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Paclitaxel and other Anticancer Taxoids were Found in Oak Leaves

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Abstract

Diterpene alkaloid paclitaxel (with the trade name Taxol) and other taxoids are well-known anticancer drugs originally extracted from different *Taxus* spp. (Gymnosperm). The significant demand for paclitaxel prompted researchers to investigate taxoids in alternative species. Finding taxans in certain angiosperms like *Corylus avellana* and some endophyte fungi of *Taxus* and non-*Taxus* species brought the hypothesis that genes involved in paclitaxel production may have horizontally been transferred from *Taxus* sp. to other plants in their vicinity, through the mediation of microorganisms. The common habitats of certain oak species, i.e., *Quercus brantii*, *Q. macranthera*, and *Q. castaneifolia* with taxane-bearing plants in conserved regions of Iranian forests, promoted us to investigate the presence of taxoids in these species. The existence of paclitaxel, 10-deacetyl baccatin III, baccatin III, 10-deacetyl paclitaxel, cephalomannine, 7-epi 10 deacetyl paclitaxel, and 7-epi paclitaxel in oak leaf extracts was shown by HPLC and their structure were confirmed through LC-MS analysis. The cytotoxic potential of crude extracts was tested against Human Embryonic Kidney (HEK 293T) and Hepatocellular carcinoma (HepG2) cell lines. The results suggest that oak leaf can be introduced as a novel source of taxanes both for therapeutic utilization and as new precursors for upcoming paclitaxel semi-synthesis approaches.

Keywords: Cephalomannine; 10 deacetyl baccatin III; Hepatocellular carcinoma cells; Human Embryonic Kidney cell line; Paclitaxel; *Quercus* sp.

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Introduction

Paclitaxel is a diterpene alkaloid that binds to the internal surface of microtubules and prevents their dynamic instability, particularly in the G2 / M transition in the cell cycle (Leung & Cassimeris, 2019). Currently, it is used as an effective drug in chemotherapy to inhibit cell division in breast and ovarian cancer cells (Zhao et al., 2016). Moreover, the performance of paclitaxel in treating leukemia, lymph node, lung, colon, head, and neck cancers has been evaluated, and its effects on AIDS and Alzheimer's disease are currently being investigated (Exposito et al., 2009; Hoffman & Shahidi, 2009; Sato et al., 2021). Paclitaxel and other taxanes, i.e., cephalomannine and baccatin III have been predominantly detected as secondary metabolites of *Taxus* sp. (Yu et al., 2018). Total chemical synthesis, semisynthesis of paclitaxel precursors, and the paclitaxel (Yukimune et al., 1996). Dependence on plant resources, high cost, and very low yields are fundamental limitations of these strategies (Soliman & Raizada, 2013).

With the increasing demand for paclitaxel and the shortage of plant resources, there is an urgent need to find other alternative resources. Finding paclitaxel in *Taxomyces andreanae*, an endophyte of the Pacific yew, opened a new window for searching for other potential sources for paclitaxel (Stierle et al., 1993). Although the isolation of endophytic microorganisms is a comparatively simple and fascinating process, but paclitaxel detection in isolates is laborious (Mohammadi Ballakuti et al., 2022). Moreover, despite several studies showing that fungi can produce paclitaxel independently of the plant, there remains a doubt that the minute amounts of taxanes in endophytic fungi are residual taxanes synthesized by the host tree (Xiong et al., 2013).

Over the past two decades, the hazel plant and its cell culture have been explored as potential alternative sources of taxanes (Bestoso et al., 2006; Ottaggio et al., 2008). The best advantage of hazel cells compared to yew cells was the sustainability of their cultures, which allowed researchers to increase taxane production through various physicochemical manipulations (Ghanati

et al., 2011; Safari et al., 2012; Bemani et al., 2013; Jamshidi & Ghanati, 2017).

Interestingly, some genera close to *taxus*, e.g., *pseudotaxus* and *austrotaxus*, do not have paclitaxel but produce other D-rings lacking taxanes (Gueritte-Voegelein et al., 1987). The existence of paclitaxel from an angiosperm, *Corylus avellana*, was first reported by Hoffman (1998). Moreover, a wide range of endophyte fungi have been found. This suggests that the biosynthetic pathways of taxanes are evolving in parallel, and taxanes are likely to be found in many plants, even in phylogenetically separated ones. Therefore, this question arises: considering the tracing of taxanes in the yew plant of gymnosperms and recently in the hazel plant of angiosperms, is it possible to find these compounds in other plant species? It has been suggested that the ability to produce taxanes can be achieved through horizontal gene transfer from *Taxus* to non-*Taxus* species, facilitated by endophytes (Heinig et al., 2013). In specific conserved areas of Iranian forests, oak trees coexist with *C. avellana* trees in their vicinity (Keivan Behjou & Sefidi, 2015). Additionally, taxanes have been identified in several endophytes associated with *C. avellana* (Mohammadi Ballakuti et al., 2022). Consequently, we hypothesize that oak trees may acquire genes responsible for taxol production from *C. avellana*. The presence of taxoids in *Quercus* species was investigated in the current study.

Materials and Methods

Chemicals

All HPLC-grade solvents were purchased from Merck (Germany). Other analytical reagents, including Coomassie Brilliant Blue G250 and MTT, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Authentic taxoid standards, including paclitaxel and its derivatives, were obtained from ChromaDex (USA).

Plant materials

All methods were carried out in accordance with the relevant institutional, national, and international guidelines and legislation. Besides, they were discussed and approved by the

Research Ethics Committee of Tarbiat Modares University. The plant samples were collected from natural oak forests in the Alborz and Zagros Mountains of Iran. No permissions or licenses were needed to collect leaves and ripen fruit samples. Nevertheless, sampling was conducted in the least quantities, and intensive care was taken so as not to damage the trees. The plants were first identified and authenticated by the authors based on the Colorful Flora of Iran (Ghahraman, 1979) and then confirmed by Dr. Vali-Allah Mozaffarian of the Institute of Forests and Rangelands Research, Tehran, Iran.

Oak leaves were collected from natural forests in the Alborz and Zagros Mountains of Iran. Samples were taxonomically identified using Ghahraman's Colorful Flora of Iran and verified by Dr. Vali-Allah Mozaffarian (Institute of Forests and Rangelands Research, Tehran, Iran). The identified species were *Quercus brantii*, *Q. macranthera*, and *Q. castaneifolia*. Voucher specimens (13981-TMUH, 13982-TMUH, and 13983-TMUH) were deposited in the herbarium of Tarbiat Modares University.

