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Evaluation of antioxidants activity of some tree barks grown in Libya (Al Jabal Al Akhdar) Pinus halepensis Mill, Pistacia lentiscus L, Juniperus phoenicea L

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Abstract

The aim of the present study investigated anti-oxidant activity of various methanol-water extracts from tree barks grown in Libya (Al Jabal AL Akhdar) by four anti-oxidant assays FRAP. DPPH. H_2O_2 and Metal chelating activity. Additionally, these antioxidant activities were compared with BHA, BHT as reference antioxidants. Tree bark has a large, diverse class of compounds, many with antioxidant properties. This study showed FRAP inhibitory activity of the *Pinus halepensis* bark extracts 56.8%, while *Juniperus phoenicea L* 57.6%, and *Pistacia lentiscus L* bark extracts was 69.2% The DPPH radical scavenging in the bark extracts exerted an inhibition of 66. 8%, 62.3% ,74.6% for *Pinus halepensis*, *Juniperus phoenicea* and *Pistacia lentiscus L* respectively. While H_2O_2 activity shows variation, ranging from 67. 8 to 81. 3%. The Metal chelating activity of the barks extract was 59% in *Pinus halepensis* and 57% in *Juniperus phoenicea L* bark extracts furthermore *Pistacia lentiscus L* was 67.8% in addition the Metal chelating activity of BHT and BHA 92%, 94% respectively. The high antioxidant activity of bark was founded in *Pistacia lentiscus L* bark extracts.

Keywords: Antioxidant activity; *Pinus halepensis*; *Pistacia lentiscus* L; *Juniperus phoenicea* L

1. Introduction

Al Jabal AL Akhdar region (Libya) has highest species diversity and having distinct environmental characteristics associated with evergreen forest along with the Mediterranean from the Atlas Mountains to the Levant, and it has an environment similar to other regions in Southern Europe such as Italy, the Greek islands and Turkey [1]. The number of plant species reach up of 1100 species from the total of plant species in Libya (2000 species) with about 75 species of plants that grow only in AL Jabal AL Akhdar and have been served for as basis of traditional medicinal systems for thousands of years [2]. *Pinus halepensis* is a steno-Mediterranean species distributed along the coasts and in the islands, it prefers warmer

Calcareous areas like Libya, Italy, Algeria, Greece, Morocco, and Turkey, where it also succeeds in colonizing the less hospitable rocks because of its springiness *Juniperus phoenicea* L is a small tree that is native to the northern lands bordering the Mediterranean Sea from Portugal to Palestine. It is also native to North Africa found in Libya, Algeria, Morocco and Canary Islands [3]. This plant species is a conspicuous constituent of the vegetation of the Mediterranean basin, particularly in Al-Jabel Al-Akhdar region. It constitutes about 80% of the total number of the trees and evergreen shrubs that exist in Al-Jabal Al-Akhdar area [4] *Pistacia lentiscus* L. is an evergreen shrub of the family *Anarcadiaceae*. This dioecious species can reach 3 m in height and grows in many Mediterranean countries [5]. All trees have stems and branches composed of an internal wood tissue this wood is covered by bark with a percentage of 9 to 15% of the stem

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volume [6] Tree barks is morphologically diverse, which could be the result of specific ecological strategies. This diversity might also be the result of adaptation to environmental conditions, fire resistance, or insect attacks. This morphological diversity and its causes remain poorly understood [7]. The bark is very promising technological raw material and classic bio refinery object because of its unique bark's biomass chemical composition and possibility to get many different products with the added value of individual or group of compounds of the synergistic biological activity, raw materials to produce various materials [8]. Several types of plant materials, such as vegetables, fruits, seeds, hulls, wood, bark, roots and leaves have been examined as potential sources of antioxidant compounds. The crude extract of bark contains a wide variety of phenolic compounds. Studies on the investigation of its bark and characterization have been reported [9]. Polyphenols: Plants produce varying forms of polyphenols, a large, diverse class of compounds, many with antioxidant properties structurally; polyphenols all have one or more aromatic (phenolic) rings with different structural elements [10].

