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Original Article

Growth Inhibitory Properties of *Scaevola spinescens* R.Br. Leaf Extracts against the Acne Vulgaris causing Bacterium *Cutibacterium acnes*

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ABSTRACT

Introduction: Acne vulgaris is a skin conditions that affects most adolescents and may also afflict adults. Medicinal plant extracts may provide leads for the development of new topical and/or oral therapies for acnes vulgaris, yet many traditional medicine plants are yet to be screened for growth inhibitory activity against *Cutibacterium acnes* (the major bacterial cause of acne). **Materials and Methods:** Methanolic and aqueous *Scaevola spinescens* R.Br. leaf extracts were investigated by disc diffusion and liquid dilution MIC assays against *Cutibacterium acnes* (a significant bacterial cause of acne). Toxicity was determined using *Artemia franciscana* nauplii bioassays. **Results:** Methanolic and aqueous *S. spinescens* leaf extracts displayed noteworthy bacterial growth inhibitory activity against *C. acnes* growth. The aqueous *S. spinescens* leaf extract had particularly good antibacterial effects against *C. acnes*, with an LD MIC value of 344 µg/mL. Similar, albeit slightly higher LD MIC values were noted for the aqueous methanolic *S. spinescens* leaf extract against *C. acnes* (LD MIC = 875 µg/mL). The methanolic and aqueous *S. spinescens* leaf extracts were nontoxic in the *Artemia fransiscana* bioassay, with LC_{50} values substantially >1000 µg/mL. **Conclusion:** The lack of toxicity of the methanolic and aqueous *S. spinescens* leaf extracts and their noteworthy growth inhibition of *C. acnes* indicate their potential as treatments to alleviate acne vulgaris. Further studies are warranted to isolate and identify the active components and to determine their antibacterial mechanism.

Keywords: Goodeniaceae, Maroon bush, Prickly fan flower, Acne vulgaris, Skin infection, Skin inflammation, *Cutibacterium acnes*.

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INTRODUCTION

Acne vulgaris (generally referred to as acne) is skin condition that is characterised by seborrhea (red scaly and inflamed skin), papules (small tender bumps), pimples/pustules (papules with pus at the tip), nodules (large painful subcutaneous lumps), whiteheads (closed plugged pores), blackheads (open plugged pores) and cystic lesions (painful, pus-filled subcutaneous lumps).[1] Acne most frequently affects skin with a high number of oil glands, including the face, chest and back, although it may also occur on most other parts of the body. The presence of prolonged acne (particularly in teenagers) can lead to anxiety and low self-esteem, and may lead to social isolation and mental health issues if not addressed. Whilst susceptibility to chronic acne is genetic in most cases (~80 %), particularly in individuals that produce high levels of androgens, personal hygiene, diet, stress and cigarette smoking may also contribute chronic acne. Notably, subcutaneous infections with the anaerobic bacteria *Cutibacterium acnes* (previously called *Propionobacterium acnes*) also trigger acne by inducing the release of pro-inflammatory cytokines.[2]

Treatments for acne vary, depending on the severity of the condition. For mild cases of acne, topical application of anti-acne creams are most frequently used, whereas both oral therapies and topical treatments may be used for severe cases of acne. A wide variety of medications may be used, including alpha hydroxyl acids, azelaic acid, benzoyl peroxide, isotretinoids, keratolytic soaps, retinoids and salicylic acid.[3] Hormonal therapy (anti-androgens and/or androgen receptor antagoniosts) may also be used to treat severe chronic acne. Additionally, antibiotics are useful in controlling chronic acne as they inhibit *C. acnes* infections, thereby blocking the development of inflammatory acne symptoms.[3] However, none of these remedies are free of toxicities and unwanted side-effects. Additionally, prolonged use of broad-spectrum antibiotics is not recommended as it can result in the development of antibiotic-resistant *C. acnes* (as well as other pathogenic bacteria), especially to the commonly used tetracycline and macrolide classes of antibiotics.[4] The use of complementary and alternative therapies that inhibit the growth of *C. acnes* is also relatively common in many regions of the world.[5-6] The development of new acne vulgaris therapies from traditional plant-based medicines has potential and several studies have begun to screen traditional medicines against *C. acne*.[6-8] However, many other plants are yet to be screened against *C. acnes* for growth inhibitory activity.

