PHYTOCHEMICAL, ANTIBACTERIAL, ANTIOXIDANT AND PHYTOTOXICITY SCREENING OF THE EXTRACTS COLLECTED FROM THE FRUIT AND ROOT OF WILD MT. ATLAS MASTIC TREE (*PISTACIA ATLANTICA* SUBSP. *KURDICA*)

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Abstract. The goals of this research were to analyze the phytochemical, antioxidant, antibacterial, and allelopathic activities of extracts collected from *P. atlantica* subsp. *Kurdica* fruit and root. The fruit extract showed the highest phenolic (5.20 mg GAE/g dry extract) and flavonoid contents (1.01 mg QR/g dry extract). Qualitative and quantitative analysis of the diverse, biologically active elements from crude extracts displayed different compounds with varying molecular weights using gas chromatography–mass spectrometry. Likewise, both extracts possess unique phytochemical constituents. Additionally, the fruit extract has strong antioxidant and antibacterial properties compared to the root extract. The results of the allelopathic assay revealed strong and significant effects of both extracts on germination, seedling growth and root length for the test crops and weeds. According to the bioactivity analysis, the result of the variable importance in the projection (VIP) revealed that fruit extracts possessed a stronger biological activity than root extracts. The principal component analysis (PCA) plot detected a relationship between different constituents and biological activeness of both extracts and divided them into four clades. The results of this study showed that *P. atlantica* subsp. *Kurdica* may serve as a powerful natural antioxidant, antibacterial and herbicide source.
Keywords: *Mt. Atlas mastic tree, GC/MS, total phenolic and flavonoid contents, bioactivity analysis, radical scavenging, allelopathy*

Introduction

Isolation and recognition of phytochemical constituents from several innate sources can be interesting to scientists (Wheelwright, 1974). The World Health Organization (WHO) focuses on the advancement of native medicaments and ethnomedicines, conducting many investigations on natural therapies in order to search alternative medicines and innovative phytochemical mediators to control eczema, paralysis, diarrhoea, throat infections, jaundice, asthma and stomach pain and kidney stones (Borrelli and Izzo, 2000). North of Iraq, an area with high plant diversity, appears to be excellent for these studies. One of the plants with several industrial and medicinal properties, is the mastic. The *Pistacia* genus is held by the family Anacardiaceae; this genus comprises 11 shrub species belonging to the order Sapindales. *P. atlantica* is a

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long-lived and dioecious plant, with different sorts of blooms. The trees rise up to 7 m in height, with branches spreading and growing erect to form a thick crown. It originates from Asia and the Mediterranean (Rougemont, 1989). In the north of Iraq, *P. atlantica* grows in natural forests, where it is used for several traditional meals and remedies, typically utilizing its gum and aromatic fruits.

Different phytochemical compounds, such as essential oils, phenolic, flavonoid compounds and several fatty acids like eicosanoic, linolenic, palmitic, lignoceric, palmitoleic, pentadecanoic, hexadecanoic and octadecanoic have been recognized in *P. atlantica* (Adams et al., 2009; Trabelsi et al., 2012). Due to their abundances in these bioactive compounds, *P. atlantica owns* some pharmacological characteristics and beneficial usages. Likewise, microorganism resistance is a contest for health and nourishment manufacture, and the search for substitute natural antimicrobial mediators endures (Soković et al., 2010). Contamination and illness as a result of multidrug-resistant (MDR) bacteria in both community and hospital situations have been difficult for many decades, for example, methicillin-resistant *Staphylococcus aureus* (MRSA) is presently unaffected by several antibiotic remedies (Dubey et al., 2013). Different organs like stem, leave and bark, as well as components from *P. atlantica*, demonstrated significant allelopathy and antimicrobial (Tahir et al., 2019). Nevertheless, there has been no information of extracts obtained from the *P. atlantica* subsp. *Kurdica* root, as alternative spontaneous products to reduce the free radical and to control the weeds and bacteria. Established along the request for natural products as an antiradical, and to control microorganisms and weeds, the current investigation was conducted to screen the ethanolic extract composition, antioxidant, antibacterial, and allelopathic activities in fruit and root of *Pistacia atlantica* subsp. *Kurdica.*

Materials and methods

Sampling site

The plant materials were collected in the Sarga mountain in the municipality of Sharbazher, Sulaimani, Iraq (Altitude: 35.85759, Longitude: 45.59086 and level sea: 1352.1 masl). Fruits and roots of *P. atlantica* subsp. *Kurdica* were acquired at 11:00 A.M. in August 2018. The plant was characterized and authenticated at the College of Agricultural Sciences of the University of Sulaimani by the taxonomist Assistant Professor Dr Rupak Abdulrazaq. A voucher specimen (NRT 03) was prepared. The plant materials were dried at 41 °C for eight days.

Chemicals agent

Ethanol and methanol, potassium acetate, Folin–Ciocalteau reagent, gallic acid and quercetin, aluminium chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS) were purchased from Sigma-Aldrich (Munich, Germany).

