Open Access
Full Length Article

ORIGINAL ARTICLE

Formulation of Anti-Acneic Medicated Cream Using Mandarin (*Citrus reticulata*) Seeds

Areeba Shakeel[1], Ayesha Zaman[1], Rehmat Zaman[2]*

[1]Department of Biosciences, COMSATS University Islamabad, Park Road, Islamabad, Pakistan.
[2]Welwrd Pharmaceuticals, Industrial Area Hattar, Haripur, KP, Pakistan.

ABSTRACT

Acne is an inflammatory skin condition and is caused by *Propionibacterium acnes*. *Citrus reticulata* seeds are used for their antioxidant and antimicrobial activity around the world as traditional medicine. The objective of this study was to test the antibacterial activity of *Citrus reticulata* seeds and the anti-oxidant effect of the seeds on acne patients. The antibacterial susceptibility assay of the water and ethanolic extracts of *Citrus reticulata* was performed by the agar-well plate method. The extracts were assayed on microbial solutions of *Staphylococcus aureus, MRSA, E. coli, Klebsiella,* and *P.acnes* the results showed that seed extracts have essential therapeutic activities. For the study of the efficiency against acne, female volunteers from the age group 20-25 were selected for testing of the 8 prepared samples. Results were calculated by the questionnaire filled out by the volunteers. Most of the samples showed positive results against acne without irritation, peeling, and drying of the skin. The results, hence, showed that *Citrus reticulata* seeds can be used as a topical treatment for acne patients.

Keywords	*Address of Correspondence	Article info.
Citrus reticulate, Antibacterial Activity, Antioxidant.	*Zaman_scholar@yahoo.com*	*Received: October 28, 2023* *Accepted: December 23, 2023*

Cite this article: *Shakeel A, Zaman A, Zaman R. Formulation of Anti-Acneic Medicated Cream Using Mandarin (Citrus reticulata) Seeds. RADS J Biol Res Appl Sci. 2023; 14(2):117-126.*

INTRODUCTION

Almost 85% of teenagers and young adults under the age of 24 years, experience some degree of acne[1]. Acne vulgaris is indicated by the formation of open and closed comedones, papules, pustules, nodules, and cysts. The term acne is a derivative of "acme" (Greek) which means "prime of life". It is a pleomorphic disorder, can appear at any time during life, and may cause severe psychological problems and scars[2]. Main factors involved in the development of acne: I) Increased sebum production. II) Abnormal keratinization of the sebaceous duct and comedone formation III) an inflammatory immune response in which *Propionibacterium acnes (P. acnes)* plays an important role[3]. In recent decades, the amplitude of acne vulgaris has significantly increased and this increase can be associated with malnutrition, lifestyle change, and environmental factors. Soreness, Itching, and pain are physical symptoms of Acne, but it mainly affects the quality of life[4]. It is associated with low self-esteem, impaired perception of well-being and self-image, humiliation, fear of rejection, social withdrawal, anger, problematic family relations, depression, and anxiety[5].

Topical medications are used for acne treatment. A topical medication is a prescription that is applied to a particular place on or in the body (The word topical derives from the Greek topikos, "of a place"). Types of topical medications are solutions, Lotion, Cream, and Ointment[6,7]. BPO alone works like oral antibiotics. It has greater activity than topical (iso)tretinoin against inflammatory lesions. Low strength (2·5% or 5%) BPO is recommended because BPO causes initial local irritation[8,9]. Tretinoin, adapalene, and isotretinoin are topical retinoids and a medical prescription is required for their use in treatment. They work for both

comedonal and inflammatory acne because they are anti-inflammatory in action[10]. Topical antibiotics seem to be to act directly on *P. acnes* and reduce inflammation. Topical antibiotics include clindamycin, erythromycin, and tetracycline. Topical antibiotics are frequently combined with other products such as topical retinoids or BPO for severe acne[11]. Combinations of topical treatments with different mechanisms of action work better than single agents for the treatment of acne. BPO inactivates tretinoin, therefore these two agents should not be applied simultaneously; if used in combination one should be applied in the morning and one at night[12].

