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Development and evaluation of herbal formulation AKIGTU01 and AKIGCL03 against acne producing microbes

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Abstract: *Acne vulgaris* is a common global skin disease affecting teenagers and adults and exerting serious psychological impacts which includes everlasting scarring, reduced self-image, depression and anxiety. One of the suspected causative agent of acne is *Propionibacterium acnes*; a Gram positive anaerobic organism which lives in skin hair follicle and openings. Treatments currently available for acne include use of oral antibiotics, hormones, isotretinoin and also physical treatments like lesion removal and photo-therapy. All these are associated with risks and none is completely satisfactory.Therefore, natural alternatives are gaining greater research support but lacks sufficient studies. In our study we have isolated *Propionibacterium acnes* from infected individuals and tested the effect of certain chemicals and herbs/ vegetable extracts against it. There anti-acne property was studied and compared with commercially used antibiotics including Clinagel (Clindamycin phosphate), Vibramycin (Doxycycline), Erythromycin, Novidat (Ciprofloxacin) and Amoxil (Amoxicillin). Results indicate that some of the selected herbs and chemicals showed good activity against *Propionibacterium acnes* synergistic to the antibiotics when used alone or in combination. Findings of this research can play an important role in natural product based drug discovery for the treatment of *Acne vulgaris*.

Keywords: *Acne vulgaris*, *Propionibacterium acne,* herbs, chemicals, anti-acne.

INTRODUCTION

Acne is the most widespread skin problem affecting 70-87% of the world population (Dreno *et al*., 2003). Teenagers and young adults are included in high risk group for acquiring this disease (White, 1998). 80% of teenagers having acne can carry on acne problem to their adulthood (Bhate and Williams, 2013). Approximately 50 million people in US are suffering from acne problem, 85% of whom are at the age of 12-25 years (Sidbury and Paller, 2000). *Propionibacterium acnes* characterized as an anaerobic, Gram-positive bacterium is believed to play a major role in development of acne. Acne is characterized by different areas of scaly red skin (seborrhea), pinheads (papules), blackheads and whiteheads (comedones), large papules (nodules) and sometimes scarring. Severe acne is usually inflammatory; however, it may also be non-inflammatory.

Current treatment methods against acne vulgaris include either application of benzoyl peroxide, retinoids and antibiotics such as erythromycin or clindamycin topically or use of medications like retinoids and antibiotics of tetracycline and macrolide classes orally. For severe acne problems, combinational treatments are normally employed. Although antibiotics are capable of inhibiting *P. acnes*, but their extensive use has resulted in antibiotic resistance warranting the development of novel therapeutic agents (Nelson *et al*., 2016, Adler *et al*., 2017, Kayiran *et al*., 2020, Karadag *et al*., 2021). In this respect, natural ingredients which have been used as traditional medicines including different parts of plants, spices and condiments and certain minerals could be investigated as novel anti-acne agents. Drugs used against Acne vulgaris mostly produce adverse effects and therefore, medicinal plants might be considered as reliable sources for development of new drugs (Abdulhussein and Al-Awsi, 2019).

There are certain chemicals that have been used in acne treatment either as an antibacterial and or as an exfoliating agent for skin resurfacing which includes benzoyl peroxide, salicylic acid, alpha-hydroxy acids, retinol, tea tree oil, sulfur, glycolic acid, lactic acid, mandelic acid, kojic acid, phenol and tricholoroacetic acid (Castillo and Keri, 2018). It is envisaged that combined formulation of herbs and chemicals could produce effective anti-acne agents with low side effects and higher efficacy.

The present research deals with antibacterial evaluation of certain herbs, chemicals and their possible combined formulations against *Propionibacterium acnes*. The organism was isolated from the affected individuals, cultured and subjected to the treatment with selected antibiotics, herbs and chemicals. Results of this study will provide information for the isolation of potent compounds from tested herbs against *Propionibacterium acnes* and

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could possibly lead to the discovery of novel drug/ cosmeceutical formulations for the treatment of acne.

MATERIALS AND METHODS

The glassware used was Pyrex types and thoroughly washed by chromic acid followed by deionized water and then autoclaved. During the study following equipments were used: Incubator model number MIR 153 (sanyo USA), Microscope model LX 400 (labomed USA) with camera, autoclave sterilizer model HVE-50 (Hirayama Japan), whatman filter paper 1, sterile swab sticks (sterile EO), glass desiccators (Germany), refrigerator model ARB-884VCM (whirlpool USA), disposable syringes, mortar and pestle (Germany). All antibiotic tablets and chemicals (tables 1, 2) were gifts from chemical and pharmaceutical companies located in Pakistan. Herb and vegetables were purchased from local markets (table 3).

