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Evaluation of Synergistic Activity of Clay and Herbal Medicine against Pyogenic Acne Bacteria

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ABSTRACT

Objective: The aim and objective of the present study was to investigate and evaluate anti-acne efficacy of various clay and herbal extracts which possess antibacterial activity against the acne causing bacteria. **Method:** In the present investigation, acne causing bacteria [*Propionibacteium acne* (n=20), *Staphylococcus aureus* (n=20) and *Staphylococcus epidermidis* (n=20)] were isolated from suspected persons voluntarily. These isolates were identified and confirmed by VITECK® 2 (BioMerieux, France). The confirmed isolates were treated with Neem extract, Turmeric extracts and 4 different types of clays with different concentrations (Yellow, Orange, Black, and Brown) and their synergistic activity was also studied by Disc Diffusion method. Interpretation of the results was according to CLSI guidelines. **Results:** The results of Neem herb (Table 1) showed promising antibacterial activity when the extract was prepared in petroleum ether whereas in the case of turmeric, methanolic extract at a concentration of 200mg/ml showed highest antimicrobial activity against all the isolates of three pyogenic bacteria. Yellow clay, when used in combination with turmeric, showed promising antibacterial activity (Table 4) and this inhibitory effect is also greater that the antibiotic which was used as a control (Gentamycin). **Conclusion:** Natural actives have a better if not comparable inhibitory action against acne causing bacteria when compared with antibacterial activity of clay. The combinatorial effect of natural agents and clay showed profound antibacterial activity. Hence, this therapy may be one of the best choices for dermatologist to treat the acne vulgaris.

INTRODUCTION

Acne is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory (papules, pustules and nodules) and no inflammatory (comedones, open and closed) lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* are common pus-forming microbes responsible for the development of various forms of acne vulgaris. Common therapies that are used for the treatment of acne include topical, systemic, hormonal, herbal and combination therapy. [1] Acne vulgaris is generally characterized by formation of seborrhea, comedones, inflammatory lesions and presence of bacteria *Propionibacterium acnes, Staphylococcus epidermidis* and *Staphylococcus aureus* in the follicular canal and sebum production. [2]

P. acne, a gram-positive an anaerobic pathogen, plays an important role in the pathogenesis of acne. It has been described as an obligate anaerobic microorganism. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. On the contrary, *S. epidermidis*, an aerobic organism, usually involves in superficial infections within the sebaceous unit. When the chemicals produced by *P. acnes* destroy the cellular structure of skin cells, *Staphylococcus aureus*, grows causing acne lesions. These factors provide a potential target for treatment. *P. acnes, S. epidermidis* and *S. aureus* are the target sites of antiacne drugs.[3]

Many allopathic drugs and their combination therapies are used in the treatment of acne vulgaris. But the problem with these drugs and their combination therapies is that they have recorded side effects. With the excessive use of antibiotics for long periods has led to the increased resistance in acne causing bacteria. To overcome the problem of antibiotic resistance, essentials oils and medicinal plant extracts have been extensively studied as an alternative. Herbs are safe, efficacious and multifunctional. Herbal therapies, which have been in use from ancient times for the treatment of acne, include various herbal extracts, oil and their ayurvedic formulation. The introduction of novel herbal formulations for the treatment of acne may produce many advantages over previously used therapies. These herbal drugs are effective against a variety of Gram-positive and Gram-negative bacteria. In addition to herbal medicines, Natural clays have been used to heal skin infections since the earliest recorded history. The ingredients in topical

acne treatments, particularly herbs and naturally derived compounds have received considerable interest as they have fewer adverse effects than synthetic agents. [4]

Owing to this fact in the present study, Neem and Turmeric extracts which have been traditionally used as antimicrobial and anti-inflammatory agents were examined along with and 4 different types of clays (Yellow, Orange, Black, and Brown) for antimicrobial activities against *P. acne, S. epidermidis* and *Staphylococcus aureus* and their synergistic activity was also studied.

MATERIALS AND METHODS

Bacterial strains: A total of 60 bacterial isolates of *Propionibacteium acne* (n=20), *Staphylococcus aureus* (n=20) and *Staphylococcus epidermidis* (n=20) were screened and identified on the basis of morphological, biochemical and cultural characteristics. Further confirmation was carried out by using VITEK®2 Compact identification system. In the procedure, isolated colonies were selected from the primary plate. Theses colonies were transferred aseptically into 3.0ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) into clear plastic (polystyrene) test tubes (12mm× 75mm). This homogenous suspension was then checked for MacFarland No. 0.05 to 0.63 using calibrated VITECK® 2 DENSICHEK (BioMerieux, France). These suspension tubes and ID cards (Fig.1) were placed into the VITECK® 2 Cassette. The cassette is finally loaded into the VITECK® 2 systems attached to the workstation. (Fig. 2-4) Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator.

