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ANTIEPILEPTIC ACTIVITY OF RUBIADIN ISOLATED FROM THE ROOTS OF *RUBIA CORDIFOLIA* IN MICE

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ABSTRACT: Epilepsy is a disorder of the central nervous system. A seizure occurs when the brain's nerve cells misfire and generate a sudden uncontrolled surge of electrical activity in the brain. Seizures can be controlled with modern medicines and surgical techniques and are found to have so many side effects. Natural products from folk remedies are an alternative source of anti-epileptic drugs with better safety and efficacy profiles. Rubiadin is an anthraquinone glycoside isolated from roots of *Rubia cordifoia* (Manjishta) known for several pharmacological activities. The present study was aimed to assess the anti-epileptic activity of isolated rubiadin from *Rubia cordifolia*. The evaluation of anti-epileptic activity was carried out with maximal electric shock (MES) and Pentylene Tetrazole (PTZ) models. The epileptic seizure was induced in mice of either sex and the challenged animals treated with whole *Rubia cordifolia* root extract & isolated Rubiadin suspension at two doses 100 mg & 250 mg respectively. The isolated Rubiadin suspension at 250 mg dose showed a significant reduction in MES and PTZ induced an epileptic seizure, which is compared with whole plant extract in mice.

INTRODUCTION: Human beings have relied on natural products as a resource for drugs for thousands of years. Plant-based drugs have formed the basis of traditional medicine systems that have been used for centuries in many countries such as Egypt, China, and India [1]. Phytotherapy still plays an important role in the management of diseases, mainly among low-income populations of African and Asian extraction. In developing countries like Nigeria, the World Health Organization (WHO) recommended the initiation of programs designed to use medicinal plants more effectively in traditional health care systems [2].

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Traditional medicine in many parts of the world relies on the use of a wide variety of plants species [3]. Epilepsy is the third most common neurological disorder after stroke and Alzheimer's disease. Although new antiepileptic drugs have been available since the late 1980s, refractoriness to treatment is still an important issue in epilepsy care. Currently available anticonvulsant drugs can control epileptic seizures efficiently in about 50% of the patients and lead to an improvement in another 25% whereas the remainders do not benefit significantly [4].

Furthermore; undesirable side effects of the drugs used clinically often render treatment difficult; so that demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is an investigation of naturally-occurring compounds, which belong to new structural classes.

The WHO estimated that approximately 80% of people with epilepsy live in developing countries, and most of them do not get adequate medical treatment [5]. The introduction of each new antiepileptic drug into the market raises valid expectations in patients and physicians for more effective treatment of epilepsy. Although safety, tolerability, and interactions are important, better efficacy is a crucial feature for a new antiepileptic drug [6]. The medicinal plant is important sources of new chemical entities with potential therapeutic effects[7].

In the modern system of medicine, barbiturates, hydantoin, phenytoin and diazepam are widely used to control the seizure but, these drugs have various limitations in the form of serious side effects, need plasma drug concentration monitoring and interacting with several drugs [8]. Indian system of medicine enlists many medicinal plants and herbal formulations which act on CNS. In Ayurveda, plants like *Crocus sativus* L, *Cymbopogon martinii* roxb, *Hibiscus rosa sinensis*, *Bacopa monnieri*, *Commiphora mukul*, *Datura metal*, *Gossypium herbaceum*, *Ferula asafoetida*, *Cymbopogon citratus etc*. are used to control epilepsy [9]. Ethyl acetate (EA) fraction of Radix Rubiae inhibits cell growth and promotes terminal differentiation in cultured human keratinocytes, which strongly suggest its antipsoriatic activity [10]. The Apoptotic effect of *Rubia cordifolia* extracts (30 mg/ml) on HEp-2 cells confirmed by fluorescent and transmission electron microscopy based on morphological and ultrastructural changes [11]. *Rubia cordifolia* role in supporting heart health is evidenced by traditional and reported activities which show that it act as potent diuretic, calcium channel blocker, antiplatelet, antidiabetic, anti-inflammatory, antistress, immunomodulator *etc*. [12]

