Antibacterial and Anticancer Properties of *Boswellia carteri* Birdw. and *Commiphora molmol* Engl. Oleo-Resin Solvent Extractions

Jiayu Zhang^{1,2}, Isaac Biggs^{1,3}, Joseph Sirdaarta^{1,3}, Alan White¹, Ian Edwin Cock^{1,3*}

¹School of Natural Sciences, Griffith University, Brisbane, AUSTRALIA.

²School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, CHINA.

³Environmental Futures Research Institute, Griffith University, Brisbane, AUSTRALIA.

ABSTRACT

Background: *Boswellia carteri* Birdw. (frankincense) and *Commiphora molmol* Engl. (myrrh) oleo-resins have traditionally been used for the growth inhibition of bacteria associated with skin diseases, urinary tract infections, respiratory infections, wound healing, dental disease and gastrointestinal disorders. Despite this, many bacterial pathogens are yet to be screened for susceptibility to frankincense and myrrh extracts. This study was undertaken to test extracts prepared from frankincense and myrrh oleo-resins for the ability to inhibit microbial and cancer cell growth. **Materials and Methods:** Frankincense and myrrh resins were extracted and tested for antimicrobial activity using modified disc diffusion and MIC methods. Inhibitory activity against *CaCo2* and HeLa cancer cell lines was evaluated using colorimetric cell proliferation assays. Toxicity was evaluated using an *Artemia franciscana* nauplii bioassay. The most promising inhibitory extracts were investigated using non-targeted GC-MS head space analysis (with screening against a compound database) for the identification and characterisation of individual components in the crude plant extracts. **Results:** The frankincense and myrrh extracts displayed broad spectrum antibacterial activity, inhibiting the growth of all of the bacteria screened against. The frankincense extracts were generally more potent growth inhibitors than were the corresponding myrrh extracts. The growth inhibition of the methanolic, ethyl acetate and hexane frankincense extracts against

Salmonella salford was particularly noteworthy, with MIC values of 462, 258 and 293 µg/mL respectively. The frankincense and myrrh extracts were both approximately equally effective against gram positive and gram negative bacteria. The majority of the extracts also proved effective at blocking the proliferation of the colorectal cancer cell line *CaCo2* and HeLa cervical cancer cell growth, although the aqueous and ethyl acetate frankincense extracts generally had the greatest efficacy (IC₅₀ values 1600-1900 µg/mL). All extracts were non-toxic, with LC₅₀ values substantially >1000 µg/mL.

Conclusion: These studies validate traditional usage of frankincense and myrrh in the treatment of several bacterial illnesses and some cancers, and indicate that they are safe for therapeutic usage.

Key words: Frankincense, Myrrh, Antibacterial, Anti-proliferative, Anti-cancer, *Artemia*, Toxicity.

Correspondence:

Ian Edwin Cock, School of Natural Sciences, Griffith University, Brisbane, AUSTRALIA.

Contact No: + 61 7 37357637

E-mail: I.Cock@griffith.edu.au

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INTRODUCTION

Natural plant resins have been valued since before written history for their therapeutic properties. The soft odoriferous oleo-resins of *Eucalyptus* spp. (known as kinos) have been used by Australian Aborigines for thousands of years and are particularly well known for their antiseptic and wound healing properties.^{1,2} *Pistacia lentiscus* resin (mastic gum, Arabic gum, tears of Chios) is used in Middle Eastern folk medicine to treat a wide variety of maladies including digestive problems, colds and influenza, snake bite, dental problems and as a general antiseptic.³ *Myroxylon balsamum* var. *pereirae* resin (balsam of Peru) has antimicrobial activity and is used as a tincture for wound healing, in cough suppressant/throat lozenges, as a skin ointment and for the treatment of haemorrhoids.⁴ Elemi resin obtained from the tree *Canarium luzonicum* (native to the Philippines) is used to treat bronchitis, catarrh, coughs and colds, skin disorders and for its antiseptic and wound healing properties.⁵ Copaiba resin obtained from several South American *Copaifera* spp. has anti-inflammatory, anticancer and antiseptic uses.⁴ However, perhaps the best known and most recognised for their useful therapeutic properties are the oleo-resins known as frankincense and myrrh which are derived from *Boswellia* spp. and *Commiphora* spp. respectively.

Three main *Boswellia* spp. (*B. carteri* Birdw., *B. frereana* Birdw. and *B. serrata* Roxb.) account for nearly all of the global production of frankincense resin.⁶ The resin is harvested by cutting incisions through the papery bark of mature trees and allowing the resinous exudates to seep out and harden into the orange-brown resin commonly known as

frankincense.⁷ A wide variety of therapeutic uses have been attributed to frankincense, including the treatment of skin diseases, urinary tract infections, respiratory infections, to aid in wound healing, as well as for digestive and gastrointestinal disorders.⁸ Many of these conditions are related to bacterial infections and several studies have reported the growth inhibitory properties of frankincense against panels of pathogens. One study screened *B. serrata* extracts for the ability to inhibit the growth of an extended panel of microbial pathogens associated with skin infections.⁹ That study tested 18 aerobic and 9 anaerobic bacterial strains (including antibiotic resistant strains), as well as 3 fungal strains. The *B. serrata* extracts inhibited the growth of 10 of the 18 aerobic bacteria, and 5 of the 9 anaerobic bacterial strains tested. Furthermore, the MIC values reported in that study were as low as 1 µg/mL against some *Streptococcus* spp., *Corynebacterium* spp., *Clostridium* spp., *Propionibacterium* spp. and *Porphyromonas* spp. In contrast, none of the fungal species tested was affected by the *B. serrata* extracts. In another study, essential oils produced by steam distillation of frankincense resins were reported to inhibit the growth of reference bacterial strains of *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*, as well as the fungal pathogen *Candida albicans*, with MIC values indicative of moderate growth inhibitory activity.¹⁰ Another study reported growth inhibitory activity of a frankincense extract against a broader panel of bacterial species,¹¹ although the value of that study was limited as the extract was only tested at very high concentrations (25-100 mg/mL), making it difficult to

compare this activity to that of other antibacterial chemotherapies. A recent publication also reported that frankincense extracts were potent inhibitors of the growth of several bacterial species associated with initiating rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis, with MIC values as low as 60 µg/mL against some bacteria.¹²