Citrus reticulata (commonly known as Kinnow or Mandarin) is a member of the family *Rutaceae* or Rue, which comprises about 140 genera and 1,300 species [12]. The plants of these fruits are large evergreen shrubs or small trees, with spiny shoots, reaching 5–15 m tall on average[13]. The seeds are small, pointed at one end, and could be few or many. Pakistani Kinnow varieties have 20-25 seeds per fruit[14]. According to the Food and Agriculture Organization (FAO), the total Citrus producer countries in the world are 142[15]. Brazil, China, Japan, Mexico, Pakistan, USA, and some countries of the Mediterranean region, are the leading *Citrus* producers which include, oranges, grapefruit, tangerines and mandarins, and lemons and limes[16]. According to an estimate, Pakistan is the 1st largest producer of Kinnow mandarin producing almost 95 % of the total Kinnow production of the world [17]. *C. reticulata* is widely consumed around the world for its appetizing qualities, but it also has therapeutic values that have long been used in traditional medicine[18]. Nutritionally, Citrus fruits contain phytochemicals that improve health[19]. They are also used in the cosmetic industry as starting material in the formulation of new fragrances and chemicals[20]. Only about 10% of the fruit is processed for juice extraction while the peel, pulp, and seeds are generated as waste during formulation of various products, which are mostly discarded[21]. Recent studies showed that *Citrus* by-products are a source of bioactive compounds and citrus seeds have been reported to be an attractive source of antioxidants, which are considered valuable, as they provide components, for further applications in food, cosmetic, and pharmaceutical industries[19]. Wild *Citrus* fruits and their seeds are rich in polyphenols. Among the many different classes of polyphenols, phenolic acids (Gallic acid and limonoids) and flavonoids are mostly found in the seeds and peels[22].

Limonoids, have an extremely bitter taste and have anticarcinogenic/ chemopreventive activities. The seed contains different types of limonins i.e. deacetylnomilin, deacetylnomilinic acid, ichangin, limonins, nomilin, andobacunone[23]. Limonins isolated from *C. reticulata* seeds have been demonstrated to have antibacterial, antifungal, and antimalarial activity**.** Flavonoids are secondary metabolites that are produced by plants that promote growth and development. Naringin is the most abundant flavonoid, found in the Mandarin seed. Naringin is of great interest to those in the food and pharmaceutical industries because of its demonstrated antioxidant, anti-inflammatory, anti-ulcer, and cholesterol-lowering effects, as well as its possible beneficial effects on several chronic conditions[24].

The objective of this study was to find out the antimicrobial activity of *C. reticulata* seeds, to calculate the efficacy of a formulated anti-acne cream using *C. reticulata* seeds, and comparative analysis of the formulated cream with a local cream.

MATERIALS AND METHODS

Microbial Cultures

Microbial cultures engaged in this study are *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (*MRSA*), *Escherichia coli*, *Propionibacterium acnes*, and *Klebsiella*. These microbial cultures were obtained from the Industrial Biotechnology Lab, Department of Biotechnology, International Islamic University, Islamabad. *P. acnes* was also used in this study.

Collection of Fruits and Seeds

Mature fruit samples of *C. reticulata* were collected from the gardens of Sargodha City, Pakistan. The fruits were selected after inspection of infections and damages. The selected fruits were then washed with 70% ethanol to remove dirt and other pesticidal remains. The fruits were washed with distilled water and cut using a knife; the seeds were then collected manually.

Drying and Crushing of Seeds

The seeds were washed individually with sterile distilled water and dried under subdued light. The seeds were

divided into three sections, (1) Dried whole seeds were milled using an electric grinder and sieved to pass through a 60-mesh screen. (2) Seeds were decorticated manually and winnowed. The kernels were then milled as described above to pass through a 60-mesh screen. The crushed seeds were washed again to remove the remains of the seed coat. (3) The hull of the seeds was separated and crushed using an electric grinder. The crushed seeds obtained were then stored in a refrigerator at 4°C.

Figure 1. (A) Dried Mandarin Whole seeds, (B) Dried Kernels, (C) Dried Hulls.

Preparation of Extracts of the Seeds of *C. reticulata*

A quantity of 5 g of finely ground powder of whole seeds was added to 100 mL of water and shaken properly. Similarly, 5 g of finely ground whole seed powder was added to 70% ethanol and then shaken. The above process was repeated for both finely ground kernels and hull powder. The suspension obtained was shaken and then the flasks were covered with aluminum foils. The 6 suspensions prepared were put in a rotating incubator at 30°C for 130 rpm. After 24 h the flasks were removed from the incubator. Using a filter paper the extract was separated from the mixture. Four dilutions of both water and ethanol extracts with sterile distilled water were prepared i-e 250 µL extract and 750 µL of sterile distilled water, 500 µL extract and 500 µL of sterile distilled water, 750 µL extract and 250 µL sterile distilled water and 100 µL of extract with no dilutions.

Determining the Antimicrobial Activity

Preparation of Microbial Dilutions

A wire loop was sterilized on a Bunsen burner until red hot. The wire loop was cooled and using the wire loop, a small portion of the microbe was taken and gently streaked on quarter parts of the agar plates. The process was repeated for every bacterium culture employed i-e *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (*MRSA)*, *Escherichia coli*, *Propionibacterium acnes*, and *Klebsiella*. The microbes were then placed in an incubator for 24 hours to allow them to grow. For every bacterium, culture microbial solutions were prepared with varying diluting factors. 900µl saline distilled water was added to each Eppendorf tube using a pipette with a sterile tip. Eppendorf tubes were marked as 10^0, 10^{-1}, 10^{-2}, 10^{-3}. A streak of microbe was taken from the prepared culture and mixed with the saline distilled water in the Eppendorf marked as 10^0. For serial dilutions of the microbial solutions, 100µl of solution from the Eppendorf marked as 10^0 was taken and added to the next tube marked as 10^{-1}. Similarly, further dilutions of 10^{-2} and 10^{-3} were prepared to get varying concentrations. The above procedure was repeated for each microbial culture. The final microbial solution was 1000µl in 10^{-3} for every strain. The solution was stored in a refrigerator at 4°C.