Scaevola spinescens R.Br. (family Goodeniaceae; commonly known as currant bush, maroon bush and prickly fanflower) is a rigid, scrubby bush that grows in arid regions of the Australian continent. The First Australians used *S. spinescens* leaves as a medicine to treat a variety of conditions, including many illnesses caused by pathogenic bacteria.[9-10] An infusion of the roots was used to treat stomach pain and urinary disorders. A decoction of crushed stem was used to treat boils, rashes and several skin disorders. The whole plant was burnt and the fumes inhaled to treat colds. Leaves and twigs were steamed and sores were treated by exposure to this steam. Surprisingly, the antimicrobial properties of *S. spinescens* have not been extensively studied. Whilst several studies have reported that *S. spinescens* leaf extracts have noteworthy antiviral activity against human cytomegalovirus (CMV) [11] and MS2 bacteriophage,[12-13] few studies have yet screened extracts prepared from this plant against bacteria. Indeed, we only a few studies have reported antibacterial activity for *S. spinescens* leaf extracts, and these screened the extracts against limited panels of bacteria.[13-15] The current study screened *S. spinescens* solvent extractions for the ability to inhibit *C. acnes* growth, and quantifies the antibacterial potency.

MATERIALS AND METHODS

Plant Source and Extraction

The *Scaevola spinescens* R.Br. leaves used in this study were provided by Jeannie Cargo of Outback Books (a commercial supplier of *Scaevola spinescens* tea) as pre-dried and coarse milled whole plant material. The leaf material was stored at -30°C until use. The dried leaves were freshly ground to a coarse powder prior to extraction. Individual 1g quantities of the ground fruit were weighed separately into tubes and 50 mL of methanol (AR grade, Ajax Fine Chemicals, Australia) or sterile deionised water were added. The fruits were extracted in each solvent for 24 hr at 4°C with gentle shaking. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying in a vacuum oven at 50°C. The resultant dry extracts were weighed and redissolved in 10 mL of deionised water (containing 0.5 % DMSO), and passed through a 0.22 µm filter (Sarstedt) to remove particulates. The extracts were then stored at 4°C until use.

Qualitative Phytochemical Studies

Phytochemical analysis of the *S. spinescens* leaf extracts for the presence of cardiac glycosides, alkaloids, saponins, tannins, flavonoids, phenolic compounds, phytosterols, flavonoids and triterpenoids was achieved as previously described.[16-18]

Antibacterial Screening

Test Bacterial Strains

All media and other materials was supplied by Oxoid Ltd., Australia unless otherwise specified. A reference strain of *Cutibacterium acnes* (ATCC6919) were purchased from American Type Culture Collection, USA. The *C. acnes* stock was cultured using a thioglycollate liquid media (Oxoid ltd., Australia) under induced anaerobic conditions through the use of anaerobic jars and AnaeroGen™ 3.5 L atmospheric generation systems (Thermo Scientific) at 37°C for 72 hr.

Evaluation of Antibacterial Activity

Antibacterial activity screening of the *S. spinescens* leaf extracts was achieved using modified disc diffusion assays.[19-21] Briefly, 100 µL of each individual bacteria was grown separately in 20 mL of the appropriate broth until an approximate count of 10^8 cells/mL was reached. A volume of 100 µL of the bacterial suspension was spread onto nutrient agar plates and the extracts were tested for antibacterial activity using 6 mm sterilised filter paper discs. Discs were infused with 10 µL of the individual extracts, allowed to dry, and placed onto the inoculated plates. The plates were left to stand at 4°C for 2 hr before incubation. The plates were incubated under induced anaerobic conditions at 37°C for 72 hr. The diameters of the inhibition zones (ZOIs) were measured to the closest whole millimetre. The assays were completed three times, each with internal triplicates (n = 9). Mean values (± SEM) are reported in this study. Ampicillin (10 µg) and vancomycin (5 µg) discs were obtained from Oxoid Ltd., Australia and used as positive controls to compare antibacterial activity. Filter discs infused with 10 µL of distilled water (containing 0.5 % DMSO) were used as negative controls.

