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ORIGINAL ARTICLE

Antioxidant activity and polyphenol composition of *Pistacia terebinthus* fruit from Tessala (Western Algeria)

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Abstract

Background: Consumption of traditional herbal beverages has been generally increased in the last decades, Terebinth coffee, known as ''menengic coffee'' in Turkish, is one of the most consumed herbal coffees in Turkey, turpentine tree is one of the components of the Mediterranean bush, particularly in Algeria, known as Betoum el Kiffan is largely used as food and in traditional medicine. **Aims**: In this study, Total phenol, flavonoid content, and antioxidant activity of three extracts of *Pistacia trebinthus* fruit growing in Algeria was measured using radical scavenging activity tests and metal-related tests including, ferric-reducing antioxidant power (FRAP). The chemical composition profile of the fruits and the coffee brands was identified by thin-layer chromatography, the effects of roasting method of this fruit was rivaled also. **Materials and Methods:** The total phenolic content of the extracts was determined using the Folin-Ciocalteu method. All extracts of the terebinth fruits and coffee brands displayed a high DPPH scavenging effect. **Results:** The results of the ferric-reducing antioxidant power show that the reduction capacity is proportional to the increase in the concentration of the samples. All the extracts of the plant exhibit antioxidant activities lower than those of the reference product besides the infusion extract of the *P. terebinthus* roasted coffee, which is the most active with an optical density of 1.68 nm at a concentration of 400 µg/mL. The chromatography results show that the various extracts of *Pistacia terebinthus* fruit carry a large number of polyphenols, in particular the carboxylic acids phenols. **Conclusions**: The plant can be considered as a coffee substitute and opens up promising avenues for the food and pharmaceutical industry in Algeria.

Keywords: Antioxidant, *Pistacia terebinthus*, Coffee, FRAP, polyphenol.

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1 Introduction

Aromatic and Medicinal Plants (MAPs) are of considerable importance. These plants are indeed employed to cure various diseases and for culinary applications, as they serve to perfume the environment and the human body. Those reasons that MAP are considered as potential sources of income for local communities and natural sources of secondary metabolites used for a healthy diet, in traditional medicine, and a wide range of industrial applications [1, 2]. Consumption of traditional herbal beverages, such as chicory, galangal root, and ginger tea, has been widely increased in the last decades due to their distinctive aroma[3].

Coffee is consumed in large quantities around the world and especially in Algeria where it is consumed after roasting. Furthermore, many coffee substitutes known as herbal coffee have been used around the world depending on regions and traditions to reduce negative side effects, such as high caffeine content responsible for certain gastrointestinal disorders, due to excessive coffee consumption. This has resulted in herbal products that are produced and consumed similar to coffee beans[4].

Terebinth coffee, known as ''menengic coffee'' in Turkish, is one the most consumed herbal coffees in Turkey. It is an oily brown-colored powder produced from the dried and roasted fruits of terebinth or turpentine tree (*Pistacia terebinthus L., Anacardiaceae*), a small bushy tree from North Africa, the plant owes its name to the oleoresin which it contains known under the name of Turpentine de CHIO. It is the first turpentine known by Dioscorides and this name was later extended to the oleoresin of conifers. This tree is one of the components of the Mediterranean bush, particularly in Algeria, where there are large stands, especially in the Tessala forest, known under the name of Betoum el Kiffan in Arabic [3, 5, 6].

The terebinth has been used since Antiquity in traditional medicine besides the use of leaves, galls, and resin of *P. terebinthus* is significantly high in the Mediterranean, like stimulants, diuretics, and astringents. Dried fruits are a source of oil of medicinal grade; the fruits are also used fresh or mixed to prepare certain pastries, dishes (Red Tirshyat) coffees, and drinks, especially in Turkey [7].

In Iran, a preparation named Galiyya is used in the traditional pharmacopeia, which is a mixture of roasted unpolished wheat

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kernels, roasted turpentine fruit, and a small mixture of apricot seeds probably. In the region of Gocer, another preparation is made from *P. terebinthus* fruit, *cannabis sativa*, and *Triticum aestivum* [8]. Different parts of *P. terebinthus* have been also reported to have several biological activities and ethnopharmacological utilizations in Turkey [9]. However, there is no data about the composition and the antioxidant potential of *Pistacia terebinthus* fruit growing in Algeria. Thus, the purpose of this work was to investigate total phenolic and total flavonoid contents, the antioxidant properties, and the composition profile of *Pistacia terebinthus* coffee.

