Research Article

Phytochemical and Antioxidant Activities of Oils of *Acacia arabica* and R*addiana* from the Hoggar Region (Southern Algeria)

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ABSTRACT

Two forest species "*Acacia arabica* and *Acacia raddiana*" originating Ahaggar (W. Tamanrasset) is a bioresource containing several active compounds such as oils and tocopherols which require special incentive for their development and preservation. The main objective of this study is to evaluate the phytochemical potential by GC and HPLC oils seeds of both species , as well as highlighting their antioxidant power test DPPH ° (1,1- diphenyl- 2- pycryhydrazyl). The results obtained show that the yields of the extracted seeds by petroleum ether oils range from 12 % and 9 % for *A. arabica* and *A. raddiana*. The physicochemical parameters corresponding to standard vegetable oils. GPC revealed that these oils are rich in polyunsaturated fatty acids Omega 6 and Omega 9 (74,58, 79,58 %) with a predominance of linoleic acid (C18: 2). In addition, HPLC showed the oils elevate levels of tocopherols (vitamin E) varying respectively from 71.47 ± 1.32 and 86.49 ± 0.34 mg/100g oil for A. *arabica* and *A. raddiana*. The chemical composition of oils exerted a strong antioxidant activity at low concentrations (EC50: 0.13 and 0.06 mg AO / ml DPPH), which have reduced 50% free radical DPPH ° on DPPHH with a time of Average reaction (24 and 27 min). From these results it appears that the phytochemicals and their antioxidant properties of these oils can be interesting to research opportunities for rational exploitation.

Keywords: Acacia arabica, Acacia raddiana, Analysis phytochemicals, Antioxidant power.

INTRODUCTION

In recent years the interest in vegetable oils woody species in relation to their therapeutic properties, has increased significantly. Scientific research in the food, pharmaceutical, cosmetics and other industries have been developed for the extraction, identification and quantification of their fatty acids and antioxidant compounds.[1-6] Each oil extracted from plants in its specificity, it exhibits various physicochemical properties that are widely exploited, such as unsaturated fatty acids, which protect and nourish soften skin. Their regular application restores radiance and suppleness to the skin and prevents premature aging.[7,8] Vegetable oils come in many cosmetic and therapeutic preparations, they are good thinners certain vitamins (Vit E) and essential oils , which attribute to their different degrees remarkable antioxidant properties, which lead to their use as preservatives natural. However, the intensive use of chemicals such as food bio- preservatives, cosmetic or pharmaceutical to cause adverse effects on human health. It is for these reasons research new molecules taking into account other criteria efficiencies has become indispensable. In this context the objective of this work is focused. The seeds of both native woody species in arid regions Hoggar "*Acacia arabica* and *Acacia raddiana*" have been the object of this phytochemical study. A chemical extraction by organic solvents and various phytochemical analyses by GC and HPLC for fatty acids and tocopherols seed oils were made. However, the antioxidant activity of test oils were performed by the method of free radical DPPH° (1,1-diphenyl -1-picrylhydrazyl).

MATERIALS AND METHODS

Plant materials

The plant material is made from the seeds of *Acacia arabica* and *raddiana* harvested in June 2010-2011 in the areas of Hoggar (Tegnouenen, Tessenaouene and Anfeeg) Tamanrasset wilaya located in the extreme south Algeria. Both species collected were identified by the services of the National Institute of Forestry Research and conservation of forests Tamanrasset (INRFT and ASG).

Chemical extractions oils

The seeds were sorted, washed, dried and crushed by a mechanical crusher Retch type, in order to reduce the area of contact with the extraction solvent. The seed oils of powders of different species were extracted using a soxhlet by low polarity and apolar solvents (petroleum ether, hexane and acetone), to determine the solubility towards its solvents and extraction time. The solvent is separated from the oil by rotary evaporator; the yield of the seed oil is calculated in percentage (Table 1).

Analysis of physicochemical parameters

The physicochemical properties of oils *A. arabica* and *A. raddiana* were performed according to the AOCS[9] and AFNOR[10] methods, five replicates were made for each parameter.

