

Screening of selected Herbal plants for Anti Acne Properties

Pandey Chetana*, Karadi. R.V, Bhardwaj K Lokesh, Sahu K Amit

Department of Pharmacognosy, KLE University's College of Pharmacy, Belgaum, Karnataka

Abstract

The objective of this study was to evaluate the *in-vitro* and *in-vivo* anti-acne effects of ethanolic and aqueous extract of rhizomes of selected *Curcuma* species (*Curcuma aromatica*, *Curcuma amada*, *Curcuma zedoaria*) and bark of *Adina cordifolia* against *Propionibacterium acnes*. Minimum inhibitory concentration to inhibit the growth of *P.acne* of ethanolic extract of *Curcuma spp.* And *Adina cordifolia* were found to be 125 µg/ml and aqueous extract of *Curcuma spp* and *Adina cordifolia*. were found to inhibit the growth of *P. acne* at concentration 250 µg/ml. 140 µg of heat killed *P. acnes* injected in the ears of rats by subcutaneous route. Ear thickness was measured as an index of inflammatory strength using vernier caliper upto 35 days but there was no significant change after 10th day.

*Corresponding author, Mailing address:

Pandey Chetana

E-mail: chetana.pandey1@gmail.com

Key words:

Propionibacterium acnes, *Curcuma aromatica*, *Curcuma amada*, *Curcuma zedoaria*, *Adina cordifolia*.

How to Cite this Paper:

Pandey Chetana*, Karadi. R.V, Bhardwaj K Lokesh, Sahu K Amit "Screening of selected

Herbal plants for Anti Acne Properties", Int. J. Drug Dev. & Res., April-June 2012, 4(2): 216-222

Copyright © 2012 IJDDR, Pandey Chetana et al.

This is an open access paper distributed under the copyright agreement with Serials Publication, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Article History:-----

Date of Submission: 31-03-2012

Date of Acceptance: 17-04-2012

Conflict of Interest: NIL

Source of Support: NONE

Introduction

Acne vulgaris, a chronic inflammatory disorder in adolescent consist of the pilosebaceous follicles, characterized by comedones, papules, cyst, nodules and often scars, chiefly on face, neck etc. The microorganism involved include *Propionibacterium acnes* (*P. acne*) and *Staphylococcus epidermidis*. The inflamed glands caused by stress, hereditary factors, hormones, drugs and bacteria. Cause of acne includes the action of sebum synthesized and secreted by the androgen-sensitive sebaceous glands, Increase in hormones called androgens in both girl and boy during puberty, Hormonal change related to pregnancy or starting or stopping birth control pills, stress, skin irritation and Heredity¹.

Propionibacterium species are inhabitants of the skin and usually are nonpathogenic. As a result, they are common contaminants of blood and body fluid cultures. *Propionibacterium* resembles *Corynebacterium* in morphology and arrangement. *Propionibacterium* acne is the pleomorphic, Gram-positive, non-spore forming anaerobic to aero-tolerant diphtheroid bacillus that produces propionic acid, as its name suggests, it also has the ability to produce of fermentation. *P. acne* and *P. granulosum* may also isolated from the gastrointestinal tract².

Thus, the germ theory of this century enabled the eradication of most microbial infection through the use of antibiotics and anti-viral drugs. Thus, the rapid and accurate analysis of *P. acne*, an invading organism, is needed.

The medication have several adverse effects like birth defects, erythema, photosensitivity, allergic dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression etc. Many remedies have been employed to treat acne from long period. Most of the remedies were taken from plants and proved to be useful, scientifically established except for a few plants and some proprietary composite herbal drugs. The cosmetics available in the market are not reasonable for everyone thus an effort has been made to study their properties for anti acne activity and to incorporate these extracts in the formulations. The product may be cost effective. This has given rise to stimulation in the search for investigating natural resources showing anti-acne activity³⁻⁵.

Ayurveda, the traditional system of Indian medicine described a number of plants which are utilized in skin care preparations. In the present study we have selected different plants, *Curcuma aromatica*, *Curcuma amada*, *Curcuma zedoaria* and *Adina cordifolia* which have been utilized by the villager for acne or the other skin disorders. In this view the survey of literature has been carried out and in Ayurveda, it has been mentioned that these drugs are

useful in skin disease. Hence, the present work has been selected to give the scientific validity to traditional claims⁶⁻⁹.

