Antibacterial activity of *Calycotum villosa* (Poiret) Link extracts

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ملخص

الأجزاء الهوائية المجففة (الاوراق، السيقان والازهار) من *Calycotum villosa* (Poiret) Link جمعت من جبل Edough (عنابة، شمال الجزائر)، خلال فترة الإزهارفي مارس 2010 . تم إعداد المستخلصات الخام من الأجزاء الهوائية باستخدام مذيبات مختلف القطبية (CH_2Cl_2 وMeOH). وقد تم التحقق من الخصائص الكيميائية للنبات والمضادة للجراثيم. أظهرت النتائج وجود بعض العائلات الكيميائية بما في ذلك القلويدات، الفلافونيدات، الستيرولات والتربينات الثلاثية. وقد تبين أن استخراج المستخلص الخام لل CH_2Cl_2 كشف عن نشاط قوي ضد *Klebsiellap neumoniae* و *Acinetobacter*. المقاومة للأدوية. وتدعم هذه الدراسة إمكانية استخدام *Calycotum villosa* (Poiret) Link لقتل البكتيريا المسببة لبعض الأمراض مثل الالتهاب الرئوي.

كلمات البحث: *Calycotum villosa* (Poiret) Link ، المركبات الطبيعية، النشاط المضاد للبكتيريا، المستخلصات الخام.

Résumé

Les parties aériennes (feuille, tige, et fleur) de *Calycotum villosa* (Poiret) Link ont été recueillies au niveau de la montagne de l'Edough(Annaba, Nord Algérien), pendant la période de floraison en Mars 2010. Des extraits bruts des parties aériennes ont été préparés en utilisant des solvants de divers polarités (CH_2CI_2 et MeOH). Les propriétés phytochimiques et antibactériennes préliminaires des extrait sont été étudiés. Les résultats ont montré la présence des familles chimiques suivantes: alcaloïdes, flavonoïdes, stérols et triterpènes. On a trouvé que l'extrait brut de CH_2CI_2a révélé une forte activité contre les bactéries multi-résistantes suivantes: *Acinetobacter sp.* et *Klebsiella pneumoniae*. Cette étude appuie l'utilisation potentielle de l'extrait de *Calycotum villosa* pour éliminer les bactéries causant des maladies infectieuses comme la pneumonie.

Mots-clés: *Calycotum villosa* (Poiret) Link, composés naturels, activité antibactérienne, extraits bruts.

Abstract

Dried aerial parts (leaf, stem, and flower) of *Calycotum villosa* (Poiret) Link were collected from Edough mountain (Annaba, North of Algeria), during the flowering period in March 2010. Crude extracts of the aerial parts were prepared using solvents of various polarity (CH_2Cl_2 and MeOH). Preliminary phytochemical and antibacterial properties of the extracts were investigated. The results showed the presence of some chemical families including alkaloids, flavonoids, sterols and triterpenes. It was found that the CH_2Cl_2 crude extract revealed a strong activity against *Acinetobacter sp.* and *Klebsiella pneumoniae* drug-resistant bacteria stains. This study supports the potential use of *Calycotum villosa* extracts to kill bacteria causing some infectious diseases such as pneumonia.

Keywords: *Calycotum villosa* (Poiret) Link, natural compounds, antibacterial activity, crude extracts.

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1. INTRODUCTION

Pharmacological industries have developed a number of antibiotics in the last three decades. However, resistance to these drugs by microorganisms has increased [1]. The problem with this microbial resistance is overgrowing and the perspective for use of antimicrobial drugs is still challenging. Therefore, actions should be taken to solve this problem by controlling the use of antibiotic, better understanding the genetic mechanisms of resistance and to developing new drugs.

During recent years, herbal medicines have become increasingly popular in some parts of the world, while in other parts it has always been an essential element of healthcare system. In Algeria, traditional medicine takes an important part to cure diseases in the general population habits. The northeast of Algeria possesses an extremely rich and varied flora, characterized by its originality on the systematic scheme (numerous endemic plants) and its wide use in folk medicine. These characteristics make the flora study of great scientific interest in the field of phytochemistry [2].