Determination of soluble sugar and protein content

Soluble carbohydrates were extracted from leaf tissues using phosphate buffer (50 mM, pH 6.8) followed by centrifugation (12,000 ×g, 20 min). The supernatant was analyzed using the phenol-sulfuric acid method, with glucose as standard (0–30 µg mL⁻¹). Protein content was determined using the Bradford assay, with bovine serum albumin as the standard (Soleimani et al., 2019).

Phenolic compounds

The leaves were washed, wiped with tissue paper, and dried. The dried samples (0.2 g) were homogenized in 3 mL methanol followed by centrifugation at 12,000 ×g, 10 min. The supernatant was divided into two portions. Half mL of 1 N Folin-Ciocalteu reagent was added to 0.5 mL of the first portion, and the mixture was left for 2–5 min at ambient temperature, followed by adding 1 mL of 20 % Na₂CO₃. After 10 min incubation at ambient temperature, the absorbance

was measured at 730 nm, and total phenolics were determined based on a standard curve of gallic acid (Jamshidi et al., 2016).

The second portion of the above-mentioned supernatant was treated with polyvinylpyrrolidone (PVP) followed by precipitation of tannins by centrifugation at 12,000 ×g, 10 min. Non-tannin phenolics in this supernatant were measured by Folin-Ciocalteu reagent as described above and were determined using a standard curve of Tannic acid at 730 nm. Tannins were calculated by subtracting non-tannin phenolics from total phenolics (Makkar Harinder et al., 1993).

Phlobaphenes (flavan-4-ols) were extracted with BuOH: HCl (70:30, v: v) for 1 h at 37 °C. Then, the absorbance was read at 565 nm, and the phlobaphene content was expressed as the absorbance per g of the sample's dry weight (Wu et al., 2020).

For extraction and measurement of anthocyanins, the dried leaves were homogenized in a mixture of MeOH: HCl (99:1 v/v) and centrifuged at 12,000 ×g for 15 min. The supernatant was kept in darkness overnight. Anthocyanin content was determined by measuring the absorbance at 550 nm using an extinction coefficient of 33,000 cm⁻¹ M⁻¹ compounds (Jamshidi et al., 2016).

Paclitaxel and certain taxoids

The dried leaves were pulverized and suspended in methanol and then filtered. The filtrate was air-dried and re-dissolved in a mixture of dichloromethane (CH₂Cl₂) and water (H₂O) in a 1:1 ratio (v/v), followed by centrifugation at 12,000×g. The dichloromethane phase was collected and dried under filtered air. The residue was then re-dissolved in HPLC-grade MeOH and filtered again through a 0.45-µm syringe filter before being used for further chemical analysis and bioassays. Qualitative and quantitative assays were performed using an HPLC system (Waters, e2695, USA) equipped with a C18 column (Perfectsil Target ODS3, 5 µm, 250 × 4.6 mm, MZ-Analysentechnik, Mainz, Germany). Elution was carried out at a flow rate of 0.8 mL/min; the mobile phase consisted of water (containing 0.1% CH₃CN) and methanol. Taxanes were eluted using

a gradient method: for the first 30 minutes, there was a linear gradient increasing from 40% to 78% MeOH, followed by isocratic elution with 78% MeOH from 30 to 40 minutes, and finally, from 40 to 45 minutes, there was a decrease in MeOH concentration back to 40%. The retention time and peak area of genuine taxane standards were used for the identification and quantification of taxanes (Sigma-Aldrich, USA; ChromaDex, USA). To ensure accuracy, each peak was reinforced by injecting its corresponding standard (Mohammadi Ballakuti et al., 2022).

The qualitative analysis of paclitaxel and other taxanes was validated using an Agilent 6410 HPLC system integrated with a Triple Quad ion trap mass spectrometer featuring an electrospray ion source. The column utilized was an Eclipse C18 with a particle size of 3.5 μm , a length of 100 mm, and a width of 4.6 mm. The solvent flow rate and injection volume were set at 350 $\mu\text{L}/\text{min}$ and 50 $\mu\text{L}/\text{min}$, respectively. Mobile phase A was composed of 0.1% formic acid in a mixture of distilled water and methanol (70:30 v/v), while mobile phase B contained 0.1% formic acid in methanol. An isocratic flow rate of 1 mL/min was maintained for 5 minutes, after which the gradient commenced at 100% A and gradually transitioned to 100% B over 25 minutes, followed by an isocratic phase of 100% B for an additional 10 minutes, culminating in a total run time of 40 minutes. Mass spectra were collected in production mode, and the identification of taxanes in the samples was confirmed through ESI/MS in production mode based on structurally diagnostic ions observed in the LC-MS spectra (Mohammadi Ballakuti et al., 2022).

Evaluation of leaf extract cytotoxicity

Human Embryonic Kidney (HEK 293T) and Hepatocellular carcinoma (HepG2) cell lines were sourced from the Pasteur Institute of Iran. These cell lines are widely utilized in biopharmaceutical and fundamental medical research, as well as in therapeutic applications (Shaw et al., 2002; Samarakoon et al., 2016; Venkatachalapathy et al., 2021). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS), along with

penicillin and streptomycin. Subsequently, they were plated in a 96-well plate at a density of 7×10^3 cells per well and incubated for 24 hours at 37 °C in a humidified environment with 5% CO₂. The growth medium was then replaced with serum-free DMEM. Oak leaves were extracted using 70% methanol, and serial dilutions were prepared in a complete growth medium to obtain the desired concentrations. Various concentrations of leaf extracts, ranging from 10 $\mu\text{g}/\text{mL}$ to 1000 $\mu\text{g}/\text{mL}$, were introduced to the wells and incubated for 48 hours. Control wells contained untreated cells. Following incubation, the media and leaf extracts were discarded, and 10 μL of MTT (3-(4,5-dimethylthiazole-2-yl)-5,3-carboxymethoxyphenyl-2, 4-sulfophenyl-2H-tetrazolium) along with 100 μL of lysis buffer (comprising 20% sodium dodecyl sulfate and 50% dimethylformamide in 30% phosphate-buffered saline) were added to each well and incubated for 4 hours at 37 °C. Afterward, 100 μL of dimethyl sulfoxide (DMSO) was added to each well, and the absorbance was measured using a microplate reader (ELx800, Biotek, USA) at a wavelength of 570 nm. The absorbance of the untreated cells was designated as 100% viability (Alipour et al., 2017).

$$\% \text{ Viability of cells} = [(A - B) / A] \times 100$$

In this context, A represents the optical density of the control cells, while B denotes the optical density of the treated cells. The lethal concentrations (LC50) were determined using Graph Pad Prism software version 5.04.

Statistical analysis

All experiments were performed in an independent triplicate of each sample. One-way ANOVA was used to analyze differences among treatments, and significance was considered at $p \leq 0.05$, as determined by LSD using SPSS software (version 22, Chicago, IL, USA).

