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Original Article

Investigating the Pharmacognostic Potential of Indian *Terminalia* Spp. in the Treatment and Prevention of Yersiniosis

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ABSTRACT

Introduction: *Yersinia enterocolitica* is a major cause of food poisoning through contaminated meat products, causing the acute gastrointestinal disease yersiniosis. Many *Terminalia* spp. have documented therapeutic properties as general antiseptics, inhibiting the growth of a wide variety of bacterial species. Despite this, Indian *Terminalia* spp. extracts have not been tested for the ability to inhibit the growth of *Y. enterocolitica*. **Methods:** *T. arjuna, T. catappa* and *T. chebula* extracts were extracted by maceration and the extracts were investigated by disc diffusion assay for growth inhibitory activity against a clinical strain of *Y. enterocolitica*. The MIC values of the extracts were determined to quantify and compare their efficacies. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *T. chebula* fruit extracts displayed potent growth inhibitory activity in the disc diffusion assay against *Y. enterocolitica*. The methanolic and ethyl acetate *T. chebula* fruit extracts were particularly potent growth inhibitors, with MIC values of 85 and 64 µg/mL respectively. The aqueous fruit extract also displayed good growth inhibitory activity against *Y. enterocolitica*, albeit with a higher MIC value (653 **µg/mL).** The *T. arjuna* branch extract was moderately active (3000 µg/mL). All other extracts were either low efficacy, or completely devoid of growth inhibitory activity. All Indian *Terminalia* spp. extracts were nontoxic (LC_{50} values <1000 **µg/mL)** in the *Artemia franciscana* bioassay. **Conclusions:** The lack of toxicity and the potent growth inhibitory bioactivity of the T. chebula extracts against *Y. enterocolitica* indicates their potential as medicinal agents in the treatment and prevention of yersiniosis.

Keywords: *Terminalia arjuna, Terminalia catappa, Terminalia chebula, Yersinia enterocolitica*, Enterobacteriaceae, antibacterial activity, food poisoning, Ayurveda.

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INTRODUCTION

The genus *Yersinia* (*Y.*) contains more than a dozen validly described species[1] of facultative anaerobes widespread within the environment. Members of the genus are also widely-associated with animal populations. Many of these bacteria are clinically significant as they can be pathogenic to humans. The zoonotic nature of these bacteria poses a unique set of challenges in the treatment and prevention of associated disease as they can persist in non-human hosts until they opportunistically infect susceptible people. Diseases caused by *Yersinia* spp. vary in severity and mortality, ranging from the highly devastating bubonic and pneumonic plagues (*Yersinia pestis*) to the gastrointestinal-distressing yersiniosis (*Yersinia enterocolitica*).[2]

Yersinosis is an acute gastrointestinal infection responsible for over 100,000 cases of illness annually in the United States.[3] Typically characterized by combinations of abdominal pain, fever or diarrhoea, infection commonly originates from the ingestion of contaminated food or water. Indeed, approximately 90% of all reported cases originate from food/water sources.[4] Prevention is further complicated as *Y. enterocolitica* can grow at 4 °C and thus refrigeration does not provide sufficient protection from illness.[5] Though rarely life-threatening, the economic drain caused through temporary incapacitation from yersiniosis means that the probing for effective treatment strategies is of particular importance. One such strategy involves utilizing natural resources such as plants with documented antibacterial capabilities. Therefore, these relatively untapped reservoirs may provide novel treatment options for yersiniosis.

