

SHORT COMMUNICATION

Antimicrobial activity of *Tephrosia purpurea* (Linn.) Pers. and *Mimusops elengi* (Linn.) against some clinical bacterial isolates

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Abstract: Plants used in traditional medicinal systems have proved to be reliable sources of antimicrobial compounds. Two native plants, *Tephrosia purpurea* (Linn.) Pers. (Fabaceae) and *Mimusops elengi* (Linn.) (Sapotaceae) were screened for their antimicrobial activity. Preliminary testing of antimicrobial activity of *T. purpurea* against 3 standard cultures (*Staphylococcus aureus* [NCTC 6571], *Pseudomonas aeruginosa* [NCTC 10662], *E. coli* [NCTC 10418] and one clinical isolate of *Candida* spp. was performed with water extracts of leaves, pods and roots using the 'disc diffusion bioassay'. Subsequently, the antimicrobial activity of ethanolic root and leaf extracts against the above three standard isolates and clinical isolates of two strains of *Staphylococcus*, two strains of *Pseudomonas* and nine coliforms were tested using the 'well method'. The active extracts were subjected to the Minimum Inhibitory Concentration (MIC) agar dilution method, to determine the minimum inhibitory concentration of each extract. Further, the effect of plant maturity was tested on the antimicrobial activity of *T. purpurea*. In addition, ethanolic extracts were prepared from the bark of *M. elengi* and tested for its antimicrobial activity against the above bacterial isolates.

Ethanolic root extracts of *T. purpurea* were found to be active against *P. aeruginosa*, two other *Pseudomonas* strains and two coliform strains. Ethanolic leaf extracts and all the water extracts showed no activity against any of the isolates. The bark extract of *M. elengi* showed activity against three *Staphylococcus* isolates including *S. aureus*. The MIC of ethanolic root extracts of *T. purpurea* and bark extract of *M. elengi* were both found to be 128mg/L. There were no differences between the antimicrobial activities of the extracts of *T. purpurea* plants at different maturity levels.

Keywords: Antimicrobial activity, coliforms, *Mimusops elengi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Tephrosia purpurea*.

INTRODUCTION

Herbal medicines are employed in a wide variety of health related applications ranging from treatment of common colds to treatment of cancer¹. Encouraging results from research in the recent past on medicinal properties of plants, have led to scientists searching for plant derived drugs. Plant species used in various traditional medicinal systems have been reliable and convenient sources to select plants to be analysed for medicinal properties².

Various chemical compounds synthesised in plants give them their medicinal properties. Lately, higher plants have been screened extensively for antimicrobial compounds¹⁻⁴. Antimicrobial compounds can be broadly defined as compounds capable of destroying or inhibiting the growth of microorganisms.

T. purpurea (Linn.) Pers (Fabaceae) is a perennial herb which is known as *pila* in Sinhala, *kolinji* in Tamil, wild indigo in English, and *Sharpunkha* in Sanskrit. This plant shows a pantropic distribution. It is common in India, Sri Lanka, Malay Peninsula, China and Hawaii. In Sri Lanka, it is more common in the low country, in both wet and dry zones⁵. *T. purpurea* grows up to a height of about 30-60 cm. This plant is extensively exploited as a medicinal plant. In the Ayurveda system, *T. purpurea* is referred to as *Sarwa wran vishapaha* which implies that it can heal any type of wound. It is also believed to be very effective in the treatment of inflammation and enlargement of spleen and liver, therefore, it is also called *plihāri* or *plihāśathru* (*plihā* = spleen)⁶. The roots and sometimes the whole plants are used in medicine⁷.

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A decoction of roots is used to treat dyspepsia, cough, asthma, fever, ulcers, skin diseases, flatulence, colic, and as a blood purifier and an anthelmintic. Wound healing potential of *T. purpurea* has also been identified⁸.

M. elengi (Linn.) (Sapotaceae) is a perennial tree distributed in India, Sri Lanka, Malay Peninsula, and Andaman Islands⁵. The plant is commonly known as *Munamal* in Sinhala, *Makil* in Tamil, Spanish Cherry in English and *Sinha kesara* in Sanskrit, and is widely used in traditional medicinal systems. A decoction is prepared from the bark, which can be used to treat diseases of the gum and teeth. It is also used to treat the diseases of the bladder and urethra. It gives relief from fever, and increases female fertility⁷. Flowers and fruits are used to prepare lotions for the treatment of sores. Anti-ulcer activity⁹ and hypotensive activity¹⁰ have been detected in the bark extract of *Mimusops*. Leaves have shown antibacterial activity⁴.

