

# Growth Inhibitory Activity of Indian *Terminalia* spp. against the Zoonotic Bacterium *Bacillus anthracis*

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## ABSTRACT

**Introduction:** Anthrax is a zoonotic disease caused by the soil bacterium *Bacillus anthracis*. It has an extremely high mortality rate if untreated. *Terminalia* spp. have a long association with the treatment of various ailments, including bacterial infections although they have not been tested for the ability to inhibit the growth of *B. anthracis*. **Methods:** Solvent extracts were prepared using *Terminalia* spp. known to inhibit microorganism growth. The antibacterial potential of the extracts were investigated by disc diffusion assay to determine the growth inhibitory potential against an environmental strain of *B. anthracis*. Their MIC values were calculated to quantify and compare their relative efficacies. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** Extracts prepared from several Indian *Terminalia* spp. displayed potent antibacterial activity in the disc diffusion assay against *B. anthracis*. The methanolic *T. chebula* fruit extract was particularly effective at inhibiting microbial growth, with MIC values against *B. anthracis* of 166 µg/mL (<2 µg impregnated in the disc). The aqueous *T. chebula* extract, as well as *T. catappa* and *T. arjuna* methanolic extracts, were also good growth inhibitors with MIC's generally <2500 µg/mL (<25 µg impregnated in the disc). All other plant extracts

were either inactive or of only low inhibitory activity. None of the extracts were deemed toxic, with all recorded LC<sub>50</sub> values substantially >1000 µg/mL.

**Conclusion:** The potent growth inhibitory activity of the methanolic *T. chebula* fruit extract against *B. anthracis* indicates its potential in the treatment and prevention of anthrax. Furthermore, due to its low toxicity, its use may extend to all forms of the disease (cutaneous, inhalation or gastrointestinal) and may extend to live stock as well as humans.

**Key words:** Terminalia, Antibacterial activity, *Terminalia chebula*, *Terminalia catappa*, *Terminalia arjuna*, Anthrax, Traditional medicine.

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## INTRODUCTION

*Bacillus anthracis* is a sporulating gram-positive bacterium and the causative agent of the zoonotic disease anthrax. Present ubiquitously in soils worldwide, *B. anthracis* is dissimilar to other organisms within the *Bacillus* genus in that it produces the lethal anthrax toxin. While linked to several bioterrorism attacks and highly fatal to humans, the bacterium predominately affects feeding ruminants through the inhalation of endospores.<sup>1</sup> Under environmental stresses such as nutrient deprivation, vegetative cells divide to metabolically inactive spores that can remain dormant for prolonged periods of time.<sup>2,3</sup> Once these are internalised, the spores revert and subsequently produce the anthrax toxin which ultimately leads to death. After a period of time, spore production once again proceeds and improper handling of the infected carcasses can result in the zoonotic transfer to people. Indeed, the primary mode of human infection is through the ingestion, inhalation or direct contact of spores to skin abrasions originating from contaminated animal sources.<sup>4</sup> Human infection of anthrax can be divided into three forms: cutaneous (contact of spores to skin lesions), gastrointestinal (ingestion) and pulmonary (inhalation of spores). Cutaneous anthrax, the most common form of the disease, is characterised primarily through the formation of visible black eschars.<sup>5</sup> Although the most prevalent, it is also the most easily treated and if discovered early is rarely fatal.<sup>6</sup> Gastrointestinal anthrax is caused through the consumption of contaminated meat and occurrence is rare in developed nations and more frequent in countries without stringent quality control programs. Symptoms include vomiting of blood, severe diarrhoea and lesions from the oral cavity to the cecum.<sup>7</sup> Pulmonary anthrax is the most deadly form of the disease and is historically associated with bioterrorism, although infection can also occur through industrial contact (wool mills, tanneries etc). Symptoms include respiratory failure, multi-organ failure and shock, and if left untreated have up to a 90% mortality rate.<sup>8</sup>