In the Indian traditional a system of medicine, various herbs have been used to treat epileptic seizures. *Rubia cordifolia,* belongs to family Rubiaceae, commonly known as Indian Madder and Manjistha in Sanskrit. It is perennial, herbaceous prickly climber with long and cylindrical root with a thin red bark. Various parts of *Rubia cordifolia* have been suggested in the Indian system of medicine for a number of diseases. The roots and stems are well known source of Anthraquinones. It is a species of flowering plant in the coffee family, Rubiaceae [13]. *R. cordifolia* is an important medicinal plant commonly used in the traditional and Ayurvedic system of medicine for treatment of different ailments. It is highly valuable plant in Ayurvedic system of medicine used for treatment of various skin diseases [14, 15].

Rubiadin is a major component of *R. cordifolia* Linn. and found to possess hepatoprotective and antioxidant property [16, 17]. The prime objective of the present research was to explore and establish the possible antiepileptic potential of rubiadin from the root of *R. cordifolia.*

MATERIAL AND METHODS:

Collection and Authentification of Plant Material: *Dried roots of Rubia cordifolia Linn* were collected from the local market Ghaziabad, Uttar Pradesh, India and authenticated by NISCARE New Delhi. A voucher specimen was deposited at the Raw material Herbarium & Museum, Delhi (RHMD) CSIR-NISCAIR Reference No. – NISCAIR/RHMD/Consult/2013/2267/48.

Extraction and Isolation of Rubiadin: The roots of *Rubia cordifolia* were subjected to a fine powder, and the 500g powder was extracted with ethanol. After complete extraction the extract was concentrated and dried under reduced pressure to the dryness in flash evaporator. After drying extract was weighed and calculated the percentage yield.

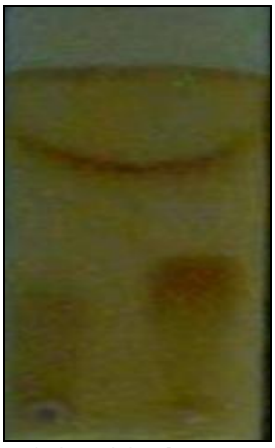

FIG. 1: TLC OF ISOLATED RUBIADIN

Separation and Purification:

Thin Layer Chromatography: Thin layer chromatography of ethanolic extract of *R. cordifolia* was performed using silica gel 60 as

stationary phase on a glass plate. The ethanolic extract was spotted on TLC and eluted with Methanol: Ethyl acetate (1:1) as solvent system. The TLC was developed by Iodine. A major spot (yellow-orange) appeared with R_f 0.5 **Fig. 1** after which it was poured into the column.

Column Chromatography: Silica gel was suspended in the required solvent and left for approximately 2 h to swell after which it was poured into the column. The ethanolic extract of 1g was suspended in the minimum amount of the particular solvent in which it would dissolve and filtered to remove impurities and any large particles which cause diffusion problems whilst developing the column. This fraction was applied to the top of the column using a pipette with great care as not to disturb the top of the column.

After application, the solvent flask was raised to facilitate solvent flow into the column and was run using gravitational force. The column was left to run overnight at a flow rate of 0.5 ml/min.

Preliminary Phytochemical Screening of *Rubia cordifolia*: Roots of *Rubia cordifolia* was tested qualitatively for the presence of various phytochemicals like glycosides, alkaloids, triterpenoids, proteins, tannins, and phenols.

TABLE 1: PRELIMINARY PHYTOCHEMICAL TESTS OF ETHANOLIC EXTRACT OF *RUBIA CORDIFOLIA*

S. no.	Test	Ethanolic extract
1	Alkaloids	+
2	Glycosides	+
3	Carbohydrates	+
4	Flavonoids	-
5	Triterpenoids & steroids	+
6	Saponins	+
7	Proteins & amino acids	+
8	Tannins	+
9	Fixed oils & Fats	-
10	Mucilage	-

Animals: Swiss albino mice weighing 18-25 g were used for the present study. The animals were maintained under controlled conditions of temperature (22 ± 2º), humidity (50 ± 5%) and 12 h light-dark cycles. The animals were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They were fed with commercial diet and water *ad libitum*. Laboratory animal care was taken as per Our Institutional Animal Ethics Committee (IAEC), Sanskar College of Pharmacy and Research (Formerly Shree Ganpati Institute of Technology), Ghaziabad, India (Registration No. 1251/PO/ReBi/S/09/CPCSEA).

