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Original Article

In vitro Callus Induction and Analysis of Essential Oil Constituents in Leaves and Callus of *Pistacia atlantica* subsp. *kurdica*

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Abstract

In order to optimize callus induction in *Pistacia atlantica* Desf. subsp. *kurdica* Rech. f., the effect of different plant growth regulator (PGR) combinations were studied. Inflorescence axis explants were cultured on WPM medium supplemented with 1, 2 and 3 mg/L of TDZ or 1, 2 and 3 mg/L of BA, in combination with 1mg/l different auxins: 2, 4-D, NAA and IBA . Callus initiation was observed in all media evaluated and the highest percentage (100%) was observed in medium containing 1 mg/L BA or TDZ and 1 mg/L NAA. Additionally, anthocyanin, total phenolic compound of callus and immature fruits were measured. Anthocyanin content of callus and immature fruits were 79.42 ± 4.63 and 61.71 ± 3.81 mg cyanidin-3-glucoside/g fresh weights, respectively. Total phenolic content of callus and fruits were 4.91 ± 0.492 and 5.1 ± 0.780 mg gallic acid/g fresh weights. GC-MS analysis of essential oil constituents of *P. atlantica* subsp. *kurdica* callus and leaves showed 8 kinds of substances in callus and 13 in leaves. Main composition of callus extract were alpha pinene (89.19%), camphene (1.09%) and Bicyclo[3.1.1] heptane, 6,6-dimethyl-2-methylene (3.40%) and it was alpha Pinene (15.63%), gamma elemene (33.84%) and caryophyllene (9.26%) in leaves.

Key words: Callus, *Pistacia atlantica* Desf. subsp. *kurdica* Rech. f., GC-MS, Anthocyanin, Phenolic compound

Introduction

Wild pistachio (*Pistacia* L.) is a genus of plants in Anacardiaceae family. Large populations of *Pistacia atlantica* Desf. subsp. *Kurdica* Rech. f. grow wild in the Kurdistan province of Iran. *P. atlantica* subsp. *kurdica*, with the local name of Baneh, is a medicinal and food stuff plant. Baneh nuts are used by the natives after grinding and mixing with other nuts. Its gum is also used in the production of chewing gum [1]. Species of wild pistachio have special traits such as resistance to tubers and root nematode and tolerance to drought condition. It can be used as rootstock for the commercial varieties of pistachio (*Pistacia vera* L.) [2]. In addition, wild pistachio is a suitable plant to create green space, forest cover, turpentine production and different industrial uses.

Biological effects including anti-atherogenic, hypoglycemic, anti-inflammatory, antipyretic, antifungal, antimicrobial, anti-viral, insecticide and anticancer activities for *Pistacia* species has been established [3]. Plant cell and tissue culture has been used as a strategy to improve tree through somaclonal variation, germplasm conservation, and genetic transformation for production of secondary metabolites. Most research has done so far on this genus was focused on the species *P. vera* and less emphasis has been placed on other important wild species. However, most works on *P. atlantica* were focused on gum biological and antioxidant activities [3]. Regeneration of *P. vera* L. from seed or seedling tissues has been the most successful in vitro method to date [2, 4-6]. An efficient protocol for inducing direct shoot organogenesis from mature leaf explants of *P. vera* L. and the subsequent regeneration of plantlets has been

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reported [7]. The effects of the mineral composition, agar and sucrose concentration of the culture medium, on in vitro development of isolated embryos of *P. vera* L. has been investigated [8]. There have also been several previous reports on plant regeneration from seedling explants of other wild pistachio species [2,9]. Embryogenic mass was produced from kernels of mature fruits of *P. atlantica* cultured in liquid Murashige and Skoog (MS) media, supplemented with 100 mg/l casein hydrolysate, 100 mg/l l-ascorbic acid, and benzylaminopurine (BAP) [2] and mature somatic embryos germinated on the maturation medium without growth regulators and developed into plantlets.

Phytochemical analysis is essential to make good use of any medicinal plant. The essential oils of *Pistacia atlantica* Desf. subsp. *mutica* Rech. f. were analyzed by capillary GC and GC/MS and results showed that three major constituents of female leave were a-pinene, Δ-cadinene and myrcene, and for male leave were a –pinene, Δ-cadinene and ß-pinene and for fruit were a-pinene , ß-pinene, myrcene, and camphene[10]. However, none of the published papers proposed a protocol for callus induction and in vitro production of active compounds from callus.