Results

Statistical analysis revealed differences in the total soluble carbohydrate levels among the oak species studied (Fig. 1A). The leaves of *Q. brantii*

exhibited the highest carbohydrate content, followed closely by *Q. castaneifolia* and *Q. macranthera*, respectively (Fig. 1A).

The total soluble protein levels in the leaves were measured at 0.59%, 0.70%, and 0.65% of the dry weights for *Q. brantii*, *Q. castaneifolia*, and *Q. macranthera*, respectively (Fig 1B). The total soluble phenolic content in *Q. brantii* was found to be significantly higher, by 130%, compared to *Q. castaneifolia* and *Q. macranthera* (Fig 1C). Similar patterns were noted in the tannin levels among these species (Fig 1D). Significant

differences were observed between the content of phlobaphens (flavan-4-ols derived from the oxidation of tannins) in the leaves of three oak species. The highest phlobaphen content was observed in *Q. castaneifolia*, while *Q. macranthera* and *Q. brantii* were respectively 74% and 50% of castaneifolia (Fig 1E).

While the anthocyanin content of *Q. castaneifolia* was significantly higher than two other species (ca. $\times 2$), no significant difference was detected between the anthocyanin contents of *Q. macranthera* and *Q. brantii* (Fig 1F).

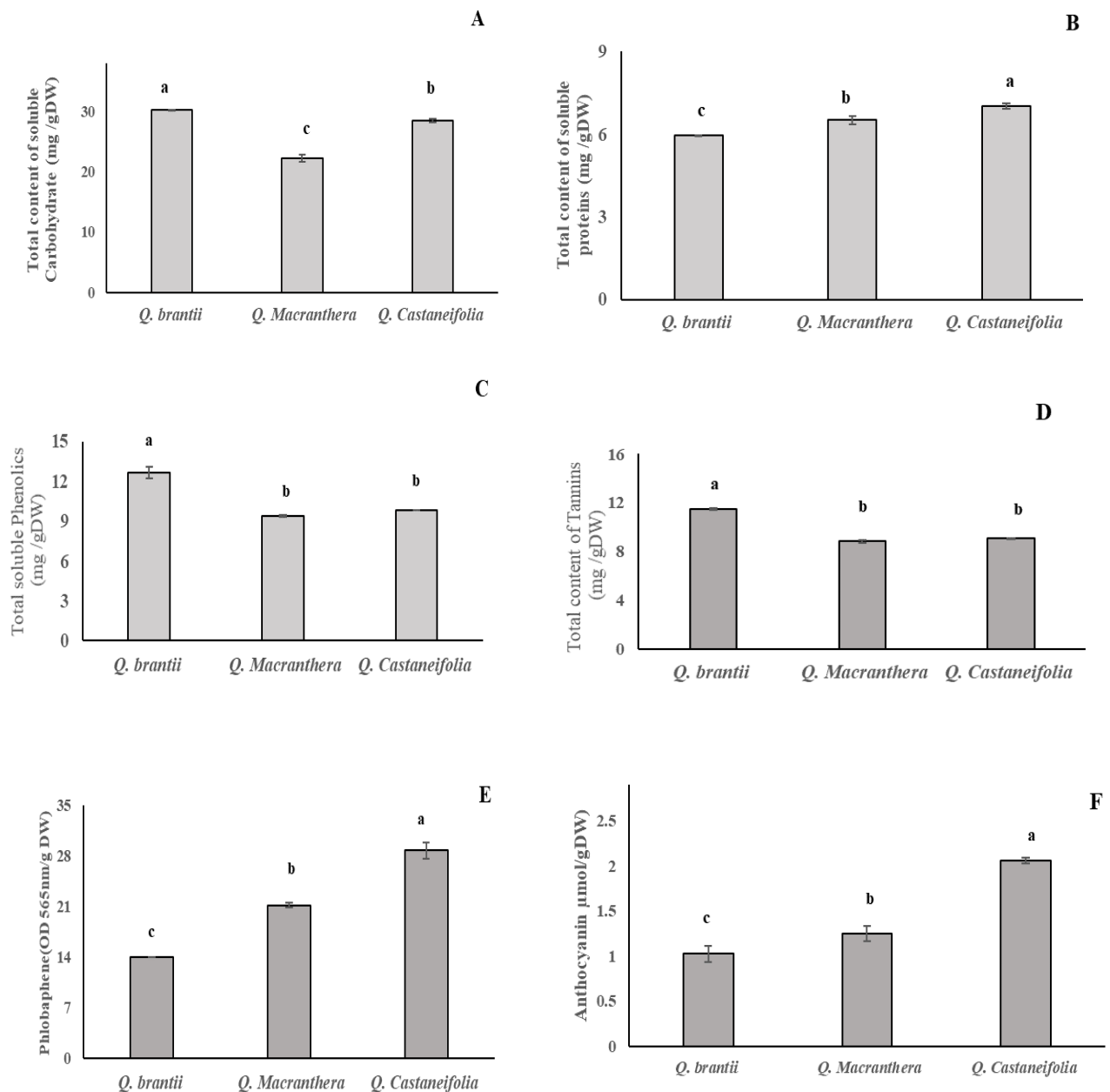


Fig 1 Total content of major primary and secondary metabolites of Quercus leaves. Total soluble carbohydrates (A), soluble proteins (B), soluble phenolics (C), tannins (D), phlobaphen (E), and anthocyanin (F). Data are mean $\pm$ SD, $n = 3$. Bars with different letters are significantly different at $p \leq 0.05$, according to the LSD test.

Paclitaxel was identified in the leaf extracts of all oak species examined using HPLC (Fig 2 A-B). The presence of taxoids was structurally validated through LC-MS analysis, with the (M+H) peaks measured at 545 m/z for 10-deacetyl baccatin III,

832 m/z for cephalomannine, 854 m/z for paclitaxel, and 7-epipaclitaxel (Fig 1S).

Except for baccatin III, which was found solely in *Q. castaneifolia*, various other taxoids were detected to varying degrees in the leaf extracts of all species studied (Table 1).

