An examination of the antimicrobial and anticancer properties of *Garcinia cambogia* fruit pericarp extracts

C. Hart¹, I. E. Cock^{1,2*}

ABSTRACT

Background: *Garcinia cambogia* (synonym *Garcinia gummi-gutta*) is commonly known as brindleberry and Malabar tamarind. It has received considerable recent interest due to its potential in the prevention and treatment of obesity and obesity related diseases. The fruit pericarp also has traditional uses in the treatment of a wide variety of diseases and medical conditions, yet these have received relatively little investigation to date. **Methods:** *Garcinia cambogia* fruit extracts were investigated for their ability to inhibit the growth of a panel of bacteria of medicinal importance. The extracts were also tested for their ability to block the proliferation of the CaCo_2 and HeLa human carcinoma cell lines. **Results:** *Garcinia cambogia* methanolic, aqueous and ethyl acetate extracts displayed broad spectrum antimicrobial activity, inhibiting the growth of between 16 (64 %; ethyl acetate extract) and 22 (88 % aqueous extract) of the 25 bacterial species tested. Strong inhibitory activity was detected with minimum inhibitory concentration (MIC) values $<1000 \mu\text{g/ml}$ against many bacteria. All extracts were effective against both Gram negative and Gram positive bacteria, although Gram negative bacteria were more sensitive. All extracts displayed anti-proliferative activity against CaCo_2 and HeLa carcinoma cells, yet were non-toxic in the *Artemia franciscana* bioassay, with LC_{50} values greatly in excess of $1000 \mu\text{g/ml}$. **Conclusion:** The inhibitory bioactivity against a range of microbes, the anti-proliferative activity against CaCo_2 and HeLa cells, as well as the lack of toxicity, indicate the potential for *G. cambogia* in the discovery and development of new natural pharmaceuticals.

Key words: Brindleberry, CaCo_2 , HeLa, Anti-proliferative activity, Anticancer activity, Antibacterial activity, Toxicity.

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INTRODUCTION

Garcinia (Family Clusiaceae) is a large diverse genus of approximately 240 species. Several *Garcinia* spp. have been reported to have high antioxidant contents. Possibly the best known species is *Garcinia mangostana* (purple mangosteen). Recent studies have reported the therapeutic potential of *G. mangostana* fruit pericarp and linked these properties to its antioxidant capacity.¹ In particular, antibacterial and anticancer activities were reported. The West African species, *Garcinia kola* (bitter kola), is well known for its use in a variety of traditional medicine systems to treat bronchitis, throat infections, coughs and colds, as well being used as a general antiseptic, anti-parasitic and purgative.² Laboratory studies have also demonstrated the high free radical scavenging activity³ and Ebola inhibitory effects² of *G. kola* fruit. Several other *Garcinia* species also have high antioxidant capacities and free radical scavenging activities.

Epidemiological studies have shown that a diet rich in antioxidants is associated with a decreased incidence of chronic diseases.⁴ High antioxidant levels have also been shown to act as a preventative against the development of degenerative diseases such as cancer,⁵ cardiovascular diseases and neural degen-

eration,⁶ diabetes and obesity⁷. Phenolic compounds are generally strong antioxidants.⁸ Their primary action involves the protection of cell constituents against oxidative damage through the scavenging of free radicals, thereby averting their deleterious effects on nucleic acids, proteins, and lipids in cells⁸. Phenolics interact directly with receptors or enzymes involved in signal transduction⁹, clearly indicating that they play a specific role in human physiology. Therefore, examination of the therapeutic properties of other high antioxidant *Garcinia* species is warranted.