C*alycotum villosa* (Poiret) Link belongs to the Leguminosae family also called "El Gendoul" in arabic, is a shrub with long thorns, it grows in the Mediterranean region and prefers siliceous well watered soils[3]. In the medicinal uses this plant was reported as being antitumoral agent[4]. and efficient for the treatment of furuncle, cutaneous abscess and chilblain in the Sicilian folk medicine[5].

As a part of a systematic research study on the constituents and antimicrobial potential of Algerian natural plants, we have already reported the phytochemical and biological propertiesof *Rosmarinus officinalis* [6], *Retama raetam* [7] and *Cyclamen*

africanum [8]. In the present study, we are reporting a phytochemical screening of *C. villosa* polar and nonpolar extracts followed by an assessment of antibacterial activity against different drug-resistant bacteria species.

2. MATERIALS AND METHODS

2.1. Plant material

Aerial parts of *C. villosa* were collected in March 2010 from Edough Mountain. The plant was authenticated by Dr. Samah DJEDDI (Biology Department, Badji Mokhtar University). A voucher specimen has been stored in the Herbarium of the Biology Department, University of Badji Mokhtar, under the code Ann-BV 2009/14.

2.2. Extraction and detection of chemical groups[2,9].

The air dried plant material (250 g) was first extracted with CH_2Cl_2 for 24 h and then with MeOH for 24 h. The dichloromethane and methanolic solutions were evaporated in the rotary to dryness and the resulting crude extracts were stored at 2–4°C for further use.

- ***Extraction procedure for alkaloids***

Dried and powdered sample was extracted with MeOH in Soxhlet apparatus. The solvent was evaporated to dryness and the crude residue was taken up in a 2% aqueous HCl. The aqueous acidic phase was extracted with CH_2Cl_2 and then evaporated to obtain a crude alkaloid mixture.

- ***Extraction procedure for flavonoids***

Air dried aerial parts of the plant were extracted in Soxhlet extractor using MeOH (70%). The concentrated MeOH extract was suspended in H_2O and extracted twice with ethyl acetate. The aqueous solution is extracted for three times by n-butanol to give the crude flavonoid extract.

- ***Identification of sterols & triterpenes***

A residue of an ether extract was dissolved in 0.5 mL acetic anhydride and then in 0.5 mL of chloroform. Then, onemL of concentrated sulphuric acid was added (Libermann-Burchards reaction) at the contact zone of the two liquids and a brownish red ring is formed indicating the presence of sterols & triterpenes.

- ***Identification of tannins***

0.5g of plant material was added to 10 mL of distilled water to prepare water extract, and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

- *Identification of coumarins*

The residue of ether extract is dissolved after dryness in hot water. The solution is divided into two equal volumes: one of which contains the reference, and the second is made alkaline with 0.5 mL of 10 % ammonia solution. The occurrence of an intense fluorescence under UV light indicates the presence of coumarins and derivatives.

2.3. Antibacterial activity

The disc diffusion method was used for the determination of antimicrobial activity of the CH_2Cl_2 and MeOH crude extracts [10]against some pathogenic microorganisms isolated directly from patients at the "Centre Hospitalo-Universitaire, Ibn Rochd" Annaba, Algeria: *Staphylococcus aureus* (Gram positive), *Acinetobacter sp., Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeroginosa* and *Serratia marcescens* (Gram negative).The bacterial species were cultured overnight at 37ºC in Mueller-Hinton medium (BIO-RAD Perpignan, France). Suspensions of the tested micro-organisms (0.1 ml of 10^7-10^8cells/ml) were spread over the surface of Petri plates. The inoculate were stored at 4 ºC for further use. Filter paper discs (Whatman n° 1; 6.0 mm in diameter) were impregnated with 20µl of each extracts and placed on the inoculated agar plates. Standard antibiotic Amikacin 1mg/ml was used in order to control the sensitivity of the test organisms. The plates containing the bacteria were incubated for 24 h at 37 ºC. The resulting diameter of the inhibition zones (IZD) have been measured in millimetres [11]. All experiments were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening of C. villosa

The present work is focused essentially on the phytochemical and antibacterial screening of *C. villosa* extracts,to the best of our knowledge, no phytochemical study of this plant has been reported before.

The first chemical investigation of *C. villosa* extracts revealed the presence of the following phytochemical groups: alkaloids, flavonoids and sterols & triterpenes. Tannins and coumarins groups were not detected in the analyzed samples (**Table 1**).

