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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Preparations based on minerals extracts of *Calicotome villosa* roots and bovine butyrate matter: Evaluation in vitro of their antifungal activity

Préparations à base d'extraits minéraux de racines de Calicotome villosa *et de matière butyrique bovine : évaluation in vitro de leur activité antifongique*

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Calicotome villosa;
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Tablet;
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Summary
Objective. — The use of preparations based on minerals extracts of *Calicotome villosa* and butter is born from the misuse of drugs without specific microbiological analyzes. Seventeen different preparations were performed. The antibacterial and antifungal activities were determined on five bacteria and two fungi strains respectively.
Material and methods. — *C. villosa* ashes are obtained by incineration of roots plant at 498 °C for 4 hours. They are analyzed to determine the shape of the particles and the mineral constituents by scanning electronic microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) techniques respectively. The effectiveness of preparations or tablets is measured in solid medium. It allows to measure the diameter of the inhibition zone for the antibacterial activity as well as the diameter of mycelia growth and the critical values (MIC, MFC, IC_{50} and IC_{90}) for the antifungal activity. Finally, the results are compared to the activity of a commercial positive control aiming to give value of the observed activity.
Results. — SEM observations reveal the presence of nanoparticles agglomerated with size of about 50 nm. The EDX analyzes indicate the presence of Fe, Na, Al, Mg, Si, K, Ca, O_2 and C. Among all the results, the preparation (B_s + A) or (B_{sd} + A) can completely inhibit the growth of two

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fungal pathogens. The activity of the preparation is faced with the activity of the synthetic fungicide nystatin.
Conclusion. — The efficacy of the preparation (B_s + A) or (B_{sd} + A) is higher than that of nystatin against *Aspergillus sp.* and *Fusarium sp.* The preparation could serve as natural antifungal for the pharmaceutical industry.

MOTS CLÉS
Calicotome villosa ;
Sels minéraux ;
Matière butyrique ;
Tablette ;
Activité antimicrobienne

Résumé
Objectif. — L'utilisation de préparations à base d'extraits minéraux de *Calicotome villosa* et de beurre est née de l'emploi abusif, sans analyses microbiologiques précises, de médicaments. Dix-sept préparations différentes ont été pratiquées. Les activités antibactériennes et antifongiques ont été déterminées respectivement sur cinq bactéries et deux champignons.
Matériel et méthodes. — Des cendres de *C. villosa* sont obtenues par incinération des racines de la plante à 498 °C pendant 4 heures. Elles sont analysées, pour déterminer la forme des particules et les constituants minéraux présents, par les techniques de la microscopie électronique à balayage (MEB) et spectroscopie à rayons X à dispersion d'énergie (EDX) respectivement. L'efficacité des préparations ou tablettes est mesurée en milieu solide. Elle permet de mesurer le diamètre de la zone d'inhibition pour l'activité antibactérienne, ainsi que le diamètre de la croissance mycélienne et les valeurs critiques (CMI, CMF, IC_{50} et IC_{90}) pour l'activité antifongique. Enfin, pour donner une valeur à l'activité observée, les résultats sont comparés à l'activité d'un témoin commercial positif (nystatine).
Résultats. — Les observations MEB mettent en évidence la présence de nanoparticules agglomérées de taille d'environ 50 nm. Les analyses EDX indiquent la présence de Fe, Na, Al, Mg, Si, K, Ca, O_2 et de C. Parmi l'ensemble des résultats, la préparation (B_s + A) ou (B_{sd} + A) est susceptible d'inhiber totalement la prolifération de deux champignons pathogènes. L'activité de la préparation est confrontée à l'activité de la nystatine, fongicide de synthèse.
Conclusion. — L'efficacité de la préparation (B_s + A) ou (B_{sd} + A) est supérieure à celle de nystatine contre *Aspergillus sp.* et *Fusarium sp.* La préparation pourrait servir d'antifongique naturel pour l'industrie pharmaceutique.

Introduction

Several plant extracts have been used traditionally as antifungal or antibacterial agents against human or vegetable pathogens. It is based especially on their beneficial effect of organic compounds, but rarely with mineral nature. For instance, the valorization of the mineral stems from the production of energy from wood or agricultural waste incineration is quantitatively limited. The industrial applications are frequently known and for example, the use of ashes obtained from waste or incinerated palms as a partial substituent of Portland cement is certainly promising, although this technology is still under development [1,2]. Indeed, some vegetable ashes were added in culture medium of pathogens. In the Mediterranean area, particularly in the Southern part, people have been exploiting wood prepared from the roots of heather, myrtle, mastic and broom. Despite recent progress in the bioprospecting of natural products by antimicrobial properties, the incidence of mortality increased to an alarming rate with nearly 30% in invasive aspergillosis, about 39% in invasive candidasis, and up to 70% in *Fusarium* infections [3].

Calicotome villosa is a Mediterranean drought deciduous shrub with mature, green stems and the researchers reported that crude *Calicotome* essential oil or its major compounds inhibit completely the radial growth of the fungi and yeast [4]. However, *C. villosa* (Poiret) link at 50—150 cm spiny shrub with yellow flowers during the spring season grow especially in the northern part of Africa mainly in Algeria, Cyprus, Spain and Greece [5—8]. This deciduous shrub called popularly in Algeria, ''Gandoul'' where leaves and stems of this plant were reported previously, but its roots have not yet been investigated [9]. Medicinal uses have been reported only for *C. villosa* (Poiret) Link as antitumoral agent and for the treatment of furuncle, cutaneous abscess and chilblain in the Sicilian folk medicine [10,11]. Moreover, this spiny broom is used in the diets of all herbivores in the Mediterranean area [12].

The ashes generated from wood are exploited with precaution because of pH, as fertilizer to poor soil. Besides, this matter, specifically the one of *C. villosa*, is used for prophylactic purposes for drying wound or in mixture with animal fatty matter for the treatment of some skin infections. In addition, the essential oils are susceptible to a synergistic action with antibiotics or minerals. However, the essential oils are very active substances with some precautions in the use, particularly their application on skin affected by lesions.

The use of antifungal combination therapy is a new clinical approach and considerable work has been undertaken on the assessment of interactions of antifungal agents in vitro [13,14]. In fact, several methods were used to clarify the drug combinations studies for the treatment of fungal infections such as the high-throughput synergy screening (HTSS); however, mixing two or more effective drugs with