Terminalia is one of the most useful genera of therapeutic plants globally. The genus consists of approximately 200-250 species of flowering trees, many of which have uses in several traditional medicinal systems.[6] Whilst numerous therapeutic properties are known for *Terminalia* spp., the antibacterial activity has been particularly well reported. Extracts prepared from the fruit of the Australian species *Terminalia ferdinandiana* (Kakadu plum) have potent growth inhibitory activity against an extensive panel of pathogens including bacteria associated diarrhoea and dysentery[7] as well as the bacterial triggers of rheumatoid arthritis (*Proteus mirabilis*)[8] and multiple sclerosis (*Acinetobacter baylyi* and *Pseudomonas aeruginosa*).[7] Leaf extracts from the same species have also been shown to inhibit growth of the same bacteria, as well as a microbial trigger of ankylosing spondylitis (*Klebsiella pneumoniae*).[8] Similarly, African *Terminalia* spp. have also been shown to be effective bacterial growth inhibitors. *Terminalia stenostachya* and *Terminalia spinosa* have strong antibacterial activity against a broad spectrum of medicinally important bacteria including several *Mycobacterium* spp., *Enterococcus faecalis*, *Staphylococcus aureus*, *Vibrio cholera*, *Bacillus anthracis*, *K. pneumoniae*, *Salmonella typhi*, *P. aeruginosa* and *Escherichia coli.*[9] Recent studies have demonstrated the growth inhibitory activity of *Terminalia sericea* and *Terminalia prunioides* against pathogenic[10-12] and food spoilage bacteria.[13]

The Indian *Terminalia* spp. are used in several traditional medicinal systems (including, Ayurveda Siddha and Unani) and their therapeutic uses have been extensively documented. Many of the Indian *Terminalia* spp. are used to treat various diseases (Table 1) and numerous recent investigations have reported on their antimicrobial properties. Leaf and branch extracts of *Terminalia arjuna* have antibacterial activity against a wide panel of microbes.[14,15] *Terminalia chebula* has traditional uses in Ayurveda for the treatment of numerous diseases and conditions[16] and has potent antibacterial activity.[14] A recent study even highlighted their potential in the prevention and treatment of the endospore forming bacterium *Bacillus anthracis.*[27] *Terminalia alata*, *Terminalia bellirica* and *Terminalia catappa* also have broad spectrum antibacterial activity.[15] However, despite the relative wealth of antibacterial studies for

Table 1: The medicinal usage, common names and known constituents of the Indian *Terminalia* species investigated in this study.

Plant Species	Part Utilized in This Study	Common Name/s	Traditional Medicinal Uses	Known Constituents	References
Terminalia chebula	fruit	Chebulic Myroblan, Black Myroblan, Haritaki, Inknut	Used externally to treat fungal infections and cutaneous wounds and in the prevention of inflammation of the mucosal membrane of the mouth. Used internally as a laxative and is known for its purgative effects. Known to have uses in the treatment of asthma and coughs.	Terflavin B and chebulinic acid	6
Terminalia arjuna	branch	Arjuna, Koha, White Marudah	Treatment of cardiovascular disorders as well as anti-inflammatory properties. Known to aid in the elimination of cholesterol. Also an analgesic and an antioxidant.	Triterpenoids, flavonoids, tannins, gallic and ellagic acid, sitosterol, proanthocyanidins	6
Terminalia catappa	fruit	Indian almond, tropical almond, umbrella tree	Therapeutic effects for liver related diseases, anticancer activity as well as effective in the blocking of HIV reverse transcriptase. Additionally known to have antidiabetic benefits.	Flavonoids (including kaempferol, quercetin), tannins, saponins and phytosterols	6

the Indian *Terminalia* spp., there is a lack of studies screening *Terminalia* spp. for the ability to inhibit *Y. enterocolitica* growth, and thus their therapeutic value for the prevention and treatment of yersiniosis. This study was undertaken to examine the ability of selected Indian *Terminalia* spp. with extensive usage in Ayurvedic medicine for the ability to inhibit *Y. enterocolitica* growth.

MATERIALS AND METHODS

Plant source and extraction

The *Terminalia chebula* Retz. (fruit), *Terminalia arjuna* Roxb. Wight & Arn. (branch) and *Terminalia catappa* L. (fruit) plant materials utilized in this study were provided by Dr. Paran Rayan, Griffith University. Voucher samples of all plant specimens are deposited at the School of Natural Sciences, Griffith University. The plant materials were thoroughly desiccated in a Sunbeam food dehydrator and the dried materials stored at -30°C. Prior to usage, the materials were thawed and ground into a coarse powder. Individual 1 g amounts of the material were then weighed into separate tubes and 50 mL of deionised water, methanol, chloroform, hexane or ethyl acetate were added. All solvents used were analytical-reagent grade and were obtained from Ajax (Australia). The ground plant materials were separately extracted in each solvent for 24 hours at 4 °C through gentle shaking. The extracts were then filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resulting extracts were weighed and suspended in 10 mL sterilized deionised water containing 1 % DMSO.