Material and Method

2.1 Extraction^{10,11}

The air-dried rhizomes of *Curcuma aromatica*, *Curcuma amada* and *Curcuma zedoaria* and bark of *A.cordifolia* were reduced to coarse powder, separately, and subjected to soxhlet extraction using soxhlet. The solvents used were (70%) ethanol. Fresh material subjected to cold maceration with chloroform water.

2.2 Animal Selection

Wistar albino mice of either sex (25-30gm) were selected for acute toxicity studies and Sprague-Dawley male rats (180-220gm) were selected for *In-vivo* anti-acne activity. The animals were acclimatized to standard laboratory conditions of temp (25^o±2^oC) and maintained on 12:12 h light: dark cycle. Procured from the animal house, Sri. Venkateshwara Enterprises, Bangalore (CPCSEA Reg. No.276), They were provided water *ad libitum*. The animal care and experimental protocols were in accordance with CPCSEA/IAEC guidelines.

2.3 Acute Oral Toxicity¹²

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) revised draft guidelines 423 B ("Up and Down" method) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.4 Assessments of Anti acne *In-vivo* Models¹³⁻¹⁶

Based on the pilot screening the following protocol was carried out. In pilot screening 6 rats were taken under study which showed that the granulomatous inflammation remain constant from day 6th day to 10th day (Table No.1).

Table 1: Protocol study for in-vivo anti acne activity on male Sprague Dawley rats

Groups	Induction of Acne	Treatment
1) Normal control	No induction	Vehicle
2) control (acne induced)	Heat killed P. acnes in PBS	Vehicle
3) Treated with Standard	Heat killed P. acnes in PBS	Clindamycin 200mg/kg b.w.,p.o.
4) Treated with AIE <i>C.aro</i>	Heat killed P.acnes in PBS	AIE <i>C.aro</i> 200mg/kg b.w.,p.o
5) Treated with AqE <i>C.aro</i>	Heat killed P.acnes in PBS	AqE <i>C.aro</i> 200mg/kg b.w.,p.o
6) Treated with AIE <i>C.ama</i>	Heat killed P.acnes in PBS	AIE <i>C.ama</i> 200mg/kg b.w.,p.o
7) Treated with AqE <i>C.ama</i>	Heat killed P.acnes in PBS	AqE <i>C.ama</i> 200mg/kg b.w.,p.o
8) Treated with AIE <i>C.zedo</i>	Heat killed P.acnes in PBS	AIE <i>C.zedo</i> 200mg/kg b.w.,p.o
9) Treated with AqE <i>C.zedo</i>	Heat killed P.acnes in PBS	AqE <i>C.zedo</i> 200mg/kg b.w.,p.o
10) Treated with AqE <i>A.cord</i>	Heat killed P.acnes in PBS	AqE <i>A.cord</i> 200mg/kg b.w.,p.o
11) Treated with AIE of <i>A. cord</i>	Heat killed P.acnes in PBS	AqE <i>A.cord</i> 200mg/kg b.w.,p.o

Thus, depending on the protocol given, the animals were divided into 11 groups containing 6 in each and kept in metabolic cages. All animals had free access to regular rat show and drinking water *ad libitum* during the study.

2.4.1 Induction of acne by *Propionibacterium acnes*

The acne like inflammatory model was produced in the ears of male Sprague Dawly rats (180-220g) by subcutaneous injection of heat-killed bacteria (65°C for 30 min).

2.4.2 Measurement of ear thickness

Ear thickness was measured as an index of inflammatory strength and acne. Thickness was measured by using a vernier calliper. Thickness was measured once every day for the first week of induction, then every other day until 35th day.

2.4.3 Histopathology

On the 10th day after the induction of acne, three animals from each group were sacrificed and ears were excised and fixed in 10% formalin (pH 7.2) and then embedded in paraffin and thick sections were taken to stain using hematoxylin-eosin dye and mounted in diphenyl xylene and observed for the changes. And other 3 animals were observed till 35th day.