The antioxidant activity of the extracts was evaluated by several in vitro test systems including the scavenging activity tests based on radical formation against 2,2-diphenyl-1-picrylhydrazyl (DPPH), as well as metal-related antioxidant activity tests including metal-chelation capacity, ferric reducing antioxidant power (FRAP) tests. Total phenol and flavonoid contents in the extracts were calculated using Folin–Ciocalteau and $AlCl_3$ reagents, respectively. The chemical composition profile of the fruits and the coffee brands was identified by thin-layer chromatography.

2 Materials and Methods

2.1 Plant material and coffee

The plant material consists of ripe fruits that were harvested at the mountain of Tessala in September 2019. The identification of the species was made by Dr. Ferkous Houssem botanist of the pharmacy department of the University of Sidi bel abbes, After harvesting, a batch of the plant material was cleared of debris, spread out, and dried at room temperature, in the shade and protected from light, then reduced into powder using a Fritsh® knife mill and stored in glass jars. Coffee brands produced by different companies were purchased from the local markets.

2.2 Extraction

The precise method of extraction naturally depends on the texture and water content of the plant material and the type of metabolite to be isolated. The extraction of metabolites from the fruit of *P. terebinthus* was carried out with some modifications according to Pakravan *et al.* [10]. Fruit of the plant samples were washed with distilled water and reduced to powder. 20 g of powder was mixed with 500 mL of methanol, which was left at room temperature for 3 days. This maceration process is a better option to extract polyphenolic compounds because high temperatures applied with decoction can destroy bioactive compounds. The final product, which has the consistency of honey was dissolved in distilled water and stored at + 40 °C. Part of the powder was rapidly roasted within 10 minutes at 50 °C and then infused to obtain the terebinth coffee. In the same conditions, the brand of coffee purchased were prepared as a mixture of Robusta and Arabica coffee. Different extracts were coded as follows: A- MeOH fruit of *P. terebinthus* –B; *P. terebinthus* roasted coffee; C- Coffee Arabica purchased.

2.3 Estimation of total phenols

The total phenolic content of the methanolic extracts was determined using the Folin-Ciocalteu method, the absorbance A of the resulting blue color solution was measured using a UV-vis spectrophotometer (Shimadzu) at 760 nm wavelength.

A volume of 0.2 mL of each extract was mixed with 1.5 mL of Folin-Ciocalteu (10%). After 5 minutes, 1.5 mL of sodium carbonate solution (6%) was added. The mixture was incubated at room temperature in the dark for 2h and the absorbance is read at 760 nm with a spectrophotometer. Gallic acid is used as a reference standard.

The total phenolic content was expressed in milligrams of gallic acid equivalents per gram of dried plant material (mg GAE / g dry weight).

2.4 Estimation of total flavonoids

The determination of the flavonoid content by the colorimetric method was made according to the method used by Kim *et al.* [11]. The total flavonoid content of each sample was measured using an aluminum trichloride assay. Absorbance was measured at 415 nm wavelength using a UV-vis spectrophotometer (Shimadzu). The procedure was carried out by the appropriate dilution of each extract (1 mL) and the standard (catechin) (20, 40, 60, 80,100 mg / L) in a 10 mL volumetric flask containing 4 mL of distilled water. At the instant t = 0, 0.3 mL ($NaNO_2$) at 5% (W / v) was introduced. after 5 minutes of reaction, 0.3 mL of 10% $AlCl_3$; 6 minutes later, 2 mL of 1M NaOH. Then the reaction mixture was diluted with 2.4 mL of distilled water and stirred vigorously. The absorbance was determined at 415 nm wavelength using a (Shimadzu) spectrophotometer.

2.5 Thin layer chromatography

Thin layer chromatography is a very successful analytical method in the pharmaceutical industry. 1 g of powdered drug with 10 mL of methanol brought to a water bath at 60 °C. Using an eluant system formed by - ethyl acetate - formic acid - acetic acid – water at the proportions 67.5: 7: 7: 17.5 rutoside, quercetin, gallic acid, and catechin were used as the reference solution. 5µl of each solution stripped by glass capillaries Revelation: the plate was examined under UV 365 and UV 254 and after Spraying a Reagents with anis-aldehyde (universal revelation) [12].

