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Original Article

Therapeutic Potential of *Arctium lappa* L. Root Extracts to Inhibit Gastrointestinal Bacterial Pathogens and Treat Gastrointestinal Disease

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ABSTRACT

Introduction: An increase in antibiotic resistance and a corresponding decrease in antimicrobial discovery have directed researchers towards alternative therapies, including plant based medicines. However, synergistic combinations of plant extracts with conventional antibiotics may be a far more effective approach in overcoming resistance and potentiating the activity of antibiotics that are otherwise ineffective against resistant bacterial strains. **Materials and Methods:** The antibacterial activity of *Arctium lappa* L. root extracts was investigated by disc diffusion and quantified by liquid dilution and solid phase MIC assays. The extracts were also combined with a range of conventional antibiotics and tested against various microbial triggers of autoimmune diseases. The ΣFIC values obtained from these assays were used to determine the class of combinational effects. Toxicity was evaluated by *Artemia* nauplii mortality and HDF cytotoxicity assays. **Results:** Methanolic and ethyl acetate *A. lappa* root extracts showed good inhibitory activity against several gastrointestinal bacterial pathogens. They were particularly good inhibitors of *S. sonneii* and *B. cereus*, with MIC values in the range 150-250µg/mL. The aqueous extract was also a noteworthy inhibitor of *B. cereus* growth. Of further interest, some combinations of the *A. lappa* root extracts and conventional antibiotics potentiated bacterial growth inhibition compared to the individual components. Four synergistic and five additive interactions were noted. Notably, no antagonistic interactions were evident, indicating that all combinations could be used without decreasing the antibacterial activity of the components. All extracts were non-toxic in the ALA and HDF assays. **Conclusion:** *Arctium lappa* root extracts have potential as inhibitors of bacterial gastrointestinal pathogens. Furthermore, extract components may also potentiate the activity of some antibiotics that are relatively ineffective alone. Isolation and identification of these compounds may be beneficial in drug design against several gastrointestinal bacterial pathogens.

Keywords: Burdock root, Synergy, Conventional antimicrobials, Interaction, Medicinal plants, Diarrhoea, Gastrointestinal pathogens, Drug combinations.

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INTRODUCTION

The World Health Organization (WHO) has estimated that nearly nine million children under the age of five die every year as a result of diarrhoea.[1] Despite that report being more than a decade old, very little has changed in the interim and diarrhoea remains the leading killer of children globally, accounting for approximately 9% of all deaths among children under the age of five.[2] This translates into more than 1400 young children dying each day, or about 530,000 children a year. To exacerbate this problem, many bacteria have developed resistance to conventional antibiotics, rendering them of little use against some diarrhoea causing pathogens.[3] There is an urgent need to develop new treatment options to combat these diseases through the development of novel drugs.

Plants have long been used in traditional healing systems to treat diarrhoea. These traditional medicines may be given as single component therapies or they may be prescribed in combination to target the multiple negative effects of diarrhoea (loose stools, cramps, loss of electrolytes and fever). The activity of several herbal preparations used traditionally to treat diarrhoea and other gastrointestinal diseases have already been validated by rigorous

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scientific evaluation. This is particularly true for plant medicines used in traditional Indian healing systems (including Ayuverda, Siddha, Unani)[4-6] and in Traditional Chinese Medicine (TCM).[7,8] In contrast, the potential of many other medicinal plants globally for alleviating the symptoms of diarrhoea and inhibiting the pathogenic causes has been relatively neglected.

Arctium lappa L. (family Asteraceae; commonly known as burdock, greater burdock, lappa, thorny burr, beggar's buttons) is a medicinal plant that is native to temperate regions of Europe and Asia, although it has been widely naturalised and is now common globally. *Arctium lappa* roots have been used for hundreds of years as traditional medicines by multiple European, Asian and North American cultures[9] for a variety of purposes including to improve the immune system and to enhance metabolism,[10] as well as for its anti-inflammatory,[11-14] anti-cancer,[15,16] and anti-biabetic properties.[17,18] Many of these illnesses are caused by bacterial pathogens and several studies have reported that *A. lappa* leaf extracts inhibit the growth of *Bacillus subtilis*, *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*.[19] However, MIC values were not determined in that study so it is not possible to compare the activity with other studies. Notably, whilst the roots are generally used medicinally, they have been relatively neglected in antibacterial studies. Instead the leaf is most frequently examined. A recent study from our group reported that *A. lappa* root extracts had noteworthy anti-bacterial activity against several microbial triggers of autoimmune inflammatory diseases, including *P. mirabilis, P. vulgaris* and *A. baylyi*, with the methanolic and ethyl acetate extracts having particularly good activity.[20] This study was undertaken to expand on the earlier antibacterial studies and investigate the antimicrobial effects of *A. lappa* root extracts and their ability to potentiate the growth inhibitory properties of conventional antibiotics against some bacterial pathogens that cause diarrhoea and gastrointestinal disease.

