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The Antimicrobial Activities of *Psidium guajava* and *Juglans regia* Leaf Extracts to Acne-Developing Organisms

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Abstract: This study aims to present the *in vitro* inhibitory effect of *Psidium guajava* and *Juglans regia* leaf extracts on the main developer of acne lesions, *Propionibacterium acnes* (*P. acnes*), and other organisms that are isolated from acne lesions. Thirty-eight subjects (males and females) who had various types of acne were enrolled in the study. The contents of the acne lesions were cultured and the frequency of *P. acnes* (alone and with *Staphylococci spp*.) was 47%, whereas the frequencies for *Staphylococcus aureus* and *Staphylococcus epidermidis* were 13% and 24%, respectively. The antimicrobial activities of *Psidium guajava* and *Juglans regia* leaf extracts, determined by disk diffusion method (zone of inhibition), were compared to tea tree oil (TTO), doxycycline and clindamycin antibiotics. The zones of inhibition due to the *Psidium guajava* and *Juglans regia* leaf extracts ranged from 15.8–17.6 mm against *P. acnes*, 11.3–15.7 mm against *S. aureus* and 12.9–15.5 mm against *S. epidermidis*, respectively. These zones of inhibition were significantly higher than those of TTO and equivalent in case of *Staphylococci spp.*, but less in case of *P. acnes*, to those obtained from doxycycline or clindamycin. It can be concluded that *Psidium guajava* and *Juglans regia* leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities.

Keywords: *Psidium guajava*; *Juglans regia*; Acne; *Propionibacterium acnes.*

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Introduction

The rapid emergence of drug-resistant organisms necessitates the continuous search of new antimicrobial substances. Natural products may act as an alternative for antibiotics and chemotherapeutic agents in certain circumstances.

Acne vulgaris is a common skin disorder forming lesions that result from an inflammatory condition, which usually requires medical treatment. The development of an acne lesion is mostly due to the presence of *Propionibacterium acnes* (*P. acnes*), although other organisms, such as *Staphylococci*, have been isolated (Marples, 1974). These organisms increase the inflammatory reaction that may be initiated by increase in sebum production, free fatty acids from sebum itself and abnormal keratinization of the sebaceous canal (Tucker *et al*., 1980). Currently, the treatment of acne involves topical administration of antibiotics (e.g. clindamycin), agents such as benzoyl peroxide, tea tree oil (TTO) or systemic antibiotics (e.g. tetracycline). The TTO and benzoyl peroxide treatments have many unwanted side effects, such as skin dryness, pruritus, stinging and burning sensations (Bassett *et al*., 1990). Because the numbers of antibiotic-resistant organisms are increasing, the need of new alternatives is essential.

Psidium guajava L. (Myrtaceae) and *Juglans regia* L. (Juglandaceae) extracts were found to have some degrees of antimicrobial activity (Alkhawajak, 1997; Holetz *et al*., 2002; Arima and Danno, 2002). However, the latter studies were not specific to certain pathological conditions. This study aims to present the *in vitro* inhibitory effect of *Psidium guajava* and *Juglans regia* leaf extracts on the main developer of acne lesions, *P. acnes*, and other organisms that are isolated from acne lesions. In addition, the antimicrobial effects of these natural products on organisms isolated from clinical specimens were also evaluated.

Materials and Methods

Plant Materials and Extraction

Juglans regia L. leaves were collected in May 2001 from trees grown in Jerash, Jordan. A voucher specimen is deposited at the University of Petra, Amman, Jordan. The leaves were air-dried (500 g) and extracted with acetone : H_2O (7 : 3, 5 L). The extracted material was evaporated in vacuum to 1.5 L, filtered to remove the precipitated chlorophyll, concentrated and defatted with petrol. After shaking with ethyl acetate, the water residue (46 g) was subjected to column chromatography on Sephadex LH-20 (3.8×50 cm) with MeOH-H_2O 1 : 1 (12 L) until the eluted material became colorless. The tested extract was then eluted with acetone-H_2O 7 : 3 (5 L) and freeze-dried (7.0 g).