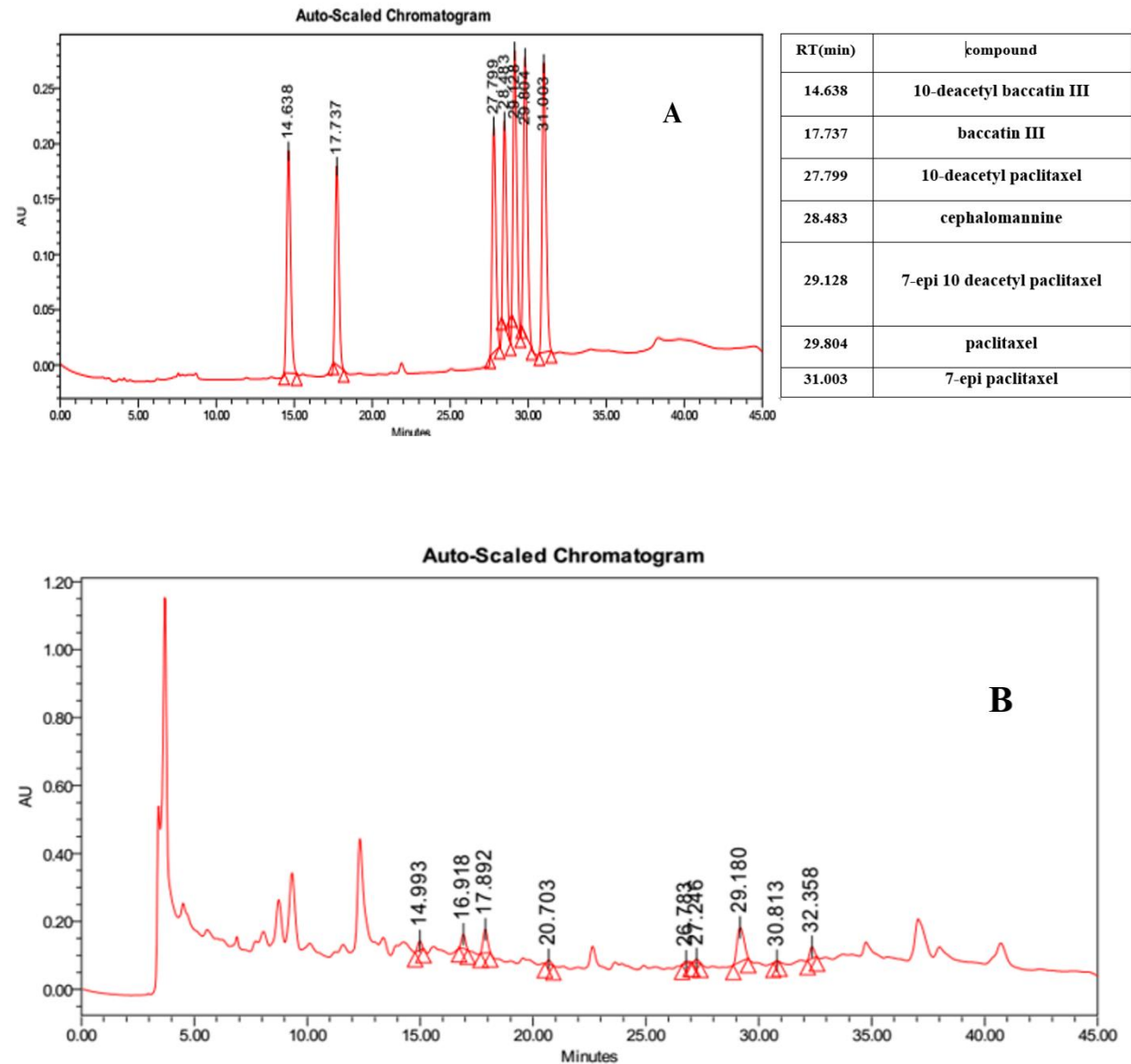


Fig 2 HPLC and LC-MS chromatograms of paclitaxel and other taxoids. Taxoids mixture standards (A) and taxoids in oak leaf extract (B) detected by HPLC. LC-MS analysis of standard taxanes and *Quercus* sp. (C-H). The [M+H] peak calculated at, 545 m/z for DAB, 587 m/z for baccatin III, 812 m/z for 10 deacetylpaclitaxel and 7-epi 10-deacetyl paclitaxel, 832 m/z for cephalomannine, 854 m/z for paclitaxel and 7-epi paclitaxel. The samples were dissolved in methanol and injected using a spray flow of 1µL/min.

Table 1: Distribution of taxoids in three Iranian Quercus species, screened by HPLC and LC-MS analysis

Quercus sp.	Taxanes						
	10-deacetyl baccatin III	baccatin III	10-deacetyl paclitaxel	cephalomannine	7-epi, 10 deacetyl paclitaxel	paclitaxel	7-epi paclitaxel
<i>Q. castaneifolia</i>	+	+	ND	+	+	+	+
<i>Q. brantii</i>	+	ND	+	+	+	+	+
<i>Q. macranthera</i>	+	ND	+	+	ND	+	ND

Cephalomannine, paclitaxel, and DAB were identified in all three oak species (Fig 3). The highest concentration of cephalomannine was found in *Q. brantii*, measuring 27 μ g. g DW⁻¹, which was significantly greater than that in *Q. castaneifolia* and *Q. macranthera*, with differences of approximately 90 times and 3 times, respectively (Fig 3A). Paclitaxel was also present in all three oak species, with the highest level recorded in *Q. brantii* at 4.78 μ g. g DW⁻¹. The levels of paclitaxel in *Q. macranthera* and *Q. castaneifolia* were considerably lower, accounting for 11.5% and 0.8% of the concentration found in *Q. brantii* (Fig 3B). A concentration of about 0.5 μ g. g DW⁻¹ of DAB was detected across the oak species, showing no significant variation among them (Fig 3C).

7-epi 10-deacetyl paclitaxel was detected in *Q. brantii* (0.77 μ g. g DW⁻¹) and *Q. castaneifolia*

(2.44 μ g. g DW⁻¹) but not in *Q. macranthera* (Fig 3D).

Similarly, 7-epi paclitaxel was detected in *Q. brantii* (1.36 μ g. g DW⁻¹) and *Q. castaneifolia* (0.42 μ g. g DW⁻¹), but not in *Q. macranthera* (Fig 3E).

10-deacetyl paclitaxel was not found in *Q. castaneifolia*, but was measured as 0.30 and 0.39 μ g. g DW⁻¹ of leaves of *Q. brantii* and *Q. macranthera*, respectively (Fig 3F). Baccatin III was detected only in *Q. castaneifolia* (1.37 μ g. g DW⁻¹).

Various concentrations of crude leaf extract from oak species were prepared and utilized for the MTT assay on HEK 293T and HepG2 cell lines. Both cell lines exhibited significant damage following a 24-hour treatment with the oak leaf extracts (Fig. 4).