Results

In the present study, the whole plants of *Curcuma aromatica*, *Curcuma zedoaria*, *Curcuma amada* and *Adina cordifolia* were collected from the local areas

of Uttrakhand and authenticated from Dept. of Botany, Govt. Post Graduate College, Pithoragarh. The voucher specimen of the same plants has been deposited in the Herbarium of Dept. of Botany, Govt. Post Graduate College, Pithoragarh.

After the effective extraction, the solvents were distilled off. The extract was then concentrated on water bath. Percentage yield was calculated. In the present study i.e 200 mg/ kg b.w. of the extracts were selected, randomly, after the acute toxicity studies.

The acne-like inflammatory model was produced in the ears of rats by subcutaneous injection of 140 µg of heat killed *Propionibacterium acnes*. Ear thickness was measured as an index of inflammatory strength, using a micro indicator once every others day for the first week, then every other day until the 35th day.

The result of the extracts were comparable with standard. The data resulted from anti-acne effect of ethanolic and aqueous extracts of Rhizomes *C. aromatica*, *C. zedoaria*, *C. amada* and bark of *A. cordifolia* showed that ethanolic extracts of the above drugs decreased the inflammation in rats ear.

On the 10th day there was a significant decrease (p<0.01) in inflammation (0.189±0.0056, 0.192±0.0024, 0.221±0.027, 0.269±0.0214) by ethanolic extract of *C. zedoaria*, *C. aromatica*, *C. amada* and *A. cordifolia* respectively. In aqueous extract there was significant (p<0.05) decrease in inflammation (0.280±0.0056, 0.294±0.0260, 0.339±0.0047, 0.376±0.0370) *C. zedoaria*, *C. aromatica*, *C. amada* and *A. cordifolia* respectively.

Thus the Table (2) reveals that the maximum inflammation on ear was on 3rd day in all groups. In test group on 5th day there was a sudden decrease in inflammation which was constant till 10th day and at around 20th day the inflammation reduced and came to normal. The observations were made till 35th day where the thickness was found to be normal.

Histological changes

As per the preliminary studies carried out in animals, the thickness of the ear (inflammation) was observed on 10th day. The thickness was found to be constant between 6th to 10th days. Hence, histopathology of the ear was assessed on 10th day. In histopathology study it was found that accumulation of neutrophils on

inflammatory lesions site with subsequent rupture of the follicle and formation of a pustule in the dermis and the transmigration of lymphocytes into the wall of the follicle associated with increasing spongiosis of the follicular epithelium (Figure 1). During 24-72 hours, the accumulation of neutrophils within the follicle led to its distension and subsequent rupture. There was a localized loss of the granular layer in the region of the eventual rupture. This shows the difference of normal and acne- induced ear section. The treated ear section showed no infiltration in the case of standard drug which was similar to the normal (Figure 1). The histopathology study supports the results shown in (Table No.2).

Table 2: Effect of Clindamycin (standard), AqE *C.aro*, AIE *C.aro*, AqE *C.ama*, AIE *C.ama*, AqE *C.zedo*, AIE *C.zedo*, AqE *A. Cord*, AIE *A. cord*.

SL. No.	GROUP	Mean thickness ±SEM					
		Day1	Day3	Day5	Day6	Day7	Day10
1	Control	1.436±0.016	1.369± 0.012	1.271± 0.021	1.265± 0.026	1.265± 0.023	1.265± 0.020
2	Clindamycin	1.351±0.0054***	1.270± 0.0031***	0.1932±0.0018***	0.1052±0.0048***	0.1052±0.0047***	0.1052±0.0046***
3	AqE <i>C.aro</i>	1.411±0.0010*	1.336± 0.0042*	0.306±0.0040*	0.294±0.0263*	0.294±0.02618*	0.294±0.0260*
4	AIE <i>C.aro</i>	1.389±0.0038**	1.303±0.0040**	0.263±0.0014**	0.192±0.0027**	0.192±0.0025**	0.192±0.0024**
5	AqE <i>C.ama</i>	1.416±0.0016*	1.339±0.0041*	0.394±0.0021*	0.339±0.005*	0.339±0.0048*	0.339±0.0047*
6	AIE <i>C.ama</i>	1.391±0.024**	1.315±0.023**	0.269±0.022**	0.221±0.029**	0.221±0.026**	0.221±0.027**
7	AqE <i>C.zedo</i>	1.409±0.0022*	1.329±0.0017*	0.296±0.0024*	0.280±0.0058*	0.280±0.0057*	0.280±0.0056*
8	AIE <i>C.zedo</i>	1.357±0.0051**	1.293±0.0026**	0.251±0.0019**	0.189±0.0059**	0.189±0.0058**	0.189±0.0056**
9	AqE <i>A. cord</i>	1.420±0.0180*	1.340±0.0150*	0.399±0.0190*	0.376±0.0374*	0.376±0.0371*	0.376±0.0370*
10	AIE <i>A. cord</i>	1.399±0.0410**	1.321±0.0170**	0.281±0.0230**	0.269±0.0217**	0.269±0.0215**	0.269±0.0214**