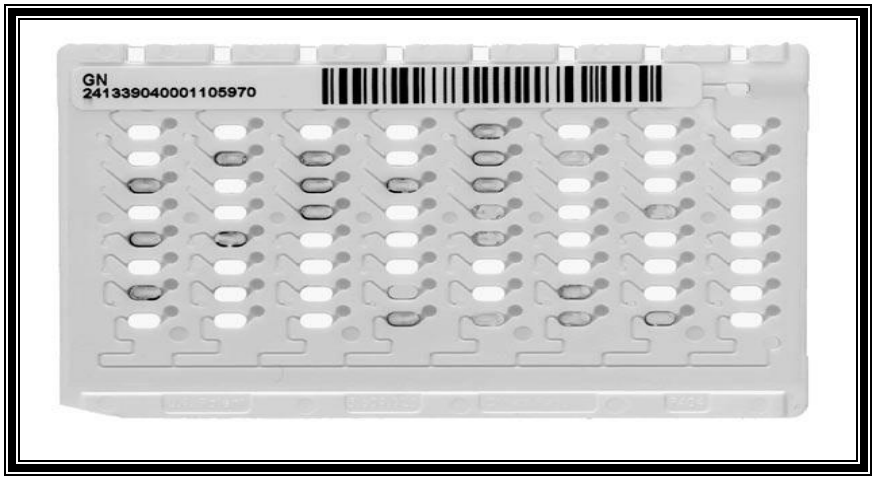

Figure 1: VITEK® 2 GN colorimetric identification card

Figure 2: VITEK® 2 compact instrument and workstation

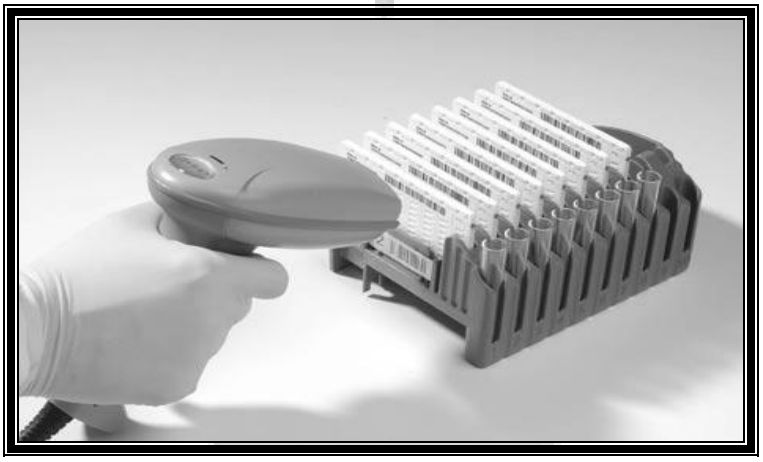

Figure 3. VITEK®2 Compact Cassette Loaded with 10 Cards and Suspension Tubes and Bar Code Scanner for Data Entry

Figure 4. VITEK®2 Cassette Loaded with Cards and Suspension Tubes Being Loaded

Into the Automatic Transport System

Antibacterial activity:

To check the presence of antimicrobial substance, the antimicrobial susceptibility tests were performed by standard disc diffusion method [5]. In this method, antibiogram patterns were studied for different extracts of Neem, and Turmeric viz petroleum ether extract, benzene extract, chloroform extract, methanol extract and aqueous extract for comparing three concentrations 1250µg/disc (50mg/ml), 2500µg/disc (100mg/ ml) and 5000µg/disc (200mg/ml) based on available literature [6,7,8]. Empty sterile discs having a diameter of 6mm were impregnated with 25ml of each serial dilution of extract solution. These impregnated discs, now contain different concentration (1250, 2500, 5000mg/disc) respectively of extract and then incubated for 15min for proper diffusion of extract and placed onto nutrient agar surface spread with 0.1ml of bacterial culture (standardized to 0.5 McFarland standards (106 cfu. Ml^{-1}). The plates were incubated at 37°C for 12–14 h. The experiments were carried out in triplicate. The results (mean value n = 3) were recorded by measuring the zone of growth inhibition around the discs. Control discs contained DMSO only. For comparison, standard antibiotic gentamycin inhibiting bacterial cell wall biosynthesis was included in the assay. The antibacterial spectra showing zone of inhibition in millimeters was recorded according to CLSI guidelines.

Synergistic activity of clays and herbal extracts:

Four varieties of clay (yellow, orange, black and brown) with different dilutions were prepared (50mg/ml, 100mg/ml and 200mg/ml). They were individually tested against the above said acne causing bacteria by disc diffusion method. Later on, the dilution which was showing highest antibacterial activity was mixed with best suited dilution of herbal extracts and results were recorded on the basis of zone of inhibition.

RESULTS AND DISCUSSION

Acne can have important negative psychosocial consequences for the affected individual like diminished self-esteem, social withdrawal due to embarrassment and depression. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated

with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling etc. (9)

However, the potential of higher plants as sources for new drugs is still largely unexplored. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine.

