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Original Research Manuscript

AN EVALUATION OF ANTIMICROBIAL EFFICACY OF ACNANO AGAINST SOME ACNE CAUSING MICROORGANISMS

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ABSTRACT

This study was undertaken to determine the antimicrobial susceptibility of Acnano (a polyherbal nano-emulsion) against acne causing microbes Propionibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis along with some other dermal disease causing microbes such as Pseudomonas aeruginosa, Morganella morganii, Enterobacter cloacae, Escherichia coli, Citrobacter braakii, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus vulgaris, yeast such as Saccharomyces cerevisiae and fungi Candida albicans and Aspergillus niger. The agar well diffusion method, approved by NCCLS with the modification, was used. Student t test was performed using One way Analysis of Variance (ANOVA). This study showed that Acnano possesses potent antimicrobial activity against bacteria as well as fungi.

Key Words : *Acnano, antibacterial susceptibility, P. acnes, S. aureus, S. epidermidis*

Introduction

Acne is an inflammatory skin disease characterized by pimples on the face. It affects individuals of all races covers 85% of teenagers, 42.5% of men, and 50.9% of women between the ages of 20 and 30 years[1,2]. Spontaneous regression usually occurs after age 20, but some patients may continue suffering during adult life[3].

In 2001, the global market for prescription acne products was estimated to be two billion dollars and the non-prescription market was estimated at two to four times of that size[4].

Lot of products are being developed to combat acne such as topical retinoids, benzoyl peroxide, salicylic acid etc. but due to high prevalence of antibiotic-resistant strains of *Propionibacterium acnes*, topical antibiotics are no longer effective as monotherapy[5]. Keeping this antibiotic resistance in consideration, Venus Medicine Research Centre, Baddi (India) has developed an anti-acne herbal nano-emulsion which is a perfect blend of essential oils and lemon effective against mild, moderate as well as chronic acne as well as other skin diseases including fungal diseases. It is an aromatic, clear, transparent light golden yellow viscous nano-emulsion which contains *Melaleuca alternifolia oil, Rosmarinus officinalis* oil, *Mentha arvensis* oil along with *Citrus limon.*

This study was undertaken to determine the antimicrobial susceptibility of Acnano against acne causing microbes i.e., *Propionibacterium acnes, Staphylococcus aureus & S. epidermidis* and other microorganisms in comparison with standard Adapalene (0.1%) .

Material and Methods

Acnano (Batch No. RND09H01, Mfg August 2009) was procured from Venus Medicine

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Research Centre, Baddi (India). It was kept at room temperature away from direct light & moisture. Adapalene was purchased from Enaltec Labs Pvt. Ltd., Navi Mumbai - 400614, India.

Selection of microorganism :

All the microorganisms (*Staphylococcus aureus, S. epidermidis & Propionibacterium acnes, Pseudomonas aeruginosa, Morganella morganii, Enterobacter cloacae, Escherichia coli, Citrobacter braakii, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus vulgaris, A. niger, C. albicans* and *S. cerevisiae*) used in the present study were Microbial Type Culture Collection (MTCC No. as per tables – 2, 3 and 4), purchased from Institute of Microbial Technology, Sector 39-A, Chandigarh – 160036, India.

Media and reagents:- Muller Hinton agar, Nutrient Broth, Sabouraud dextrose agar, Blood agar base, Barium chloride and Sulphuric acid.

Preparation of Reagents/Buffers/Standards

Muller Hinton agar, Sabouraud dextrose agar, Blood agar base medium and Nutrient broth were prepared as per manufacturer's (Hi Media Laboratories) instructions. Sterilized by autoclaving at 15 lbs pressure and 121°C for 15 minutes.

