Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes*

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The use of plants to treat skin ailments has strong support in the current trend of drug discovery. *Propionibacterium acnes*, an anaerobic pathogen, plays an important role in the occurrence of acne. The present study was conducted to evaluate the antimicrobial and antioxidant activities against *P. acnes* and cytotoxic effects of 48 medicinal plants grown in South Africa. The broth dilution and DPPH radical scavenging methods were used to determine antibacterial and antioxidant activities, respectively. Cytotoxicity was determined on mouse melanocytes (B16-F10). The ethanolic bark extract of *Acacia galpinii* Burtt Davy. (Leguminosae) exhibited the lowest minimum inhibitory concentration of 62.5 μg/mL. Excellent antioxidant activity was shown by *Aspalathus linearis* (Burm.f.) R.Dahlgren (Leguminosae), *Combretum apiculatum* Sond. (Combretaceae), *Harpephyllum caffrum* Bernh. ex Krauss (Anacardiaceae) and *Sclerocarya birrea* Hochst. (Anacardiaceae), with 50% radical scavenging activity (EC_{50}) at concentrations ranging from 1.6 μg/mL to 3.5 μg/mL. *Greyia sutherlandii* Hook. & Harv. (Greyiaceae) also exhibited good antioxidant activity with an EC_{50} value of 7.9$\pm$0.23 μg/mL. *A. linearis, G. sutherlandii* and *S. birrea* showed low toxicity with 50% viability of cells (EC_{50}) at concentrations of 125.09$\pm$0.71 μg/mL, 107.85$\pm$1.53 μg/mL and 92.07$\pm$0.09 μg/mL, respectively. The extracts of *A. linearis, G. sutherlandii* and *S. birrea* showed good antibacterial and antioxidant activities and low toxicity. Therefore, these plants can be considered as possible anti-acne agents and warrant further investigation.

Introduction

Acne, one of the most common disorders of the skin, is a polymorphic disease with non-inflammatory (blackhead or whitehead) and inflammatory (papules, pustules, or nodules) aspects and a wide spectrum of severity. Acne can have a significant impact on the psychosocial and physical aspects of life. It affects up to 85% of adolescents to some extent but is less common among infants. Its prevalence has been estimated to be 95–100% in male adolescents and 83–85% in female adolescents.[1-4]

Propionibacterium acnes, a Gram-positive anaerobic bacterium, is a normal component of the microbiota of human skin. *P. acnes* causes an increase in the secretion of sebum from sebaceous glands, which is accompanied by the thickening of the epidermis at the outlet to the pilosebaceous follicles. As a result, there is an obstruction to the flow of sebum outwards, and a comedone develops. Colonisation of the follicles with *P. acnes* and the host's inflammatory response play a pivotal role in the development of typical inflammatory papulopustular lesions.[5] In an anaerobic environment, the bacteria secretes nucleases, nuraminidases, hyaluronidases, acid phosphatises, lecithinases and other lipases. As a result of the action of these enzymes, the sebum content changes and reactive oxygen species may be released from the damaged follicular walls. Reactive oxygen species may also be the reason for the progression of inflammation in the pathogenesis of disease.[6]

Conventional drugs commonly used in acne treatment – such as tetracycline, erythromycin, mynocycline and metronidazole – act as antioxidants and antibacterials. Benzoyl peroxide, a topical agent for the treatment of acne, shows the ability to induce an inflammatory reaction mediated by reactive oxygen species in addition to its antibacterial activity.[6] These drugs also have various known side effects. The topical antibiotics can lead to dryness, redness and irritation of the skin, as well as hypopigmentation while oral antibiotics have age restrictions, can cause gastrointestinal disorders and increase the risk of venous thromboembolism.[5]

Herbal medicines are an important part of African tradition and also have very deep roots in the treatment of dermatological ailments. Ethnobotanical studies have documented the use of plants by traditional healers for the treatment of various skin ailments.[7] Different plant parts commonly used as cosmetics or face masks, known as *umemezis*, are widely used in southern Africa for skin problems like inflammation, wounds, burns, eczema and puberty acne.[8]

Because many skin disorders like atopic dermatitis and acne are associated with inflammation and the release of free radicals, which lead to oxidative and cellular damage and bacterial infections such as *P. acnes*, the presence of antioxidant and antimicrobial agents can explain the effectiveness of plants in the treatment of skin infections. In order to develop the therapeutic and drug potential of these plants, it is important to know whether they have any cytotoxic effects. Therefore, ethanol extracts of selected plants were evaluated for their antibacterial and antioxidant activities and cytotoxicity.

Limitations in the usage of some drugs and the prevailing side effects of the various chemically derived compounds have led to the search for alternative herbal agents to treat acne. The aim of this study was to test the effect of selected plant extracts on the pathogenic bacteria *P. acnes*, and to identify which plant extracts could be considered as possible anti-acne agents.