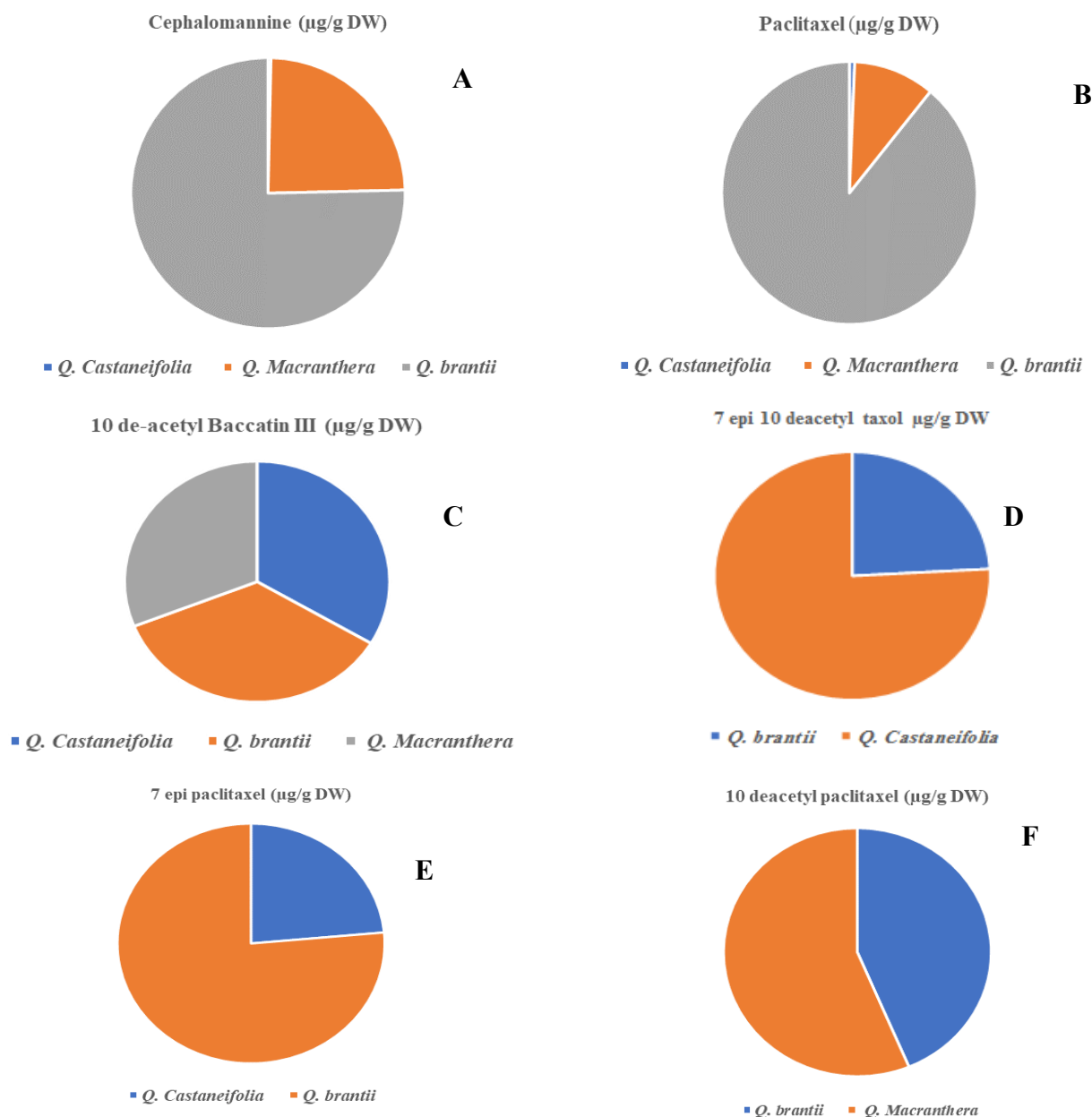


Fig. 3 Relative amounts of different taxanes in *Quercus* species. DAB, cephalomannine, and paclitaxel Baccatin III were detected in all examined *Quercus* sp.. In contrast, baccatin III was only detected in *Q. castaneifolia*, it is not shown, and 10 Deacetyltaxol was detected in *Q. brantii* and *Q. macranthera*. Data are means of three independent repetitions and are expressed as µg/g DW.

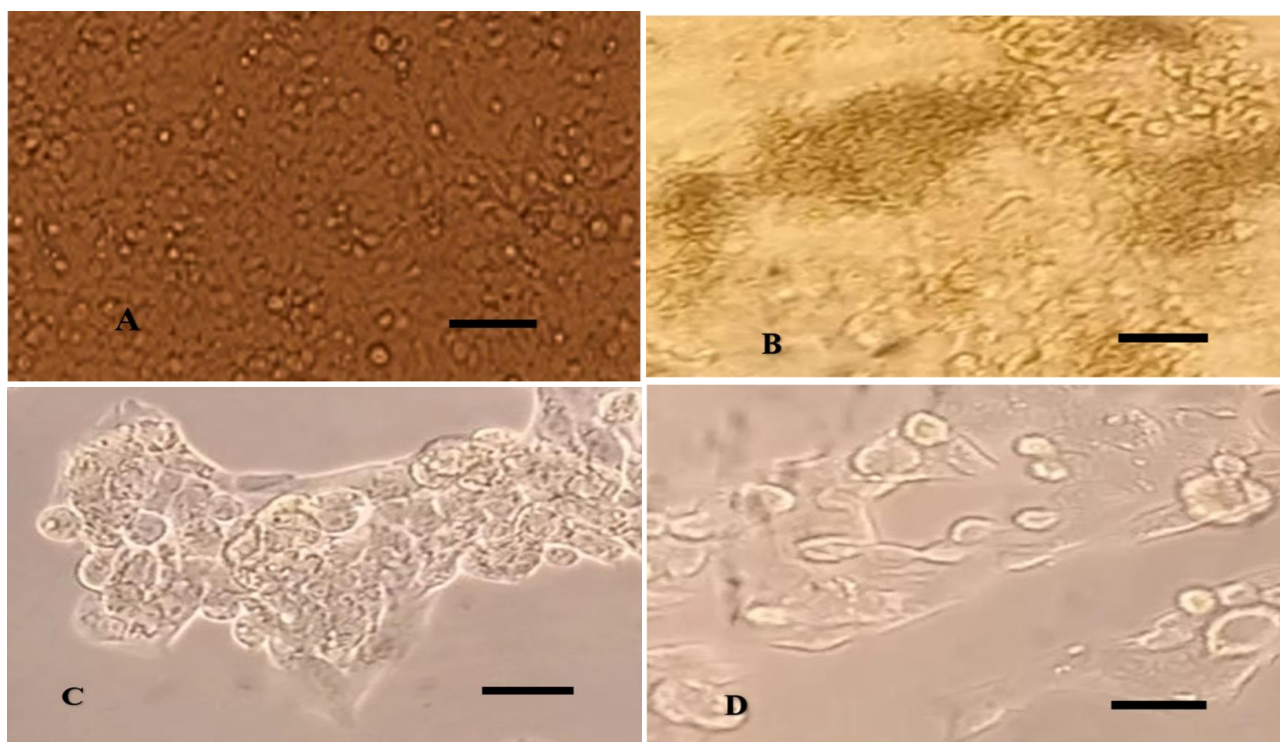


Fig. 4 Morphology of HEK 293T (A, B) and HepG2 (C, D) cells before and after treatment with leaf oak extract. Scale bar 100 μm .

