Determination of polyphenolic constituents and biological activities of bark extracts from different Pinus species

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Abstract

BACKGROUND: The most common commercially available pine bark extract is Pycnogenol, which has been reported to have cardiovascular benefits and enhance microcirculation. The present study was conducted to determine the chemical composition of four pine bark extracts, assess their biological activities and to compare the results with Pycnogenol.

RESULTS: The Pinus species were analysed by LC and LC-MS; extracts of P. brutia and P. nigra showed higher levels of phenolic components compared to P. sylvestris and P. pinea. In particular, P. brutia contained extremely high concentrations of taxifolin (18.5%). The highest radical scavenging activities were attained with P. pinea (88.6%), P. nigra (87.2%) and P. brutia (86.4%) bark extracts. Additionally, anticarcinogenic effects of the extracts and their kinetics were determined in four cell lines including human prostate (PC-3, DU 145, LNCaP) and breast adenocarcinoma (MCF7) by the MTT assay. Cell viability was reduced to 40% by extracts of P. pinea, and P. sylvestris in PC-3 cells showing a similar effect like the positive control, CPT-11.

CONCLUSION: Pinus species other than P. maritima definitively possess high biological activities, and therefore present a huge potential to be utilised in the food and the pharmaceutical industries.

INTRODUCTION

Recently, there has been increasing interest in discovering new natural antioxidant agents. Many naturally occurring compounds found in plants, herbs and spices have been shown to possess these properties, including extracts prepared from pine bark. Although the use of pine bark dates back to ancient times, it has recently found wide application in the fields of nutrition, health and medicine. Pine bark extracts contain numerous phenolic compounds such as catechin, epicatechin, taxifolin and phenolic acids. The structures of catechin and taxifolin are shown in Fig. 1. They have received considerable attention because of their antimutagenic, anticarcinogenic and high antioxidant activities. The most commonly commercially available pine bark extract is Pycnogenol, a standardised extract of French maritime pine bark (Pinus maritima), which is probably the most studied phenolic tree extract containing proanthocyanidins. Pycnogenol has been reported to have cardiovascular benefits, the ability to enhance microcirculation by increasing capillary permeability, strong free radical scavenging activity against reactive oxygen and nitrogen species, the potential to regenerate the ascorbyl radical and to protect endogenous vitamin E and glutathione from oxidative stress. Pycnogenol also accelerates wound healing processes and is a potent active ingredient for the treatment of minor injuries.

Although French maritime pine bark has been intensely investigated in the past, there is comparably less information available in the literature in regard to other pine bark species. In vitro antioxidant activities of a commercial Pinus radiata bark extract and fractions of ethanolic extracts of Pinus radiata have been studied. Another project focused on a commercial Pinus sylvestris bark extract, which was analysed by a modified lipid peroxidation inhibition assay. Additionally, antioxidant efficacies of six pine species (P. pinea, P. brutia, P. radiata, P. halepensis, P. attenuata, P. nigra) were also studied.

The aim of this research study was to develop liquid chromatography–mass spectroscopy (LC/MS) method to determine the chemical composition of four pine species commonly found in Turkey (P. brutia, P. sylvestris, P. nigra and P. pinea). By determining their radical scavenging abilities and cytotoxic activities using in vitro assays, the relationship between observed biological properties and the phenolic content of the extracts could be evaluated. Despite increasing interest in naturally occurring...
antioxidants and anti-cancer agents this correlation has not been studied previously, to the best of our knowledge.

MATERIALS AND METHODS

Plant material

Pine bark specimens were collected from four different locations in Turkey: P. nigra from Bursa (40° 29′ 44.7″ N, 29° 08′ 15.6″ E, altitude, 570 m); P. pinea from Aydın-Cine (37° 32′ 30.1″ N, 28° 08′ 35.6″ E, altitude, 520 m); P. brutia from İzmir-Delioemer (38° 10′ 17.0″ N, 27° 03′ 46.7″ E, altitude, 120 m); and P. sylvestris from Eskişehir-Musaozu (39° 41′ 46.2″ N, 30° 19′ 22.4″ E, altitude, 920 m). The samples were collected between June and August 2006. The specimens were dried at room temperature, ground by using a conventional grinder and stored at +4 °C.