The present study describes the procedure for the callus induction of *P. atlantica* subsp. *kurdica* using different growth regulators. Standard protocol for inflorescence axis culture has been reported and chemical composition of callus and leaves extract have been compared using GC-MS.

Material and Methods

Explant and media

Inflorescence axis of wild female pistachio tree (*P. atlantica* subsp. *kurdica*) collected in the middle of March in Kurdistan province, Iran. Explants were washed thoroughly under tap water for 30 min, and then immersed in 70% ethanol for 30-40 seconds. The explants were further sterilized with 1% calcium hypochlorite for 15 minutes and were washed three times (5, 10 & 15 minutes) with autoclaved distilled water to remove all the traces of calcium hypochlorite. The inflorescence axis segments (1.0–1.5 cm) were excised aseptically and transferred to sterile agar-solidified medium (20 ml) in Petri dishes. The culture medium was Woody Plant Medium (WPM) basal salts [11], 3% (w/v) sucrose, 0.6% (w/v) agar supplemented with different plant growth regulators (PGRs); pH (5.8) was adjusted with 1 N NaOH before autoclaving at 121 °C for 15 min. 150mg/l acid citric was added before pH adjustment. All cultures were incubated at 23 ± 2 °C, 16 h photoperiod with a photon flux density of 35 µmol m− 2 s− 1and 50–60% relative humidity.

In first set of experiments, explants were placed on WPM containing three concentrations of TDZ combined with 1mg/l of different auxins (2, 4-D), (NAA) and (IBA).

In second set of experiments, explants were cultured on WPM containing three concentrations of BA combined with 1 mg/l of different auxins (2, 4-D, NAA, IBA). Explants were incubated in darkness at 23 ± 2 °C for two weeks. Calli started initiating after two weeks and data were collected during six weeks. Fresh and dry weight of callus mass and percentage of callus induction were measured. To measure dry weight, calli were wrapped in aluminum foil and kept at 75° C for 48 hours.

Statistical analysis of data in the form of factorial in a complete randomized design in five replications was done. Comparison of means was done with LSD test at 5% level of significance ($P < 0.05$) using SAS software. Graphs were plotted with Excel software.

Quantification of total anthocyanin

Quantification of total anthocyanin of callus was done according to the method described by Wrolstad *et al*. [12] on a UV-Vis spectrophotometer (JENWAY-6505., U.K) using buffer systems, i.e. potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer , pH 4.5 (0.4 M). A 500 mg of fresh callus was macerated with 80% methanol in a pre-chilled pestle and mortar. Then, sample was transferred to a vial and centrifuged at 10,000 rpm for 15 min on a refrigerated centrifuge. Final volume (25 ml) was prepared by adding 80% methanol to the supernatant. Final samples were prepared by taking 5 ml volume of sample and diluting separately with pH 1.0 and pH 4.5 buffers (5 ml each). Absorbance was measured at 520 and 700 nm using 1-cm-path-length cuvettes in a UV-Vis spectrophotometer. Anthocyanin content was calculated and expressed as cyanidin-3-glucoside mg/ kg-1 fresh weight (FW), using extinction coefficient (ε) of 29,600 and molecular weight of 449.2. The final concentration of anthocyanin was calculated based on total volume of the extract and weight of sample using.

Absorbance (A) = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5
Total anthocyanin (mg kg-1) = A*MW*DF*vol made up /έ*weight of sample×1000

Extraction of phenolic compounds

Folin Ciocalteu reagent from Merck (Darmstadt, Germany) was used and a standard calibration curve was prepared using different concentrations of Gallic acid in ethanol (0.05, 0.1, 0.25, 0.5 mg/ml). Callus extracts were prepared in acetone: distilled water: acetic acid (70: 29.5: 0.5 mL/100ml) by mortar and pestle. After centrifugation (3000 rpm for 15 min at 4 °C, Herolab, HiCen 21C, Germany), the supernatant was collected. A diluted extract of each plant extract and standards was mixed with Folin Ciocalteu reagent (0.5:1.0 ml) and aqueous Na2CO3 (10 ml, 1M). The mixture was allowed to stand for 120 min and the absorbance was measured by colorimetric at 750 nm. For each sample, three replicate assays were performed. The total phenolic content was calculated as Gallic acid equivalent (GAE) by the following equation: T=C*V/M. T is the total phenolic content in mg/g of the extracts as GAE, C is the concentration of Gallic acid established from the calibration curve in mg/ml, V is the volume of the extract solution in ml and M is the weight of the extract in g.