Psidium guajava L. leaves were collected from trees grown in Sudan (07/2001) and identified in comparison with authentic *Psidium guajava* obtained from the University of Jordan/Amman. A voucher specimen is deposited at the University of Petra. Two kilograms of air-dried material were extracted with acetone : H_2O (7 : 3, 12 L), evaporated in vacuum to 1.5 L, filtered to remove the precipitated chlorophyll, concentrated and defatted with petrol. After shaking with ethyl acetate, the water residue (200 g) was subjected to column

chromatography on Sephadex LH-20 (3.8 × 50 cm) with MeOH-H_2O 1 : 1 (12 L) until the eluted material became colorless. The tested extract was then eluted with acetone-H_2O 7 : 3 (5 L) and freeze-dried (19 g).

Isolation of Microorganisms from Acne Lesions

Thirty-eight males and females, with ages ranged from 18–22 years with various types of acne were enrolled in the study. At the time of obtaining the specimens, the subjects had neither local nor systemic antibacterial treatment for three days. The lesion, a relatively new papule, was swabbed with ethyl alcohol, pierced with sterile lancet to allow the contents out, and inoculated on two blood agar plates for aerobic and anaerobic cultures. A Gram-stained smear was made for every swabbed lesion. Cultured plates were examined after 24 and 72 hours of incubation. *P. acnes* was identified by colonial morphology, Gram stain and indole test. *Staphylococci* isolates were also identified similarly as *P. acnes*, with the addition of coagulase test.

Clinical Isolates

Several organisms, isolated from clinical specimens (urine, pus and blood) and identified by conventional methods and API 20, were used in the study. The sensitivities of these isolates to the extracts were also tested.

Antimicrobial Sensitivity Test

Sterile 6.0 mm diameter blank discs were used to impregnate the different extracts. Discs were freshly prepared and used immediately. No special treatment was made when discs were impregnated with different concentrations (w/v) of *Psidium guajava* and *Juglans regia* leaf extracts. For TTO (Provital, S.A. Spain), 10%, 15% and 20% (v/v) concentrations were used after mixing with Tween-20 (Sigma, USA) in a 1 : 4 (TTO: Tween-20) ratio. Previous studies have mentioned that TTO antimicrobial activity is higher when terpinen-4-ol content is greater than 35% (Priest, 1994), and more efficient if mixed with polysorbate 20 (Priest, 1994). Control discs impregnated with Tween-20 revealed no zone of inhibition. In addition, doxycycline (5 μg) and clindamycin (2 μg) commercial discs were used as controls.

The sensitivity of *P. acnes* was performed on Muller-Hinton agar containing 5% sheep blood or serum. Sensitivity of all other organisms was performed on Muller-Hinton agar. A suspension of the organisms was made in broth. A sterile swab was soaked in the suspension and streaked on the surface of the plate before the discs were organized on the plates. The plates were incubated as previously described, and the antimicrobial activity for each disc was measured by the diameter of the zone of inhibition. The sensitivity testing was repeated two to three times and the average of zone of inhibition was taken for that specific isolate.

Statistical Analysis

Results are expressed as mean of zone of inhibition ± standard error (SE) of several isolates of *P. acnes*, *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) from several subjects. Statistical significance between the groups was determined using analysis of variance (if more than two) and paired Student's t test (between two groups).

Results

Acne Swabs and Cultures

In this study, the frequency of *P. acnes* obtained from acne lesions was 47%. In 61% of the cases (11/18), *P. acnes* was accompanied by *Staphylococci spp*. (Table 1). In 72% of the cases, inflammatory cells with or without necrotic tissue were observed while screening the stained slides from the acne swabs.

Acne Isolates

The sensitivity of *P. acnes* isolates was tested to TTO, *Juglans regia*, *Psidium guajava* leaf extracts, doxycycline and clindamycin (Fig. 1). Thirty-five percent of the *P. acnes* isolates were resistant (zone of inhibition was < 7 mm) to 15% and 20% of TTO, whereas all isolates were sensitive to 10%, 15% and 20% of *Juglans regia* and 10% of *Psidium guajava* leaf extracts with only 7% showing less than 10 mm zone of inhibition. The increase in the zone of inhibition between the groups, excluding doxycycline and clindamycin (Fig. 1) was statistically significant ($p < 0.002$). In addition, the zones of inhibition due to *Juglans regia*, *Psidium guajava* leaf extracts, doxycycline and clindamycin were significantly different than 15% and 20% of TTO ($p < 0.02–0.001$).

