Chemical study of two natural substances extracted from *Medicago sativa* grown on different soils and analysis of their effects on the growth of some pathogenic bacteria

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Abstract: To identify new phytotherapeutic molecules economically accessible and with an effective biological activity we are interested to *Medicago sativa* leaves extracts. Our first objective is to analysis the edaphic factor effect on the chemical characters of flavonoids and saponins. This could make easier a better control of the aspects of these extract with a view to a pharmaceutical preparation. The second objective is to determinate their antibacterial effect on some bacterial strains known for their pathogenicity in humans. The results show that the soil has an impact on the chemical composition of plant extracts.The microbial tests translate significant effects. Among the sensitivity degrees vary bacterial strains and plant extracts. These results have been encouraged to promote *Medicago sativa* on an appropriate soil and it is necessary to conduct additional tests to confirm the use of these substances to treat the bacterial infection.These preliminary tests allow us to justify the use of this species in traditional pharmacopoeia suggesting that their leaves possess an interesting antibacterial compounds.

Keywords : *Medicago sativa*, actives substances, flavonoïds, saponins, antibacterienne activity impact du facteur édaphique.

Introduction

To cope with the appearance of the microorganisms, that show a resistance to antibiotics, due to an excessive and uncontrolled use of those molecules, the scientists are confronted to seek for new effective antibacterial substances and broad-spectrum. One strategy for this research is to explore the plants used in traditional medicine. Medicinal plants inexhaustible source of active compounds, are nowadays the main source and basis of numerous pharmaceutical specialties. However, large number of other plant species occurring in nature, in enormous quantities and with interesting therapeutic properties, remains untapped and the man has not yet been able to take advantage of them. In order to enhance and streamline their use and to identify more natural substances with effective and potential therapeutic properties, we are interested in *Medicago sativa* study, one fabaceae more known for his interest in animal feed for its use in public health care although its use in traditional medicine dates back hundreds of years it was used by herbalists to treat various infectious diseases (Bouvyer, 2007). The literature study revealed that exempt for studies on the chemistry of organs, this species has been no investigation on its biological activity. The goals this study is to:

-Achieve a chemical-screening of the plant for determining the principal compounds with a therapeutic interest.
- Determine the chemical profiles of flavonoids and saponins extracted from leaves of Medicago sativa from different native soils. The interest is to assess the impact of edaphic factor on their variability. This could facilitate a better control of the qualitative and quantitative aspects of these active substances in order of pharmaceutical preparations. -
-Conduct an analysis of the antibacterial activity in order to identify molecules with potential antibacterial properties.

Materials and Methods

1- Plant material

It consists of leaves of *Medicago sativa*. This plant family Fabaceae species is very rich in protein, vitamins and trace elements (Bouvyer, 2007). This plant is healthy and ecological, with specific bacteria living in symbiosis on its root system; it fixes atmospheric nitrogen necessary for its growth and requires no chemical fertilizer.

The leaves of this plant were harvested before the flowering stage in the Annaba region (North-eastern Algeria) in four culture stations with different soil characteristics. After drying in the dark at ambient temperature, the leaves are detached and powdered to be used to obtain different extract.

2- Microbial strains

The microbial support used is composed of:

- 3 Strains of Reference: *Escherichia coli (ATCC); Staphylococcus aureus* (ATCC); *Pseudomonas aeruginosa* (ATCC).

-9 Bacterial strains frequently isolated in hospital and often implicated in many diseases in humans.

3 - Screening Chemical

It consists in detecting the different families of compounds existing in the leaves by precipitation reactions or coloring using reagents specific to each class of compounds. The results are noted positive (+) or negative (-).

4- Solutions used

-The decoction is obtained from an aqueous decoction of powdered leaves (20g) mixed with distilled water (1 L) and brought to boiling for 15 minutes. The decoction of 2% obtained was filtered and concentrated on a rotary evaporator.

-Crude extracts flavonoids and saponins: After highlighting the different families of compounds in the leaves of *Medicago sativa*, we targeted these two families. Their preponderance in the leaves and their possible antimicrobial activities presumed encouraged us to submit them to our investigation. Their extraction was conducted using the method described by Lee *& al.,* (1995) for flavonoids and method of Applebaum & *al.,* (1969) for the saponins. For microbiological testing and chromatographic study, the crude extracts were recovered respectively with DMSO (Dimethylsulfoxide) and acetone.