Current antibiotic strategies include the combination of both intravenous and oral antibiotics, including ciprofloxacin, doxycycline and penicillin G.<sup>9</sup> While considered the most effective method of treatment, there is always an inherent risk that the bacterium may develop an antibiotic resistance to one or more of these antibiotics. Indeed, strains with naturally conferred resistances to penicillins and cephalosporins have been previously isolated.<sup>10</sup> Furthermore, a tailored, multi-drug resistant strain of *B. anthracis* may be used in bioterrorism which would decrease available treatment options. Therefore the synthesis of new drugs or discovery of antibacterial compounds in pre-existing natural assets is vital to ensure viable treatment options are available. The antimicrobial properties of medicinal plants have long been recognised by many cultures and the identification of the active compounds offers potential in the development of new anti-*B. anthracis* agents. Thus the investigation of natural assets provides an opportunity to discover compounds effective in the treatment of anthrax.

The genus *Terminalia* encompasses approximately 200-250 species of flowering trees and has an extensive association with usage in traditional medicinal systems.<sup>11</sup> The antibacterial activity of this genus has been extensively reported. Extracts prepared from the fruit of the Australian species *Terminalia ferdinandiana* (Kakadu plum) have potent growth inhibitory activity against an extensive panel of pathogenic bacteria including bacteria associated with diarrhoea and dysentery<sup>12</sup> as well as the bacterial triggers of rheumatoid arthritis (*Proteus mirabilis*)<sup>13</sup> and multiple sclerosis (*Acinetobacter baylyi* and *Pseudomonas aeruginosa*).<sup>14</sup> 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*).<sup>15</sup> Similarly, African *Terminalia* spp. are potent bacterial growth inhibitors. *Terminalia stenostachya* and *Terminalia spinosa* have strong antibacterial activity against a broad spec-

trum of medicinally important bacteria including several *Mycobacterium* spp., *Streptococcus faecalis*, *Staphylococcus aureus*, *Vibrio cholera*, *Bacillus anthracis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*.<sup>16</sup> Recent studies have demonstrated the growth inhibitory activity of *Terminalia sericea* and *Terminalia pruinoides* against pathogenic<sup>17</sup> and food spoilage bacteria.<sup>18</sup> Many Indian *Terminalia* spp. have a history of therapeutic uses, many of which are related to microbial infections (Table 1). Numerous recent investigations have reported on their antibacterial properties. Leaf and branch extracts of *Terminalia arjuna*, have antibacterial activity against a wide panel of microbes.<sup>19,20</sup> *Terminalia chebula* has traditional uses in Ayurveda for the treatment of numerous diseases and conditions<sup>21</sup> and has potent antibacterial activity.<sup>19</sup> Similarly, *Terminalia alata*, *Terminalia bellirica* and *Terminalia catappa* have broad spectrum antibacterial activity.<sup>20</sup> However, despite the wealth of antibacterial studies for *Terminalia* spp., there is a lack of studies screening *Terminalia* spp. for the ability to inhibit *B. anthracis* growth. Indeed, a literature search only found a single study which reported *B. anthracis* growth inhibitory activity for *T. stenostachya* and *T. spinosa*<sup>16</sup> and a further study which reported lack of inhibitory activity for *Terminalia glaucescens*.<sup>22</sup> Our study was undertaken to examine the ability of selected Asian *Terminalia* spp. with extensive usage in Ayurvedic medicine for the ability to inhibit *B. anthracis* growth.

