Significance of topical propolis in the treatment of facial acne vulgaris

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Received 26 November 2014
Accepted 29 April 2015

Egyptian Journal of Dermatology and Venereology 2015, 35:29–36

Background
Acne is a common skin disorder affecting the pilosebaceous unit, arising commonly during adolescence and causing psychological stress. The pathogenesis of acne is attributed to multiple factors. Clinically, it is characterized by the presence of comedones, inflammatory papules, pustules, and sometimes nodules and cysts. Propolis has been attracting the attention of researchers because of its antimicrobial, antioxidant, antiviral, and antifungal properties.

Objectives
The aim of this study was to evaluate the clinical and bacteriological significance of topical propolis extracts in the treatment of facial acne vulgaris.

Patients and methods
This study included 40 patients with facial acne vulgaris. The patients were classified into two groups: group I included 20 patients who were treated with a topical solution of ethanolic extract of propolis, and group II included 20 patients who were treated with a topical solution of ethanol only and served as the control group. Patients were evaluated clinically to assess the efficacy of therapy after treatment. Bacteriological examination was carried out before and after treatment to assess the antimicrobial effect of propolis.

Results
There was a highly significant clinical efficacy of topical solution of ethanolic extract of propolis in the treatment of acne vulgaris. There was a highly significant bacteriological efficacy of topical solution of ethanolic extract of propolis on gram-positive aerobic (*Staphylococcus epidermidis*) and gram-positive anaerobic bacteria (*Propionibacterium acnes*).

Conclusion
Topical propolis is a promising, effective, well-tolerated, safe, and alternative medication for acne vulgaris. It has anti-inflammatory and antibacterial properties. Further studies are needed for its application in different skin diseases.

Keywords:
acne, antimicrobial, topical propolis

Egypt J Dermatol Venereol 35:29–36

1110-6530

Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, affecting nearly 85% of young people [1]. The pathogenesis of acne is attributed to multiple factors. One of them is the proliferation of *Propionibacterium acnes* within the follicle, stimulating cytokine production through toll-like receptors resulting in inflammatory lesions [2,3]. Different treatment modalities have been reported for acne vulgaris based on the type and severity of the disease.

Bee products have been used in Egypt and Greece in folk medicine since antiquity. Propolis is a lipophilic, hard, brittle material when cold, but becomes soft, pliable, gummy, and very sticky when warm [4]. The chemical composition of propolis is subject to variations based on the source of the plant and region [5].

The constituents of Egyptian propolis are mainly resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%) such as phenolic compounds and flavonoids. Propolis contains some minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn, and Fe and some vitamins such as A, B1, B2, B6, C, and E, as well as a number of fatty acids [4]. Propolis has been attracting the attention of researchers due to its various biological activities and therapeutic properties. Several studies have highlighted the antimicrobial, antiviral, antifungal, antioxidant, and anti-inflammatory properties of propolis extract in some skin diseases [6–8].

The aim of this study was to evaluate the clinical and bacteriological significance of topical propolis extracts in the treatment of facial acne vulgaris.

Patients and methods

This study was carried out on 40 patients with facial acne vulgaris. They were selected from the Outpatient Clinics

of Dermatology and Venereology Department, Tanta University Hospitals. Patients of both sexes were included in the study. Their ages ranged from 13 to 24 years. The IRB/Ethics committee approval has been acheived before the start in this study in Tanta University.

The patients were classified into two groups: group I included 20 patients with facial acne vulgaris, who were treated with a topical solution of ethanolic extract of propolis, and group II included 20 patients with facial acne vulgaris, who were treated with a topical solution of ethanol 80% only and served as the control group. All patients were treated for 8 weeks or until clinical improvement. Patients undergoing systemic treatment for acne vulgaris were excluded from the study.

All patients were subjected to complete history taking and thorough general and dermatological examinations. The classification is based on the severity into four grades as described by Adityan *et al.* [9]. The classification is based on the number of lesions on half of the face.

(1) Grade I (noninflammatory): 0–5 comedones, and/or occasional papules.
(2) Grade II (mild inflammatory): 6–20 papules, comedones, and/or few pustules.
(3) Grade III (moderate inflammatory): 21–50 predominant pustules, nodules and/or abscesses.
(4) Grade IV (severe inflammatory): more than 50 lesions, mainly cysts, abscesses and/or widespread scarring.