Preparation of plant materials and extraction for callus

The in vitro plant materials were removed from their respective culture medium and washed to remove any traces of culture medium before they were air dried at 25 ± 2 ˚C until constant weight was obtained. They were then macerated to powder form with a mortar and pestle. The determination of active compound was carried out according to the method described by Leng *et al*. [13] with slight modification. One gram (1.0 g) of the macerated plant materials were then soaked with 20 ml methanol (MeOH) and allowing the homogenate to stand for 3 days. This extraction procedure was repeated three times. The combined supernatant was concentrated to 10 ml below 40 °C and 300 mbar by using a rotary evaporator (Heidolph HEI-VAP Advantage Vacuum rotary Evaporator). The solution was transferred into separatory funnel and extracted three times with 20 ml of n-hexane. After the bottom layer of n-hexane extract was collected from the funnel, they were concentrated to dryness below 40˚C. Just before injection of samples into GC-MS column, the dried extracts were eluted with 1 n-hexane: 1 diethyl ether. Since, external standard was not employed here; all the chromatograms and mass spectra of the samples were then matched with Mass Spectrometer (HP5975 MSD, EI made in USA) library for authenticity.

Preparation of plant materials and extraction for leaf and seed

The leaves and seeds of *P. atlantica* subsp. *kurdica* were cleaned under shade condition to prevent hydrolysisof the materials and to keep the natural color of the sample. Samples were dried under room temperature condition and were powdered and kept at 4 ˚C. 100 g of milled leaves and seeds separately hydro-distilled with 30% (v/v) methanol at 100˚C in a Clevenger apparatus. The essence collected and dried over anhydrous sodium sulfate (Na2SO4) stored in brown bottles and kept in refrigerator for further GC-MS analysis.

Table 1 The effect of different TDZ concentrations with auxins NAA, IBA and 2, 4-D (each 1 mg/l) on callus induction of inflorescence axis of *Pistacia atlantica* subsp. *Kurdica*

Concentrations of TDZ (mg/L)	Auxins (1 mg/L)	Percentage of callus* induction
1	NAA	65
1	2,4-D	40
1	IBA	75
2	NAA	70
2	2,4-D	40
2	IBA	45
3	NAA	40
3	2,4-D	35
3	IBA	30

* Each experimental treatment had 5 replicates containing 5 explants.

Results

Callus induction

Leaf and inflorescence axis explants were compared for callus induction. Leaf explants could not produce good quality callus and became dark-brown and finally died after a while (data not shown). However, inflorescence axis explants were observed to start calling between 2 to 3 weeks (Fig. 1a). The frequency of callus initiation from inflorescence axis explants is presented in Table 1

and 2. The maximum frequency of callus initiation was 100% in medium containing 1 mg/L BA and 1 mg/L NAA. Mediums containing low levels of BA and TDZ showed highest percentage of callus initiation(Table 1, 2). With increasing the BA and TDZ level the frequency of callus initiation were decreased. However among different auxin types the highest percentage of callus initiation was in media contained NAA. Also results showed that frequency of callus formation were higher in cytokenin BA compared to that of TDZ. Analysis of variance showed that the concentrations of BA in the medium had a significant effect on callus fresh and dry weight in inflorescence axis culture.

Table 2 The effect of different 6-BA concentrations with auxins NAA, IBA and 2, 4-D (each 1 mg/l) on callus induction of inflorescence axis of *Pistacia atlantica* subsp. *Kurdica*

Concentrations of BA(mg/L)	Auxins (1 mg/L)	Percentage of callus induction*
1	NAA	100
1	2,4-D	65
1	IBA	75
2	NAA	70
2	2,4-D	30
2	IBA	40
3	NAA	50
3	2,4-D	45
3	IBA	30

*Each experimental treatment had 5 replicates containing 5 explants.