Minimum inhibitory Concentration (MIC) Determination

The minimum inhibitory concentration for each extract was determined using two methods. A liquid dilution MIC assay was employed as it is generally considered the most sensitive bacterial growth inhibitory assay.[22] Furthermore, as microplate liquid dilution (LD) MIC assays are perhaps the most commonly used method of quantifying bacterial growth inhibition efficacy, use of this method allows for comparisons with other studies. A solid phase agar disc diffusion (DD) assay was also used in this study as this bioassay was deemed to provide a closer representation of the environment and conditions relevant to solid-phase skin systems.

Microplate Liquid Dilution MIC Assay

The MICs of the extracts were evaluated by standard methods.[22-23] Briefly, overnight bacterial cultures were added dropwise to fresh liquid broth and the turbidity was visually adjusted to produce a McFarlands number 1 standard culture. This was subsequently diluted 1 in 50 with fresh broth, resulting in the MIC assay inoculum culture. A volume of 100 µL sterile broth was added to all wells of a 96 well plate. Test extracts or control antibiotics (100 µL) were then added to the top row of each plate and 1 in 2 serial dilutions were prepared in each column of wells by transferring 100 µL from the top well to the next well in each column, etc. A growth control (without extract) and a sterile control (without inoculum) were included on each plate. A volume of 100 µL of bacterial culture inoculum was added to all wells except the sterile control wells. The inoculated plates were incubated under induced anaerobic conditions at 37°C for 72 hr. p-Iodonitrotetrazolium violet (INT) was obtained from Sigma, Australia and dissolved in sterile deionised water to prepare a 0.2 mg/mL INT solution. A 40 µL volume of this solution was added into all wells and the plates were incubated for a further 6 hr at 30°C. Following incubation, the MIC was visually determined as the lowest dose at which colour development was inhibited.

Disc Diffusion MIC Assay

The minimum inhibitory concentrations (MIC) of the extracts was also evaluated by disc diffusion assay as previously described.[24-25] Briefly, the *S. spinescens* leaf extracts were diluted in deionised water (containing 0.5 % DMSO) and tested across a range of concentrations. Discs were infused with 10 µL of the extract dilutions, allowed to dry and placed onto inoculated plates. The assay was achieved as outlined above and graphs of the zone of inhibition versus Ln concentration were plotted. Determination of MIC values were achieved using linear regression.

Toxicity Screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared in deionised water (4 mg/mL) and serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana Nauplii Toxicity Screening

Toxicity was assessed using a modified *Artemia franciscana* nauplii lethality assay.[27-28] Briefly, 400 µL of seawater containing 38 (mean 38.4, n = 125, SD 13.7) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used in the bioassay. A volume of 400 µL of the reference toxin or the diluted plant extracts were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). For each plate, a 400 µL seawater negative control was run in triplicate. The wells were assessed at regular intervals and the number of dead counted. The nauplii were deemed dead if no movement of the appendages was observed within 10 sec. After 24 hr, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95 % confidence limits for each treatment was calculated using probit analysis.

Statistical Analysis

Data is expressed as the mean ± SEM of at least three independent experiments, each with internal triplicates (n = 9).

RESULTS

Liquid extraction yields and qualitative phytochemical screening

One gram masses of the dried *S. spinescens* leaf powder were extracted separately with methanol and water, resulting in yields of 144 and 220 mg respectively (Table 1). The extracts were dried and were subsequently resuspended in 10 mL of deionised water (containing 0.5 % DMSO), resulting in the concentrations presented in Table 1. Qualitative phytochemical studies showed that the methanolic and aqueous *S. spinescens* leaf extracts had similar phytochemical profiles. Both extracts contained moderate to high levels of phenolic compounds, flavonoids and tannins, as well as lower levels of saponins.

