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ORIGINAL ARTICLES

Chemical composition and anti-microbial activity of the crude oils extracts seeds of *Acacia arabica* and *raddiana* from Hoggar South Algeria.

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ABSTRACT

The objective of this study is the highlighting of traditional spontaneous use with phytochemical potential on antibacterial activity of crude extracts of the seeds of two medicinal plants *Acacia arabica* and *Acacia raddiana* of Hoggar region in southern Algeria. After chemical extraction of crude oil seeds by hexane, the physicochemical properties especially tocopherols were measured by HPLC. The antibacterial activity of these extracts was tested *in vitro* on strains ATCC Gram+ bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and Gram- bacteria (*Escherichia coli*) by micro-dilution diffusion in solid medium (discs and wells). The results were statistically treated (MANOVA). The results showed that the extracts have a high content of tocopherols compounds and exhibit a high antibacterial effect. Micro-dilution reveals important MIC against the Gram + bacteria *B. subtilis*, *S. aureus*, *B. cereus* ($0.088 \leq MIC \leq 0.158$ mg/ml) and Gram- bacteria *E. coli* ($0.088 \leq MIC \leq 0.123$ mg/ml) respectively for crude extracts of *A. raddiana* and *A. arabica*. The antibacterial activity of crude extracts explains the importance of traditional use by local people. However, extensive studies on their pharmacological active compounds allow their use in the treatment of certain human diseases.

Key words: *Acacia arabica*, *Acacia raddiana*, phytochemical potential, tocopherols, essential oil, antibacterial activity.

Introduction

Plants oils and extracts have been used for a wide variety of proposes for many thousands of years. Recently, the essential oils and various extracts of plants have provoked interest as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the prevention of food from the toxic effects of oxidants (Siti Amirah *et al.*, 2012). In this study two medicinal plant species *Acacia arabica and Acacia raddiana* were considered. These plants are located in the Hoggar region in extreme south of Algeria. Both species have scientific names; *Acacia nilotic*a subsp *arabica* (Jansen and chard, 2005) and *Acacia tortilis* subsp *raddiana* (Grouzis and Edward, 2003).Theses two plants are widely used in traditional medicine by indigenous nomads as remedies against inflammatory diseases of the throat, colds, diarrhea and diabetes (Eldeen et *al.*, 2010; Siti Amirah *et al.,* 2012)°.

Vernacular names in Asia of *Acacia arabica* is the gum red, neb neb and balbla (Jansen and chard, 2005). In Algeria, *arabica* species is called the Dbegh. According to Hammiche and Maiza (2005), indigenous Tassili N'ajjer called *raddiana* species Thalha. In some countries of the world as Egypt, Sudan and South Africa, scientific studies are done on the use of the two species in traditional medicines, where, gums, stems, leaves, fruits and seeds which are used against several diseases: colds, bronchitis, pneumonia, blindness, diarrhea and bleeding (Watt, 1962; Fleurentin and Pelt, 1982; Eldeen et *al.*, 2005; Eldeen and Van Staden, 2007; Kaur et *al.*, 2005; Eldeen et *al.*, 2010). In Sudanese traditional medicine, the infusion of 5g powder of *Acacia nilotica* seeds in 200ml of cold water are used against colds and the treatment of inflammation of the throat (Abdelnabi et *al.*, 1992). The effect of essential oils of seed extract from *Acacia arabica* and *Acacia raddiana* have not been previously investigated.

This paper was conducted to screen the antimicrobial activity of the crude oils extracts of seeds of both *Acacia* plants, to establish a link between the importance of the traditional uses of seeds and biological effect. The chemical compositions of the essential oils were also analyzed by gas chromatography mass spectroscopy.

Materials and methods

Plant material:

Samples of seeds of *A. arabica* and *A. raddiana* were collected in June 2010 in the Hoggar region (Tegnoueuen, Tessenaouene and Anfeeg), these regions are located in southern Algeria.

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Extraction and physicochemical analysis:

The seeds of both species were sorted, crushed and placed in cellulosic Soxlhet cartridges for chemical extraction by hexane. Physicochemical evaluation of crude extracts focused on percentage of unsaponifiables acid value and the saponification value (Audigie, 1980). Tocopherols were analyzed by RT-HPLC with detector 10A.L Shimadza fluorescence and a column of silica (AFNOR, 1978).