Cell culture

Four cell lines including PC-3 (human prostate adenocarcinoma) and MCF7 (human breast adenocarcinoma), DU 145 (human prostate carcinoma), LNCaP (human prostate carcinoma) were obtained from the Foot-and-Mouth Disease Institute Cell Culture Collection (HUKUK cell bank) Ankara, Turkey. Cells were cultured in RPMI1640 (Gibco, Grand Island, NY, USA) or DMEM/Ham’s F-12 medium (Gibco) supplemented with 10% fetal bovine serum (Sigma, St Louis, MO, USA), 100 U/100 ml penicillin and streptomycin (Biochrom AG, Berlin, Germany). MTT reagent (tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) was also purchased from Sigma. Cells were grown in humidified atmosphere with 5% CO2 at 37 °C.

Preparation of pine bark extracts

Pine bark extract was obtained by the method developed by Masquelier.8 Pine bark (100 g) was ground for 1 min, at a speed setting of 2 using a mixer (Waring, Torrington, CT, USA) to obtain coarse powder, extracted with 600 mL of boiling water, and then cooled down to 20 °C. After filtration, 250 mL of liquid were collected and sodium chloride was added up to saturation and the precipitate formed was removed by filtration. Subsequently, the filtrate was extracted three times with ethyl acetate (10 mL filtrate per 1 mL ethyl acetate (v/v)). The ethyl acetate phase was collected and dried using anhydrous sodium sulfate and reduced to 1/5 of its volume in a rotary vacuum evaporator. The extract was then poured into three volumes of chloroform, while stirring mechanically. The proanthocyadins were precipitated and collected by filtration. The light beige colour powder obtained was stored at −20 °C. All chemicals were of analytical grade purity.

HPLC analysis

Sample preparation

Sample solutions were prepared by dissolving the above described extracts in 50% aqueous methanol (Merck, Darmstadt, Germany) at a concentration of 5.00 mg mL−1 in a Sonorex sonicator (Bandelin, Berlin, Germany). In case turbid solutions were obtained, they were filtered through a 0.45 μm cellulose acetate membrane filter (Phenex; Phenomenex, Torrance, CA, USA) prior to injection. For quantification experiments the same solutions were used except for P. brutia. For this sample the initial solution was diluted 1:1 with 50% aqueous methanol prior to analysis.

LC and LC−MS conditions

An HPLC method was developed in-house for the analysis of pine bark extracts. After screening different stationary and mobile phases, and evaluating the impact of temperature on the separation, optimum results were obtained using a Synergi MAX-RP column (150 × 4.6 mm, 4 μm particle size; Phenomenex). The mobile phase comprised 0.2% formic acid (Merck) in water (A) and 0.2% formic acid in acetonitrile (Merck) (B). Gradient elution was performed starting with 95A/5B, changing the composition to 90A/10B in 10 min, followed by 70A/30B in another 20 min. Each run was ended by a washing step with 10A/90B (5 min) and an equilibration period of 10 min. Detection wavelength, flow rate and column temperature were set to 280 nm, 1 mL min−1 and 30 °C, respectively. For all solutions (samples, standards) 10 μL were injected.

All LC experiments were performed on a LaChrom Elite HPLC system (Merck-Hitachi, Tokyo, Japan), equipped with L-2200 autosampler, L-2100 quaternary pump, L-2300 column oven and L-2455 DAD-detector. For LC−MS experiments an HP 1100 from Agilent (Waldbronn, Germany) was used, linked to an Esquire 3000 plus ion-tap mass spectrometer from Bruker-Daltronics (Bremen, Germany). The latter was set to 40 psi (nebuliser), 10 L min−1 (dry gas) and a dry temperature of 300 °C. Ionisation was performed in alternating ESI mode, with a split ratio of 1:4; LC conditions were the same as described above.