2.6 Determination of antioxidant activity

Phenolic compounds are the most recommended metabolites for their antioxidant activity due to the presence of many hydroxyls, which can react with these radicals. The methods chosen to measure the antioxidant activity of our extracts are as follows:

DPPH free radical trapping test

The evaluation of the antioxidant power of the extracts of our plant was determined according to the *in vitro* method of trapping the free radical DPPH that is generally the most used substrate for the rapid and direct evaluation of the antioxidant activity due to its stability in free radical form and simplicity of analysis. It

absorbs in the visible at 515 to 520 nm wavelength. For this, several dilutions were prepared from the methanolic solutions of the extracts tested (1; 0.8; 0.6; 0.4; 0.2 and 0.1 mg/mL) and 25 µl of each solution was mixed with 975 µl of a methanolic solution of DPPH at a concentration of 0.024 mg/mL. The absorbance reading was taken against a methanol blank for each concentration at 517 nm wavelength after 30 minutes of incubation in the dark and at room temperature. The positive control was represented by a standard antioxidant solution; ascorbic acid, the absorbance of which was measured under the same conditions as the samples. Percentage inhibition of DPPH free radical (I %) was calculated according to [13].

Gallic acid was employed as the reference. Inhibition of DPPH in percent (I %) was calculated as given below: I% = [(A blank - A sample)/A blank] * 100, where A blank is the absorbance of the control reaction.

Ferric-reducing antioxidant power (FRAP) assay

The reducing activity of the extracts is determined according to the Oyaizu method modified by Eshwarappa *et al.* [14] based on the chemical reaction of reduction of ferric iron (Fe^{3+}) present in the ferrocyanide complex of potassium [Fe (CN) 63-] to ferrous iron (Fe^{2+}).

This reaction is manifested by the appearance of a measurable blue color at 593 nm wavelength, so high absorbance indicates that the extract has great reducing power. The different concentrations of the extracts were dissolved in 1 mL of distilled water then 2.5 mL of the phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) were added. The mixtures were incubated at 50 °C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (TCA) (10%) was added. The whole is centrifuged at 3000 Tr/min for 10 min. In the end, a volume of 50 µl of extract at different concentrations (200, 300,400, 500, 600, 700, 800) were mixed with 1.95 mL of FRAP reagent (distilled water and 0.5 mL of $FeCl_3$, 6 H_2O (0.1%). A blank was prepared following the same steps by replacing the 0.25 mL of the extract with the solvent MeOH. The absorbance (optical density) is measured at 593 nm wavelength. Ascorbic acid is used as a positive control.

2.7 Statistical analysis

The results were statistically compared using the t comparison test and each operation was repeated 3 times in the same conditions ($p< 0.05$). The *t* test (also called T test) was used to compare the means between the two groups and there was no need of multiple comparisons as unique *P* value is observed.

3 Results

Total phenol and flavonoid contents of the extracts

Total phenol content of the extracts was calculated according to the equation (y = 0,0175x + 0,083, r2 = 0.9976) as gallic acid equivalent (mg/g extract), whilst their total flavonoid contents were determined in accordance with the equation (y = 0,0082x 0,0013, r2 = 0.9966) obtained by calibration curves as quercetin equivalent (mg g^{-1} extract). As given in Table 1, the richest extracts in total phenols and flavonoids were found to belong to the infusion extract C Coffee Arabica purchased (127 ± 4.89 mg g^{-1} extract) and the infusion extract of the B *P. terebinthus* roasted coffee (260.71 ± 0.87 mg g^{-1} extract), while the MeOH extract of fruit of *P. terebinthus* gives (63.05 ± 3.87 mg g^{-1} extract).

The antioxidant activity of the extracts is expressed as percent inhibition and inhibitory concentrations 50 [IC_{50}]. These two parameters have been used by several researchers to determine the antioxidant activity of various secondary metabolites of various plants. The results are presented in Table 2 correspond to the IC_{50} of the extracts of *Pistacia terebinthus L* and ascorbic acid. All extracts of the terebinth fruits and coffee brands displayed a high DPPH scavenging effect, the extract C Coffee Arabica purchased with absorbances of 1.31 nm, and finally, the fruit MeOH extract with an optical density of 1.02 at a concentration of 400 µg / mL. These results confirm those obtained by the DPPH test, the infusion extract of the *P. terebinthus* exhibits powerful antioxidant activity compared to the MeOH Fruit extract.

