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SHORT COMMUNICATION

Antibacterial activity of native plants from Northwest Argentina against phytopathogenic bacteria

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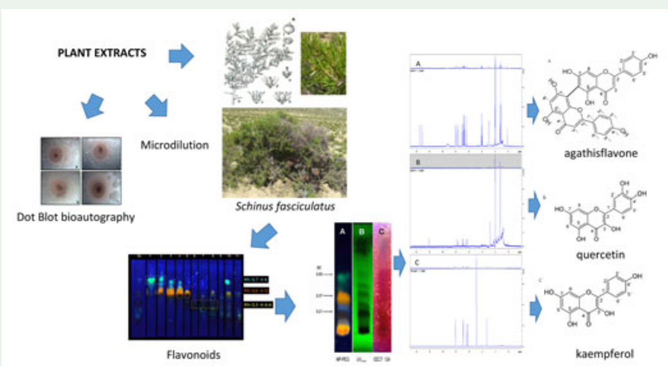
ABSTRACT

Extracts from aerial parts of medicinal plants from northwest Argentina were screened for antibacterial activity against the phytopathogenic strains namely CECT 124 (*Pseudomonas corrugata*), CECT 126 (*P. syringae* pv. *tomato*), CECT 225 (*Erwinia carotovora* var. *carotovora*), CECT 472 (*Agrobacterium tumefaciens*) and CECT 792 (*Xanthomonas campestres* pv. *vesicatoria*). Leaves and stems of *Aspidosperma quebracho-blanco*, *Schinus fasciculatus*, *S. gracilipes*, *Amphilophium cynanchoides* and *Tecoma stans* were separately extracted with solvents of increasing polarity to obtain the dichloromethane (fCH_2Cl_2), ethyl acetate (fEtAc) and methanol extracts (fMeOH), respectively. Among the thirty extracts tested, only fEtAc from leaves and stems of *S. fasciculatus* reached the IC_{50} against the five bacterial strains tested ($IC_{50} = 0.9$ mg/ml). The fEtAc from the leaves contained kaempferol, quercetin and agathisflavone which had moderate to strong antibacterial activity. This extract and its identified flavonoids showed synergic (CECT 124,126 and 792) or additive effects (CECT 472 and 225) in mixtures with Kocide 3000.



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1. Introduction

Bacterial diseases are major constraints on crop production and cause significant annual economic losses worldwide (Sampietro et al. 2016). Most phytopathogenic bacteria are Gram-negative bacilli which can enter into the leaves, stems or roots through natural openings such as the stomata or the lenticels, or be transmitted by piercing-sucking phytophagous insects (Tournas 2005). They cause a diverse range of damages on the crop plants including wilt (i.e. *Pseudomonas syringae* and *P. corrugata*), necrosis (i.e. *Xanthomonas campestris*), soft rot (i.e. *Erwinia carotovora*), and tumours (i.e. *Agrobacterium tumefaciens*) (Sampietro et al. 2016). Their control is mainly based on the preventive application of copper-containing compounds and antibiotics (Kumar et al. 2005). The appearance of resistance has led to the application of higher doses of these products which in turn often generated adverse impacts on the crop plants as well as on the environment (McLeod et al. 2017). The extensive use of antibiotics on phytopathogenic bacteria is hampered by the risk of introducing resistance to mammalian bacterial pathogens (Kumar et al. 2005). New antimicrobials, or additives to commercial ones, are needed in order to solve this problem. Plant extracts or its constituents might be used to either replace or reduce the doses of commercial biocides currently applied for control of the phytopathogenic bacteria (Sampietro et al. 2016). In this context, the aim of this work was to identify antibacterial extracts from aerial parts of native plants of Northwest Argentina and their main bioactive constituents.