## MATERIALS AND METHODS

### Plant source and extraction

The *Terminalia chebula*, *Terminalia catappa* and *Terminalia arjuna* plant materials used in this study were a gift from Dr Paran Rayan, Griffith University. Voucher samples of all plant specimens have been stored at the School of Natural Sciences, Griffith University, Brisbane Australia. The plant materials were thoroughly desiccated in a Sunbeam food dehydrator and the dried materials stored at -30°C until use. Prior to usage, the materials were thawed and ground into a coarse powder. Individual 1 g quantities of the material were weighed into separate tubes and 50 mL of methanol, deionised water, chloroform, hexane or ethyl acetate were added. All solvents were obtained from Ajax and were AR grade. The ground plant materials were individually extracted in each solvent for 24 hours at 4°C with 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 resultant extracts were weighed and redissolved in 10 mL deionised water (containing 1% DMSO).

### Qualitative phytochemical studies

Phytochemical analysis of the extracts for the presence of tannins, saponins, triterpenoids, phenolic compounds, flavonoids, phytosteroids, cardiac glycosides, anthraquinones, and alkaloids was conducted as previously described.<sup>28-30</sup>

### Antibacterial screening

#### *Environmental Bacillus anthracis* strain

An environmental strain of *Bacillus anthracis* was isolated and used in these studies. The bacterium was originally isolated from a water sample taken from Paralana hot springs (30°17'49"S, 139°44'15"E), South Australia. Isolation was achieved through successive culturing steps using a modified peptone/yeast extract (PYE) agar as previously described.<sup>31</sup> The GenBank accession number for the 16S rRNA gene sequence for the isolate is KR003287.

#### Evaluation of antimicrobial activity

The antimicrobial activity of all plant extracts was assessed using a modified disc diffusion assay as previously described.<sup>32-34</sup> Briefly, 100 µL of *B. anthracis* was grown in 10 mL of fresh PYE media until a cell count of approximately 10<sup>8</sup> cells/mL was achieved. An amount of 100 µL of bacterial suspension was spread onto nutrient agar plates. The extracts were tested for antibacterial activity using 5 mm sterilised filter paper discs. Discs were infused 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 at 30°C for 24 hours. The diameters of the inhibition zones were measured in millimetres. All measurements were rounded to the closest whole millimetre. Each assay was performed in at least triplicate. Mean values (± SEM) are reported in this study. Standard discs of chloramphenicol (10 µg) and penicillin-G (2 µg) were obtained from Oxoid Ltd. and served as positive controls for antibacterial activity. Filter discs infused with 10 µL of sterilised water were used as a negative control.

#### Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of each extract was determined as previously described.<sup>35,36</sup> Briefly, the plant extracts were diluted

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

Plant Species	Part Used in This Study	Common Name/s	Traditional Medicinal Uses	Known Constituents	References
<i>Terminalia chebula</i>	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	11, 23
<i>Terminalia catappa</i>	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	11, 24, 25
<i>Terminalia arjuna</i>	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	11, 26, 27

in deionised water and tested across a range of concentrations. Discs were infused with 10 µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was achieved 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 of each extract.

## Toxicity screening

### Reference toxin for toxicity screening

Potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) (AR grade, Chem-Supply, Australia) was prepared as a 4 mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

### *Artemia franciscana* nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay as previously described.<sup>37-39</sup> Briefly, 400 µL of seawater containing approximately 43 (mean 43.2, n = 155, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µL seawater) was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was detected within 10 seconds. Following 24 h exposure, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC<sub>50</sub> with 95% confidence limits for each treatment was determined using probit analysis.

### Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments.