Also different auxin i.e: 2, 4-D, IBA and NAA had a significant effect on callus fresh and dry weight in inflorescence axis culture, respectively ($F = 0.127$ $P < 0.01$; $F = .0023$ $P < 0.05$) ($F = 0.238$ $P < 0.01$; $F = .0054$ $P < .01$). BA concentration and auxin type interaction for both callus fresh and dry weight was not significant ($P>0.05$).

Medium with lower concentration of BA (1.0 mg/L) resulted significantly highest amount of callus fresh weight (Fig. 2a) and similar result was seen in callus dry weight (Fig. 2b). NAA at lower concentration (0.5 mg/L) showed yellowish brown, slow growing and hard callus. NAA (0.5 to 1.0 mg/L) induced green, hard and fast growing callus that significantly had more fresh and dry weight compared to IBA and 2, 4-D (Fig. 1c,d).

within medium containing cytokinin TDZ, NAA (1.0 mg/L) and IBA (1.0 mg/L) was found to be the best auxin for producing notable fresh callus (Fig. 3c).

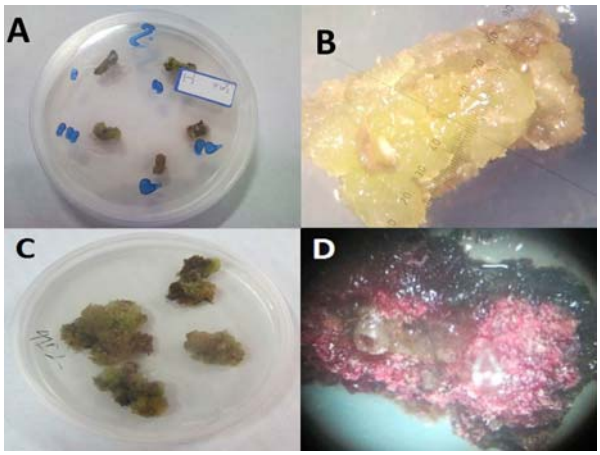


Fig. 1 Callus induction on inflorescence axis explants in *P. atlantica* subsp. *kurdica*. A) Primary stage of culture; B, C) Initiation of callus from inflorescence axis explants; D) Purple pigmented callus

However, NAA (1.0 mg/L) produced more dry weight and there was no significant difference between IBA and 2, 4-D. (Fig. 3d). With increased concentrations of TDZ (2.0 to 3.0 mg/L), the amount of fresh weight of callus significantly decreased (Fig. 3a). Lower concentration of TDZ (1.0 mg/L) produced the highest amount of dry weight (Fig. 3b).

Total anthocyanin and phenol compound

Red pigment was seen in the surface cells of the undifferentiated, friable mass forming callus (Fig.1d). The absorbance readings of the anthocyanin content of callus were repeated three times, while the average readings were taken at the wavelength of 520 nm and 700 nm and results are averages of three replicates. Based on the pH differential method the anthocyanin content for the callus of *P. atlantica* subsp. *kurdica* was 79.42 ± 4.63 mg cyanidin-3-glucoside /g fresh weights (Table 3). Anthocyanin content of the immature fruit was found to be less than the callus of *P. atlantica* subsp. *kurdica* (61.71 ± 3.81).

The standard curve for gallic acid was plotted, while the average absorbance readings of the total phenolic contents of callus were taken at the wavelength of 750 nm (Table 3). Based on the standard curve, the total phenolic content of the callus of *P. atlantica* subsp. *kurdica* was 4.91 ± 0.492mg Gallic acid/g dry weights it was 5.1 ± 0.780 in immature fruits.