Photographs of the lesions were taken from all patients and controls before starting the study and at the end of the study to evaluate the clinical efficacy and safety of the therapy (Figs. 1–9).

Bacteriological investigations

Bacteriological investigations were carried out before starting the treatment and after the treatment.

The skin was cleansed with an antiseptic agent.

Swabs were moistened with a fluid composed of 0.075 mol^{-1} sodium phosphate buffer (pH 7.9, 0.1% triton).

Swabs were taken from the most affected site of the face with a comedonal extractor.

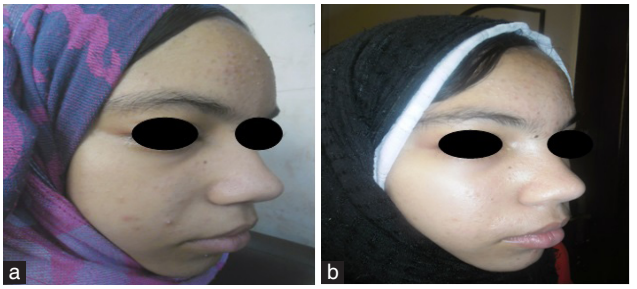
Each swab was inoculated on three plates: two plates (nutrient and blood agars) were incubated aerobically at 37°C for 24–48 h. The other blood agar plate was incubated anaerobically using microaerophilic gas pack (CampyGen; Oxoid) in jars at 37°C for 5 days.

After incubation, the plates were inspected for bacterial growth and colonies were identified by morphology of the colony.

Figure 1

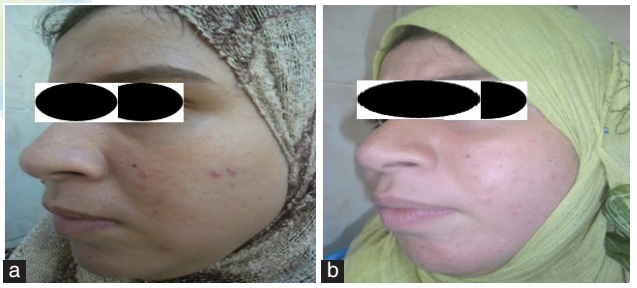
(a, b) Grade I acne patient (noninflammatory) before and after treatment with ethanolic extract of propolis (EEP) (good response).

Figure 2



(a, b) Grade II acne patient (mild inflammatory) before and after treatment with EEP (very good response).

Figure 3



(a, b) Grade II acne patient (mild inflammatory) before and after treatment with EEP (excellent response).

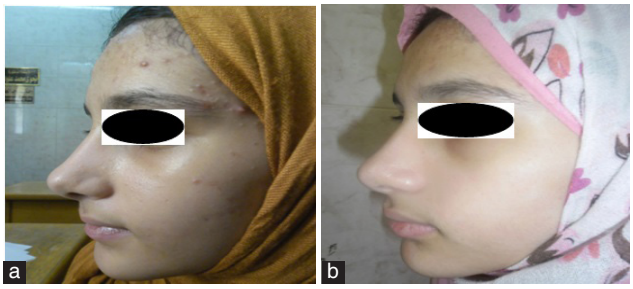
Gram stain for identifying the organism [10]

(1) The primary stain is crystal violet and the secondary stain is safranin.
(2) If the bacteria are gram positive, they retain the primary stain (crystal violet) and are not stained with the secondary stain (safranin), and appear violet/purple under a microscope. If the bacteria are gram negative, they lose the primary stain (crystal violet) and take the secondary stain and appear red when viewed under a microscope.

Biochemical reactions

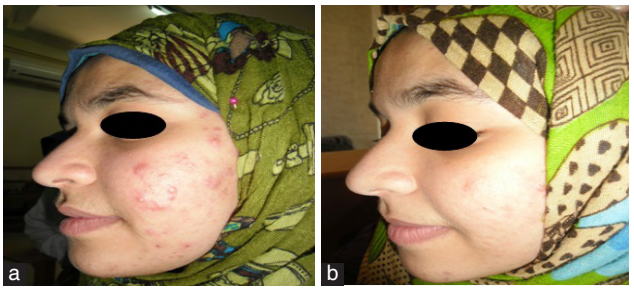
Catalase test [11]: The appearance of gas bubbles is an indicator for positive test, which is due to release of O_2.