2. Material and methods

2.1. Sampling and processing

Tree barks samples (*Pinus halepensis Mill, Pistacia lentiscus L, Juniperus phoenicea L*) were collected from Aljabal Alakhdar province. The bark samples were dried at room temperature for two weeks, and grinded to fine powder. 40 - 60 mesh and were stored in closed glass jars at room temperature until analysis.

Pinus halepensis Pistacia lentiscus L Juniperus phoenicea L

2.2. Extraction of sample

Extracted the three samples in Soxhlet extractors according to (TAPPI T 204 om88) for 6 h using methanol-water mixture (65-35% v/v).Then the extract was filtered paper Whatman No. 1, The filtrates were frozen at -84 C in ultra low temperature freezer and lyophilized for analysıs of determınatıon antıoxıdantes actıvıty,The antioxidant capacities of plant extracts were assayed with four different assays including FRAP, Metal chelating, H_2O_2, and DPPH scavenging because the antioxidant capacity cannot be fully described by a single method. The experiments were carried out in triplicate all chemicals were purchased from Sigma (Sigma Aldrich GmbH. Sternheim), Merck (Darmstadt)

2.3. Ferric Reducing Antioxidant Power assay

FRAP (ferric reducing antioxidant power) process were carried out as described by Hamad AMA et al 2019 [11] with slight modifications. Solutions having 1, 0.5, 0.25 and 0.125 g/L concentrations were obtained from the extraction samples and a 0.5 mL sample was put in the test tubes for each concentration and 4.5 ml methanol was added to them. After that, 2.5ml of 1% K3 (FeCN) 6 was added. This mixture was heated at 50 centigrade for 20 minutes in a water bath, and 2.5ml of Trichloric acid (TCA) was added to it. Firstly, the UV absorption at 520 nm was measured at 0 min. When samples were centrifuged at 4500 rpm, the samples were shifted to another test tube and 2.5 ml alcohol and 0.1% $FeCl_3$ were added to the tube, secondly the absorption was measured at 700 nm.

2.4. Radical Scavenging Activity assay

DPPH (radical scavenging activity) assay were carried out as described by Hamad AMA et al 2019[11]. With slight modification solutions at 1, 0.5, 0.25 and 0.125 g/L concentrations. They were obtained from extraction samples. 2 ml

of sample was taken in the test tubes for each concentration and 40 mM 2 ml DPPH solution was added to them. Centrifugation remained at 4500rpm for 10 minutes and UV absorbance was 520 nm.

2.5. Hydrogen peroxide scavenging assay

Hydrogen peroxide was determined according to Guder,et al 2012 [12]. 170 µl methanol-water extract was added to H_2O_2 solution, which was prepared using 40 mM phosphate solution according to the final volume, which was nearly 4 ml. After that, 230 nm absorption was determined by UV-VIS spectrophotometer.

2.6. Metal Chelating Activity assay

Metal chelating of ferrous ions by the extracts and standards was determined according to the procedure by Guder,et al 2012 [12] with slight modifications. Different extractive concentrations were prepared and 0.4ml extract was added to 50µl $FeCl_2$ solution (2 mM). The reaction was initiated when 0.2 mL ferrozine (5 mM) was added and the mixture was shaken with force. After that, the solution was left at room temperature for 10 min. Finally, UV absorption was measured at 562 nm using a spectrophotometer.

3. Results and discussion

Table1 Radical Scavenging Capacity inhibition (%)

Samples	FRAP	DPPH	H_2O_2	Metal Chelating
Pinus halepensis	56.8	66.8	72	59
Juniperus phoenicea L	57.6	62.3	67.8	57.8
Pistacia lentiscus L	69.2	74.6	81.3	67.8
BHT	90	90	90	92
BHA	87	-	-	94

The ferric reducing antioxidant power (FRAP) method is important for the evaluation of antioxidants. FRAP was associated with antioxidant potential of extracts to confirm its level [13]. In this assay, the Fe^{3+}/ Fe^{2+} transformation was investigated in the presence of samples and the absorbance values were measured at 700 nm. In addition, FRAP activity of the different bark extracts had significant variation, according to these results, we can say that especially *Pistacia lentiscus L* bark extracts have higher FRAP activity than the others.

Moreover, the researchers to determine radical scavenging activity mostly use DPPH. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components [14].