Qualitative phytochemical studies

Phytochemical analysis of the extracts was achieved as previously described [20, 21] and used to determine the presence of triterpenoids, saponins, cardiac glycosides, tannins, phytosteroids, phenolic compounds, flavonoids, anthraquinones, and alkaloids.

Antibacterial screening

Clinical *Yersinia enterocolitica* strain

The clinical isolate strain of *Yersinia enterocolitica* used in this study was supplied by Ms. Jane Gifkins of the School of Natural Sciences Griffith University, Australia. All growth studies were performed using nutrient agar (Oxoid Ltd., Australia) under aerobic conditions. Incubation was at 30 °C and the stock culture was 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 assay.[22, 23] Briefly, 100 µL of *Y. enterocolitica* was grown in 10 mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. Volumes of 100 µL of the bacterial suspension were spread onto nutrient agar plates and tested for antibacterial activity using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µL of *Terminalia* spp. extracts, allowed to dry and placed onto inoculated plates. The plates were left at 4 °C for 2 h before incubation at 30 °C for 24 h. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was performed in at least triplicate. Mean values (± SD) are reported in this study. Standard discs of chloramphenicol (10 µg) were obtained from Oxoid (Australia) and were used as positive controls to compare antibacterial activity. Filter discs impregnated with 10 µL of distilled water (containing 1% DMSO) and used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentrations (MIC) of each plant extract was determined as previously described.[24,25] Briefly, each individual plant extract was diluted in deionised water and tested across a decreasing concentration gradient. Discs were impregnated with 10 µL of the extract dilutions, allowed to dry and placed onto plates inoculated with *Y. enterocolitica*. The assay was performed in triplicate as outlined above and graphs of the zone of inhibition versus concentration were plotted. Linear ln regression was utilized to determine MIC values.

Toxicity screening

Reference toxin for toxicity screening

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

Artemia franciscana nauplii toxicity screening

Toxicity of all extracts were determined using an adapted *Artemia franciscana* nauplii lethality assay.[26,27] Briefly, 400 µL of seawater containing ~43 (mean 43.2, n = 155, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used in the bioassay. Volumes of 400 µL of reference toxin or the diluted plant extracts were transferred to the wells and incubated at 25 ± 1 °C under artificial light (1000 Lux). Negative controls (400 µL seawater) and all test treatments were run in triplicate for each plate. The wells were monitored at regular intervals

Table 2: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the plant 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	Polysteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
CFW	438	43.8	+++	+++	+++	-	+++	-	-	-	-	+++	+++	++	-
CFM	634	63.4	+++	+++	+++	-	+++	-	-	-	-	+++	+++	++	-
CFC	93	9.3	+	-	-	-	-	-	-	-	-	+	-	-	-
CFH	104	10.4	-	-	-	-	-	-	-	-	-	-	-	++	-
CFE	62	6.2	+++	++	+	-	-	-	-	-	-	+++	+++	-	-
ABW	144	14.4	++	+++	+++	-	++	-	-	-	-	+++	+++	+++	-
ABM	40	4	++	+++	+++	-	+++	-	-	-	-	+++	+++	+++	-
ABC	92	9.2	+	+	-	-	-	-	-	-	-	-	+	-	-
ABH	136	13.6	-	-	-	-	-	-	-	-	-	-	-	-	-
ABE	22	2.2	+	-	-	-	-	-	-	-	-	+	+	-	-
PFW	144	14.4	+++	+++	++	+	++	-	-	++	++	++	+++	-	-
PFM	231	23.1	+++	+++	++	+	-	-	-	+	++	++	+++	-	-
PFC	434	43.4	+	+	-	+	-	-	-	-	-	+	+	-	-
PFH	447	44.7	+	+	-	-	-	-	-	-	-	-	+	-	-
PFE	353	35.3	+	+	-	-	-	-	-	-	-	+	+	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. CFW = *T. chebula* aqueous fruit extract; CFM = *T. chebula* methanolic fruit extract; CFC = *T. chebula* chloroform fruit extract; CFH = *T. chebula* hexane fruit extract; CFE = *T. chebula* ethyl acetate fruit extract; ABW = *T. arjuna* aqueous branch extract; ABM = *T. arjuna* methanolic branch extract; ABC = *T. arjuna* chloroform branch extract; ABH = *T. arjuna* hexane branch extract; ABE = *T. arjuna* ethyl acetate branch extract; PFW = *T. catappa* aqueous fruit extract; PFM = *T. catappa* methanolic fruit extract; PFC = *T. catappa* chloroform fruit extract; PFH = *T. catappa* hexane fruit extract; PFE = *T. catap*pa ethyl acetate fruit extract.

