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*Full Length Research Paper*

# Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulata*

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**The crude alkaloids and the saponin extracts of the aerial parts of *Anabasis articulata* were examined for antibacterial and antifungal activity *in vitro* using the disc diffusion method. Activity against five bacterial strains (gram positive bacteria and gram negative bacteria) and one fungal strain is discussed. Saponin extract was active against all assayed bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumonia*, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 14028,) and a fungal strain (*Candida albicans)* with minimal inhibitory concentration (MIC) values ranging from 0.5 to 1 mg/ml. Phytochemical screening shows that this plant is particularly rich in alkaloids and saponins which might be responsible for its anticandidal activity.**

**Key words:** *Anabasis articulata*, Antibacterial activity, Antifungal activity, disc diffusion method, Saponins, alkaloids.

## INTRODUCTION

*Anabasis articulata* locally named as 'ajrem' is a wild plant widely used in Algerian traditional medicine to treat diabetes, fever, headache and skin diseases such as eczema (Hammiche and Maiza, 2006; Hmamouchi, 1999). It is taken orally after decoction in water as a single herb or with other medicinal plants. No scientific investigations concerning the pharmacological properties of *A. articulata* has been done.

The phytochemical constituents of *A. articulata* revealed the presence of saponin glycosides (Sandberg and Shalaby, 1960; Sandberg and Michel, 1962; Segal et al., 1969; Kambouche et al 2011).

In recent years, multiple resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections (Poole, 2001**)**, has forced scientists into looking for new antimicrobial substances from various sources like medicinal plants.

In the course of our study for a better valorization and in order to find some potential activities of Algerian flora, *A. articulata* was selected.

## MATERIALS AND METHODS

### Plant material

The leaves of *A. articulata* are collected from local inhabitants having knowledge of the curative properties of this plant in September 2010. The plant materials were identified and

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authenticated with assistance of Prof. Hadjadj-Aouls, M.S (Botanic Department, Oran, Algeria). Voucher specimen of this plant was deposited in the Agricultural Institute (INA) in Algeries.

### Chemical reagents

All chemicals were purchased from Sigma Aldrich (Milwau-kee, USA), Fluka (Buchs, Switzerland) and Merck (Germany).

### Phytochemical screening

Determination of major chemical groups in the plant material was carried out according to the Harborne technique (Harborne, 1984).

### Preparation of the extracts

### Extraction of saponin

Saponins contained in the leaves of *A. articulata* (100 g) were extracted with methanol in Soxhlet apparatus. The extract was concentrated, freeze-dried and re-extracted with water and butanol saturated with water. The dried crystalline butanolic extract (1.3 g) was obtained. Thin layer chromatographic analysis was performed with the method of Wagner and Bladt (1996) **.** The proportion of the developing solvent was n-butanol: acetic acid: water (40:10:50). The butanolic crystalline extract was dissolved in the developing solvent and applied to aluminum-backed plates coated with silica gel 60 F254 (layer thickness 0.2 mm, 20x20 cm; E. Merck, Darmstadt, Germany). After development, plates were air-dried, observed under UV light, sprayed with methanol : acetic acid : sulphuric acid : anisaldehyde (85:10:5:0.1) and heated at 100° C for 5 min. On heating, saponin bands turned red and were readily visualized.

### Preparation of the crude alkaloid fraction

The alkaloid extract was obtained by an acid/basic modified extraction as described by Wagner and Bladt (Wagner and Bladt, 1996) , with minor modifications (Hughes et al., 2005). *A. articulata* leaves were dried and the air-dried plant material (500 g) was extracted three times with hexane (2 L/kg) for 48 h at room temperature with occasional shaking to eliminate apolar constituents. The extract was then filtered and the residue was flooded with methanol (1.5 L/kg) using the above process. The methanol extract was then concentrated under reduced pressure and acidified with 0.5 M $H_2SO_4$. The acidic extract was washed with chloroform to remove neutral components. The aqueous acidic fraction was then made basic with ammonia (pH 10) and extracted again with chloroform until the aqueous layer was free of alkaloids. The combined chloroform extracts were evaporated. The crude alkaloid fraction as a brown residue (1.25% w/w of the dry starting material). This extract was developed by chromatography in a thin layer silica gel chromatography using chloroform: methanol (8:2) as a solvent system. Later, plates were air-dried, observed under UV light, sprayed with Dragendorf's reagents and heated at 100° C for 5 min (Wagner and Bladt, 1996)

### Microorganisms tested

The following strains of bacteria and fungi were used as test microorganisms: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis* ATCC 6633 *Pseudomonas aeruginosa,* and *Candida albicans*. These microorganisms were obtained from Microbiology Laboratory, Faculty of Science, Oran University.

