Two new cassane diterpenenoids from *Calliandra californica* Benth. (Fabaceae) with antituberculous activity

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Introduction

Baja California Sur represents an enormous resource of medicinal plants that remain unexplored for their diversity of active components. In this state the flora is diversified because of the wide variation in altitude and climate.

Fig. 1. State of Baja California Sur. México

C. californica is a medicinal plant locally known as ¨Tabardillo¨, ¨Zapotillo¨ and ¨Pelo de Angel¨ (Fig. 2). Decoction of the flower, root, or branches, together or separated, is used to treat kidney ache, cystitis, urethritis, prostate inflammation, fever, tooth-ache and cramps (1). Previous studies from the aerial parts of the plant demonstrated the presence of two new flavones: 7, 2′, 4′, 5′-tetramethoxyflavone and 5-hydroxy-7, 2′, 4′, 5′-tetramethoxyflavone. This last flavone showed activity against *Staphylococcus aureus* and *Bacillus subtilis* (2).This work deals with the bioassay-guided fractionation of the ethyl acetate extract by MDA and the structure determination of two new cassane diterpenoids that were active against *M. tuberculosis.*

Fig 2. *Calliandra californica*: *¨Tabardillo¨, ¨Pelo de Angel¨*

Materials and methods

Plant Material: *C. californica* was collected in Todo Santos, Baja California Sur, México, on 21 Mar 2002 and authenticated at the Agronomy Department of the Autonomous University of Baja California Sur (UABCS) according to Wiggins (1980). A voucher specimen was deposited at the Pharmacognosy Laboratory of the Department of Agronomy of UABCS.

***Extraction and Isolation*:**

The root of *C. californica* was exhaustively extracted successively with hexane, ethyl acetate, and ethanol at room temperature. The three crude extracts were evaluated in the antimicrobial assay against *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis,* and *Candida albicans* by ADM. The ethyl acetate extract showed the most prominent antimicrobial activity and was subjected to the bioassay-guided fractionation of their bioactive constituents (Fig. 3).

Antimicrobial Assay

The antimicrobial activity of extracts and fractions were determined by the Agar Diffusion Method (Fig. 4).

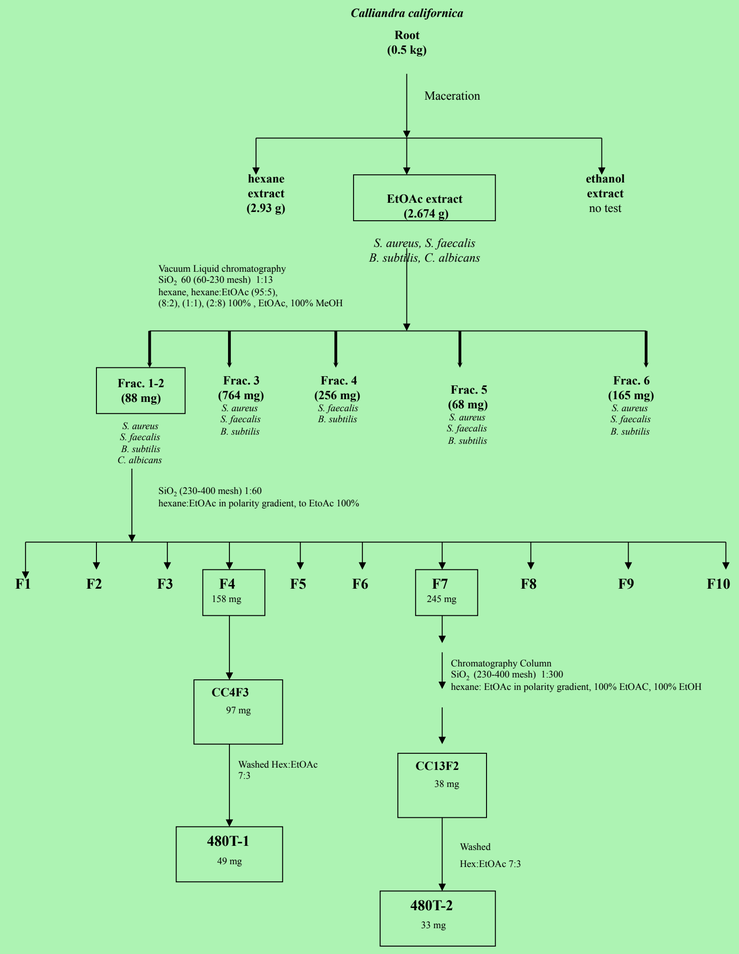


Fig. 3. Isolation of cassane diterpenoids

Bacteria Gram (+):
Staphylococcus aureus (LAEDMLP)
Streptococcus faecalis (SCRIPPS)
Bacillus subtilis (SCRIPPS)

Bacterias Gram (-):
Escherichia coli (ATCC25922)

Fungie :
Candida albicans (SCRIPPS)

Culture medium:
Dextrose Sabouraud Broth
Agar Mueller Hinton

Positive control:
Chloramphenicol (30mg)
Eritromicin (15m)
Ketoconazol (16mg)
Concentration: 2.0 mg / disk

Concentration:
2.0 mg / disk

Negative Control:
Solvents used in the preparations.

