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Phenolic Content as Antioxidant and Antimicrobial Activities of *Pistacia atlantica* Desf. (Anacardiaceae) Extract from Libya

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THE AIM of the work is to search for new, potentially biologically active extract.The chemical and biological investigations of different extracts of *P. atlantica*, resulted that the ethyl acetate extract of *P. atlantica* leaves contains a complicated mixture of phenolics, which includes mainly gallotannins as previously detected by 2D paper chromatographic screening. The isolation and identification of six polyphenoles **(1-6)**, were elucidated for the first time from this plant, including three polyphenolic acid; gallic acid **(1)**; ellagic acid **(2)**; 3,3'-dimethoxyellagic acid **(3)** and three gallotannines, 2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose, nilocitin **(4)**; 1,3-di-*O*-galloyl-β-D-$^4C_1$-glucopyranose **(5)**; 1,2,3,4,6- penta-*O*-galloyl-β-D-$^4C_1$-glucopyranose **(6)**. The structures of all isolated compounds were elucidated by conventional methods, spectroscopic analysis, including 1D and 2DNMR, ESIMS and HRESI mass as well. The search for new, potentially biologically active extract becomes much more efficient after identification of all compounds in that mixture. The *in vitro* antioxidant activity using the stable free radical DPPH (2,2-diphenyl-1-pycrylhydrazyl) method of the *P. atlantica* extracts proved that ethyl acetate extract possesses a distinct radical scavenging effect at the different concentrations and the extract completely inhibited DPPH absorbance at a concentration 77 µl, the percentage of inhibition obtained 96.4% can be considered as a full absorbance inhibition of DPPH. Also, the IC_{50} (the concentration that inhibits 50% of the absorbance of DPPH) of ethyl acetate extract of *P. atlantica* showed an IC_{50} value of 8.41±0.24 µg/ml compared with IC_{50} value of ascorbic acid 1.82±0.33 µg/ml. It could be concluded from the obtained results that the *P. atlantica* ethyl acetate extract shows a very high antioxidant capacity which is very close to the value of the reference standard used, which may be attributed to its high polyphenolic content. *P. atlantica* extracts demonstrated inhibitory effects against pathogenic microorganisms: *Staphylococcus aureus*, NCTC 7447; *Bacillus subtilis*, NCTC 10400; *Escherichia coli,* NCTC 10416; *Pseudomonas aerogenasa* NCIB 9016); *Candida albicans*, IMRU 3669. The active ethyl acetate extract shown a sensitizing effect against *Staphylococcus aureus,* 20mm inhibition zone while the methanolic extract effect against *Bacillus Subtilis* was 19.5mm inhibition zone. The present study has revealed that *Pistacia Atlantica* Desf. (Anacardiaceae) growing in Libya is capable of synthesizing and accumulating different types of phenolics, including mainly ellagitannins, gallotannins which were elucidated for the first time from this plant. It could be concluded from the obtained results that the *P. atlantica* is a promising source for bioactive compounds which have potential applications as bioactive antioxidant agents for the treatment of diseases.

Keywords: *Pistacia atlantica* Desf. (Anacardiaceae), Phenolics, gallotannines, NMR spectroscopy, Antioxidant DPPH, Antimicrobial activity.

Introduction

Natural products embrace a wide range of chemical classes among which the class of plant phenols represents the most heterogeneous one, as plant phenols are polyhydroxylated phytochemicals [1]. The phenolic compounds widely distributed in plants are tannins, which may occur as hydrolyzable tannins (formed

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in the pathway of the phenolic acids with sugar polymerization) and condensed tannins (combination of flavonoids). Since polyphenolic compounds are known to exhibit antioxidant properties [2] and can also act as direct scavenger molecules [3], they can prevent lipid peroxidation and biological damage caused by free radicals which formed under oxidative stress.

