Antimicrobial activity of some endemic Canary plants

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INTRODUCTION

Ceropegia fusca Bolle (*Asclepiadaceæ*), *Hypericum reflexum* L.f. (*Hypericaceæ* or *Guttiferæ* or *Clusiaceæ*), and *Maytenus canariensis* (loes) Künkel & Sunding (*Celastraceæ*) are three endemic species from the Canary Islands that are used in the Islands popular medicines as healing balms.

Ceropegia fusca Bolle, this is an endemic plant of the Canary Islands (Tenerife and Gran Canaria) Known by the popular names of "cardoncillo" or "mataperros".[1] Together with other *Ceropegia* specie (*Ceropegia dichotoma* Haw and *Ceropegia Kranzii* Svet), cicatrizants and vulnerary properties have been attributed to its popularity.[2] The part of this plant used for these aims is the stem. It is administered topically.

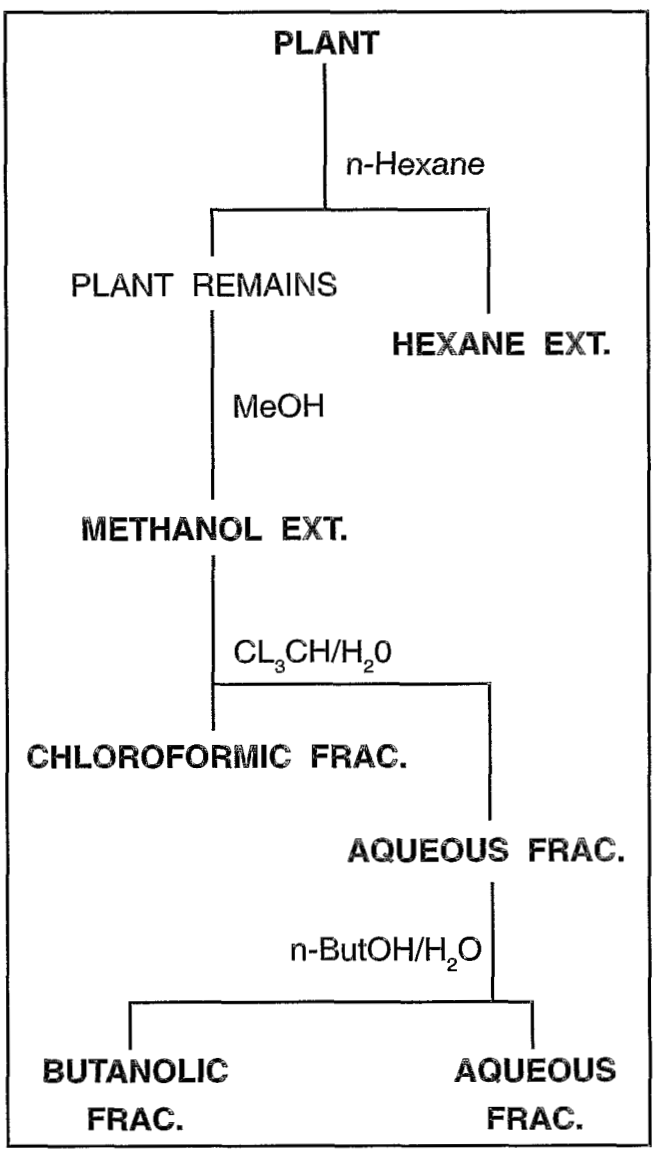
Hypericum reflexum L. f., this is another endemic plant of the Canary Islands (Tenerife, Gran Canaria, Gomera, Hierro and La Palma) known popularly as "cruzadilla" or "hierba cruz de los islenos".[1] It is employed as a vulnerary agent and for this the aerial part is employed.[3] It is used as an infusion or poultice.

Maytenus canariensis (Lœs.) K & S, a canary endemic plant (Fuerteventura, Gran Canaria, Tenerife, Gomera, Hierro and La Palma). It is the only representative of this family in the Archipelago. This plant is known by the popular name of "peralillo"[1] and some medicinal properties have been attributed to it (antirheumatic, emollient, treatment of cutaneous abscess and papillomas).[3] The parts used are the leaves and fruits and it is used as an infusion or poultice. The chemical composition of this plant was already studied and this study reported quinonic nortriterpenes and triterpenes type fridelane as the main products.[4,5]

The aim of this study is to determine their possible antimicrobial activities (antibacterial and antifungal), so as to co-relate this activity with the traditional use and, therefore, justify their employment with these objectives.