Fig.4. Agar Difusióçn Method (ADM)

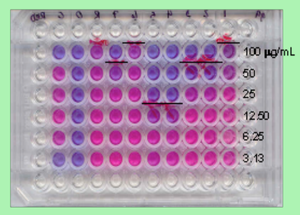
Microplate Alamar Blue Assay

In this study were used two species: *M. tuberculosis* H37Rv (ATTC Cat. No. 27294) and *M. tuberculosis* CIBIN/UMF15:099 (isolated in the Mycobacteriology Laboratory of the Centro de Investigación Biomédica del Noreste del Instituto Mexicano del Seguro Social in Monterrey, N.L., México from a patient having advanced pulmonary tuberculosis). The H37Rv strain is sensitive to streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide, but the CIBIN/UMF15:99 strain is resistant to these compounds.

The Minimum Inhibitory Concentration (MIC) of Streptomycin, was greater than 8.0, 2.0, 1.0, and 32 µg/mL, whereas using the conventional BACTEC 460-radiometric system, the CIBIN/UMF15:99 strain was resistant to the critical antimicrobial concentration of streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide. Plasticware and chemicals (reactive grade) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Growth conditions and inoculum preparation.

The *Mycobacterium* strains were cultured at 37 °C in a Middlebrook 7H9 broth supplemented with Oleic Acid-Dextrose-Catalase enrichment until log-phase growth was achieved. The inoculum for the microcolorimetric assay was prepared by diluting log-phase growth cultures with sterile Middlebrook 7H9 broth to McFarland No. 1 turbidity standard and then further diluted 1:50 to have 6 X 10^6 colony-forming units/mL. This suspension was prepared for the microcolorimetric assay on a microplate just before inoculation (Fig 5).



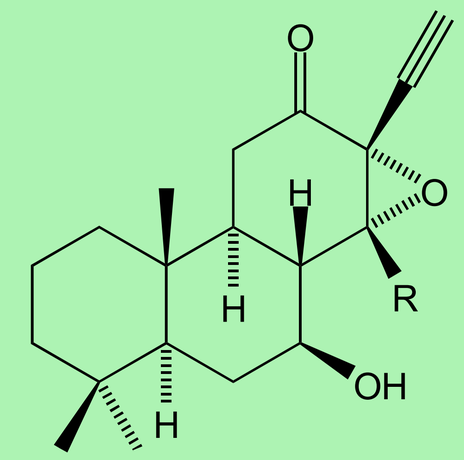
samples	MIC
1.498 TR EtOH extract	>100
2.452 TH EtOHextract	100
3.452 TS EtOH extract	100
4.480 T-1	25
5.488 TF CH_2Cl_2 extract	25
6.488 TF EtOH extract	>100
7.488 TR hexane extract	100
R, rifampicin	
O, ofloxacin	
C, controls: culture medium	

Fig 5. Antituberculosis activity Test:

Results

The ethyl acetate extract of the root of *C. californica* showed activity against *S. aureus, B. sutilis, S faecalis,* and *C. albicans*. The bioassay-guide fractionation by MDA resulted in the isolation and characterization of two new cassane-type diterpenoids **(1**, **2),** which showed relevant activity against *M. tuberculosis*. The MICs of **1** were 25 µg/mL for *M. tuberculosis* H37Rv strain and 12.5 µg/mL for the resistant clinical isolated and for **2** were 50 µg/mL for the H37Rv strain and 100 µg/mL for the resistant strain. These results indicated that the less polar compound **(1)** is the more active one.

The new cassane-type diterpenoids were characterized in the basis of extensive spectroscopic (ID and 2D NMR) analyses and by X-ray analysis for **2**.



1 R = CHO **2** R = CH_2OH

References

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2.- Encarnacion-Dimayuga, R., Ochoa, N., Anthoni, U., Christophersen, C. and Nielsen, P.H. 1994. Two new flavones from Calliandra californica. Journal of Natural Products. 57(9) 1307-1309

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