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Research Article

PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY OF *POPULUS ALBA* L. IN ALGERIA

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Abstract

The objective of the present study is to evaluate the antioxidant activity *in vitro* and the phytochemical study of the aqueous extract of the leaves of *Populus alba*. The phytochemical screening and determination of total phenolic, flavonoid contents and tannins of the leaves extract were carried out. The antioxidant potential was also established by the free radical scavenging (DPPH)and the iron reducing power (FRAP) assays. The tested extract showed their richness in phenolic compounds, terpenoids, steroids and essential oils. Thetotal phenolic contents of plant estimated by Folin-Ciocalteu method were456.11±3.87 mg Equivalent of gallic acid per gof lyophilized extract. Behind, The amount of total flavonoids was 244.5±1.57 mg Equivalent of quercetinper g of lyophilized extract. The assay by the vanillin method revealed that the concentration of condensed tannins was 261.88±4.12 mg Equivalent of catechin per g of lyophilized extract. The *in vitro* study of the antioxidant activity of the aqueous extract by the free radical scavenging assay free DPPH and the reducing power of iron presented IC50s of the order of: 43.99±0.54 and 86.78±2.15 µg/mL, respectively with comparison with as standard reference (BHT, BHA, Trolox). These results showed that the aqueous extract of the leaves of *Populus alba* is rich in bioactive substances and characterized by antioxidant power with very promising therapeutic values for the treatment of diseases.

Keywords: DPPH, Flavonoids, Phytochemical screening, Polyphenols, *Populus alba*.

INTRODUCTION

Oxidative stress is the biggest concern in the biological world due to an unsteadiness in the redox balance. This imbalance results from an overproduction of free radicals and a decrease in antioxidant defenses, leading to tissue and biomolecule damage (lipids, proteins, DNA) responsible for many chronic diseases (Belaîch *et al*., 2015 ; Smilin *et al*., 2019 ; Baldisserotto *et al*., 2019), such as cancer, cardiovascular complications and neurodegenerative disorders (Incalza *et al*., 2018). Antioxidants are simply good molecules that covert these free radicals into harmless products and thus reduce their harmful effects on our health (Gulcin, 2020). Recently, in industry, the use of synthetic antioxidant molecules has taken considerable attention but at the same time, they are known by their possible toxicity (Bammou, 2020). Now, new plant sources of natural antioxidants are sought as alternatives (Haddouchi*et al*., 2016; Tanoh *et al*., 2019), in particular polyphenol dueto their biological effects; anti-inflammatory, antibacterial, antiviral, anti-age and anticancer (Zhou *et al*., 2016; Koch, 2019). Algeria is one of the Mediterranean countries with a remarkable flora richness linked to the diversity of its ecosystems. The use of herbal remedies is an integral part of ancient traditions (Sassoui *et al*., 2020). Among which *Populus alba* (white poplar), tree of the Salicaceae family, widely used in herbal medicine (Belkhodja *et al*., 2017). This article is the fruit of a phytochemical study made on white poplar by carrying a phytochemical screening, a colorimetric assay of the various phenolic compounds (polyphenols, flavonoids and tannins), and an evaluation of the antioxidant activity *in vitro*.

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MATERIAL AND METHODS

Chemicals

All of the chemicals and reagents used in the experiments were of analytical grade. DPPH, reducing power, vitamin E, BHA, BHT, gallic acid, quercetin, vanillin.

Plant Material and Preparation of Aqueous Extract

Populus alba leaves were purchased from a herbalist in Ain El Baidha (Oran), the plant was identified in the plant cytology & ultrastructure laboratory by Professor Mrs. Helfaoui at Ahmed Ben Bella University Oran1.

Figure 1. *Populus alba* leaf with its two different sides

After the leaves were cleaned and air-dried, they ground to a fine powder and extracted with distilled water (1: 10, w/v) under the heat conditions (60 °C) during 60 min. The mixture was filtered. The obtained decoction was frozen and then lyophilized (freeze-dryer christalpha 2-4 lsc d 37520, Germany). The leaves of the white poplar were sorted and dried in the open air and at room temperature for about a week,

then they were subjected to grinding with an electric grinder 2-3 times quickly without exceeding a minute (Benahmed *et al*., 2021).