## RESULTS

### Liquid extraction yields and qualitative phytochemical screening

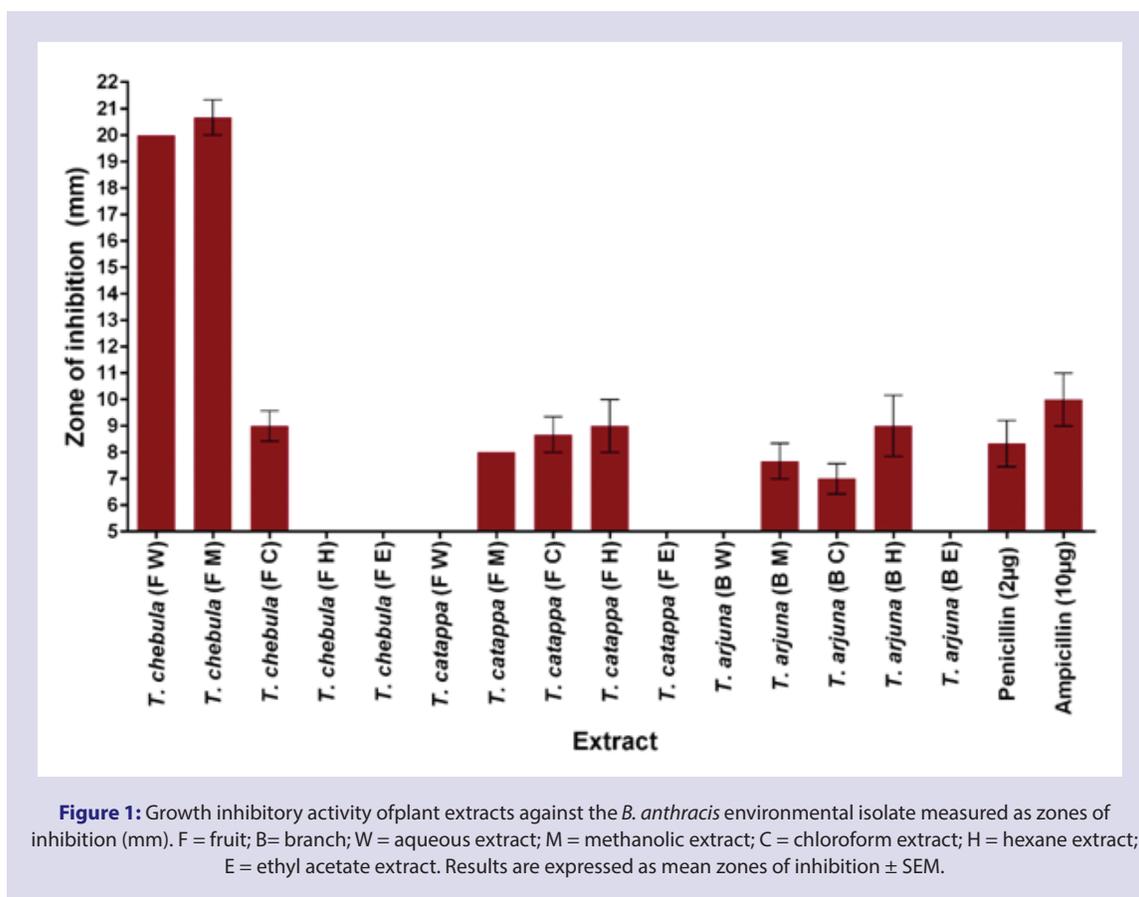
Extraction of 1 g of the various dried plant materials with the solvents yielded dried plant extracts ranging from 22 mg (*T. arjuna* leaf ethyl acetate extract) to 634 mg (*T. chebula* methanolic fruit extract) (Table 2). In general, *T. chebula* fruit extracts gave higher yields of dried extracted material compared with the corresponding extracts of the other *Terminalia* spp. The exceptions were the low polarity (chloroform and hexane) *T. catappa* fruit extracts, both of which had substantially higher amounts of extracted material than the corresponding *T. chebula* and *T. arjuna* extracts. Indeed, the *T. catappa* chloroform and hexane extracts had greater masses of extracted material than the aqueous and methanolic extracts. The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO) resulting in the extract concentrations shown in Table 2.

