ISOLATION OF TERPENIC COMPOUNDS FROM *EUPHORBIA LAGASCAE*. SCREENING OF THEIR *IN VITRO* ACTIVITY AGAINST *M. TUBERCULOSIS* AND *ST. AUREUS*

N.Duarte[1], C.Ramalhete[1], M.Martins[2], P.Abreu[3], M.Viveiros[2], L.Amaral[2], M.J.Ferreira[1]

[1]CECF, Faculty of Pharmacy, University of Lisbon, Portugal. [2]UPMM, Institute of Hygiene and Tropical Medicine of Lisbon, Portugal. [3]CQFB/REQUINTE; Faculty of Sciences and Technology, Caparica, Portugal.

INTRODUCTION

For as long as man can remember, plants have been used worldwide for the treatment of diseases. Today, many of the drugs currently used are derived from natural products or have dependent upon a natural product for their development and the recent discoveries of the antimalarial artemisinin and the anticancer agent taxol indicate the continuing importance of plant species in drug discovery.[2] In recent years, a great number of diterpenoids with lathyrane and jatrophane structures have been isolated from *Euphorbia* species, some of them possessing interesting bioactivity, namely as modulators of multidrug resistance.

In this communication, we are reporting on the isolation, structure elucidation and antibacterial activity of four terpenic compounds isolated from the methanolic extract of *Euphorbia lagascae* aerial parts (figure 1).

Figure 1 – *Euphorbia lagascae* aerial parts[1]

ABOUT TUBERCULOSIS

Tuberculosis is one of the oldest and most widespread diseases in history infecting at least two billion of the world's population, 10% of those will develop clinical illness, particularly those who also have the HIV infection. The immune suppression associated with this viral infection cause conversion of latent to active tuberculosis and provides a fertile niche for establishment of new cases. By the mid 1950s the use of *Streptomyces*-based antibiotics (streptomycin, rifampin) and other chemotherapeutic antimycobacterial agents (isoniazid, pyrazinamide, ethambutol) became of greater importance to the treatment of tuberculosis. However, the AIDS-associated strains of *M. tuberculosis* are multiple resistant to all of the common antitubercular drugs and to deal with this problem, new drugs are urgently needed.[3, 4]