Antimicrobial Susceptibility Assay

P. acnes was isolated from an acne patient using a nutrient agar medium (Figure **2**). The agar plates were labeled on the side with the date, sample, and strain name. 100µl of 10^{-3} microbial solution was added to the agar plate using a pipette with a sterilized tip. The solution poured was then gently spread on the plate using a sterilized glass rod. Six wells of 1cm with equal distance were made in the agar media. These wells were marked as Control (C), Antibiotic (AB), 25%, 50%, 75% and 100%. 100µl of distilled water was added to the well marked as C Similarly, 100µl of antibiotic was added to the well marked as AB. Aliquots of 100µl of the extract were added to its representative well. The tip of the pipette was discarded each time before introducing the extract into each well. The above process was repeated with the available five microbes. Every dish was treated as mentioned above. The Petri dishes were placed in an incubator at 30°C for 24 h. After 24 h the inhibition zone created by the seed extracts was

determined and measured. The diameter of the zone of inhibition was calculated using a scale from the edge of the well to the edge of the zone.

Figure 2. *P. acnes* isolated from acne patient.

Formulation of Anti-Acne Cream

Formulation and Application of Anti-Acne Cream Using Seed Powder

A local cream was bought from the market, in Islamabad. 1.5 g of finely ground whole seed powder was blended with 1.5 g of the cream to obtain a total 1:1 mixture. 1.5 g of whole seed powder was blended with 1.5 g of glycerin. The above procedure was repeated for finely ground kernels and hull powder. 2 volunteers who suffered from acne were selected for every sample prepared. The cream mixture was applied on the right cheek of the volunteers, the Glycerin mixture (positive control) was applied on the left cheek, the local cream was applied on the forehead and only glycerin was applied on the chin (negative control).

Formulation and Application of Anti-Acne Cream Using Seed Water Extracts

2 g of kernels water extract was measured and blended using a magnetic stirrer with 2 g of cream to obtain a 1:1 mixture of 4 g. This procedure was repeated for all the water extracts of hulls. The resultant cream was stored to settle it down. A total of 2 samples were obtained. Two volunteers who suffered from acne were selected for testing of these samples. The cream mixture was applied on the right cheek of the volunteer, the only extract was applied on the left cheek (positive control), the local cream was applied on the forehead and sterile distilled water was applied on the chin (negative control).

Formulation and Application of Anti-Acne Cream Using Seed Ethanolic Extracts

2 g of whole seed ethanolic extract was blended with 2 g of the local cream to obtain 1:1 of a total mixture of 4 g. The above procedure was repeated for the ethanolic extracts of kernels and hulls. The cream was stored to settle it down. 2 volunteers who suffered from acne were selected for the application of the prepared cream. The cream mixture was applied to the right cheek, simple ethanol extract was applied to the left cheek (positive control), the local cream was applied to the forehead and pure ethanol was applied to the chin (negative control).

Consent Form and Questionnaire

A consent form was prepared and filled out by the volunteers before the application of the formulated cream. The mixtures were applied at night and the process was repeated for four days. A questionnaire was prepared regarding the efficacy of the anti-acne cream and was filled by the volunteers. All the arithmetical analysis was executed by Microsoft Excel 2016 to calculate the percentage efficiency of the formulated samples.

RESULTS AND DISCUSSION

Seed Extraction

Washed seeds were finished to powder via grinding. After 24 hours in the rotating incubator, pure extract of the seeds was obtained through filtration. Four dilutions of the extract were prepared to give 25% for 250 mL of extract, 50% for 500 mL of extract, 75% for 750 mL of extract, and 100% for no dilutions. The water and ethanolic extracts of the seeds were prepared to examine their antimicrobial and antioxidant activities against acne.

Antimicrobial Assay

Five types of bacteria i-e *Propionibacterium acnes, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumonia,* and *Escherichia coli* were treated with the ethanolic and water extracts of whole seed, kernels, and hulls powder to investigate their antimicrobial activity.

Antimicrobial Activity of Water Seed Extracts

The well marked as C comprised saline water. Saline water has no effect on bacteria hence, no zone of inhibition was observed. The well labeled as AB contained the antibiotic

ampicillin. The antibiotic effect of ampicillin was observed on four bacteria i-e *Propionibacterium acnes, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), and Klebsiella pneumonia* (Figure **3**). Ampicillin did not show any effect against *E.coli*. Seed extracts of *C. reticulata* exhibited bactericidal activity against three of the tested microorganisms that are *S. aureus*, *Klebsiella*, and *P. acnes. E.coli* and *MRSA* showed no activity against water extracts (Table **1**).