Qualitative phytochemical studies showed that the methanolic and aqueous extracts of all species generally had a wide range of phytochemicals (Table 2). Both solvents generally extracted high levels of phenolics (particularly water soluble phenolics) for all plant materials. The methanolic and aqueous extracts generally extracted high levels of flavonoids and tannins and moderate to high levels of saponins. The ethyl acetate extracts had similar phytochemical profiles as the methanolic and aqueous extracts, although most classes of compounds were present at lower abundance. In contrast, the chloroform and hexane extracts of most of the *Terminalia* plant specimens generally only had low to moderate levels of phenolics, flavonoids and tannins. The chloroform and hexane extracts were generally devoid of detectable levels of the other classes of phytochemicals.

**Table 2: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the plant extracts**

Species	Plant Part Used	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
<i>T. chebula</i>	fruit	W	438	43.8	+++	+++	+++	-	+++	-	-	-	-	+++	+++	++	-
<i>T. chebula</i>	fruit	M	634	63.4	+++	+++	+++	-	+++	-	-	-	-	+++	+++	++	-
<i>T. chebula</i>	fruit	C	93	9.3	+	-	-	-	-	-	-	-	-	-	+	-	-
<i>T. chebula</i>	fruit	H	104	10.4	-	-	-	-	-	-	-	-	-	-	-	++	-
<i>T. chebula</i>	fruit	E	62	6.2	+++	++	+	-	-	-	-	-	-	+++	+++	-	-
<i>T. catappa</i>	fruit	W	144	14.4	+++	+++	++	+	++	-	-	++	++	++	+++	-	-
<i>T. catappa</i>	fruit	M	231	23.1	+++	+++	++	+	-	-	-	+	++	++	+++	-	-
<i>T. catappa</i>	fruit	C	434	43.4	+	+	-	+	-	-	-	-	-	+	+	-	-
<i>T. catappa</i>	fruit	H	447	44.7	+	+	-	-	-	-	-	-	-	-	+	-	-
<i>T. catappa</i>	fruit	E	353	35.3	+	+	-	-	-	-	-	-	+	+	+	-	-
<i>T. arjuna</i>	leaf	W	144	14.4	+++	+++	+++	-	++	-	-	-	-	+++	+++	+	-
<i>T. arjuna</i>	leaf	M	40	4.0	+++	+++	+++	-	+++	-	-	-	-	+++	+++	+	-
<i>T. arjuna</i>	leaf	C	92	9.2	+	+	-	-	-	-	-	-	-	-	+	-	-
<i>T. arjuna</i>	leaf	H	136	13.6	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>T. arjuna</i>	leaf	E	22	2.2	+	-	-	-	-	-	-	-	-	+	+	-	-

+++ 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.



**Table 3:** Minimum inhibitory concentration ( $\mu\text{g/mL}$ ) of the plant extracts and  $\text{LC}_{50}$  values ( $\mu\text{g/mL}$ ) in the *Artemia nauplii* bioassay

Species	Part	Extract	MIC ( $\mu\text{g/mL}$ )	$\text{LC}_{50}$ ( $\mu\text{g/mL}$ )
<i>T. chebula</i>	fruit	W	1975	2246
<i>T. chebula</i>	fruit	M	166	1883
<i>T. chebula</i>	fruit	C	9400	-
<i>T. chebula</i>	fruit	H	-	-
<i>T. chebula</i>	fruit	E	-	-
<i>T. catappa</i>	fruit	W	-	1873
<i>T. catappa</i>	fruit	M	2640	1452
<i>T. catappa</i>	fruit	C	>10000	-
<i>T. catappa</i>	fruit	H	>10000	-
<i>T. catappa</i>	fruit	E	-	-
<i>T. arjuna</i>	branch	W	-	2094
<i>T. arjuna</i>	branch	M	1608	1683
<i>T. arjuna</i>	branch	C	9200	-
<i>T. arjuna</i>	branch	H	>10000	-
<i>T. arjuna</i>	branch	E	-	-

Numbers indicate the mean MIC and  $\text{LC}_{50}$  values of triplicate determinations. - indicates no bacterial growth inhibition was evident, or that an  $\text{LC}_{50}$  value could not be obtained as the mortality did not reach 50% for any dose tested. W = aqueous extract; M = methanolic extract; C = chloroform extract; H = hexane extract; E = ethyl acetate extract.

