

A Comprehensive Review On *Rubia Cardifolia*

(Manjistha)

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ABSTRACT

Objective The objective of present revision was to scrutinize, present up to date information of *Rubia cardifolia* and its applications in medicine.

Material and Methods various search engines viz., DOAJ, Scopus, Science Direct, Google Scholar, PubMed, CAB international, SciFinder, and Web of Science were used to study the scientific literature on ethnomedicinal studies conducted on *Rubia cardifolia* in various parts of the world including India. The investigations and surveys on the *Rubia cardifolia* up to January 2018 were included in this study.

Results *Rubia Cardifolia* is a perennial herbaceous climber, belongs to family Rubiaceae commonly known as Indian Maddar, spread in hilly areas of India up to 3750m, available in the market under the trade name Manjistha. It is considered as one of the valuable herbs in Ayurveda. It possesses the phytoconstituents such as anthraquinones, glycosides, saponins, steroids, phenols, and flavonoids. It attributes various pharmacological activities as anticarcinogenic, antidiabetic, antimicrobial, anti-inflammatory, antioxidant, analgesic, neuroprotective, antimutagenic, antiglycation, gastroprotective, antiviral, hepatoprotective and many more.

Conclusion The present review is an effort to provide the detailed assessment of *Rubia Cardifolia*, its phytochemistry, and pharmacognostical properties.

Keywords phytoconstituents, pharmacology, *Rubia Cardifolia*.

1. INTRODUCTION

The family Rubiaceae comprises of about 450 genera and 6500 species and includes trees, shrubs and herbs [1]. *Rubia cardifolia* is a perennial, prickly climber with a stem, growing up to 12 m long. Leaves are ovate, lanceolate, 5-7 nerved, 2-10 cm long and 2-5 cm broad, occur in whorls of 4-6. Roots are perennial, long, cylindrical, and rusty brown in colour. Flowers are minute, perfumed, whitish or greenish yellow. Fruit is minute, glabrous, 1-2 seeded, dark purplish or blackish at maturity. The plant carries flowers and fruit during August-October [2]. It is distributed throughout the lower hills of Indian Himalayas in the North and Western Ghats in the South, and Japan, Indonesia, Ceylon, Malaysia, Peninsula, Java and Tropical Africa in moist temperature and tropical forest, up to an altitude of 3500 [3-4]. The roots of *Rubia cordifolia* is the source of a

medicine used in Ayurveda. It is an important herbal drug used in Indian system of medicine. The roots of the plant are commonly known as Manjistha and sold under the commercial name Manjith.

1.1, Vernacular names

Plant drug has number of vernacular names like Aruna, Bhandi, Bhandiralatik in Sanskrit, Mandar, Majathi in Assamme, Manjith, Manjistha in Bengali, Indian Madder in English, Manjithi in Malayalum, Manjestha in Marathi and Majit, Manjit in Hindi [5].

1.2, Taxonomic Classification of Rubia

Kingdom : Plantae

Class : Dicotyledoneae

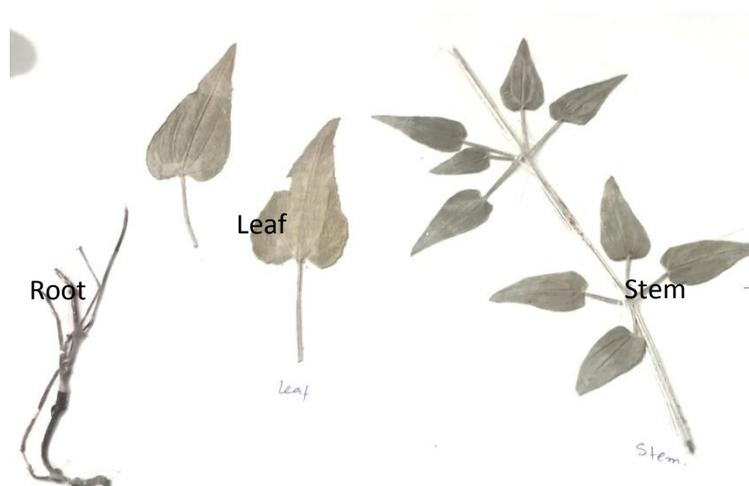
Subclass : Sympetalae

Order : Rubiales

Family : Rubiaceae

Genus : *Rubia*

Species : *cordifolia* [6].