Methods

Materials

Tetracycline, vitamin C, *p*-iodonitrotetrazolium salt and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Johannesburg, South Africa). Nutrient agar and nutrient broth were obtained from Merck

SA (Pty) Ltd (Johannesburg, South Africa). *P. acnes* (ATCC 11827) was purchased from Anatech Company South Africa (Johannesburg, South Africa). The cell culture reagents and the equipment were purchased from Highveld Biological (Johannesburg, South Africa), Labotech (Johannesburg, South Africa) and The Scientific Group (Johannesburg, South Africa). The B16-F10 mouse melanocyte cell line was obtained from Highveld Biological.

Preparation of plant extracts

Different plant parts (leaves, roots, bark and twigs) were collected from the Botanical Garden of the University of Pretoria (Pretoria, South Africa). The plants were identified by a taxonomist, Professor A.E. (Braam) van Wyk, at the H.G.W.J. Schweickerdt Herbarium of the University of Pretoria. The shade-dried plant material (80 g) was ground with a mechanical grinder, then soaked in 300 mL of ethanol and left on a shaker for 3 days. The plant material was then filtered and the solvent was evaporated under vacuum (Buchi Rotavapor, Labotech, Switzerland) to yield dry extracts. The plants were selected based on their medicinal usage as summarised in Table 1.

Antibacterial bioassay

The minimal inhibitory concentration (MIC) of the ethanolic extracts of the 48 selected plants was determined by a microdilution assay. This assay was done using the method described by Mapunya et al.[36], with slight modifications. For this purpose, *P. acnes* (ATCC 11827) was cultured from a Kwik-Stick on nutrient agar and incubated at 37 °C for 72 h under anaerobic conditions. The ethanolic extracts were dissolved in 10% dimethyl sulphoxide (DMSO) to obtain a stock solution of 2 mg/mL. The positive control (tetracycline) was dissolved in sterile distilled water to obtain a stock solution of 0.2 mg/mL. The 96-well plates were prepared by dispensing 100 μL of the nutrient broth into each well; 100 μL of the plant stock samples and positive control were added to the first row of wells in triplicate. Twofold serial dilutions were made in broth over a range to give concentrations of 3.9–500 μg/mL and 0.3–50 μg/mL for the plant extracts and positive control, respectively. The 72-h culture of bacteria was dissolved in nutrient broth and the suspensions were adjusted to 0.5 McFarland standard turbidity at 550 nm. Then 100 μL of this bacterial inoculum with 10^5–10^6 CFU/mL was added to all the wells. The wells with 2.5% DMSO and bacterial suspension without samples served as the solvent and negative controls, respectively. The plates were then incubated at 37 °C for 72 h under anaerobic conditions. The MIC (defined as the lowest concentration that showed no bacterial growth) was determined by observing the colour change in the wells after the addition of *p*-iodonitrotetrazolium salt.

Antioxidant assay

The antioxidant activity of selected plant extracts was investigated using the DPPH radical scavenging method as previously described by Du Toit et al.[37], with slight modifications. DPPH is a free radical, which is stable at room temperature and produces a violet solution in ethanol. When reduced in the presence of an antioxidant molecule, it gives rise to a colourless solution. DPPH was dissolved in ethanol to obtain a solution of 0.04% w/v.

The selected plant samples and the positive control (vitamin C) stock solutions (2 mg/mL) were serially diluted to final concentrations ranging from 0.78 μg/mL to 100 μg/mL. Ethanol and DPPH without any plant material were used as blanks while plant samples diluted in distilled water were used as controls. DPPH solution (90 μg/mL) was then added to all the wells except for the controls and allowed to react at room temperature. After 30 min, the absorbance values were measured at 515 nm using a Biotek Power-wave XS multiwell reader (A.D.P., Johannesburg, South Africa). The values were converted into the percentage antioxidant activity (AA) using the formula given below. The 50% inhibitory concentration (EC_{50}) values were then calculated by linear regression of the plots using GraphPad Prism version 4.

$$AA\% = \{Abs_{blank}(Abs_{sample} - Abs_{control}) / Abs_{blank}\}*100$$

Mouse melanocyte cytotoxicity assay

The cytotoxicity of selected plant extracts was determined following a previously described method.[36] Briefly, mouse melanocyte (B16-F10) cells were plated in complete Roswell Park Memorial Institute medium (10% foetal bovine serum and 1% gentamycin) directly in the wells of a 96-well plate (10^5 cells per well). After an overnight incubation at 37 °C in 5% CO_2 and a humidified atmosphere, extract samples and the positive control (actinomycin D) were added to the cells to give the final concentrations of plant extract and positive control of 3.13–400 μg/mL and $0.03x10^{-2}$–0.05 μg/mL, respectively. Plates were incubated at 37 °C in 5% CO_2 in a humidified atmosphere for 3 days. The toxicity effects of the extracts on the B16-F10 cells were assayed using the sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitrobenzene sulphonic acid hydrate (XTT) cytotoxicity assay. Thereafter, 50 μL of XTT reagent (1 mg/mL XTT with 0.383 mg/mL penazine methosulphate) was added to the wells and incubated for 1 h. The optical densities of the wells were measured at 450 nm with background subtraction at 690 nm. Cell survival was assessed by comparison with the controls (medium with DMSO). The EC_{50} value, which represents the concentration of plant extract that causes death in 50% of the cells, was analysed using GraphPad Prism version 4.