## Antimicrobial activity

To determine the ability of the crude plant extracts to inhibit the growth of *B. anthracis*, aliquots (10  $\mu$ L) of each extract were screened using a disc diffusion assay. The bacterial growth was inhibited by 9 of the 15 extracts screened (60 %) (Figure 1). The *T. Chebula* aqueous and methanolic fruit extracts were the most potent inhibitor of *B. anthracis* growth (as judged by zone of inhibition), with inhibition zones of  $20.7 \pm 0.7$  mm and  $20.0 \pm 0$  mm respectively. This compares favourably with the penicillin and ampicillin controls, which had zones of inhibition of  $8.3 \pm 0.6$  and  $9.6 \pm 0.6$  mm respectively. The *T. chebula* chloroform extracts also displayed good growth inhibitory activity ( $9.3 \pm 0.3$  mm), whilst the *T. chebula* hexane and ethyl acetate extracts were devoid of growth inhibitory activity. The *T. catappa* methanolic, chloroform and hexane extracts, as well as the *T. arjuna* methanolic, chloroform and hexane extracts also displayed moderate inhibitory activity. All other extracts were devoid of *B. anthracis* growth inhibitory activity.

The antimicrobial efficacy was further quantified by determining the MIC values (Table 3). The methanolic *T. chebula* fruit extract was particularly effective at inhibiting microbial growth, with MIC values against *B. anthracis* of 166  $\mu$ g/mL (<2  $\mu$ g impregnated in the disc). The aqueous *T. chebula* fruit extract, as well as the *T. catappa* and *T. arjuna* methanolic extracts were also displayed good growth inhibition with MIC's generally <2500  $\mu$ g/mL (<25  $\mu$ g impregnated in the disc). All other plant extracts were either inactive or of only low inhibitory activity.

## Quantification of toxicity

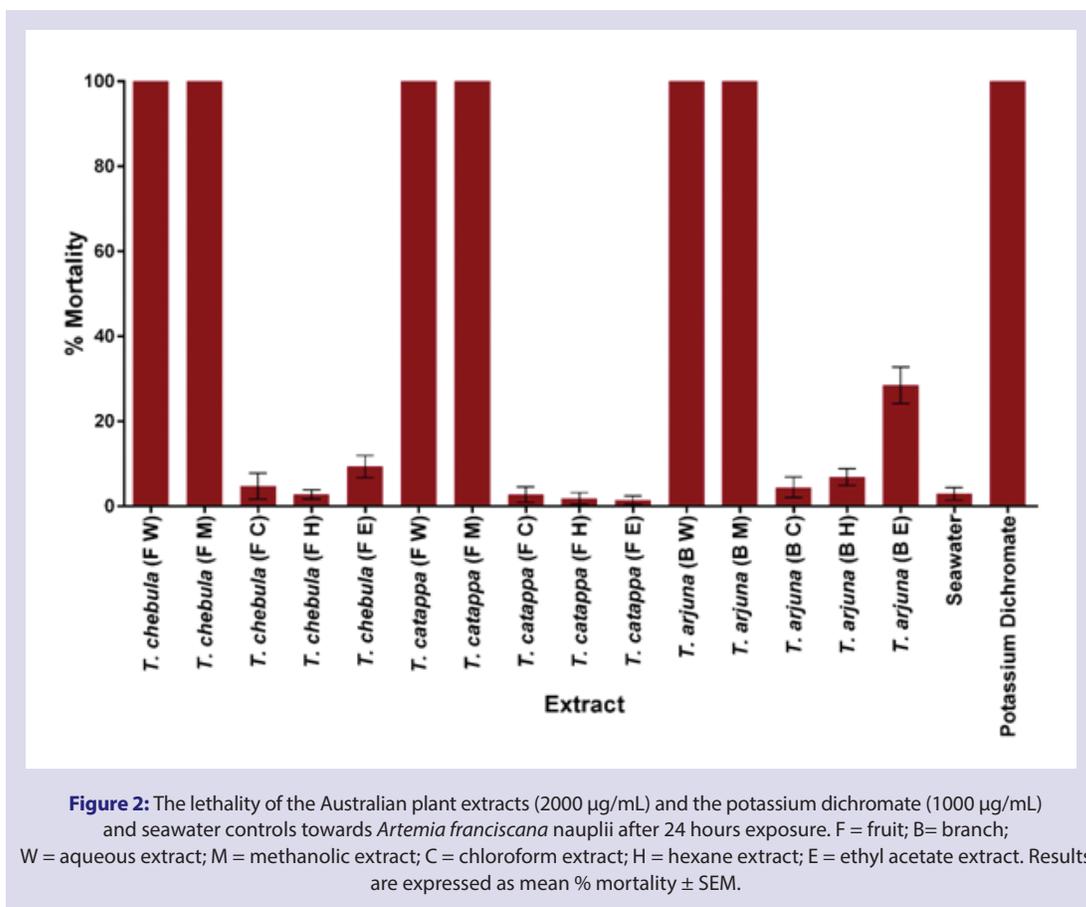
All extracts were initially screened at 2000  $\mu$ g/mL in the assay (Figure 2). For comparison, the reference toxin potassium dichromate (1000  $\mu$ g/mL) was also tested in the bioassay. The potassium dichromate reference tox-