Calibration

Calibration curves were established by dissolving 5.00 mg of (+)-catechin (Sigma) and taxifolin (Fluka, Steinheim, Russian Fed) in 5.00 mL methanol, and serially diluting this stock solution with methanol. Within the concentration range injected (1000.0 to
0.3 μg mL\(^{-1}\) the detector response was linear (\(R^2 \geq 0.9996\)), with a detection limit of less than 0.05 μg mL\(^{-1}\) (data not shown in detail).

**Antioxidant assay**

DPPH radical scavenging activity (RSA) assay was carried out to determine the activities of the extracts. The reagent is reduced by the antioxidant (AH) and serves as an indicator for the reaction DPPH\(^+\) + AH → DPPH\(^-\) + A\(^+\). The extracts were dissolved in 4 mL of methanol (final concentration of 250 μg mL\(^{-1}\)) and then added to 1 mmol L\(^{-1}\) methanolic solution of DPPH\(^-\) (Sigma). The contents were stirred vigorously for 15 s and then left to stand at room temperature for 30 min. The decrease in colourisation was measured spectrophotometrically at 517 nm using a Unicam Helios-alpha spectrophotometer (Cambridge, England). The radical scavenging activity (RSA) was calculated using the equation %RSA = 100 × [1 – (AE/A0)], where AE is the absorbance of the solution containing antioxidant extract and A0 is the absorbance of the DPPH\(^-\) solution.

**Cytotoxicity assay**

Cytotoxic effects of the samples and the standards such as taxifolin, (+)-catechin and epicatechin (Sigma) were analysed by the MTT assay which is based on the cellular reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemicals) to a blue formazan product by mitochondrial dehydrogenases of viable cells. Cell proliferation was determined by adding 0.5 mg mL\(^{-1}\) per well, prepared as a sterile stock-solution of 5 mg mL\(^{-1}\) in Dulbecco’s phosphate-buffered saline (Gibco), diluted 1:10 with medium prior to use. Medium was removed 4 h later and blue formazan crystals solubilised in 200 μL 100% dimethylsulfoxide (DMSO) per well. The amounts of blue formazan product were quantified based on the calibration curves of (+)-catechin and taxifolin (two isomers) but also catechin dimers and trimers as well as ferulic acid esters of catechin. The samples were analysed in positive and negative ionisation mode, but the results obtained in negative mode were more prominent. All of the components provided characteristic signals for [M-H]\(^-\) and [2M-H]\(^-\) ions. Apart from monomers, nine additional peaks were tentatively identified based on their UV and MS data.

The results for the two marker compounds (solutions of 0.00 mg mL\(^{-1}\)) were assayed, except for P. brutia revealed enormous variations within the species investigated (Table 1). Taxifolin varied from 0.3% in P. nigra to 18.6% in P. brutia. Sarikaki and co-workers\(^2\) analysed pine bark extract from P. maritima by HPLC and stated that main substances identified were taxifolin, catechin and ferulic acid. Romani and co-workers\(^2\) analysed the polyphenol composition of a commercial pine bark extract from P. maritima. The amount of taxifolin was reported to be 33.1 mg g\(^{-1}\) in this species, whereas our results showed a 5.6 fold higher value (186 mg g\(^{-1}\)) in a P. brutia extract. This indicates that P. brutia is an excellent source of taxifolin. In a previous study carried out by Jerez et al.,\(^5\) the total amount of procyanidins was reported as 38 mg g\(^{-1}\) extract and 60 mg g\(^{-1}\) extract for P. pinaster and P. radiata, respectively. Total amounts of just two marker compounds in our extracts were found to be 256 mg g\(^{-1}\) for P. brutia, 64.5 mg g\(^{-1}\) for P. pinea, 24.4 mg g\(^{-1}\) for P. sylvestris and 40.7 mg g\(^{-1}\) for P. nigra. When considering the amount of total procyanidins, highest yields were found in P. nigra and P. brutia (469.9 mg g\(^{-1}\) and 367.7 mg g\(^{-1}\)), which far exceed the reported values. It has to be noted that the dimer 1 (10.9 min) and ferulic acid ester of catechin (monomer ferulate 1, 20.7 min) were the most abundant procyanidin derivatives present in the bark extracts (71.9 mg g\(^{-1}\) for P. brutia, 98.8 mg g\(^{-1}\) for P. pinea, 99.3 mg g\(^{-1}\) for P. sylvestris and 269.4 mg g\(^{-1}\) for P. nigra).