Myrrh is an oleo-resin produced from several small thorny trees of the genus *Commiphora* (family Burseraceae). The main myrrh producing species is *Commiphora molmol* (synonym *Commiphora myrrha*; Yemen, Somalia, Ethiopia), although the resins of *Commiphora gileadensis* (Eastern Mediterranean, Arabian Peninsula), *Commiphora wightii* (northern India) and several other species are often also referred to as myrrh.^{13,14} Myrrh has a similar suite of therapeutic uses to frankincense, including its use in the treatment of several inflammatory and skin diseases, its use as an antiseptic, an antipyretic, a mouth wash, to aid wound healing and as a stimulant.^{15,16} Many of these conditions are related to bacterial infections and several studies have reported the growth inhibitory properties of myrrh against panels of pathogens. One study screened *Commiphora molmol* extracts for the ability to inhibit the growth of a panel of bacterial and fungal pathogens.¹⁷ That study tested myrrh for growth inhibitory activity against 7 bacterial (*Staphylococcus aureus*, *Vibrio tubiashii*, *Streptococcus* spp., *Cellulosimicrobium cellulans*, *Micrococcus luteus*, *Legionella pneumophila*, *Bacillus cereus*) and 2 fungal species (*Fusarium oxysporum*, *Aspergillus flavus*). The *C. molmol* extract inhibited the growth of all microbes screened, although it was particularly potent against *A. flavus*, *V. tubiashii*, *Streptococcus* spp., *L. pneumophila* and *B. cereus*. However, the value of that study was limited as MIC's were not determined, making it difficult to compare this activity to that of other antibacterial chemotherapies. Another study examined the growth inhibitory potential of myrrh essential oil against *S. aureus* and reported that the oil not only inhibited bacterial growth, but also inhibited biofilm formation.¹⁸ Furthermore, several myrrh compounds have been isolated and shown to have good antibacterial efficacies. Furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one have been highlighted as being particularly potent growth inhibitors, with MIC values against three bacterial and one fungal species ranging from 0.2–2.8 µg/mL.¹⁹ Similarly, several terpenoids (including the sesquiterpenoids β-elemene and T-cadinol) isolated from *C. molmol* oleo-resin inhibited the growth of a panel of pathogenic bacteria, with MIC ranging from 4–256 µg/mL.²⁰ Recently, *C. molmol* extracts were reported to inhibit the growth of some bacterial triggers of rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis.²¹

Despite the wealth of publications reporting antibacterial activity of frankincense and myrrh, many pathogens are yet to be screened against these resins. Many of the previous reports have correctly focused on bacteria associated with illness that these resins have been traditionally used to treat. However, many other bacteria which may also be affected by frankincense and myrrh remain to be studied. Furthermore, whilst frankincense and myrrh essential oils^{22,23} and several compounds isolated from these resins have growth inhibitory activity against cancer cells,²⁴ there are relatively few studies examining the anticancer properties of frankincense and myrrh extracts. This study aimed to further extend our knowledge on the antibacterial and anticancer studies of frankincense and myrrh by examining their effects on further bacterial species and on CaCo2 and HeLa carcinoma cell lines.

MATERIALS AND METHODS

Frankincense (sourced from verified *Boswellia carteri* Birdw. trees in Oman) and myrrh (sourced from verified *Commiphora molmol* Engler trees in Somalia) were obtained from Noodles Emporium, Australia and supplied as a dry resin. Voucher samples have been stored in the School of Natural Sciences, Griffith University. Prior to use, each resin was freshly ground to a coarse powder and 1 g quantities were weighed

into separate tubes. The powdered resin was extracted by standardised methods.^{25,26} Briefly, an amount of 1 g of powdered plant material was weighed into each of five tubes and five different extracts were prepared by adding 50 mL of methanol, water, ethyl acetate, chloroform, or hexane respectively. All solvents were obtained from Ajax, Australia and were AR grade. The ground dried material was extracted in each solvent for 24 hours at 4°C with gentle shaking. The extracts were filtered through filter paper (Whatman No.54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant dry extract was weighed and redissolved in 10 mL deionised water containing 1% DMSO.

Qualitative phytochemical studies

Phytochemical analysis of the frankincense and myrrh extracts for the presence of saponins, phenolic compounds, flavonoids, phytosterols, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.²⁵⁻²⁷

Antibacterial screening

Test microorganisms

All clinical bacterial strains were obtained from Michelle Mendell, Griffith University, Australia. Stock cultures of *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella Salford*, *Serratia marcescens*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were subcultured and maintained in nutrient broth (Oxoid, Australia) at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion method.²⁸⁻³⁰ Briefly, 100 µL of the test bacteria were grown in 10 mL of fresh nutrient broth until they reached a count of approximately 10⁸ cells/mL as determined by direct microscopic determination. One hundred microliters of microbial suspension was spread onto the agar plates. The extracts were tested using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µL of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Bacillus cereus*, *Citrobacter freundii*, *Pseudomonas fluorescens* and *Serratia marcescens* were incubated at 30°C for 24 hours, then the diameters of the inhibition zones were measured in millimetres. Plates inoculated with *Escherichia coli*, *Salmonella salford*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were incubated at 37°C for 24 hours, then the diameters of the inhibition zones were measured. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate and mean values were determined. Standard discs of ampicillin (2 µg) were obtained from Oxoid, Australia and served as positive controls for antibacterial activity. Filter discs impregnated with 10 µL of distilled water were used as negative controls.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of the frankincense and myrrh extracts were determined by the disc diffusion method across a range of doses.^{31,32} The plant extracts were diluted in deionised water across a concentration range of 5 mg/mL to 0.1 mg/mL. Discs were impregnated with 10 µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to calculate the MIC values.

Screen for anticancer bioactivity

Cancer cell lines

The CaCo₂ and HeLa carcinoma cell lines used in this study were obtained from American Type Culture Collection (Rockville, USA). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies), supplemented with 20 mM HEPES, 10 mM sodium bicarbonate, 50 µg/mL streptomycin, 50 IU/mL penicillin, 2 mM glutamine and 10 % foetal calf serum (Life Technologies). The cells were maintained as monolayers in 75 mL flasks at 37 °C, 5 % CO₂ in a humidified atmosphere until approximately 80 % confluent.