The root extract of *Rubia Cardifolia* has enormous pharmacological properties against various ailments such as cephalalgia, cough, diabetes, arthralgia, arthritis, discolouration of the skin, dysmenorrhoea, emmenagogue, leucorrhoea, neuralgia, pectoral diseases, pharyngitis, general debility, haemorrhoids, hepatopathy, intermittent fevers, jaundice, , ophthalmopathy, otopathy, splenopathy, strangury, slow healing of broken bones, tubercular conditions of the skin and mucous tissue, tuberculosis and urethrorrhea [7]. The roots are used for laxative, analgesic, rheumatism, dropsy, paralysis and intestinal ulcers and the dried stem is used in blood, skin and urinogenital disorders, dysentery, piles, ulcers, inflammations, erysipelas, skin diseases and rheumatism [8]. A decoction of leaves and stems is used as a vermifuge [1].

III. MACROSCOPIC AND MICROSCOPIC EXAMINATION

The stem is quadrangular, divaricately branched, prickly-hispid, Cross section of the stem showed rectangular outline with single layered epidermis covered with cuticle and pyramidal hairs. Hypodermis sclerenchymatous present at the corners of the stem. The cortex is photosynthetic chlorenchymatous, Phloem 4-6 layered, composed of sieve tubes and phloem parenchyma. Cambium ring is represented by two layers. Secondary xylem is made up of vessels, tracheids, fibers and xylem parenchyma. vessels are large and uniformly arranged. Medullary rays are uniseriate.

Leaves are glabrous and rough $3.8-9 \times 1.6-3.5$ cm long, arranged in whorls of four, ovate-lanceolate, 3-9 palmately veined. Lower leaves are larger than the upper. Leaf base is slightly cordate. The margins are with minute white prickles. Leaf section showed single layered epidermis, covered with cuticle and possesses pyramidal hairs. Palisade cells are single layered and compactly packed, spongy cells multilayered and loosely arranged. In the lower portion of the midrib 2-4 layers made of collenchymatous cells. Vascular bundles are collateral and closed, conjoint and definite in number.

Petiole 5-10 cm long with sharp, recurved prickles. Stipules absent or modified into leaves. The T.S. of the petiole showed single layered epidermis with pyramidal hairs. Below the epidermis, 3-4 layers of sclerenchymatous cells were present. The cortical cells were made of thin-walled chlorenchyma. The vascular bundle is 'C'- shaped.

Root is long, cylindrical, flexuose, smooth and reddish. The cross-section of roots showed an outer 5-7 layer of cork tissue, which occasionally contains tannin. Phellogen is not distinct, Secondary cortical cells thin-walled, red in color and polygonal in shape. Secondary xylem dish consists mainly of vessels and tracheids. Vessels are abundant and distributed uniformly. Secondary phloem forms a wide zone of reddish color consists of thin-walled, sieve elements and phloem parenchyma but lacks phloem fibers. Cambium is distinct and lacks medullary rays. The whole portion of the root is red coloured indicates the presence of anthraquinones [9].

III.PHYTOCHEMICAL STUDIES

Rubia cadifolia 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 [10]. *Rubia cardifolia* possesses different classes of bioactive compounds such as anthraquinones and their glycosides, naphthoquinones and glycosides, terpenes, bicyclic hexapeptides, iridoids [11]. *Rubia cordifolia* yielded anthraquinones namely, 1hydroxy-2 carboxy 3-methoxy anthraquinone, 1hydroxy-2 methyl 6 or 7-methoxy- anthraquinones. The other compounds oleanolic acid acetate, β -sitosterol, and scopoletin. Ten long chains saturated and unsaturated fatty acids were also known [12]. The roots of *Rubia cordifolia* produce two new anthraquinones, namely cordifoliol and cordifodiol. On the basis of spectral data analyses and chemical reactions their structures have been established as 1-hydroxy-3-ethyl-9, 10 anthraquinones (1) and 1,8-dihydroxy-11,20 (15pentyl-naphthoquinone) phenanthrene (2) [13]. The roots of *Rubia cardifolia* provide Several naphthoquinones and hydroxyanthraquinones and their glycosides [14]. Other quinones isolated were 4dihydroxy 2-methylantraquinone, 4naphthoquinone, lucid primeveroside, 1, 5-dihydroxy 2methyl anthraquinone, 3-prenyl methoxy 1, ruberythric acid anthraquinones, 2-methyl-1, 3, 6-trihydroxy-9,