Garcinia cambogia (Geartn.) Desr. is a slow-growing evergreen tree which grows in tropical areas of South Eastern and Eastern Asia, as well as in Western and Central Africa, although it is believed to have originated from the Indian subcontinent¹⁰. It is known by a variety of names including brindleberry, Malabar tamarind, pot tamarind and *Garcinia gummi-gutta*. The fruit is deeply lobed with thin green to pale yellow skin and is about the size of a grapefruit. The fruit consists of a soft white edible pulp surrounded by the pericarp. The pericarp may be used as a spice, or (in conjunction with salt) to dry and cure fish¹¹. It is also sought after for its therapeutic properties. *G. cambogia* has traditional uses for a diverse array

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of ailments, including use as a laxative and as a general antiseptic.¹² Recent interest in the therapeutic potential of *G. cambogia* has focussed on its apparent hypocholesteremic and anti-obesity effects.¹² However, scientific studies into these potential therapeutic properties have been inconclusive.¹³⁻¹⁵ Despite this lack of definitive evidence, *G. cambogia* has achieved notable recent public acclaim, at least in part due to its coverage as a weight loss aid on the popular American television show, Dr. Oz⁶ and subsequent media coverage. Much more research is required to establish the potential of *G. cambogia* in the prevention and treatment of obesity, diabetes and cholesteremic disorders.

G. cambogia contains many interesting phytochemicals, although much of the interest has focussed on hydroxyacetic acid (HCA) due to its functional inhibition of citrate lyase.¹⁷ Due to the role of citrate lyase in fatty acid biosynthesis, inhibition of this enzyme would be expected to block the synthesis and storage of triacylglycerols and result in fatty acid mobilisation from the adipose tissue. Several studies have reported this effect in rat models.¹⁸ However, similar trends have not been reported for human trials.^{13,15}

Despite the current interest in the therapeutic potential of *G. cambogia*, scientific research is focussed almost entirely on its potential anti-obesity potential. Other therapeutic properties have not been adequately explored. This study was undertaken to examine several other therapeutic properties of *G. cambogia*. In particular, the antimicrobial properties of *G. cambogia* extracts were evaluated against an extended panel of bacteria. This study also aimed to examine the anticancer properties of *G. cambogia* by testing the extracts against the CaCo₂ colorectal and HeLa cervical cancer cell lines. The toxicity of the *G. cambogia* extracts was also examined to further determine their suitability for therapeutic usage.

MATERIALS AND METHODS

G. cambogia fruit pericarp powder was bought from One Stop Nutrition, Australia as an air dried ground powder. An amount of 1 g of 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 and were AR grade. Powdered material was extracted into 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 resulting dry extracts were weighed and redissolved in 10 ml deionised water (containing 1% DMSO).

Qualitative phytochemical studies

Phytochemical analysis of *G. cambogia* extracts for the presence of saponins, phenolic compounds, flavonoids, polysteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays¹⁹⁻²¹.

Antibacterial screening

Test microorganisms

All clinical microbial strains were obtained from Michelle Mendell and Tarita Morais, Griffith University, Australia. The reference strains *Acinetobacter baylii* (ATCC33304), *Bacillus megaterium* (ATCC14581), *Escherichia coli* (ATCC8739), *Klebsiella pneumonia* (ATCC31488), *Proteus mirabilis* (ATCC21721), *Proteus vulgaris* (ATCC21719), *Pseudomonas aeruginosa* (ATCC39324), *Staphylococcus aureus* (ATCC 25923) and *Vibrio fischeri* (ATCC7744) were purchased from American Tissue Culture Collection, USA. Stock cultures of all bacteria were subcultured and maintained in nutrient broth at 4 °C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion method.²²⁻²⁴ Briefly, 100 µl of the test bacteria were grown at 37°C in 10 ml of fresh nutrient broth until they reached a count of approximately 10⁸ cells/ml (determined by direct microscopic counting). One hundred microliters of microbial suspension was spread onto the appropriate 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 were incubated at 37°C for 24 hours and then the diameters of the inhibition zones were measured in millimetres. 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 Ltd. and served as positive controls. Filter discs impregnated with 10 µl of distilled water were used as negative controls.