Inhibition of *Cutibacterium acnes* Growth

To determine the ability of the *S. spinescens* leaf extracts to inhibit the growth of *C. acnes*, 10 µL of each extract was screened using a disc diffusion assay. Bacterial growth was inhibited by both the methanolic and aqueous of both *S. spinescens* leaves (Figure 1). The inhibition of *C. acnes* growth by the aqueous extract was particularly noteworthy (as judged by zone of inhibition (ZOI), with inhibition zones of 9.0 mm, compared to 7.7 mm for the methanolic extract. Indeed, the growth inhibition by the aqueous extracts was comparable to that of the ampicillin control (10 µg), which gave 10.3 mm ZOIs. In contrast, the vancomycin (5 µg) control produced larger zones of inhibition (11.5 mm), indicating stronger antibacterial activity. However, it is noteworthy that the antibiotic controls used in this study consisted of relatively high doses of pure antibiotics. In contrast, the extracts are crude mixtures, which would contain many individual compounds. Thus, it is likely that the growth inhibitory activity of individual bioactive extract component(s) is/are particularly promising. Therefore, the methanolic and aqueous *S. spinescens* leaf extracts have potential for use in treating acne and further study is warranted.

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water (0.5 % DMSO) and qualitative phytochemical screenings of the *S. spinescens* leaf extracts.

		Methanolic extract	Aqueous extract
Mass of extracted material (mg)		144	220
Concentration of resuspended extract (mg/mL)		14	22
Phenols	Total phenols	+++	+++
Water soluble phenols	++	+++	
Insoluble phenols	+	-	
Saponins	Froth persistence	+	+
Emulsion test	+	+	
Cardiac glycosides	Keller-Kiliani Test	-	-
Triterpenoids	Salkowski Test	-	-
Phytosterols	Acetic Anhydride Test	-	-
Alkaloids	Meyer's Test	+	-
Wagner's Test	-	-	
Draggendoff's Test	-	-	
Flavonoids	Kumar Test	++	++
Tannins	Ferric Chloride Test	++	+++
Lead Acetate Test	++	+++	
Anthraquinones	Free	-	-
Combined	-	-	

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay.

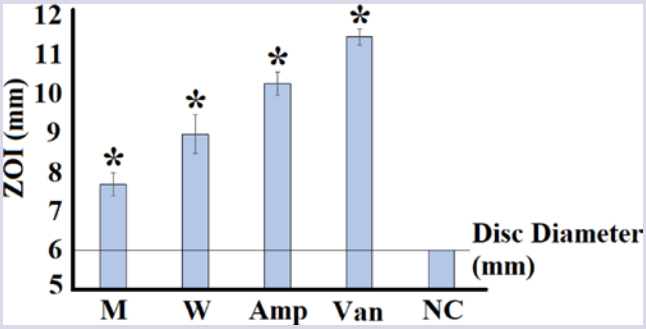

Figure 1: Growth inhibitory activity of the *S. spinescens* leaf extracts against *C. acnes* (ATCC 6919) measured as zones of inhibition (mm). M = methanolic extract; W = aqueous extract; Amp = ampicillin (10 µg); Van = vancomycin (5 µg); NC = negative control (0.5 % DMSO). Results are expressed as mean zones of inhibition ± SEM. * indicates results that are significantly different to the negative control (p<0.05).

