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Evaluation of antioxidant and antiacne property of *Rubia cordifolia*

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ABSTRACT

Acne vulgaris is the most common dermatological disorder among the individuals. The Propionibacterium acnes play a vital role in pathogenesis of acne inflammation. The P. acnes release neutrophill chemotactic factors which attracts polymorphonulcear leukocytes at site of infection. A Polymorphonulcear leukocyte induces the reactive oxygen species to create an oxidative stress which is responsible for acne inflammation. Conventional medical treatment has its own side effects besides the high cost. Ehanobotnical search reveals use of many traditional herbs in treatment of acne, which are usually free from side effects, are economical and also accessible to humans. Rubia cordifolia a well known plant traditionally being used as medicine in various skin disorders. It was thus thought worthwhile to evaluate antibacterial activity against Propionibacterium acnes by broth dilution and cup plate diffusion method. R. cordifolia extracts were also evaluated for antioxidant and lipid peroxidation inhibitory activity by 1, 1-diphenyl-2-picryl-hydrazyl and TBARs Thiobarbituric acid reactive substances method respectively. The study throws light on use of R. cordifolia in prevention and treatment of acne.

Keywords: *Rubia cordifolia, Propionibacterium acnes,* reactive oxygen species, oxidative stress, lipid peroxidation, free radical scavengers.

INTRODUCTION

Rubia cordifolia, belonging to family Rubiaceae, commonly known as Indian Maddar and Manjistha in Sanskrit is perennial, herbaceous prickly climber with long and cylindrical root with a thin red bark. Drug consists of dried root and stem and is distributed in the Himalayas and other hilly districts of India. The roots and stems are well known source of Anthraquinones, the roots have also been reported as antioxidant, antinflammatory, anticancer, immunomodulator and

hepatoprotective and are extensively used against blood, urinary and skin diseases[1, 2]. Acne is disorder of pilosebaceous unit, and generally characterized by formation of seborrhea, comedones, inflammatory lesions and presence of bacteria *propionibacterium acnes* in the follicular canal and sebum production[3]. Each of these factors provides a potential target for treatment. *Propionibacterium acnes* is the pharmacological target site of antiacne drugs[4]. *Propionibacterium acnes* , an anaerobic pathogen, plays important role in the pathogenesis of acne inflammation by inducing polymorphonulcear leukocyte and monocyte and macrophages to produce pro-inflammatory mediators. These organisms produce pro-inflammatory mediators. These organisms produce neutrophil chemotactic factors, which attract neutrophils to release inflammatory mediators such as reactive oxygen species to create an oxidative stress which is responsible for acne inflammation [5].

Long term use of antibiotics against acne is outdated because of exacerbated antibiotic resistance[6]. The objective of the study was to investigate the antibacterial and antioxidant potential of *Rubia cordifolia*, to understand their beneficial role in acne vulgaris.

MATERIALS AND METHODS

The root and stem of *Rubia cordifolia* were collected from the Pydhonie, Mumbai Market and authenticated at the Agharkar Institute (No.:Auth. 07-40) Poona. The culture of *Propionibacterium acnes* (MTCC 1951) was obtained from IMTECH (Institute of Microbial Technology) Chandigarh. The microorganism was grown using Brain Heart Infusion Broth which was procured from (Hi media Laboratories limited, Mumbai) DPPH (1, 1-diphenyl-2-picryl-hydrazyl) was procured from Sigma Aldrich Laboratories, USA. All solvents used were of LR GRADE.

Preparation of Extract and antibacterial testing

The dried powdered of *Rubia cordifolia* was extracted with methanol using soxhlet apparatus at temperature 50^0. The concentrated extract was evaporated to dryness under reduced pressure at 45^0. The antibacterial activity of extract was studied against *Propionibacterium acnes* by Cup plate diffusion method and Broth dilution method. The culture of *P.acnes* was standardized by spectrophotometric method using Mc Farland turbidity standard[7]. The Brain Heart Infusion Broth medium was used for the antibacterial assay and consisted of 1% tween and 0.03% thioglycolic acid.