MATERIALS AND METHODS

Plant source and extraction

Arctium lappa L. root material was obtained from Noodles Herbal Emporium, Australia and a voucher specimen (GU2017aALR1) was deposited in the School of Environment and Science, Griffith University, Australia. Individual 1g masses of the ground plant material were weighed into separate 50mL Falcon tubes and 50mL of methanol, deionised water, ethyl acetate, chloroform or hexane were individually added. All solvents were obtained from Ajax Fine Chemicals, Australia and were AR grade. The ground plant materials were extracted in each solvent for 24 hr at 4°C with gentle shaking. The extracts were filtered through Whatman No. 54 filter paper under vacuum and the solvent extracts were air dried at room temperature in the shade. The aqueous extracts were lyophilised by freeze drying at -50°C. The resultant dried extracts were weighed to determine the extraction yield and then dissolved in 10mL deionised water (containing 1% DMSO).

Qualitative phytochemical studies

Phytochemical analysis of the *A. lappa* extracts for the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, phytosterols, saponins, tannins and triterpenoids was achieved as previously described.[21,22]

Antibacterial screening

Conventional antibiotics

Penicillin-G (1440-1680µg/mg), chloramphenicol (≥98% purity), erythromycin (≥850µg/mg), and tetracycline (≥95% purity) were purchased from Sigma-Aldrich, Australia and used for the microplate liquid dilution assay. All antibiotics were prepared in sterile deionised water at stock concentrations of 0.01mg/mL and stored at 4°C until use. For the disc diffusion studies, ampicillin (10µg) and chloramphenicol (10µg) standard discs were obtained from Oxoid Ltd., Australia and used as positive controls.

Bacterial cultures

All bacterial strains were selected based on their ability to cause diarrhoea and gastrointestinal disease.[23-25] Reference strains of *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC O157 H7), *Shigella sonnei* (ATCC 25931), *Staphylococcus aureus* (ATCC 157293) were obtained from the American Type Culture Collection (ATCC), USA. A clinical strain of *Salmonella newport* was obtained from the School of Environment and Science teaching laboratory at Griffith University. All bacteria were cultured in nutrient broth (Oxoid Ltd., Australia). Streak nutrient agar (Oxoid Ltd., Australia) plates were tested in parallel to ensure the purity of all bacterial cultures, and for sub-culturing. All bacterial cultures were incubated at 37°C for 24 hr and were subcultured and maintained in nutrient broth at 4°C until use.

Evaluation of antibacterial activity

Antibacterial activity screening of the *A. lappa* root extracts was assessed using a modified disc diffusion assay.[26,27] Ampicillin (10µg) and chloramphenicol discs (10µg) were obtained from Oxoid Ltd., Australia and used as positive controls to compare antibacterial activity. Filter discs infused with 10µL of distilled water (containing 1% DMSO) were used as a negative control.

Minimum Inhibitory Concentration (MIC) determination

The minimum inhibitory concentration for each extract was determined using two methods. A liquid dilution MIC assay was employed as it is generally considered the most sensitive bacterial growth inhibitory assay.[28] Furthermore, as microplate liquid dilution MIC assays are perhaps the most commonly used method of quantifying bacterial growth inhibition efficacy, use of this method facilitates comparisons with other studies. A solid

phase agar disc diffusion assay was also used in this study for comparison and because it more accurately represents the growth patterns of the bacteria on solid surfaces.