Figure 4



(a, b) Grade III acne patient (moderate inflammatory) before and after treatment with EEP (excellent response).

Figure 5

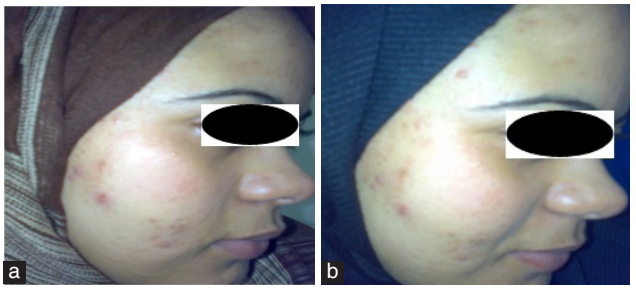


(a, b) Grade IV acne patient (severe inflammatory) before and after treatment with EEP (excellent response).

Figure 6

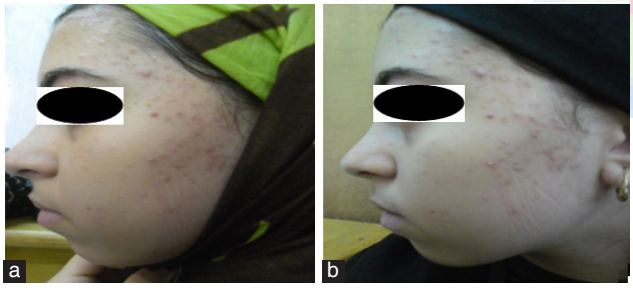
(a, b) Grade I acne patient (noninflammatory) before and after treatment with 80% ethanol (poor response).

Figure 7



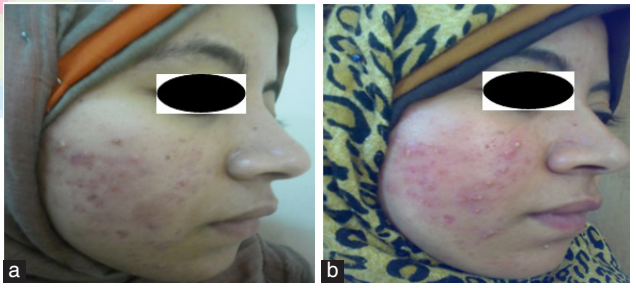
(a, b) Grade II acne patient (mild inflammatory) before and after treatment with 80% ethanol (poor response).

Figure 8



(a, b) Grade III acne patient (moderate inflammatory) before and after treatment with 80% ethanol (poor response).

Figure 9



(a, b) Grade IV acne patient (severe inflammatory) before and after treatment with 80% ethanol (poor response).

Coagulase test [11]: A positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube.

Therapeutic regimen

Preparation of topical solution of ethanolic extract of propolis [12]

The propolis was collected from beehives and prepared at National Research Centre. The collected propolis was kept in a dry place and stored at 4°C until its processing.

Propolis (500 g) was mixed with 10 l of 80% ethanol in dark brown bottle and left for 7–14 days at room temperature in a dark place. The container was shaken two to three times per day. The liquid was filtered through Whatman No. 1 filter paper, and the water was evaporated in an oven at 45°C.

The extracted weight was 120 g of propolis, and the solution was further adjusted with appropriate amounts of 80% ethanol to obtain solutions containing 10% of ethanolic extract of propolis and then stored in dark clean container for further usage.

This solution was applied to the entire face of 20 patients once daily at bed time for 8 weeks or until clinical improvement.

In the control group, 80% ethanol solution alone was applied to acne lesions of the other 20 patients once daily for 8 weeks and the results were compared.

Assessment of the clinical efficacy of propolis therapy

Clinical evaluation was carried out before starting the treatment and every week during treatment.

Patients were evaluated on the basis of the overall acne severity. The patients were evaluated for safety and tolerability. They were also asked about any complaints such as burning, erythema, edema, scaliness, and/or itching.

Treatment efficacy was categorized as follows:

(1) Excellent results: improvement in more than 75% of the lesions, as compared with pretreatment condition.
(2) Very good results: improvement in more than 50–75% of the lesions.
(3) Good results: improvement in more than 25% and less than 50% of the lesions.
(4) Poor results: improvement in less than 25% of the lesions.