Quantification of minimum inhibitory concentration (MIC)

The relative level of antimicrobial activity was further evaluated by determining the MIC values of the *S. spinescens* leaf extracts against *C. acnes* (Table 2). Notably, substantial differences were evident between the results obtained in the disc diffusion (DD) assay and the liquid dilution (LD) MIC screening assays, with substantially lower MIC values generally recorded for the liquid dilution assay (875 and 344 µg/mL for the methanolic and aqueous extracts respectively), compared to the solid phase assay (1327 and 573 µg/mL respectively). These findings may reflect the classes of molecules in the extracts. Larger and/or lower polarity molecules do not readily diffuse through agar and therefore solid phase assays may provide erroneous results for these compounds. In contrast, larger and lower polarity molecules are more suitable for testing in liquid phase assays and these assays may provide a better understanding of their antibacterial activity. Given the substantially higher MIC values measured in the solid phase assays, it is likely that the bioactive compounds are relatively large and/or nonpolar, although this remains to be verified. Alternatively, potentiating compounds (with different physiochemical properties to the inhibitory compounds) may separate as they diffuse through the agar gel, whereas they will remain together in the liquid media assays, possibly accounting for these differences.

Quantification of Toxicity

All extracts were initially screened in the *Artemia* nauplii assay at 2000 µg/mL (Figure 2). Additionally, potassium dichromate was also tested in the bioassay as a reference toxin. Potassium dichromate was rapid in its onset of mortality, promoting nauplii death within the first 3h of exposure, with 100% mortality evident within 5 hr (unpublished results). In contrast, the methanolic and aqueous *S. spinescens* leaf extracts induced substantially less than 50 % moratlity following 24 hr exposure. As 24 hr LC_{50} values >1000 µg/mL have previously been defined as nontoxic in this assay,[27-28] the methanolic and aqueous *S. spinescens* leaf extracts were deemed to be nontoxic and their LC_{50} values were not further determined.

Table 2: Disc diffusion and liquid dilution MICs against *C. acnes* growth (µg/mL) of the *S. spinescens* extracts.

Extract	MIC (µg/mL)	
	DD	LD
Methanolic extract	1327	875
Aqueous extract	573	344

DD disc diffusion; LD liquid dilution; Numbers indicate the mean DD MIC and LD MIC values of triplicate determinations. - indicates no inhibition at any concentration tested.

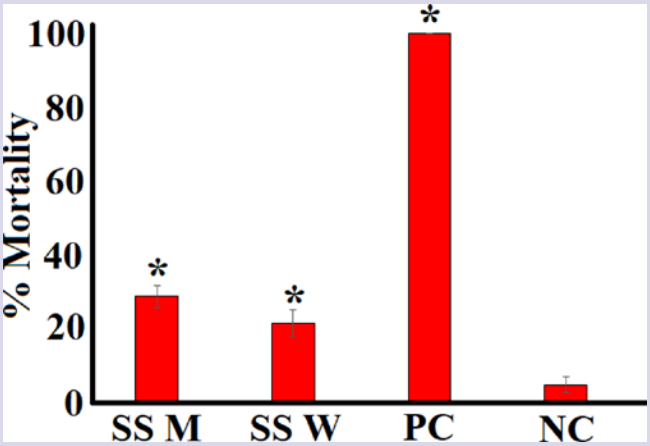

Figure 2: The lethality of the *S. spinescens* leaf extracts (2000 µg/mL), as well as the potassium dichromate (1000 µg/mL) and seawater controls towards *Artemia franciscana* nauplii after 24 hr exposure. SS = *S. spinescens*; M = methanolic extract; W = aqueous extract; PC = potassium dichromate control; NC = negative (seawater) control. * indicates results that are significantly different to the negative control ($p<0.05$). Results are expressed as mean % mortality ± SEM.

DISCUSSION

Acne vulgaris is one of the most common infectious diseases globally and the most common cause of chronic dermal inflammation globally, affecting approximately 85 % of the populatrion throughout their lives.[28] It is particularly common amongst people 12-24 years old and is often neglected by medical researchers as it has a low mortality rate. However, chronic acne vulgaris can lead to psychological issues, social isolation and emotional impairment amongst adolescents during their formative years. Additionally, ineffective therapy may cause permanent scarring and may result in lifelong self-esteem issues and depression. Whilst there are multiple therapeutic options to control acne vulgaris, none are completely effective, and all are associated with toxicities and/or unwanted side-effects.[3-4] There is a need to develop safer and more effective therapies to inhibit the growth of *C. acnes* that can be used for topical and oral treatment. Traditional plant medicines are especially promising and examination of ethnobotanical records can highlight promising plants (and plant combinations). Many traditional medicine plant have been used traditionally for hundreds (or even thousands) of years to inhibit bacterial growth, and in some case their efficacy has been verified by rigorous scientific examination. Additionally, the use of natural alternatives to inhibit the growth of acne vulgaris-inducing bacteria may be more acceptable to consumers due to their natural origin and consumer perception of safety.

