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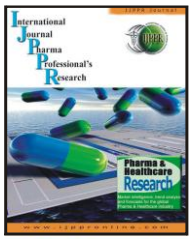
Short Communication



ANTIACNE ACTIVITY OF SOME INDIAN HERBAL DRUGS

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Abstract

Acne is a common disorder of pilosebaceous follicle which mainly affects the teenagers up to 95% boys and 83% girls due to the hormonal changes. Modern acne therapy which includes comedolytic agents, antibiotics and various anti-inflammatory agents. has many side effects due to prolonged therapy. Excessive and prolonged use of antibiotics has lead to the development of resistance in acne causing bacteria, viz , *Propionibacterium acne* and *Staphylococcus epidermidis.* In the present study certain herbal drugs such as *Camellia sinensis* (Green tea) , *Coscinium fenestratum* (Wood turmeric) , and *Quercus infectoria* (Nut galls), were chosen based on their antibacterial activity against other Gram+ve and Gram-ve bacteria. *Coscinium fenestratum* was found to be effective against *S.epidermidis*. and *Quercus infectoria* showed good activity against *P.acne.* where as *C.sinensis* was found effective against *S. epidermidis*, but did not show any activity against *P.acne.* Further research are required to isolate and characterize the active principles present in these drugs.

Key words: Propionibactrium acne, Staphylococcus epidermidis,

Introduction

Acne Vulgaris is defined as a chronic inflammatory disorder of pilosebaceous follicle,It is almost a universl disease occouring in all races and affecting 95 % of 16 year old boys 83% of 16 year old girls to some degree . The incidence of severity of acne peak at 40% in 14-17 year old girls and 35% in boys aged 16-19. [1]

Modern acne therapy has been designed to interrupt the the pathogenic pathway at one or more points. Topical or systemic therapy is available for the treatment of acne. Topical threrapy includes comedolytic agents and antibiotics and various anti-inflammatory drugs .Systemic therapy includes antibiotics, zinc and hormones. [2]

With the excessive use of antibiotics for long periods has lead to the increased reasistance in acne causing bacteria ie Propionibacterium acne and Staphylococcus epidermidis, against a number of antibiotics used of treat acne.[3] A long term therapy is required for the treatment of acne so there are more chances of occourance of adverse effects due to these medication. Some of the reported adverse effects reported are lupus erythematosus, serum sickness like reaction, autoimmune hepatitis, pigmentation of skin, alveolar bone pneumonitis.[4]. There is an increased interest among patients seeking alternative treatment for such conditions. Present study is done to study the effect of some Indian herbal drugs such as *Camellia sinensis* (Green tea) [5], *Coscinium fenestratum* (Wood turmeric) [6], and *Quercus infectoria* (Nut galls) on acne causing bacteria.

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Material and Methods

Extraction

The drugs were extracted using soxhlet apparatus with solvents such as petroleum ether chloroform, ethyl acetate, and methanol, successively. Different extracts were collected and subjected for phytochemical evaluation.

Preliminary phytochemical screening of drugs

Methanolic extracts of *C sinensis, C fenestratum, and Q.Infectoris,* were found to show good antimicrobial activity. Chemical test for the extracts were performed for different constituents. *C.Sinensis*,tested positive for Phenols and tannins. *C.Fenestratum* tested positive mainly for alkaloids where as carbohydrates, phenols and tannins were present in lesser amounts. *Q.Infectoria* tested positive mainly for phenols, where as carbohydrates were also present. **[7]**

Preliminary antibacterial screening

All the extracts were screened for antibacterial activity against two gram positive bacteria,*Bacillus subtilus*(NCIM-2063),*Staphylococcus aureus*(NCIM2075), and two gram negative bacteria *Eschrechia coli* (NCIM2065)and *klibsella pneumonae* (NCIM2957)The organism were procured from Microbial type culture collection (MTCC), Chandigarh.

Suspension containing $1x10^6$ cells/ml were prepared in saline as per Macfarlands standard.

Culture medium

Nutrient agar medium was used for the preliminary antibacterial studies

Composition	Quantity
Beef extract	3 gm
Peptone	5 gm
Agar	15 gm
Distilled water	1000 ml

Procedure

The medium was prepared by dissolving all the ingredients in distlled water and subjecting it to sterilization in a autoclave at 121ºC. The petriplates were washed thoroughly and sterilized in hot air oven at 160ºC for 1.5 hours. 30 ml of nutrient agar was seeded by organism (about 2 ml concentrations according to McFarland's standard) and poured into sterile plates and allowed to cool. Bores were made on the medium using sterile borer and 0.1 ml (5000µg/ml) of extracts we added to respective bores. 0.1 ml of Gentamycin at a concentration of 300µg /ml was taken as standard reference. The petri plates were kept in refrigerator at 4ºC for 1hr diffusion. After diffusion the petri dishes were incubated at 37±1ºC for 24 hr and zone of inhibition was observed and measured using scale.