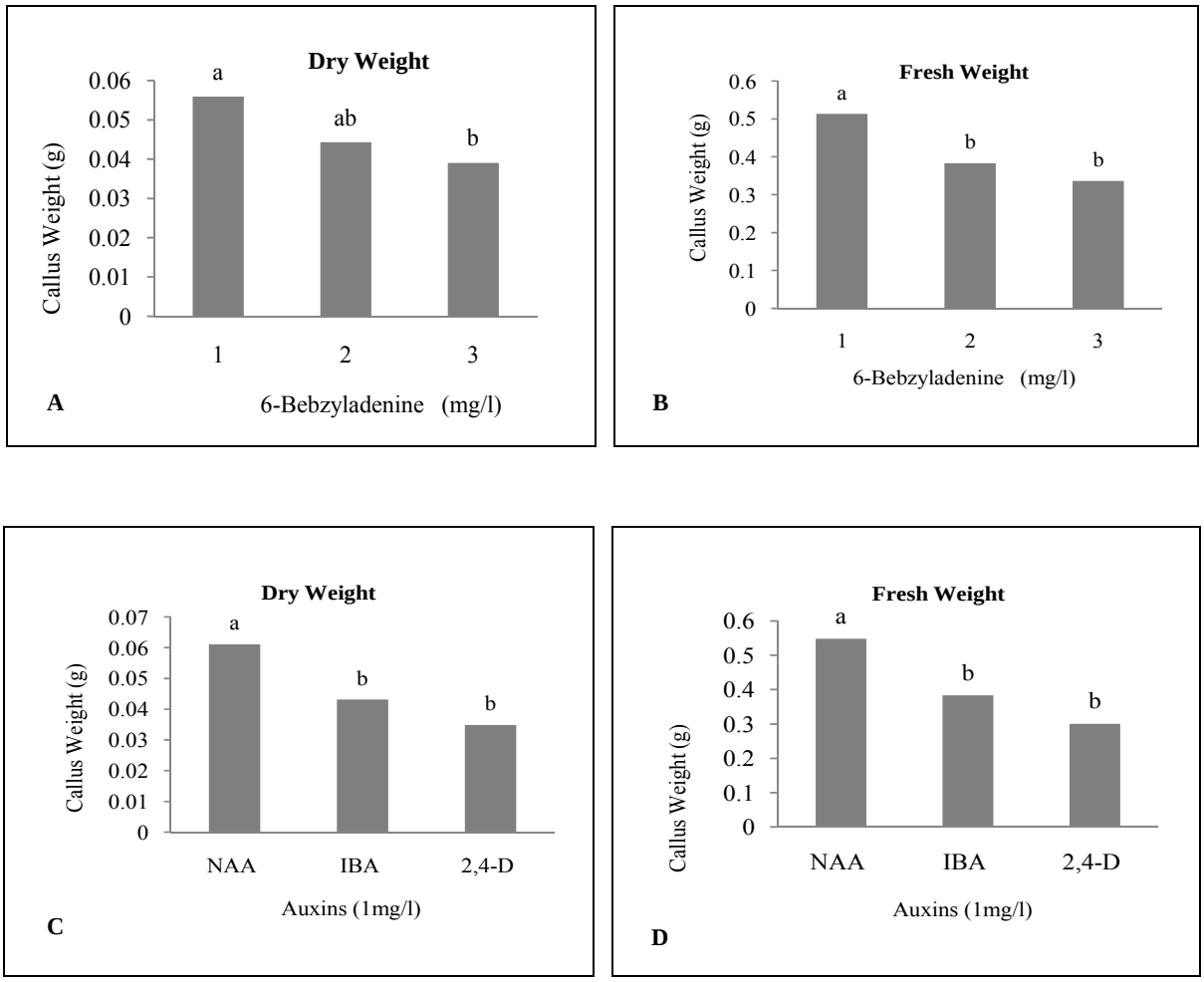
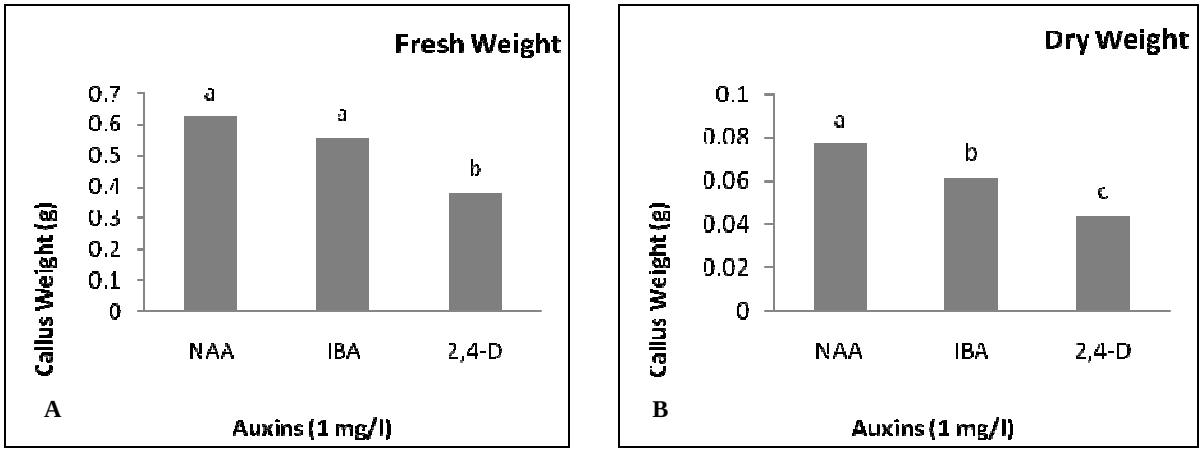