Statistical analysis

All the assays were performed in triplicate with three independent studies for each assay. EC_{50} values for antioxidant and cytotoxicity tests were derived from a non-linear regression model (curve fit) based on a sigmoidal dose response curve (variable) and computed using GraphPad Prism version 4 (GraphPad, San Diego, CA, USA).

Results

Antibacterial activity of ethanolic extracts

The antibacterial activity of the selected plants against *P. acnes* is summarised in Table 2. After the addition of *p*-iodonitrotetrazolium salt, the MIC value of the positive drug control (tetracycline) was determined to be 3.1 μg/mL. Most of the plants exhibited antibacterial activity at MIC values ranging from 62.5 μg/mL to 500 μg/mL. The ethanolic bark extract of *Acacia galpinii* Burtt Davy. (Leguminosae) exhibited the lowest MIC value of 62.5 μg/mL. Some of the plant extracts – *Aspalathus linearis* (Burm.f.) R.Dahlgren (Leguminosae), *Combretum apiculatum* Sond. (Combretaceae), *Combretum molle* Engl. & Diels (Combretaceae), *Galenia africana* L. (Aizoaceae), *Greyia sutherlandii* Hook. & Harv. (Greyiacece), *Harpephyllum caffrum* Bernh. ex Krauss (Anacardiaceae), *Ranunculus repens* L. (Ranunculaceae), *Sclerocarya birrea* Hochst. (Anacardiaceae) and *Warburgia salutaris* (G. Bertol.) Chiov. (Cancellaceae) – exhibited MIC values of 125 μg/mL. Another 28 extracts inhibited the growth of bacteria at MIC values ranging from 250 μg/mL to 500 μg/mL, whereas the remaining 10 extracts did not show any antibacterial activity, even at the highest concentration (500 μg/mL) tested. A threshold MIC value of ~100 μg/mL is suggested for rating plant extracts as having significant antimicrobial activity.[38] Therefore, the plant extracts exhibiting MIC values ranging from 62.5 μg/mL to 125 μg/mL were selected for the evaluation of antioxidant activity.

Antioxidant activity of selected extracts

Vitamin C, a widely used antioxidant compound, was used as the positive control (EC_{50} = 1.98 ± 0.005 μg/mL). The plant extracts which demonstrated excellent radical scavenging activity, comparable to vitamin C, were *A. linearis* (EC_{50} of 3.5 ± 0.5 μg/mL), *C. apiculatum* (EC_{50} of 1.6 ± 0.02 μg/mL), *H. caffrum* (EC_{50} of 2.6 ± 0.21 μg/mL) and *S. birrea* (EC_{50} of 2.06 ± 0.03 μg/mL) (Figure 1). The plant extracts of *C. molle* and *G. sutherlandii* also showed good antioxidant activity with EC_{50} values of 9.83 ± 0.8 μg/mL and 7.9 ± 0.23 μg/mL, respectively (Figure 1). *A. galpinii* and *R. repens* exhibited comparatively higher antioxidant activity with EC_{50} values of 16.05 ± 2.25 μg/mL and 24.7 ± 2.05 μg/mL, respectively. The extracts of *G. africana* and *W. salutaris* exhibited the lowest radical scavenging activity with the highest EC_{50} values of 90.92 ± 1.2 μg/mL and 111 ± 2.5 μg/mL, respectively.