5 - Determination of chemical profiles

The identification of chemical profiles of flavonoids and saponins was conducted on leaves coming from the four stations. The objective is to evaluate the impact of soil type on the variability of their chemical components. Separation and identification of substances was performed by TLC (thin layer chromatography) on silica gel G60 plates (Merck). Spots 2µl of each sample is deposited at point's pins on the edge of the plate and immersed in an appropriate eluent. After development of the chromatogram, the plate is dried at ambient temperature and then examined in the UV (λ= 254nm and 365nm). The results contain information on the number of chemical constituents their RF (retention factors of chemical constituents) and their behavior under UV light.

5-Microbiological tests

The antibacterial activity of the tested solutions was performed on filter paper discs by diffusion method on solid medium (Bauer *& al*., 1966). Muller Hinton agar is the medium used for tests of bacterial strains (Nostro *& al*., 2000). The media are inoculated with a few ml of the inoculums (10 CFU / ml) for bacterial species (Cavallo, 2006) in order to cover the whole agar surface. Filter paper discs loaded with test solution are

deposited on the surface dry of medium. After 24 h of incubation at 37 ° C, the antibacterial activity is manifested by the appearance of a halo of inhibition of bacterial growth around the discs (Bauer, 1966; Duvar 1980; Carbonelle *& al*., 1987): is considered respectively as resistant strain sensitive, highly sensitive or extremely sensitive that having a diameter D<8mm; 9mm ≥ D ≤14mm; 15mm≥ D≤ 19mm; D > 20mm. (Duraffourd *& al*., 1990; Ponce *& al.*, 2003).

Results and discussion:

Chemical Screening:

Table 1: Chemical screening of Medicago leaves

Saponins	Flavonoids	Alkaloids	Essential oils	Anthocyanes	Stérols and thriterpenes
+	+	-	-	+	+

In this work it appears that the phytochemical screening based on specific tests allowed to characterize at the Medicago leaves some families of chemical compounds (Tab.1). These tests are in agreement with the results of the literature.

Localization and characteristics of harvesting stations

Table 2: Geographical situation of the harvest stations

Stations	Latitude	Longitude	Altitude	Bioclimatic floor
Fetzara	36°48N	7°45E	0-50m	Subhumid
Aeroport	36°50N	7°48E	0-50m	Subhumid
Besbes	36°46N	7°54E	0-50m	Subhumid
Ben mhidi	36°41N	7°51E	0-50m	Subhumid

Table 3: Physico-chemical characters of soils

Stations	pH H2O	pH KCl	Texture	Organic matter %	Electrical conductivity	Total limestone %
Fetzara	7,66	6,90	sandy- slimy	3,70	2,07	14
Aeroport	7,52	6,80	slimy-clay	2,11	0,14	12,52
Besbes	7,61	6,92	slimy-clay	2,64	0,17	22,37
Ben mhidi	7,12	7,04	slimy-clay	2,26	0,12	23,47

Chemical profiles of Flavonoids and Saponins:

