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IN-VITRO ANTI-BACTERIAL AND CYTOTOXIC ACTIVITY OF *RUBIADIN*

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ABSTRACT

Studies have confirmed the medicinal potential of the *Rubiadin*. While effects of Rubiadin on some bacteria and Brine shrimp lethality using their different concentrations has not been previously explored.

Present study shows that the Pure Rubiadin exhibited antibacterial and cytotoxic activity.

The findings of present work provide need for further exploration of Rubiadin to treat microbial infections and cancer.

Keywords: Rubiadin; Antibacterial activity; Brine shrimp lethality assay

INTRODUCTION

Rubiadin, 1,3- dihydroxy-2-methyl anthraquinone has been isolated from the *Rubia cordifolia* Linn (Rubiaceae). *Rubia cordifolia* is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine **[1, 2]**. Rubiadin, isolated from the roots of *Rubia cordifolia* was found to have potent antioxidant property **[3]**. In addition, rubiadin also have been found to inhibit lipid peroxidation **[4]** and the plant *Rubia cordifolia* have been reported for anti-inflammatory **[5]**, immunomodulatory **[6]**, anticonvulsant and anxiolytic **[7]** and anti-tumor activities **[8]**. While the results of the study by Guntupalli *et al*., **[9]** strongly

indicate that rubiadin has a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats.

Although *Rubiadin* have various pharmacological uses, it has not been investigated for antimicrobial and cytotoxic activity against prominent gram-positive and gram-negative human pathogenic bacterial strains. Besides, cytotoxic activity screening of Rubiadin is also carried out with view to assess the presence of antitumor activity.

MATERIALS AND METHODS

Plant material

The Rubiadin [1,3-dihydroxy-2-methylanthracene-9,10-dione] purchased (Product code : R004, Lot. no. : T19D079; CAS No: 117-02-2) from Natural Remedies Pvt. Ltd., Bangalore. Purity of Rubiadin was determined by the manufacturer by HPLC area normalization and was certified above 94.80%.

Antibacterial assay [10, 11]

The antibacterial assay was carried out by employing 24hrs cultures of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneiumonieae*. Activity of aqueous and ethanolic extracts of *Rubiadin* was tested separately using Agar well diffusion method. The medium was sterilized by autoclaving at 120°c (16 lb/inch square). About 30 ml of the Agar medium with the respective strains of bacteria was transferred aseptically in to each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6 mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in 6mm diameter well. Antibacterial assay plates were incubated at 37 ± 2^0C for 24h. The standard disc 6 mm diameter with ciprofloxacin (100µg/disc) was used as a positive control for antibacterial activity.

Brine shrimp lethality bioassay /Cytotoxicity assay [10, 11]

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds. The bioassay was carried out against a simple zoological organism, brine shrimp nauplii. The brine shrimp lethality bioassay was carried out on the aqueous and ethanolic extracts of *Rubiadin* using standard procedure. Briefly, brine shrimp (*Artemia salina* Leach) eggs were hatched in a hatching chamber filled with fresh sea water. The chamber was kept under illumination using a fluorescent bulb for 48 hrs for the eggs to hatch into shrimp larvae. 30 mg of each extract were separately dissolved in 3 ml of DMSO, and from these 300, 160, 100, 60 and 10 µg/ml were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 16 test-tubes for each

sample. A control containing 6 ml of DMSO solvent was used for each solvent. The final volume of the solution in each test-tube was made up to 6 ml with sea water immediately after adding shrimp larvae. The test-tubes were maintained under illumination. Survivors were counted after 24 h and the percentage death at each dose was determined and LC60 values were calculated. After 24 hrs of incubation, the test tubes were inspected using a magnifying glass and the number of survivors were counted. The concentration-mortality data were analyzed statistically for the determination of LC60 values.

RESULTS

Antibacterial assay

The antibacterial activities of Rubiadin obtained by the cup plate method are presented in **Table 1**. The Rubiadin showed varying zones of inhibition at two concentrations (50 and 100 µg/ml) against two gram-positive and two gram-negative bacteria. The Rubiadin showed significant zone of inhibition against *B. subtilis E. coli S. aureus* and *K. pneumonia* with respect to the standard. The maximum zone of inhibition was obtained for at both concentrations against *S. aureus*.