10anthraquinone and 2-methyl-1, 3, 6-trihydroxy-9, 10anthraquinone 3-O α -rhamnosyl (1 \rightarrow 2)- β -glucoside. 6Methoxy geniposidic acid (iridoids) is also found along with man Justin, garancin, and alizarin [15]. The methanol extract of *Rubia cordifolia* roots contains 2 naphthoquinones [16]. The bicyclic hexapeptides RA-I and RA-II have been isolated from chloroform/methanol extract of roots of *Rubia Cardifolia* [17]. Recently two new bicyclic hexapeptides, allo RA-V and neo-RA-V, and one cyclic hexapeptide, O-Secora-V were isolated from *Rubia cardifolia* [18]. The plant also possesses dihydromollugin, Mollugin, rubilactone [19].

IV. TRADITIONAL USES

Traditionally *Rubia cordifolia* is used in the treatment of liver fluke, dysentery, maggots, wounds and intestinal worms in animals [20]. The powder of roots and fruits has been suggested by the Vedic physician, Charka for skin disorders and diseases of the spleen [8]. It is an excellent expectorant especially used in infants to relieve a cough, cold and respiratory problems [21]. In traditional Chinese system of medicine, the herb is used for abnormal uterine bleeding, internal and external hemorrhage, bronchitis, and rheumatism [22]. The stem is used in the treatment of blood disorders and spreading fever of kidneys and intestines in Tibetan system of medicine [23]. The roots are used to treat rheumatism, ulcers, inflammation, skin disease, leucoderma, dysentery, chronic fever and urinary problems in Amarkanatak region of Madya Pradesh [24]. The leaf extract is used to relieve uterine pain by tribal people of purandhar in Maharashtra [25]. Leaf extract is used as a blood purifier by Baiga tribals in Madhya Pradesh [26].

V. PHARMACOGNOSTICAL STUDIES

5.1, Hepatoprotective activity

The methanolic extract of *Rubia Cardifolia* could protect the liver against thioacetamide-induced hepatotoxicity. The activity was assessed through estimation of biochemical parameters viz. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT), the results were further supported by histopathological studies [27]. *Rubia Cardifolia* is effectual against acute and chronic hepatitis caused due to the hepatitis B virus by meddling the secretion of hepatitis B surface antigen in human hepatoma cells [28]. The aqueous methanol extract of *Rubia cordifolia* was investigated against acetaminophen and CCl₄ induced liver damage in rats. Acetaminophen produced 100% mortality at a dose of 1 g/kg in mice while pretreatment of animals with plant extract (500 mg/kg) reduced the death rate to 30%. Acetaminophen at a dose of 640 mg/kg caused liver damage analyzed by increased serum levels of SGOT and SGPT to 1447 \pm 182 and 899 \pm 201 IU/L (n =10) respectively, compared with respective control values of 97 \pm 10 and 36 \pm 11. Pretreatment with plant extract (500 mg/kg) lowered significantly (p<0.005) the serum SGOT and SGPT levels to 161 \pm 48 and 73 \pm 29.59 respectively [29]. The oral administration of rubiadin present in the extract can normalize CCl₄ induced liver damage in rats within 14 days and also prevented CCl₄-induced prolongation in pentobarbital sleeping time [30].

5.2, Antidiabetic activity

The alloxan-induced diabetic rat's administration of an alcoholic extract of roots of *Rubia cordifolia* showed significant hypoglycemic effect [31]. The antiglycation, antioxidant and antidiabetic activities of roots of *Rubia cordifolia* Linn. were investigated. Anti-AGE (Advance glycation end products) effect of *Rubia cordifolia* on glycation of guanosine with glucose and fructose was analyzed. The inhibitory effect of plant extract on glycation and fructation of guanosine in presence of reactive oxygen species generated by hydrogen peroxide, was also analyzed. In vitro antioxidant (DPPH, Superoxide anion scavenging activity, and Xanthine oxidase) and antidiabetic assays (Alpha-amylase and alpha-glucosidase) were performed to determine these activities. The UV absorbance of the glycation reactions was found to be maximum at 24 hrs. and with consecutive decreased at 48 and 72 hours. It is concluded from results that root extract of *Rubia cordifolia* has significant antiglycation, antioxidant and antidiabetic activities [32]. In streptozotocin-induced diabetic rats the aqueous root extracts of *Rubia cordifolia* was found to regulate hyperglycemia, hypertriglyceridemia, improved transaminases of liver and kidney, hypochromic microcytic anemia, and loss of body weight [33].