Minimum inhibitory concentration determination

The minimum inhibitory concentrations (MIC) of the *G. cambogia* extracts were determined by the disc diffusion MIC method across a range of doses.^{25,26} 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 anti-cancer 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 antiproliferative activity

Antiproliferation activity of the extracts was assessed as previously described.^{27,28} 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:

$$\text{Proliferation (\% untreated control)} = (A_{ct}/A_{cc}) \times 100$$

A_{ct} 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_{cc} 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 toxin for biological screening

Potassium dichromate ($K_2Cr_2O_7$) (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 *A. franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.²⁹⁻³¹ 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 was 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 serially diluted in artificial seawater for LC_{50} determination. A volume of 400 µl of seawater containing approximately 43 (mean 43.2, SD 13.8, n=80) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted to 4 mg/ml in seawater for toxicity testing, resulting in a 2 mg/ml concentration in the bioassay. A volume of 400 µl of diluted plant extract or the reference toxin was transferred to the wells and incubated at $25 \pm 1^\circ\text{C}$ under artificial light (1000 Lux). A negative control (400 µl seawater) was run in at least triplicate for each plate and all treatments were performed in at least 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 48h all nauplii were sacrificed and counted to determine the total number per well. The LC_{50} with 95 % confidence limits for each treatment was calculated using probit analysis.

Statistical analysis

Data are expressed as the mean $\pm$ SEM of at least three independent experiments. One way ANOVA was used to calculate differences between control and treated groups with a *P* value < 0.05 considered to be significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g quantities of dried plant material with various solvents yielded dried plant extracts ranging from 157 mg to 398 mg (Table 1). Methanol and water gave relatively high yields of dried extracted material (398 and 305 mg for the methanolic and aqueous extracts respectively), whilst ethyl acetate, chloroform and hexane extracted lower masses (260, 269 and 157 mg, respectively). The dried extracts were resuspended in 10 ml of deionised water (containing 1% DMSO), resulting in the extract concentrations shown in Table 1.

Table 1: The mass of dried extracted material, the concentration of extracts after resuspension in deionised water and the qualitative phytochemical screenings of solvent extractions.

Extract	Mass of Dried Extract (mg)	Resuspended Extract Concentration (mg/ml)	Total Phenolics	Water Soluble	Water Insoluble	Cardiac Glycosides	Saponins	Triterpenes	Polysteroids	Alkaloids (Meyer test)	Alkaloids (Wagners test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
Methanol	398	39.8	+++	-	++	-	-	-	-	-	-	+	-	-	-
Water	305	30.5	+++	-	++	-	-	-	-	-	-	+	-	-	-
Ethyl Acetate	260	26	++	-	+	-	-	-	-	-	-	-	-	-	-
Chloroform	269	26.9	+	-	+	-	-	-	-	-	-	-	-	-	-
Hexane	157	15.7	-	-	-	-	-	-	-	-	-	-	-	-	-

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

Figure 1: Antibacterial activity of *G. cambogia* fruit extracts and an ampicillin control (2 µg) measured as zones of inhibition (mm) against clinical bacterial strains. 1 = *A. baylii*; 2 = *A. faecalis*; 3 = *A. hydrophillia*; 4 = *C. cloaceae*; 5 = *C. freundii*; 6 = *E. aerogenes*; 7 = *E. coli*; 8 = *K. pneumoniae*; 9 = *P. mirabilis*; 10 = *P. aeruginosa*; 11 = *P. fluorescens*; 12 = *S. newport*; 13 = *S. marcescens*; 14 = *Y. enterocolatiae*; 15 = *B. cereus*; 16 = *S. salford*; 17 = *S. aureus*; 18 = *S. epidermidis*; 19 = *S. pyogenes*. Results are expressed as mean $\pm$ SEM of at least triplicate determinations.

Table 2: Minimum inhibitory concentrations (µg/ml) of *G. cambogia* fruit extracts against susceptible microbial species.