Table 1: Antibiotics included in the study

Active pharmaceutical ingredient	Drug component (mg)
Clindamycin phosphate	10 mg
Doxycyclin	100 mg
Erythromycin	250 mg
Ciprofloxacin	500 mg
Amoxicillin	250 mg

Methodology

For the collection of exudate sample, 20 healthy volunteers who were suffering from acne were selected in the 17-35 age groups. Ethical approval was taken for all protocols from Faculty of Pharmacy, Federal Urdu University for Arts Science and Technology, REF No. 050-A, dated 15th July, 2021. Samples were collected from cheeks, nose and shoulders with sterile swab stick (Sterile EO). EO is Ethylene oxide that is widely used for the sterilization of healthcare devices and instruments (Hoxey, 2006). The sterile swab stick with exudates was transported to the microbiological laboratory, inoculated and incubated in enriched medium for bacterial growth.

Exudate was transferred into 20mL sodium thioglycolate broth tubes for consecutive five days at 37°C in the incubator for good bacterial growth. Each sample from sodium thioglycolate broth medium was inoculated into the prepared 5% blood agar with the help of sterilized wire loop followed by the incubation in anaerobic condition at 37°C for five days by candle jar method (Salim *et al*., 2014). After incubation isolated colonies were examined by Gram staining method for the conformation of the microorganism (Barile, 2014).

Procedure for antibacterial activity of antibiotics against P. acnes

10 mg of selected antibiotic tablets were dissolved in 100 mL deionized water (100 ppm). Commercially available filter paper (Whatman 1) was impregnated with the prepared solution of drugs, dried and applied on surface of blood agar plates, which were already streaked with P. acnes organism. All plates were placed into glass desiccators, under anaerobic condition. After 5 days at 37°C incubation, we observed zone of inhibition. Three replicas were made for each treatment against bacteria and mean value was reported.

Procedure for antibacterial activity of herbal and vegetable extracts against P. acnes

Herbs and vegetable were soaked in methanol for two months and then sent to chemistry lab for extraction which were concentrated using rotary evaporator. 10mg of concentrated extracts were dissolved in 100mL deionized water to which commercially available filter paper discs were impregnated and the above procedure was repeated to study the inhibition of *P. acnes*.

Procedure for antibacterial activity of chemicals against P. acnes

1% solutions of the selected chemicals were used to prepare the discs. Rest of the procedure was same as above.

Procedure for studying combined inhibitory effect of herbs and chemical against P. acnes

Some prototype products were prepared using the selected herbs and chemicals. These products were tested for their antibacterial property as described above.

i) Solution "AKI"

50 ppm solution of potassium iodide was prepared in distilled water. For the preparation of product "AKI" aloe vera gel was extracted from aloe vera leaves and 200 gram of aloe vera gel was added in 100mL of 50 ppm potassium iodide solution. Aloe vera and potassium iodide solution was mix together via mixture blending for 10 minutes. After blending a disk was dipped into the mixture for one minute to soak the extract then the disk was placed in to the blood ager containing *Propionibacterium acnes* culture. Plate was incubated at 37°C for five days within anaerobic condition.

ii) Solution "CI"

1 gram of cinnamon powder was dissolved in 100mL hot distilled water and its antimicrobial activity was determined as described above.

iii) Solution "TU"

1 gram of turmeric powder was dissolved in 100mL hot distilled water and its antimicrobial activity was determined as described above.

iv) Soliution "TU"

1 gram of clove was added to 100 distilled water and then boiled for 1 hour. On cooling its antimicrobial activity was determined as described above.