Evaluation of cancer cell anti-proliferative activity

Anti-proliferative activity of the extracts was assessed as previously described.^{25, 33} Briefly, 1 mL of trypsin (Sigma) was added to the culture flasks and incubated at 37°C, 5% CO₂ for 15 min to dislodge the cancer cells. The cell suspensions were then transferred to a 10 mL centrifuge tube and sedimented by centrifugation. The supernatant was discarded and the cells were resuspended in 9 mL of fresh media. Aliquots of the resuspended cells (70 µL, containing approximately 5000 cells) were added to the wells of a 96 well plate. A volume of 30 µL of the test extracts or cell media (for the negative control) was added to individual wells and the plates were incubated at 37°C, 5 % CO₂ for 12 hours in a humidified atmosphere. A volume of 20 µL of Cell Titre 96 Aqueous One solution (Promega) was subsequently added to each well and the plates were incubated for a further 3 hours. Absorbances were recorded at 490 nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed in at least triplicate and triplicate controls were included on each plate. The antiproliferative activity of each test was calculated as a percentage of the negative control using the following formula:

$$\text{Proliferation (\% untreated control)} = (A_t/A_c) \times 100$$

A_t is the corrected absorbance for the test extract (calculated by subtracting the absorbance of the test extract in media without cells from the extract cell test combination) and A_c is the corrected untreated control (calculated by subtracting the absorbance of the untreated control in media without cells from the untreated cell media combination).

Toxicity screening

Reference toxins for biological screening

Potassium dichromate (K₂Cr₂O₇) (AR grade, Chem-Supply, Australia) was prepared as a 2 mg/mL solution in distilled water and was serially diluted in synthetic seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.³⁴⁻³⁶ Briefly, *A. franciscana* cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/L distilled water were prepared prior to use. An amount of 1 g of *A. franciscana* cysts were incubated in 500 mL synthetic seawater under artificial light at 25°C, 2000 Lux with continuous aeration. Hatching commenced within 16-18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. The extracts and positive control were also serially diluted in artificial seawater for LC₅₀ determination. A volume

of 400 µL of seawater containing approximately 38 (mean 37.8, n = 156, SD 14.6) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted in seawater for toxicity testing. A volume of 400 µL of the diluted plant extracts or the reference toxin was transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µL seawater) was run in triplicate for each plate. All treatments were performed in triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered moribund if no movement of the appendages was observed within 10 sec. After 48 h all nauplii were sacrificed and counted to determine the total number per well. The LC₅₀ with 95 % confidence limits for each treatment was calculated using probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments. One way ANOVA was used to calculate statistical significance between control and treated groups with a *P* value < 0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of frankincense and myrrh oleo-resins with various solvents yielded dried plant extracts ranging from 124 mg (aqueous frankincense extract) to 869 mg (methanolic and chloroform frankincense extracts) (Table 1). With the exception of the aqueous frankincense extract and the methanolic and chloroform myrrh extracts, all solvents extracted high yields of dried extracted material (generally 650-850 mg). The dried extracts were resuspended in 10 mL of deionised water resulting in the extract concentrations shown in Table 1. Qualitative phytochemical screening studies (Table 1) showed that all solvents extracted wide ranges and relatively large amount of phytochemicals. Furthermore, all solvents extracted similar classes of phytochemicals, with polyphenolic compounds (particularly water insoluble polyphenolic compounds), flavonoids, saponins and triterpenoids generally present in the highest levels. All extracts were generally devoid of all other classes of phytochemicals.

Antimicrobial activity

To determine the growth inhibitory activity of the myrrh extracts, aliquots (10 µL) of each extract were tested in the disc diffusion assay against panels of gram negative (Figure 1) and gram positive bacteria (Figure 2). The growth of all bacterial species was inhibited by the frankincense and myrrh extracts. The methanolic extracts of both frankincense and myrrh were generally the most potent growth inhibitors against both gram positive and gram negative bacteria (as determined by zones of inhibition), with some notable exceptions. The frankincense ethyl acetate extract was a particularly potent inhibitor of *E. coli*, *P. fluorescens* and *S. salford* growth, with equal or greater zones of inhibition than recorded for the corresponding methanolic extract. Similarly, the ethyl acetate myrrh extract was also a more potent growth inhibitor of *P. fluorescens* and *S. salford* growth than the methanolic myrrh extract. The aqueous, chloroform and hexane extracts also inhibited the growth of all of the bacterial species tested. However, it is noteworthy that whilst growth inhibition was detected for all bacteria, the zones of inhibition of some extracts against several bacterial species indicated that this inhibition was not particularly strong against these bacteria. For example, the zones of inhibition of all extracts against *S. marcescens* were generally < 7 mm (indeed, many were < 6 mm), indicating that these extracts had only low efficacy.

Table 1: The mass of dried frankincense and myrrh extracts, the concentration after resuspension in deionised water (1% DMSO) and qualitative phytochemical screenings of solvent extractions

	Frankincense					Myrrh				
	M	W	E	C	H	M	W	E	C	H
Mass of dried extract (mg)	869	124	808	869	840	181	728	642	307	162
Resuspended extract concentration (mg/mL)	86.9	12.4	80.8	86.9	84	18.1	72.8	64.2	30.7	16.2
Qualitative phytochemical screens										
Total phenolics	++	++	+	++	+	+++	+++	++	++	+
Water soluble phenolics	+	+	-	-	-	+	+	-	-	-
Water insoluble phenolics	++	++	+	++	+	+++	+++	++	++	++
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-
Saponins	+++	+	+++	+	+++	+++	+++	+	-	-
Triterpenoids	+	+	-	-	-	++	+++	++	+++	-
Phytosteroids	-	-	-	-	-	-	-	-	-	-
Alkaloids (Mayer test)	-	-	-	-	-	-	-	-	-	-
Alkaloids (Wanger test)	-	-	-	-	-	-	-	-	-	-
Flavanoids	+++	++	-	-	+	+++	+++	++	-	-
Tannins	-	-	-	-	-	-	-	-	-	-
Free anthraquinones	-	-	-	-	-	-	-	-	-	-
Combined anthraquinones	-	-	-	-	-	-	-	-	-	-

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

Table 2: The MIC values against susceptible bacteria ($\mu\text{g/mL}$), IC_{50} values ($\mu\text{g/mL}$) against CaCo2 and HeLa cancer cell lines, and the LC_{50} values ($\mu\text{g/mL}$) for *Artemia franciscana* nauplii exposed to frankincense and myrrh extracts