Extract isolation

The phytochemical of fruit and root were obtained by the Soxhlet method by placing 85 grams of dried-ground tissues of *P. atlantica* subsp. *Kurdica* in a cellulose thimble. An amount of 220 mL of ethanol (Pure ethanol) was practiced for the extraction using a standard Soxhlet method for 150 min in a Soxhlet extraction system (BÜCHI Extraction

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System Model B-811). The solvent from extracts was then expelled utilizing a vacuum a vacuum rotary evaporator.

Determination of total phenolic compounds

The Folin-Ciocalteau method was applied to determine the amount of total compounds of phenolic acids as described by Singleton et al. (1999) with little modifications. The sample test contained 0.03 mL of each extract (root or fruit), which mixed with 1.7 ml Folin-Ciocalteau (diluted ten times). After 4 min of incubation, 1.3 mL of sodium carbonate solution (10%) was complemented. The calibration curve was determined by mixing aliquots of 5, 10, 15, 20, and 25 µg/mL gallic acid solutions with the same reagents, as the sample tests. The blank contained all solutions with 0.03 mL of distilled water in the state of plant extract. All test tubes were kept for 48 min in the dark at 39 °C. The absorptions of the samples were determined with a spectrophotometer at 750 nm. Each sample was repeated three times. The quantity of phenolic compounds in samples was determined by the calibration curve ($y = 0.0267x - 0.0269$, $R^2 = 0.99$) and expressed in mg gallic acid equivalent/ g dry extract using this formula:
Total phenolic content (mg gallic acid equivalent/g dry extract) = sample concentration calculated from the calibration curve of gallic acid in mg/mL × [extract volume (mL)/weight of the dry extract (g)].

Determination of total flavonoid compounds

The $AlCl_3$ method was used by three triplicates to determine the total flavonoid content of ethanolic fruit and root extracts (Madaan et al., 2011). A stock solution of quercetin was composed by dissolving 10.0 mg quercetin in 10 mL pure methanol, then the series of standard solutions of quercetin in concentration 0.005, 0.010, 0.020, 0.040 and 0.080 mg/mL were prepared by using pure methanol. Three mL of a solution composing of 1 mL of methanol 80%, 0.33 mL of 2% $AlCl_3$, 0.07 mL of 1 M potassium acetate and 1.60 mL of autoclaved distilled water was supplemented to 40 µl of the sample extract or 1 mL of the standard solutions. The mixture was hardly agitated and incubated for 32 min at room temperature. The absorption of the reaction compound was estimated at 415 nm against the blank (contains all above reagents and 40 µL of distilled water in the state of plant extract). The equation of linear regression of the standard curve ($y = 0.0243x + 0.0005$, $R^2 = 0.99$) of the series of quercetin dilutions, was plotted by MS Excel software and exploited to calculate the total flavonoid content by using this formula:
Total flavonoid content (mg quercetin equivalent/g dry extract) = concentration of samples derived from the quercetin calibration curve in mg/mL × [volume of the extract (mL) / dry extract weight (g)].

Qualitative and quantitative screening of ethanolic extracts by GC/MS

The qualitative and quantitative screening of constituents was accomplished using GC/MS. The GC/MS analysis was completed by using Shimadzu GC-QP 2010 Ultra gas chromatograph, The GC oven temperature was stated at 40 °C and then progressively upturned to 280 °C at a rate of 15 °C min^{-1}. Helium was exploited as a healer gas; inlet pressure was 96.1 kPa; linear velocity was 47.2 cm sec^{-1}. Column flux was 1.71 mL min^{-1}; injector temperature 280 °C; injection mode: split. MS scan

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conditions: source temperature 200 °C; interface temperature 280 °C; detector gain 0.69 kV + 0.10 kV and mass range of m/z 50-800. The constituents of the extracts in different parts were set by matching the retention indices with those of recognized constituents stored in the NIST library (2005) or either those of the literature. Peak area concentrations of the constituents were calculated to based on GC peak areas.

Antibacterial test

The antibacterial activity was measured by the agar-well diffusion method (Mounyr et al., 2016). The bacteria employed in this exploration, were *Staphylococcus aureus (*ATCC® 6538P™), methicillin-resistant *Staphylococcus aureus* (MRSA: Clinical isolates), *Bacillus subtilis* (ATCC® 6633™) and *Bacillus cereus* (Clinical isolates). Test microorganisms were initially cultivated in nutrient broth at 37 °C. The antibacterial potential of *P. atlantica* subsp. *Kurdica* was studied using the ethanolic extracts dissolved in Tween-80 (1%). For each pathogenic bacterium, a suspension (10^7 CFU/mL) was first dispersed generally on the solid agar surface. At that point, a hole with a diameter of 0.60 cm has penetrated with an autoclaved cork borer, and a volume (80 µL) of the extract solution (255 mg/mL) was inserted into the well. For 15 h, the plates were incubated at 37 °C and the diameter of the suppression areas was calculated in millimeters.