1. Preparation of Mueller Hinton Agar, Sabouraud Dextrose Agar and Blood agar Plates:-

Immediately after autoclaving, it was allowed to cool in a 45 to 50°C water bath. Aseptically add 5% v/v sterile defibrinated blood to blood agar base medium. Freshly prepared and cooled medium were poured into a flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4mm corresponding to 15 to 20 ml of medium for each plate with diameter of 90 mm. The agar medium was allowed to cool to room temperature. A representative sample of each batch of plates examined for sterility by incubating at 35 °C for 24 hrs .

2. Inoculation Preparation:-

At least three to five colonies of the same morphological type are selected from overnight plates cultures on non selective agar medium. The top of each colony is touched with a loop and the growth is transferred into a tube containing 4-5 ml of sterile Nutrient broth medium to produce a suspension which match the turbidity standard of 0.5 McFarland standard.

3. Preparation of McFarland Standard:-

It is prepared by adding 0.5ml of 0.048 M $BaCl_2$ (1.172 % w/v $BaCl_2.H_2O$) to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring. Using matches cuvettes with 1 cm. path length and water a blank standard the absorbance in a spectrophotometer at wave length of 625nm. the acceptable range of standard is 0.08 – 0.13.

4. Inoculation of Test Plates :-

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removes excess inoculum form the swab. The dried surface of a MH agar plates is inoculated with bacterial culture suspension except *P. acne* by streaking the swab over the entire sterile agar surface. The blood agar plates is inoculated with *P. acne* culture suspension and sabouraud dextrose agar plates is inoculated with fungus and yeast culture suspension by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approx. 60° each time to ensure an ever

distribution of inoculum. As a final step, the rim of the agar is swabbed. Allowed the plates to dry until there is no visible surface moisture.

5. Boring in Petri-plates :-

Created two bores in petri plates by using borer which has 6mm diameter and sterilized by dry heat sterilization (DHS). Individually, 100 micro liter of each Acnano and Adapalene (0.1%) were added in two bores each in each plate. All bacterial plates and their replicates were incubated at 35^{O}C for 24 hours whereas fungal plates were incubated at 25^{O}C for 96 hours. The bacterial plates of *P. acnes* were incubated anaerobically at 35^{O}C for 24 hours. Zone of inhibition was measured by antibiotic zone reader.

Results and Discussion

Already known active constituents of individual ingredients of Acnano have been given in Table 1.

Propionibacterium acnes, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the major causal organism of acne[6,7,8]. *Propionibacterium acnes* is a relatively slow growing, typically aero-tolerant anaerobic gram positive bacterium that is linked to the skin condition acne. When a pore is blocked the bacterium overgrows and secretes chemicals that break down the wall of the pore, spilling bacteria such as *Staphylococcus aureus* onto the skin, and forming an acne lesion (folliculitis). *P. acnes* is an inhabitant of normal skin flora. It is implicated in the development of inflammatory acne by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils[9].

Zone of Inhibition (mm) of Acnano against these microorganisms was found to be better than the standard – Adapalene (0.1%)

Zone of Inhibition (mm) of Acnano against *Propionibacterium acnes* was found to be two times more than that of Adapalene (Table 2, Fig.1). Microbial efficacy of *S. epidermidis* & *S. aureus* were also better than the standard (Fig.2&3). Student t test was performed using One way Analysis of Variance (ANOVA). P value of *Staphylococcus aureus, S. epidermidis & Propionibacterium acnes.* was found to be highly significant $p<0.001$.