	Solvent Extracts				
	Methanol	Water	Ethyl Acetate	Chloroform	Hexane
Gram-negative bacteria					
<i>A. baylii</i> (clinical isolate)	1143	1380	745	-	-
<i>A. baylii</i> (ATCC33304)	2178	2206	843	-	-
<i>A. faecalis</i> (clinical isolate)	-	-	-	-	-
<i>A. hydrophillia</i> (clinical isolate)	1447	724	488	-	-
<i>B. megaterium</i> (ATCC14581)	2533	906	428	-	-
<i>C. cloaceae</i> (clinical isolate)	328	615	76	-	-
<i>C. freundii</i> (clinical isolate)	-	-	-	-	-
<i>E. aerogenes</i> (clinical isolate)	-	4452	3730	-	-
<i>E. coli</i> (clinical isolate)	1087	1143	-	-	-
<i>E. coli</i> (ATCC14581)	1633	1293	-	-	-
<i>K. pneumoniae</i> (clinical isolate)	387	311	1146	-	-
<i>K. pneumoniae</i> (ATCC31488)	915	1768	-	-	-
<i>P. mirabilis</i> (clinical isolate)	425	659	126	-	-
<i>P. mirabilis</i> (ATCC21721)	627	802	116	-	-
<i>P. vulgaris</i> (ATCC21719)	557	473	296	-	-
<i>P. aeruginosa</i> (clinical isolate)	217	346	727	-	-
<i>P. aeruginosa</i> (ATCC39324)	733	981	-	-	-
<i>P. fluorescens</i> (clinical isolate)	1485	1267	583	-	-
<i>S. newport</i> (clinical isolate)	1737	1492	-	-	-
<i>S. marcescens</i> (clinical isolate)	4265	3770	-	-	-
<i>V. fischeri</i> (ATCC7744)	512	394	728	-	-
<i>Y. enterocolatiae</i> (clinical isolate)	1286	1084	1563	-	-
Gram-positive bacteria					
<i>B. cereus</i> (clinical isolate)	2040	1438	620	-	-

Continued

<i>S. salford</i> (clinical isolate)	-	-	-	-	-
<i>S. aureus</i> (clinical isolate)	1389	1086	627	-	-
<i>S. aureus</i> (ATCC25923)	1558	1147	-	-	-
<i>S. epidermidis</i> (clinical isolate)	735	428	993	-	-
<i>S. pyogenes</i> (clinical isolate)	-	-	-	-	-

Numbers indicate the mean ($\pm$ SEM) MIC values of at least triplicate determinations. - indicates no growth inhibition.

Figure 2: Antibacterial activity of *G. cambogia* fruit extracts and an ampicillin control (2 μ g) measured as zones of inhibition (mm) against ATCC reference bacterial strains. 1 = *A. baylii* (ATCC33304); 2 = *E. coli* (ATCC14581); 3 = *K. pneumonia* (ATCC31488); 4 = *P. mirabilis* (ATCC21721); 5 = *P. vulgaris* (ATCC21719); 6 = *P. aeruginosa* (ATCC39324); 7 = *V. fischeri* (ATCC7744); 8 = *B. megaterium* (ATCC14581); 9 = *S. aureus* (ATCC25923). Results are expressed as mean $\pm$ SEM of at least triplicate determinations.

Figure 3: Anti-proliferative activity of *G. cambogia* fruit extracts against CaCo2 cancer cell lines measured as percentages of the untreated control cells. NC = untreated control; M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract. Results are expressed as mean percentages $\pm$ SEM of at least triplicate determinations. * indicates values that are significantly different to the untreated control ($P < 0.05$).

Figure 4: Anti-proliferative activity of *G. cambogia* fruit extracts against HeLa cancer cell lines measured as percentages of the untreated control cells. NC = untreated control; M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract. Results are expressed as mean percentages $\pm$ SEM of at least triplicate determinations. * indicates values that are significantly different to the untreated control ($P < 0.05$).

Figure 5: The lethality of *G. cambogia* fruit extracts (2000 $\mu\text{g/ml}$) and the potassium dichromate control (1000 $\mu\text{g/ml}$) 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; NC = untreated (seawater) control. Results are expressed as mean $\pm$ SEM of at least triplicate determinations.