Ability of radical scavenging extracts by DPPH assay

The antioxidant ability of both extracts was determined using the method, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging as defined by Shimada et al. (1992) with some amendments. Two mL (6×10^{-5} M) of DPPH solution was mixed with 20 µL sample. A control with methanol and a DPPH solution have also been implemented. The samples and control were incubated in the dark at 23 °C for 31 min and absorbed against a blank containing only methanol at 517 nm. The triple experiment was conducted. The inhibition percentage of samples were calculated from the data of absorbances by the formula:
% inhibition = [(Absorbance of control - Absorbance of the sample) / Absorbance of control] × 100.

Radical scavenging ability of extracts by ABTS radical cation

The free radical scavenging activity of root and fruit extract was set by 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS) radical cation decolorization assay (Re et al., 1999). ABTS radical cation was made by the mixing of ABTS (7 mM) in water and 2.45 mM of potassium persulfate (1:1). The mixed solution was hoarded in the dark at 21 °C for 15 h before use. For obtaining an absorbance of 0.700 ± 0.002 at 734 nm, the ABTS radical cation solution was made weaker with methanol in ratio 1:37.50. Seven µL of root or fruit extract was added to 2.993 mL of diluted ABTS radical cation solution. A control solution contained 7 µL of methanol and 2.993 mL of diluted ABTS radical cation solution. After 8 min of incubation, the absorbance was read against the blank (methanol) at 734 nm. All tests have been repeated three times. Percent of inhibition was calculated using the formula:
ABTS cation scavenging (%) = [(Absorbance of control-Absorbance of the sample)/Absorbance of control] × 100.

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Allelopathy assay

The allelopathic power of the root and fruit extracts was performed on the seed germination parameters, of four plant species: wheat (*Triticum aestivum* L. variety Aras), barley (*Hordeum vulgare* L. variety Alkher), wild mustard (*Sinapis arvensis* L.), and narbon vetch (*Vicia narbonensis* L.*)*. Plant seed was sterilized with NaClO (0.90%) for 12 min and washed with autoclaved distilled water six times. Five replicates, each comprising of 15 seeds, were exercised for each of the control, 1.10 and 2.20 mg/mL plant extracts, using sterile Petri dishes (0.90 cm diameter) padded with three-sterile filter papers (Whatman). The plant extracts were mixed in a distilled water-acetone mixture (98:2) (Tahir et al., 2018). Nine mL of distilled water/acetone (control) or 9 mL different doses of the two plant extracts (1.10 or 2.20 mg/mL) were added to each Petri dishes. In order to prevent loss of moisture and microbial contamination, the dishes were covered with parafilm and deposited in a dark incubator at 19 °C for 7 days. In the eighth day, the germination percentage [%G = (Total germinated seeds/15) × 100] and seedling growth were measured. A seed was considered as a germinated seed when the appearance of the radicle become obvious (*Figure 1*).

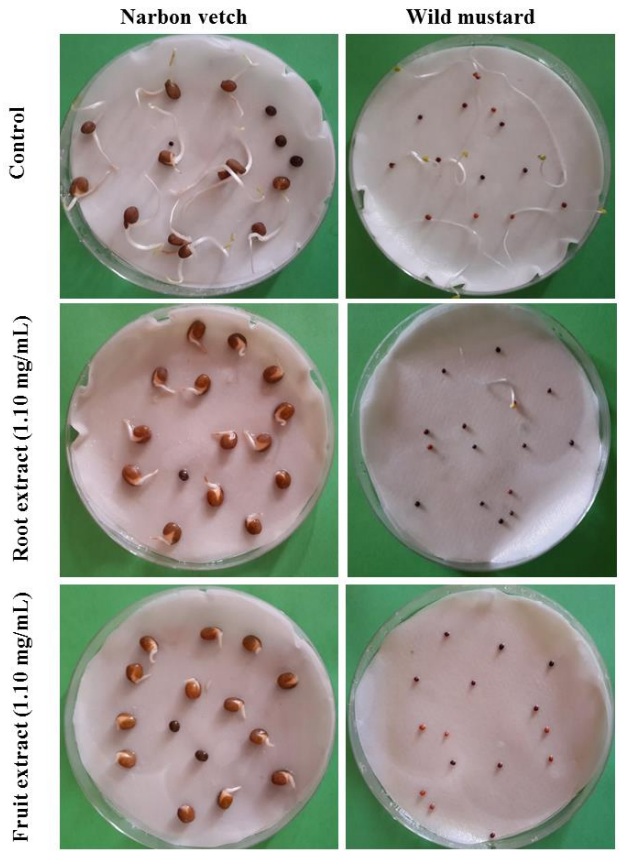

Figure 1. *Allelopathy effects of P. atlantica subsp. Kurdica fruit and root extracts on the germination parameters of two weeds: narbon vetch and wild mustard*

Statistical data analysis

Data on bio-activities were submitted to the analysis of variance (One way-ANOVA) using XLSTAT 2016 software. Means were matched by Duncan's new multiple range

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test at the 5% significance level. The data was exposed to a PCA analysis using the software XLSTAT 2016 to define the relation between chemical components and inhibitory effects.