***P<0.001, **P<0.01, *P<0.05

Graph 1: Effect of Clindamycin (standard), AqE *C.aro*, AIE *C.aro*, AqE *C.ama*, AIE *C.ama*, AqE *C.zedo*, AIE *C.zedo*, AqE *A. Cord*, AIE *A. cord*

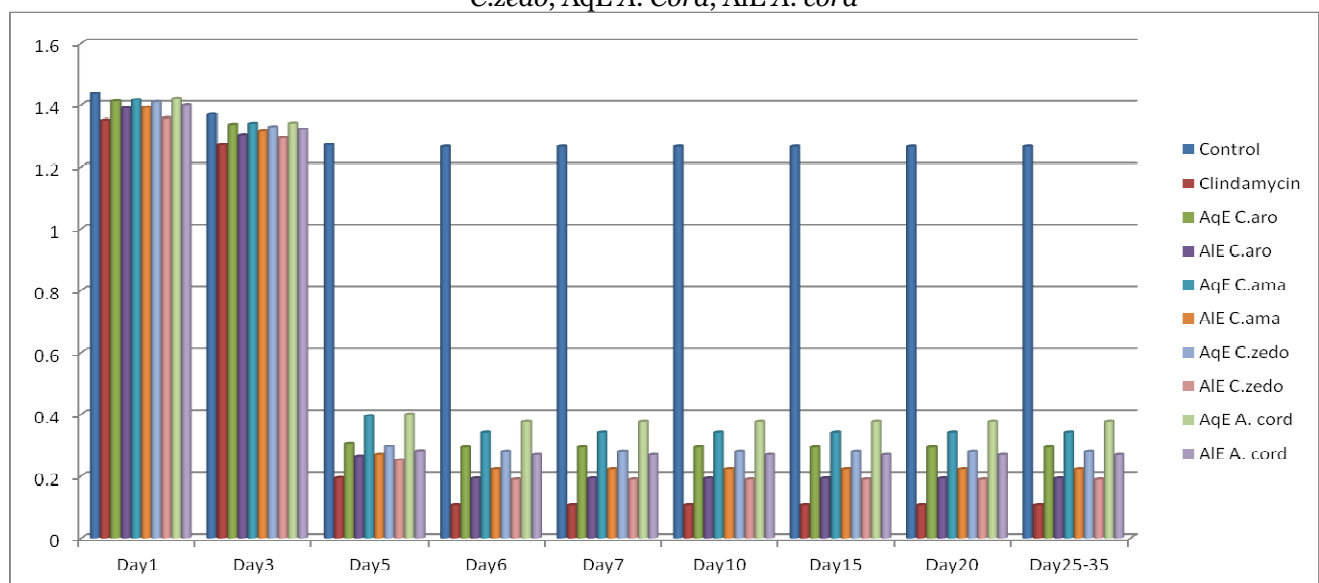
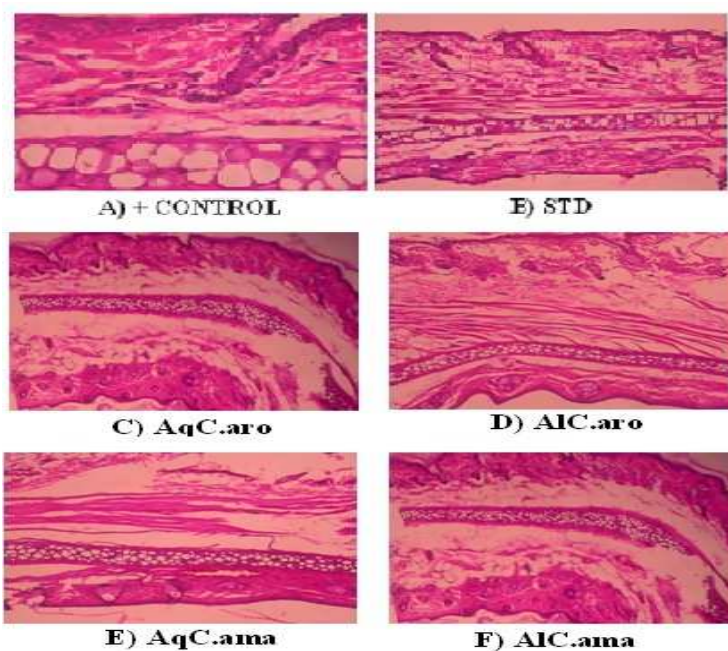