The current study was designed to determine the antimicrobial activity of *T. purpurea* and *M. elengi* on some bacterial isolates. Further, the effect of maturity of the plants on the antimicrobial activity of *T. purpurea* was also investigated.

METHODS AND MATERIALS

Microbial strains: Three standard cultures (*Staphylococcus aureus* [NCTC 6571], *Pseudomonas aeruginosa* [NCTC 10662], *E. coli* [NCTC 10418]) and clinical bacterial isolates (fixed cultures on sterile filter paper strips, which were stored in the freezer at -70 °C) from various tissue samples of patients received at the Microbiology Laboratory of the Faculty of Dental Sciences, University of Peradeniya, were used. The clinical isolates included, 2 strains of *Staphylococcus*, 2 strains of *Pseudomonas* and 9 strains of coliforms. Bacterial filter paper strips were pressed on different media, in order to revive cultures, viz. *Staphylococcus* strains on solidified Blood Agar and all the other isolates on solidified Mac Conkey Agar. The plates were incubated at 37°C for 24 h. The resulting colonies of the clinical isolates were tested for the confirmation of their identity by performing the Coagulase test for *Staphylococcus* strains and the Cytochrome Oxidase test for *Pseudomonas* strains¹¹. In addition to the above, one *Candida* isolate was also used.

Plant material:

a) *T. purpurea* samples for the preparation of water extracts: Mature plants of *T. purpurea* growing along

the roadside of Kuliypitiya (Kurunegala District) were uprooted carefully. They were washed well with water to remove dirt and debris.

b) *T. purpurea* samples for the preparation of ethanolic extracts: Mature seeds of *T. purpurea* were obtained from plants growing along the roadside of Kuliypitiya (Kurunegala District). The seeds were sown in a seed bed, at the Agricultural farm at Meewathura (Kandy District). The seed bed was watered regularly, until the seedlings were 3 wks old. A soil mixture was prepared by mixing loamy soil from the Agricultural farm at Meewathura, with sand and coir dust in a 3:1:1 ratio and 40 cement pots (diameter: 30 cm, height: 22 cm) were filled with the prepared soil mixture. Two to three wks old *Tephrosia* seedlings from the seed bed were transferred into each pot. The pots were kept in a plant house. They were watered daily with 2L of water per pot.

Harvesting of *T. purpurea* was done at two different stages of maturity; viz 3 months and 5 months; (before and after flowering and fruit set respectively). At each harvesting stage, 20 plants were uprooted and washed well with water to remove the soil.

c) *M. elengi* bark samples for the preparation of ethanolic extracts: Bark of a mature *M. elengi* tree, from Kuliypitiya (intermediate zone), was peeled using a knife, and taken to the laboratory. Bark segments were cut into pieces (1 cm x 0.5 cm).

Preparation of extracts:

a) Water extracts for preliminary testing of *T. purpurea*: Mature leaves, pods and roots of freshly harvested plants were separated. Samples of 20 g each were taken from the above 3 plant parts and from a combination of the above. Each sample was boiled in 80 mL of distilled water and a final volume of 10 mL was obtained by evaporation of water, according to Ayurvedic practice. The extracts were filtered using Whatman No.1 filter paper, and transferred into sterile glass vials.

b) Ethanolic extracts of *T. purpurea*: Preparation of ethanolic extracts from 3 month old and 5 month old *Tephrosia* plants was done separately. Harvested *T. purpurea* plants were washed again with water, and allowed to dry at room temperature (27±1°C). The roots were separated from the plants. Leaves were picked separately. The separated plant parts of *Tephrosia* were cut into small pieces (1 cm x 0.5 cm). Fifty grams from each sample were soaked in 250 mL of 99% ethanol for 24 h at room temperature (27±1°C). The plant parts were subjected to the vacuum infiltration technique

with the same aliquot of ethanol at room temperature for 1 h¹². The resulting ethanolic extract was filtered using Whatman No.1 filter paper. This was repeated with 3 changes of ethanol for *Tephrosia* plant parts and 4 changes for *Mimusops* bark¹³. The ethanolic filtrates were concentrated at 40°C using a rotary evaporator to obtain extracts devoid of ethanol. They were freeze-dried and transferred into sterile glass vials, and were stored in the freezer, at -70°C.