Results and discussion

Screening of the polyphenolic constitutions

The results of the phytochemical contents of the fruit and root ethanolic extracts are detailed in *Figure 2*. The polyphenolic constitutions were considered by the Folin–Ciocalteu method and there was not significant variance ($p < 0.05$) among all tested samples for total phenolic content (TPC), while significant differences were noticed between the extracts collected from fruit and root for the flavonoid content (TFC). The maximum values of phenol and flavonoid compounds with 5.20 mg gallic acid equivalents/g and 1.01 mg quercetin equivalents per gram of dry extract, were registered by fruit extract. The outcome showed that fruit extract was rich in polyphenolic compounds. The TPC amount in both extracts was varied from the earlier assessments in methanolic, water, butanol and ethyle acetate extracts of the same species and another plant part (Ben Ahmed et al., 2017; Benamar et al., 2018; Tahir et al., 2019). Likewise, the variation between our results and other researchers in the quantity of polyphenolic compounds in *P. atlantica* may be contributed to a growing region, growing season, growth stage. solvent, a method of extraction and plant organ.

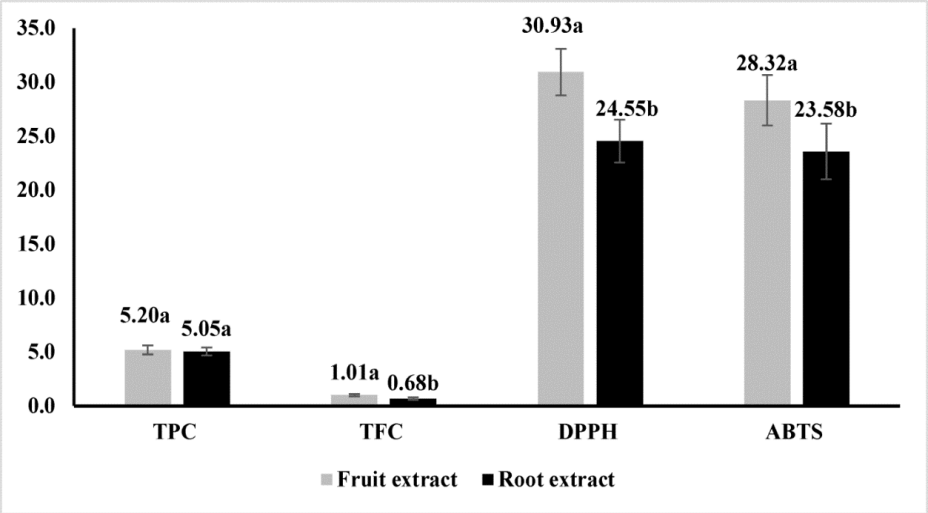

Figure 2. *Total phenolics (TPC in mg gallic acid equivalent/g dry extract), total flavonoids (TFC in mg quercetin equivalent/g dry extract) contents and antioxidant potentials (%) in P. atlantica subsp. Kurdica fruit and root extracts. The letters attached to each bar by the different letters mark substantial differences according to Duncan's multiple-range test. Each value is an average of three replicates*

GC/MS analysis of the extracts

Chromatographies analysis of the extracts allowed 26 compounds to be detected (*Table 1*). Several classes of compounds, including essential oil, polyphenol, and aliphatic compounds, were noticed in these extracts. Among its 18 components detected

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in the fruit extracts, 6 constituents dominated the composition of this extract, with a content of 72.83%. Constituents: 2-isoamyl-6-methylpyrazine (38.57%), oxalic acid, hexadecyl 2-methyl phenyl (9.26%), (NB)-O-[(diethyl boryl oxy)(ethyl) boryl]-caprolactone oxime (7.19%), pyrogallol (6.78%), 3-cyclopentylpropionic acid, 3-methyl phenyl ester (5.62%), and benzoic acid, 3-hydroxy-benzoic acid (5.41%) were the predominated components. Among the detected fractions, seventeen compounds were limited to fruit extracts. As displayed in the GC/MS analysis of the root extracts, 9 compounds were determined. Among them, five constituent concentrations were ranged from 5 to 37%. The major ones were 2-fromyl-3-benzyl-3-cholestanol (36.97%), l-(+)-ascorbic acid 2,6-dihexadecanoate (15.98%), cis-9-hexadecenal (9.83%), alpha-amyrenone (6.34%) and 1-heptacosanol (5.62%). Eight of ten compounds were constricted to this organ. Variations in the extract composition among the different tissues can be expounded by the volatility and biotransformation of monoterpenes, which often occur in several metabolic pathways leading to a mixture of products and, which may undergo spontaneous auto-oxidation (McGarvey and Croteau, 1995). To our awareness, there are no works on the chemical composition, antioxidant, antibacterial and allelopathic activities of the ethanolic extracts from *P. atlantica* subsp. *Kurdica* root. Hence, our findings are of attentiveness. In the previous works of *P. atlantica*, various compounds of several phytochemical clades have been known. Most of the predominated components found from different parts of *P. atlantica* subsp. *Kurdica*, have made it as a fountain of pharmaceutical and industrial usages. For instance, 2-isoamyl-6-methylpyrazine is used as foodstuffs (Xu-Yan et al., 2012). Studies revealed that pyrogallol had antibacterial properties averse to food-borne gram-positive (Han and Wang, 2017). Compound l-(+)-ascorbic acid 2,6-dihexadecanoate recognized as an antioxidant food additive (Cort, 1974). Previous investigations using α-amyrenone have proved its pharmacological potential as a new healing tool for the controlling of painful and inflammatory sicknesses (Quintão et al., 2014).