Follow-up was carried out every 2 weeks for 2 months after the therapy for assessment of any recurrence or complications.

Results

Clinical results

In group I, there were three male (15%) and 17 female patients (85%), with a ratio of 1 : 5, whereas in group II there were eight male (40%) and 12 female patients (60%), with a ratio of 2 : 3.

As regards the grades of severity of acne, in group I there were four patients (20%) of grade I, 10 patients (50%) of grade II, four patients (20%) of grade III, and two patients (10%) of grade IV, and in group II there were five patients (25%) of grade I, 11 patients (55%) of grade II, three patients (15%) of grade III, and one patient (5%) of grade IV.

Evaluation of clinical efficacy of treatment

The clinical efficacy of topical treatment was as follows: group I showed excellent results in 15 patients (75%), very good results in two patients (10%), and good results in three patients (15%). The control group (group II) showed poor results in all patients. There was highly significant clinical efficacy in group I than in group II (Table 1).

The clinical efficacy of propolis in group I in relation to acne grade severity was follows: among patients with grade I severity of acne, one patient (25%) showed excellent response and three patients (75%) showed good result, and among patients with grade II severity of acne eight patients (80%) showed excellent results and two patients (20%) showed very good results. All patients with grade III and grade IV showed excellent results. There was significant improvement in patients of grade II, III, and IV than in patients of grade I. This shows that more significant improvement was found in inflammatory lesions than in noninflammatory lesions (Table 2).

Safety assessment

Relatively mild dermatological complaints were reported in both treatment groups, with no significant difference. In group I, six patients (30%) reported mild itching during application, whereas 14 patients (70%) showed skin tolerability. In group II, eight patients (40%) reported mild itching, one patient (5%) complained of erythema, and 11 patients (55%) showed skin tolerability. Overall, tolerability (62.5%) was obtained for both treatment groups (Table 3).

Assessment of patients during the follow-up period

In group I, there was no recurrence within 2 months during follow-up in 10 patients (50%). Recurrence occurred in less than 1 month in six patients (30%) and within 1–2 months in four patients (20%).

Table 1 Comparison between group I and II as regards clinical efficacy of treatment

Clinical efficacy	Studied groups [*n* (%)]			χ^2-test	
	Group I (*n* = 20)	Group II (*n* = 20)	Total (*N* = 40) [*n* (%)]	χ^2	*P*-value
Poor	0 (0)	20 (100)	20 (50)	55.452	<0.001**
Good	3 (15)	0 (0)	3 (7.50)		
Very good	2 (10)	0 (0)	2 (5)		
Excellent	15 (75)	0 (0)	15 (37.50)		
Total	20 (100)	20 (100)	40 (100)		

**Highly significant (*P* < 0.001).

Table 2 Relation between clinical efficacy of propolis in group I and acne grades of severity

Acne grades (group I)	Clinical efficacy [*N* (%)]			
	Good	Very good	Excellent	Total
I	3 (75)	–	1 (25)	4
II	–	2 (20)	8 (80)	10
III	–	–	4 (100)	4
IV	–	–	2 (100)	2
Total	3 (15)	2 (10)	15 (75)	20
χ^2-test				
χ^2	14.717			
P-value	0.023*			

*Significant (*P* < 0.005).

Table 3 Comparison between the two groups as regards tolerability and safety

Safety assessment, post-therapy complaints	Groups [*n* (%)]			χ^2-test	
	Group I (*n* = 20)	Group II (*n* = 20)	Total (*N* = 40)	χ^2	*P*-value
Tolerability	14 (70)	11 (55)	25 (62.50)	2.034	0.362
Erythema	0 (0)	1 (5)	1 (2.50)		
Mild itching	6 (30)	8 (40)	14 (35)		
Total	20 (100)	20 (100)	40 (100)		

Bacteriological results

The culture on blood agar showed *Staphylococcus epidermidis* as rapidly growing bacterial colonies, circular and pinhead, and convex with entire margins (white or cream colored), whereas *P. acnes* was seen as slow-growing bacterial colonies, nonspore forming, and polymorphic (white and opaque, Figs. 10 and 12).

The culture on nutrient agar showed *S. epidermidis* as circular bacterial colonies, 2–3 mm in diameter with a smooth shiny surface (Fig. 11).

