Activity of extracts from two *Eysenhardtia* species against microorganisms related to urinary tract infections

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The genus *Eysenhardtia* comprises 14 species[1,2] and some of its members, including *Eysenhardtia polystachya* and *E. texana*,[1,3] have been used in traditional medicine for the treatment of kidney and bladder infections.

E. polystachya is a tree used widely in traditional Mexican medicine as a herbal remedy and is native to north-eastern Mexico, especially in the Nuevo Leon and Tamaulipas states.[4] The *E. texana* tree is also known as kidneywood because of its use for kidney and bladder complaints. This tree is distributed throughout south-central USA and northern and central Mexico.[5]

Previous studies on *Eysenhardtia* species have highlighted the hypoglycaemic activity of *E. polystachya*[6] In another study, Wächter *et al*. isolated two flavanones together with a known flavanone from a methanol-dichloromethane extract obtained from the aerial parts of *E. texana*. These compounds show activity against *Staphylococcus aureus* and *Candida albicans*.[7]

The aim of the present study is to evaluate the antimicrobial activity of methanolic extracts of *E. polystachya* and *E. texana* against nine species of bacteria and one species of yeast related to urinary tract infections.

Plants were selected following information received and focused on the Fabaceae used in Mexican traditional medicine. Dried leaves of *E. polystachya* and *E. texana* (Fabaceae) were collected in Nuevo Leon state in Mexico. Marco Antonio Guzmán authenticated the plants and voucher specimens are kept in the herbarium of the Facultad de Ciencias Biológicas, Universidad Autonoma de Nuevo León (voucher herbarium specimen number: Guzman 024246). The local ethical committee approved the study.

Leaves from *E. polystachya* and *E. texana* were harvested, cleaned, air-dried and stored in the dark. The leaves of each plant were extracted with methanol at room temperature for seven days. The filtrates obtained were evaporated to dryness using a rotary evaporator. Stock solutions of organic extracts were prepared in methanol at a concentration of 25 mg/mL. The yield of dried extract from the fresh plant material was 6.2 % for *E. polystachya* and 7.4% for *E. texana*.

Clinical isolates of *Escherichia coli*, *Proteus mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *S. epidermidis* and *C. albicans* were used. Using 24-h cultures, a cell suspension was prepared and turbidity was adjusted to McFarland 1 standard corresponding to 10^6 colony forming units (cfu)/mL.

The agar well-diffusion method was used as an initial screening assay of antimicrobial activity,[8] pouring 50 µL methanolic extract at a concentration of 25 mg/mL. C. Rivas medium was used.[9] Gentamicin (40 µg/mL) was used as the positive control and water as the negative control. Plates were incubated under aerobic conditions at 37°C for 24 h. All tests were carried out in triplicate.

The minimum inhibitory concentration (MIC) was evaluated on plant extracts that showed antimicrobial activity using the broth microdilution method at eight concentrations for each extract (6250, 3125, 1565, 780, 390, 195, 100 and 50 µg/mL). Bacteria were also incubated in culture medium alone to assure viability of the strains. The MIC was defined as the lowest concentration of compound that completely inhibited growth of the organism in the microdilution wells. *S. aureus* ATCC 29213 was included as a control strain.

Phytochemical screening was performed using biochemical tests. The Dragendorff method detected alkaloids; Shinoda's and sulphuric acid tests for flavonoids; the Salkowski test for sterols and methyl sterols; Baljet for terpenoid lactones; potassium permanganate and Br_2/CCl_4 for carbon-carbon double bonds; ferric chloride for phenolic compounds; 2,4-dinitrophenylhydrazine for carbonyl groups; and the Molisch test for carbohydrates. Saponins were detected by haemolytic activity, coumarins with the sodium hydroxide test, and triterpenes and steroids with the Liebermann Burchard test.