Plocama pendula Ait. (*Rubiaceæ*) is the last species of this work, and it has been chosen based on its chemical composition, since the presence of anthraquinonic type principals, make us think in the presence of an antimicrobial type activity.[7]

Scheme 1
Preparation of extracts
(in bold types are shown extracts to assay)

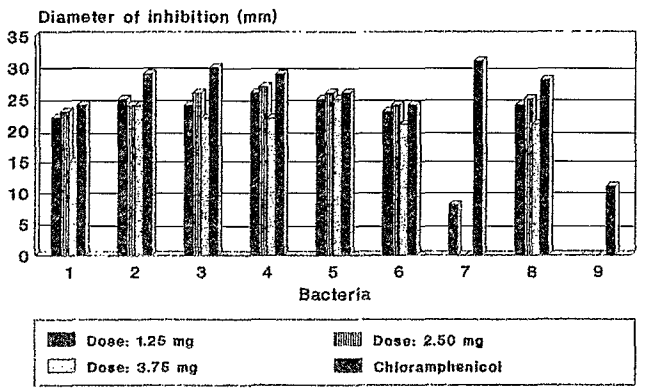


This is the only species of the genus and an endemic plant of Canary Archipelago known popularly as "balo".[1,2,6] A shrub with a deep smell in its branches. There is little information about its uses. This species was studied before from a chemical point of view reporting anthraquinonic substances as the products of interest.[7]

An antimicrobial study was carried out by the dilution and difusion method.[6,8]

Fig. 1

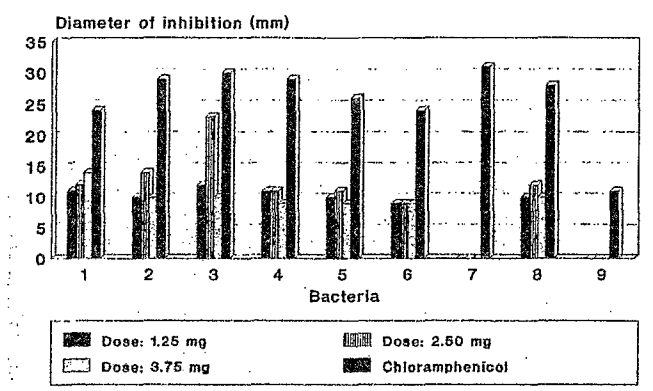
Hypericum reflexum (Methanolic Extract)



(1): *B. cereus*; (2): *B. subtilis*; (3): *B. bronchiseptica*; (4): *M. luteus*; (5): *S. aureus*; (6): *S. epidermidis*; (7): *E. coli.*; (8): *K. pneumoniæ*; (9): *P. æruginosa.*

Fig. 2

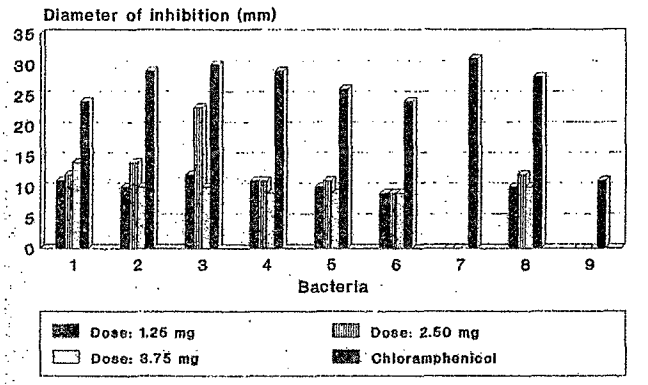
Hypericum reflexum (Chloroformic Extract)



(1): *B. cereus*; (2): *B. subtilis*; (3): *B. bronchiseptica*; (4): *M. luteus*; (5): *S. aureus*; (6): *S. epidermidis*; (7): *E. coli.*; (8): *K. pneumoniæ*; (9): *P. æruginosa.*

Fig. 3

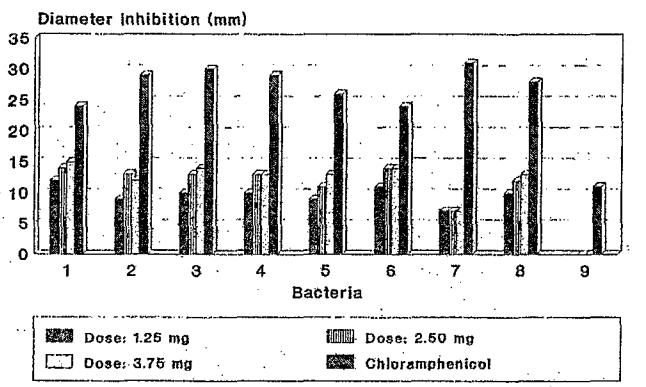
Hypericum reflexum (Butanolic Extract)