The sensitivity of *S. epidermidis* isolates to TTO, *Juglans regia*, *Psidium guajava* and doxycycline is presented in Fig. 2. Eighty-one percent of *S. epidermidis* isolates were resistant to 15% and 20% of TTO, whereas 12.5% of the isolates were resistant to *Juglans regia* leaf extracts. The increase in the zone of inhibition between the groups excluding doxycycline (Fig. 2) was statistically significant ($p < 0.001$). In addition, the zones of inhibition due to *Juglans regia*, *Psidium guajava* leaf extracts and doxycycline were significantly different than 15% and 20% of TTO ($p < 0.02$).

Table 1. The Results of the Cultured Swabs Obtained from Acne Lesions

No. of Subjects	*P. acnes*	*P. acnes* + *S. aureus*	*P. acnes* + *S. epidermidis*	*S. aureus*	*S. epidermidis*	No Growth
38 (100%)	7 (18%)*	4 (11%)	7 (18%)	5 (13%)	9 (24%)	6 (16%)

*The frequency of obtaining such organisms from acne lesions.

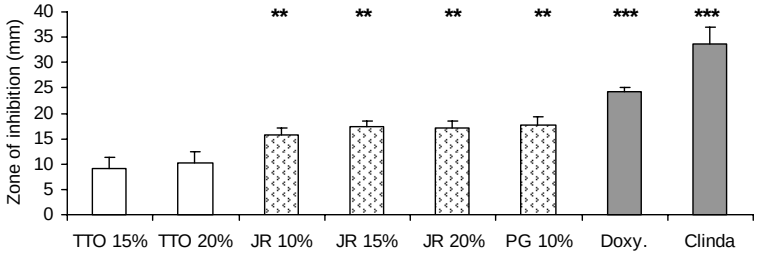

Figure 1. The antimicrobial effect of TTO, *Juglans regia* (JR), *Psidium guajava* (PG) leaf extracts, doxycycline and clindamycin to *P. acnes* isolated from acne lesions is presented by the mean (± SE) of zone of inhibition. The increase in the zone of inhibition between the groups excluding doxycycline and clindamycin was significant ($p < 0.002$). The zones of inhibition due to JR, PG leaf extracts, doxycyline and clindamycin were signifcantly different than 15% and 20% of TTO ($^{**}p < 0.02$, $^{***}p < 0.001$).

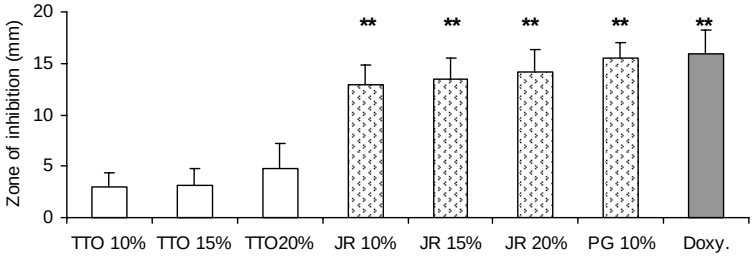

Figure 2. The antimicrobial effect of TTO, *Juglans regia* (JR), *Psidium guajava* (PG) leaf extracts, doxycycline and clindamycin to *S. epidermidis* isolated from acne lesions is presented by the mean (± SE) of zone of inhibition. The increase in the zone of inhibition between the groups excluding doxycycline was significant ($p < 0.001$). The zones of inhibition due to JR and PG leaf extracts, doxycyline and clindamycin were signifcantly different than 15% and 20% of TTO ($^{**}p < 0.02$).

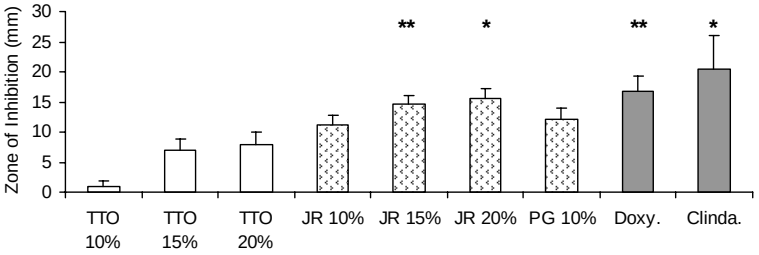

Figure 3. The antimicrobial effect of TTO, *Juglans regia* (JR), *Psidium guajava* (PG) leaf extracts, doxycycline and clindamycin to *S. aureus* isolated from acne lesions is presented by the mean (± SE) of zone of inhibition. The increase in the zone of inhibition between the groups excluding doxycycline and clindamycin was significant ($p < 0.007$). The zones of inhibition due to 15%, 20% of JR, doxcycline and clindamycin were signifcantly different than those of 15% and 20% of TTO ($^{*}p = 0.053$, $^{**}p < 0.03$).