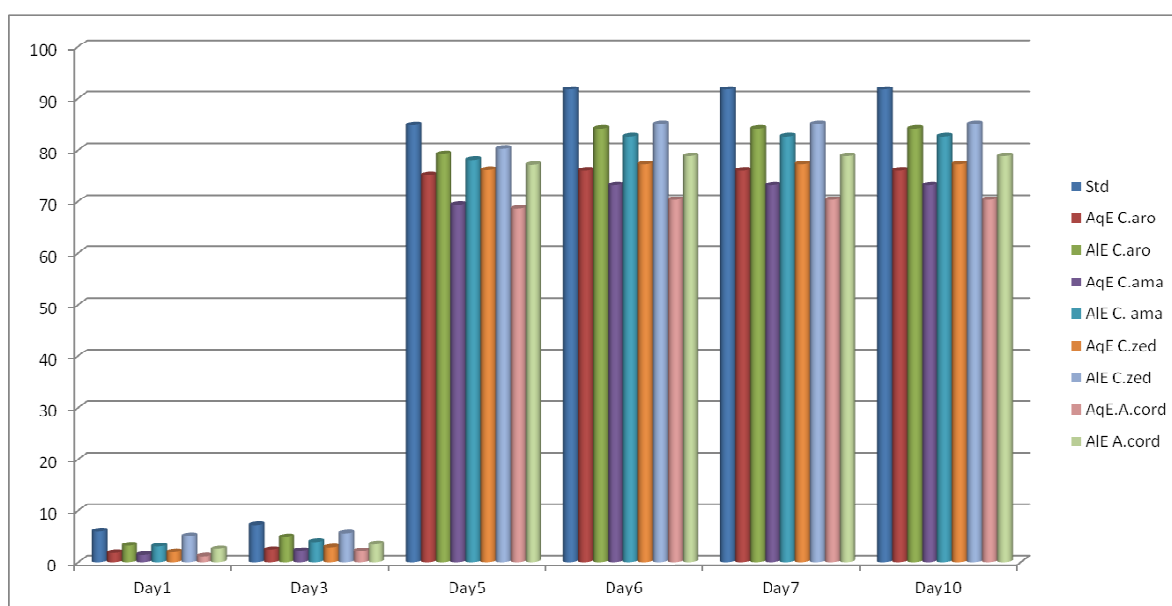


Table 3: Percentage inhibition of *P. acne* induced granulomatous inflammation treated with std. AqE *C.aro*, AIE *C.aro*, AqE *C.ama*, AIE *C.ama*, AqE *C.zedo*, AIE *C.zedo*, AqE *A. Cord*, AIE *A. cord*

