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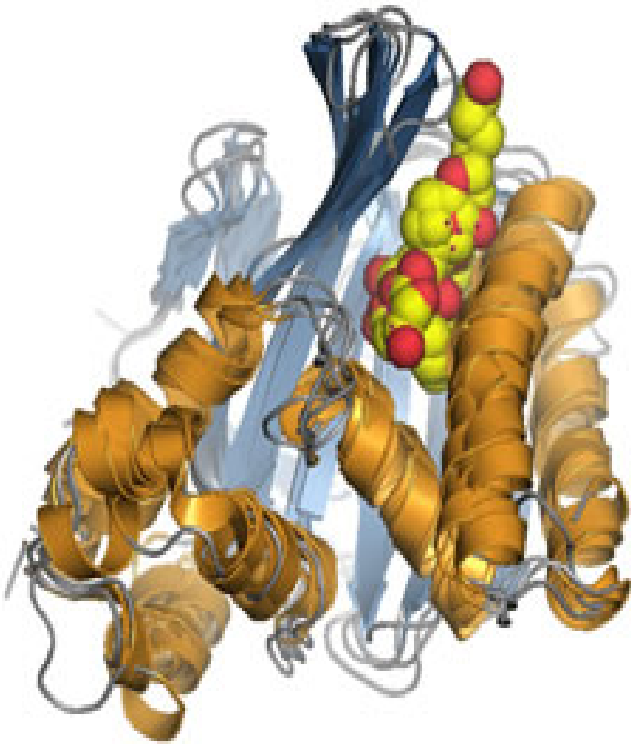

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Phenolic content and antioxidant activity of extracts from *Acacia arabica* Barks

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Abstract. *Acacia Arabica plant is well known in the region of North African Sahara for its efficiency in the traditional treatment of several diseases such as diarrhoea, eczema, tonsillitis and conjunctivitis. This work was aimed to determine the total phenolic content and antioxidant activity of methanol:water (8:2) and acetone:water (7:3) extracts of barks of Acacia Arabica growing in Tamanrasset (Algeria). Total phenolic contents of the extracts were determined by the Folin-Ciocalteu method and their antioxidant activities were evaluated using Phosphomolybdenum, reducing power and DPPH (1,1-diphenyl-2-picrylhydrazine) methods. The highest phenolic content was obtained from acetone:water extract. For the evaluation of antioxidant activity and for Phosphomolybdenum and Reducing power methods, it was found that the methanol:water extract is more effective than acetone:water extract who has shown more effectiveness by the DPPH method. These results suggest that Acacia Arabica may act as a chemopreventive agent, providing antioxidant properties.*

***Key Words*:** *Acacia arabica*; total phenolic; antioxidant activity; Phosphomolybdenum assay; RPA; DPPH

1. Introduction

Human body is exposed to a large number of foreign chemicals everyday [1]. The most of which are man-made and the inability to properly metabolize them negatively affects the health by the generation of free radicals. Oxidative damage originates from an increase in free radical production either by exogenous radicals such as radiation, pollution and cigarette smoking. Other sources are endogenous sources, such as inflammation, the respiratory burst and xenobiotic killing[2-4].

Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in various plants [5]. They can reduce the access of oxidants and other deleterious molecules due to their ability to scavenge oxygen-nitrogen-derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases [6]. Based on accumulative evidence, in recent decades, tremendous interest has considerably increased in finding natural substances (i.e. antioxidants) present in

foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects. On the other hand, polyphenols, used as natural antioxidants, are gaining importance, due to their health benefits for humans, decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation. Natural antioxidants are also in high demand for application as nutraceuticals/functional food/bio-pharmaceuticals because of consumer preferences.

Traditionally, *Acacia* species have long been used for the treatment of skin, stomach and tooth problems. *Acacia arabica* (Lam.) Willd. syn. Acacia *nilotica* (L.) Del. (Mimosaceae). Commonly known as Amoura, Taggart, or Bagarowa has been recognized worldwide as a multipurpose tree.