Phytochemical studies (Table 1) show that methanol and water extracted the widest range and largest amount of phytochemicals in this study. Both extracts showed moderate to high levels of total phenolics and insoluble phenolics (water soluble phenolics were below the threshold of detection), as well as low to moderate levels of flavonoids. Similar classes of phytochemicals were detected in the ethyl acetate and chloroform extracts, although at substantially lower levels. Alkaloids were not detected in any of the extracts. None of the phytochemical classes were detected in the hexane extract.

Antibacterial activity

Aliquots (10 μl) of each extract were tested in the disc diffusion assay against panels of clinical isolated bacterial strains (Figure 1) and reference bacterial strains (Figure 2). The methanolic, aqueous and ethyl acetate extracts displayed broad spectrum inhibitory activity against the clinical bacteria strains (Figure 1). Both Gram positive and Gram negative bacteria were susceptible. The number of susceptible Gram negative

clinical isolates inhibited was higher for the aqueous extract (11 out of 14 Gram negative bacterial species tested; 79 %) than in the methanol extract (10 out of 14 Gram negative bacteria tested; 71%). Similarly, the ethyl acetate extract was also an effective at inhibiting growth of the bacterial clinical isolates, inhibiting the growth of 9 of the 14 Gram negative bacteria tested (64 %). The methanolic, aqueous and ethyl acetate extracts were also effective inhibitors of the Gram positive clinical isolates, each inhibiting 2 of the 5 Gram negative clinical isolates tested (40 %). The growth of none of the clinical isolates was inhibited by the chloroform or hexane extracts.

The extracts were similarly effective at inhibiting the growth of the reference bacterial strains (Figure 2). The methanolic and aqueous extracts inhibited the growth of all Gram positive and Gram negative reference strains tested (100 %). The ethyl acetate extract also displayed broad spectrum antibacterial activity against the reference bacterial strains, inhibiting the growth of 5 of the 7 species tested (71 %). Both Gram posi-

Table 3: The IC₅₀ (µg/ml) of CaCo2 and HeLa cells exposed to *G. cambogia* fruit extracts.

Cell Line	Solvent Extracts				
	Methanol	Water	Ethyl Acetate	Chloroform	Hexane
CaCo ₂	85.7 ± 4.4	94.2 ± 7.4	115.4 ± 7.5	190 ± 7.7	2280 ± 187
HeLa	43.1 ± 3.6	48.2 ± 4.0	67.5 ± 5.3	CND	CND

Numbers indicate the mean (± SEM) IC₅₀ values of at least triplicate determinations. CND indicates that an IC₅₀ determination was not possible as the number of cells was > 50 % of the untreated control at all doses tested.

Table 4: LC₅₀ (95 % confidence interval) for *Artemia franciscana* nauplii exposed to *G. cambogia* extracts, the reference toxin potassium dichromate and a seawater control.

Extract	LC ₅₀ (µg/ml)	
	24 hours	48 hours
Methanol	3260	1575
Water	2344	1658
Ethyl Acetate	4382	3218
Chloroform	-	-
Negative Control (seawater)	-	-
Positive Control (potassium dichromate)	198	32

-denotes values that were not obtained as ≥ 50 % mortality was not obtained at this time point at any concentration tested.

tive and Gram negative reference bacterial strains were inhibited by the ethyl acetate extract to approximately the same extent (4 out of 7 Gram negative bacterial species tested (57 %), compared to 1 of the 2 reference Gram positive bacterial species (50 %)).