			Frankincense					Myrrh				
			M	W	E	C	H	M	W	E	C	H
MIC ($\mu\text{g/mL}$)	Gram negative bacteria	<i>Citrobacter freundii</i>	1879	2826	3667	3280	3924	2071	>10,000	>10,000	1203	3111
		<i>Escherichia coli</i>	814	3167	2727	637	2637	1637	>10,000	7584	984	2857
		<i>Pseudomonas fluorescens</i>	2214	2826	1845	1486	1583	2282	>10,000	>10,000	855	>10,000
		<i>Serratia marcescens</i>	>10,000	>10,000	>10,000	>10,000	>10,000	3867	>10,000	>10,000	>10,000	>10,000
		<i>Salmonella salford</i>	462	4528	258	3622	293	4286	>10,000	8320	504	4865
	Gram positive bacteria	<i>Bacillus cereus</i>	1856	>10,000	2750	2750	2124	1479	>10,000	>10,000	>10,000	7387
IC_{50} ($\mu\text{g/mL}$)		<i>Staphylococcus aureus</i>	926	3274	3167	2625	>10,000	1042	>10,000	>10,000	8467	>10,000
		<i>Staphylococcus epidermidis</i>	1143	>10,000	3528	2855	3283	1227	>10,000	>10,000	7964	>10,000
		CaCo2 cells	CND	1818	1902	-	-	-	3290	4040	2960	-
LC_{50} ($\mu\text{g/mL}$)		HeLa cells	CND	1679	CND	-	1583	-	CND	3090	2500	CND
		<i>Artemia franciscana</i> nauplii	>10,000	4406	>10,000	>10,000	>10,000	2538	>10,000	>10,000	>10,000	-

Numbers indicate the mean MIC, IC_{50} and LC_{50} values of triplicate determinations. - indicates no inhibition. CND = although significant inhibition of proliferation was evident, an IC_{50} value could not be determined as inhibition of cell proliferation did not exceed 50% at any dose tested.

The relative level of antimicrobial activity was further evaluated by determining the MIC values (Table 2) for each extract against the bacterial species which were shown to be susceptible by disc diffusion assays. Several of the extracts were effective at inhibiting microbial growth at low concentrations, with MIC values against the bacterial species that they inhibited generally $< 2000 \mu\text{g/mL}$ ($< 20 \mu\text{g}$ impregnated in the disc), indicating the good antimicrobial activity of these extracts. These

MIC values compare favourably with the dosages of the pure standard ampicillin, which was tested using $2 \mu\text{g}$. The methanolic frankincense and myrrh extracts were the most consistently potent, achieving low-middle range MIC values ($\leq 2000 \mu\text{g/mL}$) against several species. Indeed, the methanolic frankincense extract recorded MIC's below $2000 \mu\text{g/mL}$ against all bacterial species except *P. fluorescens* (MIC of $2214 \mu\text{g/mL}$). The *S. salford* growth inhibitory activity of the frankincense

Table 3: GC-MS analysis of the frankincense methanolic and aqueous extracts, elucidation of empirical formulas and putative identification of each compound

Putative Identification	Empirical Formula	Molecular Mass (Da)	Retention Time (min)	FM	MM
1,3,5,5-Tetramethyl-1,3-cyclohexadiene	C ₁₀ H ₁₆	136	10.868	✓	
Methyl N-hydroxybenzenecarboximidoate	C ₈ H ₉ NO ₂	151	11.198	✓	✓
2,4(10)-Thujadiene	C ₁₀ H ₁₄	134	12.501	✓	
(1S,3S,5S)-1-Isopropyl-4-methylenebicyclo[3.1.0]hexan-3-yl acetate	C ₁₂ H ₁₈ O ₂	194	12.835	✓	
6-Methyl-5-heptene-2-one	C ₈ H ₁₄ O	126	13.961	✓	
cis-p-Mentha-2,8-dien-1-ol	C ₁₀ H ₁₆ O	152	14.136	✓	
2-Menthene	C ₁₀ H ₁₈	138	14.299	✓	
Propanoic acid, 2,2-dimethyl-, propyl ester	C ₈ H ₁₆ O ₂	144	14.52		✓
Myrtenylisovalerate	C ₁₅ H ₂₄ O ₂	236	14.579	✓	
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	C ₁₀ H ₁₈ O	154	14.991	✓	
p-Cymene	C ₁₀ H ₁₄	134	15.26	✓	
2-Ethyl-1-hexanol	C ₈ H ₁₈ O	139	15.389		✓
Cineole	C ₁₀ H ₁₈ O	154	15.497	✓	✓
γ-Terpineol	C ₁₀ H ₁₈ O	154	15.94	✓	
α-Pinene	C ₁₀ H ₁₆	136	16.436	✓	
Toluene	C ₇ H ₈	92	16.971	✓	
1,1-Dimethyl-decyl-mercaptan	C ₁₂ H ₂₆ S	202	17.219	✓	
p-Cymenene	C ₁₀ H ₁₂	132	17.454	✓	
Linalool	C ₁₀ H ₁₈ O	154	17.764	✓	
Nonanal	C ₉ H ₁₈ O	142	17.89		✓
2,6-Dimethylphenol	C ₈ H ₁₀ O	122	17.97		✓
1,2-Dimethyl-1-cyclohexene	C ₈ H ₁₄	110	18.011	✓	
Thujone	C ₁₀ H ₁₆ O	152	18.368	✓	✓
Dehydrosabinene ketone	C ₉ H ₁₂ O	136	18.466	✓	
Camphen-6-ol	C ₁₀ H ₁₆ O	152	18.672	✓	
2,4(10)-Thujadiene	C ₁₀ H ₁₄	134	18.819	✓	
(1S,3S,4S,5R)-(+)-3-Thujanol	C ₁₀ H ₁₈ O	154	18.942	✓	
(+)-Sabinol	C ₁₀ H ₁₆ O	152	19.132	✓	✓
L-camphor	C ₁₀ H ₁₆ O	152	19.304	✓	
α-Phellandren-8-ol	C ₁₀ H ₁₆ O	152	19.433	✓	
Sabina ketone	C ₉ H ₁₄ O	138	19.707	✓	
Pinocarvone	C ₁₀ H ₁₄ O	150	19.885	✓	
o-Xylenol	C ₈ H ₁₀ O	122	19.94		✓
endo-Borneol	C ₁₀ H ₁₈ O	154	20.002	✓	
Ethyl benzoate	C ₉ H ₁₀ O ₂	150	20.074		✓
Verbenone	C ₁₀ H ₁₄ O	154	20.186	✓	✓
Terpinen-4-ol	C ₁₀ H ₁₈ O	154	20.361	✓	✓
p-Cymen-8-ol	C ₁₀ H ₁₄ O	150	20.559	✓	
cis-m-Menth-8-ene	C ₁₁ H ₂₀	138	20.63	✓	
α-Terpineol	C ₁₀ H ₁₈ O	154	20.768	✓	
(-)-Myrtenol	C ₁₀ H ₁₆ O	152	20.97	✓	✓
Sabiny acetate	C ₁₂ H ₁₈ O ₂	194	21.144	✓	✓
2-Pinen-7-one	C ₁₀ H ₁₄ O	150	21.407	✓	
3,4-Dimethylbenzaldehyde	C ₉ H ₁₀ O	134	21.52	✓	✓
Carveol	C ₁₀ H ₁₆ O	152	21.615	✓	

Continued...