Gram stain

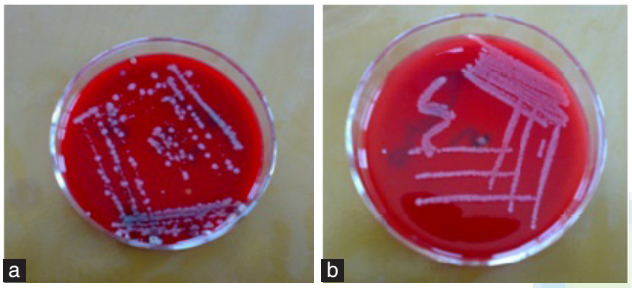
S. epidermidis is gram-positive cocci, arranged in grape-like clusters, whereas *P. acnes* is gram-positive bacteria, rod-shaped or branched, and singular, in pairs, or in groups (Figs. 13 and 14).

Biochemical reactions

S. epidermidis and *P. acnes* are catalase positive and coagulase negative (Figs. 15 and 16).

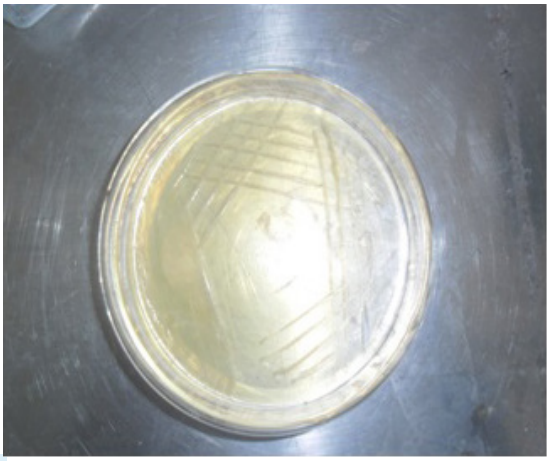
Microbial ecology of acne lesions in all patients in relation to acne grades was as follows: patients of grade

Figure 10



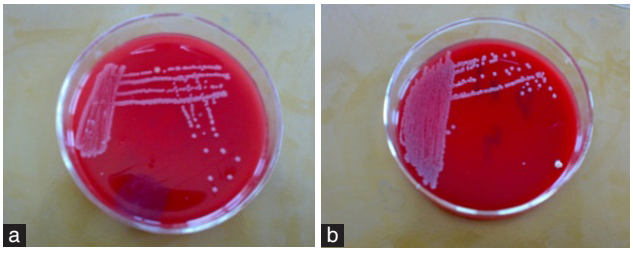
(a, b) Aerobic culture on blood agar: circular bacterial colonies, pinhead, convex with entire margins and white-to-cream colored.

Figure 11



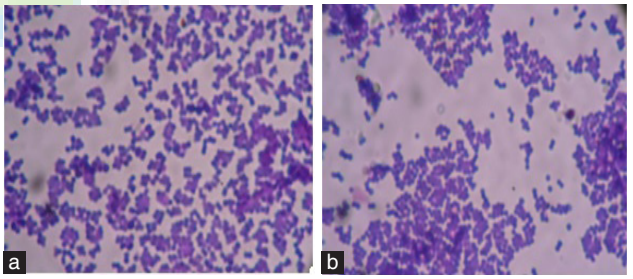
Staphylococcus epidermidis: aerobic culture on nutrient agar: circular bacterial colonies, 2–3 mm in diameter with smooth shiny surface.

Figure 12



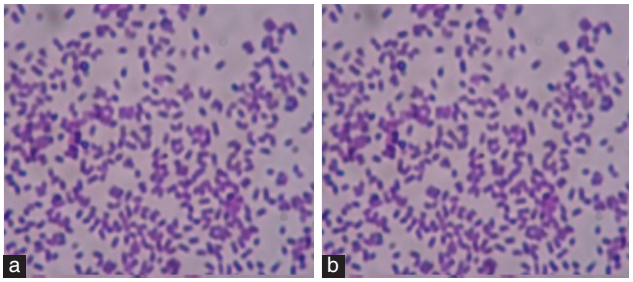
(a, b) *Propionibacterium acnes*: anaerobic culture on blood agar: polymorphic, white, and opaque.