Fig. 2 The effect of different 6-BA concentrations with auxins NAA, IBA and 2, 4-D (each 1 mg/l) on callus weight of inflorescence axis of *Pistacia atlantica* subsp. *kurdica*; A) 6-BA concentrations on dry weight of callus. B) 6-BA concentrations on fresh weight of callus. C) Auxins NAA, IBA and 2,4-D (each 1 mg/l) on dry weight of callus. D) Auxins NAA, IBA and 2, 4-D (each 1 mg/l on fresh weight of callus. The similar letters on charts showed non-significant difference between treatments. Whereas dissimilar letters showed significant difference between treatments ($p<0.05$).



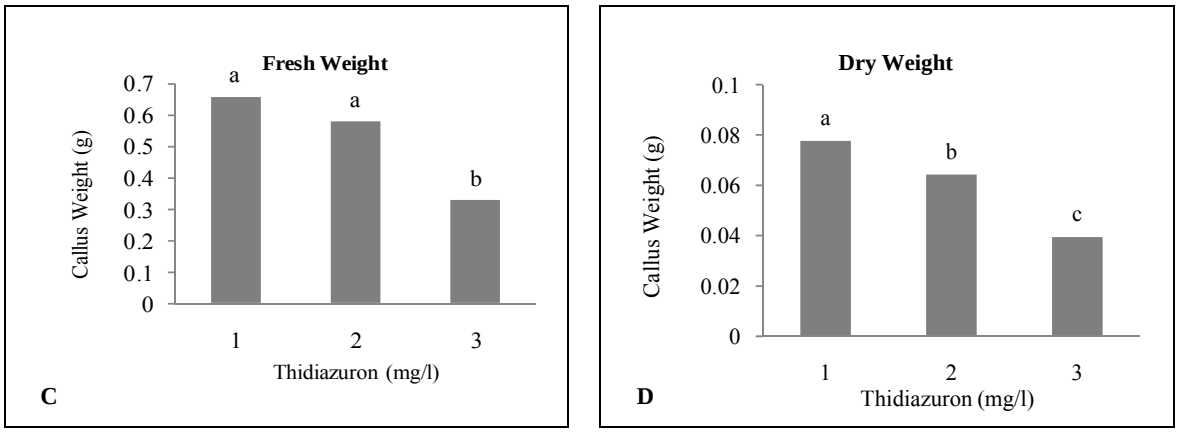


Fig. 3 The effect of different TDZ concentrations with auxins NAA, IBA and 2, 4-D (each 1 mg/l) on callus weight of inflorescence axis of *Pistacia atlantica* subsp. *kurdica* ; A) Comparison of auxins NAA, IBA and 2,4-D (each 1 mg/l) on fresh weight of callus. B) Comparison of uxins NAA, IBA and 2, 4-D (each 1 mg/l) on dry weight of callus. C) Comparison of TDZ concentrations on fresh weight of callus. D) Comparison of TDZ concentrations on dry weight of callus. The similar letters on charts showed non-significant difference between treatments. Whereas dissimilar letters showed significant difference between treatments ($p<0.05$).