Table1: Ingredients of Acnano and their anti-microbial active components

Ingredient	Active constituents
Melaleuca alternifolia oil (Tea tree oil)	Terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole etc.
Rosmarinus officinalis oil (Rosemary oil)	p-cymene, linalool, γ-terpinene, β-pinene, α-pinene, eucalyptol etc.
Mentha arvensis oil (mint oil)	Menthol
Citrus limon	Citric acid and ascorbic acid

Table 2: Antibacterial Susceptibility Test (AST) of Acnano against *Staphylococcus aureus, S. epidermidis & Propionibacterium acnes.*

Name of micro-organism	MTCC No.	Zone diameter in mm	
		Adapalene as standard (0.1%)	Acnano
Staphylococcus aureus	*737*	15.26±0.19	16.32±0.14
Staphylococcus epidermidis	435	11.85±0.11	13.82±0.15
Propionibacterium acnes	*1951*	11.23±0.12	23.39±0.10

Table 3: Antibacterial Susceptibility Test (AST) of Acnano against *Pseudomonas aeruginosa, Morganella morganii, Enterobacter cloacae, Escherichia coli, Citrobacter braakii, Klebsiella pneumoniae, Acinetobacter baumannii & Proteus vulgaris.*

Name of micro-organism	MTCC No.	Zone diameter in mm	
		Adapalene as standard (0.1%)	Acnano
Pseudomonas aeruginosa	*1688*	16.13±0.20	16.56±0.24
Morganella morganii	662	N il	12.41±0.11
Enterobacter cloacae	*441*	11.23±0.10	23.39±0.23
Escherichia coli	739	13.99±0.17	19.29±0.16
Citrobacter braakii	2690	13.79±0.14	17.26±0.18
Klebsiella pneumoniae	109	11.12±0.10	12.44±0.09
Acinetobacter baumannii	1425	13.69±0.11	14.7±0.12
Proteus vulgaris	426	Nil	12.41±0.14

Table 4: Antibacterial Susceptibility Test (AST) of Acnano against *A. niger, C. albicans & S. cerevisiae*

Name of micro-organism	MTCC No.	Zone diameter in mm	
		Adapalene as standard (0.1%)	**Acnano**
Aspergillus niger	1344	5.99±0.06	22.13±0.25
Candida albicans	227	Nil	20.78±0.31
Saccharomyces cerevisiae	170	14.19±0.38	27.17±0.29

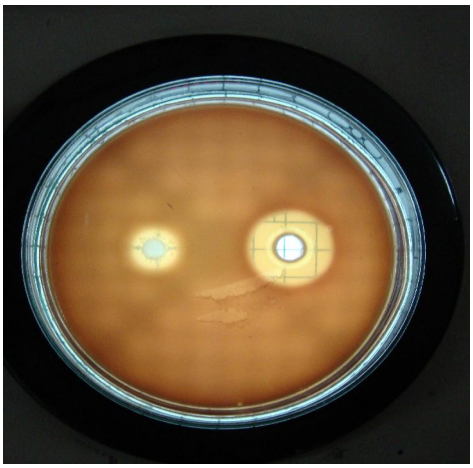

Figure 1 : AST of Acnano against *P.acnes* (MTCC No. - 1951)

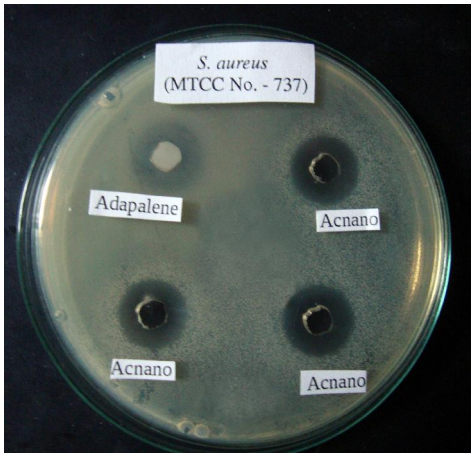

Figure 2 : AST of Acnano against *S.aureus* (MTCC No. - 737)

