following 14 days.

Experimental model: Swimming stress induced ulcer

Methanolic extract of *Rubia cordifolia*, suspended in 1% Sodium carboxy methyl cellulose (NaCMC) in distilled water in doses of 100, 200 and 400 mg/ kg and its chloroform fraction and residual fraction in doses of 50,100 and 200 mg/kg and Ranitidine, the reference drug, in the dose of 10 mg/kg were administered orally twice daily at 10:00 and 16:00 h respectively for ulcer protective studies. Control group of animals received suspension of 1% CMC in distilled water for the same administration period. The 24-h fasted mice were treated with test drug, Ranitidine or vehicle administered orally. Thirty

minutes later, they were placed inside a vertical cylinder filled with water up to a height of 10 cm. The temperature of the water was maintained at 20–25 °C. The mice were removed from the cylinders after 3 h and sacrificed. Their stomachs were removed and analyzed as described above for ulcers, gastric secretion studies and in vivo antioxidant studies respectively[4,5]. It was further evaluated for mucin content and in vivo antioxidant activity[6].

Results:

Pharmacognostical Study

The genus contains about 60 species of perennial scrambling or climbing herbs and sub-shrubs native to the Old World, Africa, temperate Asia and America. The best known species are Common Madder (*Rubia tinctorum*), Wild Madder (*Rubia peregrina*), and Indian Madder(*Rubiacordifoli*

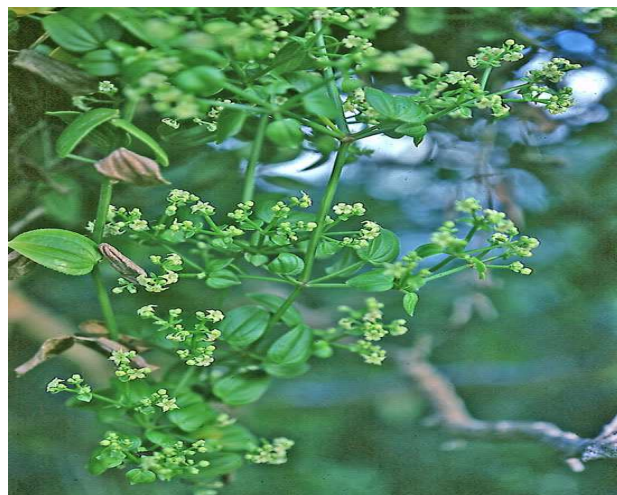


Fig.1: R.Cordifoliaplant



Fig.1a: R.Cordifolia roots

Rubia cordifolia an ayurvedic herb is a perennial, climber with very long, cylindric, flexuose roots with a thin red bark. It can grow to 1.5 m in height. 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 paniced 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. *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 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.

Microscopical study

The fine powder of the plant was warmed in water and was spread on a slide in a drop of glycerin and covered with a cover-glass. The slides were observed under the microscope with normal light (bright field) as well as under polarized light. Under polarized light calcium oxalate crystals, strach grains, lignified cells appear bright against dark background.

Observations:

Calcium Oxalate crystals: Calcium oxalate crystals are abundant with powder. The crystals are in the form of thin pointed needles, which are originally in the form of thick bundles called raphides. Due to breaking of the raphides the needles are scattered in the powder. The needles are either uniformly thin or spindle shaped. Broken needles are also seen in the powder. Due to birefringent property of the crystals, they appear bright white under black background. The individual needles are thin and pointed. They are 30µm long. Some of the needles are broken into fragments (**Figure 2 and 3**).

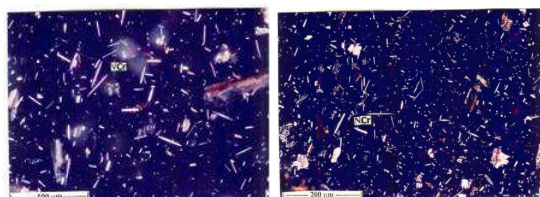
Strach grains: the strach grains are not abundant as the needles crystals, but are frequently seen in the powder. The strach grains are circular to ovoid (**Figure 4**). They exhibit T shaped dark bands which are due to birefringent property of the strach grains and are 25µm in diameter. When stained with IKI, they appear dark purple in colour (**Figure 5**).