Antioxidant spectrophotometric assay

The effectiveness of the radical scavenging associated with the site and the number of compound hydroxyl groups. Most components with the OH group in *ortho* position of the ring, showed the greatest powerful scavengers of DPPH radical while the constituents with more of OH in the structure were the most powerful ABTS radical scavengers (Baba and Malik, 2014). The antioxidant ability of different extracts of fruit and root was conducted using DPPH and ABTS assays as shown in *Figure 2*. Then, a lower percentage value designates a lower antioxidant activity. As reported in the DPPH method, the antioxidant activity was ranged significantly from 24.55 to 30.93%. Fruit extract had the maximum DPPH scavenging activity (30.93%), while the lowest action (24.55%) was stated by root extract. In the ABTS radical, the scavenging activities in the various extracts were difference significantly and ranged from 23.58 to 28.32%. The fruit extract exhibited the maximum antiradical ability by ABTS. Interestingly, the consequences of antioxidant activities revealed that fruit extract appears to heavily affect the radical scavenging ability. These findings suggested that the antioxidant powers of fruit extracts can be attributed to their high flavonoid quantity. Our results of antiradical ability are lower than the output (87.00-97.50%) obtained by Hatamnia et al. (2014) and Hatamnia and Malekzadeh (2015). Several factors could be clarified this variation like the genetic variability of the plant, harvesting time of the samples, a method of extraction and the effect of the climate.

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Table 1. *GC/MS analysis of different extracts acquired from fruit and root of P. atlantica subsp. Kurdica*

No.	Compounds name	Fruit	Root
1	Tetradecane	0.86	–
2	Pentadecanoic acid	4.53	–
3	9,12-octadecadienoic acid	1.64	6.53
4	Cis-9-hexadecenal	–	9.83
5	1-heptacosanol	–	5.62
6	Tetrahydrocyclopenta[1,3]dioxin-4-one	3.26	–
7	Pyrogallol	6.78	–
8	Benzoic acid, 3-hydroxy-benzoic acid	5.41	–
9	Elemol	0.98	–
10	6-deoxy-D-galactose	4.02	–
11	Beta-eudesmol	1.47	–
12	Dichloroacetic acid, tridec-2-ynyl ester	3.57	–
13	2-isoamyl-6-methylpyrazine	38.57	–
14	3-cyclopentylpropionic acid, 3-methylphenyl ester	5.62	–
15	3-pentadecyl-phenol	2.85	–
16	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.73	–
17	2,2-dimethyl-3-vinyl-bicyclo[2.2.1]heptane	0.71	–
18	Oxalic acid, hexadecyl 2-methylphenyl	9.26	–
19	(NB)-O-[(diethylboryloxy)(ethyl)boryl]-caprolactone oxime	7.19	–
20	Alpha-tocopherol-beta-D-mannoside	1.55	–
21	l-(+)-ascorbic acid 2,6-dihexadecanoate	–	15.98
22	2-fromyl-3-benzyl-3-cholestanol	–	36.97
23	Cis-13-octadecenoic acid	–	4.39
24	Dibenzo[a,h]cyclotetradecene, 2,3,11,12-tetraethyl-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15	–	4.78
25	22-alpha-hydroxy-3,4-secostict-4(23)-en-3-oic acid	–	4.56
26	Alpha-amyrenone	–	6.34