Phytochemical analysis of plant

For phenolics, the total polyphenol content of the aqueous extracts is estimated by the Folin-Ciocalteu reagent according to the colorimetric method of Singleton and Rossi, (1965).0.1 ml of plant extract are mixed with 0.5 ml of Folin-Ciocalteu reagent (1: 10 v / v) .The mixture is stirred using a vortex and then incubated for 5 min in the dark and at room temperature. Then, 1.5 ml of the aqueous solution of anhydrous sodium carbonate (2% w / v) is added and then stirred. After incubating the mixture for one hour, the absorbance is measured at 765 nm. The total polyphenol contents are expressed in micrograms of gallic acid equivalent per milliliter of extract (µg EAG / ml of extract). Concerning flavonoids, The dosage of flavonoids in extracts is based on the formation of a complex between Al^{+3} and the flavonoids. The method of Topçu *et al*. (2007) is used with some modifications for a determination on a 96-well microplate. To 50 µl of plant extract, 130 µl MeOH were added and then mixed with 10 µl of CH3COOK solution (1M), then a volume of 10 µl of 10% solution (Al (NO3) 3, 9H2O) was added .After 40 minutes of incubation, the absorbance measurement was carried out at 415 nm against a blank prepared by replacing the reagents with MeOH (50 µl extract and 150 µl MeOH). Flavonoid contents were expressed in micrograms of quercetin equivalent per milliliter of extract (µg EQ / ml of extract), calculated from a line prepared using different concentrations of quercetin. The condensed tannins are measured in the presence of concentrated sulfuric acid by a colorimetric method according to Price *et al*., (1978). They depolymerize and by reaction with vanillin, turn into anthocyanidols of specific red color. A volume of 0.05 ml of the suitably diluted extract is mixed with 3 ml of the vanillin / methanol solution (4%), and then 1.5 ml of concentrated sulfuric acid is added. The mixture is stirred and then incubated at room temperature for 15 minutes. Absorbance is measured at 500 nm against a blank containing pure methanol. The tannin contents are estimated in mg catechin equivalents per 100 gram of the weight of lyophilized dry extract (mg EC / 100g extract), with reference to a standard range of catechin.

Phytochemical screening

In order to demonstrate the different chemical classes of secondary metabolites present in the aqueous extract of the species tested, several characterization reactions were established. This qualitative phytochemical screening carried out is based on precipitation or coloring reactions using specific reagents which allowed us a preliminary identification of chemical substances.

Flavonoids: 1 ml of 10% lead acetate (H2O) is added to 1 ml of extract. Their presence is indicated by a yellowish green color (Yahyaoui *et al*., 2017).

Tannins: 2.5 ml of extract are added with 0.5 ml of 2% iron perchloride, MeOH (FeCl3), a precipitate develops: greenish brown for the catechetical tannins or blue black for the gallic tannins (Yahyaoui *et al*., 2017).

Coumarins: 2 ml of extract are added with 3 ml of 10% sodium hydroxide, H_2O with stirring, a yellow color appears (El Yahyaoui *et al*., 2017).

Terpenoids: 2.5 ml of the sample are added to 1 ml of chloroform and 1.5 ml of concentrated sulfuric acid. The formation of a red or purple ring indicates their presence (N'Guessan *et al*., 2009).

Steroids: 2.5 ml of acetic anhydride and 500 µl of concentrated sulfuric acid are added to 2.5 ml of extract, then the mixture is left to react for 20 minutes. The blue-green appearance indicates the presence of steroids (N'Guessan *et al*., 2009).

Saponins: 3 ml of distilled water are added with 1 ml of extract then stirred vigorously for 2 minutes. Then the mixture is left to stand for 20 minutes. The formation of persistent foam indicates the presence of saponosides (Yodav and Agarwala, 2011).