Table 3 Total phenol (mg.g-1 dry weight) and total anthocyanin (mg.kg-1 fresh weight) of callus and immature

Tissue	Anthocyanin(mg.kg^{-1} FW)	Total Phenol(mg.g^{-1} DW)
Callus(Inflorescence axis)	79.42 ± 4.63	4.91 ± 0.492
Immature fruit	61.71 ± 3.81	5.1 ± 0.780

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil was extracted by the hydrodistillation from the leaves and dried callus, and the constituents were analyzed by GC/MS. The chemical constituent of the essential oil, retention time index (Rt) and quantitative percentage of the compounds from leaves and dried callus are presented in Table 4 and 5. A total of 13 compound being identified in the essential oil of leaves with total percentage of 81.38. The combinations of alpha Pinene (15.63%), gamma elemene (33.84%), caryophyllene (9.26%) with 58.73% in total were the main constitute of the essential oil. Other compounds such as Camphene, Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene, Beta Pinene, Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-, 3-Hexen-1-ol benzoate, Spathulenol, Globulol, Ledol, Phytol and Oleic acid with total percentage of 22.68, ranging from 1.09 to 3.51 percent of essential oil. The eight compounds being identified in the essential oil from calli induced on inflorescence axis. The main components were alpha pinene (89.19%), camphene (1.09%) and Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene (3.40%) comprising 93.68% of essential oil. other compounds such as caryophyllene, gamma elemene, spathulenol, globulol and bornyl acetate representing1.02% of the essential oil and ranging from 0.12 to 0.33 percent.

Discussion

We reported here high frequency callus induction of *P. atlantica* subsp. *kurdica* from inflorescence axis explants. Callus induction is not well developed for *Pistacia* genus. Tissue browning, frequently observed in callus derived from mature explants of some woody plants [14]. Tissue browning is caused by phenolic oxidation which is very often in cultures of many tree species [14]. The products of phenolic oxidation are toxic to callus tissue and cause tissue browning 12–24 hours after each passage of explants [15]. For *P. atlantica* subsp. *kurdica* the leaf explants became dark after callus induction and callus did not grow. We used different explants and it appeared that inflorescence axis is a good source for callus induction.

Table 4 Components identified in the essential oil of the leaves of *Pistacia atlantica* subsp. *kurdica*

Compound No.	Compound Name	Retention Time (Rt)	Percentage (%)
1	Alpha Pinene	7.30	15.63
2	Camphene	7.76	1.77
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	8.70	2.15
4	Beta Pinene	8.66	2.31
5	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	20.40	3.51
6	Caryophyllene	25.47	9.26
7	Gamma.-Elemene	28.20	33.84
8	3-Hexen-1-ol, benzoate	30.58	1.34
9	Spathulenol	30.87	3.28
10	Globulol	31.08	2.44
11	Ledol	31.33	1.96
12	Phytol	46.39	2.80
13	Oleic Acid	47.01	1.09

Table 5 Components identified in the essential oil of the callus inflorescence axis of *Pistacia atlantica* subsp. *kurdica*

Compound No.	Compound Name	Retention Time (Rt)	Percentage (%)
1	Alpha Pinene	7.37	89.19
2	Camphene	7.91	1.09
3	Bicyclo[3.1.1]heptane, 6,6-Dimethyl-2-methylene	8.75	3.40
4	Caryophyllene	25.40	0.13
5	Gamma.-Elemene	28.13	0.33
6	Spathulenol	30.85	0.12
7	Globulol	31.07	0.12
8	Bornyl acetate	20.45	0.32

Concentrations and type of auxin and cytokinin play an important role in callus induction. In this work we checked the effect of different cytokinins and auxin concentration on callus induction from immature inflorescence axis. WPM medium supplemented with different concentrations of plant growth regulators TDZ, BA, 2, 4-D, IBA and NAA used for callus induction. Results indicated that all combinations of growth regulators were able to induce callus from inflorescence axis explants, While the rate of callus induction and callus fresh and dry weights were significantly different.