Antimicrobial Activity of Whole Seed (S) Water Extract

Whole seed water extract showed antimicrobial activity against the three above-mentioned microbes (Figure **3**). *S. aureus* showed the highest sensitivity to water extract of whole seed of *C. reticulata* with a zone of inhibition of 0.2cm with E_{50}, 0.3cm with E_{75}, and 0.3cm with E_{100}. *P.acnes* also showed some sensitivity against water extract of whole seed with a zone of inhibition of 0.2cm with E_{50}, 0.2cm with $E_{75,}$ and 0.3 with $E_{100.}$ *Klebsiella* showed the least antibacterial sensitivity to water extract of whole seed of *C. reticulata* with a zone of inhibition of 0.1cm with E_{50}, 0.2cm with E_{75}, and 0.3cm with E_{100} (Table **1**).

Antimicrobial Activity of Kernels (K) Water Extract

Kernels showed no antimicrobial activity against any of the microbes. No zone of inhibition was observed against any dilutions of the kernels water extract (Figure **3**). Hence, bacteria do not show any sensitivity against kernels water extract (Table **1**).

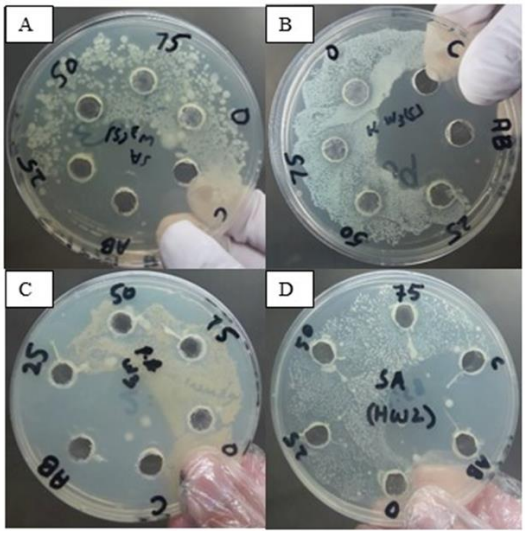

Figure 3. (A) Whole seed water extract against *S. aureus*, (B) Water Whole seed extract against *Klebsiella*, (C) Whole seed against *P.acnes*, (D) Water Hull extract against *Streptococcus aureus.*

Antimicrobial Activity of Hulls (H) Water Extract

Hull water extract showed the highest antimicrobial activity against *S.aurues.* Overall among other extracts, the highest sensitivity was showed by *S. aureus* against water extract of hulls with a zone of inhibition of 0.2cm for $E_{50,}$ 0.3cm for $E_{75,\ and}$ 0.4cm with E_{100} (Table **1**).

Table 1. Antibacterial activity of Water Seed Extracts where S= whole seed, K= kernels, and H= hulls.

Microbe	Water Extracts	Control (cm)	Antibiotic (cm)	E_{25} (cm)	E_{50} (cm)	E_{75} (cm)	E_{100} (cm)
Propionibacterium acnes	S	-	2.5	-	0.2	0.2	0.3
	K	-	2.4	-	-	-	-
	H	-	2.3	-	-	-	-
MRSA	S	-	2.1	-	-	-	-
	K	-	2.0	-	-	-	-
	H	-	2.1	-	-	-	-
Streptococcus aureus	S	-	2.4	-	0.2	0.3	0.3
	K	-	2.3	-	-	-	-
	H	-	2.3	0	0.2	0.3	0.4
Klebsiella	S	-	2.4	-	0.1	0.2	0.3
	K	-	2.3	-	-	-	-
	H	-	2.2	-	-	-	-
E.coli	S	-	-	-	-	-	-
	K	-	-	-	-	-	-
	H	-	-	-	-	-	-

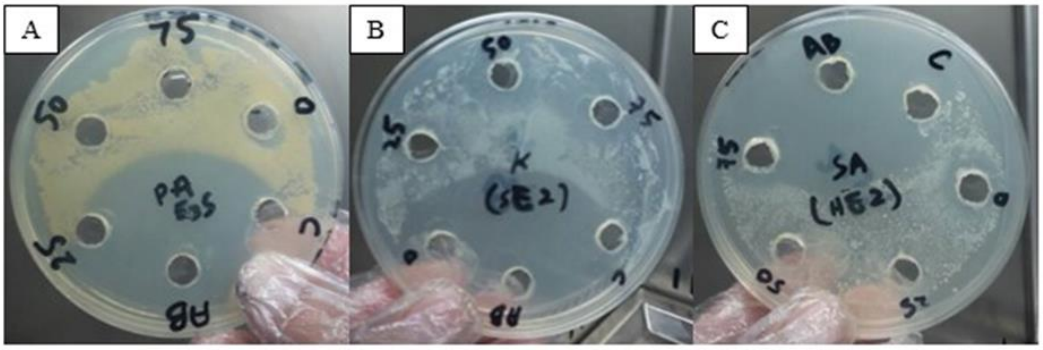

Figure 4. (A) Ethanolic Whole seed extract against *P. acnes*, (B) Ethanolic Hull extract against *Klebsiella* (C) Ethanolic Hull extract against *S. aureus.*

Table 2. Antibacterial Activity of Ethanolic Seed Extracts where S= whole seed, K= kernels, and H= hulls.