Free quinones: A few drops of sodium hydroxide (10%, H2O) with 5 drops of HCl are mixed with 1 ml of the extract. The appearance of a yellow, red or purple color explains their presence (Dohou *et al*., 2003).

Essential oils: 2 ml of extract are added to 100 µl of NaOH (10%) and 100 µl HCl (10%), the formation of a white precipitation confirms the presence of essential oils (Cahyono, 2015).

***In vitro* Antioxidant potential assays**

DPPH free radical scavenging activity: The 2,2-diphenyl-1-picrylhydrazyl molecule (MW = 394.33 g / mol) is a free radical stable to a purple color in solution in methanol or ethanol. It has a characteristic absorption at 517 nm (Miguel-Chávez, 2017). Mix 100 µL of different concentrations of the plant extract with 900 µL of a methanolic solution of DPPH (6mg: 100ml). The absorbance reading is taken at 517nm after allowing the reaction to incubate in the dark for one hour. The extracts are tested against a blank which contains the methanolic solution of DPPH incubated under the same conditions as the samples. α-tocopherol, BHA, BHT are used as positive controls (reference substances), at the same chosen concentrations and under the same operating conditions. The antiradical activity is determined by the formula

DPPH radical scavenging activity (%) = (A control - A sample) / (A control) x 100

Ferric Reducing Antioxidant Power « FRAP »: This power is determined by the method of Oyaizu, (1986) with a slight modification. Reducing power is the ability of the antioxidants in the extract to reduce the ferric iron Fe^{3+} in the ferricyanide complex to ferrous iron Fe^{2+}. The reduced form gives a blue-green color proportional to the reducing power of the extract (Nadour, 2015).10 µl of extract is mixed with 40 µl of PBS phosphate buffer solution (0.2M; PH = 6.6) and 50 µl of a 1% solution of potassium ferricyanide [K3Fe (CN) 6]. The mixture is incubated in a water bath at 50 ° C for 20 minutes, then 50 µl of 10% trichloroacetic acid (TCA) are added to stop the reaction, 40 µl of distilled water and 10 µl of ferric chloride (FeCl3.6H2O) to 0.1%. Absorbance is measured at 700 nm.

RESULTS AND DISCUSSION

From calibration curves of gallic acid, quercetin and catechin it can be seen that *Populus alba* has a concentration of total polyphenol, total flavonoid and condensed tannin in the order of 456,11±3,87 µg EAG/ml of extract, 244,55±1,57 µg EQ/ml of extract and 261,88±4,12 mgEC/100g of extract respectively (Table 1).

Table 1. The levels of phenolic compounds in the aqueous extract of *Populus alba*

Aqueous extract	Total polyphenols (µg EAG/ ml E)	Total flavonoids (µg EQ/ml E)	Condensed tannins (mg EC/ 100g E)
Populus alba	456.11±3.87	244.55±1.57	261.88±4.12