Table 3: Continued

cis-p-mentha-1(7),8-dien-2-ol	C ₁₀ H ₁₆ O	152	21.955	✓	
4(10)-Thujen-3-ol, acetate	C ₁₂ H ₁₈ O ₂	194	22.185	✓	
p-Cumic aldehyde	C ₁₀ H ₁₂ O	148	22.278	✓	
D-Carvone	C ₁₀ H ₁₄ O	150	22.392	✓	
(-)-Bornyl acetate	C ₁₂ H ₂₀ O ₂	196	23.672	✓	✓
p-Cymen-7-ol	C ₁₀ H ₁₄ O	150	23.738	✓	
Thymol	C ₁₀ H ₁₄ O	150	24.022	✓	✓
(-)-Myrtenol	C ₁₀ H ₁₆ O	152	24.923	✓	
Tricyclo[7.1.0.0[1,3]]decane-2-carbaldehyde	C ₁₁ H ₁₆ O	161	25.064		✓
Limonene oxide	C ₁₀ H ₁₆ O	152	25.252	✓	
(1S,3S,4S,5R)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-3-ol	C ₁₀ H ₁₈ O	144	25.605	✓	
Isobutyl 3-hydroxy-2,2,4-trimethylpentanoate	C ₁₂ H ₂₄ O ₃	216	25.724		✓
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	C ₁₂ H ₂₄ O ₃	216	26.465	✓	
Copaene	C ₁₅ H ₂₄	204	26.719	✓	
Guaia-1(10),11-diene	C ₁₅ H ₂₄	204	27.297	✓	✓
1-Ethyl-5,5-dimethyl-1,3-cyclopentadiene	C ₉ H ₁₄	122	28.364	✓	
Caryophyllene	C ₁₅ H ₂₄	204	28.572	✓	
γ-Elemene	C ₁₅ H ₂₄	204	29.04		✓
(3S,4aR,5S,8aS)-4a,5-Dimethyl-3-(prop-1-en-2-yl)-2,3,4,4a,5,6-hexahydronaphthalen-1(8aH)-one	C ₁₅ H ₂₂ O	218	29.67	✓	
cis-murola-3,5-diene	C ₁₅ H ₂₄	204	29.825	✓	
Humulene	C ₁₅ H ₂₄	204	30.016	✓	
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	C ₁₅ H ₂₄	204	30.298	✓	
epsilon-Murolene	C ₁₅ H ₂₄	204	30.75	✓	✓
β-Longipinene	C ₁₅ H ₂₄	204	31.135		✓
Eudesma-4(14),11-diene	C ₁₅ H ₂₄	204	31.142	✓	
(+)-Ledene	C ₁₅ H ₂₄	204	31.402	✓	✓
2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206	31.67	✓	✓
γ-Murolene	C ₁₅ H ₂₄	204	31.928	✓	
Benzoic acid, 4-ethoxy-, ethyl ester	C ₁₁ H ₁₄ O ₃	194	32.066		✓
Ethyl 4-ethoxybenzoate	C ₁₁ H ₁₄ O ₃	194	32.15	✓	
Cubenene	C ₁₅ H ₂₄	204	32.408	✓	
Sabinylisobutanoate	C ₁₄ H ₂₂ O ₂	194	32.695	✓	
α-o-Menth-8-ene-4-methanol	C ₁₅ H ₂₆ O	222	32.799		✓
5-Cyclodecen-1-ol, 4,10-bis(methylene)-7-(1-methylethyl)-, (1R,5E,7S)-	C ₁₅ H ₂₄ O	220	32.962	✓	
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1a-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]	C ₁₅ H ₂₄ O	220	33.555		✓
Epicurzerenone	C ₁₅ H ₁₈ O ₂	230	34.089		✓
Epicubenol	C ₁₅ H ₂₆ O	222	34.623	✓	
Isospathulenol	C ₁₅ H ₂₄ O	220	34.608		✓
γ-Eudesmol	C ₁₅ H ₂₆ O	222	34.692		✓
tau-Cadinol	C ₁₅ H ₂₆ O	222	34.856	✓	✓
2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	C ₁₅ H ₂₄ O	220	35.431	✓	✓
Azunol	C ₁₅ H ₁₈	198	35.505	✓	
α-Phellandrene	C ₁₀ H ₁₆	136	37.448	✓	
Squalene	C ₃₀ H ₅₀	410	39.411	✓	
1-Propylpentyl laurate	C ₂₀ H ₄₀ O ₂	312	40.954	✓	

FM = methanolic frankincense extract; MM = methanolic myrrh extract.

Figure 1: Antibacterial activity of (a) frankincense and (b) myrrh extracts measured as zones of inhibition (mm) against gram negative bacteria. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; A = ampicillin control (2 µg). Results are expressed as mean $\pm$ SEM of triplicate determinations.

Figure 2: Antibacterial activity of (a) frankincense and (b) myrrh extracts measured as zones of inhibition (mm) against gram positive bacteria. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; A = ampicillin control (2 μ g). Results are expressed as mean $\pm$ SEM of triplicate determinations.

Figure 3: Anti-proliferative activity of (a) frankincense and (b) myrrh extracts against CaCo₂ cancer cell lines measured as percentages of the untreated control cells. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Cis = Cisplatin (50 mg/mL). Results are expressed as mean percentages $\pm$ SEM of triplicate determinations. * indicates results that are significantly different to the untreated control ($p < 0.01$).

Figure 4: Anti-proliferative activity of (a) frankincense and (b) myrrh extracts against HeLa cancer cell lines measured as percentages of the untreated control cells. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Cis = Cisplatin (50 mg/mL). Results are expressed as mean percentages $\pm$ SEM of triplicate determinations. * indicates results that are significantly different to the untreated control ($p < 0.01$).

Figure 5: The lethality of (a) frankincense and (b) myrrh extracts towards *Artemia franciscana* nauplii after 24 hours exposure. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; PC = potassium dichromate control (1000 µg/mL); NC = seawater control. Results are expressed as mean $\pm$ SEM of triplicate determinations.

Figure 6: Head space gas chromatograms of 0.5 µL injections of methanolic (a) frankincense and (b) myrrh extracts. The extracts were dried and resuspended in methanol for analysis.