Microbe	Ethanol Extracts	Control (cm)	Antibiotic (cm)	E_{25} (cm)	E_{50} (cm)	E_{75} (cm)	E_{100} (cm)
Propionibacterium acnes	S	-	2.6	-	0.2	0.2	0.3
	K	-	2.1	-	-	-	-
	H	-	2.0	-	-	-	-
MRSA	S	-	1.9	-	-	-	-
	K	-	2.1	-	-	-	-
	H	-	2.1	-	-	-	-
Streptococcus aureus	S	-	2.0	-	-	-	-
	K	-	2.3	-	-	-	-
	H	-	2.3	-	-	0.2	0.3
Klebsiella	S	-	2.0	-	-	-	-
	K	-	2.3	-	-	-	-
	H	-	2.4	0.2	0.3	0.6	0.8
E.coli	S	-	-	-	-	-	-
	K	-	-	-	-	-	-
	H	-	-	-	-	-	-

Antimicrobial Activity of Ethanolic Seed Extracts

The well labeled as C contained saline water. Saline water has no effect on bacteria hence, no zone of inhibition was observed. The well labeled as AB contained the antibiotic ampicillin. The antibiotic effect of ampicillin was observed on four bacteria i-e *Propionibacterium acnes, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), and Klebsiella pneumonia.* Ampicillin did not show any effect against *E.coli*. Seed extracts of *C. reticulata* exhibited bactericidal activity against three of the tested microorganisms that are *S. aureus*, *Klebsiella*, and *P. acnes. E.coli* and *MRSA* showed no activity against water extracts.

Antimicrobial Activity of Whole Seed (S) Ethanolic Extract

The whole seed showed antimicrobial activity against *P.acnes* only with a zone of inhibition of 0.2cm with E_{50}, 0.2cm with E_{75}, and 0.3cm with E_{100}. *P.acnes* showed approximately similar sensitivity against ethanolic and water extracts of whole seeds.

Antimicrobial Activity of Kernels (K) Ethanolic Extracts

Kernels showed no antimicrobial activity against any of the microbes. No zone of inhibition was observed against any dilutions of the Kernels Ethanolic Extract. Hence, bacteria do not show any sensitivity against kernels of ethanol extract. The seed kernels hence do not involve any type of antimicrobial activity against available microbes.

Antimicrobial Activity of Hulls (H) Ethanolic Extracts

Ethanolic extracts of hulls showed antimicrobial activity against *Klebsiella* and *S. aureus. Klebsiella* showed the highest sensitivity to ethanol extract of seed hulls of *C. reticulata* with a zone of inhibition of 0.2cm with E_{25}, 0.3cm with E_{50}, 0.6cm with E_{75}, and 0.8cm with E_{100} (table **2**). *Klebsiella* showed overall the highest sensitivity against ethanolic extracts of seed hull among other ethanolic and water extracts. *S. aureus* showed the least sensitivity to ethanol extract of seed hulls of *C. reticulata* with a zone of inhibition of 0.2cm with E_{75} and 0.3cm with E_{100} (Table **2**).

Volunteer Feedback About Powdered Whole Seed, Kernels, And Hulls

6 volunteers were selected for the three samples formulated with powdered seed and the result is given in Table **3**. The formulated cream containing whole seeds did not cause any side effects, irritation, peeling, or dryness. It produced positive results in reducing acne from the skin, as well as providing a fine texture, and was effective against sunburn and suntan. Hulls and Kernels also produced positive results against acne treatment but some amount of irritation and dryness was also observed. The glycerin mixture of the powdered seeds also showed positive. However, Glycerin and the local cream showed no effects and did not have any activity in reducing acne. Powdered Hulls showed the most effectiveness against acne about 81%. Powdered hulls were observed to be overall better than the other 8 samples. The least effectiveness was shown by powdered kernels and was about 66%. Whole seeds powdered formulated cream showed almost 79% effectiveness against acne.

Side effects were not observed in any of the samples provided. Hulls water extract was found to be more effective than the formulated cream. Other extracts showed almost similar effectiveness to the formulated cream. No effect was shown by either distilled water or the local cream. Almost similar effectiveness was observed in the case of water extracts formulated cream i-e 75% for whole seed, 76% for kernels, and 73% for hulls.