and the number of dead were counted. The nauplii were classified as dead if no movement was detected within 10 seconds. After 24 h, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was determined using probit analysis.

Statistical analysis

Data is expressed as the mean ± SD of at least three independent experiments.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extractions of 1 g of various dried *Terminalia* spp. materials with various solvents resulted in dried plant extracts ranging from 22 to 144 mg (*T. arjuna* branch extracts), 62 to 634 mg (*T. chebula* fruit extracts) and 144 to 447 mg (*T. catappa* fruit extracts) (Table 1). Methanolic and aqueous *T. chebula* extracts provided considerably greater yields of extracted material relative to the ethyl acetate, chloroform and hexane counterparts. Interestingly, the opposite trend was evident for the *T. catappa* fruit extracts, with the lower polarity chloroform and hexane extracts yielding higher masses of extracted material than in the higher polarity extracts. Substantially lower yields were recorded in all *T. arjuna* branch extracts. The dried extracts were resuspended in 10 mL of deionised water (containing 1 % DMSO), resulting in the concentrations presented in Table 2.

Qualitative phytochemical studies showed that the aqueous and methanolic extracts generally had a wide range of phytochemicals (Table 2). All *Terminalia* spp. generally contained substantial levels of phenolics, especially water soluble phenolics. Additionally, these extracts generally resulted in high levels of tannins and flavonoids and moderate to high levels of saponins. Similarly, the ethyl acetate extracts had comparable phytochemical profiles to the methanolic and aqueous counterparts. However, most classes of compounds were present in low abundances. Conversely, the hexane and chloroform extracts of most of the *Terminalia*

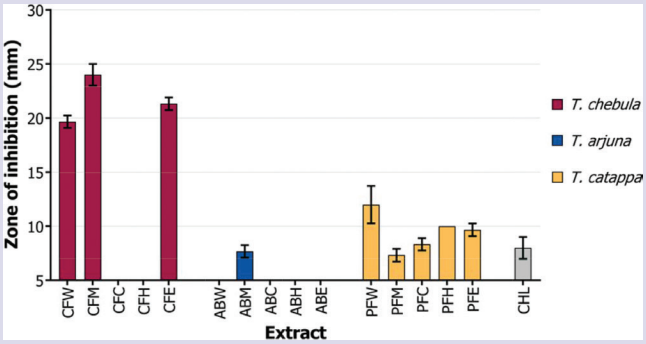

Figure 1: Growth inhibitory activity of *T. ferdinandiana* plant extracts against the *Y. enterocolitica* clinical isolate measured as zones of inhibition (mm). CFW = *T. chebula* aqueous fruit extract; CFM = *T. chebula* methanolic fruit extract; CFC = *T. chebula* chloroform fruit extract; CFH = *T. chebula* hexane fruit extract; CFE = *T. chebula* ethyl acetate fruit extract; ABW = *T. arjuna* aqueous branch extract; ABM = *T. arjuna* methanolic branch extract; ABC = *T. arjuna* chloroform branch extract; ABH = *T. arjuna* hexane branch extract; ABE = *T. arjuna* ethyl acetate branch extract; PFW = *T. catappa* aqueous fruit extract; PFM = *T. catappa* methanolic fruit extract; PFC = *T. catappa* chloroform fruit extract; PFH = *T. catappa* hexane fruit extract; PFE = *T. catappa* ethyl acetate fruit extract; CHL = chloramphenicol (10 µg). Results are expressed as mean zones of inhibition ± SD.

spp. typically only had low to moderate levels of phenolics, tannins and flavonoids and were mostly devoid of detectable levels of the other phytochemical classes.