Table 1: Results of total phenols and flavonoids content

Extracts	Total phenols GAE/g extract [1]	Flavonoids content QE/g extract [2]
MeOH extract of fruit of *P. terebinthus*	63.05 ± 3.87	37.03 ± 1.25
Infusion extract of the B *P. terebinthus* roasted coffee fruit	260.71 ± 0.87	112.30 ± 3.21
Infusion extract C Coffee Arabica purchased	127 ± 4.89	68.01 ± 2.09

[1] Data expressed in mg equivalent of gallic acid (GAE) to 1 g of extract.
[2] Data expressed in mg equivalent of quercetin to 1 g of extract.

Thin-layer chromatography

Our results show that the various extracts of *Pistacia terebinthus* contain a large number of polyphenols, in particular the acids phenols, catechins, and flavonoids, at UV-254 nm all flavonoids show fluorescence quenching. While at UV-365 nm light depending on type and structure, flavonoids exhibit a dark yellow, green or blue fluorescence, which is intensified and changed by spraying various reagents also flavonoid extracts often contain phenol carboxylic acids (caffeic acid, chlorogenic acid) which form blue fluorescent areas.

Table 2: Results of the antioxidant activity of the extracts by DPPH

Extracts	IC50 (mg/mL)
MeOH extract of fruit of *P. terebinthus*	63.05 ± 3.87
Infusion extract of the B *P. terebinthus* roasted coffee fruit	260.71 ± 0.87
Infusion extract C Coffee Arabica purchased	127 ± 4.89
Ascorbic acid	0.12 ± 0.06

4 Discussion

According to our results, we observed a clear predominance of phenolic acids in the thin layer chromatography plate. On the other hand, these results confirm those observed by Karacan & Çağran [7], who affirmed that the extract of fruit is rich in terpenes including alpha-pinene tocopherols and carboxylic phenol acids. These are thought to be two phenol acids, namely caffeoylquinic acids, which come in a variety of forms: 3,5-caffeoylquinic and 4,5-caffeoylquinic requiring NMR analysis to confirm the exact structure [7,15]. Phenolic compounds are involved in several aspects of plant physiology, lignification, regulation of growth, and interactions with certain microorganisms or parasites.

The total phenolic content in *P. terebinthus* fruit is comparable to the values reported in the literature, all extracts of the terebinth fruits and coffee brands displayed a high concentration of polyphenols and show also a high DPPH scavenging effect [3] the coffee extracts usually exhibited a better activity in the test methods applied for the establishment of *in vitro* antioxidant activity. It is interesting to point out that the terebinth coffees produced after roasting of the powdered fruits have shown greater activity in these tests as compared to the fruits MeOH extract. According to the literature, a few studies on the antioxidant activity of the terebinth fruits have been reported up to date. The qualitative and quantitative variations of phenolic compounds can be particularly marked during the life of the plant, or by certain treatment such as roasting. A suggestion can be made that the roasting process may cause an elevation in the antioxidant activity of the fruits. This richness in phenolic compounds of the genus Pistacia is indicated by Decandia *et al.* [16], who state that Mediterranean shrubs are generally rich in phenolic compounds. Several reports have shown the close relationship between total phenolic content and the antioxidant activity of plants.

The richness in phenolic compounds explains the use of this plant in traditional medicine. These compounds are widely known for their antiviral, antispasmodic, anti-mitotic, hypocholesterolemic, anti-inflammatory, anti-hypertensive, and antimicrobial activities, believed to be responsible for significant antioxidant activity, particularly in fruit extracts of *P. terebinthus* growing in Algeria, which can be used as substitute of coffee.

5 Conclusions

The results obtained in this study clearly showed that the biological activities of *Pistacia trebinthus* fruit, on the whole, are appreciated, the antioxidant effect *in vitro* was achieved using two tests: DPPH and FRAP and the effects generated by the extracts were important especially for the infused roasted fruit powder. However, further studies to isolate and identify active compounds, especially phenolic, and *in vivo* studies are needed. Moreover, the plant can be considered as a coffee substitute and opens up promising avenues for the food and pharmaceutical industry in Algeria.