Microplate liquid dilution MIC assay

The MICs of the extracts were evaluated by standard methods.[29-31] All plates were incubated at 37°C for 24 hr. p-Iodonitrotetrazolium Violet (INT) was obtained from Sigma-Aldrich, Australia and dissolved in sterile deionised water to prepare a 0.2mg/mL INT solution. A 40µL volume of this solution was added into all wells and the plates were incubated for a further 6 hr at 37°C. Following incubation, the MIC was visually determined as the lowest dose at which colour development was inhibited.

Disc diffusion MIC assay

The Minimum Inhibitory Concentrations (MIC) of the extracts was also evaluated by disc diffusion assay as previously described.[32,33] Graphs of the zone of inhibition versus Ln concentration were plotted and MIC values were achieved using linear regression.

Sum of Fractional Inhibitory Concentration (ΣFIC) assessment

Interactions between the *A. lappa* root extracts and the conventional antibiotics were examined by determination of the sum of Fractional Inhibitory Concentrations (ΣFIC) for each combination.[28] The FIC values for each component (a and b) were calculated using the following equations where a represents the plant extract sample and b represents the conventional antibiotic:

$$FIC(a) = \left(\frac{MIC[a \text{ in combination with b}]}{MIC[a \text{ independently}]} \right)$$

$$FIC(b) = \left(\frac{MIC[b \text{ in combination with a}]}{MIC[b \text{ independently}]} \right)$$

The ΣFIC was then calculated using the formula ΣFIC = FIC(a) + FIC(b). The interactions were classified as synergistic (ΣFIC ≤0.5), additive (ΣFIC >0.5-1.0), indifferent (ΣFIC >1.0-4.0) or antagonistic (ΣFIC >4.0).[28]

Toxicity screening

Two assays were used to assess the toxicity of the individual samples. The *Artemia* nauplii Lethality Assay (ALA) was utilised for rapid preliminary toxicity screening, whereas the MTS cellular proliferation assay was used to determine a cellular evaluation of toxicity.

Artemia franciscana Kellogg nauplii toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared in deionised water (4mg/mL) and serially diluted in artificial seawater as a reference toxin. Toxicity of the *A. lappa* extracts, reference toxin and conventional antibiotics was assessed using a modified *Artemia franciscana* nauplii lethality assay.[34,35] The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.

Cellular viability assay

All extracts and conventional antibiotics were screened for toxicity towards normal human primary dermal fibroblasts (HDF; ATCC PCS-201-012).[36,37] The HDF cells were cultured and screened in Dulbecco's Modified Eagle Medium (DMEM; ThermoFisher Scientific, Australia), supplemented with 10% foetal calf serum (Life Technologies), 50µg/mL streptomycin (Sigma-Aldrich, Australia) and 50 IU/mL penicillin (Sigm-Aldricha, Australia). All extracts were screened at 200µg/mL with incubation at 37°C and 5% CO_2 in a humidified atmosphere following standard protocols.[25] Following the incubation, 20µL of Cell Titre 96 Aqueous One solution (Promega) was added to each well and the plates were incubated for a further 3 hr. Absorbances were recorded at a test wavelength of 540nm and a blank wavelength of 690nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed three time, each with internal triplicates (n=9). Triplicate controls were included on each plate. The % cellular viability of each test was calculated using the following formula:

$$\% \text{ cellular viability} = \frac{\text{Abs test sample} - (\text{mean Abs control} - \text{mean Abs blank})}{(\text{mean Abs control} - \text{mean Abs blank})}$$

Cellular viability ≤50% of the untreated control indicated toxicity, whereas extracts or controls with >50% untreated control viability were deemed to be non-toxic.

Statistical analysis

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

RESULTS

Liquid extraction yields ranged from 23mg (*A. lappa* ethyl acetate root extract) to 469mg (aqueous *A. lappa* root extracts) (Table 1). Qualitative phytochemical screening (Table 1) showed that the higher polarity solvents (methanol and water) extracted the greatest mass and widest diversity of phytochemical classes.

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the *A. lappa* root extracts.

Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/mL)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
M	257	25.7	+++	+++	+++	-	-	-	-	-	-	+++	++	-	-
W	181	18.1	+++	+++	++	-	+	-	-	-	-	+++	++	-	-
E	17	1.7	+	+	+	-	-	-	-	-	-	+	-	-	-
C	103	10.3	-	-	+	-	-	-	-	-	-	-	-	-	-
H	28	2.8	-	-	+	-	-	-	-	-	-	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. W = aqueous extract; M = methanolic extract; C = chloroform extract; H = hexane extract; E = ethyl acetate extract.