Pistacia, a genus of flowering plants from the family Anacardiaceae, contains about twenty species, among them five species are more popular including *P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus* [4], that are native to all of Africa, and southern Europe, warm and semi desert areas across Asia. Different parts of these species have been used in traditional medicine for various purposes like tonic, aphrodisiac, antiseptic, antihypertensive and management of dental, gastrointestinal, liver, urinary tract, and respiratory tract disorders. *P. atlantica* is a species of *Pistacia* tree known by the English common name 'mastic' tree and as the Persian turpentine tree. In Arabic, it is called (Butm or Butum), *P. atlantica* is a deciduous tree growing up to 7 m (23 ft) tall with branches spreading and growing erect to form a dense crown [5]. Genus *Pistacia* is characterized by the presence of a wide range of diverse compounds such as flavonoids, triterpenes, sterols and phenolic compounds. The phenolic compounds have been detected in different parts of these species, gallic acid**,** 3-(8-pentadecenyl)-phenol; 3,4,5-tri-*O*-galloyl quinic acid and 1,2,3,4,6-Pentagalloyl glucose from *P. vear*, *P. lentiscus*. *P. atlantica* fruits [6]; and from *P. lentiscus* isolated digallic acid [7]; monogalloyl glucose [8]. The present study aims at the isolation and identification of polyphenols from *Pistacia atlantica* Desf (Anacardiaceae) leaves as well as evaluation of its biological activities. During the current study, we isolated and identified an additional six phenolic compounds (1–6) from the ethyl acetate extract of *P. atlantica* leaves. All compounds have been characterized for the first time from leaves of *Pistacia atlantica.*

Results and Discussion

The fresh leaves of dried powdered *P. atlantica* was extracted by successive solvent using Soxhlet extractor, beginning with n-hexane, followed by ethyl acetate and finally with methanol. The received active ethyl acetate extract was subjected to a series of column and preparative paper chromatographic separations to isolate compounds **(1 – 6)**. Compounds **4** and **5** have not been described before from this species.

Structure Elucidation

In this study, the isolation and identification of the major polyphenolic metabolites, particularly phenolic acids and gallotannines of *P. atlantica* were carried out for the first time. Successive column chromatographic separations resulted in isolation of three polyphenolic acids **(1–3)** which identified as; gallic acid [9]; ellagic acid [10]; 3,3'-dimethoxyellagic acid [11], respectively, on the basis of their chromatographic properties (R_f-values, fluorescence) under UV-light and their responses towards specific spray reagents for tannins ($FeCl_3$, KIO_3). The structures of these three metabolites were confirmed by spectroscopic analysis, including 1D, 2DNMR, and by negative ESIMS, HRESI mass as well. Two new ellagitannins **(4,5)**;2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose, nilocitin **(4);** 1,3-di-*O*-galloyl-β-D-$^4C_1$-glucopyranos **(5)** carried out for the first time from *P. atlantica* and 1,2,3,4,6-penta-*O*-galloyl-β-D-$^4C_1$-glucopyranose **(6)** has been previously isolated from *P. lentiscus* fruits [12].

Compound 4, was obtained as an off-white amorphous powder (75 mg) showed a blue color with $FeCl_3$, and a pink color with KIO_3 on PC indicative of gallotannins. It possessed UV spectral data λ_{max}: 274 nm. and negative ESIMS spectrum showing $[M-H]^-$ ion peak at 483 mU corresponding to *Mr* = 484, identical with those reported for digalloyl glucoses. Complete acid hydrolysis of compound **4**, yielded gallic acid and glucose (CoPC, UV spectral data, ^1H,^{13}C-NMR and ESIMS analysis); On controlled acid hydrolysis **4** yielded an intermediate **4a**, which was separated by Prep. PC, using BAW as solvent. **4a** was then subjected to chromatographic, UV-spectral and negative ESI-MS analysis proving its identity to mono-galloyl glucose (CoPC, UV spectral data, ^1H,^{13}C-NMR and ESIMS analysis). The site of attachment of the two galloyl moieties in the molecule of **4** were then determined through ^1H-NMR and ^{13}C-NMR analysis (DMSO-d_6, room temperature) the data were in agreement with the achieved structure of **4** as 2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose or nilocitin [13] **(Fig. 1).**

Compound 5 was isolated as an off-white amorphous powder (45 mg) showed a blue color with $FeCl_3$, and a pink color with KIO_3 on PC indicative of gallotannins. It possessed R_f-values, UV spectral data one band at λ_{max} 278 nm; and negative ESI-MS spectrum showing peak at ([M-