c) Ethanolic extracts of *M. elengi*: Fifty gram samples were subjected to the above method of extraction using ethanol. The extracts were evaporated and freeze-dried and stored until further use as for *Tephrosia*.

Bioassays:

a) Disc diffusion bioassay with water extracts of *T. purpurea*: Broth cultures were prepared by inoculating, isolated pure standard cultures of *S. aureus* [NCTC 6571], *P. aeruginosa* [NCTC 10662], *E. coli* [NCTC 10418] and *Candida* spp. to Brain Heart Infusion Broth (BHIB) and incubating at 37°C overnight.

20 mL of sterile molten Mueller Hinton Agar (MHA) at 45 °C was pipetted out into a sterile Universal bottle, to which 50 µL of one of the prepared broth cultures was added and the bottle was capped. The contents were mixed thoroughly, poured immediately into a sterile Petri dish, and allowed to set. Subsequently, each plate was marked into 5 partitions on the reverse side. The partitions were labeled as L (leaves), P (pods), R (roots), C (combination) and DW (distilled water). This procedure was repeated with all 4 microbial isolates in triplicate. Antibiotic testing filter paper discs (6 mm in diameter) were saturated with the prepared water extracts of *T. purpurea* (about 150 µL), by placing a drop at a time on the disc using a micropipette and allowing it to absorb. A control disc was prepared using distilled water. The discs were placed on the corresponding labeled partition of each Petri dish and incubated at 37°C overnight, and the zone of inhibition around each disc was measured after 18-24 hrs.

b) 'Agar diffusion' bioassay with ethonolic extracts of *T. purpurea* and *M. elengi*: *Tephrosia* root extracts-3 months old (R1), 5 months old (R2), *Tephrosia* leaf extracts-5 months old (L), and *Mimusops* bark extract (M) were tested using the 'Agar diffusion' bioassay for their antimicrobial activity.

Molten Mueller Hinton Agar (MHA) was poured into 16 sterile 90 mm Petri dishes (15 mL per dish) and allowed to solidify. Equal volumes (0.25 mL) of each broth bacterial culture (McFarland series), was pipetted

on to an MHA plate separately, and spread with a sterile glass spreader. They were allowed to dry at 44 °C for 10 min. Using a sterile 8 mm cork borer, wells were cut on each MHA plate. The wells were named with the code names of the four extracts (R1, R2, L, and M) and as SDW (sterile distilled water) and D (Dimethylsulfoxide - DMSO).

30 mg of each of the freeze dried leaf extracts and the bark extract was dissolved in sterile distilled water, while similar quantities of the root extracts were dissolved in DMSO (since the root extracts were not completely soluble in water). These solutions were then loaded into the corresponding wells of each Petri dish, using a micropipette. The wells named as W and D were loaded with sterile distilled water and DMSO respectively. The plates were subsequently incubated at 37°C overnight, and the zone of inhibition around each well was measured. This procedure was repeated 3 times.

Determination of the Minimum Inhibitory Concentration (MIC) of antimicrobial extracts:

a) Preparation of bacterial cultures with similar cell densities: Extracts which showed significant antimicrobial activity at the activity test were tested for their MIC. Broth cultures of bacteria with their turbidity equal to that of the standard McFarland 0.5 solution were prepared as follows.

The isolated bacterial colonies were streaked on their respective media and incubated at 37°C overnight. A loopful from an isolated colony was taken to a sterile inoculating needle. It was smeared on the inner wall of a sterile screw-cap tube, containing 10 mL of sterile normal Saline. Subsequently, the tube was capped and vortexed for several seconds to dissolve the bacterial culture well. The turbidity of the broth culture obtained was made similar to that of the McFarland 0.5 standard solution by dissolving more of the colony or diluting with more normal Saline. This was repeated with all the bacterial isolates, to obtain broth cultures with more or less similar cell densities and stored under refrigeration (4°C) for use on the same day.