Xylem bundles: Another characteristic feature of the powder is xylem bundles which are seen in the powder as short, thick or thin bundles. These bundles consist of broken pieces of xylem elements, especially vessels. The xylem bundles are 250-450µm long and 150-250µm thick (**Figure 6**)

Powder also contains broken fragment vessel walls which possess prominent dense, multiserial, alleviate circular boarder pits. The pits are 8µm in diameter. They seem to be

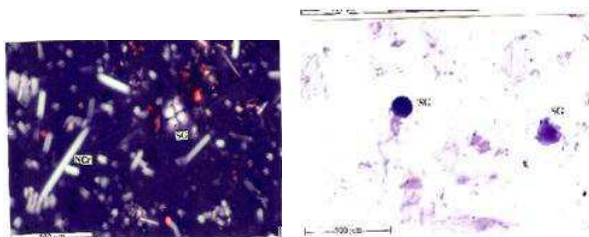
vestured pits with minute outgrowths with in the pit cavity (Figure 7).

Fig. 2 and 3 Calcium oxalate raphides needles as seen under the polarized light



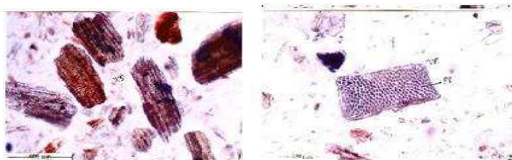
Where, Ncr-needle crystal

Fig. 4 and 5 Starch grains (stained with iodine) and needle crystals



Where, Ncr- needle crystals, SG-starch grains

Fig. 6 and 7 Thick cylindrical bundles of xylem elements and broken wall of a vessel element showing dense circular pits seen in the powder



Where, XB- Xylem bundles, Pi- pits, Ve- vessel element a broken fragment

Physicochemical Constants Ash Value

Table 1: Table showing Total ash, Acid Insoluble Ash and Water soluble Ash

S.no.	Parameter	Standard Reading % w/w (NMT)	Practical Yield % w/w (NMT)
1.	Total Ash	12	10.5*
2.	Acid Insoluble Ash	0.5	0.5*
3.	Water Soluble Ash	-	5.5*

*Average of three values

Extractive Values

Table 2: Extractive Values of *R. cordifolia* root Powder

S.no.	Parameter	Practical Yield % w/w (NMT)
1.	Methanol Soluble Extractive	10.5*
2.	Chloroform Soluble Extractive value of Methanol Extract	12.6 *

*Average of three values

Phytochemical Study: Nature of Extract

Table 3: Showing the colour and nature of the extract

S.no.	Name of the extract	Nature	Colour
1.	Methanol	Semi-solid	Reddish Brown
2.	Chloroform fraction	Sticky	Dark Reddish
3.	Residual Fraction	Non-sticky, hard	Reddish Brown

Preliminary Phytochemical screening of the extract

Table 4: Phytochemical Investigation of *R. cordifolia* root powder.

S. n o.	Test	Methanol Extract	Chloroform Fraction	Residual Fraction
1.	Alkaloids	+	++	+
2.	Carbohydrates	+	-	+
3.	Glycosides	+	+	+
4.	Flavanoids	-	-	-
5.	Saponins	+	+	-
6.	Proteins & amino acids	+	-	-
7.	Triterpenoid & steroids	+	+++	-
8.	Tannins	+	+	-
9.	Fixed oils and Fats	-	-	-
10.	Mucilage	-	-	-

”+”, “+++”, “+” = Present, “-” = absent

Thin layer chromatography profile:

Table5: Table showing TLC of successive extract of *R. cordifolia* Linn.

Compound	R _f value	Result		
		Methano	Chloroform Fraction	Residual Fraction
Terpenoids	0.78	+	+	-
Alkaloids	0.42	+	+	+
Steroids	0.69	+	+	-

HPTLC Analysis:

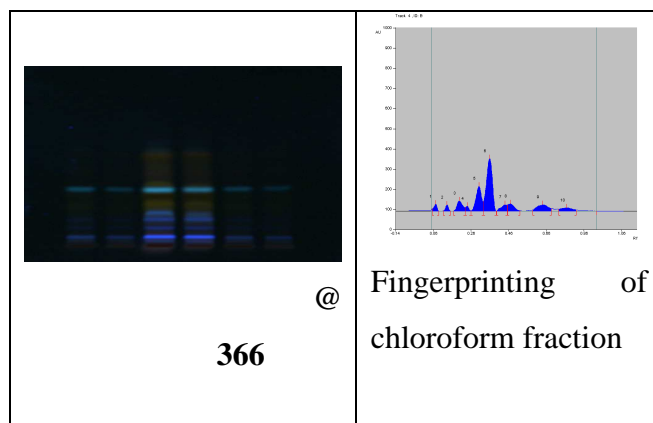
Methanolic extract, its chloroform and residual fractions were subjected for HPTLC studies using solvents of varying polarity. Solvent system Toluene : Ethyl acetate (85:15 V/V) was selected for better separation of the components for the HPTLC fingerprinting. The R_f values of the separated components and their amount as evident by percentage peak area. The derivatization of HPTLC plate using anisaldehyde sulphuric acid reagent showed colour changes from pink to menginea of component spot on HPTLC plate when heated at 110°C. The chloroform fraction of total methanolic extract showed the maximum concentration of triterpenoides and their glycosides as compared to the residual fraction. The number of components separated from the each extracts and their R_f values and their percentage area are represented in

Tables--.