5.3, Anticarcinogenic activity

The root extract of *Rubia cordifolia* possesses anticancerous activity against MDA-MB-231 breast cancer cell lines using Sulforhodamine B assay [34]. The administration of Mollugin in Mice showed inhibition of passive cutaneous anaphylaxis and protection of mast cell degranulation and also showed inhibition against lymphoid leukemia (P338) [35]. The ethanolic extract of *Rubia cordifolia* exhibited strong inhibition of human cervical cancer cell line thus can be a source of powerful pharmacophore for treatment of disease like cancer [36]. The presence of cyclic hexapeptides and quinones in *Rubia cordifolia* exhibited a momentous anticancer activity against various proliferating cells [37]. The derivatives of RA-V like Alkyl ether and ester exhibit momentous effects against P388 lymphocytic leukemia, human nasopharynx carcinoma, and MM2 mammary carcinoma cells [38]. Several secondary plant metabolites were isolated from roots of *Rubia cordifolia* and rhizomes of *G. glabra* by using Cayman COX (ovine) inhibitory screening assay, few molecules showed potent COX-2 inhibitory activity against cancer [39].

5.4, Neuroprotective activity

The extract of *Rubia cordifolia* showed significant protective activity on neurodegeneration and shows improvement in memory retention activity when compared with A β 25-35 induced group of albino mice [40]. *Rubia cordifolia* exhibit neuroprotective activity by preventing the reduction and increasing GSH levels by inducing GCLC (c-glutamylcysteine ligase) expression, reducing oxidative stress levels by direct scavenging, and decreasing iNOS expression, which may be attributed to the GSH and vitamin C content of the plant [41].

5.5, Antimicrobial activity

The methanolic extract of *Rubia cordifolia* roots showed antibacterial activity against all the three Gram-positive bacteria and four Gram-negative bacteria and also exhibit antifungal activity against *Candida*

albicans. The methanolic extract of *Rubia cordifolia* roots showed activity against three out of four bacteria which showed resistance to antibiotics used. The aqueous extract of roots of *Rubia cordifolia* exhibit antibacterial activity only against two Gram-positive bacteria [42]. *Rubia cordifolia* extract of roots showed Antimicrobial activity against three Gram-positive (Staphylococcus epidermidis, Staphylococcus aureus, and Bacillus cereus) and three Gram-negative (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi) bacteria [34]. The root extracts constituents of *Rubia Cardifolia* such as anthraquinones and flavonoids suppressed the activity of phytopathogens of Gossypium, While as Sitosterol and daucosterol possess antibacterial activity [43]. *Rubia cordifolia* root extract exhibit inhibition against the bacterial pathogens like Vibrio alginolyticus, Pseudomonas aeruginosa, Shigella sps, Plesiomonas shigelloides and Vibrio parahaemolyticus and showed a highest antimicrobial effect against Pseudomonas aeruginosa and Plesiomonas shigelloides [44]. In the study, emodin and physcion were isolated as the most active constituents from *Rubia cardifolia* show antimicrobial activity [45].

5.6, Anti-oxidant activity

The methanolic and aqueous extracts of root, stem, and leaf of *Rubia cordifolia* possessed free radical scavenging potential with the highest amount possessed by aqueous and methanol extract of root with an IC50 value of 41.00 ($\mu\text{g/ml}$) and 61.95 ($\mu\text{g/ml}$) respectively [46]. The Strong antioxidant activity exhibited by Rubiadin, a dihydroxy anthraquinone isolated from an alcoholic extract of *Rubia cordifolia* prevents lipid peroxidation induced by FeSO₄ and t-butyl hydroperoxide (tBHP) in a dose-dependent manner. The percentage 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 [47]. The in vitro antioxidant activity of methanolic extract of roots and rhizomes of *Rubia cordifolia* showed an anticholinergic activity on rats which may be attributed to antioxidant activity and presence of phenolic compounds [48].