2. Results and discussion

The leaf extracts from the *Schinus* species showed the highest yields in dry matter after extraction with dichloromethane (3.03%, *S. fasciculatus*; 2.89%, *S. gracilipes*), ethyl acetate (2.08%, *S. fasciculatus*; 2.51%, *S. gracilipes*) and methanol (11.37%, *S. fasciculatus*; 11.25%, *S. gracilipes*). The yields indicated that polar constituents were extracted in higher amounts than middle or low polarity compounds (Table S1). The same was reported for other *Schinus* species where the polar solvents yielded four or five times more dry leaf matter than non-polar solvents (Salem et al. 2018). The yields also suggested that the antibacterial activity of the aerial parts of the investigated plants was mostly provided by middle polarity compounds. From 46 IC_{50} values recorded, 23 were obtained for fEtAc extracts (Table S2). Among the thirty extracts assayed, only the fEtAc extracts from leaves and stems of *S. fasciculatus* reached the IC_{50} on the five bacterial strains ($IC_{50} = 0.9–3.7$ mg/ml). They also were the only ones that suppressed the growth of all the bacterial strains by dot blot assays (DI =1.9–4.8 mm) (Table S3). TLC analyses and bioautographies of the leaf and stem extracts of *S. fasciculatus* indicated antibacterial activity at relation-to-front values of 0.21 (B_1), 0.37 (B_2) and 0.55 (B_3) on all the bacterial strains. The inhibitory zones matched with the presence of flavonoid compounds (Figure S1). A bioassay guided isolation of B_1, B_2 and B_3 led to the antibacterial fractions F_6, F_4 and F_1, respectively. UV-VIS and NMR analyses indicated that kaempferol and a dimer of apigenin named agathisflavone were the main constituents of F_1 and F_6, respectively, while quercetin was one of the constituents of F_4 (Table S4, Figure S2 and S3). Dot blot assays indicated that agathisflavone, quercetin and kaempferol inhibited all bacterial strains, with diameters in the ranges of 2–3 mm, 2–5 mm and 3–4 mm, respectively. Kaempferol was the flavonoid with the strongest antibacterial activity in microdilution assays (MIC =250 μg/ml, CECT 124 and 472; MIC =500 μg/ml, CECT 126 and 792; MIC =2000 μg/ml, CECT 225), followed by quercetin (MIC =1000 μg/ml, CECT 124, 126 and 792; MIC =2000 μg/ml, CECT 472 and 225) and agathisflavone (MIC =2000 μg/ml, all strains) (Table S5). Based on previous reports, the isolated flavonoids showed strong (MIC $<$500 μg/mL) and moderate (MIC =600–1500 μg/mL) antibacterial activity which varied not only with the flavonoid assayed but also with the bacterial strains tested (Sampietro et al. 2016). The mechanisms of action of flavonoids and Kocide 3000 potentiated (synergized) each other on CECT 124, 126 and 792, and did not interact (additive effect) in the growth inhibition observed for CECT 472 and 225 (Table S6). The reasons of these joint actions are hard to unravel because both the flavonoids and Kocide 3000 were reported to exert their antibacterial effect by multiple cellular targets of action. Kocide 3000, as other cupric agents, release copper ions which catalyse the production of deleterious reactive oxygen species (ROS), and forms extremely stable complexes with several cellular components (Stevenson et al. 2013). Quecetin and kaempferol tested on human pathogenic bacteria such as *Staphilococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, damaged the membrane fluidity and potential which in turn should inhibit the activity of several enzymes and should reduce ATP synthesis, membrane transport and bacterial motility (Cushnie and Lamb 2005; Tsuchiya 2010; Calderón-Montaño et al. 2011; Plaper et al. 2003). In the case of agathisflavone, it was reported as a neuroprotective and antioxidant agent but not as an antibacterial compound (dos Santos Souza et al. 2018).

3. Conclusion

The leaf and stem extracts of *S. fasciculatus* showed the widest antimicrobial activity on the bacterial strains tested. Quercetin, kaempferol and agathisflavone are antibacterial constituents of the leaf extract. The combination of cupric agents and the identified compounds or the leaf extract may be a new strategy for control of bacteria such as *A. tumefaciens*, *P. corrugata*, *P. syringae*, *E. carotovora* and *X. campestris*.

Disclosure statement

No potential conflict of interests was reported by the authors.

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