Table 1: Medicinal use of plants selected for present study

Plant Name	Medicinal use
Acacia caffra Willd.	Treatment of blood disorders, infantile abdominal disorders[7]
Acacia galpinii Burtt Davy.	As a demulcent[8]
Acacia mellifera Benth.	Treatment of coughs, gastrointestinal ailments, malaria, pneumonia, stomach aches, sterility, skin diseases[9]
Aloe arborescens Mill.	Used in cosmetics; treatment of X-ray burns, stomach aches[7]
Aloe barbadensis Mill.	As an antioxidant; used in cosmetic application, wound healing[7,10,11]
Aloe ferox Mill.	Treatment of ophthalmia, venereal sores[7]
Aloe sessiliflora Pole-Evans.	Believed to promote menstruation; as enemas[7,12]
Anchusa capensis Thunb.	Used as a mutagenic and neurotoxin, and in traditional phytomedicine[13]
Annona senegalensis Pers.	Treatment of dermatological diseases and ophthalmic disorders[14]
Arbutus unedo L.	As anti-diarrhoeal, astringent, antioxidant, urinary antiseptic, depurative; treatment of diabetes and hypertension[15-18]
Aspalathus linearis (Burm.f.) R.Dahlgren	Used for alleviation of infantile colic, allergies, asthma, dermatological problems[12]
Barleria albostellata C. B. Clarke	As antibacterial, antifungal, anti-inflammatory, antioxidant and for acne inhibition[19]
Barleria repens Nees	As antibacterial, antifungal[19]
Broussonetia papyrifera (L.) Vent.	Treatment of stomach pains, ill-defined abdominal pains[20]
Buxus macowanii Oliv.	Treatment of gout, malaria, rheumatism, skin disorders[13]
Carpobrotus edulis (L.) Bolus	Treatment of infections of the mouth and throat, eczema, wounds and burns[12]
Ceratonia siliqua L.	As anti-diarrhoeal, antitussive, diuretic; treatment of warts[21]
Combretum apiculatum Sond.	Treatment of conjunctivitis, stomach disorders[7]
Combretum molle Engl. & Diels	As a anthelmintic; treatment of coughs, fever, stomach ailments, wounds[7]
Cotyledon orbiculata L.	Treatment of earache, toothache, epilepsy, boils and inflammation[7,12]
Cryptocarya woodii Engl.	Treatment of diarrhoea[22]
Dahlia imperialis Roezl	Treatment of skin ailments like rashes, grazes, infected scratches[23]
Datura stramonium L.	Treatment of abscesses and wounds; relieves asthma and reduces pain; remedy for boils[7,12]
Dichrostachys cinerea (L.) Wight & Arn.	Treatment of body pains, elephantiasis, sores and skin ailments, toothache[7,12]
Diospyros lycioides Desf.	Chewed and used as a toothbrush, and to ease body pains[7]
Dodonaea viscosa Jacq.	As antipruritic in skin rashes and fungal skin diseases[12]
Erythrophleum lasianthum Corbishley.	Treatment of fever, general body pains, headaches, intestinal spasms, migraines[7,12]
Euclea divinorum Hiern.	As chewing sticks for toothache, headaches; as a purgative; bark infusion is used to enhance appetite[7]
Euclea natalensis A.DC.	Treatment of bronchitis, chronic asthmas, pleurisy, toothache, urinary tract infections[7]
Galenia africana L.	Treatment of asthma, coughs, skin diseases, eye inflammation, venereal sores, wounds[24]
Gomphocarpus fruticosus R.Br.	Treatment of headache and tuberculosis and to relieve stomach pain and general aches in the body[7,12]
Greyia flanaganii Bolus.	To ward off sickness[25]
Greyia sutherlandii Hook. & Harv.	As emetics for biliousness[7]
Harpephyllum caffrum Bernh. ex Krauss	As blood purifiers, for facial saunas, skin washes; to treat acne and eczema[7,12]
Heteropyxis natalensis Harv.	Treatment of colds, bleeding gums, nosebleeds; as a vermifuge[7,12]
Hyaenanche globosa Lamb.	Treatment against vermins[13]
Knowltonia vesicatoria Sims.	Treatment of headaches, toothaches, skin blisters[12]
Magnolia grandiflora L.	Treatment of abdominal discomfort, blood pressure, dyspnoea, epilepsy, heart disturbances, infertility, muscle spasm[26,27]
Myrsine africana L.	As anthelmintics, blood purifier[28]
Parinari curatellifolia Planch. ex Benth.	Treatment of ailments of the eye or ear, pneumonia[29,30]
Ranunculus repens L.	Treatment of muscular aches, rheumatic pains, sores[31]
Rhus lancea L.f.	As antibacterial and antifungal[32]
Sclerocarya birrea Hochst.	Treatment of diarrhoea, inflammation, skin ailments, stomach ailments, malaria, ulcers[7,12]
Sideroxylon inerme L.	As skin lightener; to treat fevers; to treat gall sickness in stock[7,12,33]
Symphytum officinale L.	Treatment of arthritis, bruises, insect bites, inflamed bunions, wounds, skin conditions, nosebleeds, sunburn, rheumatism[34]
Warburgia salutaris (G. Bertol.) Chiov.	Treatment of influenza, rheumatism, malaria, venereal diseases, headaches, toothaches, dermatological disorders, gastric ulcers[7,12]
Zanthoxylum capense Harv.	Treatment of colic, stomach aches, toothaches, fever, epilepsy[7,12,35]

Table 2: Minimum inhibitory concentrations (MICs) for antibacterial activity of extracts against *Propionibacterium acnes* determined by microdilution assay