Multiple plant species have been used traditionally to treat acne, including (but not limited to) *Allium cepa* L. (onion), *Allium sativum* L. (garlic), *Aloe vera* L., *Camellia senensis* (L.) Kuntze (tea plant), *Cannabis sativa* L. (cannabis, marijuana), *Echinacea purpurea* (echinacea), *Eucalyptus globus* Labill. (blue gum), *Lavendulla angustifolia* Mill. (lavender), *Portulaca oleraceae* L. (common purslane), *Salvia officinalis* Spenn. (rosemary), and *Thyme vulgaris* L. (thyme) [as reviewed in 29]. Several of these have already been tested for the ability to inhibit the growth of *C. acnes*, although the growth inhibitory properties of many of these are yet to be verified. Substantially fewer studies have screened Australian plants for the ability to inhibit the growth of *C. acnes*, although several recent studies have begun to examine the potential of Australian plants to inhibit *C. acnes* growth. In particular, *Terminalia ferdinandiana* Exell. fruit extracts were recently reported to be good inhibitors of *C. acnes* growth (as well as several other skin pathogens).[30-31] Similarly, the anti-*C. acnes* activity of several Australian *Syzygium* spp.[32] and *Acronychia acidula* F. Muell. fruit extracts has also been documented.[33]

This study examined the growth inhibitory properties of methanolic and aqueous *S. spinescens* leaf extracts against *C. acne* (the main bacterial cause of acne vulgaris). *Scaevola spinescens* R.Br. was selected for screening in this study due to its ethnobotanical uses to treat a variety of pathogenic diseases,[9-10] as well as previous reports of noteworthy antibacterial activity against other bacterial pathogens of clinical relevance to humans.[13-15] Additionally, *S. spinescens* leaves have been used therapeutically by the Frist Australian for thousands of years without reports of toxicity and are generally considered to be safe. Our study confirmed the potential of *S. spinescens* leaf extracts for inhibiting the growth of the main acne vulgaris causing bacteria, *C. acnes*. The aqueous extract was the most promising growth inhibitor, with an LD MIC value of 344 µg/mL measured, compared to an LD MIC of 875 µg/mL for the methanolic extract. Studies using extracts prepared from other plants have reported comparable or considerably higher MIC values as signifying potent inhibitory activity. Extracts produced from *T. ferdinandiana* (commonly known as Kakadu plum) leaves were reported to be potent inhibitors of *C. acnes* growth, with an MIC value of 625 µg/mL.[30] In contrast, an LD MIC of 344 µg/mL was determined for the aqueous *S. spinescens* leaf extract against *C. acnes* in our study. This represents approximately double the potency compared to the *T. ferdinandiana* study. Another study screened methanolic and aqueous *Acronychia acidula* F.Muell. (commonly known as lemon aspen) against *C. acnes* and reported noteworthy inhibitory activity for the aqueous extract (MIC = 1455 µg/mL), although the methanolic extract was completely ineffective against that bacterium.[33] Notably, the MIC value of the *S. spinescens* leaf extracts reported in our study (particularly the aqueous extract) indicates that these extract was more potent than the extracts tested in the other studies. Thus, the use of the aqueous *S. spinescens* leaf extract to treat acne vulgaris is promising and further testing is warranted.