Physical parameters

Physical analyzes were measured by the refractive index, density and percentage of phospholipids. The refractive indices of the different oils were measured by Abbe refractometer at 20°C ± 1°C. The densities were

determined using a 10 ml pycnometer at the temperature 20°C ± 1°C. Phosphatides oils were solubilized by the solvent acetone at 4°C for 2 hours, and then filtered through a pre-weighed filter paper, dried in an oven at 40°C. Once the paper is cooled, its weight is determined (Table 1).

Analysis of chemical parameters

The acidities of the different oils are measured by titration of test 1g of oil taken with KOH (0.1 N) in presence of a phenolphthalein indicator. For saponification samples were treated with 0.1 N alcoholic KOH and a few drops of the phenolphthalein by boiling under reflux for 1 hour. Then titrated with sulfuric acid H_2SO_4 0.2N. The iodine value of oil is determined by the reagent Wij (ICl). After the samples were titrated with sodium thiosulfate Na_2SO_3 (0.1 N) until the solution is colorless.

Fatty acid analysis by GC

According to the methods of AFNOR[10], the technique consists of 0.2 g of the oil in 5 ml of alcoholic solution of 3 ml of 2N KOH more solvent isooctane (2,2,4-trimethylpentan) and 1g $NaSO_41H_2O$ (sodium hydrogen sulfate monohydrate), agitation by a vortex and decant a few minutes. 0.5 ml of supernatant with 1.5 ml of isooctane is deposited in the vial for injection into the chromatograph. Device: Perkin Elmer Auto -System XL.

Column: Capillary BPX70. Column temperature: 140°C for 5 min and 180°C for 15 min.

Injection Temperature 200 ° C

Detection temperature 280 ° C

FID Detector

N_2 gas flow (15 ml / S).

HPLC Analysis of tocopherols

Extractions cold solvent by maceration in petroleum ether for 24 hours oils powders of each sample were made. After filtration on filter paper, the filtrates are evaporated to dryness and cold to remove the solvent. 1 g of the oil is diluted in 25 ml of pure solvent isooctane (2,2,4-triméthylpentan). Then, the vial is filled with 1.5 ml of each dilution to injection. The peaks of each sample are compared to the peaks of the reference vitamin E according to DIN EN9936 - solution. The HPLC apparatus is RF- 10A Shimadzu × L, with a fluorescence detector.

Test the antioxidant activity by DPPH°

Evaluation of anti-radical potential is achieved by the method described by Scherer and Godoy[11], two approaches are applied: firstly determining the reduction of DPPH° radical to a reference time, it is defined by % RSA: Radical Scavenger Activity at λ = 517 nm with a Shimadzu UV - visible spectrometer, and secondly monitoring of the kinetics of the reduction. The calculated parameters are the antioxidant activity: Percent inhibition of DPPH:

$\%\ DPPH° = (A_0 - A_1/A_0) \times 100$

A_0: absorbance of DPPH° white;

A_1: Absorbance of the test sample;

IC_{50}: Concentration of antioxidant to reduce 50% of DPPH ° on DPPHH;

TIC_{50}: Time taken to reach an antioxidant concentration equal to IC_{50};

Antiradical efficiency: EAR = 1 / (IC50 × TIC_{50}).

Statistical Analysis

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

RESULTS

Percentage yield of oil seeds

The yield of oil from seeds of both Acacia species varies with the extraction solvent (Figure 1). More no polar solvent is more solubilization of oil increases, which explains that the petroleum ether had the best performance against other solvents.

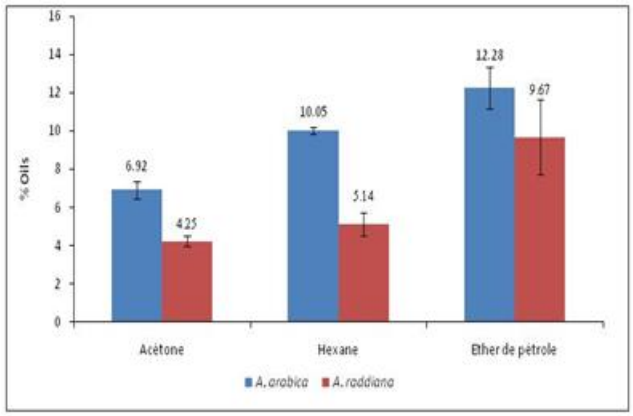

Figure 1: Oil yield of both species acacia

Physicochemical Parameters oils

The physicochemical properties indicate that both are non-drying oils, their refractive indices, and the density of iodine indexes obtained show that unsaturated fatty acids was confirmed by the following gas chromatography. In addition, it was noted that the percentage of unsaponifiable are significant (Table 1).