Table 2: Chemicals included in the study

Chemical	Company and Lot No./Cat No.
Acetic acid	(Merck) Lot.no. K31811963
Diethyl ether	(Merck) Lot No. K11286826
Ethanol	(Merck) Cat No. 1009832511
Ammonium hydroxide	(Merck) Product No. 104282500
Hydrogen peroxide	(Merck) Lot No. K20562500406
Calcium carbonate	(Merck) Lot No. K10571769
Nitric acid	(Merck) Cat No. 1004562510
n- hexane	(Merck) Lot No. K24252068732
Oxalic acid	Riedel-de HaenLot No. 6098
Sodium bicarbonate	(Merck) Cat No. 1.06329
Sodium carbonate	M &BProduct No. 66524
Phenol	AldrichCat No. 24,232-2
Salicylic acid	BDHProduct No. 30038
Starch	(Merck) Cat no. 7472558
Potassium Ferro cyanide	BDH Product No. 29610
Potassium cyanide	BDHProduct No. 29603
Tartaric acid	(Merck) Cat No. 8.43377.0100
Sulphuric acid	(Merck) Lot No. 8247987
Glycerin	KCiPharma
Potassium iodide	(Merck) Cat No.5040.018B180940
Iodine pure	(Merck) Cat NO. 2759827
Dithiozone	(Merck) Cat No. 1163489
Mercury (II) sulphate	(Merck) Lot No. K74859658
Mercuric iodide	BDHProduct No. 29165
Sodium iodide	(Merck) Lot No. K3365782 303
Sodium azaide	(Merck) Lot No. K36141 236
Ascorbic acid	(Merck) Cat No. 1.500074
Potassium carbonate	(Merck) Lot No. K19578824
Magnesium sulphate	(Merck) Lot No. K29313367
Potassium bromide	(Merck) Cat No.1049050500
Potassium chloride	(Merck) Lot No. TA684235
Boric acid	(Merck) Lot No. K20295860
Calcium chloride	(Merck) Cat No. TD274080
Zinc acetate	BDHProduct No. 685780
Ammonium chloride	(Merck) Batch No. A437342
Iron 2 sulphate	(Merck) Lot No. TA1118465 211
Sodium thioglycolate	(Sigma-Aldrich) EC Number 206-696-4
Nutrient agar	(Merck) Lot No. VM 100650
EDTA	(Merck) Cat No. 84211006

v) Solution "G"

1 gram garlic was meshed in morter pestle to which 100mL distilled water was added. The solution was then assayed for antimicrobial response.

vi) Solution "VT"

1 gram vitex seeds were meshed in morter pestle to which 100mL distilled water was added. The solution was then assayed for antimicrobial response.

RESULTS

Microscopic Identification

Acne exudates were inoculated in sodium thioglycolate broth for five days in anaerobic condition. After five days turbid medium showed bacterial growth. Growth was examined through microscopy taken from blood agar as well as sodium thioglycolate broth which presented single, Gram positive, anaerobic bacilli on glass slides confirming the presence of *Propionibacterium acnes* which are Gram positive, endosporic anaerobic rod shaped microorganisms (Figure 1).

Clinagel gel was used as control whereas remaining four antibiotics (Doxycyclin, Erythromycin, Ciprofloxacin and Amoxicillin) were used to study the anti-acne effect against *P. acnes*. Prominent inhibitory effect was observed for all the tested antibiotics as mentioned in table 4.

Among different herbs and vegetables selected for the study, only garlic, clove, turmeric, cinnamon and vitex showed good inhibition of *P. acnes* (table 5). Garlic was most effective in inhibiting the growth with a zone of

inhibition 31 mm followed by cinnamon measuring 25 mm. Clove and turmeric showed similar activity with zone of inhibition of 24 mm while smallest inhibitory zone was produced by vitex at 21 mm.

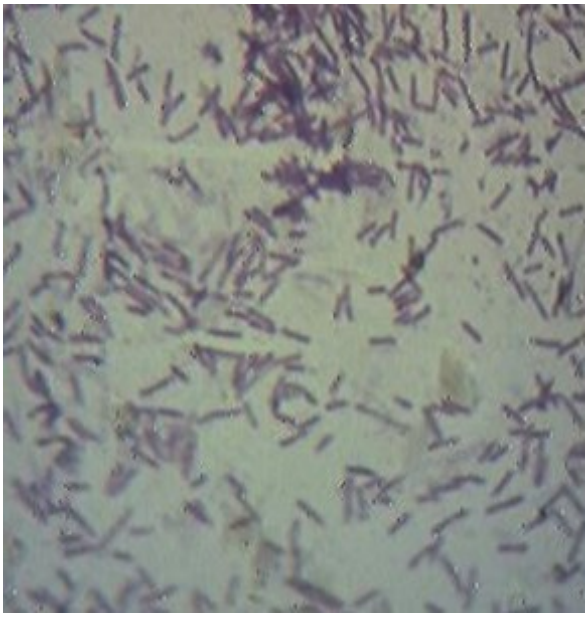

Figure 1: Microscopic identification of *Propionibacterium acnes*

Several experiments were performed to check the combine effects of different herbs, and chemicals with various combinations and concentrations. Since potassium iodide showed best inhibitory response among all the chemicals tested, therefore different products containing varying concentration of potassium iodide and herbs showed promising results. On comparing zone of inhibitions of these products with the selected antibiotics, prototype formulation AKIGTU01 and AKIGTU03 showed highest inhibitory effect (Table 7).