Table 1: Phytochemical analysis of *C. villosa* extracts

Crude extracts	*alkaloids*	*coumarins*	*flavonoids*	*sterols & triterpenes*	*tannins*
CH_2Cl_2	++++	-	+	+	-
MeOH	++	-	++	+	-

When the chemical profile of the studied plant is compared to the previously studied species it appears similar, Aberkane et al. [12] reported the presence of 04 flavonoids (Glucopyranosyl chrysin type), *Calycotome villosa* subsp. *intermedia* collected from Moroccocontains two flavonoid belonging to the same type: chrysin-7-O-(β-D-glycopyranoside) and chrysin-7-O-β-D-[(6"-acetyl) glycopyranoside] [13], more resent study showed that one alkaloid as well as a paraben derivative were extracted from the same species [14].

***3.2. In vitro antimicrobial effects of C. villosa* extracts**

The antibacterial test was carried out on both dichloromethane (non polar) and methanolic (polar) crude extracts and was evaluated using disk diffusion method by measuring inhibition zone diameters [10]. Amikacin (1000 mg l^{-1}) was included as positive control. The methanol crude extract didn't show any antibacterial activity against all bacteria strains tested, while the dichloromethane crude extract showed a strong antimicrobial activity against *K. pneumoniae* (20.5 ± 2.7 mm) as well as *Acinetobacter sp.* (15.7 ± 1.3 mm) (**Figure 1**). The extract showed a moderate antibacterial effect against *E. coli* (12.9 ± 0.9 mm), *P. aeruginosa* (13.1 ± 2.3 mm) and *S. marcescens* (10.2 ± 0.3 mm)and no effect against *P. mirabilis (***Table 2**).

Table 2: Antimicrobial activity of *C. villosa* extracts expressed as diameter of the inhibition zone in mm in the disk diffusion assay

Microorganisms	***CH_2Cl_2 extract*** *(1000 mg l^{-1})*	***Sensitivity****	***MeOH extract*** *(1000 mg l^{-1})*	***Sensitivity****	***Amikacin*** *(1000 mg l^{-1})*
Acinetobacter sp.	15.7±1.3	++	<6	-	15.0 ± 0.02
E. coli	12.9±0.9	+	6.3±0.2	-	18.2 ± 0.07
K. pneumoniae	20.5±2.7	+++	8.1±1.1	-	23.5± 0.05
P. mirabilis	07.5±0.1	-	8.9±2.5	-	14.5 ± 0.02
P. aeruginosa	13.1±2.3	+	<6	-	18.1 ± 0.06
S. marcescens	10.2±0.3	+	8.8±0.2	-	17.0 ± 0.01
S. Aureus	06.4±1.5	-	8.1±1.3	-	28.0 ± 0.9

*the sensitivity of the different strains was classified by the diameter of the inhibition zone [11]:

-: diameter less than 8 mm; not sensitive; +: sensitive; diameter 9–14 mm; ++: very sensitive; diameter 15–19mm.+++: extremely sensitive for diameter larger than 20mm.

It should be mentioned that there are no background antibacterial study on *C. villosa* extracts.

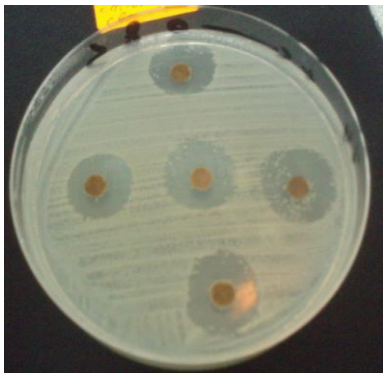

Figure 1: **Sensitivity of *Acinetobacter sp.* Against *C. villosa* CH_2Cl_2 extract**

4. CONCLUSION

This preliminary screening showed interesting results and indicated the antimicrobial potential of *C. villosa*. In light of these experiments, it could be concluded that the non polarextract (CH_2Cl_2 extract) exhibited interesting antibacterial activity against some pathogenic strains.

The antimicrobial activity of *C. villosa* could be attributed to various phytochemical constituents (flavonoid and alkaloid compounds) present in the crude extracts. Further work on the types of bioactive components could reveal the exact potential ofthe plant to inhibit several pathogenic microbes and encourage the development a novel broad spectrum herbal antimicrobial formulation in the future.

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