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References

[1] Arnal-Schnébelen, B., Goetz, P., & Grassart, E. Les plantes médicinales. 1st ed. Springer. 2009: pp. 253

[2] Cortes, D. (2017). Metabolitos Secundarios Activos: Los Medicamentos Que Nos Proporciona la Naturaleza. Diego Cortes. Available at: https://books.google.dz/books?id=tu4ptAEACAAJ

[3] Orhan, I. E., Senol, F. S., Gulpinar, A. R., Sekeroglu, N., Kartal, M., & Sener, B. (2012). Neuroprotective potential of some terebinth coffee brands and the unprocessed fruits of Pistacia terebinthus L. and their fatty and essential oil analyses. *Food Chemistry*, *130*(4), 882-888. https://doi.org/10.1016/j.foodchem.2011.07.119

[4] Secilmis, S. S., Yanık, D. K., & Gogus, F. (2015). Processing of a novel powdered herbal coffee (Pistacia Terebinthus L. Fruits Coffee) and its sensorial properties. *Journal of Food Science and Technology*, *52*(7), 4625-4630. https://doi.org/10.1007/s13197-014-1475-7

[5] Lapie, G., & Maige, A. Flore forestière illustrée, comprenant toutes les espèces ligneuses de l'Algérie et les espèces ligneuses les plus répandues en Tunisie, au Maroc et dans le midi de la France; pour la détermination facile, sans l'emploi de mots techniques, de toutes les espèces décrites. 1st ed. Paris. 1914.

[6] Perrot, E., Mascré, M., Régnier, J., Crété, P., & Weitz, R. (1943). Matières premières usuelles du règne végétal: thérapeutique, hygiène, industrie. Available at: https://books.google.dz/books?id=-XaZzQEACAA

[7] Karacan, M., & Çağran, F. (2009). Multielement determination in fruit, soaps, and gummy extract of Pistacia terebinthus L. by ICP OES. *Turkish Journal of Biology*. *33*(4):311–318. Available at : https://journals.tubitak.gov.tr/biology/abstract.htm?id=10380

[8] Cappers, R.T.J. (2018). Digital atlas of traditional food made from cereals and milk. University of Groningen Library. (Groningen Archaeological Studies). Available at: https://books.google.dz/books?id=5Oi9DwAAQBAJ

[9] Yeşil, Y., Çelik, M., & Yılmaz, B. (2019). Wild edible plants in Yeşilli (Mardin-Turkey), a multicultural area. *Journal of Ethnobiology and Ethnomedicine*, *15*(1), 1-19. https://doi.org/10.1186/s13002-019-0327-y

[10] Pakravan, S., Hajimoradloo, A., & Ghorbani, R. (2012). Effect of dietary willow herb, Epilobium hirsutum extract on growth performance, body composition, haematological parameters and Aeromonas hydrophila challenge on common carp, Cyprinus carpio. *Aquaculture Research*, *43*(6), 861-869. https://doi.org/10.1111/j.1365-2109.2011.02901.x

[11] Kim, D. O., Chun, O. K., Kim, Y. J., Moon, H. Y., & Lee, C. Y. (2003). Quantification of polyphenolics and their antioxidant

capacity in fresh plums. *Journal of Agricultural and Food Chemistry*, *51*(22), 6509-6515. https://doi.org/10.1021/jf0343074

[12] Sherma J., & Fried, B. Handbook of Thin-Layer Chromatography. 3rd Ed. Taylor & Francis. 2003: pp. 461. ISBN: 9780824708955.

[13] Hamlaoui, I., Bencheraiet, R., Bensegueni, R., & Bencharif, M. (2018). Experimental and theoretical study on DPPH radical scavenging mechanism of some chalcone quinoline derivatives. *Journal of Molecular Structure*, *1156*, 385-389. https://doi.org/10.1016/j.molstruc.2017.11.118

[14] Eshwarappa, R. S., Lakshmikantha, R. Y., Subaramaihha, S. R., Subbaiah, S. G., Surendranath, A. R., & Dhananjaya, B. L. (2015). Antioxidant activity of insect gall extracts of Pistacia integerrima. *Acta Scientiarum Polonorum. Technologia Alimentaria*, *14*(4), 367–374. https://doi.org/10.17306/J.AFS.2015.4.36

[15] Ulukanli, Z., Karabörklü, S., Öztürk, B., Çenet, M., & Balcilar, M. (2014). Chemical Composition, Antibacterial and Insecticidal Activities of the Essential Oil from the Pistacia terebinthus L. Spp. Palaestina (B oiss.)(Anacardiaceae). *Journal of Food Processing and Preservation*, *38*(3), 815-822. https://doi.org/10.1111/jfpp.12035

[16] Decandia, M., Sitzia, M., Cabiddu, A., Kababya, D., & Molle, G. (2000). The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in goats fed woody species. *Small Ruminant Research*, *38*(2), 157-164. https://doi.org/10.1016/S0921-4488(00)00145-0

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