5.7, Anti-inflammatory activity

The ethanolic extract of *Rubia cadifolia* at different concentration 200 $\mu\text{g/ml}$ to 1000 $\mu\text{g/ml}$ shows activity in the range of 4.34% to 18.55%. The anti-inflammatory activity was carried out by using carrageenan-induced rat paw edema method. The extract shows prominent anti-inflammatory activity as compared to that of standard (Ibuprofen gel) [49]. The methanolic extract of roots of *Rubia cordifolia* in rats was aimed to investigate the analgesic and anti-inflammatory effect. *Rubia cordifolia* (100-300 mg/kg, p.o.) was evaluated for its antiinflammatory activity by carrageenan-induced rat paw edema. *Rubia cordifolia* (100-300 mg/kg, p.o.) exhibited significant ($P < 0.05$) reduction in the paw edema produced by the carrageenan [50]. The study of *Rubia cordifolia* for the anti-inflammatory activity in rats with carrageenan paw edema showed significant anti-inflammatory activity at a dose of 10 and 20 ml/kg of the water extracts as compared to that of phenylbutazone (100 mg/kg) [51].



5.8, Wound healing activity

The alcoholic extract and the hydrogel of *Rubia Cardifolia* were studied for the estimation of its healing efficiency on excision wound model in mice. A different formulation of alcoholic extract applied on the excision wound surface as a single dose. Wound area and histopathology were used to evaluate the effect on wound healing. The effect produced by gel, in terms of wound contracting ability, wound closure, a decrease in surface area of the wound, tissue regeneration at the wound site and histopathological characteristics were significant ($p < 0.01$) in treated mice [52]. The root extract of *Rubia cordifolia* was reported as a wound healer in experimental models an effectual wound healing standard [53]. Several drugs are described in the Ayurveda for their wound healing properties under the term 'vranaropaka'. *Rubia cordifolia* was also found to be effective in experimental models for wound healing property [53]. Polyherbal combination of *Rubia cordifolia*, *C.asiatica*, *T.belerica*, *P.zeylanica*, and *W.somnifera* was formulated and assessed of wound healing. Healing was assessed based on physical parameter namely, wound contraction, a period of epithelisation and histological study. It promotes contraction and epithelisation of excision wound [54].

5.9, Gastroprotective activity

The activity of *Rubia cordifolia* against experimentally induced gastric ulcer and compare activity with its fractions by employing aspirin plus pylorus-ligated ulcer screening model in Wistar rats. The study confirmed that chloroform fraction showed the significant activity at lower doses compared to plant extract. The mechanism can be attributed to decrease in gastric acid secretory activity along with strengthening of the mucosal defensive mechanism by prostaglandin synthesis and antioxidant potential [55]. The extract of *Rubia cordifolia* exhibited significant protection against gastric ulcers in all the models of rats compared to ranitidine. In polyherbal formulations, the ulcerogenic effect in rats showed significantly lesser ulcer result even at a very high dosage as compared to that of aspirin [56].

5.10, Antimutagenic activity

Different root extracts of *Rubia cordifolia* and their antimutagenic activity was estimated in Ames Histidine reversion assay using Salmonella typhimurium against mutations induced by direct-acting mutagen 4-nitro-o-phenylenediamine (NPD) and against S9-dependent mutagen 2-Aminofluorene (2-AF) in TA98 tester strain of S. typhimurium. The root extract of *Rubia cordifolia* was fractionated into highly polar methanol extract (RME) and comparatively less polar chloroform extract (RCE). RME was found to be quite effective in inhibiting the mutagenicity of 2AF, i.e. indirect-acting mutagen, in comparison to the mutagenicity induced by direct-acting mutagen i.e. NPD. The inhibitory activity against NPD was 41.69% and 58.21% at the maximum dose tested ($2.5 \times 10^3 \mu\text{g}/0.1 \text{ ml}$) during coincubation and preincubation mode of experiments, respectively. RME significantly inhibited 2AF-induced histidine revertants by 70.71% and 71.70% respectively during coincubation and preincubation mode of experiments with IC_{50} of $500 \mu\text{g}/0.1 \text{ ml}$ in the preincubation mode of the experiment. Chloroform extract (RCE) reduced the mutagenicity of NPD by 59.04% in the preincubation mode of the experiment at the maximum tested dose of $2.5 \times 10^3 \mu\text{g}/0.1 \text{ ml}$ with IC_{50} of $664 \mu\text{g}/0.1 \text{ ml}$. RCE

completely inhibited the 2AF mutagenicity, as about 99% inhibitory effect was recorded in both coincubation and preincubation mode of treatments [57].