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. Most of the extracts were effective at inhibiting microbial growth at low concentrations, with MIC values against the bacterial species that they inhibited often < 1000 µg/ml (< 10 µg impregnated in the disc), indicating the potent antimicrobial activity of these extracts. These MIC values compare favourably with the dosages of the pure standard ampicillin (2 µg per disc). The ethyl acetate extract was particularly potent, achieving MIC values as low as 76 µg/ml (0.8 µg per disc against *C. cloaceae*). Several MIC values below 100 µg/ml were also noted for the ethyl acetate extract against other bacterial species. The methanolic and aqueous extracts also had good antibacterial efficacies, with MIC values < 1000 µg/ml for many bacteria. The *G. cambogia* fruit extracts generally displayed lower efficacies towards Gram positive bacteria than Gram negative bacteria. Indeed, MIC values against Gram positive bacteria were generally > 1000 µg/ml (Table 2). Of the Gram positive bacterial species tested, only *S. epidermidis* was highly susceptible to the *G. cambogia* extracts, with MIC values < 1000 µg/ml for the methanolic, aqueous and ethyl acetate extracts.

Inhibition of cancer cell proliferation

G. cambogia fruit extracts were serially diluted to test their ability to inhibit CaCo₂ colorectal carcinoma cell growth (Figure 3). All extracts displayed potent inhibitory activity when screened undiluted, with cell proliferation inhibited to as low as 3.8 ± 1.2% of the untreated control cell growth for the methanolic extract. The aqueous, ethyl acetate and

chloroform extracts were very effective at inhibiting CaCo₂ proliferation (to < 10% of untreated cell proliferation). The hexane extract also significantly inhibited CaCo₂ cell proliferation, albeit to a lesser extent (to approximately 40% of the untreated cell proliferation). Inhibition of proliferation was dose dependent with the anti-proliferative activity decreasing at lower concentrations (Table 3).

The *G. cambogia* extracts were similarly effective inhibitors of HeLa cervical carcinoma cell growth (Figure 4). All extracts displayed potent inhibitory activity when screened undiluted. Indeed, the methanolic and aqueous extracts inhibited 100% of the untreated control cell growth. The ethyl acetate, chloroform and hexane extracts were also effective at inhibiting CaCo₂ proliferation (to < 65 % of untreated cell proliferation). As for the CaCo₂ screening, inhibition of HeLa proliferation was dose dependent (Table 3).

The level of anti-proliferative activity was further evaluated by determining the IC₅₀ values (Table 3) for each extract. The methanolic, aqueous and ethyl acetate extracts were particularly good inhibitors of both CaCo₂ and HeLa cell proliferation with an IC₅₀ generally < 100 µg/ml, indicating their potential for cancer therapeutic development. The HeLa cells were most susceptible, with IC₅₀ values as low as 43.1 µg/ml (against the methanolic extract).

Quantification of toxicity

G. cambogia fruit extracts were diluted to 4000 µg/ml (to give a bioassay concentration of 2000 µg/ml) in artificial seawater for toxicity testing 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 *G. cambogia* extracts were slower at inducing mortality, with ≥ 12 hours needed for mortality induction. Despite the slower onset of mortality, methanol,

water and ethyl acetate extracts all induced mortality significantly above that of the artificial seawater control (Figure 5). In contrast, the chloroform and hexane extracts were non-toxic, with mortality levels similar to those seen for the seawater control. Table 4 shows the extract and control toxin concentrations required to achieve 50% mortality (LC_{50}) at various times. As toxicity of crude plant extracts has been defined as 24 h LC_{50} values < 1000 $\mu\text{g/ml}$,^{29,31} the measured LC_{50} values indicate that all *G. cambogia* fruit pericarp extracts were non-toxic.

DISCUSSION

The current study reports on the antimicrobial and anticancer activities of various *G. cambogia* fruit extracts, and on their toxicity. Bacterial growth was inhibited in both Gram positive and Gram negative bacteria by the *G. cambogia* fruit extracts, although the Gram negative bacteria were generally more susceptible. The ability of plant extracts to inhibit the growth of both Gram positive and Gram negative bacteria has been previously reported for other plants that have a history of medicinal usage for the treatment of microbial diseases. The antiseptic properties of the *Eucalypt* spp.³² *Leptospermum* spp.^{33,34} and *Syzygium* spp.^{26,35,36} have been studied extensively and shown to inhibit the growth of a wide variety of bacteria. However, the greater susceptibility of the Gram negative bacterial species towards the *G. cambogia* fruit extracts is not worthy. This is in contrast to other previous studies which have reported a greater susceptibility of Gram positive bacteria towards solvent extracts for South American,³⁷ African,^{38,39} and Australian³⁴ plant extracts, although other examples of plants having a greater effect on Gram negative bacteria have also been reported.^{40,41}