As shown in Table 2, many of the dominant constituents were catechin derivatives (dimers at 10.9, 11.7 and 19.4 min; trimers at 13.9 and 14.6 min; ferulic acid ester of monomers at 20.7 and 21.1 min) and isomers of taxifolin (23.3 and 23.8 min). As no standard compounds for these constituents were available, they were quantified based on the calibration curves of (+)-catechin and taxifolin (for taxifolin isomers).

Previously, it has been stated that reversed-phase HPLC could achieve good separation of individual procyanidins from monomers to trimers compared to normal-phase HPLC.\(^10\) As already stated, we accomplished the separation of the aforementioned constituents on reversed-phase with much better

**RESULTS AND DISCUSSION**

**Variation of proanthocyadins**

Prior to studying the biological activities of different pine bark extracts all specimens were investigated for their chemical composition. For this purpose sample solutions were prepared in 50% aqueous methanol, and analysed by an in-house developed HPLC assay (Fig. 2). By using C-12 column material and an acidic mobile phase, all major constituents were well resolved within 30 min. The individual signals were assigned by co-chromatography with standards at hand and comparison of UV spectra. Based on these results, two compounds could be identified in the extracts [(+)-catechin and taxifolin]. In order to obtain quantitative results, the two unambiguously identified compounds were used to establish calibration curves; correlation coefficients higher than 0.9996 confirmed linearity of the detector signal in the tested range.

The HPLC method developed was then directly used for LC–ESI–MS studies, which enabled not only identification of (±)-catechin and taxifolin (two isomers) but also catechin dimers and trimers as well as ferulic acid esters of catechin. The samples were analysed in positive and negative ionisation mode, but the results obtained in negative mode were more prominent. All of the components provided characteristic signals for [M-H]\(^-\) and [2M-H]\(^-\) ions. Apart from monomers, nine additional peaks were tentatively identified based on their UV and MS data.

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Previously, it has been stated that reversed-phase HPLC could achieve good separation of individual procyanidins from monomers to trimers compared to normal-phase HPLC.\(^10\) As already stated, we accomplished the separation of the aforementioned constituents on reversed-phase with much better
Table 2. Identified components in pine bark extracts by LC–ESI–MS∗

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Expected mass (amu)</th>
<th>Observed mass</th>
<th>Proposed structure</th>
<th>Pinus brutia</th>
<th>Pinus pinet</th>
<th>Pinus sylvestris</th>
<th>Pinus nigra</th>
<th>Pycnogenol®</th>
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<tr>
<td>10.9</td>
<td>578</td>
<td>577 [M-H]−</td>
<td>Dimer 1</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>11.7</td>
<td>578</td>
<td>577 [M-H]−</td>
<td>Dimer 2</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>13.1</td>
<td>290</td>
<td>289 [M-H]−,</td>
<td>Catechin</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>13.9</td>
<td>866</td>
<td>865 [M-H]−</td>
<td>Trimer 1</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>14.6</td>
<td>866</td>
<td>865 [M-H]−</td>
<td>Trimer 2</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>18.1</td>
<td>592</td>
<td>591 [M-H]−</td>
<td>Methylated dimer</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
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<tr>
<td>19.4</td>
<td>578</td>
<td>577 [M-H]−</td>
<td>Dimer 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
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<tr>
<td>20.7</td>
<td>466</td>
<td>465 [M-H]−,</td>
<td>Monomer ferulate 1</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>21.1</td>
<td>466</td>
<td>465 [M-H]−</td>
<td>Monomer ferulate 2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>23.3</td>
<td>304</td>
<td>303 [M-H]−</td>
<td>Taxifolin 1</td>
<td>++++++++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23.8</td>
<td>304</td>
<td>303 [M-H]−</td>
<td>Taxifolin 2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
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</table>