Table 1: Antibacterial activity of methanolic extracts of selected drugs and gentamycin

S. No	Plant extracts	Zone of Inhibition in mm			
		B.subtilus	E.Coli	K.Pneumonae	S.aureus
1	Quercus infectoria	25	24	23	23
2	Coscinium fenestratum	18	12	17	16
3	Camelia sinensis	23	22	24	22
4	Gentamycin	30	27	27	27

Antibactrial activity of extracts against *Staphylococcus epidermidis*

Staphylococcus epidermidis a one of the bacteria reported to be associated with acne [8]. in the present study the methanolic extracts were tested against *Staphylococcus epidermidis* to establish their antiacne activity. The extracts were tested for antibacterial activity using well diffusion and Broth dilution method.

Staphylococcus epidermidis MTCC-435 was purchased from microbial type culture collection and gene bank, Chandigarh. The freeze dried microorganism was revived by suspending it in 0.9% NaCl and kept at 37±1ºC for ½ hr.The suspension of *S. epidermidis* was streaked on sterile nutrient agar and incubated at 37±1ºC for 24 hr. Colonies were observed on nutrient agar.

Zone of inhibition of methanolic extracts at a concentration of 5000µg/ml were calculated as per the procedure described above and results were tabulated.

Table 2: Antibacterial activity of methanolic extracts against *staphylococcus epidermidis*.

Organism	Zone of Inhibition in mm			
	Quercus infectoria	Coscinium fenestratum	Camelia sinensis	Gentamycin
staphylococcus epidermidis	18	22	18	23

Minimum inhibitory concentration of methanolic extracts are also calculated as per the procedure described by [9]

Table 3. Minimum inhibitory concentration of methanolic extracts against *staphylococcus epidermidis*

Organism	Concentration in µg/ml of extract against *S.epidermidis*			
	Quercus infectoria	Coscinium fenestratum	Camelia sinensis	Gentamycin
Staphylococcus epidermidis	500	400	1000	50

Antibactrial activity of extracts against *Propionibacterium acnes*

Propionibacterium acne is an anaerobic diptheroid which is involved in pathogenesis of acne [10]. In the present study the antibacterial screening against *Propionibacterium acnes* was performed using Broth dilution method.

Propionibacterium acnes, MTCC 1951 was purchased from microbial type culture collection and gene bank, Chandigarh. The freeze dried microorganism was revived by shaking it with nutrient yeast glucose broth and kept at 37±1ºC in a anaerobic jar for 24 hr.

In the present study Rabbit blood agar is used for maintaining the cultures and Nutrient yeast extract glucose was used to find out the MIC of extracts.

Culture medium

Rabbit blood agar medium

To prepare rabbit blood agar medium all the ingredients except the blood were dissolved and the solution was sterilized at 121º for 15 minutes. It was then cooled to 50º and 5% sterilized difibrinated rabbit blood was added. The revived culture of *P.acnes* was streaked on rabbit blood agar plates using a loop, the plates were then kept in incubator fo

48 hr at 37±1ºC. After 48 hr colonies of the organism were observed.

Minimum inhibitory concentration was found out using liquid cultures of Nutrient yeast extract glucose broth in which a loop full of revived cultures were transferred and kept in anaerobic jar gas pack and indicator strips . The jar was kept in incubator for 48 hr at 37±1ºC. After 48 hr colonies of organism were observed as turbidity.

Table 3. Minimum inhibitory concentration of methanolic extracts against *P.acnes.*

Organism	Concentration in µg/ml of extract against *S.epidermidis*			
	Quercus infectoria	**Coscinium fenestratum**	**Camelia sinensis**	**Gentamycin**
Propionibacterium acnes	500	1000	--------	100

Results and Discussion

In the present study, the preliminary antibacterial screening of methanloic extracts of *Q.Infectoria ,C.fenestratum, and C.sinensi*s were found to be effective against *E.Coli, K.Pneumonae, S.aureus and B.subtilus*. The extracts were then screened for their activity against acne causing bacteria *Staphylococcus epidermidis and Propionibacterium acnes* using well diffusion and MIC techniques.

C.fenestratum was found to be most effective against *S.epidermidis.* of all the the three extracts with the least MIC of 400 µg/ml where as *C.sinensis* was found to be least effective with the highest 1000 µg/ml concentration.

In case of activity of extracts against *P.acne* ,methanolic extract of *Q.Infectoria* was found to be most effective with least MIC of 500 µg/ml followed by *C.fenestratum* which was effective at a dose of 1000 µg/ml but surprisingly *C.sinensis* did not show any activity against *P.acne.* though it was effective against *S.epidermidis.*

Conclusion

C.fenestratum an alkaloidal drug was effective against aerobic acnegenic bacteria *S.epidermidis.* and tannin containing drug *Q.Infectoria* was found effective against anaerobic acnegenic bacteria *P.acne.* Further research is needed to isolate and the characterize the novel compounds present in these drugs which can help to formulate effective dosage forms to combat this disease.

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