in was rapid in its onset of mortality, inducing nauplii death within the first 3 hours of exposure and 100% mortality was evident following 4-5 hours (results not shown). The aqueous and methanolic extracts of all *Terminalia* spp. were toxic in the *Artemia* nauplii bioassay, with > 50% mortality rates at 24 h. The mortality for all other extracts was not significantly different to the mortality seen for the seawater control.

To further quantify 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 3 shows the LC<sub>50</sub> values of the extracts towards *A. franciscana*. No LC<sub>50</sub> values are reported for the chloroform, hexane and ethyl acetate extracts of any *Terminalia* spp. as <50 % mortality was seen for all concentrations tested. All other extracts were determined to be nontoxic, with LC<sub>50</sub> values substantially greater than 1000  $\mu$ g/mL following 24 h exposure. Extracts with an LC<sub>50</sub> of greater than 1000  $\mu$ g/mL towards *Artemia* nauplii have been defined as being nontoxic.<sup>40</sup>

## DISCUSSION

Many *Terminalia* spp. have a history of therapeutic usage to treat microbial infections and numerous recent investigations have reported on their antibacterial properties. *T. arjuna* leaf and branch extracts have antibacterial activity against a wide panel of microbes.<sup>19, 20</sup> *T. chebula* also has a tradition of use in Ayurveda for the treatment of numerous diseases and conditions.<sup>21</sup> *T. chebula* has also been reported to display potent antibacterial activity against a microbial panel.<sup>19</sup> Similarly, *T. alata*, *T. bellirica* and *T. catappa* have also been reported to have broad spectrum antibacterial activity.<sup>20</sup> Interestingly, whilst *B. anthracis* was not tested in any of the previous studies, several reports indicate strong growth



inhibition of the related bacterial species *Bacillus subtilis*. *T. catappa* was a particularly potent growth inhibitor, with an MIC of 5 µg/mL reported.<sup>41</sup> A different study reported growth inhibition of 2 *Bacillus* spp. including *B. subtilis* at doses as low as 100 µg/mL.<sup>42</sup> Similarly, inhibition of *B. subtilis* growth was reported for *T. arjuna* at 1000 µg/mL.<sup>43</sup> However, that study did not report MIC values, making it difficult to compare efficacy between the extracts. *B. subtilis* is closely related to *B. anthracis* with approximately 95% 16S rRNA sequence homology.<sup>44</sup> Indeed, under current taxonomic standards, bacteria with >97% 16S rRNA sequence homology are generally classified as a single species. It is therefore perhaps not surprising that the Indian *Terminalia* spp. extracts screened in our study displayed growth inhibitory activity towards *B. anthracis*.