5.11, Antiviral activity

The anti-rotaviral effect of *Rubia cordifolia* aerial part (RCAP) and its cytotoxicity toward MA-104 cells was evaluated using the WST-8 assay. Colloidal gold method and real time polymerase chain reaction (qPCR) assay were used to confirm the findings of the antiviral assay. Then, 4', 6-diamidino-2-phenylindole (DAPI) staining method was subsequently used to investigate the mode of death among the cells. And. It was shown that both the viability of MA-104 cells and the viral load were reduced with increasing concentration of the extract. DAPI staining showed that virus-induced apoptosis was the cause of the low cell viability and viral load, an effect which was accelerated with incubation in the aqueous herbal extract. The major compounds identified to exhibit this activities were Xanthopurpurin and Vanillic Acid. This study showed that RCAP extract effectively inhibited rotavirus multiplication by promoting virus-induced apoptosis in MA-104 cells [58].

5.12, Nephroprotective activity

The hydro alcoholic extract of *Rubia cordifolia* significantly decreases the cisplatin-induced nephrotoxicity as inferred from the tissue antioxidant status in the drug administered animals. A significant change was observed in serum creatinine and urea levels. Lipid peroxidation in the kidney and liver tissues was also significantly reduced extract treated animals [59]. An effective column-switching countercurrent chromatography (CCC) protocol combining stepwise elution mode was successfully developed for simultaneous and preparative separation of antioxidative components from ethyl acetate extract of traditional Chinese herbal medicine *Rubia cordifolia* show nephroprotectivity [60].

5.13, Anti-proliferative activity

The antiproliferative activity of *Rubia cordifolia* extract tested on A431 cells (epidermal carcinomoid cells) and 3T3 fibroblast cells. It was observed that a fraction of *Rubia cordifolia* significantly inhibited the incorporation of [3H]-thymidine, induced by foetal bovine serum, in a dose-dependent manner. It also inhibited the PMA (phorbol 12- myristate 13-acetate) induced expression of c-fos genes in A-431 cells. It appears that inhibition of DNA synthesis depends for its antiproliferative properties [61].

5.14, Cardioprotective activity

Nowadays combination therapy (hypolipidemic, diuretic, calcium channel blocker, vasodilator, antiplatelet) are common in patients with cardiac dysfunction. Chances of drug interaction and adverse consequences arise with combination therapy. *Rubia cordifolia* an individual plant with multiple activities is essential to support heart health [62].



5.15, Radioprotective effect

The alcoholic extract of root of *Rubia cordifolia* provides significant protection against radiation-induced lipid peroxidation, hemopoietic injury, and genotoxicity. The protection appears to be through its antioxidant, metal chelation and anti-inflammatory property [63]. The aqueous extract of *Rubia cordifolia* effectively prevents Single strand breaks induced in plasmid pBR322 DNA following ionizing radiations [64].

5.16, Anti-acne activity

Rubia cordifolia showed a significant inhibitory activity against *Propionibacterium acnes* standardized culture. The evaluation was carried out by broth dilution method; suggested Minimum Inhibitory Concentration of *Rubia cordifolia* extract was 600 µg/ ml. The evidence of anti-acne activity of *Rubia cordifolia* was further supported by Cup-plate method [65]. *Rubia Cordifolia* exhibits anti-acne activity due to a rich fraction of anthraquinone in a gel formulation against *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Malassezia furfur* in comparison with standard Clindamycin gel [66].

5.17, Nootropic and Anti-stress activity

The Alcoholic extract of roots of *Rubia cordifolia* enhance brain γ -amino-n-butyric acid (GABA) levels and decreased brain dopamine and plasma corticosterone levels. Acidity and Ulcers caused due to the cold restraint stress were inhibited by the alcoholic extract. Animals treated with alcoholic extract spent more time in open arm in elevated plus maze model, which antagonized scopolamine-induced learning and memory mutilation [31].

5.18, Anti-ESBL (Extended Spectrum β -Lactamase) activity

The study attempted to explore the antibacterial properties of *Rubia cordifolia*, against ESBL (Extended Spectrum β -Lactamase) producing urinary E.coli. Different E.coli strains were isolated from urine samples collected from patients using standard methods. All the isolates were tested for different antibiotics and screened for their ESBL production based on NCCLS guidelines. Total 7 different ESBL producing E.coli were obtained. All the seven isolates were tested against the ethanolic extract of *Rubia cordifolia* using Kirby Bauer method and were found to be inhibited variably by the extract. Thus suggests inhibitory properties of the extract against ESBL producing E.coli [67].