Figure 7: Monoterpenoid components detected in the aqueous and methanolic frankincense extracts: (a1) 2,4(10)-thujadiene, (a2) *cis*-p-mentha-2,8-dien-1-ol, (a3) myrtenylisovalerate, (a4) cineole, (a5) γ -terpineol, (a6) α -pinene, (b1) 1,2-dimethyl-1-cyclohexene, (b2) thujone, (b3) (1*S*,3*S*,4*S*,5*R*)-(+)-3-thujanol, (b4) (+)-sabinol, (b5) L-camphor, (b6) α -phellandren-8-ol, (c1) sabina ketone, (c2) pinocarvone, (c3) endo-borneol, (c4) verbenone, (c5) terpinene-4-ol, (c6) *cis*-menth-8-ene, (d1) α -terpineol, (d2) (-)-myrtenol, (d3) sabinyl acetate, (d4) 2-pinen-7-one, (d5) 3,4-dimethylbenzaldehyde, (d6) *cis*-p-mentha-1(7),8-dien-2-ol, (e1) 4(10)-thujen-3-ol, acetate, (e2) *p*-cymic aldehyde, (e3) D-carvone, (e4) (-)-bornyl acetate, (e5) *p*-cymen-7-ol, (e6) thymol, (f1) limonene oxide, (f2) sabinylisobutanoate, (f3) α -phellandrene, (f4) linalool.

Figure 8: Sesquiterpenoid components detected in the aqueous and methanolic frankincense extracts: (a) copaene, (b) guaia-1(10),11-diene, (c) caryophyllene, (d) cis-muurolo-3,5-diene, (e) humulene, (f) epsilon-muuroloene, (g) eudesma-4(14),11-diene, (h) (+)-ledene, (i) gamma-muuroloene, (j) cubenene, (k) epicubenol, (l) tau-cadinol, (m) azulol, (n) gamma-elemene and (o) beta-longipinene.

ethyl acetate and hexane extracts, and of the myrrh chloroform extract was also noteworthy, with MIC values 258, 293 and 504 µg/mL respectively.

Inhibition of cancer cell proliferation

The frankincense and myrrh extracts were tested against 2 cancer cell lines (CaCo₂ colorectal carcinoma cells, Figure 3; HeLa cervical cancer cells, Figure 4) to determine their ability to inhibit cancer cell growth. The aqueous and ethyl acetate extracts of both frankincense and myrrh displayed potent inhibitory activity against CaCo₂ cells, with proliferation inhibited to <50 % of the untreated control cell growth (Figure 3). In addition, the myrrh chloroform extract was also a potent inhibitor of CaCo₂ cell proliferation, inhibiting greater than 90 % of cellular proliferation (compared to the untreated control). Generally, the myrrh extracts were more potent inhibitors of CaCo₂ cell proliferation than the corresponding frankincense extracts. The methanolic frankincense extract also inhibited CaCo₂ cell proliferation, although the level of inhibition was only 24% when compared to the untreated controls.

Also noteworthy, the frankincense chloroform and hexane extracts induced a significant increase in cellular proliferation compared to the untreated control cells. Inhibition of CaCo₂ proliferation by frankincense and myrrh extracts was dose dependent, with the level of inhibitory activity decreasing at lower concentrations. The dose dependent induction of proliferation by the chloroform and hexane extracts was not evaluated in this study.

The frankincense and myrrh extracts were also shown to affect the proliferation of HeLa cells, albeit with lower efficacy than against the CaCo₂ cells (Figure 4). For the frankincense extracts, the hexane extract was the most potent inhibitor of HeLa cell proliferation, inhibiting growth by approximately 95% of the untreated control cell growth. The aqueous extract was also a potent anti-proliferative agent, inhibiting growth by approximately 90%. The methanolic and ethyl acetate frankincense extracts also significantly inhibited HeLa proliferation, albeit to a lesser extent. The aqueous, ethyl acetate and chloroform myrrh extracts also inhibited HeLa growth. Inhibition of HeLa proliferation by frankincense

and myrrh extracts was dose dependent, with the level of inhibitory activity decreasing at lower concentrations.

The relative level of anti-proliferative activity was further evaluated by determining the IC_{50} values (Table 2) for each extract against the $CaCo_2$ and HeLa cells. In general, the frankincense extracts were better anti-proliferative agents than the myrrh extracts. Indeed, the frankincense methanolic extract displayed IC_{50} values substantially $<2000 \mu\text{g/mL}$ against both $CaCo_2$ and HeLa cells. The frankincense ethyl acetate (against $CaCo_2$ cells) and hexane extracts (against HeLa cells) also had IC_{50} values substantially $<2000 \mu\text{g/mL}$. Despite displaying significant inhibitory activity, IC_{50} values could not be determined for the frankincense methanolic extract (against both cell lines), the frankincense ethyl acetate extract (against HeLa cells), nor the aqueous and hexane myrrh extracts (against HeLa cells) as the levels of inhibition did not exceed 50% at any dosage tested.

Quantification of toxicity

The frankincense and myrrh extracts were screened for toxicity in the *Artemia* nauplii lethality bioassay. For comparison, the reference toxin potassium dichromate was also tested in the bioassay. Potassium dichromate was rapid in its induction of mortality, with mortality evident within 4 hours of exposure (unpublished results). The frankincense and myrrh extracts were slower at inducing mortality, with ≥ 12 hours needed for mortality induction. Despite the slower onset of mortality, all extracts except the myrrh hexane extract induced mortality significantly above that of the artificial seawater control (Figure 5). Table 2 shows the extract and control toxin concentrations required to achieve 50% mortality (LC_{50}) at various times. As toxicity of crude plant extracts has previously been defined as 24 LC_{50} values $<1000 \mu\text{g/mL}$,³⁶ the measured LC_{50} values indicate that all of the frankincense and myrrh extracts were nontoxic.

Non-targeted GC-MS headspace analysis

As the methanolic frankincense and myrrh extracts displayed the broadest, and often the most potent bacterial and cancer growth inhibition (as determined by MIC and IC_{50} ; Table 2), they were deemed the most promising extracts for further phytochemical analysis. Optimised GC-MS parameters were developed and used to examine the phytochemical composition of these extracts. The resultant gas chromatograms for the methanolic frankincense and myrrh extracts are presented in Figures 6a and Figure 6b respectively. Major peaks were present in the methanolic frankincense extract at approximately 15.3, 17.5, 19.1, 20.3, 21.0 and 21.4 min. Numerous overlapping peaks were also evident throughout all stages of the chromatogram. In total, 74 unique mass signals were noted for the frankincense methanolic extract (Table 3). Putative empirical formulas and identifications were achieved for all of these compounds. A very different chromatographic profile was noted for the methanolic myrrh extract (Figure 6b). Major peaks were present in that chromatogram at approximately 11.1, 14.4, 19.5, and 34.1 min, although numerous smaller peaks were also evident throughout the chromatogram. In total, 34 unique mass signals were noted for the methanolic myrrh extract (Table 3). Putative empirical formulas and identifications were achieved for all of these compounds.