Antibacterial activity

Aliquots (10 µL) of each extract were tested in the disc diffusion assay against some bacterial pathogens that cause diarrhoea and gastrointestinal disease (Figure 1). The mid to high polarity extracts were generally substantially better bacterial growth inhibitors than the lower polarity extracts. Indeed, the methanolic and aqueous *A. lappa* root extracts possessed broad-spectrum inhibitory activity, inhibiting the growth of all of the bacterial species tested. The ethyl acetate, chloroform and hexane extracts each also inhibited the growth of one or two bacteria. *Shigella sonneii* was particularly susceptible to the *A. lappa* root extracts, with ZOIs of 12.4, 9.5 and 10.2mm for the methanolic, aqueous and ethyl acetate extracts respectively. *Bacillus cereus* was similarly susceptible to the methanolic, aqueous and ethyl acetate *A. lappa* root extracts, with ZOIs of 10.8, 9.3 and 8 mm respectively. The methanolic *A. lappa* root extract also displayed noteworthy inhibition of *S. newport* (ZOIs of 8.2mm). Substantially smaller ZOIs indicative of only moderate to low antibacterial activity were noted for the methanolic and aqueous extracts against *E. coli* and *S. aureus*. All of the bacterial pathogens tested in our study were susceptible to the ampicillin and chloramphenicol controls. However, the *A. lappa* root extracts were screened at relatively low levels in our study. For example, as 10µL of the ethyl acetate extract (1.7µg/mL) was infused into the test discs, this extract was tested at 17µg/disc. The control antibiotics tested in this study were pure compounds and were screened at relatively high doses (10µg/disc). In contrast, the extracts contained crude mixtures of compounds and the active components would be expected to

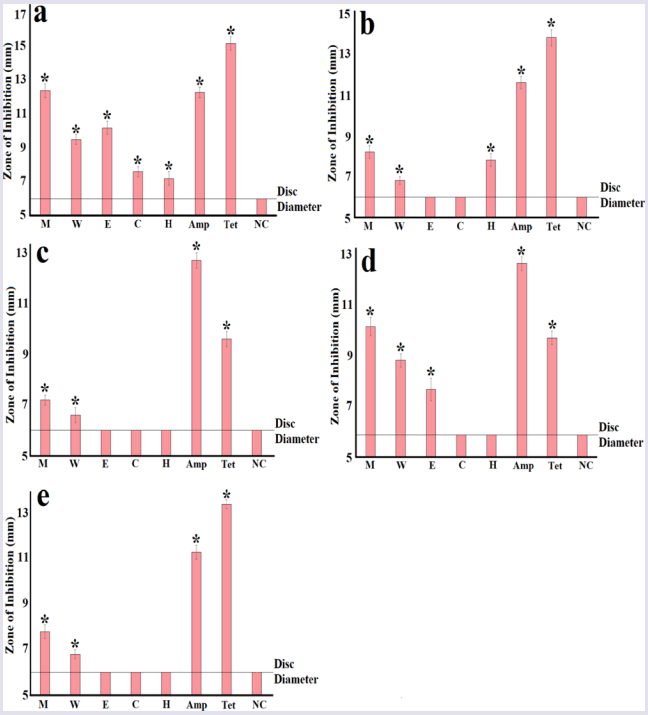

Figure 1: Antibacterial activity of *A. lappa* root extracts against (a) *S. sonneii* (ATCC 25931); (b) *S. newport* (clinical isolate); (c) *E. coli* (ATCC O157 H7); (d) *B. cereus* (ATCC 14579); and (e) *S. aureus* (ATCC 157293), measured as zones of inhibition (mm). M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform; H = hexane extract; Amp = ampicillin (10µg); Tet = tetracycline (10µg); NC = negative control (nutrient broth). Results are expressed as mean zones of inhibition of at least six replicates ± SEM; * indicates results that are significantly different to the negative control (p<0.01).

Table 2: Disc diffusion (DD) and liquid dilution (LD) MIC values (µg/mL) for the *A. lappa* root extracts against some bacterial pathogens.