H]$^-$ ion at m/z =483mU corresponding to *Mr* = 484, to be a digalloyl glucose. Complete acid hydrolysis of compound **5**, yielded gallic acid and glucose (CoPC, UV spectral data, ^{1}H-NMR analysis). The site of attachment of the two galloyl moieties in the molecule of **5** were then determined through ^{1}H-NMR and ^{13}C-NMR analysis ESIMS as well as. The ^{1}H-NMR spectrum of **5** revealed two aromatic proton singles, at δ 6.98 and 6.97 ppm assignable to the two existing galloyl moieties. The spectrum also showed in the sugar region, two clearly resolved downfield proton resonances, the most down field of which was found to resonate at δ 5.6 ppm (*d*, *J*=8 Hz), attributable to a β-configurated anomeric glucose proton. The second downfield sugar proton resonance was recognized at δ 5.03 ppm (*t*, *J* =8Hz). The significant downfield shifts, recognized for these two sugar resonance (in comparison with the resonances of the corresponding protons in free β-glucose) indicated that the hydroxyl groups, geminal to these protons are galloylated, thus proving the structure to be 1,3-di-*O*-galloyl-β-D- $^4C_1$glucopyranose.^{13}C-NMR spectrum of **5**, exhibited carbon resonances which were in accordance with this structure. The β-anomeric carbon was recognized from the resonance at δ 94.1 ppm. The most downfield sugar resonance located at δ 78.2 ppm is obviously due to the galloylated sugar carbon, C-3. Galloylation at C-3 also followed from the upfield shifts of the resonances of C-2 and C-4, compared to the resonances of the corresponding carbons in the spectrum of free β-glucose due to β-effects . The presence of two galloyl moieties in **5** follows from the two carboxylic carbonyl carbon resonances, recorded in this spectrum at δ 165.4 and 166.2 ppm. Other chemical shifts of the resonances of the remaining galloyl and glucose carbons in this spectrum were in agreement with the achieved structure of **5** as 1,3-di-*O*-galloyl-β-D-$^4C_1$ glucopyranose as **Fig. 1.**

Biological Activities

Determination of Radical Scavenging activity by DPPH Assay

The evaluation of the antioxidant activity of methanolic and ethyl acetate extracts of *P. atlantica* were done through in *vitro* assays by DPPH (2,2-diphenyl-1-pycrylhydrazyl) radical scavenging activity, the extracts of *P. atlantica* showed high values for absorbance inhibition at the 3 different concentrations used, (19, 38, 77µl) (**Fig. 2)**, ethyl acetate and methanolic extracts possess a distinct radical scavenging effect at concentration 77 µl, the percentage of inhibition obtained 96.4% and 53.7% respectively, the ethyl acetate extract completely inhibited DPPH absorbance which can be considered as a full absorbance inhibition of DPPH. Also, the IC_{50} (the concentration that inhibits 50% of the absorbance of DPPH), was determined from the graph plotted for the % inhibition against concentration in µg/ml for ascorbic acid and *P. atlantica* ethyl acetate extract which found to be 1.82±0.33 µg/ml and 8.41±0.24 µg/ml respectively. It could be concluded from the obtained results that the *P. atlantica* ethyl acetate extract shows a very high antioxidant capacity which is very close to the value of the reference standard used, which may

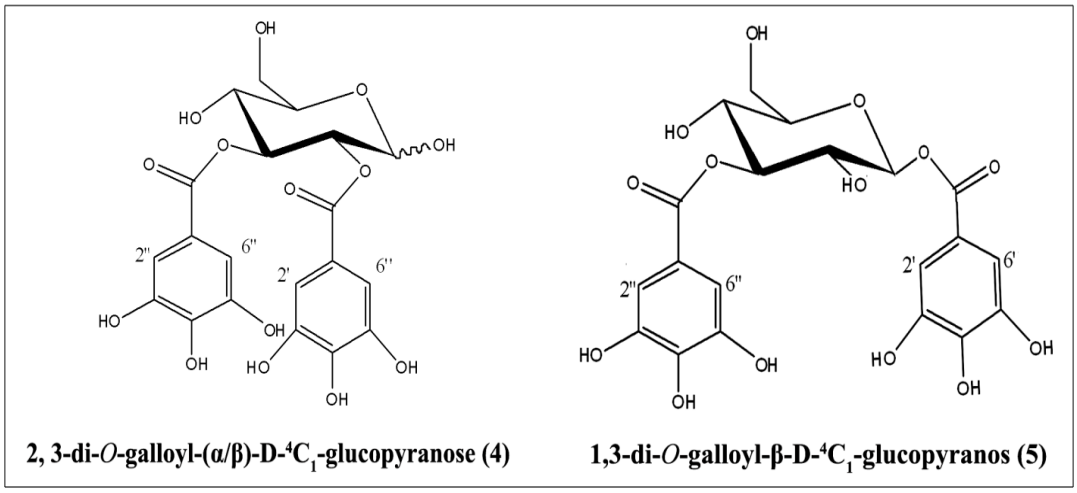

Fig. 1. Chemical structures of compounds (4) and (5).

be attributed to its high polyphenolic content.