Sl. No.	Test Material	Percentage Inhibition (%)					
		Day1	Day3	Day5	Day6	Day7	Day10
1	Std	5.92	7.23	84.79	91.68	91.68	91.68
2	AqE <i>C.aro</i>	1.74	2.41	75.13	76.01	76.01	76.01
3	AIE <i>C.aro</i>	3.27	4.82	79.20	84.13	84.13	84.13
4	AqE <i>C.ama</i>	1.39	2.19	69.30	73.12	73.12	73.12
5	AIE <i>C. ama</i>	3.13	3.94	78.01	82.52	82.52	82.52
6	AqE <i>C.zed</i>	1.88	2.92	76.11	77.20	77.20	77.20
7	AIE <i>C.zed</i>	5.01	5.55	80.25	85.00	85.00	85.00
8.	AqE. <i>A.cord</i>	1.11	2.11	68.60	70.27	70.27	70.27
9.	AIE <i>A.cord</i>	2.57	3.50	77.10	78.73	78.73	78.73

Graph 2: Histogram showing the effect of test materials on percentage inhibition of inflammation *P. acne* induced acnes in rat's ear



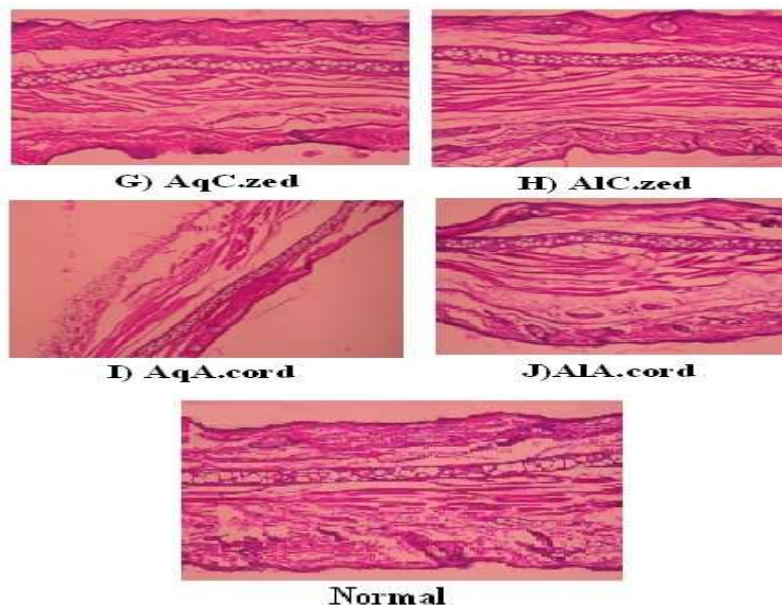


Figure No.1 Histological changes observed in the ear of rats

Discussion

Acne vulgaris is a chronic inflammatory disease results in the formation of inflamed and/or non-inflamed eruptions *Propionibacterium acnes* are the anaerobes, in the skin which grow in the sebaceous region.

Various antibiotics like tetracycline, Clindamycin, and erythromycin etc and other drugs like benzoyl peroxide are used for acne treatment. The various drawbacks of synthetic drugs are different side effects and resistant developed towards these drugs.

Herbal therapy is required to overcome the above drawbacks and treat the acne. So in the present study four plants (*C. aromatica*, *C. amada*, *C. zedoaria* and *A. cordifolia*) were selected for the anti acne activity. The preliminary phytochemical study was carried out according to standard literature. This revealed that they contain various phytoconstituents which can be responsible for the anti acne activity. The extracts were subjected to antimicrobial activity against *Propionibacterium acnes* (1 µg/ml). The MIC obtained from the various extracts against *P. acnes* suggests that the ethanolic extracts of the plants showed significant antimicrobial activity.

Dried rhizomes of *Curcuma* species and bark of *A. cordifolia* were powdered. The results of powdered

crude drug material and their extracts were compared with the literature^{85,86}. As per literature review various phytoconstituents like curcuminoids, alkaloids, triterpenoids and flavonoids are proved to possess antibacterial activity^{53,62,75}.

The acne like inflammatory activity was carried out by measuring the ear thickness and histopathological studies of the ear. Ethanolic extracts of all the four plants showed significant reduction in the ear thickness. It seems that the increased ear thickness and inflammation caused due to various biochemicals, *viz.* various kinins, histamine and 5-HT is significantly reduced. Curcumin and its derivative are the active anti-inflammatory constituents. The anti-inflammatory activity of curcumin may be due to its ability to scavenge oxygen radicals which has been implicated in inflammation process. All the four plants have proved to possess anti-inflammatory activity¹⁰⁴⁻¹⁰⁶.