∗Quantification of catechin derivatives and taxifolin in different Pinus species; assignment based on MS results, quantification based on calibration data of (+)-catechin (n = 3) and taxifolin (ND, not detected; − = 0; + ≤ 1%; ++ ≤ 4%; +++ ≤ 7%; ++++ ≤ 10%; ++++++ ≤ 15%; +++++++ ≤ 20%).

resolution (Fig. 2). Moreover, no broad or unresolved signals due to the interference with high molecular weight oligomers (tetramers and higher) were observed. Pycnogenol® has been reported to contain oligomeric proanthocyanidins with chain lengths between 2 and 12 monomeric units.11 The compounds identified in the present study were up to trimers only, a deviation which might be associated with the extraction method used. By observing that dimers eluted ahead of monomers and trimers, it was inferred that the elution order is not related to the degree of polymerisation. This conclusion is in accord with previously published data.10
Radical scavenging activities

Procyanidins are considered to be superior antioxidants because of the higher number of target sites for free radicals. Several studies were conducted which report excellent radical scavenger properties of Pycnogenol® extracts. In this study, samples were screened for their radical scavenging capacities by DPPH which is a stable free radical with a characteristic absorption at 517 nm. The highest radical scavenging activities were attained with P. pinea (88.6%), P. nigra (87.2%) and P. brutia (86.4%) bark extracts, followed by P. sylvestris (78.5%) whereas the commercial Pycnogenol® showed the lowest value (58.3%) (Fig. 3). Considering antioxidant activities, comparably lower radical scavenging activities can be explained in regards to the lower total proanthocyanidins identified for P. sylvestris and Pycnogenol® which were 211.2 and 12.8 mg, respectively.

Anti-cancer activities

Cytotoxic effects of the samples and the standards, together with a positive control (CPT-11), were analysed by the MTT assay. The cytotoxic effects in terms of cell death were determined by treating PC-3, MCF7, DU 145 and LNCaP cell lines with 10 µg mL⁻¹ doses of the samples for 24 and 48 h.

All investigated samples showed a similar inhibition profile in terms of cell viability in PC-3 cells. An increase in cell proliferation was observed at the end of 24 h treatment, followed by a decrease after 48 h. P. sylvestris bark extract showed the highest growth inhibition which in return revealed 141% to 38% cell viability after 48 h of treatments, similar values were obtained for P. pinea bark extract after 48 h with 39% (Fig. 4). On the other hand, cell viability values for P. nigra were 161% and 51% at 24 and 48 h, respectively. P. brutia (173%, 65%) and Pycnogenol® (127%, 94%) were less effective. Considering standard compounds, epicatechin caused the lowest cell viability with 115% and 21% after 24 and 48 h of treatments, followed by (+)-catechin with values of 110% and 33% whereas values obtained for taxifolin were higher (126% and 48%). Although low cell viabilities were attained for extracts of P. sylvestris, P. pinea and P. nigra at the end of 48 h at a concentration of 34.5 µmol L⁻¹, Rao and co-workers reported that pure phenolic compounds such as catechin, quercetin and dioxyflavanone showed IC₅₀ values at concentrations higher than 200 µmol L⁻¹. They also stated that epicatechin failed to reduce proliferation in PC-3 cells up to the concentration range of 200 µmol L⁻¹. Considering CPT-11, it should be noted that values obtained for the positive control were 81% and 36% for the respective treatments. These data suggest that extracts of P. sylvestris and P. pinea are much more potent in activation and inhibition of the growth of PC-3 in comparison to the rest of the extracts and Pycnogenol®.

P. sylvestris and P. brutia extracts revealed similar inhibition profiles in terms of cell viability against MCF7 cells showing a decrease from 88% to 83% and 112% to 97% for the respective extracts at the end of 24 and 48 h (Fig. 5). The remaining extracts, standard compounds and the positive control showed a similar trend but the differences were not significant. These results indicate that none of the tested samples had the ability to inhibit the growth of MCF7 cells.