Composition of fatty acids in oils

Oils *Acacia arabica* and *Acacia raddiana* are mainly composed of Geometric isomerism of cis unsaturated fatty acids (Figure 2). Therefore this composition with no problem digestibility and assimilation by the body.

Tocopherol composition of oils

Analysis of tocopherols by HPLC showed that the oils of the two species have a high content of α, β, and γ-tocopherols (Figure 3).

Table 1: Physicochemical parameters oils of two Acacia species

Physicochemical properties of oils	*Acacia arabica*[b]	*Acacia raddiana*[a]
% Water content	0,21±0,004	0,32±0,003
Color	Gren yelow	yelow
Refraction index η^{20}_{D}	1,4586 ± 0,0005	1,4626 ± 0,004
% Dry matter	69,13 ± 1,76	67,31±0,05
Specific gravity g/ml	0,845 ± 0,001	0,799 ± 0,007
Viscosity mPa.s	28,67 ± 0,57 [a]	14,17±0,28 [a]
% phospholipides	1,85 ± 0,76[a]	0,70 ± 0,17[b]
% of unsaponifiables	3,23 ± 0,22	3,36 ± 0,21
Acid value mg KOH/g	10,93 ± 1,16	1,4 ± 0,26
Saponification value	184,26 ± 1,55	168,53 ± 0,90
Indice de peroxyde $meqO_2$/Kg	20 ± 0,86[a]	14,16 ± 0,76[b]
Iodine value $meqO_2$/Kg	144,50 ± 0,50[a]	155,26 ± 1,16[b]

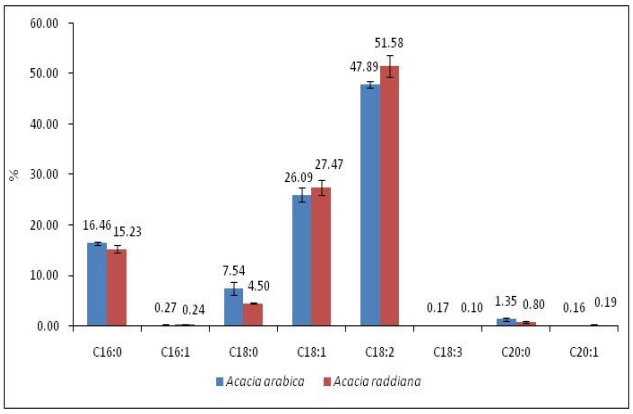

Figure 2: Fatty acid composition of oils *A. arabica* and *raddiana.*

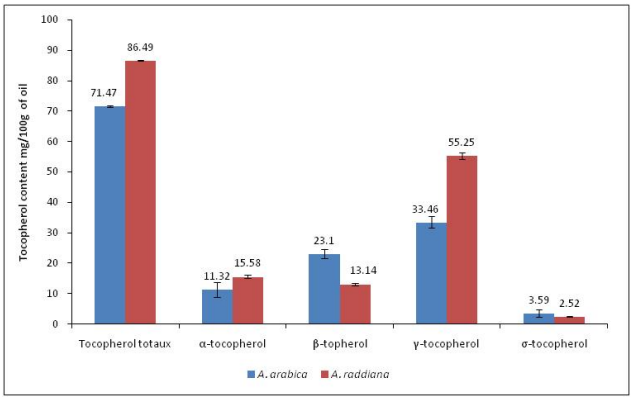

Figure 3: The composition of tocopherols oils Acacia

Antioxidant activity of oils

Of the three compounds tested gallic acid and of the oils *A. raddiana* have disclosed a higher anti -radical activity with a reaction time considered as an intermediate for the oils as acacia does not exceed 30 minutes. On the other, gallic acid, which is a pure compound to present a low response time means that the reduction reaction of free radical DPPH is faster in the presence of this acid (table 2).