Antimicrobial Activity of Extracts.

Antimicrobial activity of the tested extracts was determined by the agar well diffusion method [14]. In *vitro* antibacterial activity of the extracts of *P. atlantica* leaves were studied against five

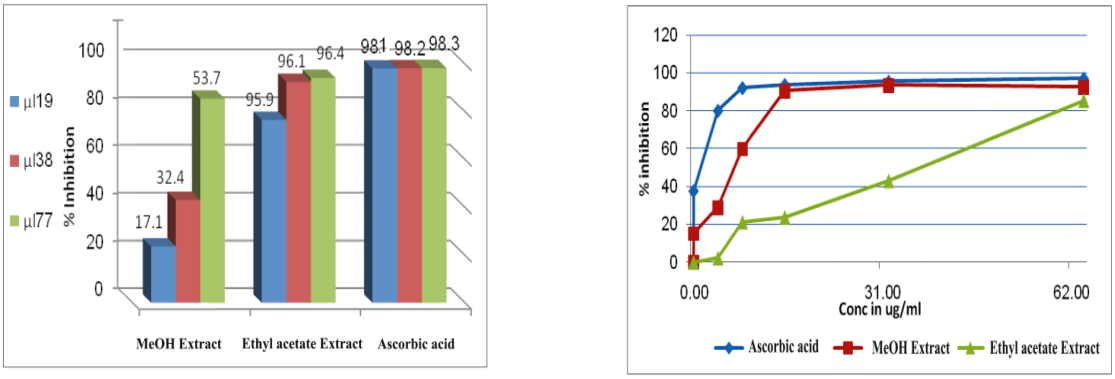

Fig. 2. Antioxidant activity of the two extracts compared to ascorbic acid at (19, 38 and 77µl).

bacteria strain and fungi. The tested organisms were Gram- negative bacteria (*Escherichia coli* NCTC-10416, *Pseudomonas aerogenasa* NCIB 9016), Gram-positive bacteria (*Bacillus subtilis*, NCIB-3610 and *Staphylococcus aureus*, NCTC-7447) and unicellular fungi as (*Candida albicans* IMRU 3669). The ethyl acetate extract shown a sensitizing effect against *Staphylococcus aureus,* 20mm inhibition zone while the methanolic extract effect against *Bacillus Subtilis* was 19.5mm inhibition zone. The organisms were tested against the activity of 50 mg/ml of the extract ethyl acetate and methanol results are depicted in the Table1.

TABLE 1. *In vitro* antibacterial activity of the extracts of *P. lentiscus* and *P. atlantica.*Antibacterial activity of leaves of ethyl acetate and methenolic extracts (50 mg/ml) of Pistacia atlantica.

Test Organisms / Sample No.	Mean Diameter of inhibition Zone (mm)				
	***Bacillus Subtilis* NCIB-3610**	***Escherichia coli* NCTC-10416**	***Pseudomonas Aeruginosa* NCIB-9016**	***Staphylococcus Aureus* NCTC-7447**	***Candida Albicans* IMRU-3669**
Ethyl acetate extract	17	14	13	16	-
Methanol extract	19.5	13	19	20	18.5

Experimental

General

NMR spectra were acquired in DMSO-d_6 on a Jeol ECA 500MHz NMR spectrometer,^{1}H at 500 MHz and ^{13}C 125MHz. Standard pulse sequence and parameters were used to obtain one-dimensional. ^{1}H chemical shifts (δ) were measured in ppm, relative to TMS, ^{13}C - NMR chemical shifts to DMSO-d_6 by adding 39.5 ppm and two dimensional COSY, HSQC and HMBC spectra. FTESI-MS spectra were measured on a Finnigan LTQ-FTMS (Thermo Electron, Bremen, Germany) (Department of Chemistry, Humboldt-Universität zu Berlin). UV recording was made on a Shimadzu UV–Visible-1601 spectrophotometer. Paper chromatographic analysis was carried out on Whatman 1 MM and 3 MM, using solvent systems: (1) H_2O; (2) 6% AcOH; (3) BAW (n-BuOH–AcOH–H_2O, 4:1:5, upper layer).