Preparation of Rubiadin Suspensions: Suspensions of Rubiadin were prepared by using 125 mg and 500 mg rubiadin with Carboxy methylcellulose (CMC), polysorbate -80, methylparaben, propylparaben and purified water q.s.to 50 ml.

Drug and Chemicals: Pentylenetetrazole, Phenytoin and other chemicals used in the present study were obtained from Sun Pharmaceuticals, India Ltd., Gamma-aminobutyric acid (GABA) and dopamine used in the estimation is obtained from Sigma Biochemical's, USA. All the solvents used for the extraction processes are of laboratory grade and they are purchased from local firms and were used in the present study.

Acute Toxicity Study: Acute toxicity was conducted on Swiss albino mice weighing between 18-25 g using staircase/up and down method. The acute oral toxicity study was carried out according to OECD guidelines 423. First, one animal is dosed with 100 mg/kg body weight. If an animal dies, a much lower dose is tested. If the animal survives, then two more animals are dosed, after 48 h observation of the first animal.

If survives, then the main test should be terminated. If an animal dies, two more animals are dosed and observed. The study was performed with an initial dose of 100 mg/kg body weight. The dose sequences followed were 100, 200, 500, and 1000 mg/kg body weights. Animals did not show any significant toxicity at 1000mg/kg.

Assessment of Antiepileptic Activity:

PTZ Induced Convulsions: In this experiment, carboxymethyl cellulose (CMC, 1%) and diazepam (4 mg/kg) were used as a vehicle and positive control, respectively. *Rubia cordifolia* plant extract at the dose of 100 mg/kg, b.w. & Rubiadin suspension (100 mg & 250 mg) were administered orally to the animals three days before the PTZ induction at the dose of 80 mg/kg, b.w. (minimal dose needed to induce convulsions). Then after three days, PTZ was injected i.p. to induce clonic-tonic convulsions in mice. After induction of PTZ,

the following parameters (onset of jerks, the onset of clonus, Straub's tail and extensor) were observed carefully and blindly with the help of digital stopwatch.

Maximal Electro Shock (MES) Induced Seizures in Swiss Albino Mice: In this method, the animals were randomized into 6 groups (6 mice/group). Maximal electroshock seizure model was used to evaluate the anticonvulsant activity of the test samples in this study. Seizures were induced in mice by delivering electroshock of 50 mA using an electro-convulsometer through a pair of ear clip electrodes. The animals (n=6) received the vehicle CMC treated as vehicle control and test samples orally. The standard group received phenytoin (25 mg/kg) i.p and tested after 30 min for MES induced seizure response. Before delivery, the current output was checked by a multimeter. After the electric stimulation occurrence, the duration of phases was noted, and HLTE (Hind Limb Extension) phase was compared with the control group. The decrease in duration of HLTE was calculated as a protective action.

Statistical Analysis: All results were expressed as mean ± SD. For analyzing the variations in the observation of seizures between plant treated mice and control, students "t-test" was used. Data were analyzed using Dunnett's tests. $P<0.05$ was considered statistically significant in all cases.