It is widely distributed throughout arid and semi-arid zones of the world. *Acacia arabica* has been proved as effective medicine in treatment of malaria; sore throat (aerial part) and toothache (bark) [7-12] have tested the anti-fertility activity of Acacia *arabica* pods and nuts.

The methanolic extracts of *Acacia arabica* barks have been claimed against HIV-PR [13,14]. Currently, one group of researchers has tested the antiplasmodial activity of A. *nilotica* ethyl acetate extract against different chloroquine resistant and sensitive strains of *Plasmodium falciparum* [15]. The fresh plant parts of this species have been reported to be most active against Hepatitis C virus [16]. It is an important multipurpose tree that has been used extensively for the treatment of various diseases, e.g. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma [17].

The aim of this study was to determine the total phenolics and flavonoids content and to investigate the in vitro antioxidant and anti radical capacities of methanol-water and acetone-water extracts from *Acacia* arabica barks which are collected from the region of Tamanrasset in the south of Algeria. The extracts were prepared from dried and powdered barks. The total phenolic and flavonoids contents were measured from plant extracts using Pholin-Ciocalteu method, the antioxidant activity was examined using tow antioxidant assays: Ferric Reducing and Phosphomolybdenum assays and the free radical scavenging was examined using the DPPH test.

2. Materials and methods

Plant material

Acacia Arabica barks were collected from the village named Amsel 30 km from Tamanrasset city center on September 2009. The barks were air dried until dryness at room temperature and in darkness, then were stocked in tissue bags untill use.

Chemicals and reagents

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland), Sigma-Aldrich (Steinheim, Germany and Merck (Germany).Riedel-dhaen,Prolabo.

Sample preparation and extraction

The plant material was milled to a fine powder using a coffee-grinder. The phenolics from samples were isolated by a modified version of the method described by Djerridane et al (2006) [18]. Two amounts of 80 g of fine ripe barks powder were macerated in methanol:water (80:20, v/v) and acetone:water (70:30, v/v) separately for 24 h at room temperature. The crude preparations were filtered, and the residues re-extracted twice with the same hydrau-methanolic and hydrau-acetonic solvents for 24 h at room temperature. The extracts were filtered. The filtrates were combined. After removing the methanol and acetone

under vacuum at 40 °C, the Phenolic compounds were then extracted three times with ethylacetate (1:1, v/v). The three organic phases were combined; the residual water in the ethylacetate was eliminated with anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The extracted phenolics were dissolved in methanol and then filtered using filter paper. Methanolic solutions of phenolic C(methanol:water) and C(acetone:water) extracts were stored inside freezer until analysis. The storage conditions (time and temperature) were the same for the two extracts.

Determination of total phenolic and flavonoid contents

Determination of total phenolic content (TPC)

The concentration of total phenolics (TPC) was determined by the Folin–Ciocalteau colorimetric method [19]. The total phenolic content (TPC) was expressed as gallic acid equivalents (GAE) in mg/100g dry plant material. The concentration of phenolic compounds was calculated according to the equation obtained from standard gallic acid graph.

Determination of total flavonoids content (TFC)

The total flavonoid content (TFC) was determined according as the aluminum chloride colorimetric method described of Chang et al (2002) based on the method of Woisky and Salatino (1998) [20,21].with slight modifications and results were expressed as mg rutin equivalents (RE) per g of dry weight. The method is based on the quantification of yellow color produced by the interaction of flavonoids with $AlCl_3$ reagent. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard rutin graph.

Determination of antioxidant activity

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). Extract solution (2ml), phosphate buffer (2ml, 0.2M, pH 6.6) and potassium ferricyanide (2ml, 10mg/ml) were mixed, and then incubated at 50°C for 20 min. Trichloroacetic acid (2ml, 100mg/l) was added to the mixture. A volume of 2ml from each of the above mixtures was mixed with 2ml of distilled water and 0.4ml of 0.1% (w/v) ferric chloride in a test tube. After 10min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated a high reducing power.

Phosphomolybdenum assay

The antioxidant activity of samples was also evaluated by Phosphomolybdenum assay using the method of Prieto et al., (1999) with some modifications. 0.4ml of sample solution was mixed with 4ml of reagent solution containing (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in vials. The vials were capped and then incubated in water bath at 95°C for 90 min. After the incubation, samples were cooled to room temperature and absorbance of the mixture was measured at 765 nm against a blank. A typical blank solution containing 4ml of reagent solution and appropriate volume of the same solvent was used for the extract.