Antibacterial activity of different extracts

The tested extracts of *P. atlantica* subsp. *Kurdica* fruits and roots held the antibacterial potent versus Gram-positive than Gram-negative bacteria. The cause of the increased sensitivity of Gram-positive bacteria than Gram-negative bacteria could be related to their differences in cell membrane structures. The antimicrobial potentials of *P. atlantica* subsp. *Kurdica* fruit and root extracts were determined primarily by the disc diffusion technique against different Gram-positive bacteria strains, which commonly encountered in infectious diseases. As stated in *Figure 3*. It can be noted that both plant extracts, revealed varying grades of antibacterial potential against the four bacterial strains tested. The effects of both extracts varied considerably from all bacteria tests except methicillin-resistant *S. aureus*. The zones of fruit extract inhibition against four types of bacteria were ranged from 3.38 to 7.35 mm. The ethanol fruit extracts exhibited the strongest anti-*S. aureus* activity with an inhibition zone diameter of 7.35 mm and a feeble activity against methicillin-resistant *S. aureus* (6.40 mm). The extracts of root presented a relatively moderate activity principally against all bacterial species with the inhibition diameters ranged from 3.38 to 7.13 mm. This extract of plant parts showed a small activity mainly against *B. subtilis* (3.38 mm) and the maximal inhibition zones against *B. cereus*. It has been described in this section that the zone of inhibition could

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be importantly affected by the structure of crude extracts that are a combination of phyto-components which may impact the diffusion power of the active elements. Similar results have been obtained from an investigation carried on *P. atlantica* subsp. *Kurdica* leaf, stem and bark by Tahir et al. (2019).

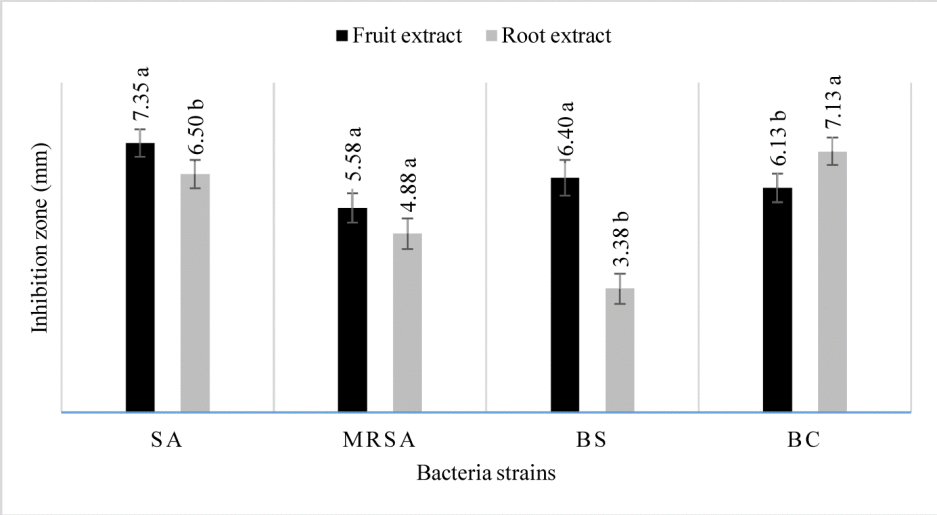

Figure 3. *Inhibition zones (mm) of the fruit and root extracts against four different species of bacteria. SA: S. aureus, MRSA: methicillin-resistant S. aureus, BS: B. subtilis, BC: B. cereus. The letters attached to the numbers of each bar by the different letters refer to substantial differences based on Duncan's multiple-range test. Each value is an average of three replicates*

Responses of crop and weed species to different extracts

All the applied concentrations of fruit extracts significantly blocked the germination and growth of the test plant species (*Table 2*). Fruit extracts at 2.20 mg/mL were more effective than fruit extracts at 1.10 mg/mL. The highest preventing of germination percentage (81.36% on wild mustard), root length (95.06% on narbon vetch) and shoot growth (88.34% on barley) were recorded by fruit extracts at a concentration of 2.2 mg/mL, while the lowest values of blocking of germination percentage (3.41% on bread wheat), root elongation (59.67% on wild mustard) and shoot length (56.20% on wild mustard) were reported by fruit extracts at 1.1 mg/mL concentration. All the doses of root extracts significantly inhibited the germination and growth of test crops and weeds. Root extracts at 2.20 mg/mL concentration were comparatively more toxic. The results presented that fruit and root extracts seemed more active in preventing bread wheat and narbon vetch germination. In addition, both extracts had more phytotoxic power against root elongation of all test species because of its directness contact with the extracts. Overall, the reduction of germination parameters was attributed to the presence of some allelochemical compounds in both extracts such as alpha-amyrenone, benzoic acid, 3-hydroxy-benzoic acid, pentadecanoic acid and -(+)-ascorbic acid 2,6-dihexadecanoate (Javaid et al., 2006; Ferreira et al., 2017). In addition, these allelochemical compounds modify the central vascular cylinder, layers of cortex cells and the mitochondrial metabolism, thereby altering some physiological and metabolic reactions related with growth of the plants (Gatti et al., 2010). Similarly, Tahir et al. (2019) found that the bark, stem and leaf extracts of *P. atlantica* subsp. *Kurdica* significantly decreased the growth of some crops and weeds.