Furthermore, reactive oxygen species (ROS), i.e., hydrogen peroxide, singlet oxygen, hydroxyl and superoxide radicals, have crucial acts such as in energy production, phagocytosis, intercellular signal transfer, cell growth regulation and important biological compounds synthesis [15]. Although hydrogen peroxide is not very reactive, it can show toxic property to cell due to their transformation hydroxyl radical in the cells. By this way, H_2O_2 scavenging activity is very important for antioxidant defense in cell or food systems [12].

Iron and other transition metals such as copper, chromium, cobalt, vanadium, cadmium, arsenic, and nickel support oxidation owing to their catalyst properties on free radical reactions. These transition metals donate single electrons during redox reaction between them. When some compounds chelate metals, their pro-oxidant activity diminishes by reducing their redox potentials and stabilizing the oxidized form of the metal [16].

The highest radical scavenging activities were attained with *pinus pinea* (88.6%), *pinus nigra* (87.2%) and *pinus brutia* (86.4%) bark extracts, followed by *pinus sylvestris* (78.5%) [17]. The RSA (radical scavenging activity) values of 7 supercritical fuid extraction (SFE) extracts were determined ,the highest RSA was attained with *pinus pinea* (81.0%) while *pinus parvifora* showed the lowest activity (31.9%) In addition, SFE extracts of *pinus sylvestris* (58.4%, 46.4%) and *pinus nigra* (53.7%, 52.3%) harvested from Turkish and Germany respectively [18].

Pinus halepensis showed the highest percentage of antioxidant activity 79.93% [19]. Moreover, *Pinus halepensis* extracts are important sources of bioactive compounds possessing important antioxidants, antihemolytic and Geno protective effects [20].

Bark extracts from other Pine species have been reported to possess potent antioxidant activity. Extracts of *Pinus sylvestris, Pinus pinea*, and *Pinus massoniana* barks showed cytotoxic activity against human cancer cell lines. In addition, *pinus sylvestris* and *pinus massoniana* bark extracts reduced the production of several inflammatory mediators , *pinus pinea* and *Pinus densiflora* bark extracts have been found very promising for diabetes treatment due to their inhibitory effects on glucose absorption and carbohydrate-hydrolysing enzymes, respectively. , all these data show that bark extracts from different pine species have remarkable biological effects, thus making pine bark a valuable raw material for the food and pharmaceutical industries [17].

The high phenolic content and the important antioxidant activities of *P. lentiscus* extract could be a useful source of natural products and may be increasingly important for human consumption, prevention of damages caused by oxygen free as well as for the agro-food, cosmetic and pharmaceutical industries.[21]. The results exhibited the highest inhibition activity of *J. phoenicea* against H_2O_2.

The Nano extract of *J. phoenicea* can be used effectively in the production of potential antioxidant, antiparasite antimicrobial and anticancer [22].

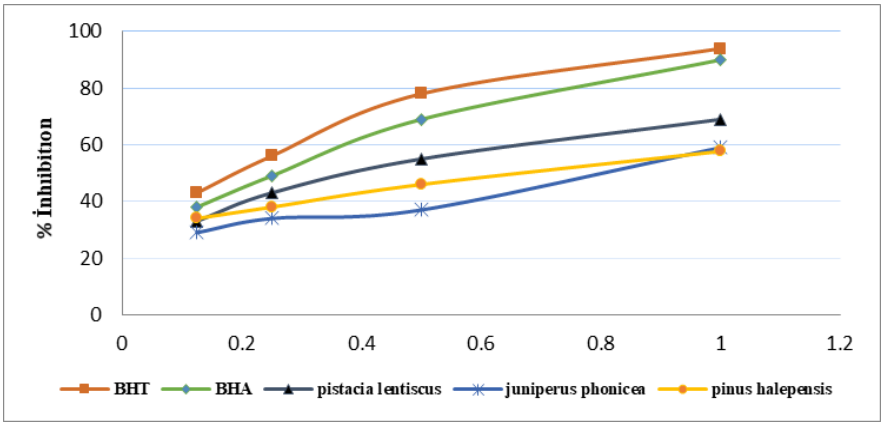

Figure 1 FRAP (ferric reducing antioxidant power)