Antimicrobial activity

To determine the ability of the *Terminalia* spp. fruit and branch extracts to prevent *Y. enterocolitica* growth, 10 µL of each extract was screened using a disc diffusion assay. Bacterial growth inhibition was evident in 9 of the 15 extracts screened (60%) (Figure 1). The *T. chebula* aqueous, methanolic and ethyl acetate extracts were the most potent inhibitors of growth (as judged by zone of inhibition), with inhibitory zones of 19.7 ± 0.6, 24 ± 1.0 and 21.3 ± 0.6 mm respectively. These are significantly better inhibition than the chloramphenicol control (10 µg), which had an inhibitory zone of 9 ± 0.6 mm. Similarly, all *T. catappa* extracts tested had comparable *Y. enterocolitica* growth inhibition to the chloramphenicol control. Of these, the aqueous *T. catappa* proved the most effective extract, with an inhibitory zone of 12 ± 1.7 mm. The antimicrobial efficacy was further quantified by determining the MIC values (Table 3). The *T. chebula* extracts were the most potent inhibitors of *Y. enterocolitica* growth, with MIC values of 653, 85 and 64 µg/mL for the aqueous, methanolic and ethyl acetate extracts respectively (~0.6-6.5 µg infused into the disc). The *T. arjuna* methanolic extract showed low to moderate activity (3000 µg/mL). All other extracts were inactive, with MIC > 10,000 µg/mL.

Quantification of toxicity

All extracts were initially screened in the assay at 2000 µg/mL (Figure 2). Potassium dichromate was also tested as a reference toxin in the bioassay. Potassium dichromate was rapid in its induction of mortality, with nauplii death evident within the first 3 h of exposure and 100 % mortality evident within 4-5 h (unpublished results). The methanolic and aqueous extracts of all *Terminalia* spp. displayed apparent toxicity in the *Artemia* nauplii bioassay, with ≥50% mortality rates at 24 h. The mortality for all other extracts were not significantly different to that observed in the seawater control.

Table 3: Minimum inhibitory concentration (µg/mL) of the plant extracts and LC_{50} values (µg/mL) in the *Artemia nauplii* bioassay.

Extract	MIC (µg/mL)	LC_{50} (µg/mL)
CFW	653	2246
CFM	85	1883
CFC	-	-
CFH	-	-
CFE	64	-
ABW	-	2094
ABM	3000	1683
ABC	-	-
ABH	-	-
ABE	-	-
PFW	>10000	1873
PFM	>10000	1452
PFC	>10000	-
PFH	>10000	-
PFE	>10000	-

Numbers indicate the mean MIC and LC_{50} values of triplicate determinations. - indicates no bacterial growth inhibition was evident, or that an LC_{50} value could not be obtained as the mortality did not reach 50% for any dose tested. CFW = *T. chebula* aqueous fruit extract; CFM = *T. chebula* methanolic fruit extract; CFC = *T. chebula* chloroform fruit extract; CFH = *T. chebula* hexane fruit extract; CFE = *T. chebula* ethyl acetate fruit extract; ABW = *T. arjuna* aqueous branch extract; ABM = *T. arjuna* methanolic branch extract; ABC = *T. arjuna* chloroform branch extract; ABH = *T. arjuna* hexane branch extract; ABE = *T. arjuna* ethyl acetate branch extract; PFW = *T. catappa* aqueous fruit extract; PFM = *T. catappa* methanolic fruit extract; PFC = *T. catappa* chloroform fruit extract; PFH = *T. catappa* hexane fruit extract; PFE = *T. catappa* ethyl acetate fruit extract.