In broth dilution method [8] the medium was poured in the test tubes which were then sterilized by autoclave using 15 lb pressure at 121^0 for 30 m. Using sterile pipettes exact amount of extract was added to the medium. A stock solution of 100 mg/ml each extract was made and diluted. As indicated in the (Table1) to obtain a final volume of 10 ml.The tubes were then inoculated with 0.05 ml of standardized culture. The tubes were incubates at temperature 37^0 for 48 h and observed for growth by comparing the turbidity produced. The lowest concentration that can inhibit the growth was considered as the Minimum Inhibitory Concentration (MIC).

The cup plate diffusion method [9] involved sterilization of petriplates, seeding of medium, inoculation and incubation. The plates were sterilized by dry heat in an oven at 160^0 for 1 h. The medium was sterilized by autoclave at 120^0 (15 lb/in2). About 30 ml of molten agar with bacteria

was transferred aseptically into each petriplate. After solidifying, wells of 10 mm were bored aseptically using sterile cork borer. The wells were filled with different concentrations of extract (10 mg, 20 mg, and 40 mg) and control, then plates were placed in an McIntosh Fildes anaerobic jar and evacuated using vacuum. The jar was then filled with nitrogen gas and then kept in an incubator at 37^0 for 48 h. The zone of inhibition was measured; clindamycin was used as a positive control.

Table1: Results Of Zone Of Inhibition Of *Rubia Cordifolia* Extract

Sr.No.	Test material	Zone of inhibition in (mm)
1.	Vehicle control (methanol)	-
2.	10mg	17
3.	20mg	20
4.	40mg	25
5.	Positive control(Clindamycin)	29

Fig 1) zone of inhibition of 20 mg and 10 mg ext. of *R.cordifolia*

Fig 2) zone of inhibition of 40 mg ext. of *R.cordifolia* and methanol control

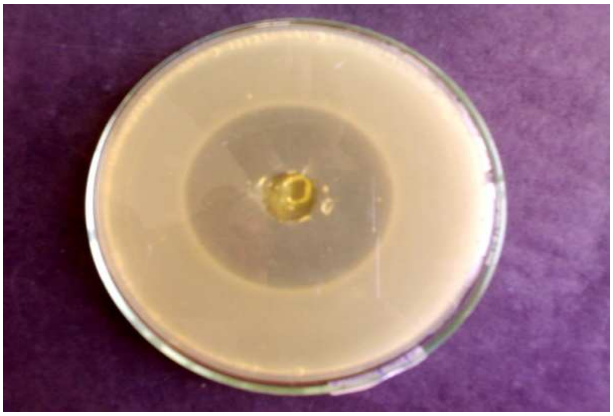

Fig 3) zone of inhibition of Clindamycin standard

Free radical scavenging activity

The activity was assayed spectrophotometrically by DPPH method.[10,11] The reduction in absorbance of DPPH solution (2 mg/ml) was monitored at 516 nm after addition of various concentrations of test extracts to DPPH reagent and maintaining these solutions were maintained at room temperature for 5 m before taking the reading. The EC_{50} values for test material was calculated from the calibration curves of concentration of extract (µg/ml) verses % reduction in absorbance after subjecting to linear regression between 10–80 %. This activity was expressed as effective concentration at 50% (EC_{50}) that is the concentration of the test solution required to give a 50% reduction in absorbance of the test solution as compared to that of blank solution. Ascorbic acid was used as a positive control.

The lipid peroxidation inhibitory activity

The activity was studied by the method [12] The reaction mixture contained mice liver homogenate (0.2 ml, 10% w/v) in 0.15 M KCl, KCl (0.1 ml, 150 µM), Tris buffer (0.4 ml, pH 7.5) and various concentrations of test extracts. *In vitro* lipid peroxidation was initiated by addition of $FeSO_4$. $7H_2O$ (0.1 ml, 10 µM) and ascorbic acid (0.1 ml, 100 µM). The reaction mixture was incubated at 37^0 for 1 h. After the incubation period, reaction was terminated by addition of thiobarbituric acid (TBA- 2 ml, 0.8%) and by heating the contents for 15 min. for development of coloured complex. The lipid peroxides formed were measured as thiobarbituric acid reacting substances (TBARs) by method [12]. The tubes were then centrifuged at 4000 rpm for 10 m. and cooled. The % inhibition of lipid peroxidation was determined by comparing the results of test compound with those of controls not treated with extracts by monitoring the colour intensity at 532 nm Curcumin was used as a positive control. The results were expressed as IC_{50} value that is the concentration of extract required for 50 % inhibition of production of lipid peroxides.