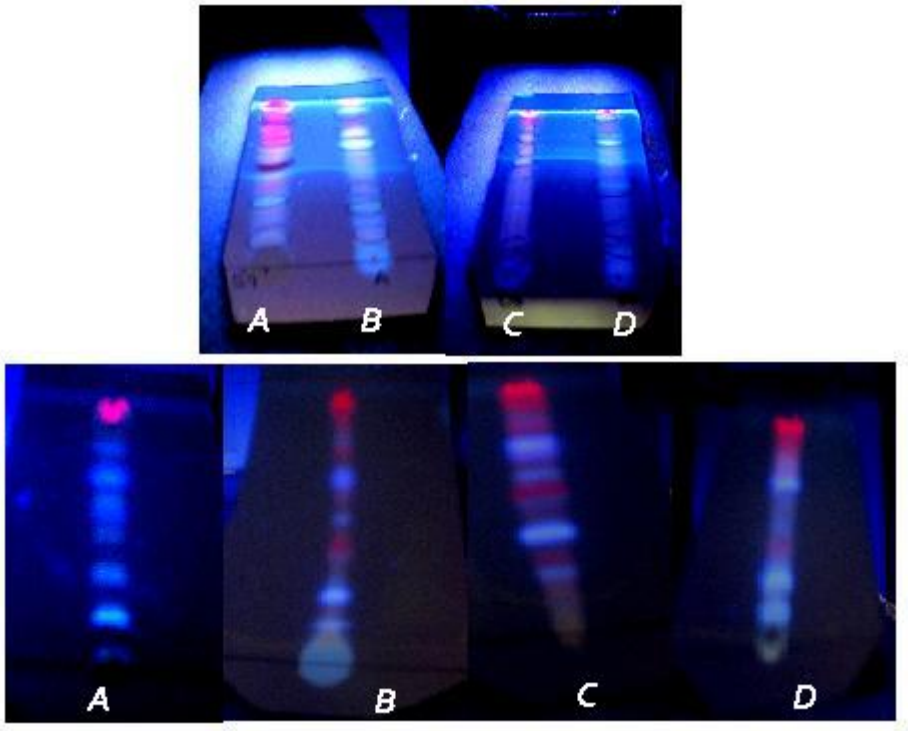

Figure1: Chromatograms observed under UV light at 366 nm (A:Benmhidi;B:Aeroport;C:Besbes;D:Fetzara)

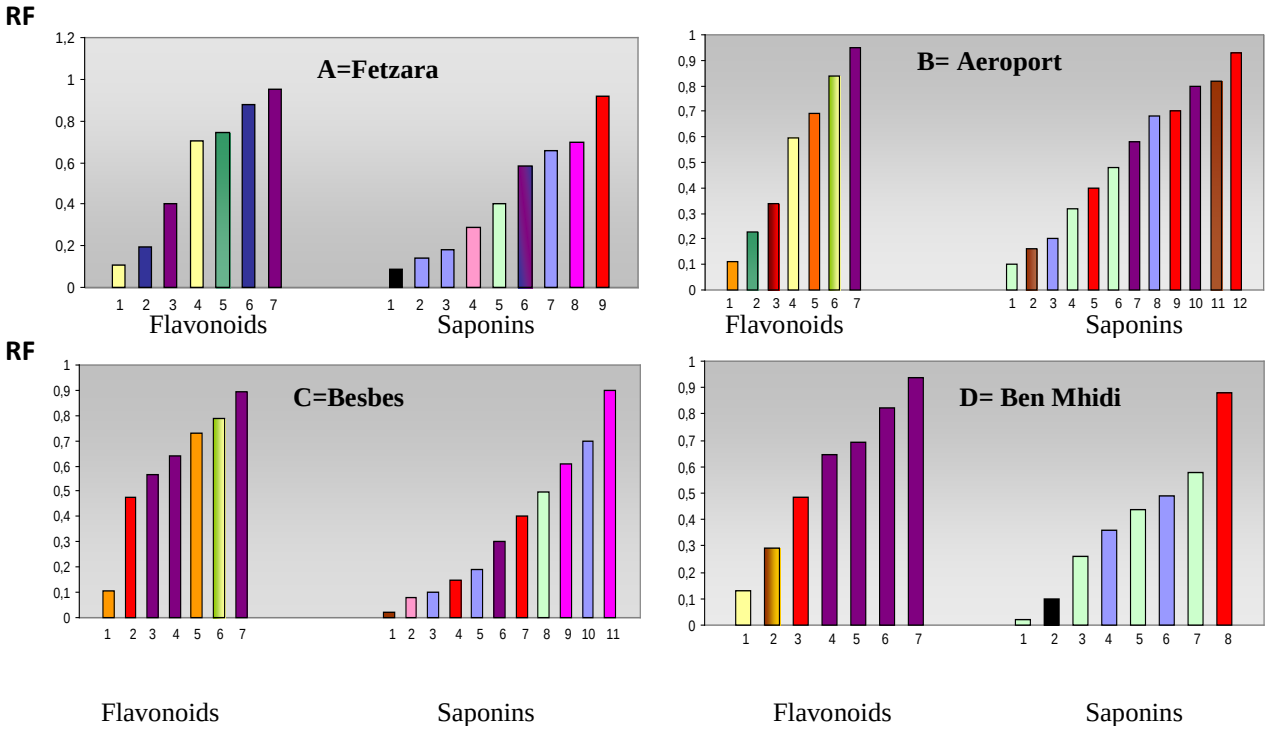

Figure 2: Chromatogram profiles of Medicago leaves crud extracts of different studied stations (Chemical component and its RF and color respective).

The results of chromatography visualized under UV light (fig.1) shows, for each soil type a wealth in chemical compounds flavonoids and saponins. However, the comparison of figures 2 (a, b,c,d.) representing their respective numbers, their colors and their RF indicate that these compounds vary qualitatively and quantitatively from soil to soil. These results indicate that although there is a geographic unit of the source of plant material (Tab. 2) the chemical composition of flavonoids and saponins is still dependent on soil physicochemical characteristics (Tab.3).