The ethanolic extracts of the seeds showed comparatively less effectiveness against acne. Whole seed and Kernels ethanolic extracts were to some extent effective against acne. Hulls Ethanolic Extract did not show any effectiveness against acne. However, all samples helped in improving the texture of the skin and reducing sun damage. Severe side effects were not observed in any case, although, whole seed extract showed irritation to some extent. Other side effects like the peeling of skin and dryness were not observed in any formulation. The Extracts showed similar results as the formulated cream. Ethanol and local cream did not show any effectiveness against acne. The highest effectiveness was shown by kernels of ethanolic extract and was about 79%. Whole seed extract showed 69% and the least effectiveness among the other 8 samples as well was shown by Hulls Ethanolic extract i-e 63%.

Table 3. Questionnaire Results of Formulated Cream Samples, where WS+G+C= Whole, SK=Kernels and SH=Hulls, W=Water, E= Ethanol, C=Cream and G=Glycerin.

Sample Type	Effect on Acne	Positive Effects	Side Effects	Overall Effect
WS+G+C	+ve	Freshen skin and reduce sun damage	-ve	Satisfactory
SH+G+C	+ve	Freshen skin and reduce sun damage	Minor irritation and dryness	Good
SK+G+C	+ve	Freshen skin	Minor irritation	Fair
WS(W)+C	+ve	Freshen skin and reduce sun damage	-ve	Good
SH(W)+C	+ve	Freshen skin and reduce sun damage	-ve	Good
SK(W)+C	+ve	Freshen skin and reduce sun damage	-ve	Satisfactory
WS(E)+C	+ve	Reduce sun damage	Minor irritation	Satisfactory
SH(E)+C	+ve	Freshen skin and reduce sun damage	-ve	Fair

SK(E)+C	+ve	Freshen skin and reduce sun damage	-ve	Satisfactory
Glycerin	-ve	-ve	-ve	No effect
Water	-ve	-ve	-ve	No effect
Ethanol	-ve	-ve	-ve	No effect
Cream	-ve	-ve	A minor increase in pimples	No effect

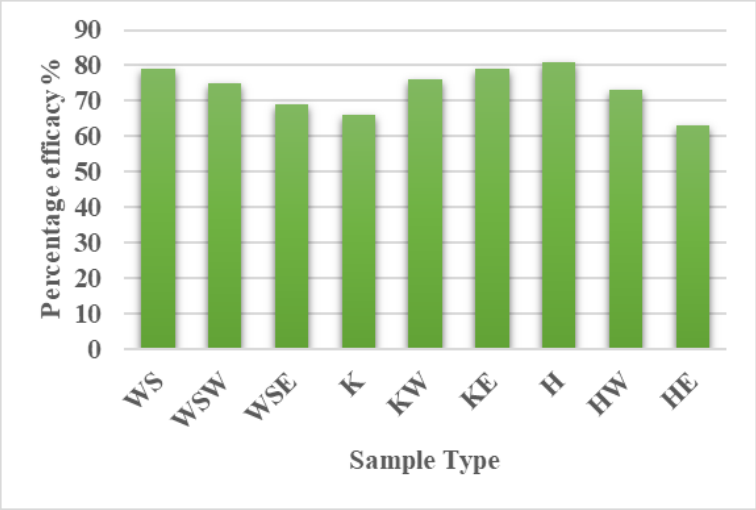

Figure 5. Percentage efficacy of Samples prepared where WS= whole seed, WHW= Whole seed water extract, WSE= Whole seed ethanolic extract, K=Kernels, KW=Kernels Water extract, KE= Kernels Ethanolic Extract, H=hulls, HW=Hulls Water Extract and HE= Hulls Ethanolic Extract