Plant Material

Fresh leaves of *Pistacia atlantica* Desf (Anacardiaceae) were collected on May, 2016 from wadi sawfajjin, Beni walid, Libya, the located at 180 kilometers North West of Tripoli. The plant was identified according to Ali, et al. [15]. A flowering voucher specimen is deposited in the herbarium of the Benghazi University, Libya.

Preparation of Extract

The fresh leaves of dried powdered *P. atlantica* (2 Kg) were extracted by successive solvent extraction using Soxhlet, beginning with n-hexane, followed by ethyl acetate and finally with methanol, (three times extraction, each for 8h with 2 L solvent). The three extracts were filtered through filter paper and evaporated to dryness under reduced pressure at 40°C in a rotavapor and further used for screening purpose. The dried extracts weights were calculated to give n-hexane (35 g), ethyl acetate (59 g) and methanol (65g), respectively. The three extracts were preliminary investigated for their biological activity.

Isolation and Identification of Phenolics

The two dimension paper chromatography of the ethyl acetate extract using the solvent systems (1) and (3), respectively, revealed the presence of polyphenolic components. The concentrated ethyl acetate extract (59 g) was chromatographed on a polyamide S6 and repeated Sephadex LH-20 column fractionation. The polyamide column (120×7.5 cm) and eluted with H_2O followed by H_2O–MeOH mixtures of decreasing polarities to yield six subfractions **(I–VI),** then started with H_2O followed by isocratic elution from 10% to 100 % H_2O/MeOH. Following removal of the solvents six subfractions were individually collected and subjected to two dimensional paper chromatography (2DPC). Compound **1** (74 mg) was isolated pure from 2.50 g of fraction II. Compounds **2** (48 mg) and **3** (86 mg) were individually separated purely by fractionation of 2.6 g of fraction III over Sephadex LH-20 column using a H_2O/MeOH mixture of decreasing polarity for elution. Compound **4** (75 mg) was individually isolated pure from 2.3g of fraction IV by fractionation on a Sephadex LH-20 column and 40% aqueous MeOH for elution, followed by preparative paper chromatography (prep. PC), using BAW system for final purification. Compound **5** (45 mg) was individually separated pure from 200 mg of fraction V by (prep. PC), using n-BuOH water saturated as solvent. Compound **6** (38 mg) was isolated pure from 3.5g of fraction VI by fractionation on a polyamide column using (methanol: toluene: H_2O) (60: 38: 2), followed by preparative PC, using BAW as solvent. Their chemical structure have been established by conventional methods of chemical and physical analysis and confirmed by ^1H and ^{13}C NMR spectroscopy.

New compound; 2,3-di-O-galloyl-(α/β)-D-$^4C_1$ glucopyranose or nilocitin (4), was obtained as an off-white amorphous powder (75 mg) showed a blue color with $FeCl_3$, and a pink color with KIO_3 on PC indicative of gallotannins. R_f-values: 60 (H_2O), 65 (AcOH-6),50 (BAW),UV spectral data λ_{max},: 276 nm. it possess negative ESIMS spectrum $[M-H]^-$ ion peak at 483 mU corresponding to *Mr* = 484, identical with those reported for digalloyl glucoses. Complete acid hydrolysis of compound **4**, yielded gallic acid and glucose (CoPC, UV spectral data,^1H,^{13}C-NMR analysis); On controlled acid hydrolysis **4** yielded only, one intermediate **4a** which was separated by Prep.PC, using BAW as solvent which proved its identity as a monogalloyl glucose. spectral data of **4**:1D-^1H NMR (DMSO-d_6) δ (ppm): α-glucose moiety:5.45 (1H*, d, J*=3.3 Hz, H-1),4.92 (1H, *dd, J*=8 & 3.3 Hz, H-2) ,5.78 (1H*, t,J*=8 Hz,H-3),3.1-4.0 (*m*, H-4,5,6); β-glucose moiety:4.98 (1H*, d, J*=7.5 Hz, H-1),5.08 (1H*, t, J*=7.5Hz, H-2),5.41(1H*, t, J*=7.5Hz, H-3), 3.1-4.0 (*m*, H-4,5,6).Galloyl moieties in α- and β-anomers 6.87(2H,*s*), 6.81(4H,*s*), 6.79(2H, *s*).^{13}C-NMR Spectral Data (DMSO-d_6)δ (ppm): α-glucose moiety 89.3 (C-1), 72.2 (C-2), 72.2 (C-3), 68.3 (C-4), 72.2 (C-5), 60.6 (C-6) , β-glucose moiety 94.5 (C-1), 73.1 (C-2), 75.5 (C-3), 68.3 (C-4), 76.7(C-5), 60.6(C-6). Galloyl moieties in α- and β-anomers; 120.64, 121.38, 121.42 (C-1' α/ β',1'' α/β), 109.97 (C-2',6'α/β, 2'',6'' α/β), 145.64 (C-3',5'α/β,3'',5''α/β), 138.67, 138.9 (C-4'α/β,4''α/β),164.8,165.2,165.4,165.5 (C=O α/β, C'=O α/β).