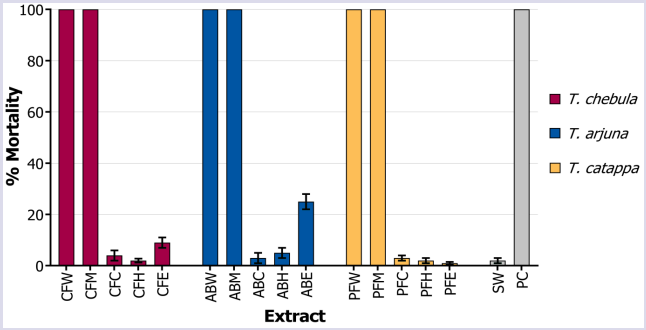

Figure 2: The lethality of the Australian plant extracts (2000 µg/mL) and the potassium dichromate (1000 µg/mL) and seawater controls towards *Artemia franciscana* nauplii after 24 hours exposure. CFW = *T. chebula* aqueous fruit extract; CFM = *T. chebula* methanolic fruit extract; CFC = *T. chebula* chloroform fruit extract; CFH = *T. chebula* hexane fruit extract; CFE = *T. chebula* ethyl acetate fruit extract; ABW = *T. arjuna* aqueous branch extract; ABM = *T. arjuna* methanolic branch extract; ABC = *T. arjuna* chloroform branch extract; ABH = *T. arjuna* hexane branch extract; ABE = *T. arjuna* ethyl acetate branch extract; PFW = *T. catappa* aqueous fruit extract; PFM = *T. catappa* methanolic fruit extract; PFC = *T. catappa* chloroform fruit extract; PFH = *T. catappa* hexane fruit extract; PFE = *T. catappa* ethyl acetate fruit extract. PC = potassium dichromate control; NC = negative (seawater) control. Results are expressed as mean % mortality ± SD.

To further enumerate the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay. Table 2 shows the LC_{50} values of the extracts towards *A. franciscana*. No LC_{50} values are reported for the chloroform, hexane or ethyl acetate extracts of any *Terminalia* spp. as ≤50% mortality was seen across concentrations tested. All other extracts were deemed nontoxic as they yielded LC_{50} values significantly greater than 1000 µg/mL following 24 h exposure. Extracts with LC_{50} values of ≥1000 µg/mL towards *Artemia* nauplii are deemed to be nontoxic as previously reported [28].

DISCUSSION

Terminalia spp. have been used for a broad range of medicinal purposes by traditional healers from a wide variety of ethnic and cultural groupings. The best documented of these are the traditional Indian medicinal systems, particularly the Ayurveda. Ayurvedic practitioners utilise various *Terminalia* spp. for a wide variety of medicinal purposes including abdominal and back pain, coughs and colds, conjunctivitis, diarrhoea and dysentery, fever, headache, heart disorders, inflammation, leprosy, pneumoniae, sexually transmitted diseases, worms, wounds, haemorrhages, ulcers, and as a general tonic.[6] Many of these diseases are caused by microbial pathogens, indicating the potential of these plants as antimicrobial agents. Indeed, recent investigations have reported on their antibacterial properties of several Indian *Terminalia* spp. extracts prepared from several parts of *T. arjuna* have antibacterial activity against a wide panel of microbes.[6, 16, 17] *T. chebula* is also used in Ayurvedic medicine for the treatment of numerous microbial diseases and conditions[6,18] and displays potent antibacterial activity against a panel of microbes.[16] *T. catappa* has also been reported to have broad spectrum antibacterial activity.[17]

The growth inhibitory activity of the *T. chebula* extracts against *Y. enterocolitica* is particularly noteworthy for the development of future antibiotic chemotherapeutics against the gastrointestinal disease yersiniosis. Potent *Y. enterocolitica* growth inhibitory activity was evident in the *T. chebula* fruit extracts. The methanolic and ethyl acetate extracts were the most potent growth inhibitors, with MIC values substantially <100 µg/mL.