### Preparation of test samples

The dried alkaloids extracts and the saponin fraction were dissolved in sterile dimethylsulfoxide (DMSO) at selected concentrations.

### Disc diffusion method

To investigate the antimicrobial activity of the extracts, disc diffusion method is used (Collins and Lyne, 1970; Nascimento, 2000). The agar gel (MHA for bacteria and SDA for fungi) is treated with the appropriate microorganism suspension (each microorganism was inoculated at a concentration of *ca* $10^6$ colony forming units per ml for bacteria and $10^4$ spore/ml for fungal strains) and the antimicrobial activity of extracts that hepenetrate into the agar by diffusion is measured. The assays are based on the use of sterile discs filter paper (6 mm diameter) impregnated with 20 µl of the extract solution to be examined and allowed to dry at room temperature. A sterile disc impregnated with DMSO is used as negative control. After incubation for 24 h at 37°C for bacteria plates, while fungi plates are incubated for 24 h at 25°C, all plates were observed for zone of growth inhibition and the diameter of these zones was measured in millimeters. All experiments were performed in triplicates.

## RESULTS

The qualitative chemical analysis of the aqueous extract of *A. articulata* showed that preliminary alkaloid tests were positive for both tertiary and quaternary alkaloids according to Mayer's and Dragendorff's reagents. Moreover, the screening for saponin component showed positive results with $FeCl_2$ and $HgCl_2$. The quantitative chemical analysis exhibited the presence of alkaloids and saponin with percentage 1.25 and 1.3, respectively. After development of butanol extract in plates of silica gel and observed under UV light, four saponin glycosides were detected; saponin bands turned red and were visualized.

The antimicrobial activities of *A. articulata* saponin and alkaloids extracts against microorganisms examined in the present study and their potency were quantitatively assessed by the presence or absence of inhibition zones and zone diameters (Table 1), MIC(minimal inhibition concentration) value is deducted. Our results showed

**Table 1.** Antimicrobial activity of *Anabasis articulata* extracts.

| Concentrations (mg/ml) | Extracts | Inhibition zone (mm)* Microorganisms | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E. co*** | ***S. a*** | ***K p*** | ***B.s*** | ***P. a*** | ***C. a*** |
| 5 | Saponin | 17.8 | 16.3 | 12.5 | 14.1 | 21.1 | 13 |
| | Alkaloids | 10.1 | 12.4 | 8.5 | 11 | 13.3. | – |
| 2.5 | Saponin | 15.2 | 13.2 | 10.1 | 13.4 | 16.1 | 10.8 |
| | Alkaloids | 8.5 | 12 | 8.9 | 10 | 10.5 | – |
| 1 | Saponin | 10.3 | 13.3 | 9.2 | 10 | 12.5 | 9.3 |
| | Alkaloids | 8.1 | 7.6 | 7.8 | 9.5 | 8.5 | – |
| 0.5 | Saponin | 7 | 10.9 | 7 | 7.8 | 10.2 | 8.8 |
| | Alkaloids | – | – | 6.1 | 6.2 | 7.4 | – |

**: Values are the mean of tree replicates.*
*–, not active*
*E. co: Escherichia coli* ATCC 25922
*S. a: Staphylococcus aureus* ATCC 6538
*K. p: Klebsiella pneumonia*
*B. s: Bacillus subtilis* ATCC 6633
*P. a: Pseudomonas aeruginosa* ATCC 14028
*C. a: Candida albicans*

that the saponins extract exhibited a higher degree of antimicrobial activity against all bacterial strain tested as compared with alkaloids extract.

In the light of our findings, this activity was more pronounced with saponin extract. However, the alkaloid extract of *A. articulata* had no antimicrobial activity against *C. albicans.*

Maximal inhibition zones and MIC values for the microorganisms sensitive to the saponin extract of *A. articulata* were in the range of 7–21.1 mm, 0.5 – 1 mg/ml respectively (Table 1).

## DISCUSSION

Most antibacterial medicinal plants attack Gram-positives trains while few are active against Gram-negative bacteria (Herrera et al, 1996; Meng et al., 2000; Scrinivasan et al. 2001). Interestingly, our current finding shows a remarkable antimicrobial activity on a larger range of Gram- negative antibiotic-resistant isolates in *A. articulata*.

Otherwise, the major chemical component responsible for this effect would be □-sitostérol steroids (Kambouche et al. 2011) in *C. albicans* (MIC 0.5 mg/mL), since alkaloids did not present any anticandidal effect.

The results may suggest that saponin extract of the *A. articulata* possess compounds with antibacterial and anticandidal properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases.

However, antimicrobial potential of saponin isolated β -sitostérol steroids is necessary to examine to confirm its high activities.

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