According to our results, the determination of the total polyphenols obtained from the aqueous extract of *Populus alba* shows that their content is high in comparison with other polyphenolic compounds. A study carried out by Tawfeek *et al*., (2019) on the same plant genus, they indicated that the leaves of *Populus alba* have a high content of total polyphenols compared to that found in our studies extract.The work carried out by Medina-Villar *et al*. (2015) and Tàlas-Nebehaj*et al*., (2019) approved the richness of *P.alba* in polyphenols. The concentration of flavonoids obtained in the aqueous extract of *Populus alba* is higher than that recorder in other species such as *Populus nigra* by some authors (Arab *et al*., 2018). Tawfeek *et al*., (2019) revealed that their *P.alba* extract is particularly very rich in these substances compared to our species extract analyzed. Our extract is rich in tannins, this is in agreement with the studies carried out by Trémolieres and Carbinier, (1985) and Khurilenko *et al*., (2013), on the other hand, the values recorded by Arab *et al*., (2018) in the other poplar species: *P.nigra* are lower. The amount of phenolic compounds and flavonoids in plant extracts varies from plant to plant, this is probably due to geographic location, harvest season, climatic and environmental conditions, plant maturity and shelf life (Gheffour *et al*., 2015). In addition, several studies have shown that the levels of phenolic compounds in a plant are closely related to the complexity of this group of compounds, the extraction methods and the concentration of the solvent used. Indeed, several constraints make it difficult to extract phenolic compounds from plant tissue. The presence in plant cells of several types of enzymes can modify phenolic compounds, in particular polyphenol oxidases and glyosidases. In addition, the method of drying the plant can be a factor in the deterioration of the structure of polyphenol (Ribéreau-Goyau, 1968). Other parameters can influence the rate and mature of phenolic compounds, including the nature of the solvent, particle size, and extraction time (William and Douglas, 2006). The variation in tannin level could be due to environmental conditions (Fahmi *et al*., 2013), and depends on degradability, is likely to be influenced by the mode of cultivation, the phenological stage (over time, the plants tend to harden through lignification and their nutritional value declines, the nature of the soil (arid soils are sandy, infertile and poor in nutrients), the climate at the time of sampling (temperature and rainfall), as well as by constitution of the sample (stems, leaves-flowers ratio). The treatment of these samples for the purpose of experimentation (method of drying, grinding and storage) is also likely to vary the results (Rira, 2006). Preliminary evaluation of the phytochemical composition of the leaves of the plant *Populus alba* made it possible to demonstrate the presence of some chemical groups and the results observed (Table 2). The phytochemical screening results summarized in table 2 shows that the aqueous extract of *Populus alba* is rich in flavonoids, catechetical tannins; saponins, and essential oils, and that coumarins are scarce. While, it is poor in free quinones and steroids. Our results on flavonoids are consistent with those obtained by Tawfeek *et al*., (2019); Grezici *et al*., (2017); Cherif-Haouat *et al*., (2013) and Khurilenko *et al*., (2013) which confirms their presence in the plant. As well as essential oils are strongly present which is in agreement with the results observed by the researchers (Belkhodja *et al*., 2016).

Our aqueous extract of *Populus alba* studied is rich in terpenoids, which is in agreement with the study conducted by Cherif-Haouat*et al*., (2013).Boumeghar and his collaborators, (2019) indicates the presence of hydrolysable tannins in the methanolic extract of *Populus alba* in the buds which are absent in this present work but on the other hand our aqueous extract is rich in catechic tannins which are not revealed by researchers (Cherif-Haouat*et al*., 2013).The negative results obtained on steroids do not agree with those illustrated by Tawfeek *et al*., (2019). Saponins are strongly present in the aqueous extract of *Populus alba* while Merghache*et al*., (2018) demonstrated that the other genus: *Populus nigra* lacks these compounds and free quinones, which are generally absent in the aqueous extract studied.

Table 2. Results of the phytochemical test of the aqueous extract of *Populus alba*

Chemical groups	Presence or absence	Observations
Flavonoids	+++	Yellowish green color.
Tannins	+++	Greenish brown precipitate (catechetical tannins).
Coumarins	+	A little yellow color.
Terpenoids	+++	Appearance of a red ring.
Steroids	-	Absence of desired color.
Saponins	+++	Strong appearance of foam.
Free quinones	-	Lack of color expected.
Essential oils	+++	White precipitate.

(+): presence; (-): absence.

Evaluation of antioxidant activity *in vitro*:

Trapping of the DPPH radical: Inhibition of the radical DPPH is expressed as IC50; this parameter is defined as the effective concentration of the extract capable of trapping 50% of the DPPH in the reaction mixture, more the value of IC50 is low, more the antioxidant activity of a compound is high (Hebi and Eddouks, 2016).