Brine shrimp lethality bioassay (Cytotoxicity assay)

Rubiadin showed positive results indicating that the test sample is biologically active. Following the procedure of Meyer, the lethality of Rubiadin of the brine shrimp was evaluated. The results of the brine shrimp lethality after 24 hrs exposure to Rubiadin and the positive control, vincristine sulphate is summarized in **Figure 1**. Plotting of log of concentration (log C) of versus percent mortality (% Mortality) for Rubiadin showed an approximate linear correlation shown in figure 2. In this bioassay Rubiadin revealed prominent cytotoxicity with the LC60 values of 317.44 ug/ml.

Table 1: Antibacterial assay of Rubiadin

Sample Name	Dose in µg/ml	Zone of inhibition in mm			
		S. aureus	*B. subtilis*	*E. coli*	*K. pneumonia*
Control (DMSO)	0.1	NA	NA	NA	NA
Rubiadin	50	19.0012 ± 0.0001	19.3360 ± 0.6774	16.760 ± 0.6774	17.4690 ± 0.674
	100	36.0102± 1.0000	33.360 ± 0.6774	39.6600± 0.6774	32.43 ± 0.2974
Standard (Ciprofloxicin)	100	64.6670 ± 0.6774	63.660 ± 0.6774	46.0001 ± 1.0000	40.0020 ± 1.0000

(-) No zone of inhibition detected. Values are means ± SEM from three readings.