Extract	*S. sonneii*		*S. newport*		*E. coli*		*B. cereus*		*S.aureus*	
	DD MIC	LD MIC	DD MIC	LD MIC	DD MIC	LD MIC	DD MIC	LD MIC	DD MIC	LD MIC
M	286	156	746	526	>5000	>5000	258	174	3266	2947
W	1450	1186	>5000	>5000	>5000	>5000	525	470	>5000	>5000
E	218	183	-	-	-	-	386	256	-	-
C	2455	1800	-	-	-	-	-	-	-	-
H	1680	1218	878	655	-	-	-	-	-	-
Controls										
Penicillin-G	ND	-	ND	-	ND	-	ND	-	ND	-
Erythromycin	ND	-	ND	-	ND	-	ND	-	ND	-
Tetracycline	ND	1.25	ND	-	ND	2.5	ND	2.5	ND	1.25
Chloramphenicol	ND	2.5	ND	0.33	ND	-	ND	2.5	ND	2.5

M = methanol extract; W = water extract; E = ethyl acetate extract; C = chloroform extract; H = hexane; DD = disc diffusion; LD = liquid dilution; - indicates no inhibition at any dose tested.

contribute a relatively low % of the molecules in those mixtures. Therefore, the ZOIs measured in our study indicate that the mid to high polarity *A. lappa* root extracts may have potential in the treatment of treating gastrointestinal bacterial infections and the potency of the extracts should be further quantified.

Quantification of Minimum Inhibitory Concentration (MIC)

The relative antimicrobial strength of the extracts was further evaluated by determining the MIC values using two methods: the liquid dilution MIC assay and the disc diffusion MIC assay (Table 2). Consistent with the antibacterial screening assays, the mid to higher polarity methanol, aqueous and ethyl acetate *A. lappa* root extracts were the most effective at inhibiting the growth of the bacterial pathogens. The MIC values of the conventional antibiotic controls were only determined for the liquid dilution assay. Pre-prepared commercial susceptibility discs containing set amounts of antibiotics loaded were used for the disc diffusion assay and thus the zones of only single doses were recorded. Chloramphenicol was the most versatile antibiotic as it inhibited all bacteria tested except *E. coli*. Notably, the *E. coli* strain used in these studies was completely resistant to all other antibiotics. Notably, all bacteria were completely resistant to penicillin and erythromycin. Additionally, the *S. newport* strain tested in our study was completely resistant to all antibiotics except tetracycline. However, even where MICs were determined for the conventional antibiotics against the bacterial pathogens, nearly all MICs were substantially >1µg/mL. As MIC values >1µg/mL for pure antibiotics indicates resistance in this assay,[32,38-40] these bacteria were considered resistant to those conventional antibiotics. The only exception was the inhibition of *S. newport* by chloramphenicol (MIC = 0.33µg/mL), which indicates that that bacterium is highly susceptible to that antibiotic.

The MIC values determined for the *A. lappa* root extracts compare relatively well between the disc diffusion and liquid dilution assays. All bacterial species were susceptible to the methanolic and ethyl acetate extracts, although the inhibition was only noteworthy (<1000µg/mL) against the *S. sonneii, S. newport* (for the methanolic extract only) and *B. cereus*. The *B. cereus* strain tested in our study was also particularly susceptible to the aqueous extract, with an MIC value of 470µg/mL. Therefore, the *A. lappa* root extracts (particularly the methanolic and ethyl acetate extract) may be useful in the prevention and treatment of bacterial-induced diarrhoea and gastrointestinal disease.

Fractional Inhibitory Concentration (FIC) assessment

Combinations of the *A. lappa* root extracts with conventional antibiotics against the bacterial pathogens were tested to determine the classes of interactions for these combinations (Table 3). ΣFIC values could not be determined for many of the combinations as one or both of the components in the combination were ineffective against the tested bacterium when tested alone. Of the 25 effective combinations, the majority (16) were non-interactive (~ 64%). Whilst these combinations have no additional benefit over the individual monotherapies, the lack of antagonism indicates that taking these therapies in combination would not have detrimental effects. This is important information as allopathic and complementary therapies are often taken concurrently. Four synergistic combinations were also noted (two each against *S. sonneii* and *B. cereus*). Interestingly, all of these synergistic combinations contained tetracycline as the antibiotic component in combination with either the methanolic or ethyl acetate extracts. Therefore, it is likely that mid polarity

Table 3: ΣFIC values for the *A. lappa* root extracts and conventional antibiotic combinations against susceptible bacteria.