DPPH radical scavenging activity

Scavenging Radical activity of DPF extracts against stable $DPPH^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl hydrate) was determined using the method of Brand-Williams, Cuvelier, & Berset,(1995) [22]. One ml of DPPH solution was mixed with equal volume of the extract solution in phosphate buffer (pH 7.4). The mixture was slightly shaken and kept in dark for 30 minutes. When $DPPH^{\bullet}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light-yellow) were measured at 517nm. The antioxidant activity of the extract was expressed as an IC50 value defined as the

concentration (in mg/l) of the extract that inhibited the formation of DPPH radicals by 50% [23].

3. Results and discussion

Total phenolic and flavonoid contents

Total phenolic content (TPC)

Phenolic compounds contribute to the overall antioxidant activities of plants mainly due to their redox properties. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals. The total Phenolic content of the two extracts is listed in Table 1 The higher phenolic content TPC was found in the extract C(acetone:water).

Total flavonoid content (TFC)

Flavonoids are the most common and widely distributed group of plant phenolic compounds, characterized by a benzo-γ-pyrone structure. It is ubiquitous in fruits and vegetables. Most of the flavonoids possess strong antioxidant properties following chain breaking mechanism. The higher flavonoid content was found in the extract C(acetone:water). (table 1)

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities for extracts studied

Samples	TPC Gallic acid eq. (mg/g)	TFC Rutin eq. (mg/g)	Reduction power AEAC(mM)	Phosphate Molybdate AEAC (mM)	IC_{50} DPPH ($x10^{-2}$g/l)
C(acetone:water)	275,751	0,784	3,452	2,319	9,16
C(methanol:water)	259,286	0,214	7,053	2,748	10,96
Gallic acid	-	-	0,0012	0,00087	-
Vit E	-	-	-	-	34

Determination of antioxidant activity

Reducing power assay

Compounds with reducing power indicate that they are electron donors, and have ability to reduce the oxidized intermediates that are formed as a result of lipid peroxidation processes thus they can act as primary and secondary antioxidants [24]. Extracts of *Acacia arabica* have considerable reduction potential when extract reacts with potassium ferricyanide (Fe^{3+}) it converts potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride and form ferric ferrous complex that has an absorption maximum at 700 nm. This assay is relatively simple and inexpensive. The reducing power of the extracts was expressed as number of ascorbic acid equivalent and compared to the Gallic acid reducing value. The results were shown in Table 1, the highest reducing power was found in the C(methanol:water) extract.

Phosphomolybdenum assay

Phosphomolybdenum method is utilized for spectrophotometeric quantitation of total antioxidant capacity. In the presence of antioxidant compounds molybdenum Mo (VI) is reduced to form green phosphate/Mo(V) complex. In Phosphomolybdenum method extracts showed excellent antioxidant activity that was comparable to that of gallic acid. The

antioxidant activity of extracts and standard was listed in table 1, the highest reducing value was found in the C(methanol:water) extract.

DPPH radical scavenging activity

DPPH$^{\bullet}$ radical is a stable lipophilic free radical which has been generally used for estimating antioxidant activity of food and medicine materials. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radial by hydrogen donation. It is visually noticeable as a colour change from purple to yellow [23]. In the current study, the two extracts showed excellent activity comparing to the vitamine E activity, the IC_{50} values are showen in the table 1, the highest activity is in the C(acetone:water) extract. It was observed that the free radical scavenging activity increased with the increase of phenolic compound content. It was already reported that positive correlation between free radical scavenging activity and total phenolic compound. The linear relation between phenolic content and antioxidant activity indicates that the phenolic compounds might be the major contributors towards the free radical scavenging activities of plant extracts.

4. Conclusion

On the light of the results obtained from the above study, it can be suggested that *Acacia arabica* barks could be used as a easily accessible source of natural antioxidant, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc. For the future, more studies are needed on the isolation and identification of antioxidant components in this plant. Safety and toxicity studies are also needed for utilization of *Acacia arabica* and its components in different antioxidant pharmaceutical formulations.