TABLE 2: EFFECT *RUBIA CORDIFOLIA* PLANT EXTRACT AND RUBIADIN SUSPENSION ON PTZ INDUCED CONVULSION IN MICE

Treatment	Dose (mg/kg)	Animal	The onset of Jerks (Sec)	Straud tail (Sec)	The onset of Clonus (Sec)	Extensor (Sec)
Untreated	Untreated	6	43.5 ± 2.6	60.2 ± 7.5	71.3 ± 4.5	168.0 ± 9.1
Diazepam	4 mg/kg	6	242.7 ± 30.7	333.3 ± 117	No	No
Plant Extract	100 mg/kg	6	38.5 ± 3.2**	51.8 ± 11.6**	147.7 ± 9.9**	244.2 ± 10**
Rubiadin suspension	100 mg/kg	6	40.3 ± 2.4**	57.5 ± 7.6**	140.8 ± 13.2**	249.2 ± 9.9**
Rubiadin suspension	250 mg/kg	6	97.8 ± 12.2**	90.3 ± 7.7**	152.5 ± 7.3**	344.3 ± 13.4**

Values are mean ± SD (n=6); **statistically significant ($P<0.05$) compared to untreated

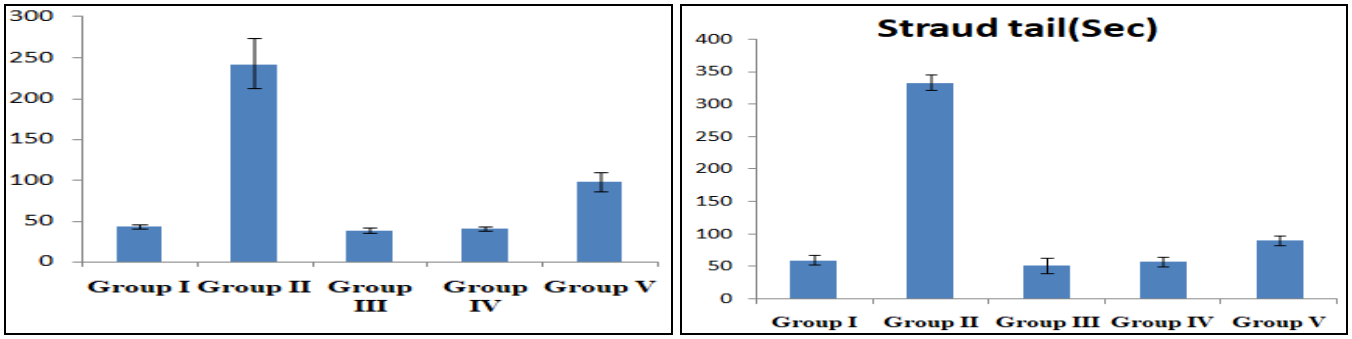

FIG. 2 AND 3: EFFECT OF *RUBIA CORDIFOLIA* EXTRACT & RUBIADIN SUSPENSION ON PTZ INDUCED ONSET OF JERK (A) AND STRAUB TAIL (B) IN MICE. Group I-Vehicle control (Untreated) (CMC 1%); Group II- Diazepam (Treated Group); Group III – Plant extract 100mg/Kg BW; Group IV- Rubiadin Suspension 100mg/ kg BW; Group V- Rubiadin Suspension 250mg/ kg BW.