5.19, Anti-arthritic activity

The ethanolic extract of *Rubia cordifolia* exhibits a significant anti-arthritic activity which was statistically similar to aspirin [68].

5.20, Spasmolytic activity

The Crude extract of *Rubia cordifolia* suppressed the spontaneous contractions of guinea- pig atria, rabbit jejunum and rat uterus in a concentration dependent manner (0.1-3 mg/ml). In rabbit aorta, it inhibited

norepinephrine (10 μ M) and KCl (80 mM) induced contractions. Replacement of physiological salt solution with calcium free solution abolished the spontaneous movements of rabbit jejunum. However, the addition of calcium (25 μ g/ml) in the tissue bath restored the spontaneous movements. When the tissues were pretreated with plant extract (1 mg/ml) or verapamil (0.5 μ g/ml) addition of calcium failed to restore spontaneous contractions. These results indicate that the plant extract exhibits spasmolytic activity similar to that of verapamil suggestive of the presence of calcium channel blocker like constituent(s) in this plant [69].

5.21, Immunity enhancing activity

The ethanolic extracts of the whole plant of *Rubia Cardifolia* were tested for many immunity enhancing activity using a murine model. The active compound present in the extract enhanced both cell-mediated and humoral immunity. Administering the extracts to rats that were given the immunosuppressive drug, phosphamidon showed significant restoration in immunity [70].

5.22, Anti-convulsant Activity

Triterpenes isolated from *Rubia Cardifolia* inhibited seizures induced by maximum electric shock, electrical kindling and various chemoconvulsants in rats. Brain GABA and serotonin (5-HT) contents were raised by triterpenes reveals its anticonvulsant property [71].

5.23, Diuretic activity

The diuretic activity of the hydroalcoholic extract of roots of *Rubia cordifolia* in rats to support its folklore claim. Four groups of rats were treated with vehicle (normal saline: 25 ml/kg), furosemide (20 mg/kg) and two doses of extract (286 mg/kg and 667 mg/kg body weight) orally. Urine excreted was collected up to 5h post-treatment and analyzed for urine volume, Na⁺, K⁺, Cl⁻ and creatinine content. The extract showed a significant ($p < 0.01$) and dose dependent increase in urine volume and electrolyte excretion. Both doses of extract showed less influence on creatinine clearance than furosemide. The result indicates that hydroalcoholic extract of roots of *Rubia cordifolia* possesses potent diuretic activity [72].

5.24, Sexual Performance and Virility activity

A number of herbal drugs including *Rubia cardifolia* have been validated for their effect on sexual behavior and fertility and can, therefore, serve as the basis for the identification of new chemical leads useful in sexual and erectile dysfunction [73].

5.25, Anti-HIV activity

Ninety-two extracts were prepared from 23 plants. Anti-HIV activity was measured in a human CD4⁺ T-cell line, CEM-GFP cells infected with HIV-1NL4.3. Nine extracts of 8 different plants significantly reduced viral production in CEM-GFP cells infected with HIV-1NL4.3. *Aegle marmelos*, *Argemone mexicana*, *Asparagus racemosus*, *Coleus forskohlii*, and *Rubia cordifolia* demonstrated promising anti-HIV potential [74].

5.26, Anti-lead nitrate activity

Lead nitrate administration induced a significant ($P<0.001$) increase in LPO, significant ($P<0.001$) depletion of CAT and GSH in renal tissues. It also showed a significant ($P<0.001$) reduction in macrophage yield, the viability of macrophage, phagocyte index, serum immunoglobulin level, and PFC in the kidney. The combination treatment with *Rubia cordifolia* observed a significant ($P<0.001$) reversal of lead nitrate-induced toxicity on oxidative stress and immunological parameters [75].

VI.CONCLUSION

Rubia Cardifolia is important medicinal herb with a variety of medicinal properties. The biologically active compounds from different solvent extracts of root, stem, and leaves in Rubia cordifolia revealed the presence of anthraquinones, glycosides, saponins, steroids, phenols, and flavonoids etc. The Chemical property of these compounds in Rubia Cardifolia is responsible for various protective activities.

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