All extracts showed a similar inhibition profile against DU 145 cells where a decrease was observed in the cell viability from 24 to 48 h except for Pycnogenol®. With the latter, cell viability remained at the same rate during the observed period. In contrary, standards and CPT-11 showed an increasing trend from 24 to 48 h (Fig. 6). But above all, none of the tested samples inhibited the growth of DU 145 cells significantly.

Although the extracts of P. sylvestris, P. brutia and P. nigra caused more pronounced inhibition of the growth of LNCaP cells after 48 h compared to 24 h, the overall activity of the extracts at a concentration of 10 µg mL⁻¹ was not significant. After 48 h, cell viability even increased for the remainder of the tested materials, including the positive control (Fig. 7). These results indicate that none of the samples had the potential to inhibit the growth of LNCaP cells at the tested concentrations.

Brusselmann and co-workers, investigating the induction of cancer cells by flavonoids, stated that inhibition of lipogenesis was associated with induction of apoptosis and reported lipogenesis values as 74.6% for taxifolin and 91.6% for catechin in LNCaP cells. These findings are in accordance with our results, where proliferative effects of taxifolin and catechin were observed against LNCaP, MCF7 and DU 145 cells, but on the other hand slight inhibitions were obtained with the extracts of P. sylvestris and P. brutia for MCF7, P. brutia for DU 145 and P. nigra for LNCaP. These observations might be related to synergistic effects of components in the extracts as suggested by Packer et al., stating that Pycnogenol® displayed higher biological activity than individual components found in the product.

CONCLUSION

Results presented here touch upon the fact that Pinus species other than P. maritima also possess remarkable biological activities
Figure 4. Growth inhibitory and cell death effects of the extracts, the standards and Pycnogenol® on PC-3 cells. To assess the effect of the samples on exponentially growing PC-3 cells, $6 \times 10^4$ cells mL$^{-1}$ were placed in 96-well plate and the next day the cells were treated either with ethanol as control or a $10 \mu$g mL$^{-1}$ dose of all the samples and compared with CPT-11 in complete medium. After 24 and 48 h of treatments, cytotoxic effects of samples were analysed by the MTT assay. The data were obtained from three independent assays using three wells for each assay. Cytotoxic effects and/or growth inhibition was calculated as % cell viability.

Figure 5. Growth inhibitory and cell death effects of the extracts, the standards and Pycnogenol® on MCF7 cells.

and, therefore, might be interesting alternatives for commercial applications. In particular, the bark of *P. brutia* can be an effective source of taxifolin as it contains considerably high amounts of this secondary metabolite. Extracts of *P. pinea*, *P. brutia* and *P. nigra* showed strong radical scavenging activities, also possessing high amounts of total phenolic compounds identified (280.0 mg g$^{-1}$, 367.7 mg g$^{-1}$, 469.9 mg g$^{-1}$ extract, respectively). *P. sylvestris* extract, containing significantly lower amounts of the identified compounds (211.2 mg g$^{-1}$), showed the highest growth inhibition in PC-3 cells. These findings suggest that some other compounds or a combination of them might be responsible for the actual inhibitory activity. Our group is currently performing further activity-guided studies to clarify this question. Additionally, it was observed that extracts revealed high radical scavenging activities especially at 24 h treatments in PC-3 cells but not in other cell lines tested. The activity represented might decrease oxygen radical levels by scavenging and this might have promoted the PC-3 and LNCaP cell proliferation at shorter periods of exposures. However, the 48 h exposures that neutralised radicals required as resources for the cell, did result in growth inhibitory function on cells which were eventually sensitive to ROS formation.

**ACKNOWLEDGEMENTS**

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Biological activities of pine bark extracts

Figure 6. Growth inhibitory and cell death effects of the extracts, the standards and Pycnogenol® on DU 145 cells.

Figure 7. Growth inhibitory and cell death effects of the extracts, the standards and Pycnogenol® on LNCaP cells.

REFERENCES