Plant name	Common name	Family	Voucher no.	Part used	MIC μg/mL
Acacia caffra	Cat thorn	Leguminosae	PRU 90700	Leaves	250
Acacia galpinii	Monkey thorn	Leguminosae	PRU 16209	Bark	62.5
Acacia mellifera	Blackthorn	Leguminosae	PRU 078373	Leaves	250
Aloe arborescens	Krantz aloe	Aloaceae	MN 5	Leaves	500
Aloe barbadensis	Aloe vera	Aloaceae	PRU 118947	Leaves	Na†
Aloe ferox	Red aloe	Aloaceae	PRU 110308	Leaves	Na†
Aloe sessiliflora	Lebombo aloe	Aloaceae	PRU 118948	Leaves	Na†
Anchusa capensis	Cape forget-me-not	Boraginaceae	Not available	Leaves	Na†
Annona senegalensis	White custard apple	Annonaceae	PRU 074974	Bark	250
Arbutus unedo	Strawberry tree	Ericaceae	PRU 6211000	Leaves	500
Aspalathus linearis	Rooibos	Leguminosae	PRU 110523	Leaves	125
Barleria albostellata	Grey barleria	Acanthaceae	PRU 096399	Leaves	500
Barleria repens	Small bush violet	Acanthaceae	PRU 081712	Leaves	250
Broussonetia papyrifera	Paper mulberry	Moraceae	PRU 51221	Leaves	500
Buxus macowanii	Cape box	Buxaceae	PRU 110526	Leaves	Na†
Carpobrotus edulis	Sour fig	Azioaceae	PRU 096398	Leaves	Na†
Ceratonia siliqua	Carob tree	Leguminosae	SM 95502	Leaves	Na†
Combretum apiculatum	Red bushwillow	Combretaceae	PRU 110531	Leaves	125
Combretum molle	Velvet bushwillow	Combretaceae	EP 81	Leaves	125
Cotyledon orbiculata	Pig's ear	Crassulaceae	PRU 096402	Leaves	Na†
Cryptocarya woodii	Cape laurel	Lauraceae	PRU 064439	Leaves	250
Dahlia imperialis	Dahlia	Asteraceae	PRU 3311010	Leaves	500
Datura stramonium	Thorn apple	Solanaceae	MN 8	Leaves	500
Dichrostachys cinerea	Sickle brush	Leguminosae	PRU 096403	Leaves	500
Diospyros lycioides	Blue brush	Ebenaceae	PRU 118949	Twigs	Na†
Dodonaea viscosa	Hopbrush	Sapindaceae	PRU 096404	Leaves	500
Erythrophleum lasianthum	Swazi ordeal tree	Leguminosae	PRU 110525	Leaves	250
Euclea divinorum	Magin gwarra	Ebenaceae	AJ 64	Leaves	250
Euclea natalensis	Natal guarri	Ebenaceae	PRU 95059	Leaves	250
Euclea natalensis	Natal guarri	Ebenaceae	NL 22	Roots	250
Galenia africana	Kraalbos	Aizoaceae	SM 93723	Leaves	125
Gomphocarpus fruticosus	Milkweed	Asclepiadaceae	MN 1	Leaves	250
Greyia flanaganii	Kei bottlebrush	Greyiaceae	P. Van Wyk 2274	Leaves	250
Greyia sutherlandii	Natal bottlebrush	Greyiaceae	PRU 118946	Leaves	125
Harpephyllum caffrum	Wild plum	Anacardiaceae	PRU 118950	Leaves	125
Heteropyxis natalensis	Lavender tree	Myrtaceae	PRU 096405	Leaves	250
Hyaenanche globosa	Hyaena poison	Euphorbiaceae	SM 95499	Leaves	250
Knowltonia vesicatoria	Blister-leaf	Ranunculaceae	PRU 096499	Roots	250
Magnolia grandiflora	Magnolia	Magnoliaceae	PRU 2651000	Leaves	250
Myrsine africana	African boxwood	Myrsinaceae	SM 95503	Stalks	500
Parinari curatellifolia	Mobola plum	Chrysobalanaceae	PRU 096215	Bark	250
Ranunculus repens	Creeping buttercup	Ranunculaceae	PRU 096416	Leaves	125
Rhus lancea	Karee	Anacardiaceae	PRU 110530	Leaves	250
Sclerocarya birrea	Marula	Anacardiaceae	NH 1910	Bark	125
Sideroxylon inerme	White milkwood	Sapotaceae	PRU 96216	Bark	250
Symphytum officinale	Comfrey	Boraginaceae	PRU 096414	Leaves	250
Warburgia salutaris	Pepper-bark tree	Cancellaceae	PRU 110529	Leaves	125
Zanthoxylum capense	Small knob wood	Rutaceae	PRU 096406	Leaves	Na†