New compound; 1,3-di-*O*-galloyl-β-D-$^4C_1$ glucopyranose (5), was obtained as an off-white amorphous powder **(45 mg)** showed a blue color with $FeCl_3$, and a pink color with KIO_3 on PC indicative of gallotannins. *Rf*-values: 65 (H_2O), 73 (AcOH), 38 (BAW); UV spectral data one band at λ_{max} in MeOH : 278nm . Negative ESI-MS spectrum showing peak at $[M-H]^-$ ion at m/z = 483mU corresponding to *Mr* = 484, to be a digalloyl glucose ion. On complete acid hydrolysis, yielded glucose and gallic acid (CoPC and ^1H, ^{13}C-NMR). 1D-^1H NMR spectrum allowed the definition of the glucose protons as follows:5.6 (1H, *d, J*= 8Hz, anomeric H-1).5.03 (1H*, t, J*=8 Hz, H-3), 3.2-3.7 (sugar protons overlapped with water protons, H-2,4,5,6). Galloyl moiety: 6.97(2H*, s*, H-2' and H-6'), 6.99 (2H*, s*, H-2'' and H-6''). ^{13}C-NMR spectral analysis, afforded a spectrum containing double signals for most of the glucose and galloyl carbons. Resonances were assigned by

comparison with the ^{13}C NMR data, reported for similar galloyl glucoses. Glucose moiety : 94.1(C-1), 77.1(C-2), 78.2(C-3), 70.5(C-4), 71.6(C-5), 61.1(C-6), Galloyl moieties:120.3,119(C-1',1''), 110.1 , 110, 109, 108 (C2',6'and C2'',6'') , 145.8, 145.7 (C3', 5'and C3'',5''), 138.7,139.6(C-4',4''), 165.4, 166.2 (C = O).

Biological Assays

Radical Scavenging Effect

Radical scavenging activity of plant extract against the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich Chemie, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) using UV spectral data at $\lambda_{max:}$ 517nm Estimation was done to the method of Molyneux *et al*. [16]. Radical scavenging activity of the extract was measured by slightly modified method of Brand-Williams, Cuvelier, and Berset, C. [17]. Where the extract solution was prepared by dissolving 0.025 g of the dry extract in 10 ml of methanol. The solution of DPPH in methanol (6×10^{-5} M) was freshly prepared, before UV measurements. Three ml of this solution were mixed with 9 different concentrations of the samples. The resulting solutions were kept in the dark for 30 min at room temperature and then the decrease in absorbance was measured. Absorbance of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition } = [(AB-AA)/AB]\times100$$

where: AB is the absorbance of blank sample and AA is the absorbance of the tested samples.

IC_{50} value: the concentration of the substrate that causes 50% loss of the DPPH activity (color), were calculated for the standard and the extract from a graph plotted for the % inhibition against the concentration in µg/ml.

Antimicrobial Activity of Extracts.

Test organisms

Bacteria

i. Gram-positive bacteria: *Staphylococcus aureus*, NCTC-7447); *Bacillus subtilis*, NCIB-3610.

ii. Gram-negative bacteria: *Escherichia coli* NCTC-10416, *Pseudomonas aerogenasa* NCIB 9016

Fungi

Unicellular fungi: *Candida albicans*, IMRU 3669

Microbial cultures were grown on nutrient agar and potato dextrose agar for bacteria and fungi respectively and maintained at 4°C in a refrigerator for further studies.