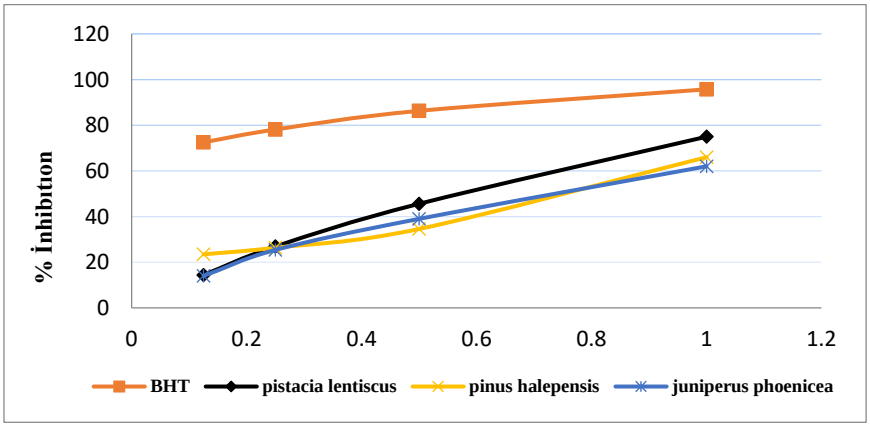

Figure 2 DPPH (radical scavenging activity)