Preparation of extract solutions:

Solutions extracts were prepared using DMSO (dimethyl sulfoxide, 1mg/ml). In this is concentration, this solvent has no effect on the growth of bacteria used in the *in vitro* experimenting unlike some other solvents.

Antimicrobial screening:

A panel of four pathogenic bacteria used in this study including three Gram positive bacteria, *Bacillus cereus* ATCC6633, *Bacillus subtilis* ATCC11778, *Staphylococcus aureus* ATCC33862 and one Gram negative bacteria *Escherichia coli* ATCC25922. All bacteria were obtained from microbiology department of Pasteur Institute of Algiers. The evaluation of the antimicrobial potency extracts of *Acacia* was carried out by three different methods:

-Microdilution method to determine minimum inhibitory concentrations (MIC) described by Thornsberry *et al*. (1983). 1 ml of each dilution of extract was mixed with 8.5 ml of nutrient broth and inoculated with 0.5 ml of bacterial suspension 10^6 cfu/ml. Then, serial dilutions were made from 10^{-1} to 10^{-8}. The cultures were incubated at 37° C for 24 h. Bacterial growth was estimated and compared to the control.

-The disc diffusion method was employed for the determination of antimicrobial activity of the essential oils, according to the methods suggested by the national community for clinical laboratory standard (NCCLS, 2001). The suspension of tested microorganisms (10^6 cfu/ml) was uniformly swabbed on agar plates (Mueller Hinton agar) for bacteria. Steril blank discs (6 mm in diameter, Whatman paper) were individually impregnated with 15 µl of pure crude oils and placed onto the inoculated agar plate. The plates were inverted and incubated at 37°C for 24 h.

Antibacterial activity was evaluated by measuring diameter of the resulting zone of inhibition against the tested bacteria in millimeters.

The latter method has been well development wells in the MH agar culture medium and fills approximately 15µl of each dilution of the extract according to the protocol described by Thornsberry *et al*. (1983) and Eloff *et al*. (1998).

Statistical Analysis:

Statistical analysis of data was made by MANOVA (* $P < 0.05$) according to the Newman-Keuls test.

Results:

Antibacterial activity:

Table 1: Antibacterial activity oils seed of *Acacia arabica* and *Acacia raddiana* by measuring diameter of inhibition and MIC.

Bacterial strains	*Acacia Arabica extracts*			*Acacia raddiana extracts*		
	Disk (mm)	Well (mm)	MIC [mg/ml]	Disk (mm)	Well (mm)	MIC [mg/ml]
B. cereus ATCC6633	$14,8 \pm 0.57^{b}$	$15,36 \pm 0.47^{c}$	≤ 0.158	$15,3 \pm 0.67^{c}$	$20,92 \pm 0.63^{b}$	≤ 0.105
B. subtilis ATCC11778	$20,7 \pm 0.97^{a}$	$19,3 \pm 0.44^{b}$	≤ 0.158	$17,6 \pm 0.54^{b}$	$21,02 \pm 0.57^{b}$	≤ 0.105
S. aureus ATCC33862	$14,76 \pm 0.55^{b}$	$19,4 \pm 1.2^{b}$	≤ 0.126	$15,6 \pm 0.54^{c}$	$22,3 \pm 0.44^{b}$	≤0. 088
E. coli ATCC25922	$21,1 \pm 0.74^{a}$	$25,06 \pm 0.75^{a}$	≤ 0.123	25.06 ± 0.36^{a}	25.96 ± 2.94^{a}	≤ 0.088

[a, b, c] homogen groups according to Newman-Keuls test at $P<0.05$.

The results measured by the methods of disk and wells confirmed the MIC found by microdilution varying in the range 0.088-0.158 mg/ml (Tab. 1).

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Physicochemical characterization:

The results show that the seeds of two species of *Acacia* have yields of 5 to 10% of crude oils whose color varies from yellow to greenish yellow (Tab. 2).

Table 2: Essential oils composition of two *Acacia Arabica* and *Acacia raddiana* seeds using chemical extraction by hexane.