Figure 13



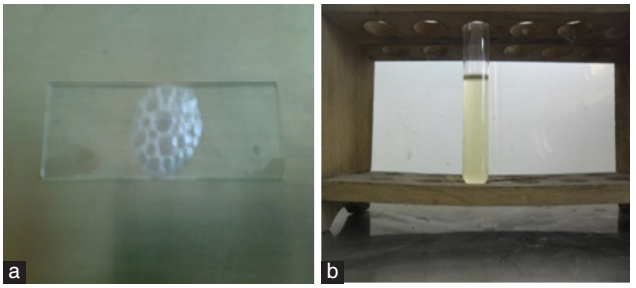
(a, b) *Staphylococcus epidermidis*: heavy and light colonization: gram-positive cocci, arranged in grape-like clusters, gram stain (×100).

Figure 14



(a, b) *Propionibacterium acnes*: heavy and light colonization: gram-positive bacteria, rod-shaped or branched, singular, in pairs, or in groups, gram stain (×100).

Figure 15



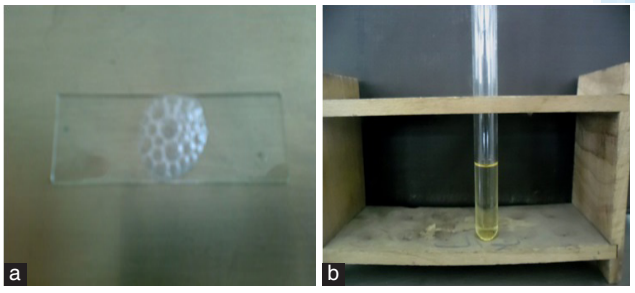
(a, b) Biochemical reactions (catalase and coagulase tests): *Staphylococcus epidermidis*: catalase positive (gas bubbles) and coagulase negative (no gelling of plasma).

I and grade III had lesions that were mainly colonized by *P. acnes*, in patients of grade II, colonization by P. acnes and *S. epidermidis* were nearly equal, while patients of grade IV had lesions that were mainly colonized by *S. epidermidis*.

Bacteriological examination results before treatment were as follows: in group I, 50% of lesions were colonized by *P. acnes* and 50% of lesions were colonized by *S. epidermidis*, and in group II, 70% of lesions were colonized by *P. acnes* and 25% by *S. epidermidis*, whereas only one lesion showed no bacterial growth (5%). Bacteriological examination results after treatment were as follows: in group I, lesions of 17 patients (85%) showed no growth, lesions of only one patient (5%) showed *P. acnes* colonization, and that of two patients (10%) showed *S. epidermidis* colonization; and in group II, lesions of two patients (10%) showed no growth, lesions of 14 patients (70%) were colonized by *P. acnes*, and lesions of four patients (20%) were colonized by *S. epidermidis*. There was highly significant decrease in bacterial growth after treatment in group I, with highly significant increase in patients with no growth compared with those with growth after treatment, whereas in group II there was no significant difference in bacterial growth after treatment compared with that before treatment (Table 4).

The clinical assessment of treatment in patients of group I in relation to bacteriological examination after treatment was as follows: 15 patients (75%) showed no growth. All these patients showed excellent results during treatment, and five patients (15%) who had growth, showed good and very good results during treatment (Table 5).

Figure 16



(a, b) Biochemical reactions (catalase and coagulase tests): *Propionibacterium acnes*: catalase positive (gas bubbles) and coagulase negative (no gelling of plasma).

Discussion

Acne vulgaris is a common skin condition encountered by dermatologists. Rationale use of available treatment options based on the type and severity of the disease is considered a key component of successful acne therapy. Since ancient times, propolis has been used as traditional monastic, folk, and natural medicine in many countries. Propolis is a resinous substance that bees collect from exudates of plants to seal holes in the beehives [13].

A wide range of pharmacological properties were demonstrated for this substance, including antimicrobial, antiviral, antifungal, antioxidant, antitumor, and anti-inflammatory [6]. Thirty-nine constituents were identified in the Egyptian propolis. One of the first and the most important biological property of propolis is the antimicrobial activity, especially against bacteria. Propolis antibacterial activity has been mainly correlated with flavonoids and phenolic acids. Studies have been performed to evaluate this property against a large panel of gram-positive aerobic or anaerobic bacteria, which either displayed much lower activity against the gram-negative ones or displayed no activity [14].