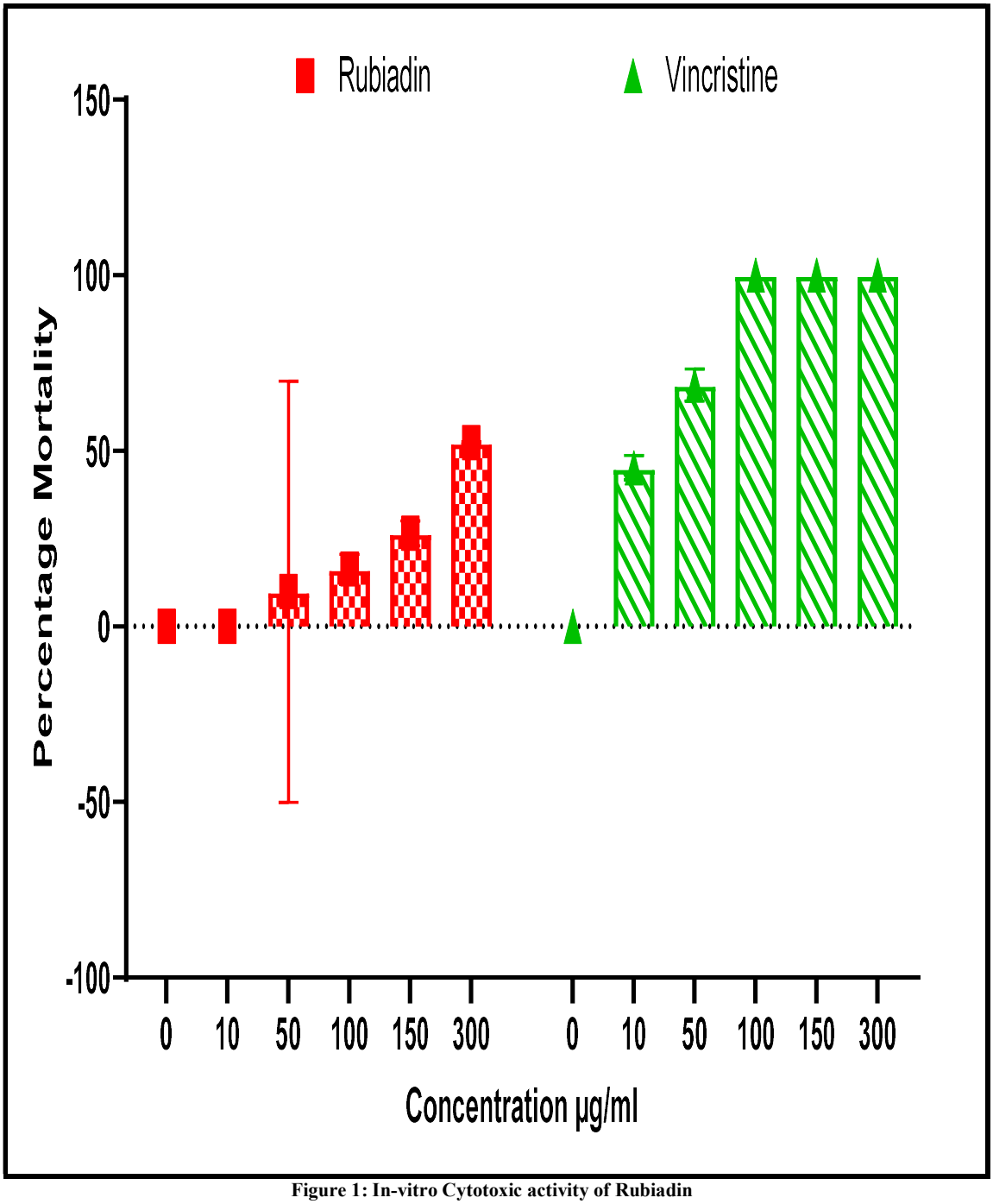

Figure 1: In-vitro Cytotoxic activity of Rubiadin

Values are means ± SEM from three readings.