Antimicrobial assay

The methanolic and ethyl acetate extracts leaves of *P. atlantica* were evaluated for antibacterial activity against five bacteria strain and fungi using the agar diffusion technique [18], 1 mg/ml solution in dimethyl formamid was used. The DMF showed no inhibition zones [19]. The tested organisms were Gram- negative bacteria (*Escherichia coli* NCTC-10416, *Pseudomonas aerogenasa* NCIB 9016), Gram-positive bacteria (*Bacillus subtilis*, NCIB-3610 and *Staphylococcus aureus*, NCTC-7447) and unicellular fungi as (*Candida albicans* IMRU 3669). Microbial cultures were grown on nutrient agar and potato dextrose agar for bacteria and fungi respectively and maintained at 4°C in a refrigerator for further studies.

Conclusion

The present study has revealed that *Pistacia Atlantica* Desf. (Anacardiaceae) growing in Libya is capable of synthesizing and accumulating different types of phenolics, including mainly ellagitannins, gallotannins which were elucidated for the first time from this plant. It adds to the previously characterized isolation of two new compounds 2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose **4**; 1,3-di-*O*-galloyl-β-D-$^4C_1$-glucopyranose **5**. According to these results, it could be concluded that the *P. atlantica* ethyl acetate extract shows a very high antioxidant capacity which is very close to the value of the reference standard used. *In vitro* antibacterial activity of the extracts of *P. atlantica* leaves were studied against five bacteria strains and fungi, ethyl acetate extract shown a sensitizing effect against *Staphylococcus aureus,* 20 mm inhibition zone while was 19.5mm of methanolic extract against *Bacillus Subtilis*

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References

1. Haslam E., Lilley T. H., Cai Ya, Martin R. and Magnolato D., Traditional Herbal Medicines: The role of polyphenols, *Planta Medica;* **55**(1), 1 (1989).

2. Mishra K., Ojha H., Chaudhury N., Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chemistry* **130**, 1036–1043 (2012).

3. Bouchet N., Barrier L. and Fauconneau B., Radical scavenging activity and antioxidant properties of tannins from Guiera senegalensis (Combretaceae), *Phytotherapie. Res.,* **12** (3), 159-162 (1998).

4. Alma M.H., Nitz S., Kollmannsberger H., Digrak M., Efe F.T., Yilmaz N., Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish Pistachio (Pistacia vera L.). *Journal of Agricultural and Food Chemistry;* **52**(12), 3911–3914 (2004).

5. Martinez J. J. I., Impact of a gall-inducing aphid on Pistacia atlantica Desf. Trees. *Arthropod-Plant Interactions;* **2**(3), 147-151 (2008).

6. Abdelwahed A., Bouhlel I., Skandrani I., Study of antimutagenic and antioxidant activities of Gallic acid and 1, 2, 3, 4, 6-pentagalloylglucose from Pistacia lentiscus. Confirmation by microarray expression profiling. *Chemico-Biological Interactions*. **165**(1), 1–13 (2007).

7. Bhouri W., Derbel S., Skandrani I. et al., Study of genotoxic, antigenotoxic and antioxidant activities of the digallic acid isolated from Pistacia lentiscus fruits. *Toxicology in Vitro.*; **24** (2), 509-515 (2010).

8. Romani A., Pinelli P., Galardi C., Mulinacci N., Tattini M., Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of Pistacia lentiscus L. *Phytochemical Analysis;* **13**(2), 79–86 (2002).

9. Nawwar M.A.M., Sahar A. M. Hussein and Merfort I., NMR spectral analysis of polyphenols from Punica granatum, *Phytochemistry,* **36**(3), 793-798 (1994).

10. Sahar A. M. Hussein, Barakat, H.H., Merfort, I. and Nawwar, M.A.M., Tannins from the leaves of Punica granatum, *Phytochemistry,* **45** (4), 819-823 (1997).

11. Sahar A. M. Hussein, Flavonoid and methoxyellagic acid sodium sulphates from *Frankenia laevis* L. *Die Pharmazie* **59** (6), 484-487 (2004).