DISCUSSION

Our results indicate that the anti-acne activity of selected herbs were similar to the antibiotics used in our study. Herbal treatment has no side effects and has been used from time immemorial as home based remedy for a variety of ailments; therefore, it is proposed that formulations using mixture of these herbs can be used as an alternative therapy to treat acne.

Although several antibiotics are used against acne, there are number of reports on antimicrobial resistance by acne-causing specie over recent years. Apart from that, adverse effects associated with benzoyl peroxide, retinoids, isotretinoids, azelaic acid, and salicylic acid and other widely used anti-acne agents could not be ignored (Nasri *et al*. 2015). This necessitates the development of novel therapeutic agents with high efficacy and low side effect profiles. In this approach, the present study reports the antibacterial efficacy of few crude herbal extracts and certain chemicals against acne-causing bacterial specie. The safety of chemicals that produced good anti-acne effect was studied. It is reported that most of the chemicals that we used in our study has been previously utilized for other therapeutic effects (Baumann et.al. 2018, Mazzarello *et al*. 2019, Dhase *et al*. 2014, Anzengruber *et al*. 2019, Smith *et al.* 2020, Privitera *et al*., 2017).

Table 3: Herbs & vegetables included in the study

Name of Herbs or vegetable	Family name	Botanical Name
Neem (leaves)	Meliaceae	Azadirachtaindica
Neem (Seeds)	Meliaceae	Azadirachtaindica
Aleo Vera gel	Asphodelaceae	Aloe Barbadensis Miller
Aak Flower	Apocynaceae	Calotropisprocera
Aak Milk	Apocynaceae	Calotropisprocera
Lemon tree leaves	Rutaceae	Citrus
Lemon	Rutaceae	Citrus
Sapodilla tree leaves	Sapotaceae	Manilkarazapota
Basil Leave	Lamiaceae	Ocimumbasilicum
Cinnamon	Lauraceae	Cinnamomumverum
Honey	-	-
Turmeric	Zingiberaceae	Curcuma longa
Mustard Leaves	Cabbage family	Brassica juncea
Carrot	Umbelifers	Sativus
bitter melon	Cucurbits	Momordicacharantia
Cucumber	Cucurbits	Cucumissativus
Beet	Amaranthaceae	Beta vulgaris
Fenugreek leaves	Fabaceae	Trigonellafoenum-graecum
Spinach	Amaranthaceae	Spinaciaoleracea
coriander leaves	Apiaceae	Coriandrumsativum
Mint leaves	Lamiaceae	Mentha
Aleo Vera Leaves	Asphodelaceae	Aloe Barbadensis Miller
Clove	Myrtaceae	Syzygiumaromaticum
Apple juice	Rosaceae	Malusdomestica
Peach	Rosaceae	Prunuspersica
Rose water	Rosaceae	Rosa
Sandalwood	Santalaceae	Santalum album
Onion	Amaryllidaceae	Allium cepa
Garlic	Amaryllidaceae	Allium sativum
Ginger	Zingiberaceae	Zingiberofficinale
Vitex	Verbenaceae	Vitexagnus-castus L
Cardamom	Zingiberaceae	Elettariacardamomum
Lettuce	Asteraceae	Lactuca sativa

Anti-acne effects of certain chemicals were observed against *Propionibacterium acnes* (table 6). We found highest inhibition response from potassium iodide, followed by mercuric iodide, iodine and sodium iodide. Hydrogen peroxide. sodium carbonates, sodium bicarbonate, potassium carbonate and potassium bromide

also showed noticeable inhibition against *Propionibacterium acnes* as mentioned in table 4.