†*Na, not active at the highest concentration (500 μg/mL) tested.*

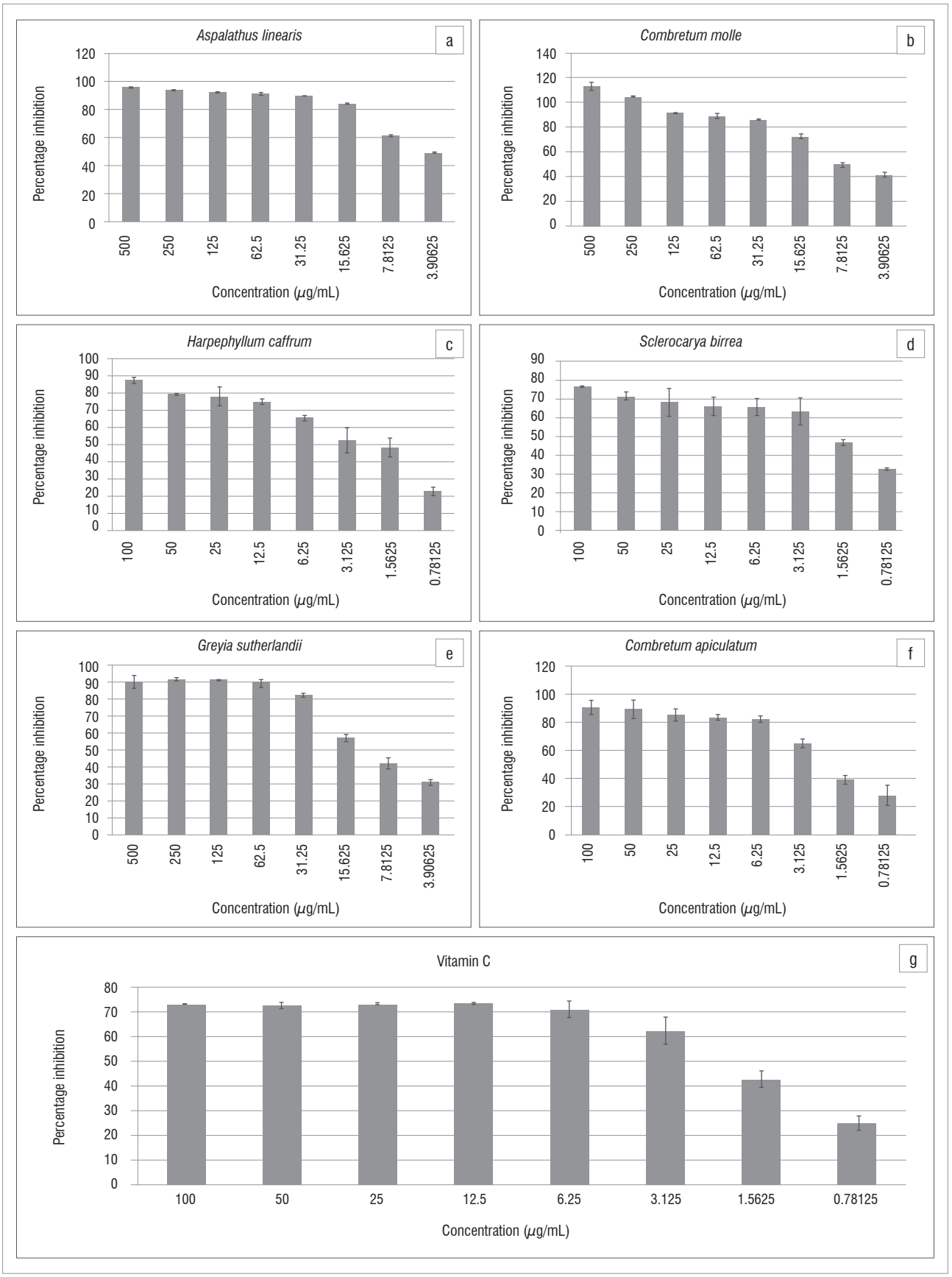

Figure 1: The DPPH radical scavenging activity of the potential extracts and the positive control (vitamin C): (a) *Aspalathus linearis* (EC_{50} = 3.5 ± 0.5 μg/mL), (b) *Combretum molle* (EC_{50} = 9.83 ± 0.8 μg/mL), (c) *Harpephyllum caffrum* (EC_{50} = 2.6 ± 0.21 μg/mL), (d) *Sclerocarya birrea* (EC_{50} = 2.06 ± 0.03 μg/mL), (e) *Greyia sutherlandii* (EC_{50} = 7.9 ± 0.23 μg/mL), (f) *Combretum apiculatum* (EC_{50} = 1.6 ± 0.02 μg/mL) and (g) vitamin C (EC_{50} = 1.98 ± 0.005 μg/mL).

Cytotoxicity of selected extracts

Cytotoxicity was assessed on the plant extracts which demonstrated EC_{50} values of ≤10 μg/mL for radical scavenging activity. The plant extracts of *A. linearis*, *G. sutherlandii* and *S. birrea* showed low toxicity with 50% viability of cells (EC_{50}) at concentrations of 125.09±0.71 μg/mL, 107.85±1.53 μg/mL and 92.07±0.09 μg/mL, respectively (Figure 2). During a previous study by our research group, the leaf extract of *H. caffrum* showed toxicity to B16-F10 cells at a concentration of 100 μg/mL.[39] The plant extract of *C. molle* showed moderate toxicity with an EC_{50} value of 48.83±0.21 μg/mL, whereas *C. apiculatum* was found to be the most toxic with an EC_{50} value of 12.15±0.03 μg/mL and was found to be lethal to almost all cells at the highest concentration of 400 μg/mL. Actinomycin D, the positive control, showed an EC_{50} value of $4.5x10^{-3} \pm 0.5x10^{-3}$ μg/mL (Figure 2).

Discussions

Plant extracts were explored for antibacterial activity against *P. acnes*. Similar to our findings, the ethanolic extract of *Coscinium fenestratum* (Gaertn.) Colebr. (Menispermaceae) inhibited the growth of *P. acnes* at an MIC value of 46 μg/mL.[40] According to Tsai et al.[41], methanolic extracts of *Rosa damascena* Mill (Rosaceae), *Eucommia ulmoides* Oliv. (Eucommiaceae) and *Ilex paraguariensis* A. St.-Hil. (Aquifoliaceae) inhibited the growth of *P. acnes* at MIC values of 2000 μg/mL, 500 μg/mL and 1000 μg/mL, respectively. To the best of our knowledge, the present study is the first scientific report of the antibacterial activity of all the selected plants against *P. acnes*. However, some of the plants used in this study have been previously reported to be active against other pathogens. In another study, leaf extracts of *A. linearis* showed zones of inhibition against *Bacillus cereus*, *Micrococcus luteus* and *Candida albicans* of 7.0 mm, 6.4 mm and 8.5 mm, respectively.[42]