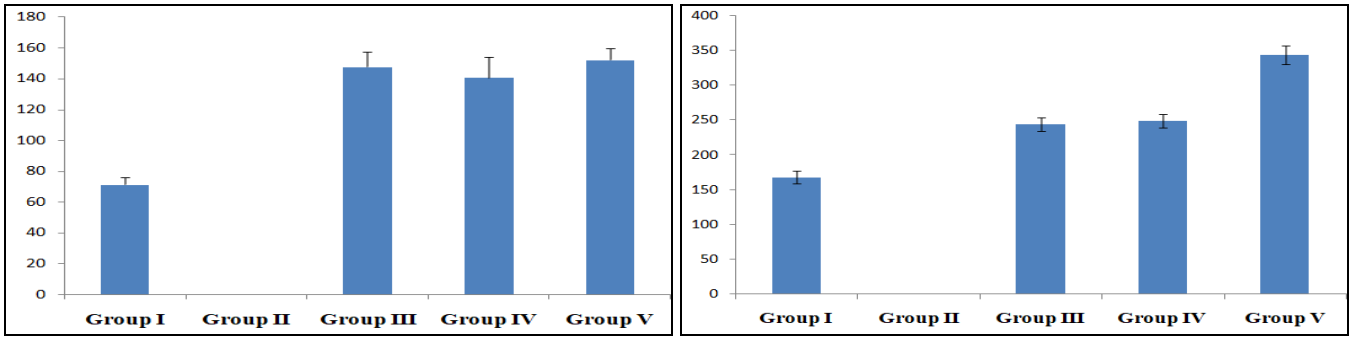

FIG. 4: EFFECT OF *RUBIA CORDIFOLIA* EXTRACT AND RUBIADIN SUSPENSION ON PTZ INDUCED CLONUS IN MICE. Group I-Vehicle control (Untreated) (CMC 1%); Group II- Diazepam (Treated Group); Group III – Plant extract 100mg/Kg BW; Group IV- Rubiadin Suspension 100mg/ kg BW; Group V- Rubiadin Suspension 250mg/ kg BW.

FIG. 5: EFFECT OF *RUBIA CORDIFOLIA* EXTRACT AND RUBIADIN SUSPENSION ON PTZ INDUCED EXTENSOR IN MICE. Group I-Vehicle control (Untreated) (CMC 1%); Group II- Diazepam (Treated Group); Group III – Plant extract 100mg/Kg BW; Group IV- Rubiadin Suspension 100mg/ kg BW; Group V- Rubiadin Suspension 250mg/ kg BW.

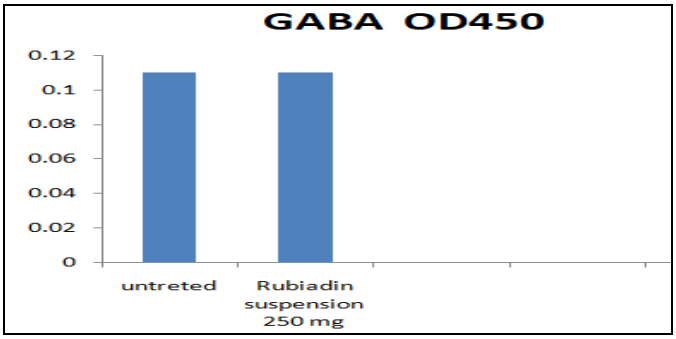

FIG. 6: ESTIMATION OF GABA IN BRAIN HOMOGENATE

TABLE 3: EFFECT OF *RUBIA CORDIFOLIA* EXTRACT & RUBIADIN SUSPENSION ON MES INDUCED SEIZURE IN MICE

S. no.	Group	Dose (mg/kg)	Hind Limb Extension (Mean ± SEM)
1	Vehicle control	Oral	12.9 ± 0.45
2	Phenytoin	25 (i.p.)	2.7 ± 0.32**
3	Plant ext.	100 (oral)	11.5 ± 0.72**
4	Rubiadin suspension	250 (oral)	4.3 ± 0.28**
5	Rubiadin suspension	100 (oral)	8.6 ± 0.81**

Values are mean ± SD (n=6); **statistically significant (P<0.05) compared to Vehicle control.