Whilst an examination of the phytochemistry of the *Terminalia* spp. was beyond the scope of our study, a commonality of this genus is their relatively high levels of a number of tannin components including exifone (4-galloylpyrogallol), ellagic acid dehydrate, trimethyl ellagic acid, chebulic acid, corilagen, castalagin and chebulagic acid.[6,8-10] Gallotannins have been reported to inhibit the growth of a broad spectrum of bacterial species[29] through a variety of mechanisms including binding cell surface molecules including lipoteichoic acid and proline-rich cell surface proteins,[30,31] and by inhibiting glucosyltransferase enzymes.[32] Ellagitannins have also been reported to exert antibacterial activity via several mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[29, 31]

It is likely that other phytochemical classes also contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies indicate that polyphenolics, flavonoids, saponins, and terpenes were present in most *Terminalia* spp. extracts. Terpenoids have been previously reported to have potent broad spectrum antibacterial activity[33] and therefore may contribute to the inhibitory activity against *S. pyogenes*. Many studies have also reported potent antibacterial activities for a wide variety of flavonoids.[34] Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism of *Y. enterocolitica* growth inhibition.

Importantly, our findings also demonstrate that the Indian *Terminalia* spp. extracts tested in our study were nontoxic towards *Artemia franciscana* nauplii, with LC_{50} values substantially > 1000 µg/mL. Extracts with LC_{50} values > 1000 µg/mL towards *Artemia* nauplii are defined as being nontoxic.[28] Whilst our preliminary toxicity studies indicate that these extracts may be safe for use as *Y. enterocolitica* growth inhibitors, studies using human cell lines are required to further evaluate the efficacy of these extracts.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide

LC_{50}: The concentration required to achieve 50 % mortality

MIC: minimum inhibitory concentration

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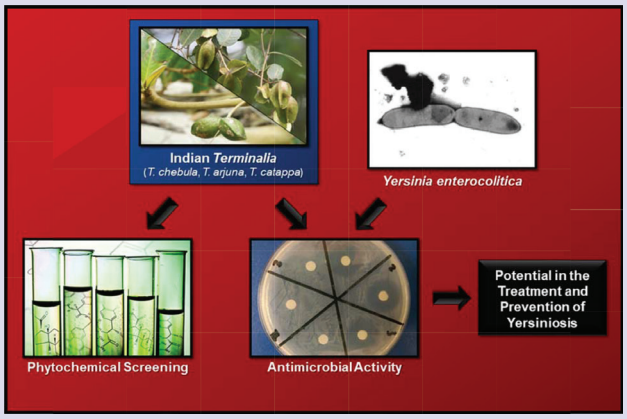
PICTORIAL ABSTRACT



SUMMARY

- Aqueous, methanolic and ethyl acetate *T. chebula* extracts were potent inhibitors of *Y. enterocolitica* growth.
- The *T. chebula* methanolic and ethyl acetate extracts were particularly potent with MIC values of 85 and 64 µg/mL respectively.
- The *T. arjuna* branch extract was moderately active (3000 µg/mL).
- All other extracts were either low efficacy, or completely devoid of growth inhibitory activity.
- All Indian *Terminalia* spp. extracts were nontoxic in the *Artemia* nauplii assay.

ABOUT AUTHORS

Dr. Mitchell Henry Wright is a postdoctoral researcher at Oregon Health & Science University in Portland, Oregon (USA) where he works on investigating Mn(III) transformations in aquatic systems. Specifically, his research focuses on manganese oxidation/reduction by bacteria and how these organisms influence the geochemical cycling of the metal. His previous postdoctoral posting involved investigating the potential of Australian native plants in the treatment and prevention of various pathogenic bacteria. This has resulted in several publications between both disciplines.

Dr. Anthony Carlson Greene is a senior lecturer and researcher at Griffith University, Brisbane Australia. He obtained his PhD in Microbiology from the University of New South Wales and focuses on extreme environments, Bioremediation and Geomicrobiology. His specific interests include the microbial ecology of thermophilic, saline and alkaliphilic environments and the mechanisms and industrial potential of extremophilic bacteria contained therein.

Dr Ian Edwin 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.