The lowest LC50 of oak leaf extract against both examined cell lines belonged to *Q. brantii* (Table 2).

Table 2: LC50 of leaf extract of Iranian oak species against HEK 293T and HepG2 cell lines.

Quercus sp.	LC50 ($\mu\text{g. mL}^{-1}$)	
	HEK 293T	HepG2
<i>Q. castaneifolia</i>	439.7 $\pm$ 20.1	396.1 $\pm$ 15.9
<i>Q. brantii</i>	409.5 $\pm$ 18.7	333.6 $\pm$ 11.6
<i>Q. macranthera</i>	510.9 $\pm$ 35.8	380.6 $\pm$ 25.4

Discussion

The oak tree is a prominent species found in the natural forests of Iran. The leaf extracts from various oak species exhibit significant levels of soluble sugars and proteins, although there are variations among the species. Despite their high carbohydrate and protein content, oak leaves are not utilized for livestock feed due to their elevated levels of phenolic compounds, particularly anthocyanins and tannins, which greatly diminish their palatability. Nevertheless, oak plants have historically demonstrated their worth as a source of compounds with therapeutic properties. Species of *Quercus* have exhibited antioxidant, anti-inflammatory, antimicrobial, and anticancer effects attributed to their phytochemical makeup (Burlacu et al., 2020). Local inhabitants have traditionally used extracts from the leaves and

seed hulls of various oak species, especially Persian oak (*Q. brantii*), for the treatment of gastric issues, diarrhea, and wound healing (Safary et al., 2009; Ebrahimi et al., 2012).

Despite the identification and characterization of numerous bioactive compounds derived from plants, a significant number of plant species remain uninvestigated for potential new drug leads. Furthermore, the potent activity observed in a crude plant extract may stem from the presence of either unexpectedly potent compounds or a combination of several weakly active compounds that work additively or synergistically (Atanasov et al., 2015). Consequently, oak extracts may be utilized to discover other valuable pharmaceutical compounds, including paclitaxel.

The detection of paclitaxel and other taxoids in the leaves of *Q. brantii*, *Q. macranthera*, and *Q. castaneifolia* presents a significant new application for these species. Notably, *Q. brantii* exhibited the highest concentration of paclitaxel at 4.78 µg per gram of dry weight, which is 6.5 times greater than the maximum level of paclitaxel previously recorded in hazel leaves (Hoffman & Shahidi, 2009).

Cephalomannine was the second taxoid identified in all three oak species. This compound has been recognized as a promising therapeutic agent for glioblastoma, the most prevalent malignant primary brain tumor (Qiao & Kondo, 2018). The highest concentration of cephalomannine was observed in *Q. brantii*, measuring 27 µg/g DW, which is significantly greater than that found in hazel leaves, approximately 170 times higher (Hoffman & Shahidi, 2009).

DAB serves as a vital precursor for paclitaxel. An intermediate step in the biosynthesis of paclitaxel involves the acetylation of 10-deacetylbaaccatin-III (10-DAB) to form baaccatin-III. This process is facilitated by a crucial rate-limiting enzyme known as 10-deacetylbaaccatin III-10-O-acetyltransferase (DBAT). The reaction is entirely reliant on the presence of 10-DAB and is specifically targeted at the 10-hydroxyl group of the taxane ring. DAB was found in all three oak species studied at nearly identical concentrations; however, Baaccatin III was exclusively identified in *Q. castaneifolia*. This discrepancy may be attributed to varying levels of DBAT activity across different species (Li et al., 2017).

10-deacetyl paclitaxel (10-DAP) was identified in both *Q. brantii* and *Q. macranthera*, serving as a precursor for the semi-synthesis of paclitaxel. Similar to other taxanes, 10-DAP exhibits anticancer properties, as evidenced by its ability to inhibit tumor growth in a mouse model of lymphocytic leukemia (McLaughlin et al., 1981).

Additionally, 7-epi-10-deacetylpaclitaxel was found in *Q. brantii* and *Q. castaneifolia*, with its concentration in the latter being nearly three times greater than that in *Q. brantii*. This compound has been demonstrated to induce apoptotic cell death in HepG2 cells by generating reactive oxygen

species (ROS) and activating the MAPK pathway (Subban et al., 2017). Furthermore, 7-Epipaclitaxel, the primary derivative of paclitaxel, was detected in the leaf extracts of *Q. brantii* and *Q. castaneifolia*, with its concentration in *Q. brantii* being almost three times that of *Q. castaneifolia*. Compared to paclitaxel, 7-epipaclitaxel exhibits greater stability and cytotoxicity, as it has been shown to induce cell death, alter mitochondrial membrane potential, and cause chromatin condensation in squamous cell carcinoma of the head and neck (Kumar et al., 2021).

The extract derived from oak leaves demonstrated cytotoxic effects on both the cancer cell line (HepG2) and the embryonic cell line (HEK 293T). The observed cytotoxicity of the oak leaf extract may be partially linked to its taxoids. The extraction and purification of various taxoids from oak leaves, along with an assessment of their toxicity, were beyond the scope of the current study and warrant further research. Additionally, the cytotoxicity of phenolic compounds and their potential additive or synergistic effects with taxoids should also be considered (Atanasov et al., 2015). Given the findings presented, oak leaves may be recognized as a promising new source of taxanes for therapeutic applications and as a novel precursor for the semi-synthesis of paclitaxel.

The distribution of paclitaxel in both plants and endophytic fungi, along with the potential for horizontal gene transfer related to paclitaxel biosynthesis between these two closely associated groups, has been a topic of ongoing discussion (Heinig et al., 2013; Kumar et al., 2021; Yang et al., 2014). Recently, the taxadien-5 α -ol-O-acetyltransferase gene, a crucial component of the paclitaxel biosynthetic pathway, was successfully isolated from *Corylus avellana* L. through the nested-PCR technique (Raeispour Shirazi et al., 2021). In silico characterization and phylogenetic analysis of this gene demonstrated significant homology with its orthologs found in *Quercus suber*, *Juglans regia*, *Morus notabilis*, and *Ziziphus jujube*. Notably, these plant species, while belonging to different families, were positioned on the same branch of the phylogenetic tree (Raeispour Shirazi et al., 2021).