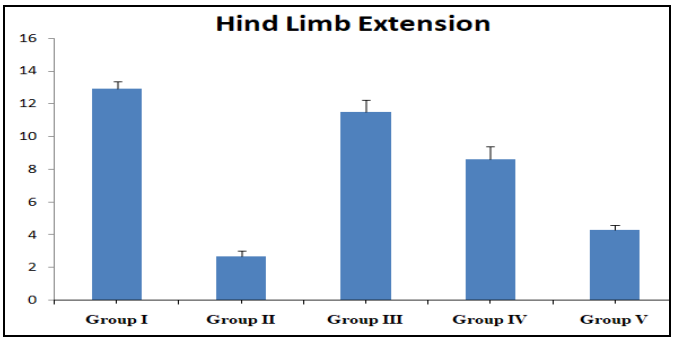

FIG. 7: EFFECT OF RUBIA CORDIFOLIA EXTRACT AND RUBIADIN SUSPENSION ON MES INDUCED SEIZURE IN MICE. Group I-Vehicle control (Untreated) (CMC 1%); Group II-Phenytoin (Treated Group); Group III – Plant extract 100mg/Kg BW; Group IV- Rubiadin Suspension 100mg/ kg BW; Group V-Rubiadin Suspension 250mg/ kg BW.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening of *Rubia cordifolia*: The phytochemical screening of *Rubia cordofolia* extract revealed the presence of glycosides, alkaloids, flavonoids, tannins, phenols, terpenoids, and sterol. The results of column chromatography given a yellow fraction and this is purified by recrystallization (yield was 0.2%) for further studies

PTZ Induced Epilepsy: The anticonvulsant activity of a compound is generally assessed by its ability to prevent the convulsions, to delay the onset of seizures and also shorten the duration of convulsions, spread of seizure discharge through neural tissue within the brain and central nervous system or elevating the seizure threshold. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures, whereas the PTZ test represents a valid model for human generalized myoclonic and also absence seizures. Prevention of seizures induced by PTZ and MES in laboratory animals is the most commonly used preliminary screening test to characterize potential anticonvulsant drugs.

In this study, the onset of action in the untreated group was 43.5 ± 2.6, while it was significantly delayed by 457.93% in the positive control (diazepam) group (242.7 ± 30.7). Moreover, rubiadin suspension at higher concentration (250 mg/kg, p.o.) showed a significantly delayed the onset of action by 124.83% as compared to the untreated group. Besides, the whole plant extract (at 100 mg/kg, p.o.) and rubiadin suspension at lower concentration (at 100 mg/kg, p.o.) per se did not show any effects on delaying the onset of jerking seizures movement.

Additionally, inflation of tail due to the mechanical contraction of the dorsal sarco-cosygeus muscle and electrical stimulation of spinal cord elicited tail elevation such as Straub tail reaction (STR) or simply called as Straub tail.

This Straub tail was initiated at 60.2 ± 7.5 seconds in the untreated group, while it was significantly delayed by 453.65% in the positive control group (333.3 ± 11.5 sec).

Further, rubiadin suspension at higher concentration (250 mg/kg, p.o.) showed a significant delayed STR by 50% as compared to the untreated group. Besides, the whole plant extract (at 100 mg/kg, p.o.) and rubiadin suspension at lower concentration (at 100 mg/kg, p.o.) per se did not show any effects on delaying the inflation of the STR. Other convulsive parameters like, onset of clonus convulsive episode was significantly reduced by 97.48%, 113.88%, and 107.15% in the rubiadin suspension 100 mg/kg, rubiadin suspension 250 mg/kg, and whole plant extract group at 100 mg/kg, respectively as compared to the untreated group (71.3 ± 4.5).

On the other hand, the duration of extensor period was also significantly reduced by 48.33%, 104.94%, and 45.36% in the rubiadin suspension 100 mg/kg, rubiadin suspension 250 mg/kg, and whole plant extract group at 100 mg/kg, respectively as compared to the untreated group (168.0 ± 9.1) as shown in **Table 2**.

The result obtained in the present study showed that the rubiadin suspension 250 mg was able to delay PTZ- induced seizures, and it is probable that it may be interfering with GABAergic mechanism to exert its effect. GABAergic system plays a major role in the pathophysiology of cerebral ischemia, epilepsy, sleep, and mood disorders. Estimation of GABA level in brain tissues will be a great tool in neuroscience research.

In the present work, the results show minimal alteration in the level of GABA **Fig. 6**.

MES Induced Epilepsy: The MES test is the most frequently used animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures /"Grandmal".

Based on the results of MES, it was found that the vehicle control group showed hind limb extension as 12.9 ± 0.45. Further, the positive control (phenytoin 25 mg/kg. i.p.) group revealed 79.07% inhibition of hind limb extension as compared to the vehicle control group. Moreover, the hind limb extension was inhibited by 33.33%, 66.67%, and 10.85% in the rubiadin suspension 100, 250 and plant ext. 100 mg/kg. oral, respectively with respect to the vehicle control group.