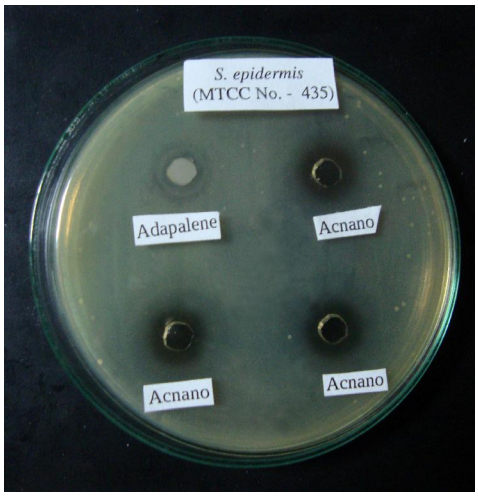

Figure 3 : AST of Acnano against *S.epidermidis* (MTCC No. - 435)

Figure 4 : AST of Acnano against *S.cerevisiae* (MTCC No. - 170)

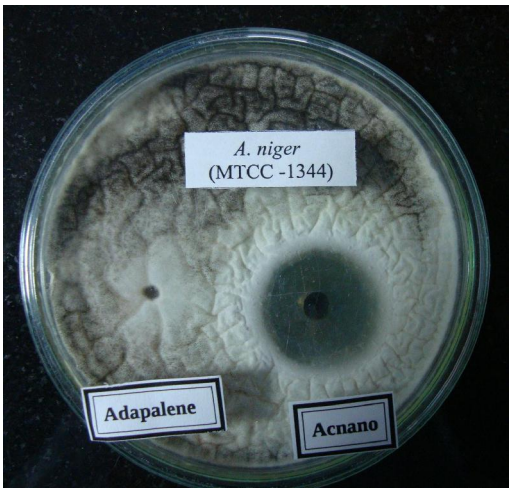

Figure 5 : AST of Acnano against *A.niger* (MTCC No. - 1344)

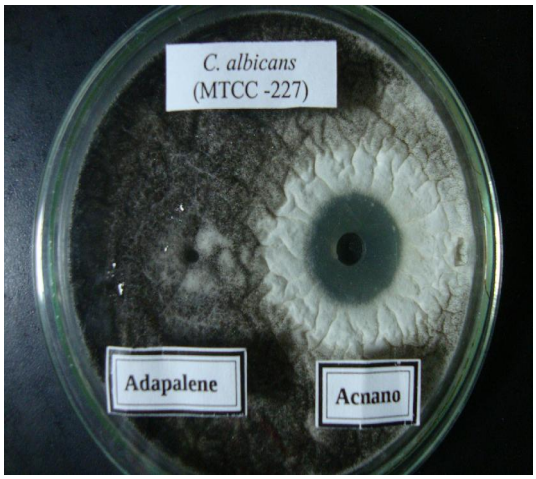

Figure 6 : AST of Acnano against *C.albicans* (MTCC No. - 227)

Besides main causal organism of acne, some other organisms such as *Pseudomonas aeruginosa, Morganella morganii, Enterobacter cloacae, Escherichia coli, Citrobacter braakii, Klebsiella pneumoniae, Acinetobacter baumannii & Proteus vulgaris* have also been associated with skin disorders[10,11,12,13,14,15,16, 17]. Efficacy of Acnano against these organisms was found to be better than that of Adapalene (0.1%) in all the microorganisms studied (Table 3).

Antifungal effects of Acnano were also seen. *Aspergillus niger, Candida albicans and Saccharomyces cerevisiae* are the most common fungi that causes skin diseases[18, 19, 20, 21,22]. To verify that Acnano, besides being anti-acne product, can cure other skin related diseases also, antibacterial susceptiblity of Acnano against these fungi and yeast, was seen. Zone of inhibition of Acnano against *Aspergillus niger,* was found to be 4 times more than that of Adapalene (Fig.5). Efficacy of Acnano against *Saccharomyces cerevisiae* was found to be almost twice of Adapalene (Fig.4). Efficacy of Adapalene against *Candida albicans* was found to be nil but was well exhibited by Acnano (Table 4, Fig.6).

All these results show that Acnano is potent against bacteria, fungi and yeast under study with particular mention of causal organisms of Acne .

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