WPM medium supplemented with 1mg/l BA and NAA produced the highest fresh and dry weight of the callus. The highest callus induction was obtained in the presence of NAA with 2 mg/l TDZ or l BA with 2 mg/l TDZ. Similar results reported in *P. vera* [16]. Tilkat *et al*., [16] showed that the media with 1 and 2 mg/l TDZ combined with 1mg/l NAA and IAA as well as 1 and 2 mg/l BA produced callus from mature leaf explants of *Pistacia vera*. In another work different IAA/BAP ratio were used and results showed that the media having a, 1.0:4.0, IAA/BAP combinations induced callusing tissues at high percentage from leaflet with petioles of *P. vera* [16]. Cotyledonary explants of *P. vera* in the presence of 2.0 mg/l each of 2, 4-D and Kin and 3% sucrose produced maximum amount of callus with desired characteristics [17]. Embryogenic mass from kernels of mature fruits of *P. atlantica* subsp. *kurdica* cultured in liquid MS medium supplemented with 0.5-4 mg/l BAP was differentiated directly from the explants after 3 weeks [2]. Using the same medium, they produced somatic embryos after a few subcultures.

In vitro anthocyanin production is well documented in many plants [18]. Callus initiated from inflorescence axis explants of *P. atlantica* subsp. *kurdica* was a purple-red-colored. There was no report on anthocynin in this species and we measured the anthocaynin content of callus and compared to that of its fruit. Cell culture and callus tissues of a large number of plants accumulate anthocyanin in vitro [19, 20, 21, 22, 23, 24, and 25]. The amount of anthocyanin was relatively high in the callus of this species without application of any stimuli. Higher anthocyanin in in vitro condition have been reported for other plants such as Vitis, Ipomaea, Glehinia, and Vaccinum [10, 26, and 27]. Anthocyanin accumulation in other plants were enhanced by high sucrose concentration [28, 29], low NH4+ nitrogen concentration, [30, 31] and high osmotic potential in the culture medium [32].

Therefore it is possible to enhance anthocyanin induction in this plant by different treatments.

The total phenolic content for the fruit of *P. atlantica* subsp. *kurdica* was not significantly different from the callus culture. The phenolic contents of the tuber of I. batatas were higher than those of the callus [33]. A very different amount of total polyphenol in unripe, ripe and dried ripe samples of *Pistacia vera* L. using acidic methanol extract was reported [34]. The total phenolic content in *P. vera* were very higher than that of our study and showed that the main constituent of total polyphenol was anthocyanin. In the Folin Ciocalteu method different solvent such as methanol, acetone, ethanol and boiling water could be used to extract phenolic compounds from plants tissues [35] and extraction with different solvents affect yield of total polyphenol content [36].

Results in our study show the composition of the essential oils of *P. atlantica* subsp. *kurdica* in the order of retention time. The major constituent of *Pistacia Lentiscus* (L.) Moench [37], *P. atlantica* subsp. *kurdica* [38], *Pistacia mutica* Fisch. & C.A.Mey. [39] and *P. vera* [40] was α-pinene, ranging from 21.70% to 78.90% for *P. lentiscus*, 25.25% for *P. mutica*, 97.20% for *P. atlantica* subsp. *kurdica* and 75.62% for *P. vera*. In our study α-pinene was the major compound in both callus and leaves. ß-Pinene also has been reported in all analytical data from the literature [38 and 40] and from our results in leaf extract, ranging from 1.26 to 38.70 percent. Camphene is present in all reported data, ranging from 0.41 to 6.23 [38, 39, and 40]. Caryophyllene is reported for *P. lentiscus* [36] as 2.04%. Gamma Elemene was present in our study (0.33% for callus and 33.84% for leaf) and reported in fruit of *P. mutica* (0.21%) [39]. The monoterpene alpha-pinene was found to be present in the callus cultures of floral axis Smyrnium perfoliatum [41]. α-pinene, β-pinene, 1,8-cineole, and camphor in the callus of Rosmarinus officinalis L. were reported [42]. The high accumulation level of α-pinene in the callus of inflorescence axis of *P. atlantica* subsp. *kurdica* was interesting. Chemical profiles of the callus extract were apparently more similar to that of leaves; however, some compounds were detected in callus which were different from leaves.

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