Table 3. Antioxidant activity of *Populus alba* leaves by the DPPH test

	DPPH (CI50 µg /ml)
Populus alba	43,99±0,54
BHA	6.14±0.41
BHT	12.99±0.41
α-Tocopherol	13.02±5,17

The results obtained in table 3 show that the three standard antioxidants: BHA, BHT and α-tocopherol having a powerful antioxidant activity with an IC50 of the order of 6,14±0,41 µg/ml, 12,99±0,41 µg/ml and 13,02±5,17 µg/ml repectively. In comparison with these values, the aqueous extract of the leaf of *Populus alba* shows less activity with an IC50 equal to 43, 99±0, 54 µg/ml. Tawfeek *et al*., (2019) found an IC50 of 27,45±2,15µg/ml for the leaves of *P.alba* and an IC5O of 33,00±1,00 µg/ml for the stems. These values are relatively high compared to our results. Grezici *et al*., (2017) showed that anti-radical activity of *P.alba* is due to its chemical composition rich in essential oils (IC50= 18,05 0,38 µg/ml). Štajner *et al*., (2011); Boudkhili *et al*., (2012) ; Ahmed *et al*., (2019) and Talos-Nebehaj *et al*., (2019) confirmed the antioxidant power of white poplar. Indeed, numerous studies carried out on the antioxidant activities of plant extracts have

shown that the anti-radical capacity determined by the DPPH test is well correlated with content of phenolic compounds, known by their antioxidant activities (Zhang and Tsao, 2016). Although, the DPPH test is widely used as a method of evaluating antioxidant activity, this test is not standardized, which explain the divergence of results obtained from one job to another and minimizes the reliability of any comparison (Scherer and Godoy, 2009). In addition, these antioxidant power results vary not only by the type of extraction, subspecies and climatic conditions that directly influence the chemical composition (Ivanescu *et al*., 2018), but also by the conditions of the test (reaction, temperature, antioxidant/DPPH ratio, Ph …) (Popovici *et al*., 2009. Noipz *et al*., 2011; Costa *et al*., 2012).

Ferric Reducing Antioxidant Power « FRAP »: The reducing power of iron is considered a reliable test for studying the antioxidant activity of various compounds and plant extracts. It is used to assess the ability of the antioxidant to donate electrons (Haida and Hakiman, 2019). The intensity of the color is related with the reducing power of the sample studied (Yadav *et al*., 2012).

Table 4. Antioxidant activity of *Populus alba* leaves by FRAP test

	FRAP (CI50 µg /ml)
Populus alba	86,78±2,15
Ascorbic acid	6.77±1.15
α-Tocopherol	34.93±2.38

This capacity is inversely proportional to the IC50 value. Our result in table 4 show that the iron reducing value of ascorbic acid (6,77±1,15 µg/ml) is significantly higher than α-tocopherol (34,93±2,38 µg/ml) while the *P.alba* extract (86,78±2,15 µg/ml) has a lower value compared to these two standard antioxidants. Ahmed *et al*. (2019); Talos-Nebehaj *et al*., (2019) and Tawfeek *et al*. (2019) approved the ability of *Populus alba* to reduce iron. Different methods have been developed to measure the reducing power of an antioxidant, these technique differ essentially in the types of reactions involved. The method used to evaluate the antioxidant capacity give results which can vary significantly. These differences are mainly due to the different reactivities of antioxidant with the different indicators used (Amorati and Valgimigli, 2018). Phenolic compounds and flavonoids in particular are known for their redactor/antioxidant properties (Weidner *et al*., 2018). The spatial configuration and the number of hydroxyl groups of flavonoids can influence the various antioxidant mechanisms (Tzima *et al*., 2018).Sarian and his collaborators, (2017) approved that quercetin is an excellent reducing agent with a great antioxidant effect due to the presence in its structure: 3'-4'-catechol of the B cycle (ortho-diphenolic structure), of the group 3-OH in combination with the double bond C2-C3 adjacent to the 4-oxo function and the presence of the double bond C2-C3 in conjugation with the 4-oxo function (carbonyl).

CONCLUSION

Our results lead us to conclude that the aqueous extract of *Populus alba* contains a considerable amount of total polyphenols, flavonoids and tannins which confirms its antioxidant activity and this has been shown by the results obtained in different tests: DPPH and iron reducing power test. These results confirm that *Populus alba*as a new natural medicinal source due to itsbioactives substances.Indeed, the leaves of the plant have many therapeutic properties. It is therefore preferable to deepen and develop the phytochemical study and the various biological activities of this species.

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