The antibacterial activity of *C. apiculatum* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* was reported by Serage[43]. The acetone extract of the stem bark of *C. molle* showed antimicrobial activity against *E. coli* and *Shigella* spp. at an MIC of 50 000 μg/mL. The extract also showed inhibitory effects on the fungus *C. albicans* with complete inhibition at a concentration of 400 μg/mL.[44] In a study done by Lining et al.[45], the crude methanolic extract of *Diospyros lycioides* Desf. (Ebenaceae) showed activity against *Streptococcus mutans* and *Prevotella intermedia* at an MIC of 1250 μg/mL. In contrast, our results showed no activity of the ethanolic extract of *D. lycioides* against *P. acnes*. In another study conducted by Mativandlela et al.[46], the ethanolic extract of *G. africana* showed antimycobacterial activity against *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* at MIC values of 780 μg/mL and 1200 μg/mL, respectively. The ethanolic extract of *H. caffrum* was reported to be active against four bacterial species, namely *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae* and *S. aureus,* while an aqueous extract showed activity against *C. albicans*.[47] The acetone extracts of the bark and leaves of *S. birrea* were reported to be active against *S. aureus*, *P. aeruginosa*, *E. coli* and *Enterococcus faecalis* at MIC values ranging from 150 μg/mL to 3000 μg/mL.[48] In a study done by Motsei et al.[49], the leaf extracts of *W. salutaris* inhibited growth of *C. albicans* at MIC values ranging from 12 500 μg/mL to 25 000 μg/mL and the bark extracts showed growth of inhibition against *S. aureus*, *Staphylococcus epidermis*, *B. subtilis* and *E. coli*.[50] No reports regarding the antimicrobial activity of *G. sutherlandii* and *R. repens* were found in the literature. However, in the present study, both of these plants showed growth inhibitory activity against *P. acnes* at an MIC of 125 μg/mL. In a study conducted by Eloff and Katerere[51], the acetone and chloroform leaf extracts of *A. galpinii* inhibited the growth of *S. aureus* and *E. coli*. Similar to our findings, the ethanol bark extract of