Bacteria	Extract	Penicillin-G	Chloramphenicol	Erythromycin	Tetracycline
S. sonneii	**M**	-	*0.63*	-	**0.44**
			(ADD)		**(SYN)**
	W	-	1.38	-	1.27
			(IND)		(IND)
	E	-	*0.58*	-	**0.48**
			(ADD)		**(SYN)**
	C	-	1.75	-	1.94
			(IND)		(IND)
	H	-	1.5	-	1.33
			(IND)		(IND)
S. newport	**M**	-	1.06	-	-
			(IND)		
	W	-	3.37	-	-
			(IND)		
	H	-	1.14	-	-
			(IND)		
E. coli	**M**	-	-	-	2.34
					(IND)
	W	-	-	-	3.15
					(IND)
B. cereus	**M**	-	*0.62*	-	**0.39**
			(ADD)		**(SYN)**
	W	-	1.39	-	*0.76*
			(IND)		*(ADD)*
	E	-	*0.87*	-	**0.48**
			(ADD)		**(SYN)**
S. aureus	**M**	-	2.44	-	2.13
			(IND)		(ADD)
	W	-	1.82	-	3.21
			(IND)		(IND)

M = methanolic extract; W = aqueous extract ; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; **SYN = synergistic interaction**; *ADD = additive interaction*; IND = indifferent interaction; - = a ΣFIC could not be determined as at least one component of the combination was inactive.

component(s) potentiate the activity of tetracycline. Furthermore, an additional five combinations produced additive effects against *S. sonneii* or *B. cereus*. Nearly all of these combinations contained chloramphenicol as the antibiotic component in combination with either the methanolic or ethyl acetate extract, whilst one combination contained tetracycline and the aqueous extract (against *B. cereus*). As these combinations have enhanced effects compared to either component alone, they would be beneficial for the treatment of diarrhoea and gastrointestinal bacterial infections. Notably, none of the combinations produced antagonistic effects. Therefore, all combinations are safe to use without decreasing the activity of either component.

Quantification of toxicity

All extracts were initially screened at 1000µg/mL in the *Artemia* nauplii lethality bioassay as LC_{50} values >1000µg/mL have previously been defined as non-toxic.[24,35] Potassium dichromate was also included in the bioassay at 1000µg/mL as a positive control (Table 4). Potassium dichromate was rapid in its induction of mortality, with significant mortality noted by 4 hr of exposure (unpublished results). No LC_{50} values were determined for the ethyl acetate, chloroform or hexane extracts as <50% mortality was seen in all tested concentrations (Table 4). In contrast, LC_{50} values of 1656 and 1458µg/mL (i.e. substantially >1000µg/mL) were

Table 4: LC_{50} values determined for *A. lappa* root extracts in the *Artemia* nauplii and HDF bioassays following 24 hr exposure.

Extract	LC_{50} value (µg/mL)	
	ALA	HDF assay
M	1656	-
W	1458	-
E	-	-
C	-	-
H	-	-
PC	56	NT

- indicates that less than 50% mortality was induced by the extract at all concentrations tested. ALA = *Artemia* nauplii toxicity assay; HDF = human dermal fibroblast toxicity assay; M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; NT = Not tested.

determined for the methanolic and aqueous extracts respectively. Furthermore, all plant extracts demonstrated a lack of toxicity towards normal human primary dermal fibroblasts, with cellular viability for all tests substantially >50% of the untreated control. All extracts were therefore deemed to be non-toxic.

DISCUSSION

Recent increases in the rates of microbial resistance to clinically used antibiotics has rendered many frontline treatments to be ineffective against pathogenic diseases. This is particularly true for diarrhoea-causing pathogens. The gastrointestinal system is an ideal environment, not only for microbial growth, but also for the exchange of genetic information between different microbial strains, and even between different species. When a pathogen in this environment possesses antibiotic resistance genes, it can readily exchange those genes with other microbes and individual pathogens can accumulate resistance to multiple conventional antibiotics. There is an urgent need to develop new antibiotic therapies to treat diseases caused by these pathogens. For reasons reviewed elsewhere,[3] the previous methods of antibiotic discovery are unlikely to yield many new antibiotics in the future and medical science must explore new methods to treat pathogenic diseases. A re-examination of traditional medicine is an attractive option as many traditional medicines have been used effectively for hundreds or even thousands of years. Furthermore, this use has often been well documented, allowing for selection of traditional therapies for screening. Indeed, there has been a significant increase in published studies into traditional herbal therapies to treat pathogenic diseases in most regions of the world, although some notable species are yet to be screened against bacterial pathogens important to human health.