(1): *B. cereus*; (2): *B. subtilis*; (3): *B. bronchiseptica*; (4): *M. luteus*; (5): *S. aureus*; (6): *S. epidermidis*; (7): *E. coli.*; (8): *K. pneumoniæ*; (9): *P. æruginosa.*

Fig. 4

Maytenus canariensis (Methanolic Extract)



(1): *B. cereus*; (2): *B. subtilis*; (3): *B. bronchiseptica*; (4): *M. luteus*; (5): *S. aureus*; (6): *S. epidermidis*; (7): *E. coli.*; (8): *K. pneumoniæ*; (9): *P. æruginosa.*

MATERIAL AND METHODS

PLANT MATERIAL

The plants chosen were collected in April 1991 in Tenerife (Canary Islands-Spain); *Ceropegia fusca* Bolle in a place known as "Montaña de los Riscos" (San Isidro, 200-300 m), *Hypericum reflexum* in "Barranco de Badajoz" (Güímar, 600 m), *Maytenus canariensis* in "Barranco de Badajoz" (Güímar, 500-600 m) and *Plocama pendula* in "Barranco de San Andrés" (San Andrés, 20 m).

PREPARATION OF EXTRACTS

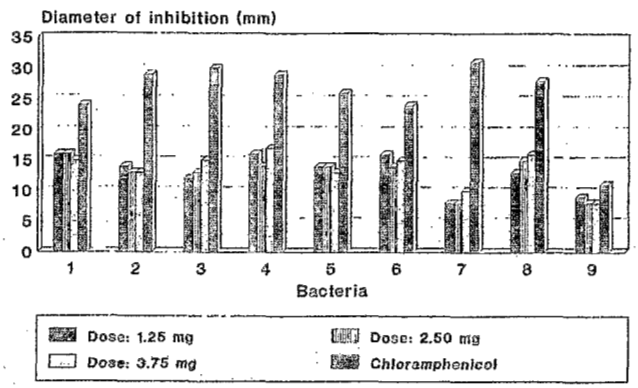
The plants, previously dried and powdered, were macerated sequentially in n-hexane and methanol, three days, each time, at room temperature. The resulting extracts being filtered, joined together and dried under pressure at a temperature below 45 °C.

Afterwards with the methanol extract a distribution Cl_3CH/H_2O was made and the chloroformic and aqueous fractions obtained. Finally the aqueous fraction was again subjected to a distribution n-ButOH/H_2O (Scheme 1).

An infusion was prepared with 100 g of leaves and 1000 ml of water and the volume reduced until finally adjusted to a concentration of 1 g/ml based on the weight of starting dry material.

Fig. 5

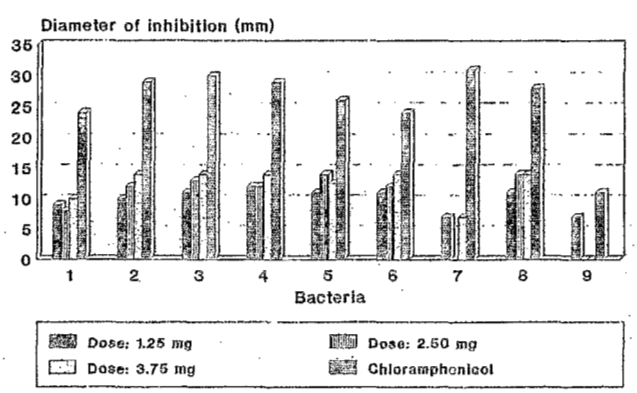
Maytenus canariensis (Butanolic Extract)



(1): *B. cereus*; (2): *B. subtilis*; (3): *B. bronchiseptica*; (4): *M. luteus*; (5): *S. aureus*; (6): *S. epidermidis*; (7): *E. coli.*; (8): *K. pneumoniæ*; (9): *P. æruginosa.*

Fig. 6

Ceropegia fusca (Hexanic Extract)



(1): *B. cereus*; (2): *B. subtilis*; (3): *B. bronchiseptica*; (4): *M. luteus*; (5): *S. aureus*; (6): *S. epidermidis*; (7): *E. coli.*; (8): *K. pneumoniæ*; (9): *P. æruginosa.*

ANTIMICROBIAL ACTIVITY

Preparation of samples: In the study of the antimicrobial activity, the different extracts and fractions were diluted in dimethylsulfoxide (DMSO). For each extracts three concentrations were preparated: 125, 250 and 375 mg/ml, and blank disks were impregnated with 10 µl of sample (final doses obtained: 1.25, 2.50 and 3.75 mg).