Whilst an examination of the phytochemistry of the *Terminalia* spp. examined in our study 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.<sup>11,13-15</sup> Gallotannin have been reported to inhibit the growth of a broad spectrum of bacterial species<sup>45</sup> through a variety of mechanisms including binding cell surface molecules including lipoteichoic acid and proline-rich cell surface proteins<sup>46,47</sup> and by inhibiting glucosyltransferase enzymes.<sup>48</sup> Ellagitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL.<sup>45</sup> Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.<sup>45,47</sup> 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 the *Terminalia* spp. extracts. Terpenoids have been previously reported to have potent broad spectrum antibacterial activity<sup>49</sup> and therefore may contribute to the inhibitory activity against *B. anthracis*. Many studies have also reported potent antibacterial activities for a wide variety of flavonoids.<sup>50</sup> Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism of *B. anthracis* growth inhibition.

The findings reported here demonstrate that all of the Indian *Terminalia* extracts tested in our study were nontoxic towards *Artemia franciscana* nauplii, with LC<sub>50</sub> values substantially >1000 µg/mL. Extracts with LC<sub>50</sub> values >1000 µg/mL towards *Artemia* nauplii are defined as being nontoxic.<sup>40</sup> Whilst our preliminary toxicity studies indicate that these extracts may be safe for use as *B. anthracis* growth inhibitors, studies using human cell lines are required to further evaluate the safety of these extracts. Furthermore, whilst the results of our study are promising, it must be noted that the growth inhibitory studies screened against vegetative cells. As *Bacillus* spp. are spore formers, further studies are required to determine whether extracts with *B. anthracis* growth inhibitory activity also affect bacterial growth from the spores.

## CONCLUSION

The results of this study demonstrate the potential of various Indian *Terminalia* spp. in the growth inhibition of *B. anthracis*. Further investigations aimed at the purification of the bioactive components are needed to assess the mechanisms of action of these agents.

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## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

## ABBREVIATION USED

**DMSO**: Dimethyl sulfoxide; **LC<sub>50</sub>**: The concentration required to achieve 50% mortality; **MIC**: Minimum inhibitory concentration; **PYE**: Peptone yeast extract.

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## PICTORIAL ABSTRACT



## SUMMARY

- Extracts of 3 Indian *Terminalia* spp. inhibited *B. anthracis* growth.
- *T. chebula* fruit methanolic extract was particularly potent, with an MIC of 166 µg/mL.
- Aqueous *T. chebula* and methanolic *T. catappa* and *T. arjuna* extracts also were good growth inhibitors (MIC's <2500 µg/mL).
- Hexane and ethyl acetate extracts were generally devoid of inhibitory activity.
- All the extracts were non-toxic in the *Artemia* nauplii assay.

## ABOUT AUTHORS



**Dr Mitchell Henry Wright:** Received his PhD in 2014, for his work investigating the manganese reduction and oxidation characteristics of environmental bacteria. He is currently a postdoctoral researcher at Griffith University, Australia, where he is working on several projects both in the areas of geomicrobiology and pharmacognosy. His present research interests are the use of biogenic manganese oxides in the bioremediation of metal-contaminated sites as well as the use of Australian native plants in the treatment and prevention of various pathogenic bacteria.



**Reece Courtney:** Is a current post graduate student in the School of Natural Sciences Griffith University, Australia under the supervision of Dr Ian Cock. His research interests include medical microbiology, pharmacognosy and medicinal plants. He has a particular interest in the medicinal properties of the genus *Terminalia*, which is the subject of his research project.



**Dr Anthony 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 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 *Scaevolaspinescens*, *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.