A concentration series of the extract was prepared¹⁴ by pipetting out different volumes from the R1 stock solution into 8 sterile Universal bottles as shown in Table 1. This procedure was repeated for all other extracts.

20 mL of molten MHA (at 50°C) was transferred into a Universal bottle with an extract concentration of 128 mg/L, using a sterile pipette and a dispenser. The bottles were capped and the contents were mixed thoroughly.

Subsequently, the medium was poured into a sterile 90 mm Petri dish which was marked into 16 partitions on the reverse side. The partitions were labeled with the names of the bacterial isolates. This was repeated with the remaining 8 concentrations (ranging from 0-64 mg/L) in universal bottles. MHA was allowed to set and the plates were dried at 44°C. A 10 µL drop each from the prepared broth bacterial cultures was placed on agar on the corresponding partition using a micropipette. After absorption of culture, they were incubated at 37°C overnight.

The entire procedure was repeated for the remaining active extracts in triplicate.

RESULTS

Activity test of water extracts–disc diffusion bioassay

The zones of inhibition observed in the disc diffusion bioassay are shown in the Table 2.

Since the average zones of inhibition were <3 mm, it could be concluded that the water extracts of *T. purpurea* had no antibiotic activity on *S. aureus*, *P. aeruginosa*, *E. coli*, and *Candida* spp.

Table 1: Volumes of the stock solution and concentration of the final solution of extract

Volume of stock solution (µL)	Concentration of the final solution (mg/L)
2560	128
1280	64
640	32
320	16
160	8
80	4
40	2
20	1
0	0

Activity test of ethanolic extracts–Agar diffusion bioassay

The zones of inhibition observed in the ‘Agar diffusion bioassay’ of the ethanolic extracts are shown in Table 3. Zone of inhibition > 3mm were considered as inhibitions resulting from considerable antimicrobial activity.

The bark extract of *M. elengi* (M) showed considerable inhibition of the three *Staphylococcus* isolates i.e. *S. aureus* [NCTC 6571] (Table 3 and Figure 1), *Staphylococcus* strain 1 and *Staphylococcus* strain 2. There was no difference between the average inhibition zones within these three isolates. No inhibition was observed on the other isolates, viz: coliforms and *Pseudomonas* spp.

The root extracts of *T. purpurea* (R1 and R2) showed considerable inhibition of the three *Pseudomonas* isolates i.e. *P. aeruginosa* [NCTC 10662] (Table 3 and Figure 2), *Pseudomonas* strain 1, *Pseudomonas* strain 2 and two of the coliform strains i.e. coliform strain 6 and coliform strain 9. There was no difference between the average inhibition zones given by *Tephrosia* root extract within the three *Pseudomonas* isolates and within coliform strains 6 and 9. No inhibition was shown by the leaf extract (L) on any of the isolates tested (Figure 2).

DMSO showed a very slight inhibition on the three *Pseudomonas* strains and the ten coliform strains (including *E. coli*). However, it was almost insignificant when compared with the inhibition by the root extracts. No inhibition was shown by DMSO on any of the *Staphylococcus* strains used.

Minimum Inhibitory Concentrations (MIC)

The inhibition of *Staphylococcus* strains was observed only in the plates containing a concentration of 128 mg/L of *Mimusops* bark extract, i.e. the highest concentration used (Figure 3). Similarly, the inhibition of *Pseudomonas*

Table 2: Average zones of inhibition given by the antibiotic discs soaked with water extracts of *T. purpurea*

Isolate	Average zone of inhibition (mm)				Distilled water
	Extract				
	Root	Leaves	Pods	Combination	
<i>S. aureus</i> [NCTC 6571]	0.0	0.0	0.0	0.0	0.0
<i>P. aeruginosa</i> [NCTC 10662]	1.0	0.0	0.0	1.0	0.0
<i>E. coli</i> [NCTC 10418]	1.0	0.0	0.0	1.0	0.0
<i>Candida</i> spp.	0.0	0.0	0.0	0.0	0.0

strains and the coliform strains 6 and 9 was observed in the plates containing a concentration of 128 mg/L *Tephrosia* root extracts. There was no inhibition of any of the isolates tested in concentrations lower than 128 mg/L.