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Relationship between chemical components and bioactivity parameters

The objective of variable importance in projection (VIP) was to identify and select the most important predictor variables. In a given model, a variable with a VIP score near or above 1 can be considered important. As displayed in *Figure 4*, the fruit extract had a high impact (VIP > 1) and it was considered as variable importance in the bioactivity assays. For reducing the multidimensional structure of the data and providing a two-dimensional map to explain the relationship and variance between the data obtained from phytochemical and bioactivity analysis of both extracts, a PCA plot was performed and two main PCAs for the analyzed two extracts accounted for 100% of the total variation, PC1 for 88.32% and PC2 for 11.68% (presented in *Figure 5*). The plot obtained from PCA using the linkage method among classes indicated the presence of four clusters:

a. Group 1 with higher concentrations of pyrogallol (C4), benzoic acid, 3-hydroxy-benzoic acid (C5), 3-cyclopentylpropionic acid, 3-methylphenyl ester (C7), Oxalic acid, hexadecyl 2-methylphenyl (C8), and (NB)-O-[(diethylboryloxy)(ethyl)boryl]-caprolactone oxime (C9). Moreover, a positive correlation with total phenolics content, total flavonoids content, inhibition growth of *S. aureus* (SA), methicillin-resistant *S. aureus* (MRSA), and *B. subtilis* (BS).
b. Group 2 with high amount of 9,12-octadecadienoic acid (C1), Cis-9-hexadecenal (C2), 1-heptacosanol (C3), l-(+)-ascorbic acid 2,6-dihexadecanoate (C10), Alpha-amyrenone (C12), high inhibition growth of *B. cereus* (BC), high germination inhibition of wheat (INW) and narbon vetch (INNV).
c. Group 3 with a high content of 2-isoamyl-6-methylpyrazine (C6), high inhibition of germination of barley (INB) and high radical scavenging activity (DPPH and ABTS).
d. Group 4 with a high concentration of 2-fromyl-3-benzyl-3-cholestanol (C11) and high blocking of germination in wild mustard (INM)

Table 2. *Inhibition percentages of various concentrations of fruit and root extracts of P. atlantica subsp. Kurdica on the germination and growth of four plant species*

		Inhibition (%)				
Plant species	**Parameters**	**Control**	**Fruit (1.10 mg/mL)**	**Fruit (2.20 mg/mL)**	**Root (1.10 mg/mL)**	**Root (2.20 mg/mL)**
T. aestivum	Germination	0.00b	3.41b	13.57ab	5.09b	22.04a
	Root length	0.00b	81.42a	88.50a	84.96a	88.50a
	Shoot length	0.00c	58.86b	64.88b	55.18b	83.61a
H. vulgare	Germination	0.00d	40.00c	79.38a	41.88c	62.81b
	Root length	0.00d	65.08c	88.83a	75.42b	91.62a
	Shoot length	0.00d	50.44c	88.34a	70.85b	89.21a
V. narbonensis	Germination	0.00b	8.18b	8.48b	8.18b	22.56a
	Root length	0.00b	93.83a	95.06a	93.83a	93.83a
	Shoot length	0.00c	63.16ab	82.30a	55.50b	79.90a
S. arvensis	Germination	0.00d	68.38c	81.36ab	75.20bc	87.68a
	Root length	0.00c	59.67b	80.66a	80.25a	85.60a
	Shoot length	0.00b	56.20a	58.21a	67.44a	74.06a

The different letters connected to each row mark substantial differences according to Duncan's multiple-range test. Each value is an average of five replicates

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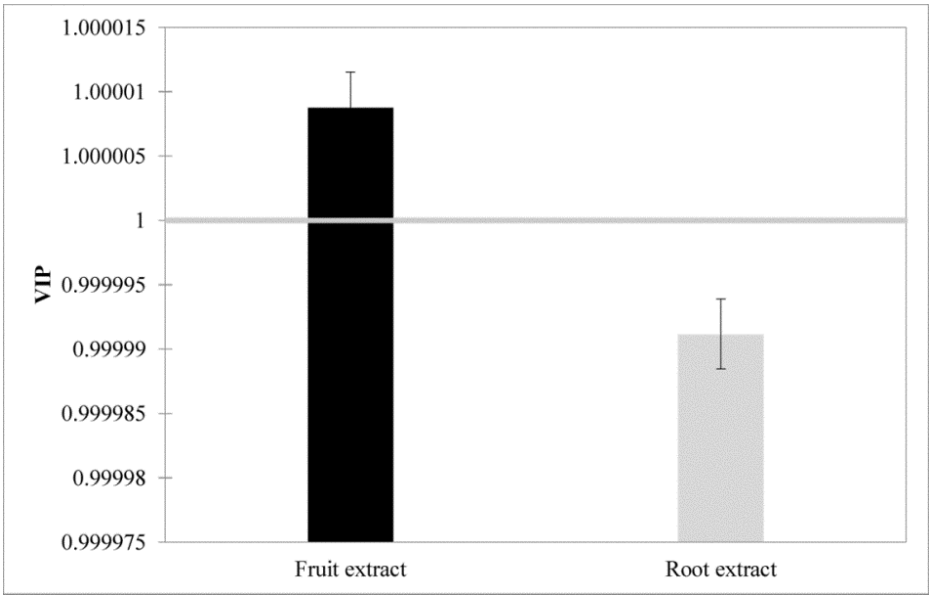