References

[1]. Santhakumari P; Prakasam A; Pugalendi KV. Modulation of oxidative stress parameters by treatment with piper leaf in streptozotocin induced diabetic rats. Indian J. Pharm. 35:373-378, 2003

[2]. Ghisseli A., Serafini M., Natella F., Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Rad. Biol. Med. 29, 1106, 2000

[3]. Davies K. J. Oxidative stress: the paradox of aerobic life Biochem. Soc. Symp. 61, 31, 1995

[4]. Haliwell B., Cross C. E. Oxygen-derived species:their relation to human disease and environmental stress. Environ. Health Perspect. 102, Suppl. 10, 5, 1994

[5]. Prakash D, Singh BN, Upadhyay G. Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). *Food Chem*; 102:1389–1393, 2007.

[6]. Ardestani A, Yazdanparast R. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chem.*; 104:21–29, 2007.

[7]. Chopra R.N., Nayar S.L., Chopra I.C: Glossary of Indian medicinal plants. C.S.I.R., New Delhi (1956): 2-23, 1999.

[8]. Jain A., Katewa S.S., Galav P.K., Sharma, P.: Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan, India. J. Ethnopharmacol; 102(2): 143-157, 2005.

[9]. Jain A.K., Shimoi K., Nakamura Y., Tomita I. And Kada T. Preliminary study on the dismutagenic and antimutagenic effect of some natural products. Curr Sci 1987; 56: 1266-1269.

[10]. Joshi S.G.: Medicinal Plants I.B.H. Delhi: 270-271, 2007.

[11]. Kubmarawa D., Ajoku G.A., Enwerem N.M., Okorie D.A.: Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol; 6(14): 1690-1696, 2007.

[12]. Chowdhury A.R., Baberji R., Mishra G. And Nigam S.K.: Chemical composition of *Acacia* seeds. J Am Oil Chem Soc; 60: 1893-1894, 1983.

[13]. Hussein G., Miyashiro H., Nakamura N., Hattori M., Kawahata T. and Otake T., Kakiuchi N. and Shimotohno K.: Inhibitory effect of sudanese medicinal plant extracts on HIV replication and HIV-1 protease protease. Phytother Res.; 13(1): 31-36, 1999.
[14]. Bessong P.O., Obi C.L.: Ethnopharmacology of HIV in South Africa - A mini review. African J.Biotechnol.; 5(19): 1693-1699, 2006.
[15]. EI-Tahir A., Satti G.M.H., Khalid S.A.: Antiplamodial activity of selected sudanese medicinal plants with emphasis on *Acacia nilotica.* J. Phytother; 13(6): 474-47, 1999.
[16]. Hussein G., Miyashiro H., Nakamura N., Hattori M., Kakiuchi N., Shimotohno K.: Inhibitory effect of sudanese medicinal plant extracts on hepatitis C virus protease. Phytother Res.; 14(7): 510-516, 2000.
[17]. Rahaman O.: A Review of Uses of Acacia Nilotica (Booni) In Alternative Medicine. www. SearchWarp.com 2010.
[18]. Djeridane, A., Yousfi, M., Nadjemi, B., Maamrim, S., Djireb, F., & Stocker, P. Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase. Journal of Enzyme Inhibition and Medicinal Chemistry, 21, 719–726, 2006.
[19]. Singleton, V. L., & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144–158, 1965.
[20]. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C., Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10, 178–182, 2002
[21] Woisky, R., & Salatino, A. Analysis of propolis: Some parameters and procedures for chemical quality control. Journal of Apicultural Research, 37, 99–105, 1998.
[22]. Brand-williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittelwissenschaft und technologie, 28, 25–30, 1995.
[23] Ruch, R.T., Cheng, S.J., Klaunig, J.E., Spin trapping of superoxide and hydroxyl radicals. Methods in Enzymology 105, 198–209, 1984.
[24]. Yen, W.-J., Chang, L.-W., & Duh, P.-D. Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. LWT, 28, 193–200, 2005.

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