Table 2. *In vitro* Antimicrobial Activity of *Juglans regia*, *Psidium guajava* and TTO to Several Clinical Isolates Determined by Diameter (mm) of Zone of Inhibition

Organism	TTO 15%	TTO 20%	*Juglans regia* 15%	*Psidium guajava* 10%	Doxycycline
Providencia stearti	10	10	20	16	0
Providencia rettgeri	0	0	15	11	0
Proteus vulgaris	0	9	0	0	0
E. coli	8	10	0	0	0
Salmonella spp.	8	10	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0
Streptococcus group C	0	0	15	10	20
Streptococcus faecalis	0	0	17	0	0
Staphylococcus aureus	0	10	10	12	20
Candida albicans	0	0	20	22	0

The sensitivity of *S. aureus* isolates to TTO, *Juglans regia*, *Psidium guajava*, doxycycline and clindamycin is presented in Fig. 3. Thirty-three percent of *S. aureus* isolates were resistant to 15% and 20% of TTO, whereas 11%–33% of the isolates showed less than 10 mm zone of inhibition to *Juglans regia* and *Psidium guajava* leaf extracts. The increase in the zone of inhibition between the groups, excluding doxycycline and clindamycin (Fig. 3) was statistically significant ($p < 0.007$). The zones of inhibition due to 15% and 20% of *Juglans regia*, doxycycline and clindamycin were signifcantly higher than those of 15% and 20% of TTO ($p < 0.03–0.053$). However, the apparent increase in the zone of inhibition (apparently dose-dependent) between the different concentrations of *Juglans regia* was not statistically significant ($p > 0.1$).

Clinical Isolates

The antimicrobial activities of *Juglans regia* and *Psidium guajava* leaf extracts were mainly against Gram-positive organisms *Providencia spp*. and *Candida albicans*. However, TTO showed more diversity but limited antimicrobial activity (Table 2).

Discussion

This study shows the antimicrobial effect of *Juglans regia* and *Psidium guajava* leaf extracts on acne-developing organisms. This effect was superior to TTO, which was recently considered to be a very good topical treatment for acne (Bassett *et al*., 1990). Gas chromatography was performed to ascertain that the used TTO in this study was of good quality. It revealed that it contains more than 35% of terpinen-4-ol.

It has been reported that the antimicrobial activities of *Juglans regia* and *Psidium guajava* leaf extracts were attributable to the presence of tannins, triterpenoids and flavonoid glycosides in the leaves themselves (Arima and Danno, 2002; Begum *et al*., 2002;

Fukuda *et al*., 2003). However, the extracts used in this study consist mainly of polyphenols including polymeric proanthocyanidins (condensed tannins) and hydrolyzable tannins, because triterpenoids, low molecular tannins, flavonoids glycosides and polysaccharides are washed out by methanol-water step (Dauer *et al*., 2003). These polyphenols are also known to have antimicrobial effects (Guyot *et al.*, 1999).

Using anti-inflammatory agents may also be helpful in reducing the inflammatory process in the acne lesions. *Psidium guajava* leaves were found to contain anti-inflammatory activity, by decreasing serum chemokines such as interleukin-8 and eosinophil cationic protein (Suzuki *et al*., 2000) and having antioxidant activity (Jimenez-Escrig *et al*., 2001). In addition, *Juglans regia* extracts also have anti-inflammatory and oxidative activities (Anderson *et al.*, 2001; Erdemoglu *et al.*, 2003). Furthermore, the polymeric proanthocyanidins and polyphenols are known to have anti-inflammatory activities. Therefore, the antimicrobial, anti-inflammatory and antioxidant activities of *Psidium guajava* and *Juglans regia* extracts may be more beneficial for treating acne.

This study shows the *in vitro* antimicrobial activities of *Juglans regia* and *Psidium guajava* leaf extracts to *P. acnes*, *S. aureus*, *S. epidermidis* and other organisms, such as *Candida albicans*. This induces a challenge to develop more studies to evaluate the antimicrobial activities of different extracted materials from the leaves of *Juglans regia* and *Psidium guajava*, followed by formulation and *in vivo* therapeutic studies against acne lesions.

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