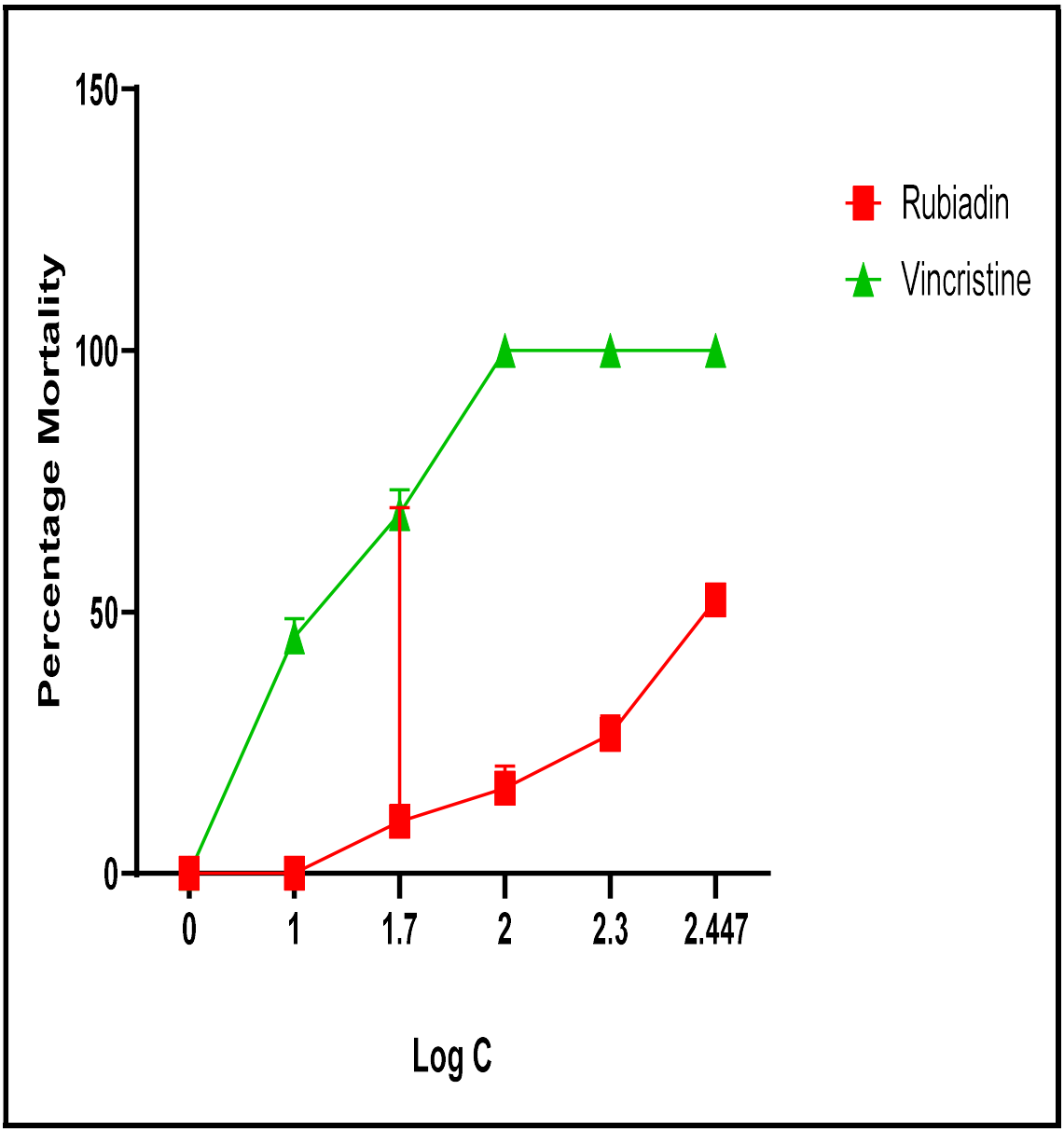

Figure 2: Graphical presentation of Log C versus percent mortality of Rubiadin

CONCLUSION

The Rubiadin have a potential to be a candidate for the investigation of cytotoxic compounds. The finding of present work provides us preliminary information that could be use as a basis for the development of Rubiadin like drugs overcome microbial infections and cancer.

REFERENCES

[1] K. R. Kirtikar, B. D. Basu, Indian Medicinal Plants vol II (International Book Distributors, Dehradun, 1980). Sec edition, p. 1305-1307.

[2] Nilambari Deshkar, Shrikant Tilloo, and Vipinchandra Pande, A Comprehensive Review of *Rubia*

Cordifolia Linn Pharmacognosy Review, 2008,2,3,124-134.

[3] Tripathi, Y.B., Sharma, M., Comparison of the antioxidant action of the alcoholic extract of *Rubia cordifolia* with rubiadin. Indian Journal of Biochemistry and Biophysics 1998.35, 313–316.

[4] Tripathi, Y.B., Sharma, M.K., Manickam, M., Rubiadin, a new antioxidant from *Rubia cordifolia*. Indian Journal of Biochemistry and Biophysics 1997.34, 302–306.

[5] Antarkar, D.S., Chinwala, T., Bhatt, N., 1983. Anti-inflammatory activity of *Rubia cordifolia* Linn. in rats. Indian Journal of Pharmacology 15, 185–188.

[6] Joharapurkar, A.A., Deode, N.M., Zambad, S.P., Umathe, S.N., 2003.Immunomodulatory activity of alcoholic extract of *Rubia cordifolia* Linn. Indian Drugs 40, 179–181.

[7] Kasture, V.S., Desmukh, V.K., Chopde, C.T., 2000. Anticonvulsant and behavioral actions of triterpine isolated from *Rubia cordifolia* Linn. Indian Journal of Experimental Biology 38, 675–680.

[8] Manohar, K., Adwankar, M.K., Chitnis, M.P., 1982. In vivo anti-cancer activity of RC-80, a plant isolate from *Rubia cordifolia* Linn. Against a spectrum of experimental tumour models. Chemotherapy 28, 291–293

[9] Guntupalli M. Mohana Rao, Chandana V. Rao, Palpu Pushpangadan, Annie Shirwaikar, Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. Journal of Ethnopharmacology, 2006, 103, 484–490.

[10] Kengar S B, Chopade A R, Wadkar GH: Evaluation of in-vitro anti-bacterial and cytotoxic activity of *phyllanthus amarus* standardized extracts, Asian Jr. of Microbiol. Biotech. Env. Sc. 2017, 19(2), 2017 : 440-443

[11] Kengar S B, Chopade A R, Wadkar GH: Cytotoxic activity of *phyllanthus fraternus*, Asian Jr. of Microbiol. Biotech. Env. Sc., 2017, 19(2), 466-467.