Methanolic extracts of *Eysenhardtia polystachya* and *E. texana* were active against *S. epidermidis* (mean inhibition: 1.75 cm for both extracts). In addition, *E. polystachya* was active against *Proteus vulgaris* (mean inhibition: 1.75 cm), *Enterbacter aerogenes* (mean inhibition: 1.75 cm) and *S. aureus* (mean inhibition: 1.25 cm). No activity was detected for methanolic extracts of *Eysenhardtia polystachya* and *E. texana* against *Escherichia coli*, *P. mirabilis*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Serratia marcescens* and *C. albicans*.

Minimum inhibitory concentrations for extracts against bacteria that demonstrated sensitivity in the initial screening were determined (Table 1). Methanolic extract of *Eysenhardtia polystachya* against the selected bacteria ranged from 390 to 3125 µg/mL and the methanolic extract of *E. texana* was active against *Staphylococcus epidermidis* at a concentration of 1565 µg/mL.

Activity of crude extracts as high as 6250 µg/mL are considered acceptable,[10,11] and thus the activity observed for *E. polystachya* against *S. epidermidis* (390 µg/mL) is quite good, considering that a crude extract was used and the active compound should be diluted. It is possible that isolation of the active compound or compounds will provide lower MIC values.

The Liebermann-Burchard, Salkowski, sulphuric acid and Wagner tests were negative for both extracts, and the Shinoda, potassium permanganate, ferric chloride and Br_2/CCl_4 tests were positive for both extracts. Baljet was positive for *E. polystachya* and negative for *E. texana*, and the Molish and sodium hydroxide tests were positive for *E. texana* and negative for *E. polystachya*. These results indicate the presence of flavonoids, carbon-carbon double bonds and phenolic compounds in both extracts. Terpenoid

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Table 1. Minimal inhibitory concentrations for gentamicin and each plant extract against selected bacteria.

	E. aerogenes	*S. aureus*	*P. vulgaris*	*S. epidermidis*	*S. aureus* ATCC 29213
Gentamicin (μg/mL)	<50	1565	390	780	780
E. polystachya (μg/mL)	3125	3125	1565	390	3125
E. texana (μg/mL)	ND	ND	ND	1565	3125

ND: not determined

lactones in *E. polystachya* and carbohydrates and coumarins in *E. texana* were also detected.

The bacteria included in this study are the most frequent causal agents of urinary tract infection. According to the results of this study, methanolic extract of *E. polystachya* has broad-spectrum effect because the antibacterial activity observed included both Gram-positive and Gram-negative organisms.

Wächter *et al.*, using a methanol-dichloromethane extract obtained from the aerial parts of *E. texana*, isolated two new antibacterial and antifungal flavanones together with a known flavanone.[7] According to the results of the present study, methanolic extracts of *E. texana* have only limited antibacterial activity (only against *S. epidermidis*). No other antimicrobial activity for compounds isolated from *E. texana* has been reported.

The present results suggest that a methanolic extract of *E. polystachya* is a good alternative to other antibacterial compounds and underlines the importance of screening plant extracts in the search for new agents.

Finally, according to phytochemical screening, the *E. polystachya* extract contains flavonoids, terpenoids, carbon-carbon double bonds and phenolic compounds. Based on these results, the isolation and characterisation of active compounds from *E. polystachya* is particularly important in light of the multidrug resistance observed in certain Gram-positive and Gram-negative bacteria,[12,13] against which only a few therapeutic options are available.

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Absence of intervening sequences (IVSs) in helix 11 region within 16S rRNA genes among more than 240 isolates of the seven *Campylobacter* species

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Thermophilic *Campylobacter jejuni* and *C. coli* are curved Gram-negative bacteria that are the most recognised cause of acute bacterial diarrhoea in the Western world. Infrequently, human illness is associated with *C. lari*, *C. upsaliensis* and *C. fetus*.[1,2] The genus *Campylobacter* belongs to the ε-subdivision of the Proteobacteria.

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