DISCUSSION

Multiple antimicrobial studies have been undertaken to support the traditional usage of frankincense and myrrh. Most often, these studies have examined the growth inhibitory potential of distillates and essential oils. Several of these have reported good growth inhibitory activity for *Boswellia* spp. One study compared essential oils produced from 4 different grades of *Boswellia sacra* Flueck. oleo-resins.³⁷ Despite being classed as different grades, potent bacterial growth inhibition was reported for all 4 oils. Indeed, MIC values of $5 \mu\text{g/mL}$ were reported for all oils against

each gram positive bacteria screened. That study also reported potent inhibition of growth for all 7 gram negative bacteria screened, with MIC values $30\text{--}80 \mu\text{g/mL}$. Similarly, another study also reported MIC values $\leq 32 \mu\text{g/mL}$ for *B. sacra* and *B. papyrifera* essential oils against a panel of 10 bacterial species.³⁸ Four fungal strains were also screened and MIC values $\leq 4 \mu\text{g/mL}$ were reported. In contrast, van Vuuren *et al.*¹⁰ screened 20 frankincense essential oils and reported only low to moderate inhibitory activity, with large variations between the different samples. For example, the MIC values of the frankincense oils against *S. aureus* ranged from $6\text{--}16 \text{ mg/mL}$ (values that indicate low activity). Whilst the growth inhibitory activity was more potent for some oils against other bacterial species (e.g. as low as 1.5 mg/mL against *B. cereus*) the variation in efficacy was evident against all bacterial species screened. That study tested oils from several *Boswellia* spp. and oils from different origins. Large variations were noted between different commercial sources of the oils of individual species. Interestingly, phytochemical studies revealed that the oils all had similar compositions, albeit with variations in quantity of the individual compounds. The profound effects of varied ratios of bioactive compounds within crude mixtures are well known³⁹ and may account for these differences.

Fewer studies have reported on the bacterial growth inhibitory properties of extracts produced from frankincense oleo-resins and many of these have also proved contradictory. One study reported MIC values $23\text{--}52 \mu\text{g/mL}$ for methanolic and aqueous *B. sacra* and *B. frereana* extracts against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Streptococcus pneumoniae* and *Corynebacterium diphtheria*.⁴⁰ In contrast, other studies have screened similar aqueous and methanolic extracts against similar bacterial species with very different results. A recent study screened *B. carteri* aqueous and methanolic extracts against several *K. pneumoniae* strains (both reference and clinical isolates) and reported that they were essentially inactive (MIC $>10,000 \mu\text{g/mL}$) against all strains.¹² Similarly, the same study also reported that several *P. aeruginosa* strains were non-susceptible to the *B. carteri* extracts, in contrast to the potent inhibition reported in the Hasson *et al.* study.⁴⁰ Our study reported a range of bacterial growth inhibitory efficacies. The extracts screened in our study displayed only low growth inhibitory towards most bacteria, consistent with the van Vuuren *et al.* study.¹⁰ However, the MIC values of the frankincense extracts against some bacteria (particularly *S. salfordi*) indicate potent inhibition similar to those reported in other studies.^{37,38} Different *Boswellia* spp. derived from different locations were tested across these studies and thus sample variations may explain these variations. Furthermore, the use of different bacterial strains and different assay conditions between the groups may have contributed to these different findings.

Similar trends are evident for myrrh essential oils and extracts. As for frankincense, much variation has been reported in studies examining the bacterial growth inhibitory properties of myrrh. One study reported for growth inhibitory activity against 7 bacterial (*Staphylococcus aureus*, *Vibrio tubiashii*, *Sterptococcus* spp., *Cellulosimicrobium cellulans*, *Micrococcus luteus*, *Legionella pneumophila*, *Bacillus cereus*) and 2 fungal species (*Fusarium oxysporum*, *Aspergillus flavus*), with particularly potent inhibition against *A. flavus*, *V. tubiashii*, *Sterptococcus* spp., *L. pneumophila* and *B. cereus*.¹⁷ However, the value of that study was limited as MIC's were not determined, making it difficult to compare this activity to that of other antibacterial chemotherapies. Another study reported potent inhibition ($30\text{--}250 \mu\text{g/mL}$) against methicillin resistant strains of *Staphylococcus aureus* by *Commiphora molmol* extracts.⁴¹ In contrast, other studies have reported low-moderate bacterial growth inhibition for *C. molmol*,²¹ *C. guidotti* and *C. myrrh* against a variety of bacterial species.⁴² The results of our study also indicate only low to moderate bacterial

growth inhibition for the methanolic, aqueous, ethyl acetate and hexane myrrh extracts against bacterial growth. However, the chloroform myrrh extract displayed potent growth inhibitory activity against *C. freundii*, *E. coli*, *P. fluorescens* and *S. salford*.

Anti-proliferative activity against CaCo₂ and HeLa carcinoma cell lines was noted for the aqueous, ethyl acetate and hexane frankincense extracts, with IC₅₀ values generally approximately 1500-2000 µg/mL. In contrast, the myrrh extracts had much lower anti-proliferative activity, with most IC₅₀ values >3000 µg/mL. These findings support and extend previous studies examining the anticancer effects of frankincense and myrrh oils. A recent study reported frankincense and myrrh extracts to inhibit cellular proliferation and be potent inducers of apoptosis in MCF-7, HepG2, HS-1 and A459 cells, with IC₅₀ values ≤60 µg/mL.⁴³ Similarly, *Boswellia carteri* essential oil induced cytotoxicity in J82 bladder carcinoma cells.⁴⁴ Furthermore, that study also reported that the *B. carteri* essential oil was specific to carcinoma cells and did not affect normal cells. Whilst that study did not determine the IC₅₀ value to quantify the effect, it examined the apoptotic mechanisms involved. Of further interest in our studies, the chloroform and hexane frankincense extracts demonstrated a stimulatory effect on CaCo₂ cell proliferation. Therefore these extracts may prove useful in accelerating wound healing and further compound isolation and identification studies are required.