The ethanolic extracts of all four plants showed significant reduction in the over all damage caused due to *P. acne* which can be seen in the histopathological studies. The literature review shows that inflammation is caused due to ROS (Reactive Oxygen Species). The ethanolic extracts of *Curcuma* species contain curcuminoids, alkaloids,

flavanoids etc. The above phytoconstituents were proved potent anti-oxidants. The presence of various phytoconstituents including the curcuminoids in *Curcuma* spp. and flavanoids, alkaloids in *A. cordifolia* showed significant antiacne properties which is supported by the antimicrobial and histopathological studies.^{54-56,63,70,72}

Conclusion

In conclusion, the presented data indicate that the administration of ethanolic and aqueous extracts of rhizomes of *C. aromatic*, *C. zedoria*, *C. amada* and bark of *A. cordifolia* decreased inflammation and showed antibacterial activity. The ethanolic extracts have potent anti-acne activity compare to aqueous extracts.

Acknowledgement

I thank Professor R.V.Karadi for his guidance and I would also like to thank the whole Pharmacognosy Department of K.L.E. University for their support.

References

- 1) *Dorland's Illustrated Medical Dictionary*. 30th ed. Saunders, 2000; 18-19.
- 2) Chen YL, Yu CK, P. acne induces acute TNF α -mediated apoptosis of hepatocytes followed by inflammatory T-cell-Mediated Granulomatous Hepatitis in Mice". *J Biomed Sci*. 1996; 349-56.
- 3) Lalla J.K, S.Y. Nandedkar, M.H. Paranjape, N.B. Talreja et al., "Clinical trials of ayurvedic formulation in the treatment of acne vulgaris". *J ethpharm* 2001; 99-102.
- 4) Kubo Isao, Hisae Muroi, Aya Kubo et al., "Naturally occurring antiacne agents". *J Nat.Prod* 1994; 9-17.
- 5) Jain A, E Basal et al., "Inhibition of Propionibacterium acnes- induced mediators of inflammation by Indian herbs". *Phytomedicine* 2003; 34-38.
- 6) Yamaguchi N et al. "In-vitro evaluation of antibacterial, anticollagenase, and antioxidant

activities of hop components addressing acne vulgaris". *Phytomed* 2009; 16(1):369-76.

- 7) Rastogi RP et al. *Compendium of Indian Medicinal Plants*. Vol.3rd. Central Drug Research Institute; Lucknow; 1980-1984; 241.
- 8) Arya VS. *Indian medicinal plants-A compendium of 500 species*. 2nd ed., Vol. 2nd, Chennai: Orient Longman Ltd; 1998; 2419.
- 9) Nadkarni AK. *Indian Materia Medica*. 3rd ed., Vol. 1st, Bombay: Popular Prakashan Limited; 1986; 413-15.
- 10) Mukherjee P.K., "Quality Control of Herbal Drugs- an approach to evaluation of Botanicals". 1st ed. Business Horizons Pharmaceutical Publications, New Delhi; 2002.
- 11) Khandelwal KR. *Practical Pharmacognosy, Techniques and Experiments*, 9th ed., Pune, Nirali Prakashan. 2002; 157-9
- 12) OECD – Organisation for Economic Co-operation and Development, Guidelines on Acute Oral Toxicity. (Revised Document, October 2000).
- 13) Toho M *et al*. "Immunohistochemical studies of the acne-like inflammatory model". *Jikken Dobutsu*. 1990; 39(4): 531-7.
- 14) De Young L.M *et al*., "Acne- like chronic inflammatory activity of P.acne preparation to stimulate the reticuloendothelial system". *J Invest Dermatol* 1985; 85(3):255-58.
- 15) Vogels G.H., Vogels W.H., "Pharmacological assay". 2nd ed. Berlin, Heiderberg, New York: Springer-Verlag; 1997.
- 16) Shuichi Higaki *et al*., "Propionibacterium acnes biotypes and susceptibility to minocycline and Keigai-rangy-to". *J of Dermatol* 2004; 43:103-07.

SJR SCImago
Journal & Country
Rank

Powered by
SCOPUS™