The HPTLC spectrums of the each extracts are shown in Figures:

PLATE:

- Track 1:** RCM-Methanolic Extract (10ul)
- Track 2:** RCM-Methanolic Extract (5ul)
- Track 3:** RCC-Choloroform Fraction (10ul)
- Track 4:** RCC-Choloroform Fraction (5ul)
- Track 5:** RCR-Residual Fraction (10ul)
- Track 6:** RCR-Residual Fraction (5ul)



Fingerprinting of chloroform fraction

Photodocumentation:

Pharmacological Study:

Swimm stress induced ulcer

It was observed that in the swimming-stress induced control group the ulcer index was 30.8±0.66 and the maximum numbers of ulcers were of the ulcer score 2,3 and 4. Methanolic extract of *R. Cordifolia* was found to produce a decrease in ulcer index in the of 400 mg/kg dose; the percentage reduction was 36.34% .Whereas chloroform fraction (RCC) found to produce significant (p<0.001) decrease in the ulcer index in all three doses; the percentage reduction being 37.6%, 43.25% and 59.66%, in the dose of 50 mg/kg, 100 mg/kg and 200 mg/kg, respectively. In case of residual fraction (RCR), there are non-significant changes in the ulcer index. Control group showed significant (p<0.001) decrease in mucin content compared to normal group. Swim-stress induced control was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione, thus leading to oxidative stress. RCM and RCC showed significant (p<0.001) reduction in lipid peroxidation and increase in the catalase and reduced glutathione, whereas increased in SOD had seen only RCC group at 200mg/kg. Ranitidine (10 mg/kg) was found to produce significant (p<0.001) reduction in ulcer index, the percentage reduction being 68.68%. No significant increase in mucin content was observed compared to the control group. It

also showed significant (p<0.001) increase in the SOD, catalase and reduced glutathione whereas

less significant (p<0.01) on lipid peroxidation. (Table: 6)

Table 6: Effect of *R. cordifolia* on Ulcer Index and antioxidant parameters in stomach of Swim-stress treated mice.

Sr. No.	1	2	3	4	5	6	7	8	9	10	11	12
Group	Normal	Control	Standard	RCM 100	RCM 200	RCM 400	RCC 50	RCC 100	RCC 200	RCC 50	RCC 100	RCC 200
Ulcer Index (%protection)	-	30.82 ±1.48	9.65±2.07* ** (68.68)	26.59±2.55 ^{NS} (13.72)	23.63±1.41** (23.32)	19.62±1.51*** (36.34)	19.23 ±3.03 *** (37.6)	17.49±1.51*** (43.25)	12.43 ±1.81* ** (59.66)	28.69 ±3.64 NS (6.91)	26.25±2.19 ^{NS} (14.82)	25.23 ±4.55 NS (18.13)
Mucin Content (µg of alcian blue/g wet tissue)	101.2±2.7	60.63 ±1.81* **	68.23 ±1.93 NS	68.83 ±1.68 NS	71.8±1.46**	80.65 ±1.36* **	71.43±2.83**	78.84±1.74***	93.85 ±1.24* **	64.8±1.85 ^{NS}	68.85 ±1.85 NS	72.4±1.2**
SOD (Units/gm of wet tissue)	215.6±4.81	99.92±2.84** *	134.7 ±6±2.93***	11.08±1.74 ^{NS}	113.3±2.33 ^{NS}	116.48 ±2.46* *	115.7±2.64* *	118.48 ±1.81* *	144.0 ±4±1.68***	103.08 ±1.95 NS	110.66 ±3.99 NS	113.7±2.99* *
AT (Units/gm of wet tissue)	50.2 ±4±1.78	15.98±0.73** *	43.64 ±1.36***	23.53±1.52*	24.67±1.28**	30.23±1.54** *	25.05 ±1.49* *	30.73±1.68** *	36.14 ±1.22* **	19.02±0.77 ^{NS}	21.29±0.69 ^{NS}	22.65 ±1.5* *
GSH (nmols/gm of wet tissue)	239.98±5.65	99.78±2.11***	143.2 ±4±3.3***	117.88 ±2.48*	122.4±2.85**	141.22 ±4.39* **	121.46 ±3.69 **	144.78 ±2.83* **	168.3 ±6±7.72***	102.6±2.08 ^{NS}	104.12 ±2.64 NS	112.82 ±3.12 NS
LPO (nmols of MDA/gm of wet tissue)	132.74±3.59	240.36 ±3.21* **	217.6 ±8±4.48**	217.88 ±2.89* *	202±2.51***	177.66 ±4.67* **	189.4 ±2±3.15***	176.58 ±3.25* **	159.72 ±2.01 ***	228.26 ±2.77 NS	223.66 ±4.2 ^{NS}	217.44 ±4.78 **