Table 3: Average zones of inhibition given by ethanolic extracts of *T. purpurea* and *M. elengi*

Isolate	Average zone of inhibition (mm)					
	Extract					
	R1	R2	L	M	D	W
<i>S. aureus</i> [NCTC 6571]	0	0	0	6.5	0	0
<i>Staphylococcus</i> 1	0	0	0	6.5	0	0
<i>Staphylococcus</i> 2	0	0	0	6.5	0	0
<i>P. aeruginosa</i>	4.5	4.5	0	0	<1	0
<i>Pseudomonas</i> 1	4.5	4.5	0	0	<1	0
<i>Pseudomonas</i> 2	4.5	4.5	0	0	<1	0
<i>E. coli</i> [NCTC 10418]	0	0	0	0	1	0
Coliform 1	0	0	0	0	<1	0
Coliform 2	0	0	0	0	<1	0
Coliform 3	0	0	0	0	<1	0
Coliform 4	0	0	0	0	<1	0
Coliform 5	0	0	0	0	<1	0
Coliform 6	5.0	5.0	0	0	<1	0
Coliform 7	0	0	0	0	<1	0
Coliform 8	0	0	0	0	<1	0
Coliform 9	4.5	4.5	0	0	1	0

(R1)-*Tephrosia* 3 months old root extract, (R2)-*Tephrosia* 5 months old root extract, (L)-*Tephrosia* -5 months old leaf extract, and (M) *Mimusops* bark extract. (D) DMSO and (W) Sterile distilled water

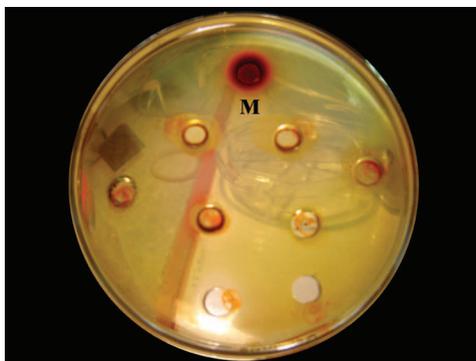


Figure 1: Inhibition of *S. aureus* [NCTC 6571] around the well, loaded with the bark extract of *Mimusops* (M) – The transparent concentric area around the well signifies the absence of bacterial growth. Brick colour around the well is due to the diffused extract. The remaining wells which were loaded with the extracts of *Tephrosia* and the two controls show no inhibition.

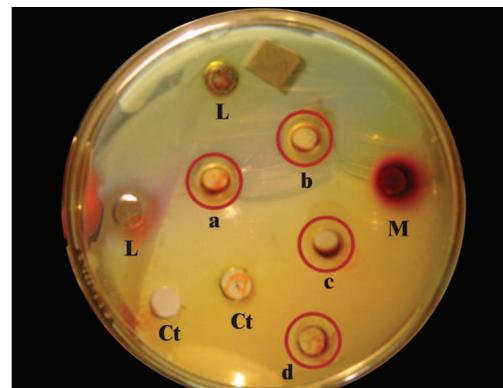


Figure 2: Inhibition of *P. aeruginosa* [NCTC 10662] by the root extracts of *T. purpurea*. [a, b, c and d marked in red circles are wells loaded with the root extracts]. The remaining wells (L) which were loaded with the leaf extracts of *Tephrosia*, bark extract of *Mimusops* (M) and the two controls (Ct) show no inhibition.

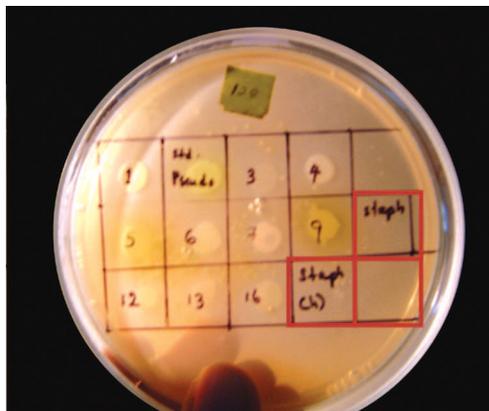


Figure 3: Plate containing 128 mg/L of *Mimosops* bark extract, showing absence of growth of three *Staphylococcus* strains on partitions marked in red. Other partitions containing all other isolates indicate growth.