12. Mei-Hui Lin, Fang-Rong Chang, Mu-Yi Hua, Yang-Chang Wu and Shih-Tung Liu, Inhibitory Effects of 1,2,3,4,6-Penta-O-Galloyl-β-D-Glucopyranose on Biofilm Formation by Staphylococcus aureus, *Antimicrobial Agents and Chemotherapy,* **55**(3), 1021–1027 (2011).

13. Nawwar M.A., Souleman A.M., Buddrus J., Bauer H., Linscheid M., Polyphenolic constituents of the flowers of Tamarix nilotica: The structure of nilocitin, a new digalloylglucose. *Tetrahedron Letters* **25**, 49–51(1984).

14. Kumar G., Karthik L., Rao K.V.B., *International Journal of Pharmaceutical Sciences and Research;* **1**(11), 51-58 (2010).

15. Ali Said S., Fernandez C., Greff S., Derridj A., Gauquelin T., Mevy J-P., Interpopulation variability of leaf morpho-anatomical and terpenoid patterns of Pistacia atlantica Desf. growing in Algeria. *Flora*.; **206**(4), 397–405 (2011).

16. Molyneux P., The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology,* **26** (2) , 211-219 (2004).

17. Brand-Williams W., Cuvelier M.-E. and Berset C., Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology,* **28**(1), 25-30 (1995).

18. Ibrahim H. S., Eldehn W.M., Abdel-Aziz H, H. A., Elaasser M. M. and Abdel-Aziz M. M., Improvement of antibacterial activity of some sulfa drugs through linkage to certain phthalazin-1(2H)-one scaffolds. *European Journal of Medicinal Chemistry* **85**, 480-486 (2014).

19. Bauer A. W., Kirby W. M. M., Sherris J. C. and Turck M., Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**, 493-496 (1966).

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المحتوى البوليفينولي، كمضادات للأكسدة و الميكروبات النشطه لمستخلص اوراق نبات البطم الاطلسي من العائلة (البطمية) الذي ينمو في ليبيا

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الهدف من هذا البحث هو البحث عن مستخلص جديد نشط بيولوجيًا لاوراق نبات البطم الاطلسي، وقد تم قياس الاثار البيولوجية لخلاصة كحول الميثانول وايثيل اسيتات لاوراق هذا النبات و مجزئاتة و بعض المركبات المفصولة منة .

اثبتت التجارب أن مستخلص الايثيل اسيتات يحتوي على خليط معقد من الفينولات، والذي يتضمن بشكل أساسي جالوتانين وتم تعريف ٦ مركبات بوليفينول (١-٦) تعرف لأول مرة من هذا النبات، بما في ذلك ثلاثة احماض بوليفينوليه وهم حمض الجاليك (١)؛ حمض الإيلاجيك (٢)؛ وحمض ٣,٣' ثنائى ميثوكسي حمض الإيلاجيك (٣) وثلاث مركبات جلاوتانين جديده.

تم توضيح هياكل كل هذه المركبات المعزولة بالطرق الحديثه والتحليل الطيفي. قد تم الحصول على مستخلص جديد نشط بيولوجيًا وأكثر فعالية بعد تحديد جميع المركبات في هذا المستخلص من حيث نشاطها كمضادات للأكسدة ذلك باستخدام الشارد الحر DPPH (٢,٢-ثنائي- فينيل-١-بيكريل هيدرازيل) مقارنة بالمرجع حامض الاسكوربيك حيث أثبتت أن مستخلص الإيثيل اسيتات يمتلك تأثير مسح جذري متميز عند تركيزات مختلفة واظهر قدرة عالية جداً كمضادات أكسدة قريبة جداً من قيمة حمض الاسكوربيك المستخدم، والذي يمكن أن يعزى إلى محتواه من المركبات البوليفينوليه العاليه التركيز.

الدراسة كشفت أن اوراق نبات البطم الأطلسي التى تنمو في ليبيا قادرة على تجميع وتراكم أنواع مختلفة من المركبات البوليفينوليه، بما في ذلك ايلاجيتانين أساسا، جالوتانين التي تم فصلها لأول مرة من هذا النبات.

يعتبر نبات البطم الأطلسي مصدر واعد للمركبات النشطة بيولوجيا التي لها تطبيقات محتملة كعوامل مضادة للأكسدة والنشطة بيولوجيا يمكن ان تستخدم لعلاج بعض الأمراض.