Physicochimical properties of oils	*Acacia arabica*	*Acacia raddiana*
seed yield in oil %	10,06 ± 1,48	5,14 ±1,65
Color	Green yellow	Yellow
Acid value mg KOH/g	10,93 ± 1,16	1,4 ± 0,26
Saponification value	184,26 ± 1,55	168,53 ± 0,90
Unsaponifiables %	3,23 ± 0,22	3,36 ± 0,21
Tocopherol content mg/100g of oil	71,47 ± 0,22	86,49 ± 0,13
α-tocopherol	11,32 ± 2,56	15,58 ± 0,52
β-topherol	23,10 ± 1,52	13,14 ± 0,36
γ-tocopherol	33,46 ± 1,88	55,25 ± 1,07
σ-tocopherol	3,59 ± 1,32	2,52 ± 0,16

There was a high acidity for crude oil of *Acacia Arabica* about ten times *Acacia raddiana*. The values of other parameters were slightly different (Tab.2).

Discussion:

Tocopherols are a group of four (α, β, γ, and σ) lipophilic antioxidants synthesized by photosynthetic organisms, occurring mainly in leaves and seeds. Their antioxidant function is attributed to inhibition of membrane lipid peroxidation and scavenging of reactive oxygen species, but also other functions have been shown in plant metabolism such as role in sugar export from leaves to phloem. The literature data on tocopherols' content and isomers' composition were mainly devoted to seed oils because of the nutritional importance of vitamin E (Szyman and Kruk, 2008.).

The antibacterial tests showed that crude extracts of two species of Acacia have significant bactericidal activity to minimal levels of 0.088-0.158 mg/ml of strains *E. coli* and *S. aureus* (Table 1), which confirms the popular practices used in the gastroenteritis treatment. Otherwise, *B. subtilis* and *B. cereus* exhibit higher MIC and lower diameters inhibitions zones compared to other bacteria (Tab.1).

These strains are known for their strength, the latter is due to sporulation implemented by these bacteria to adapt to a hostile environment (Tortora et *al.*, 2003). Indeed, daily observations of culture media have revealed an acceleration of sporulation, translated by the presence of spores after 24 to 48 hours of incubation at low concentrations of both extracts. These results are in agreement with those obtained by Abdelnabi et *al*. (1992), who worked on the fruits of *Aacacia nilotica* (*arabica*) of Sudanese origin, except that MIC found against *B. cereus*, *B. subtilis* and *E. coli* are slightly different and range from 0.4 to 1.6 mg/ml. Other studies have been conducted on different organs of plants *A. arabica* and *A. raddiana*, such as leaves, bark and roots. Crude extracts of these parts obtained by different solvents (methanol, chloroform, acetone, ethyl acetate, and petroleum ether) have also shown that extracts exhibit important antimicrobial activity with variable MIC according to the solvent extraction, organ and the origin of the plant (Kambizi and Afolayan, 2001; Eldeen *et al.,* 2005; Eldeen *et al.,* 2010).

These variations encountered in the different results may be due to the chemical composition of crude extracts from qualitative and quantitative view, and factors such as the environmental factor, the part of the plant used, the age or even the genetic factor of the plant. The physicochemical properties obtained of the two extracts validate *in vitro* biological studies tests. It was noted that the two crude oils are rich in unsaponifiables and tocopherols (Tab. 2), these components have an antibacterial effect because they are antioxidants which can play a positive role in inhibiting the growth of various bacteria.

These results are almost identical with the results of some researchers who worked on the two species of *Acacia* originated from Africa, India, Pakistan and Australia (Grindley, 1945; Gustone *et al*., 1968; Gustone et *al*., 1972, Chowdhyry *et al.,* 1983, Rathee and Kaushal, 1983, Zaka *et al.,* 1986, Brown *et al.,* 1987, Jamel *et al*., 1987; Benerji *et al*., 1988; Maity and Mandal, 1990; Kallappa *et al*., 2002). In fact, the important bioactivity of two extracts *A. arabica* and *A. raddiana* is related to the phytochemical composition of active components. Indeed, several authors (Tindale and Roux, 1969; Malan and Roux, 1975; El Mousallamy *et al*., 1991; Kirtikar and Basu, 1993; Seigler, 2003) showed that the two *Acacia* species have a wide variety of secondary metabolites such as flavonoids, polyphenols, derived polyphenols and other antioxidants that are endowed with antioxidant and antimicrobial activity.

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Conclusion:

This work has allowed us to conclude that the crude extracts of the seeds of two species of *Acacia* have antibacterial activity on the tested strains. We can say that there is a concordance between popular practices and results, other deeper biochemical and microbiological studies on the active compounds of its extracts are needed to better understand their traditional therapies properties.

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