GC-MS headspace analysis of the methanolic frankincense and myrrh extracts in our study detected a number of interesting compounds, including a wide diversity of terpenoids. Monoterpenoids were particularly prevalent, with approximately 34 monoterpenoids putatively identified in the methanolic frankincense extract: 2,4 (10)-thujadiene (Figure 7a1), cis-p-mentha-2,8-dien-1-ol (Figure 7a2), myrtenylisovalerate (Figure 7a3), cineole (Figure 7a4), monoterpenoid-terpineol (Figure 7a5), α-pinene (Figure 7a6), 1,2-dimethyl-1-cyclohexene (Figure 7b1), thujone (Figure 7b2), (1S,3S,4S,5R)-(+)-3-thujanol (Figure 7b3), (+)-sabinol (Figure 7b4), L-camphor (Figure 7b5), α-phellandren-8-ol (Figure 7b6), sabina ketone (Figure 7c1), pinocarvone (Figure 7c2), endo-borneol (Figure 7c3), verbenone (Figure 7c4), terpinene-4-ol (Figure 7c5), cis-menth-8-ene (Figure 7c6), α-terpineol (Figure 7d1), (-)-myrtenol (Figure 7d2), sabinyl acetate (Figure 7d3), 2-pinen-7-one (Figure 7d4), 3,4-dimethylbenzaldehyde (Figure 7d5), 4(10)-thujen-3-ol, acetate (Figure 7e1), p-cumic aldehyde (Figure 7e2), D-carvone (Figure 7e3), (-)-bornyl acetate (Figure 7e4), p-cymen-7-ol (Figure 7e5), thymol (Figure 7e6) limonene oxide (Figure 7f1), sabinylisobutanoate (Figure 7f2), α-phellandrene (Figure 7f3). Of these, cineole, thujone, (+)-sabinol, verbenone, terpinene-4-ol, (-)-myrtenol, sabinyl acetate, 3,4-dimethylbenzaldehyde, (-)-bornyl acetate and thymol were also present in the methanolic myrrh extract. Linalool (Figure 7f4) was present in the myrrh but not the frankincense methanolic extract.

Monoterpenes have been reported to exert a wide variety of biological effects including antibacterial, antifungal, anti-inflammatory and anti-tumour activities⁴⁵ and therefore may contribute to the bacterial growth inhibitory activity reported here. Indeed, many of the monoterpenoids putatively identified in our study have been previously reported to have potent broad spectrum antibacterial activity.⁴⁵ A wide variety of monoterpenoids including camphor, carvone, cineole, borneol, menthone, pinene, terpinene, as well as their derivatives, inhibit the growth of an extensive panel of pathogenic and food spoilage bacteria.⁴⁶ Interestingly, several of these monoterpenoids have also been reported to suppress NF-κB signaling (a major regulator of both cancer and inflammatory diseases).⁴⁷⁻⁵⁰ Thus, the terpene components may also be involved in the inhibition of cancer cell proliferation reported here. Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism(s) of bacterial growth inhibition.

Several sesquiterpenoids including copaene (Figure 8a), guaia-1(10), 11-diene (Figure 8b), caryophyllene (Figure 8c), cis-murola-3,5-diene (Figure 8d), humulene (Figure 8e), epsilon-murolene (Figure 8f), eudesma-4(14),11-diene (Figure 8g), (+)-ledene (Figure 8h), γ-murolene (Figure 8i), cubenene (Figure 8j), epicubenol (Figure 8k), tau-cadinol (Figure 8l) and azulone (Figure 8m) were also detected in the methanolic frankincense extracts. Of these, guaia-1(10),11-diene, epsilon-murolene, (+)-ledene and tau-cadinol were also present in the methanolic myrrh extract. The sesquiterpenoids γ-elemene (Figure 8n), β-longipinene (Figure 8o) were present in the myrrh, but not the frankincense methanolic extract. Previous studies have reported bacterial growth inhibitory activities for many sesquiterpenoids including cadinol,⁵¹ caryophyllene,⁵² copaene, epicubenol and cubenene.⁵³

Notably, despite triterpenoids being detected as a major component in the methanolic myrrh extract and as a minor component in the methanolic frankincense extract in the qualitative screenings, no triterpenoids (nor diterpenoids or sesterterpenoids) were detected by GC-MS headspace analysis. It is perhaps not surprising that triterpenoids were not detected as a mass range cut off of 450 m/z was used in these studies. Therefore, many triterpenoids would have molecular masses that would be near this cut off and may not be detected. However, given the diversity of other terpenoids detected, it is perhaps surprising that no di- and sesterterpenoids were detected in the extract. Due to the low polarity of these compounds, perhaps an analysis of the lower polarity extracts (chloroform, hexane) may have detected these classes of phytochemical.

The results of this study indicate that the frankincense and myrrh extracts examined in this report are worthy of further study due to their bacterial growth inhibitory activity and ability to block cancer cell proliferation. Furthermore, as extracts with LC₅₀ values greater than 1000 µg/ml in the *Artemia* nauplii bioassay have been defined as being non-toxic,³⁶ all extracts were deemed nontoxic. However, whilst these extracts have therapeutic potential, caution is needed before they can be applied to medicinal purposes and further toxicity evaluation using human cell lines is required.

CONCLUSION

The results of this study partially validate the traditional usage of frankincense and myrrh oleo-resins to treat microbial diseases and cancer, and indicate that they are worthy of further study. Bioactivity driven purifications of the active components and an examination of the mechanisms of action of these agents is required.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS USED

DMSO: Dimethyl sulfoxide; **LC₅₀:** The concentration required to achieve 50% mortality; **MIC:** Minimum inhibitory concentration.

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PICTORIAL ABSTRACT

SUMMARY

- Frankincense and myrrh extracts displayed broad spectrum antibacterial activity against gram positive and gram negative bacteria.
- Frankincense methanolic (MIC 462 µg/mL), ethyl acetate (MIC 258 µg/mL) and hexane (MIC 293 µg/mL) extracts were particularly potent against *S. salford*.
- Most extracts blocked the proliferation of CaCo₂ and HeLa carcinoma cell lines.
- Aqueous and ethyl acetate frankincense extracts were the most potent cancer anti-proliferative agents (IC₅₀ values 1600-1900 µg/mL).
- All frankincense and myrrh extracts were nontoxic.
- Phytochemical profiling highlighted the higher terpenoids contents of the methanolic frankincense and myrrh extracts.

ABOUT AUTHOR

Dr Ian Cock: 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 nearly 200 scientific publications in a variety of peer reviewed journals.