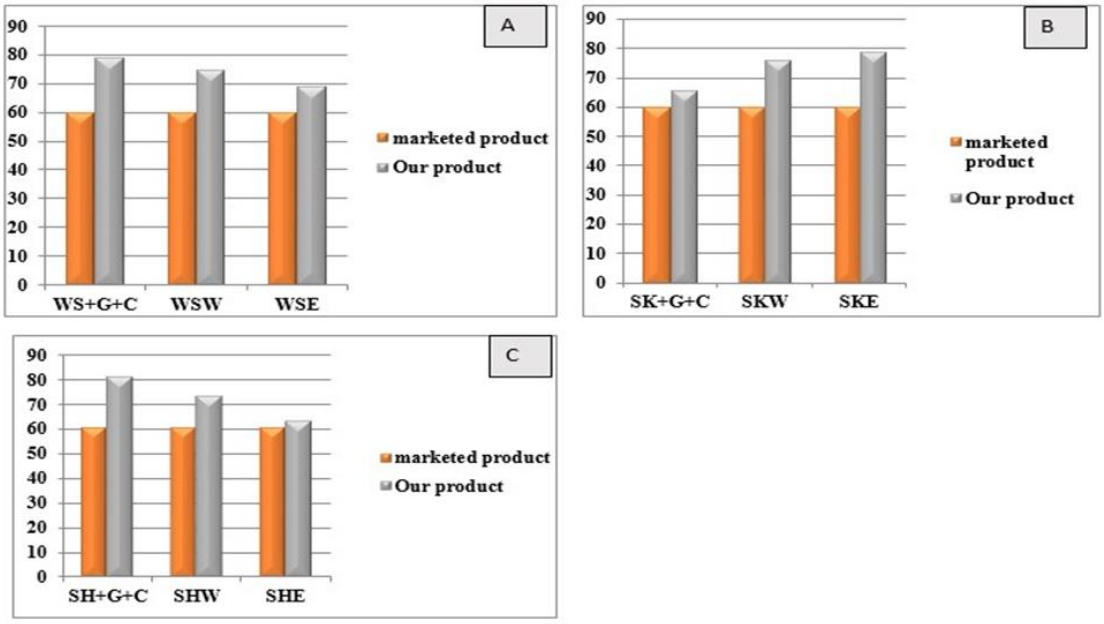

Figure 6. (A) Results of whole seed powder, whole seed water extract, and whole seed Ethanolic extract compared with marketed product, (B) Results of seed kernels powder, seed kernels water extract, and seed kernels Ethanolic extract compared with the marketed product, (C) Results of seed hulls powder, seed hulls water extract and seed hulls Ethanolic extract compared with marketed product

Comparative Studies

The sample containing Seed Hulls (SH) and Whole Seed powder (WS) showed 81% and 79% efficacy respectively with no side effects. Other samples also showed good results against acne. The samples containing Seed Hull Ethanolic Extract (SHE) and Seed Kernels (SK) Powdered showed the least efficacy against acne i-e 63% and 66% respectively. The overall results of the experiment are shown in Figure **5**. The percentage efficacy showed by the local cream was approximately 60%. Comparing the results of the formulated samples with the local sample, showed that the formulated samples showed higher efficacy than the original ones. Hence, the addition of *C. reticulata* seed samples to a local cream not only increased its efficiency against acne but also improved the texture of the skin and reduced the effect of sunburn.

The agar well diffusion method was used to find the antimicrobial activity of mandarin seeds against five microbes i.e. *Klebsiella pneumonia, MRSA, Escherichia coli, Staphylococcus aureus* (SA), and *P. acnes*. Two types of seed extracts were made for this purpose; water extract and Ethanolic extract. Different dilutions of seed extracts were prepared i.e. 25%, 50%, 75%, and 100%. Antimicrobial activity was seen in the following order: 100% > 75% > 50%. The antimicrobial activity against *P. acnes* proved that *C. reticulata* seeds can be used for our study against acne.

Inspired by these surprising features of Mandarin seeds an anti-acne was formulated using these seeds. 9 samples were prepared and applied to 18 volunteers. The survey method was used to find the efficiency of our medicated products and the results were compared with existing marketed products. Optimistic results were observed during the survey (Figure **6**). Formulated Medicated cream reduced acne, and provided sunlight protection, and long-lasting freshness. Furthermore, negative features like inflammation, redness, dryness, and scaling were reduced to a large extent. It will be an innovation in the field of cosmetics.

CONCLUSION

Antimicrobial and antioxidant properties of *Citrus reticulata* can be used in the treatment of microbial infections. Seed extracts of *Citrus reticulata* possess compounds containing antimicrobial and antioxidant properties that can be useful to control acne from the skin. According to a survey paper seed hull extract in water, whole seed extract in ethanol, and seed hull powder extract showed the best results on volunteers having acne. They help in reducing acne without causing any side effects on their skin. Finally, this study depicted that these prepared new formulations of seed extracts of *Citrus reticulata* are very good therapeutic compositions for acne.

Extraordinary results were observed by blended mandarin seed powder and its extract in existing marketed products. Because of the amazing benefits observed during this study, in the future, a novel product can be formulated. In the future, we will design new research to formulate a chemical-free dermatological product using mandarin seed. A synthetic product can never beat a natural product, as it has many long-term harmful effects. Hence, a natural product can be formulated, which will not encompass any synthetic chemicals and have natural compounds of mandarin seeds to overcome the side effects of synthetic cosmetics.

CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENT

None.

REFERENCES

1. Al-Natour SH. Acne vulgaris: Perceptions and beliefs of Saudi adolescent males. J Family Community Med. 2017;24(1):34.
2. Kawashima M, Nagare T, Katsuramaki T. Open-label, randomized, multicenter, phase III study to evaluate the safety and efficacy of benzoyl peroxide gel in long-term use in patients with acne vulgaris: A secondary publication. J Dermatol. 2017.
3. Dreno B, Martin R, Moyal D, Henley JB, Khammari A, Seité S. Skin microbiome and acne vulgaris: staphylococcus, a new actor in acne. Exp Dermatol. 2017.
4. Kubota Y, Shirahige Y, Nakai K, Katsuura J, Moriue T, Yoneda K. Community-based epidemiological study of psychosocial effects of acne in Japanese adolescents. J Dermatol. 2010;37(7):617-22.
5. Jena AK, Sahoo S. Evaluation of Associated Anxiety and Depression in Patients with Acne Vulgaris: A Hospital Based Clinico-Epidemiological Study. PARIPEX-Indian J Res. 2017;5(10).