Micro-organisms: The following's strains were used as test organisms:

Bacteria: *Klebsiella pneumoniæ* (CECT 440), *Pseudomonas aeruginosa* (CECT 110), *Staphylococcus aureus* (CECT 240), *Staphylococcus epidermidis* (CECT 231), *Bordetella bronchiseptica* (CECT 142), *Micrococcus luteus* (CECT 247), *Bacillus cereus* var. *mycoides* (CECT 193), *Bacillus subtilis* (CECT 356), *Escherichia coli* (CECT 405).

Fungi: *Candida albicans* (CECT 1001), *Candida tropicales* (CECT 1400), *Candida guillermondi* (CECT 1019), *Saccharomyces cerevisæ* (CECT 1193), *Cryptococcus albidus* (CECT 1081).

Method: The antibacterial and antifungal effects were tested by the disk-diffusion[8] and the microdilution in microplaques[9] methods.

In the first method, blank disks impregnated with DMSO were used as negative controls and disks of Chloramphenicol (30 µg-DIFCO) and Amphotericine (100 µg-PASTEUR) as positive controls.

RESULTS AND DISCUSSION

Graphic results of a diffusion-disk method are shown in Figures 2-7. Results express the diameter of inhibition in mm of the principal extracts against different germs at several doses (1.25, 2.50 and 3.75 mg). Only active extracts are shown in these figures. Likewise, in Table I the MIC values (dilution method) of those extracts, expressed in mg/ml, are presented.

From the four plants tested, only *Plocama pendula* did not show antimicrobial activity. The others were active but only as antibacterian, not showing a significantly antifungal activity. This study was carried out by the dilution and diffusion method and the results from both tests were in agreement.

Hypericum reflexum was the most powerful in all assays the highest inhibitory activity being for the germens G(+) and not the G(–). The highest activity was presented by methanolic

extract with similar values as those of chloramphenicol. The results obtained by the chloroformic and butanolic fractions were lower than those presented by methanolic extract. This fact seems to suggest that the responsible compounds of the antimicrobial activity are of different types. The hexane extract did not show any activity.

The assay of the butanolic and chloroformic fractions from methanol extracts of *Maytenus canariensis* showed that the responsible active principles of the antimicrobial activity are in the butanolic fraction because it is that which presents the largest activity. The hexane extract did not inhibit the growth of the germs.

Ceropegia fusca showed activity but only in its hexane extract.

It may be concluded that the results obtained in this study show that the three plants have an interesting antimicrobial activity, which could well justify their use in popular medicine as vulneraries, cicatrizants and antiulcerogenics agents.

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Table 1

MIC values for the different extracts

	MIC (mg/ml)					
Bacteria	A	B	C	D	E	F
Bacillus cereus	1.56	0.05	0.4	0.4	0.2	0.2
Bacillus subtilus	6.25	0.05	0.78	0.05	0.4	0.1
Micrococcus luteus	25	0.05	0.4	0.2	0.2	0.2
Staphylococcus aureus	0.78	0.05	0.2	0.05	0.05	0.05
Staphylococcus epidermidis	3.125	0.05	0.2	0.4	0.2	0.1
Bordetella bronchiseptica	3.125	1.56	3.125	0.2	0.04	0.05
Escherichia coli	12.5	1.56	6.25	6.25	1.56	1.56
Klebsiella pneumoniæ	3.125	0.05	0.78	0.1	0.05	0.1
Pseudomonas æruginosa	3.125	12.5	25	6.25	3.125	3.125

(A) = Hexane extract from *Ceropegia fusca.*
(B) = Methanolic extract from *Hypericum reflexum.*
(C) = Chloroformic fraction from *Hypericum reflexum.*
(D) = Butanolic fraction from *Hypericum reflexum.*
(E) = Methanolic extract from *Maytenus canariensis.*
(F) = Butanolic fraction from *Maytenus canariensis.*