It remains to be determined through further research whether the complex biosynthesis of taxan and the associated genes in oak are original and have evolved independently or if they have been horizontally transferred through unidentified mechanisms, such as microorganisms that coexist in their environment.

Conclusions

The current research indicates that Iranian oak leaves can serve as a novel, cost-effective, readily available, and sustainable source for paclitaxel and other taxanes, suitable for therapeutic applications, and as precursors for future semi-synthesis of paclitaxel. Each oak tree yields approximately 30,000 deciduous leaves (around 15 kg of dry weight) annually, which, according to the findings, is sufficient to extract 70 mg of paclitaxel. This presents a significant advantage over the traditional yew tree, where nearly 1 kg of bark is required to obtain just 1 mg of paclitaxel.

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- Ongoing investigations are assessing whether other non-edible parts of the oak plant and cell cultures derived from these parts contain comparable levels of taxanes. Furthermore, the results indicate that while paclitaxel was found in the leaf extracts of all examined species, the presence and concentration of other taxoids varied among them. Consequently, additional research is essential to explore the effects of climate, weather, and soil conditions on the type and quantity of taxanes to optimize the yield of the desired taxoid.
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- Conflicts of Interest**
- The authors declare no conflict of interest
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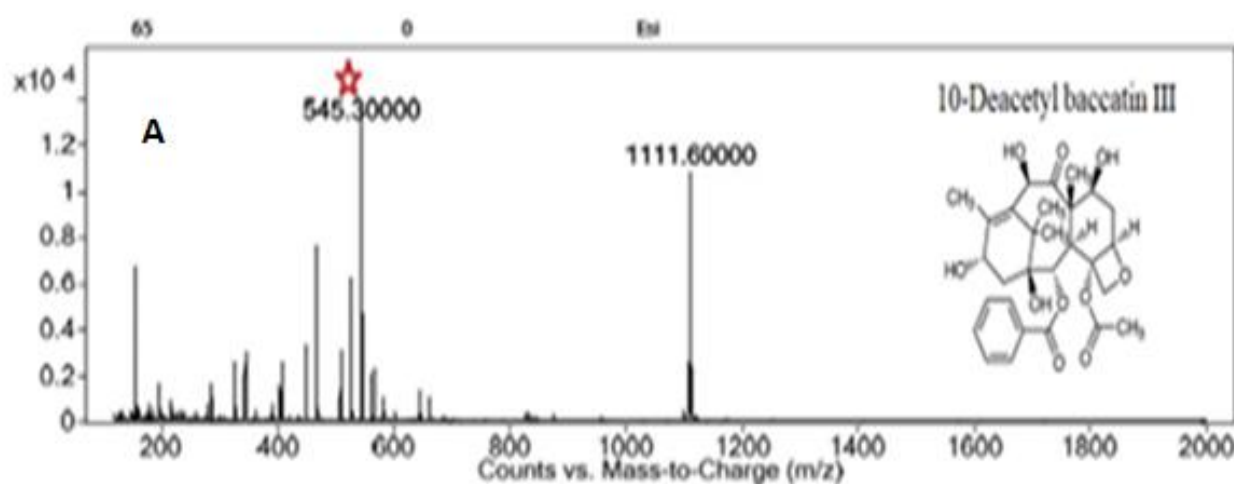
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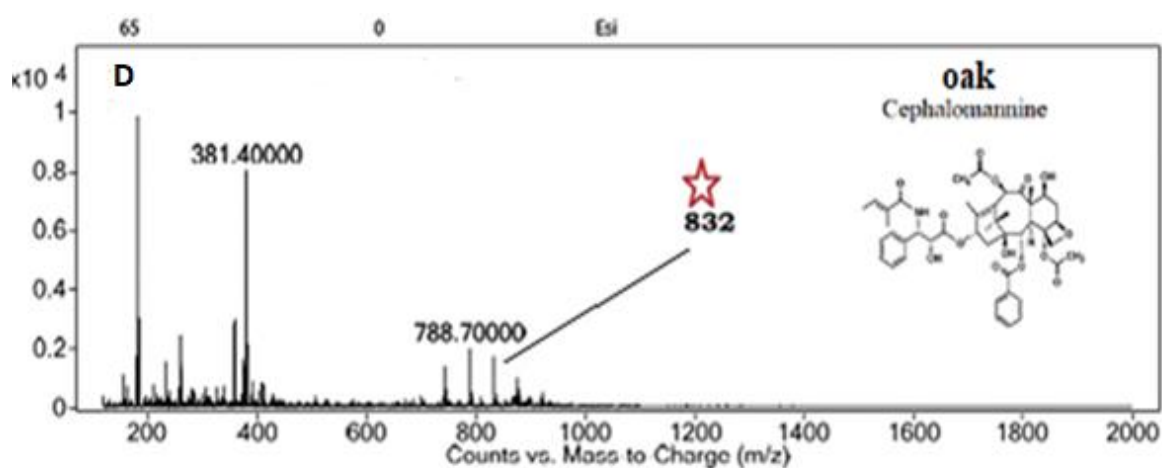
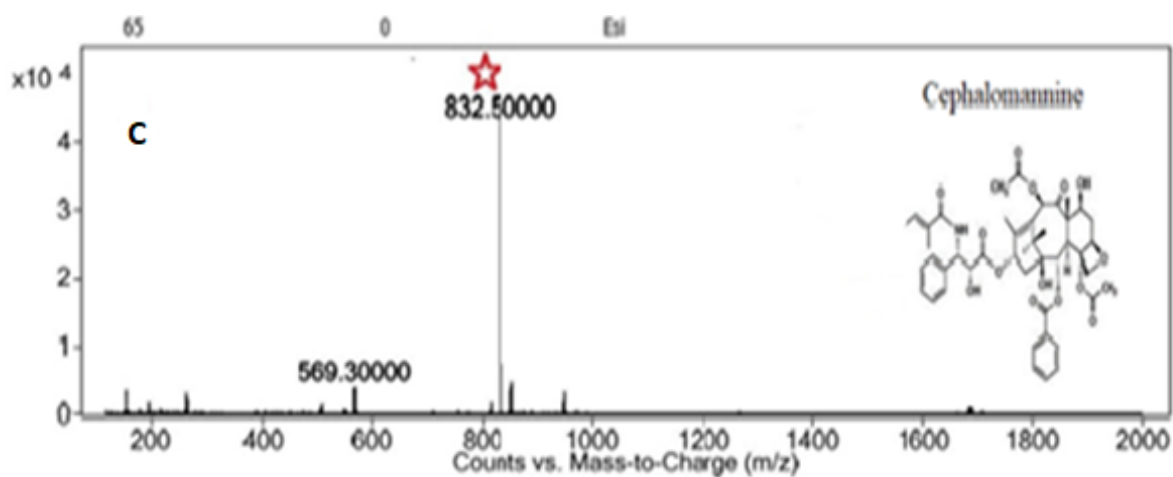
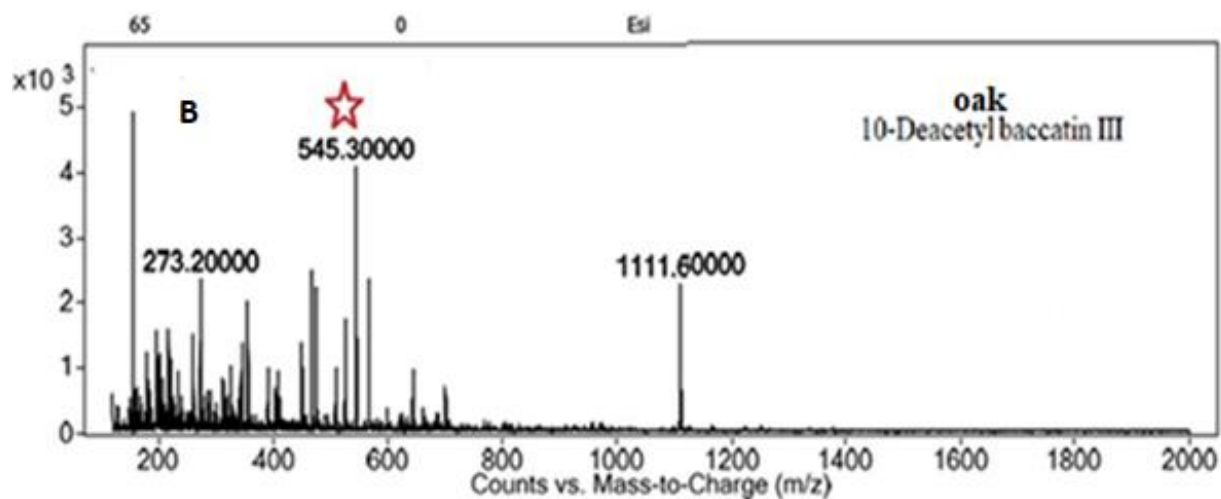
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Supplementary data