Table 2: Parameters of the antioxidant activity of oils acacia

	IC_{50} (mg AO/ml DPPH°)	IC_{50} (mg AO/g DPPH°)	TIC_{50} (min)	EAR
A. arabica	0,131	524	27,16	$0,07\times10^{-3}$
A.raddiana	0,066	264	23,14	$0,16\times10^{-3}$
Acide gallique	0,053	212	11,44	$0,41\times10^{-3}$

IC_{50} : Concentration d'antioxydant nécessaire pour réduire 50% de DPPH° ; **TIC_{50}** : Temps d'équilibre pour IC_{50} ; **EAR** : Efficacité anti-radicalaire.

DISCUSSION

Physicochemical parameters obtained are in agreement with those obtained by other researchers who worked on *Acacia arabica* and *Acacia raddiana* from India, Pakistan and Australia.[12, 1-5] The oil composition is characterized by a predominance of unsaturated fatty acids linoleic C18: 2 and oleic C18: 1 (Figure 2). According to Ha et *al.*[13] and IP et *al.*[14] these unsaturated fatty acids confer hypocholesterolemic and antiatherogenic action. The ratio of polyunsaturated fatty acids to the saturated acid is less than or equal 5. According Troegeler - Meynadier and Enjalbert [15], this contribution is highly recommended by nutritionists. With rates of about 44 and 51 % linoleic acid, which is an essential acid, are shown in the two oils Acacia species is a precursor of the unsaturated fatty acids of the omega-6 series. It is indirectly causing prostaglandin and leukotriene series 1 and 2. Excluding these substances play an important role in the nervous system, the cardiovascular balance, the immune system and allergic and inflammatory reactions. Linoleic acid also plays a role in cell permeability and its deficiency causes, among other skin aging resulting in dryness and loss of elasticity of the skin, with the appearance of wrinkles.[7,8] However, it is noted that omega -3 fatty acid levels of the two oils are negligible (0.1 to 0.17 %) (Figure 3). The other fatty acids of this series (eicosapentaenoic acid EPA and docosahexaenoic DHA) are absent in the Acacia oils. Thus, a diet based oil acacia should be complemented by an omega -3 fatty acid ratio as either vegetable oil rich in α -linolenic acid is oil rich fish EPA and DHA. HPLC analysis shows that the tocopherols acacia two oils are provided µ-tocopherol followed by β, α, 6-tocopherol (Figure 3). This richness in antioxidants including µ -tocopherol offers high stability and resistance to self oxidation of oils acacia during storage or cooking treatments is a good indicator of stability. This allowed us to say that the results of antioxidant activity obtained confirm the ability of the two oils to reduce the free radical DPPH° at low concentrations (Table 2). With this specific chemical composition of the two extracts were acacia a significant antioxidant (Table 2). Studies on the relationship between the chemical structure of the phenolic compounds and

free radical scavenger to as DPPH showed that the anti - radical activity is dependent on the number, position and nature of the substituent on the rings. These parameters are also related to the polarity of the compounds.[16-19] However, both crude acacia exhibit heterogeneity in the composition can result in different properties of polarity and chemical structures of these compounds beside their capacity for scavenging free radicals.

The observed activity may be attributed to variation of the active substances in the two oils such as acacia tocopherols and other secondary metabolites such as polyphenols, saponins, alkaloids, flavonoids and tannins.[20-24]

CONCLUSION

Oils *Acacia arabica* and *raddiana* is characterized by a specific chemical composition characterized by a significant insaturartion due to high levels of linoleic and oleic fatty acids , combined with the presence of high levels of tocopherols. This specific composition revealed an important antioxidant that gives them the nutritional, therapeutic and dietary benefits. However, the phytochemical composition of the oils also predestined for cosmetic purposes, which may attract the interest of cosmetic laboratories to incorporate these oils in cosmetic compositions.

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