Figure 4. *Variable importance in the projection (VIP) showing the importance of fruit extract*

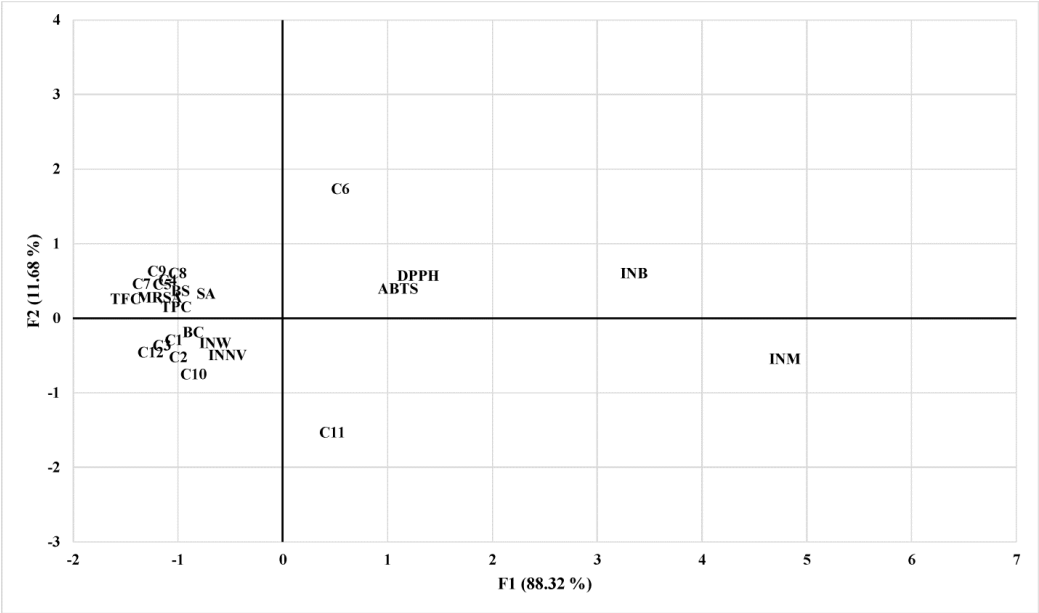

Figure 5. *PCA biplot showing the distributions of chemical compounds (12 fundamental compounds having an average concentration of at least 5.00% of the total extract composition in at least one plant tissue) and biological activity parameters. C1: 9,12-octadecadienoic acid, C2: Cis-9-hexadecenal, C3: 1-heptacosanol, C4: Pyrogallol, C5: Benzoic acid, 3-hydroxy-benzoic acid, C6: 2-isoamyl-6-methylpyrazine, C7: 3-cyclopentylpropionic acid, 3-methylphenyl ester, C8: Oxalic acid, hexadecyl 2-methylphenyl, C9: (NB)-O-[(diethylboryloxy)(ethyl)boryl]-caprolactone oxime, C10: l-(+)-ascorbic acid 2,6-dihexadecanoate, C11: 2-fromyl-3-benzyl-3-cholestanol, C12: Alpha-amyrenone, SA: S. aureus, MRSA: methicillin-resistant S. aureus, BS: B. subtilis, BC: B. cereus, TPC: Total phenolics content, TFC: Total flavonoids content, INB: Inhibition of germination in barley (average of both concentrations), INM: Inhibition of germination in mustard (average of both concentrations), INW: Inhibition of germination in wheat (average of both concentrations), INNV: Inhibition of germination in narbon vetch (average of both concentrations)*

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Conclusions

Qualitative and quantitative analysis of the diverse biologically active elements from crude extracts of *P. atlantica* subsp. *Kurdica* displayed different types of molecular weight compounds in each extract. The high radical scavenging and antibacterial capacity are detected in the ethanol fruit extract compared to the root extract. The current work determines that fruit and root extracts have significant herbicide effects on the germination and growth of seedlings in four plants. Fruit and root residues of *P. atlantica* could be disseminated on wilderness, causing to the leaching of allelochemical compounds that would prevent or decrease the seed germination of weeds. Therefore, both extracts are considered to be new natural sources of antioxidants, antibacterial and weedicides. The prospect investigates on *P. atlantica* may be conducted on the following characteristic:

a. More resources are needed to verify the primary mechanisms of *P. atlantica* extracts as inhibitory agents.
b. For making the bio-weedicide to control the weeds. It is important to purify individually the allelopathic components, which have the inhibitory properties, presenting in the *P. atlantica* extracts.

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