Microbiological tests:

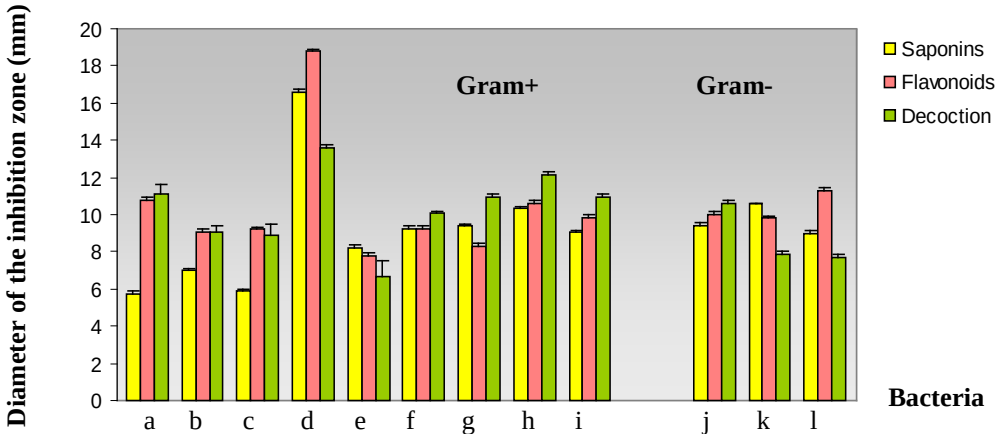

Figure 3 :Compared activity of three extracts on Gram (-)and gram (+) bacterial growth. **a**: E. Coli; **b**: E. Coli(BLSE); **c**: E. Coli (ATCC) **d**: Klebsiella oxytoca; **e**: K.pneumoniae; **f**: Serratia,sp; **g**: Pseudomonas,sp; **h**: P,aeruginosa(ATCC) ; **i** : Acinetobacter; **j**: Staphylococus aureus; **k**: S. epidermidis; **l**: S.aureus(ATCC)

Microbial tests reveal for each of three solutions studied an antibacterial activity well defined on most of the Gram+ and Gram- . The importance of this activity is variable depending on the strain (fig.3).

Klebsiela oxytoca was very sensitive towards the three solutions proved very active. Their diameters of zones of inhibition are of the order of 13,4mm, 18,7mm and 16,4mm respectively for the decoction, flavonoids and saponins. While *Klebsiela pneumonia* has proved resistant. Apart its effect on *Klebsiella oxytoca* whose sensibility towards the 3 solutions are quite similar the decoction is the average more active. With a concentration of 2% only its activity appears superior to that of flavonoids and saponins. This marked activity could be explained by the combined action of flavonoids and saponins in the decoction or by a synergy effect between the different constituents soluble in water because in the absence of complete chemical screening, we can not exclude the possibility of existence in the decoction of other molecules known for this type of activities such as: terpenoids (Ceccherell, *& al.*, 1985; Grade *&t al*., 1992;) and phenolic compounds (Slavienaka & *al*., 2005).

These sensitivity tests allowed us to determine the presence of an antibacterial activity in *Medicago sativa*. This plant is traditionally used to treat rum, digestive disorders, abscesses and other infectious diseases (Bouvyer, 2007). Therefore inhibiting the growth of bacteria tested partly responsible for these pathologies (Fauchère & April, 2002) would explain at least parts the plant using in traditional medicine.

Conclusion

It appears from this study that the phytochemical screening based on specific tests allowed to characterize some families of chemicals in relation to literature results.

The study of chemical profiles of crude extracts of flavonoids and saponins isolated from the leaves of *Medicago sativa* native soils of different shows for each substance rich in chemical compounds, however, varies

qualitatively and quantitatively from one soil to another. This shows that although there is a geographic unit of the source of plant material, homogenization chemical characteristics is all the same dependent edaphic factor.

The results of microbiological tests showed that the leaves of *Medicago sativa* possess chemical molecules with antibacterial power defined on the majority of strains tested. On Klebsiella oxytoca the effect is very potential.

Those results have been encouraged to promote *Medicago sativa* on an appropriate soil. It is necessary to conduct additional tests to confirm the specific use of the studied solutions to treat the bacterial infections caused by bacteria found to be sensitive to our extracts because the preliminary tests of bacterial activity allow us to justify the use of *Medicago sativa* in traditional pharmacopoeia suggesting that their leaves possess an interesting antibacterial compounds.

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