Arctium lappa root extracts were selected for screening in our study as this plant has a long history of medicinal use to treat diseases caused by bacterial pathogens, and previous studies have reported antibacterial activity against other bacterial pathogens.[19,20] The extracts were screened against a panel of bacterial pathogens selected as they are all associated with diarrhoea and gastrointestinal disease. *Escherichia coli* is a common trigger of diarrhoea, particularly in children.[41] *Staphylococcus* spp. (including *S. aureus*) are common causes of antibiotic-associated nosocomial diarrhoea.[42] *Bacillus* spp. (including *B. cereus*) release diarrheagenic toxins in food poisoning cases.[43] Similarly, *Shigella sonnei* can cause shigellosis,[44] whilst other food-borne strains including *Salmonella* spp. inhabit the lower gut and cause acute diarrhoea.[45]

The mid to high polarity *A. lappa* root extracts were effective at inhibiting the growth of several gastrointestinal bacterial pathogens at relatively low concentrations, with liquid dilution MIC values against the bacterial species that they inhibited generally substantially <1000µg/mL, indicating the noteworthy antimicrobial activity of these preparations. Interestingly, the extracts were effective against both Gram-positive and Gram-negative bacteria. 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 *Eucalyptus* spp.,[46] *Leptospermum* spp.[47] and *Syzygium* spp.[48,49] have been studied extensively and shown to inhibit the growth of a wide variety of bacteria. However, the susceptibility of the Gram-negative bacterial species (*S. sonneii, S. newport* and *E. coli*) towards the plant extracts is noteworthy. This is in contrast to other previous studies which have reported a greater susceptibility of Gram-positive bacteria towards solvent extracts for South American,[50] African[51] and Australian plant extracts.[46-48] The methanolic and ethyl acetate extracts were particularly good inhibitors of *S. sonneii* and *B. cereus*, with MIC values 150-250 µg/mL.

The combinational studies combining the *A. lappa* root extracts with conventional antibiotics highlighted several useful combinations with enhanced antibacterial activity compared with the inhibitory activity of either the extract or antibiotic components alone. Indeed, four synergistic and five additive interactions were noted, with all of these being against *S. sonneii* or *B. cereus*. Notably, all of these potentiating combinations contained either tetracycline or chloramphenicol as the antibiotic component, generally in combination with either the methanolic or ethyl acetate *A. lappa* root extract. The implications of these potentiating combinations include enhanced efficacy, the requirement for lower dose administration and a reduction in side effects, as well as possibly reduced antimicrobial resistance.[3] Importantly, none of the combinations produced antagonistic effects. This is an important finding as it indicates that it is safe to use the *A. lappa* root extracts and conventional antibiotics in combination without decreasing the efficacy of either component. This is an important finding as many users of herbal

and traditional medicines self-diagnose/treat, often with multiple therapies concurrently. Thus, an understanding of drug-herbal medicine interactions is important. These results indicate that all of the combinations tested are safe to use without decreasing the efficacy of either component.

Microbes have developed numerous resistance mechanisms to avoid the effects of antibiotics. One main method is through the use of Multi-Drug Resistant (MDR) efflux pumps that are encoded chromosomally and are used to rapidly remove antibiotics that have entered the bacterial cells, thus rendering them resistant to the effects of the antibiotic.[3] A single pump may allow the bacteria to escape several types of antimicrobials. When these efflux pumps are inhibited, the intracellular concentration of antibiotic will increase, allowing the treatment to once again be effective. Interestingly, many plants possess Multi-Drug Resistance (MDR) pump inhibitors in order to enhance the activity of their own natural antimicrobial compounds. Such MDR pump inhibitors become effective tools when used in combination with some previously ineffective/resistance prone antibiotic compounds and several examples have previously been reported.[3] Isoflavones isolated from *Lupinus argenteus* Pursh potentiate the activity of the natural plant antibiotic berberine as well as the synthetic fluoroquinoline antibiotic, norfloxacin as inhibitors of *S. aureus* growth.[52] That study reported that the isoflavone allows a greater concentration of berberine to occur inside the bacteria by inhibiting the efflux mechanism (MDR pump). Similarly, *Mezoneuron benthamianum* Baill. and *Securinega virosa* (Roxb. Ex Willd) Baill. extracts act as efflux pump inhibitors for fluoroquinolone, tetracycline and erythromycin in resistant strains of *S. aureus* (MRSA).[53] As a consequence, the *M. benthamianum* ethanol extract and chloroform extract of *S. virosa* reduce the MIC (Minimum Inhibitory Concentration) of norfloxacin against *S. aureus* by a factor of 4.