N=6 normal group= vehicle Na CMC 1% (10 ml/ kg, p.o.)

Values are expressed as mean \pm SEM. Control group was compared with normal group. Test and standard groups were compared with control group. Statistical comparison was performed using analysis of variance (ANOVA) followed by Turkey's test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS: non significant.

RCM100, 200, 400 - Methanolic Extract of *Rubia cordifolia* 100 , 200 , 400 mg/kg , p.o. respectively, RCC50,100, 200 – Chloroform fraction of *Rubia cordifolia* 50, 100 , 200 mg/kg , p.o. respectively, RCR50,100, 200 – Residual fraction of *Rubia cordifolia* 50, 100 , 200 mg/kg , p.o. respectively.

Discussion:

The important histological findings in case of root powder of *R.cordifolia* suggest that there is presence of calcium oxalate crystals in the form of thin pointed needles, which are originally in the form of thick bundles called raphides. Due to breaking of the raphides the needles are scattered in the powder. The needles are either uniformly thin or spindle shaped. Broken needles are also seen in the powder. Due to birefringent property of the crystals, they appear bright white under black background. It also shows frequent starch grains which are circular to ovoid. Another characteristic feature of the powder is xylem bundles which are seen in the powder as short, thick or thin bundles. These bundles consist of broken pieces of xylem elements, especially vessels. Powder also contains broken fragment vessel walls which possess prominent dense, multiseriate, alleviate circular boarder pits. These can be considered as a distinguishing characteristic for powder microscopy of the plant.

The quality control parameters for the raw materials were established with the help of several official determinations based on physico-chemical parameters. Controlled incineration of crude drugs results in an ash residue consisting of inorganic materials (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. More direct contamination, such as by sand or earth, is immediately detected by the ash value. The total ash, acid-insoluble ash, and water soluble ash were found to be within limit as specified in official books and was observed slightly higher. Likely reason for this may be due to contamination or sometimes due to unwanted parts of the drug. Different extractive values determine the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. It is used as a means of evaluating crude drugs which are not readily estimated by other means [7]. The results obtained for alcohol and water soluble extractive determinations gave an idea about the nature of compounds present. The methanol soluble extractive value of *R.cordifolia* was found to be (10.5%) and chloroform soluble extractive (12.6%) value[8]. Systematic and complete studies of crude drug include a thorough investigation of both primary and secondary metabolites derived as a result of plant metabolism. Preliminary phytochemical screening was performed for establishing the profile of extract for its nature of chemical composition. The qualitative chemical tests carried out for the identification of the nature of phyto-constituents present. Both the extracts showed the presence of Triterpenoids, glycosides and saponins. Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different classes

of natural products. Methanol extract of root (RCM), chloroform fraction (RCC) and Residual fraction (RCC) were subjected for TLC studies using solvents of varying polarity and the suitable solvent system for better separation of the components. The RF values of the separated components were recorded. RF value helps in ascertaining the number of similar type of compounds present in the extracts. The extract showed presence of anthraquinone glycosides, Terpenoids, steroids. HPTLC fingerprinting analysis was also carried out for methanolic extract and its chloroform fraction. Chloroform fraction was subjected for their presence of Triterpenoids.

Acute toxicity studies aims at establishing the therapeutic index i.e. the ratio between the pharmacologically effective dose and the lethal dose, and also to perform the primary screening. Methanol extract, its chloroform fraction and residual fraction of the plant were found to be safe up to 2000 mg/kg.