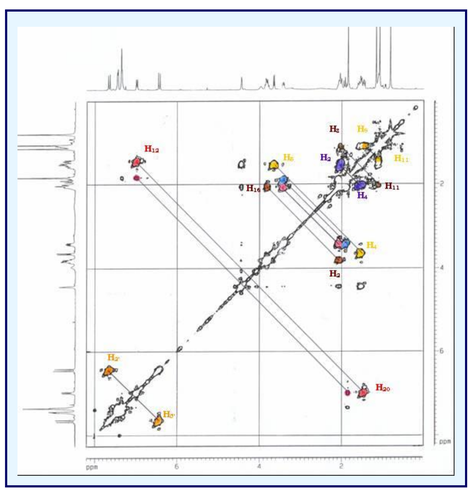

Figure 3 – ^{1}H-^{1}H COSY spectrum of **1**

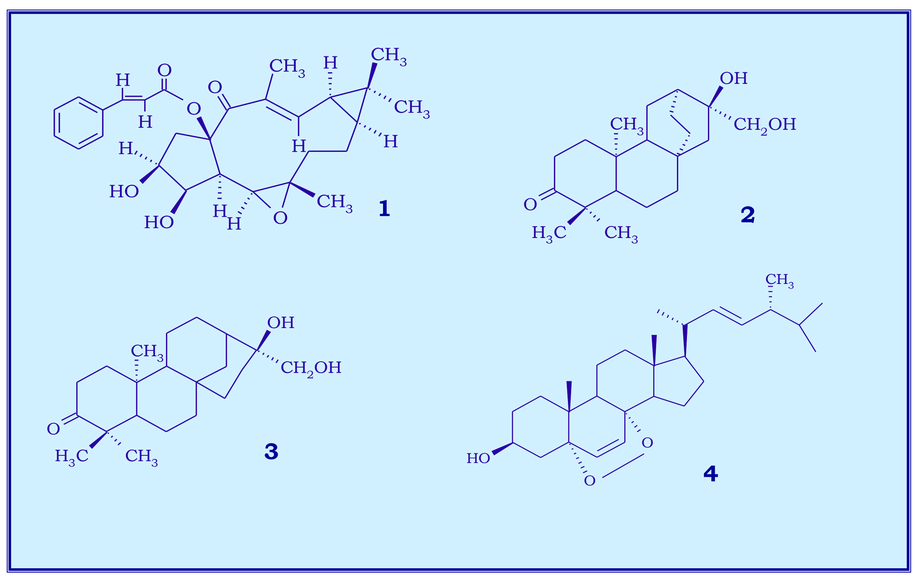

Figure 2 – Some of the terpenic compounds isolated from *Euphorbia lagascae*

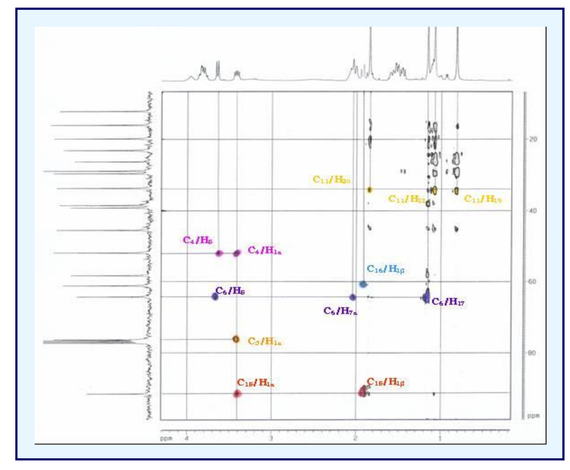

Figure 4 – Partial HMBC spectrum of **1**

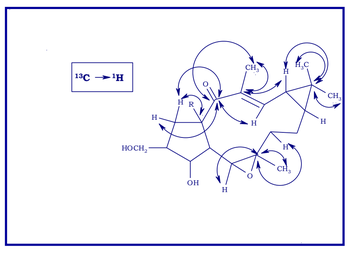

Figure 5 – Relevant HMBC correlations of **1**

MATERIALS AND METHODS

The methanolic extract of *E. lagascae* was fractionated by chromatographic methods (column, preparative and HPLC) yielding several terpenic compounds, whose structures were elucidated by physical and spectral data, ^{1}H and ^{13}C NMR spectroscopy (COSY, HMBC, HMQC and NOESY experiments as can be seen on figures 3, 4, and 5). Four of the isolated compounds: one new lathyrane type diterpene (**1**), (two known polycyclic diterpenes (**2** and **3**), and one sterol, **4**) were tested for their antibacterial activity against *Staphylococcus aureus* (ATCC 25923) by the use of the broth dilution method.[5]. The sterol (**4**) was also tested for its activity against *Mycobacterium tuberculosis* (H37RV ATCC 27294) by the use of the Bactec 960 system[6].

RESULTS AND DISCUSSION

The phytochemical study of the air-dried aerial parts of *Euphorbia lagascae* (methanolic extract) yielded a **new diterpene with the lathyrane skeleton** that was named **latilagascol** (**1**), two known polycyclic diterpenes (*ent*-16α,17-dihydroxyatisan-3-one, **2**, and *ent*-16α,17-dihydroxykaurane-3-one, **3**), as well as a known sterol named ergosterol peroxide (**4**) as can be observed on figure 2.

The reemergence of tuberculosis is a significant public health problem and there is an obvious need for new anti-tubercular drugs. **Ergosterol peroxide** (**4**) was described by Cantrell *et al*[7] as having activity against *M. tuberculosis* with a minimum inhibitory concentration (MIC) of 1 µg/ml. The results obtained in our study indicate that concentrations of ergosterol peroxide that ranged from 2.5 to 10 µg/ml had no detectable effect on the activity against the standard *M. tuberculosis*, therefore we can not reconcile our results with those of Cantrell *et al*.

The global escalation of methicillin resistant *Staphylococcus aureus* coupled to the knowledge that resistance to vancomycin, the drug of last resort, is now being detected demands that any isolated compound should be examined for such activity against *St. aureus* methicilin resistant. Unfortunately, concentrations of the four tested compounds as high as 100 µg/ml had no effect on the growth of the *Staphylococcus aureus* ATCC 25923 strain.

[1] http://www.nf-2000.org/publications; [2]Newton S, Lau C, Wright C. *Phytotherapy Research* 2000; 14: 303-22.; [3]Okunade A, Elvin-Lewis M, Lewis W. *Phytochemistry* 2004; 65: 1017-32.; [4]Mitscher L, Baker W. *Med. Res. Rev.* 1998; 18: 363-74.; [5]Kristiansen M, Leandro C, Ordway D, Martins M, Viveiros M, Pacheco T, Kristiansen J, Amaral L. *Int J Antimicrobial Agents* 2003; 22: 250-5.; [6]Scarparo C, Ricordi P, Ruggiero G, Piccoli P. *J Clin Microbiol* 2004; 42:1109-14; [7]Cantrell L, Rajab S, Franzblau S, Fronczek F, Fischer N. *Planta Med* 1999; 65:732-4