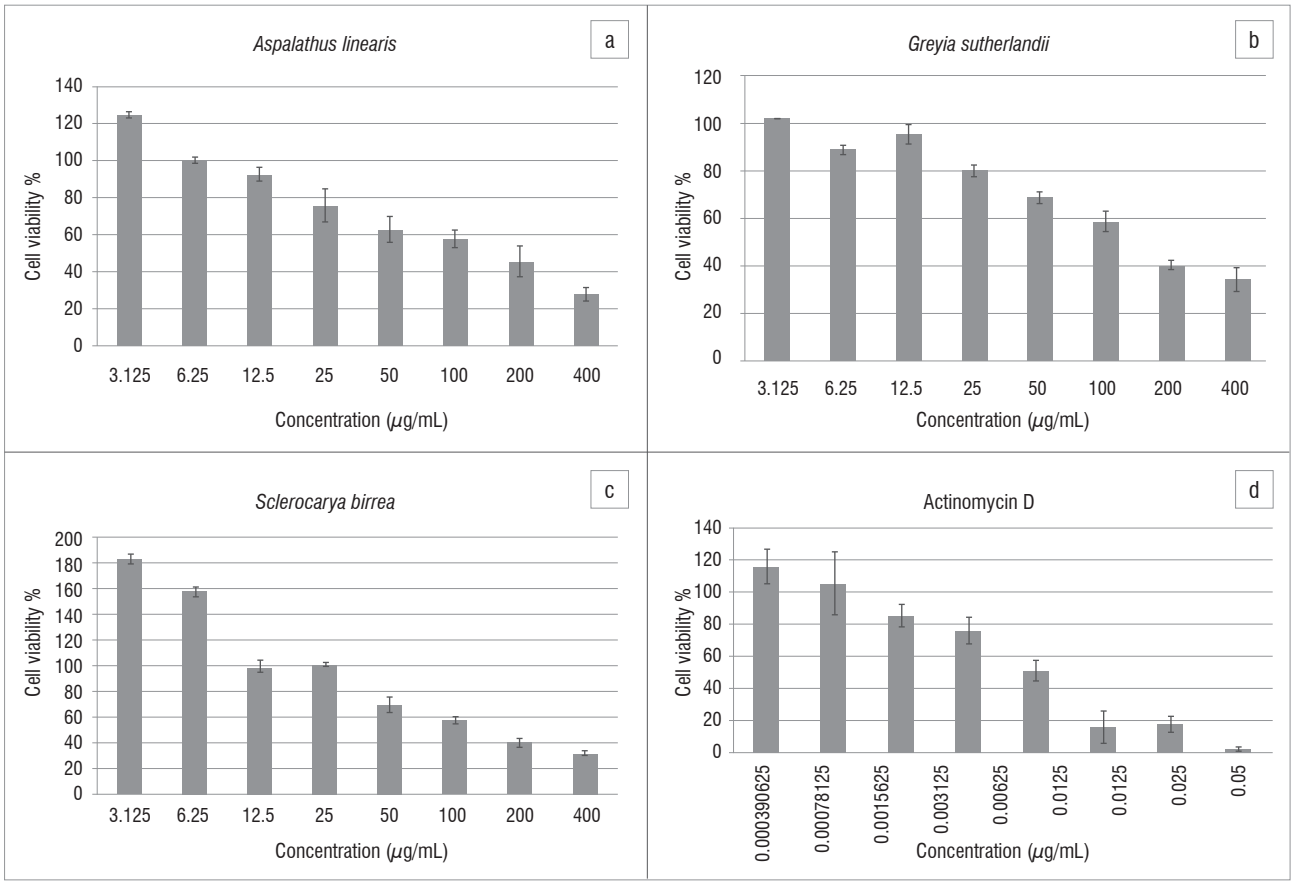

Figure 2: The cytotoxic effects of the plant extracts and the positive control (actinomycin D) on mouse melanocytes B16-F10: (a) *Aspalathus linearis* (EC_{50} =125.09±0.71 μg/mL), (b) *Greyia sutherlandii* (EC_{50} =107.85±1.53 μg/mL), (c) *Sclerocarya birrea* (EC_{50} =92.07±0.09 μg/mL) and (d) actinomycin D (EC_{50} =$4.5x10^{-3} \pm 0.5x10^{-3}$ μg/mL).

A. galpinii exhibited good inhibitory effect on *P. acnes* (MIC 62.5 μg/mL). However, no reports on the antimicrobial activity of bark extracts of *A. galpinii* were found in the literature.

Acne is associated with the production of free radicals along with the infection of *P. acnes*. Reactive oxygen species are produced as a result of the action of hydrolytic enzymes released from bacteria on the follicular walls of pilosebaceous units. Therefore, the plant extracts were evaluated for antioxidant activity along with antibacterial activity. In our study, the ethanol extracts of *A. linearis, C. apiculatum*, *H. caffrum*, *S. birrea, C. molle* and *G. sutherlandii* exhibited significant antioxidant activity with EC_{50} values of $\leq$10 μg/mL. Our results are in agreement with other researchers. During a previous study by Joubert et al.[52], the DPPH radical scavenging activity of *A. linearis* and its constituents were confirmed. The polar fractions of *C. apiculatum* showed antioxidant activity with an EC_{50} value of 3.91 μg/mL.[53] The DPPH radical scavenging activity of *H. caffrum* and *S. birrea* was confirmed by Moyo et al.[54] with EC_{50} values of 6.8 μg/mL and 5.02 μg/mL, respectively. In another study, acetone and dichloromethane extracts of *C. molle* displayed antioxidant activity after spraying with DPPH.[55] It has been reported that DPPH free radicals abstract the phenolic hydrogen of the electron-donating molecule, which could be the general mechanism for the scavenging action of flavonoids.[56] Based on the mechanism of reduction of the DPPH molecule that is correlated with the presence of hydroxyl groups on the antioxidant molecule, the antioxidant activity of the polar plant extracts in the present study can be explained as a result of the presence of their phytoconstituents (phenolics or flavonoids) which are radical scavengers with an available hydroxyl group and are known to occur abundantly in plant species.

In order to evaluate the therapeutic potential of the plants, the cytotoxicity of selected samples was tested on B16-F10 cells. To the best of our knowledge, the cytotoxicity of the extracts described in the present study is reported for the first time. However, previous researchers have documented similar cytotoxic effects on different cell lines. In a study by McGaw et al.[57], *A. linearis* showed low toxicity on vero cells and brine shrimp larvae with LD_{50} values of >1000 μg/mL. *S. birrea* showed low cytotoxicity on vero cells with an IC_{50} value of 361.24 μg/mL.[58] According to previous studies by Fyhrquist et al.[59] on the cytotoxicity of *C. molle*, the extract showed IC_{50} values of 27.7 μg/mL, 72.6 μg/mL and 42.6 μg/mL on T24 (bladder carcinoma), HeLa (cervical carcinoma) and MCF-7 (breast carcinoma) cells, respectively, while the *C. apiculatum* extract showed IC_{50} values of 65.0 μg/mL and 40.1 μg/mL for T24 and MCF-7 cells, respectively. No records of cell cytotoxicity for *G. sutherlandii* were found in the literature.

The results shown in this study prove the capability of medicinal plants as anti-acne agents, although the mode of action and in vivo studies are required to give conclusive results.

Conclusions

Based on the results obtained, it can be concluded that the ethanol bark extract of *A. galpinii* demonstrated the best activity against *P. acnes* with acceptable antioxidant activity. This plant might have other attributes that were not investigated in the present study which could be useful in the treatment of *P. acnes*. Although the plant extracts of *H. caffrum, C. apiculatum* and *C. molle* showed good antibacterial and excellent antioxidant activity, these samples also showed moderate toxicity to mouse melanocyte cells. The plant extracts of *A. linearis, S. birrea* and *G. sutherlandii* also exhibited good antibacterial and antioxidant activity but had low toxicity to the mouse melanocytes; these extracts therefore have potential as anti-acne agents, either alone or in combination.

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Authors' contributions

R.S. conducted the experiments and drafted the manuscript; N.L. supervised the work and edited the manuscript.

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