DISCUSSION

In the ayurvedic and the traditional medicinal systems of Sri Lanka, the roots of *T. purpurea* are used for the treatment of the diseases of gum, spleen, liver and skin. In both medicinal systems, the decoction is prepared by boiling the roots in water. However, according to the test results, the water extract of roots, leaves and pods of *T. purpurea* did not seem to possess considerable antimicrobial properties on any of the pathogens tested. The belief is that it is not the antimicrobial property of *Tephrosia* which is employed in traditional medicine, rather the astringent, antipyretic, anthelmintic, diuretic and laxative properties (*Harischandra Wijesinghe, personal communication*).

However, the ethanolic root extracts showed significant antimicrobial activity against the three *Pseudomonas* strains. The three strains of the coliforms tested were not inhibited by the water extract. The reason for the water extract not to be effective could be due to the heat sensitivity of the antibiotic compound, which gets destroyed during the process of boiling. Another possible reason is that the antibiotic compound is more soluble in alcohol than in water (due to higher polarity). This is supported by the fact that there was a minute zone of inhibition on both *Pseudomonas* and *E. coli* even with the water extract of the roots.

The inhibition of *Pseudomonas* and coliforms by the *Tephrosia* ethanolic root extract was however less than the inhibition of *Staphylococcus* by the *Mimosops* extract. A considerable activity of both aqueous and

alcoholic extracts of *M. elengi* against several bacterial strains including *S. aureus* has been revealed⁴. Here, the activity of the water extract has been shown to be greater than that of the ethanolic extract against *Alcaligenes faecalis*. The significant antibiotic activity shown by *Mimosops* bark extract against three *Staphylococcus* strains in the current study may bring a ray of hope for MRSA (Methicillin-Resistant *Staphylococcus aureus*) infections. Further studies will have to be carried out with the purified active compound and tested on MRSA strains for antimicrobial activity. In a similar study plant extracts of *Bidens pilosa*, *Bixa orellana*, *Cecropia peltata*, *Cinchona officinalis*, *Gliricidia sepium*, *Jacaranda mimosifolia*, *Justicia secunda*, *Piper pulchrum*, *Polygala paniculata* and *Spilanthes americana* antimicrobial activity against various pathogens including *S. aureus*, *E. coli* and *Candida* spp. has been shown. However, the extracts have been ineffective against *P. aeruginosa* and *Streptococcus* spp.¹⁵ Literature cites antimicrobial action of *T. purpurea* against acne-inducing *Propionibacterium acnes* and *Staphylococcus epidermidis*¹. However, the MIC values obtained, are much higher than the values obtained in the current study, viz 675 mg/L and 2500 mg/L respectively.

The MIC, of all the extracts exhibiting antimicrobial activity were found to be 128 mg/L; i.e. the highest concentration used. However, this value was obtained for crude extracts. For more accurate determination of the MIC, the active compound has to be isolated. A much lower MIC can be expected in such purified extracts. The MIC of Ciprofloxacin on *P. aeruginosa* (ATCC 27853) is 0.25 mg/L and that of Vancomycin on *S. aureus* (ATCC 25923) is 0.5 mg/L¹⁴. It is reported by Abu-Shanab *et al.* that the crude extracts of different plants (*Althaea officinalis*, *Mentha longifolia*, *Melissa officinalis*, *Rosa damascena*) showed antibiotic activity against MRSA with the most potent extract with an MIC in the range of 395 to 780 mg/L¹⁶. Accordingly the results of this study indicate that *T. purpurea* and *M. elengi* extracts have considerable promise to be used as antimicrobial agents. *T. purpurea* and *M. elengi* could be used to isolate new bio-active natural products that may lead to the development of new pharmaceuticals that address therapeutic needs. For the exploitation of the antimicrobial activity of these extracts, the isolation and chemical characterisation of these compounds need to be carried out. Subsequent to such studies the extracts of *T. purpurea* and *M. elengi* could be incorporated into products such as tablets, other forms of drugs, disinfectants, tooth paste etc.

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