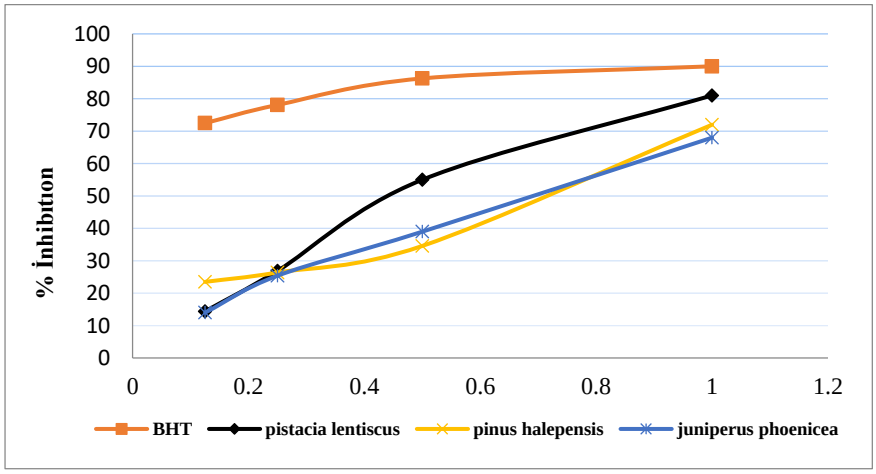

Figure 3 Hydrogen peroxide scavenging

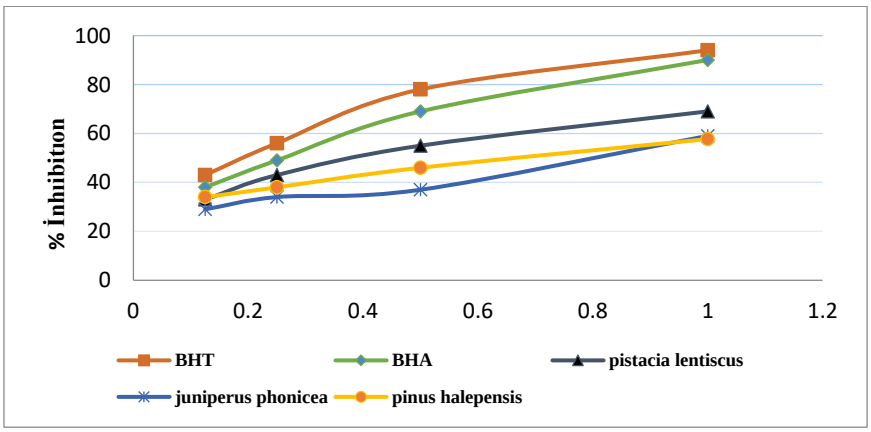

Figure 4 Metal Chelating Activities

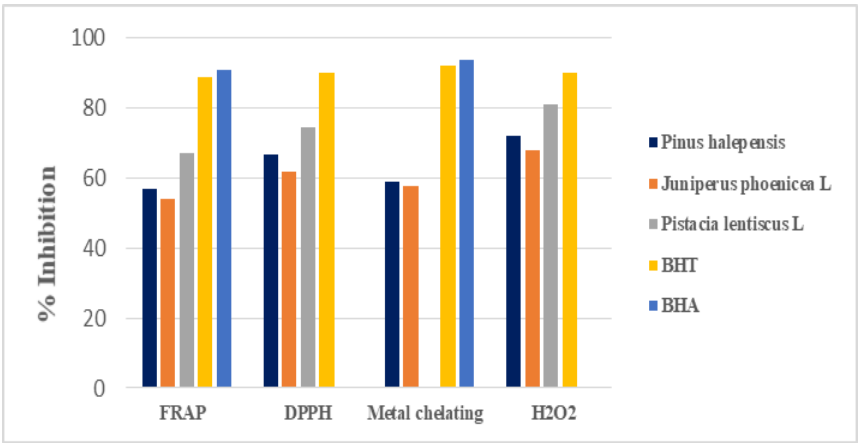

Figure 5 Radical Scavenging Capacity inhibition of bark samples

As seen in the Fig.1 FRAP inhibition activity variation, ranging from 56.8%, to 69.2%, finding that FRAP inhibition activity of the *Pinus halepensis* bark extracts 56.8%, while the FRAP inhibition for *Juniperus phoenicea L* 57.6%, and

Pistacia lentiscus L bark extractives were 69.2%, furthermore that Butylated Hydroxyl Toluene (BHT) with an activity of 90% and Butylated Hydroxyl Anisole (BHA) 87%.

The bark sample extractives were analyzed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is related to the number of electrons gained The DPPH radical scavenging (%) activity is shown in the Fig .2 *Pinus halepensis* bark extract exerted an inhibition of 66.8% and *Juniperus phoenicea* L bark extractives was 62.3%, and *Pistacia lentiscus* L bark extract 74.6 % while in addition that of BHT was 90%

H_2O_2 antioxidant activity in Fig .3 shows variation, ranging from 67.8 to 81.3%. *Pinus halepensis* bark extractives demonstrate 72.8% of H_2O_2 inhibition activity, and, while *Juniperus phoenicea* L bark extractives had H_2O_2 inhibition activity about 67.8%, and *Pistacia lentiscus* L 81.3 % while H_2O_2 inhibition activity of BHT was 90%

Accordance to Fig.4 the metal chelating activity of the barks was 59 % in *Pinus halepensis* extractives and while 57.8 % in *Juniperus phoenicea* L bark extractives furthermore metal chelating activity of *Pistacia lentiscus* L was 67.8 % in addition the metal chelating activity of BHT and BHA 92%, 94% respectively.

The bark extractives had modest antioxidant activities compared with (BHA) and (BHT). As seen in Fig 5 the *Pistacia lentiscus* L extracts were found to have the highest antioxidant activity. The antioxidant activity of the *Pistacia lentiscus* L bark extractives was lower than the findings of [17]. WHO applied the DPPH method.

The *Juniperus phoenicea* L bark extractives yielded lowest antioxidant activities due to the extraction method used FRAP, Metal chelating, H_2O_2 and, DPPH scavenging activity was (57.6 - 57.8 -.67.8 – 62.3) respectively. While antioxidant activities of *Pinus halepensis* was (56.8 - 59 - 72 - 66.8) respectively.

Furthermore, can be seen antioxidants activity FRAP scavenging activity (69.2%), Metal chelating (67.8%), H_2O_2 scavenging activity (81.3%) and DPPH scavenging activity (74.6 %) were found in *Pistacia lentiscus* L bark

4. Conclusion

According to the present results, the methanolic extracts of *P. lentiscus* bark have a considerable scavenging activity against the free radical DPPH; High ferric reducing power; Metal chelating and H_2O_2 scavenging activity this could be related to the high content of this plant on secondary metabolites. These extracts could therefore be a source of natural antioxidant molecules as an alternative to the use of synthetic antioxidants. Furthermore, these results support the large use of *P. lentiscus* in traditional medicine in Libya. For further studies, it is interesting to identify the chemical composition of the extracts and explore more biological activities of the plant.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be declared.

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