In our study, all bacterial species were resistant to penicillin-G, chloramphenicol, erythromycin and tetracycline, with only low susceptibility or complete resistance to each antibiotic. All of these antibiotics are susceptible to resistance due to efflux pumps.[53,54] A single pump can provide bacteria with resistance to a wide array of chemically and structurally diverse antibiotics and it is not uncommon for an organism to code for more than one efflux pump.[53,54] It is therefore imperative to identify agents that can block the efflux mechanism (Efflux Pump Inhibitors - EPIs) or alter the process of efflux, and in so doing, extend the life of existing antibacterial drugs. Plants produce various secondary metabolites that are used as defense mechanisms against pathogenic invaders. Some plants produce antimicrobials which, along with other compounds, inhibit the efflux of those antimicrobials from a bacterial cell. There are currently no EPI/antimicrobial drug combinations on the market, although research into identifying potential EPIs is ongoing.[53] The synergistic and additive interactions noted in our study suggest the possibility of a common EPI in the *A. lappa* root extracts that could be inhibiting a MDR efflux pump in these bacteria.

Identification of the specific components responsible for the antimicrobial activity reported in of *A. lappa* root extracts tested herein was beyond the scope of our study, although the mid to high polarity extracts were abundant in phenolics, flavonoids and tannins. Many studies have reported potent growth inhibitory activities for a wide variety of flavonoids against extensive bacterial panels.[55] Similarly, several tannin compounds have bacterial growth inhibitory activity. Gallotannins have been reported to inhibit the growth of a broad-spectrum of bacterial species[56] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins,[56,57] and by inhibiting glucosyltransferase enzymes.[58] Elligitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 63 µg/mL.[55,57] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[55,57] Thus, it is likely that multiple compounds within the tested decoctions contribute to the antimicrobial properties of these extracts.

The findings reported here also show that none of the *A. lappa* root extracts displayed significant toxicity towards *A. franciscana* or HDFs. Whilst this indicates that these decoctions are safe to use therapeutically, further toxicity studies using other human cell lines are needed to further evaluate the suitability of the decoctions for medicinal purposes. The results of this study indicate that the *A. lappa* root extracts screened in this report are worthy of further study due to their anti-pathogenic activities.

CONCLUSION

The results of this study demonstrate the potential of the *A. lappa* root extracts in inhibiting the growth of some bacterial gastrointestinal pathogens. Furthermore, extract components may also potentiate the activity of antibiotics that are otherwise relatively ineffective against those bacteria. Therefore, a combinational approach not only increases the effectiveness of these antibiotics, but may also potentially reduce the side effects and reduce the development of drug resistant pathogens. Isolation of the bioactive and potentiating compounds may be beneficial in drug design against several bacterial gastrointestinal pathogens.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALA: *Artemia* lethality assay; **DMSO:** Dimethyl sulfoxide; **EPI:** Efflux pump inhibitor; **FIC:** Fractional inhibitory concentration; **HDF:** Human dermal fibroblasts; **LC_{50}:** The concentration required to achieve 50 % mortality; **MIC:** Minimum inhibitory concentration; **MDR:** Multi-drug resistant; **ZOI:** Zone of inhibition.

SUMMARY

- *Arctium lappa* root extracts were screened for the ability to block the growth of a panel of bacteria that cause gastrointestinal disease..
- The antibacterial activity was quantified by determining the MIC values of each extract.
- The extracts were also tested in combination with conventional antibiotics and the class of interaction was determined
- Toxicity of *A. lappa* root extracts was determined using the *Artemia* nauplii and HDF cell

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