Whilst the antibacterial components of the *S. spinescens* leaf extracts were not identified in this study, a several classes of compounds were highlighted by the qualitative phytochemical analysis studies. The detection of moderate to high levels of polyphenolics, tannins and flavonoids was particularly noteworthy. Many studies have reported potent antibacterial activities for a wide variety of flavonoids.[34] This has been attributed to a variety of mechanisms, including their ability to complex with extracellular and soluble proteins, as well as bacterial cell wall proteins.[35] Similarly, multiple tannins have broad spectrum antibacterial activity via a variety of intra- and extracellular mechanisms, including the precipitation of microbial proteins.[36] Polyphenolics are toxic to micro-organisms via enzyme inhibition mechanisms, possibly through non-specific interaction with proteins, or by reaction with sulfhydryl groups.[37] It is also likely that other phytochemical classes may also contribute to the growth inhibitory properties of these extracts. Further studies to elucidate the phytochemicals in this extract (and their

potential therapeutic mechanisms) are required.

Notably, the methanolic and aqueous *S. spinescens* leaf extracts tested herein were both nontoxic towards *Artemia nauplii* and are thus likely to be safe for topical application, as well for oral usage. However, further toxicity studies using human cell lines (and subsequent *in vivo* studies) are required to confirm the safety of these extracts before they are accepted as natural therapies for acne vulgaris. Furthermore, whilst our study reported the *S. spinescens* leaf extracts to be nontoxic, it is noteworthy that all of these studies have examined acute toxicity. Pharmacodynamic and pharmacokinetic studies are required to determine the ability of the extract components to cross the skin barrier, their duration in the blood stream prior to clearance, and the urinary excretory products. Indeed, such studies are required for any formulation to ensure that their components do not accumulate and cause chronic toxicity.

CONCLUSION

The results of this study demonstrate the potential of the *S. spinescens* leaf extracts as natural antibacterial components to treat acne vulgaris. The aqueous extract was particularly potent, indicating that polar components may be responsible for the anti-*C. acnes* activity. Furthermore, the lack of toxicity of the extract indicates the suitability of that extract for topical or oral use.

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CONFLICT OF INTEREST

The author declare no conflicts of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50 % mortality; **MIC:** minimum inhibitory concentration; **ZOI:** zone of inhibition.

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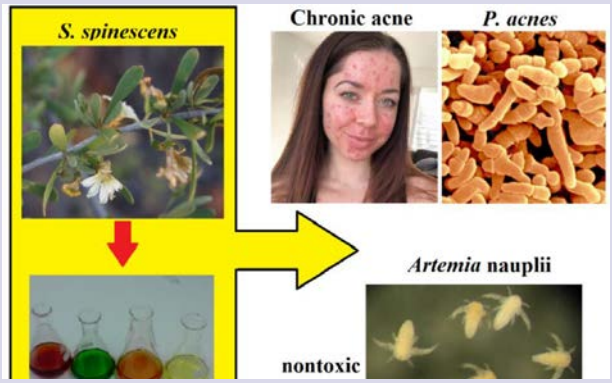
PICTORIAL ABSTRACT



SUMMARY

- Methanolic and aqueous *S. spinescens* leaf extracts were screened for the ability to block the growth of *Cutibacterium acnes*.
- The aqueous *S. spinescens* leaf extract was a particularly good inhibitor of *C. acnes* growth (LD MIC = 344 µg/mL).
- The methanolic *S. spinescens* leaf extract was also a good inhibitor of *C. acnes* growth, albeit with a slightly higher LD MIC value (LD MIC = 875 µg/mL).
- The nontoxicity of the *S. spinescens* leaf extracts was verified using the *Artemia* nauplii toxicity bioassay.

About Authors

Dr Ian Cock leads a research team in the Environmental Futures Research Institute and the School of Natural Sciences at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin including *Aloe vera*, South Asian and South American tropical fruits, as well as Australia plants including *Scaevola spinescens*, *Pittosporum phylliraeoides, Terminalia ferdinandiana* (Kakadu plum), Australian *Acacias, Syzygiums, Petalostigmas* and *Xanthorrhoea johnsonii* (grass trees). This range of projects has resulted in more than 250 scientific publications in a variety of peer reviewed journals.

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