Stress-induced ulcer better resembles clinical ulcers in chronicity severity and practicality of experiencing stress due to varietal patterns of lifestyle in day to day life and serves the most reliable model to study ulcer healing process (Jones, 2006). The incidence of swim stress-induced ulcer is predominant in the glandular part of the stomach. Stress plays an important role in aetiopathology of gastroduodenal ulceration. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucous production. Increase in gastric motility, vagal over activity, mast cell degranulation, decreased gastric mucosal blood flow and decreased prostaglandin synthesis are involved in genesis of stress-induced ulcers [9]. RCC at all doses and RCM 400mg/kg significantly ($p < 0.001$) reduces ulcer index compared to control group and also

there is significantly increase in the mucin content at similar doses.

Stress induced ulcers are due to increase in free radical generation apart from acid pepsin factors. Stress significantly induced lipid peroxidation as seen from increase in LPO levels in control group compared to normal. And there is significant decrease in LPO levels in RCC and RCM groups which suggest its protective effect. This is due to increase in the generation of reactive oxygen species (ROS) during stress leading to oxidative damage. Normally the increase in damage due to O_2^- is contained by dismutation with SOD. SOD converts the reactive O_2 to H_2O_2 , which if not scavenged by the CAT can by itself cause lipid peroxidation by increase in the generation of hydroxyl radicals. Hence decrease in CAT levels has led to increase in accumulation of these reactive products and thus, has caused increased lipid peroxidation and tissue damage. The effect is further aggravated by decreased activity of gastric peroxidases during stress [2]. Decrease level of endogenous GSH are the characteristic features of $\bullet OH$ -mediated oxidative damage of the gastric mucosa during ulceration[10]. The experimental data shows that administration of RCC at all doses exhibit significant increase in SOD, CAT and GSH levels as compared to the control animals, which suggest its efficacy in preventing free radical induced damage and gastroprotective effect.

Phytochemical findings of the methanolic extract showed presence of alkaloids, phytosterols, saponins, tannins, carbohydrates, phenolic compounds, anthraquinone glycosides, triterpenoids. The chloroform fraction shows the presence of Triterpenoids in major concentration along with that alkaloids and saponins and the residual fraction consist of alkaloids and glycoside.

Pentacyclic Triterpenoids, in addition to their anti-inflammatory properties, are also known to promote mucus secretion. Thus mucus secreting potential and subsequent wound healing effect of the extract of *Rubia cordifolia* may be linked to the presence of triterpenes. In addition, Triterpenoids are also reported to be good antioxidant, such as: α and β -amirins, oleanolic acid, ursolic acid, lupeol and glycirretinic acid.

Conclusion: Roots of *Rubia cordifolia* Linn. were used for studying Pharmacognostic, Phytochemical and Pharmacological evaluation. Microscopical study showed there was presence of calcium oxalate crystals in the form of thin pointed needles, which were originally in the form of thick bundles called raphides. It also showed frequent starch grains which were circular to ovoid. Another characteristic feature of the powder was xylem bundles which were seen in the powder as short, thick or thin bundles. These bundles consist of broken pieces of xylem elements, especially vessels. Powder also contains broken fragment vessel walls which possess prominent dense, multiseriate, alleviate circular boarder pits.

The results for total ash, acid insoluble ash and water soluble ash were found to be within range with that of standard values given in Ayurvedic pharmacopoeia.

The preliminary phytochemical tests of methanolic extract showed presence of alkaloids, phytosterols, saponins, tannins, carbohydrates, phenolic compounds, anthraquinone glycosides, triterpenoids. The chloroform fraction showed the presence of Triterpenoids in major concentration along with that alkaloids and saponins. TLC/HPTLC studies showed effective separation and presence of steroidal nucleus, alkaloids, Triterpenoids, saponins and anthraquinones.

The methanolic extract of *Rubia cordifolia* Linn. at the dose of 400 mg/kg showed reduction in ulcer index, lipid peroxidation and increase in the mucin content, CAT and reduced glutathione in stomach tissue of the swimming stress induced ulcer method, whereas SOD activity was shown in higher dose of chloroform fraction. It was proving its protective effect against swimming stress induced gastric injury. Chloroform fraction showed same result in the dose of 50 mg/kg and dose dependant increase in the activity at higher doses. Residual fraction did not show any reduction in the ulcer index and increase in mucin content also it was insignificant in antioxidant parameters. *Rubia cordifolia* found to be less potent than ranitidine (10mg/kg) but the antioxidant activity found to be more potent than ranitidine.

The above results demonstrates that chloroform fraction was more potent than parent methanol extract at lower dose. As per the phytochemical evidences the chloroform fraction mainly contains Triterpenoids, may be this activity was because of Triterpenoids. As Triterpenoids are reported as a good antiulcer and antioxidant compound. It could be concluded that *Rubia cordifolia* has both gastroprotective and ulcer healing properties.

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