6. Gold LS, Weiss J, Rueda MJ, Liu H, Tanghetti E. Moderate and severe inflammatory acne vulgaris effectively treated with single-agent therapy by a new fixed-dose combination adapalene 0.3%/benzoyl peroxide 2.5% gel: a randomized, double-blind, parallel-group, controlled study. Am J Clin Dermatol. 2016;17(3):293-303.
7. Thielitz A, Sidou F, Gollnick H. Control of microcomedone formation throughout a maintenance treatment with adapalene gel, 0.1%. J Eur Acad Dermatol Venereol. 2007;21(6):747-53.
8. Fakhouri T, Yentzer BA, Feldman SR. Advancement in benzoyl peroxide-based acne treatment: methods to increase both efficacy and tolerability. J Drugs Dermatol. 2009;8(7):657-61.
9. Williams HC, Dellavalle RP, Garner S. Acne vulgaris. Lancet. 2012;379(9813):361-72.
10. Yentzer BA, McClain RW, Feldman SR. Do topical retinoids cause acne to "flare"? J Drugs Dermatol. 2009;8(9):799-801.
11. Simonart T, Dramaix M. Treatment of acne with topical antibiotics: lessons from clinical studies. Br J Dermatol. 2005;153(2):395-403.
12. Parveen Z, Siddique S, Ali Z. Chemical composition of essential oil from leaves of seeded and seedless Citrus reticulata blanco var. kinnow. Bangladesh J Sci Ind Res. 2015;49(3):181-4.
13. Sahoo UK, Jeeceelee L, Lallinrawna S, Muthukumaran RB. Effect of Citrus reticulata Blanco leaf extract on seed germination and initial seedling growth parameters of five home garden food crops. J Exp Biol. 2015;3:6.
14. Kazmi SK, Khan S, Kabir NUR, Mirbahar AA, Raziq M, Kauser NA. Embryogenic callus induction, somatic embryogenesis, regeneration and histological studies of kinnow mandarin (Citrus reticulata blanco L.) from nucellar embryo and epicotyl region. Pak J Bot. 2015;47(1):305-10.
15. Waghmare V, Pandhure N. Induction and regeneration of callus from different explants in Citrus reticulata (Blanco). Int J Curr Res Rev. 2015;7(13):84.
16. Hasan MR, Gupta A, Hasan MN, Rejwan HM, Hasan R, Prodhan SH. Efficient regeneration system for the improvement of Kinnow mandarin (Citrus reticulata Blanco). J Biol Agric Healthcare. 2016;6:39-47.
17. Hussain M, Raja NI, Iqbal M, Iftikhar A, Sadaf HM, Sabir S, et al. Plantlet regeneration via somatic embryogenesis from the nucellus tissues of Kinnow Mandarin (Citrus reticulata L.). Am J Plant Sci. 2016;7(6):798.
18. Leng X, Song C, Han J, Shangguan L, Fang J, Wang C. Determination of the precise sequences of computationally predicted miRNAs in Citrus reticulata by miR RACE and characterization of the related target genes using RLM-RACE. Gene. 2016;575(2):498-505.
19. Wang H, Chen G, Guo X, Abbasi AM, Liu RH. Influence of the stage of ripeness on the phytochemical profiles, antioxidant, and antiproliferative activities in different parts of Citrus reticulata Blanco cv. Chachiensis. LWT Food Sci Technol. 2016;69:67-75.
20. Parveen Z, Siddique S, Ali Z. Chemical composition of essential oil from leaves of seeded and seedless Citrus reticulata blanco var. kinnow. Bangladesh J Sci Ind Res. 2015;49(3):181-4.
21. Bhatlu MLD, Singh SV, Verma AK. Recovery of naringin from kinnow (Citrus reticulata Blanco) peels by adsorption-desorption technique using an indigenous resin. Sādhanā. 2017;1-10.
22. Xi W, Zhang Y, Sun Y, Shen Y, Ye X, Zhou Z. Phenolic composition of Chinese wild mandarin (Citrus reticulata Blanco) pulps and their antioxidant properties. Ind Crops Prod. 2014;52:466-74.
23. Das DR, Sachan AK, Shuaib M, Imtiyaz M. Chemical characterization of volatile oil components of Citrus reticulata by GC-MS analysis. World J Pharm Pharm Sci. 2014;3:1197-204.
24. Hayat K. Citrus: molecular phylogeny, antioxidant properties and medicinal uses. Nova. Sci. 2014;3:235.