The present research work deals with evaluation of antibacterial activity of herbs and clay in combination. In the current study, total 60 bacterial isolates of *Propionibacteium acne* (n=20), *Staphylococcus aureus* (n=20) and *Staphylococcus epidermidis* (n=20) were screened and identified on the basis of morphological, biochemical and cultural characteristics. The further confirmation was carried out by using VITEK®2 Compact identification system. All the confirmed isolated were tested against the different extracts of Neem and turmeric. The results of Neem herb (Table 1) showed promising antibacterial activity when the extract was prepared in petroleum ether whereas in case of turmeric, methanolic extract at a concentration of 200mg/ml showed highest antimicrobial activity against all the isolates of three pyogenic bacteria (Table 2).

Natural clays have been used to heal skin infections since the earliest recorded history. In the current study, attention was drawn to a clinical use of four different types of clays (yellow, orange, black and brown clay) (Table 3) against pyogenic microbes causing pimples and acne like skin infections and rashes. These clays and others like them are interesting as they may reveal an antibacterial mechanism that could provide an inexpensive treatment for this and other skin infections, especially in global areas with limited hospitals and medical resources.

Current investigation showed that out of all the four clays, yellow clay when used in combination with turmeric showed promising antibacterial activity (Table 4) and this inhibitory effect is also greater that the antibiotic which was used as a control (Gentamycin).

Nonetheless, the observations of humans who have been ‘cured’ of illness by clay applications and the correlating photographic documentation (10,11) were the stimuli for our research into the healing mechanism of clays. In 2002, Line Brunet de Courssou reported to the World Health Organization (WHO), a summary of ~10 years of work in the Ivory Coast of Africa (East

Africa), where she documented the use of specific clay minerals as therapeutic treatment of advanced Buruli ulcer disease (10). However, she was unable to conduct additional experiments of traditional and accepted scientific studies due to lack of financial support. There have been several reports describing the antibacterial properties of natural and synthetic clay minerals (12, 13, 14, 11,15).

CONCLUSION

Thus, it was concluded from above study that natural actives have a better if not comparable inhibitory action against acne causing bacteria when compared with known synthetic antibacterial agent. The synergistic effect of natural herbs and clays will prove one of the best remedies in future for the treatment of skin infections especially pimples and acne. Their inhibitory potential on bacterial growth may be used in the development of natural drugs or cosmetics to treat acne.

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Table 1: Antimicrobial activity of Neem Extracts

Test pathogens	Mean Zone of Inhibition in mm															
	Petroleum Ether			Benzene			Chloroform			Methanol			Aqueous			Gentamycin
	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200 mg/ml	Control
Propionibacterium acne (n=20)	19	20	**22**	17	17	**18**	9	13	**12**	20	22	**18**	12	14	**21**	25
Staphylococcus aureus (n=20)	15	18	**19**	8	11	**12**	10	14	**18**	12	16	**19**	14	17	**20**	23
Staphylococcus epidermidis (n=20)	15	16	**18**	12	14	**18**	10	14	**18**	10	14	**18**	12	16	**18**	27

Table 2: Antimicrobial activity of Turmeric Extracts

Test pathogens	Mean Zone of Inhibition in mm															
	Petroleum Ether			Benzene			Chloroform			Methanol			Aqueous			Gentamycin
	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200 mg/ml	Control
Propionibacterium acne (n=20)	14	21	**22**	17	12	**18**	17	17	**19**	20	22	**26**	12	18	**24**	25
Staphylococcus aureus (n=20)	17	20	**18**	11	18	**20**	10	17	**22**	12	16	**25**	15	19	**21**	23
Staphylococcus epidermidis (n=20)	16	18	**19**	14	18	**18**	12	12	**16**	10	16	**26**	12	18	**19**	27

Table 3: Antimicrobial activity of various clays

Test pathogens	Mean Zone of Inhibition in mm												
	Yellow clay			Orange Clay			Black Clay			Brown Clay			Gentamycin
	50mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	100mg/ml	200mg/ml	50mg/ml	100mg/ml	200mg/ml	50mg/ml	Control
Propionibacteium acne (n=20)	14	16	20	10	11	10	-	-	-	-	10	10	25
Staphylococcus aureus (n=20)	16	16	19	-	-	-	-	-	-	-	-	-	23
Staphylococcus epidermidis (n=20)	15	19	21				-	-	-	-	-	-	27

Table 4: Synergistic effect of Clay and Turmeric methanolic extract

Test Pathogens	Mean Zone of Inhibition in mm				
	Yellow clay (200mg/ml) + Turmeric methanolic extract (200mg/ml)	**Orange clay (200mg/ml) + Turmeric methanolic extract (200mg/ml)**	**Black clay (200mg/ml) + Turmeric methanolic extract (200mg/ml)**	**Brown clay (200mg/ml) + Turmeric methanolic extract (200mg/ml)**	**Gentamycin**
Propionibacteium acne (n=20)	26	22	19	20	25
Staphylococcus aureus (n=20)	25	23	22	21	23
Staphylococcus epidermidis (n=20)	26	21	21	20	27