Potent anti-proliferative activity against CaCo_2 and HeLa carcinoma cells was noted for all of the *G. cambogia* fruit pericarp extracts screened in this study, with IC_{50} values generally substantially < 200 $\mu\text{g/ml}$ for the methanol, water, ethyl acetate and chloroform extracts. Despite its wide array of traditional therapeutic uses¹², the anticancer properties of *G. cambogia* were previously unreported. However, recent studies have examined the anti-proliferative properties of the taxonomically related species *G. mangostana*^{1,42} and *G. atroviridis*⁴³ and reported similar anticancer efficacies.

Whilst the antimicrobial and anticancer components of *G. cambogia* are yet to be identified, pure xanthenes isolated from the related species *G. mangostana* show promising therapeutic properties. α -Mangostin and its derivatives have been particularly well studied and have been shown to have potent inhibitory activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus typhimurium*⁴⁴. In contrast, the same study reported only low inhibitory activity towards *Escherichia coli*, *Klebsiella* spp and *Proteus* spp. More recent studies have reported *Mycobacterium tuberculosis*⁴⁵, *S. aureus* (including MRSA)⁴⁶, *Propionibacterium acnes* and *S. epidermidis*⁴⁷ inhibitory activities of mangosteen xanthenes.

Numerous studies have also documented the anticancer effects of xanthenes isolated from *G. mangostana* fruit pericarp. Several xanthenes including α , β and γ mangostin, mangostinone, 2-isoprenyl-1,7-dihydroxy-3-methoxy xanthone and garcinone E exhibit anti-proliferative^[48] and apoptotic properties⁴⁹ against human leukemia cell line HL60. Garcinone E has also been reported to have potent cytotoxic effects on HCC36, TONG, HA22T, Hep3B, HEpG2 and SK-Hep-1 hepatocarcinoma cell lines⁵⁰. The same study demonstrated that garcinone E was also effective against NCI-Hut 125, CH27 LC-1, H2891 and Calu-1 lung carcinoma cells, as well as against AZ521, NUGC-3, KATO-III and AGS gastric carcinoma cell lines. Whilst it has yet to be established whether similar compounds are present in the *G. cambogia* fruit extracts, if they are subsequently detected, they may be responsible (at least in part) for the antibacterial and anticancer properties reported here.

The results of this study indicate that the *G. cambogia* fruit extracts examined in this report are worthy of further study due to their antibacterial activity and ability to block cancer cell proliferation. Furthermore, as extracts with LC_{50} values greater than 1000 $\mu\text{g/ml}$ in the *Artemia nauplii* bioassay have been defined as being non-toxic,^{29,31} all the *G. cambogia* fruit extracts were determined to be nontoxic. Whilst the results of this study are encouraging, further studies to purify and identify the bioactive components are needed. Furthermore, studies are also required to examine the mechanisms of action of these agents. Whilst the extracts examined in this report have potential as antimicrobial and anticancer agents, caution is needed before these compounds can be applied to medicinal purposes. In particular, further toxicity studies using human cell lines are needed to determine the suitability of these extracts for these purposes.

CONCLUSION

The results of this study partially validate the traditional usage of *G. cambogia* fruit pericarp extracts in multiple traditional medicinal systems to treat bacterial diseases and cancer, indicating that they are worthy of further study. Bioactivity-driven purifications of the active components and examination of the mechanisms of action of these agents is required.

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CONFLICTS OF INTEREST:

The authors report no conflicts of interest.

ABBREVIATIONS:

DMSO: Dimethyl sulfoxide, **IC_{50} :** The concentration required to inhibit by 50 %, **LC_{50} :** The concentration required to achieve 50 % mortality, **MIC:** minimum inhibitory concentration.

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