RESULTS AND DISCUSSION

Extract of *R. cordifolia* showed a significant inhibitory activity against *P. acnes* standardized culture. The evaluation was carried out by broth dilution method; suggested MIC of *R. cordifolia* extract was 600 µg/ml . The evidence of anti-acne activity of *R. cordifolia* was further supported by Cup-plate method.

The *R. cordifolia* extract of 40 mg/ml concentration was produced a significant zone of inhibition (25 mm) as compared to clindamycin which was used as positive control (29 mm). (Fig.1, 2, 3 and Table -1).

Antioxidant activity of methanolic extract of *R. cordifolia* also showed a significant free radical scavenging activity, carried out by DPPH method. The EC_{50} value was calculated 57.54 µg/ml and R^2-0.9757 for *R. cordifolia* extract. The result was compared with ascorbic acid as standard control (EC_{50} 2.87 µg/ml, R^2- 0.9601). The methanolic extract of *R. cordifolia* showed significant lipid peroxidation inhibitory activity. The IC_{50} value of 138 µg/ml and R^2 was-0.9921 .The result was compared with Curcumin as standard (IC_{50} 50 µg/ml, R^2-0.9469). Acne vulgaris is a chronic inflammatory disease of the pilosebaceous units. The etiologic factors include increased sebum production, ductal hyperkeratosis, abnormality of the microbial flora within the pilosebaceous unit, and chemomediators of inflammation. The dermal inflammation is not due to

presence of bacteria, but from biologically active chemomediators produced by *P. acnes* and the microenvironment within the pilosebaceous unit, is probably more important than the absolute number of *P. acnes* organisms. Indeed, the major role of the sebaceous gland appears to be supplying nutrients needed by *P. acnes* and moreover, the microbiologic principle of biofilms appears to be applicable to *P. acnes* in acne [13].

The recent demonstration that the 'peroxisome proliferator activated receptors' (whose natural ligands are polyunsaturated fatty acids and theirs oxidation products), have a central role in the induction of acne, has indicated new links between free radicals and skin inflammation[14].

CONCLUSION

Our investigations have revealed *R.cordifolia* as a promising anti-acne agent because it inhibits the proliferation of *Propionibacterium acnes* and hence prevents its consequences. The result of anti-acne activity of *R. cordifolia* thus can be expressed in relation with antioxidant activity which controls the oxidative stress in acne inflammation.

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REFERENCES

[1] M. K. Shrotri, R. C. Ghavane, U. Mukunda, *Indian Drugs* **2005;** 42(1): 20-23.
[2] Indian council of medicinal reserch New Delhi, Quality standards of Indian medicinal plant. **2005**; 3:307-315.
[3] J. J. Leyden, *J. Am. Acad. Dermatol* **1995;** 32: S15
[4] L. Lever, R. Marks, *Drugs* **1990;** 29: 681- 692.
[5] A. Jain, E. Basal, *Phytomedicine* **2003**; 10: 34-38.
[6] H. E. Degroot, S.F. Friedlander, *Curr. Opin. Pediatr*. **1998**; 10: 381.
[7] A. Balows , In: Manual of Clinical Microbiology, Washington DC: 5th ed. **1991;** 1173-1183.
[8] B. J. Wadher, G. L. Bhooserddy. In Manual of Diagnostic Microbiology, 1st ed. **1995**; 62-67.
[9] Barry AL. In: The Antimicrobial Susceptibility Test: Principles and practices, Lea & Febiger, Philadelphia: **1996**; p.163-164.
[10] P. M. D'Mello, M. A. Jadhav, C. I. Jolly. *Indian Drugs* **2000**; 37: 518-520.
[11] J. L. Lamaison, A. Carnet. *Parma Acta Helv*. **1991**; 66:185
[12] H. Ohkawa, N. Ohishi, K. Yagi, *Anal. Biochem*. **1979**; 95: 351-358.
[13] C. N. Burkhart, L. Gottwald, *Skinmed* **2003**; 2(4): 222-228.
[14] S. Briganti, M. Picardo, *J. Eur. Acad. Dermatol. Venereol***. 2003**; 17(6): 663-669.