The present study aimed to evaluate the significance of topical propolis extracts in the treatment of facial acne vulgaris.

In the current study based on the clinical assessment of acne patients after treatment with topical ethanolic extract of propolis, there was significantly higher efficacy and higher significant reduction of the inflammatory and noninflammatory lesions compared with that before treatment. This indicates the anti-inflammatory

Table 4 Bacteriological examination of acne lesions before and after treatment in both groups

Groups	Bacteriological examination	Patients (N = 40) [n (%)]		χ^2-test	
		Before treatment	After treatment	χ^2	P-value
Group I (n = 20)	No growth	0 (0)	17 (85)	29.70	<0.001**
	Propionibacterium acnes	10 (50)	1 (5)		
	Staphylococcus epidermidis	10 (50)	2 (10)		
	Total	20 (100)	20 (100)		
Group II (n = 20)	No growth	1 (5)	2 (10)	0.44	0.80
	Propionibacterium acnes	14 (70)	14 (70)		
	Staphylococcus epidermidis	5 (25)	4 (20)		
	Total	20 (100)	20 (100)		

**Highly significant (P < 0.001).

Table 5 Relation between efficacy of ethanolic extract of propolis and bacteriological examination after treatment

Bacteriological examination after treatment	Clinical efficacy (group I) [*n* (%)]			
	Good	Very good	Excellent	Total
No growth	1 (5)	1 (5)	15 (75)	17 (85)
Propionibacterium acnes	1 (5)	–	–	1 (5)
Staphylococcus epidermidis	1 (5)	1 (5)	–	2 (10)
Total	3 (15)	2 (10)	15 (75)	20 (100)
χ^2-test				
χ^2	11.363			
P-value	0.023*			

*Significant ($P < 0.005$).

and antibacterial properties of propolis. The significant reduction in seborrhea and excess greasiness of the skin also confirmed the efficacy of this daily treatment of propolis. These results were in accordance with the study of Wagh [12], who stated that propolis was very effective in the treatment of facial acne as it has anti-inflammatory effect.

In this study, the tolerability was excellent. This may be due to the presence of wax and essential oils in the composition of the Egyptian propolis. Eshwar and Suma [13] stated that propolis was very tolerable in most acne patients, but mild irritation occurred in few patients. Transient local irritation and itching might be because of ethanol, which acts as an irritant on the inflamed skin sites of acne lesions.

In this study, as regards the microbial etiology of acne lesions in both groups of patients before treatment, culture of swabs reported gram-positive anaerobic *P. acnes* and aerobic *S. epidermidis*. These results were in accordance with that reported by others, who stated that a proportion of acne affected pilosebaceous follicles (inflamed and noninflamed) are generally colonized by *P. acnes* and *S. epidermidis* [15,16]. Nishijima *et al.* [17] reported that after culture of inflammatory pustules, lesions were colonized predominantly by *P. acnes* and *S. epidermidis*, which are important in the pathogenesis of acne vulgaris. They stated that these microorganisms may be the representative bacteria in any acne lesion and other bacteria species may be contaminants.

In the present study, there was a higher significant effect of ethanolic extract of propolis against *P. acnes* and *S. epidermidis* growth. This antimicrobial activity of propolis is due to its composition of flavonoids and phenolic acid esters that have antibacterial, antioxidant, and anti-inflammatory properties.

These results were in accordance with that of Eshwar and Suma [13], who stated that propolis dilution of 1 : 20 in blood agar completely inhibited the growth of *S. epidermidis* and *Corynebacterium* spp. Fokt *et al.* [18] reported that propolis was active against gram-positive bacteria. Wagh [12] also stated that propolis inhibited most of the anaerobic *Propionibacterium* spp. in a study of in-vitro activity of propolis against clinical isolates of anaerobic bacteria. Grange and Davey [19] stated that ethanolic extract of propolis inhibited the growth of anaerobic bacteria, strains of bacteroids, and *Peptostreptococcus* spp., but it was less effective against gram-positive rods of *Propionibacterium* spp.

Propolis ethanolic extract has a promising role in the treatment of acne vulgaris. It is natural, safe, tolerable, cheap, and has anti-inflammatory and antibacterial properties. Further studies are needed for its application in different skin diseases.

Acknowledgements

Conflicts of interest

None declared.

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