CONCLUSION: The Herbal drugs are always a best way to cure any disease because of their availability and fewer side effects. The isolated rubiadin, at higher dosage given better effects in both pentylene tetrazole & maximal electric shock models.

In conclusion, from the above study, rubiadin per se at 250 mg/kg may have potential anticonvulsant activity compared to the lower dose group of rubiadin and whole plant extract in Swiss albino mice compared to the untreated group.

Future Perspective: The isolated compound Rubiadin is an anthraquinone glycoside, has shown anticovulsant effect and from earlier literature review on *Rubia cordiofolia*, it has been seen that its Triterpenes part has shown anticovulsant effect. Therefore in future it might be the possibility that the combination of both isolated compounds, *i.e.* Rubiadin & triterpenes have synergestic effect for the treatment of epilepsy.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Balandrin NF, Kinghorn AD, and Farnsworth NR: Human Medicinal Agents from Plants, Eds., ACS Symposium Series 534, 1993: 2-12.
2. WHO: Drug Policies and Management: Medicinal Plants. Geneva, Switzerland: WHO Resolution 1978 31-33.
3. Geoffrey CK: Medicinal plants and the control of protozoal disease with particular reference to malaria. London: Transaction of the Royal Society of Tropical Medicine and Hygiene 1996: 605-9.
4. Schmidt D and Loscher W: Drug resistance in epilepsy: putative neurobiological and clinical mechanisms. Epilepsia 2005; 46: 858-77.
5. Reddy DS: Pharmacotherapy of catamenial epilepsy. Indian Journal of Pharmacology 2005; 37(5): 288-93.
6. Dieter S: Efficacy of new antiepileptic drugs. Epilepsy Currents Review & Critical Analysis 2011; 11(1): 9-11.
7. Farnsworth NR: Screening plants for new medicines. In: Biodiversity, Part II. National Academy Press, Washington 1989; 20(9): 83-97.
8. Sharma A, Khosla R, Mehta V and Kela A: Antiepileptic agents newer generation Indian J of Pharmacog 1996; 28(1): 1-10.
9. Warrier PK and Nambiar VPK: Indian medicinal plants: A compendium of 500 species. Published by Orient Longman Ltd. Vol. I, 1994: 218-23.
10. Zhou LL, Lin ZX, Fung KP, Che CT, Zhao M, Cheng CHK and Zuo Z: Ethyl acetate fraction of *Radix rubiae* inhibits cell growth and promotes terminal differentiation in cultured human keratinocytes. Journal of Ethno-pharmacology 2012; 142: 241-47.
11. Shilpa PN, Venkatabalasubramanian S and Devaraj SN: Induction of Apoptosis by methanolic extract of *Rubia cordifolia* L. in HEp-2 cell line is mediated by reactive oxygen species. Asian Pacific Journal of Cancer Prevention 2012; 12: 2753-58.
12. Bhatt P and Kushwaha AS: *Rubia cordifolia* Overview: a new approach to treat cardiac disorders. International Journal Drug Development & Research 2013; 5(2): 47-54.
13. Sharma SK, Kumari P, Tiwari S and Sachan K: *Rubia cordifolia* an ethnopharmacological review. American Journal of Pharmtech & Research 2016; 6(4): 1-11.
14. Nadkarni AK: Indian Materia Medica. Popular Prakashan, Mumbai, Edition 3, Vol. I, 2000.
15. Sukh D: A selection of Prime Ayurvedic plant drugs Ancient-Modern Concordance. New Delhi: Anamaya publishers 2006.

16. Tripathi YB and Sharma M: Comparison of antioxidant action of the alcoholic extract of *R. cordifolia* with Rubiadin. Indian Journal of Biochem and Biophys 1998; 12(5): 313.

17. Rao GM, Rao CV and Pushpagandan P: Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. Journal of Ethnopharmacology 2006; 103: 484-90.

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