Table 4: Zone of inhibition of antibiotics against *Propionibacterium acnes* (mm)

Treatment name	Mean (Zone of inhibition in mm)
Clinagel	24
Vibramycin	24
Erythromycin	23
Ciprofloxacin	24
Amoxil	23

As combination formulations could provide improved treatment opportunities against acne, we tested the combined effect of selected herbs and chemicals and found that 2 products exhibited good inhibitory response. Proper selection of formulations may improve the activity, decrease side effects and enhance patient compliance.

CONCLUSION

The present investigation revealed the possibility of developing commercial products with the selected herbs and chemicals for the management of acne vulgaris while rationalizing their utility in traditional medicine.

Table 5: Zone of inhibition of herbs /vegetables against *Propionibacterium acnes* (mm)

Name of Herbs or vegetables	Mean (Zone of inhibition in mm)	Name of Herbs or vegetables	Mean (Zone of inhibition in mm)
Neem (leaves)	Nil	Mint leaves	Nil
Neem (Fruit)	Nil	Aloe Vera Leaves	Nil
Aloe Vera gel	Nil	Clove	24
Aak Flower	Nil	Apple juice	Nil
Aak Milk	Nil	Peach	Nil
Lemon tree leaves	Nil	Rose water	Nil
Lemon	Nil	Sandalwood	Nil
Sapodilla tree leaves	Nil	Onion	Nil
Basil Leave	Nil	Garlic	31
Cinnamon	25	Ginger	Nil
Honey	Nil	Vitex	21
Turmeric	24	Cardamom	Nil
Mustard Leaves	Nil	Lettuce	Nil
Carrot	Nil	Fenugreek leaves	Nil
Bitter melon	Nil	*Spinach*	Nil
Cucumber	Nil	Coriander leaves	Nil
Beetroots	Nil		

Table 6: Zone of inhibition of chemicals against *Propionibacterium acnes* (mm)

Chemical Name	Mean (Zone of inhibition in mm)	Chemical Name	Mean (Zone of inhibition in mm)
Acetic acid	Nil	Iodine pure	28
Diethyl ether	Nil	Dithizone	Nil
Ethanol	Nil	Mercury (II) sulphate	Nil
Ammonium hydroxide	Nil	Mercuric iodide	29
Hydrogen peroxide	24	Sodium iodide	28
Calcium carbonate	Nil	Sodium azaide	Nil
Nitric acid	Nil	Ascorbic acid	Nil
n- hexane	Nil	Potassium carbonate	22
Oxalic acid	Nil	Magnesium sulphate	Nil
Sodium bicarbonate	21	Potassium bromide	23
Sodium carbonate	21	Potassium chloride	Nil
Phenol	Nil	Boric acid	Nil
Salicylic acid	Nil	Calcium chloride	Nil
Starch	Nil	Zinc acetate	Nil
Tartaric acid	Nil	Ammonium chloride	Nil
Glycerin	Nil	Iron 2 sulphate	Nil
Potassium iodide	30		

Table 7: Zone of inhibition against *Propionibacterium acnes* (mm)

Product Code	Composition	Mean (Zone of inhibition in mm)
AKI	Aloe vera gel + potassium iodide	2
AKICI	AKI (10ml) + CI (5ml)	24
AKITU	AKI (10ml) + TU (5ml)	22
AKICL	AKI (10ml) + CL (5ml)	24
AKIG	AKI (10ml) + G (5ml)	25
AKIVT	AKI (10ml) + VT (5ml)	22
AKIGCI01	AKI (10ml) + G (5ml) + CI (5ml)	22
AKIGTU01	AKI (10ml) + G (5ml) + TU (5ml)	29
AKIGCL01	AKI (10ml) + G (5ml) + CL (5ml)	22
AKIGVT01	AKI (10ml) + G (5ml) + VT (5ml)	--
AKIGCI02	AKI (10ml) + G (5ml) + CI (2.5ml)	--
AKIGTU02	AKI (10ml) + G (5ml) + TU (2.5ml)	--
AKIGCL02	AKI (10ml) + G (5ml) + CL (2.5ml)	--
AKIGVT02	AKI (10ml) + G (5ml) + VT (2.5ml)	--
AKIGCI03	AKI (10ml) + G (5ml) + CI (10ml)	22
AKIGTU03	AKI (10ml) + G (5ml) + TU (10ml)	29
AKIGCL03	AKI (10ml) + G (5ml) + CL (10ml)	24
AKIGVT03	AKI (10ml) + G (5ml) + VT (10ml)	20

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