Fig. 1S





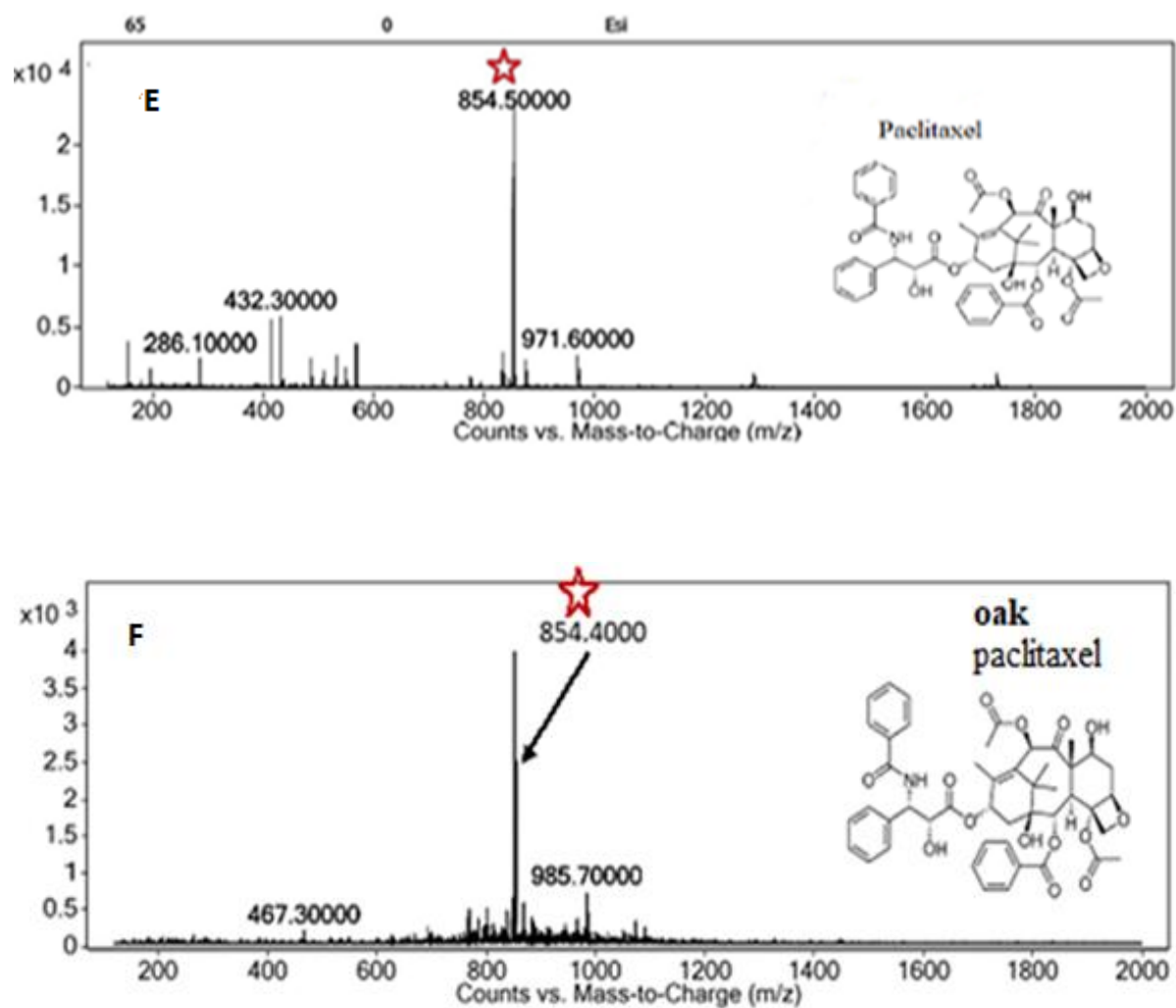


Figure 1S: LC-MS chromatograms of paclitaxel and other taxoids. LC-MS analysis of standard taxanes and of *Quercus* sp. (A-F). The [M+H]⁺ peak calculated at, 545 m/z for DAB, 587 m/z for baccatin III, 812 m/z for 10 deacetylpaclitaxel and 7-epi 10-deacetyl paclitaxel, 832 m/z for cephalomannine, 854 m/z for paclitaxel and 7-epi paclitaxel. The samples were dissolved in methanol and injected using a spray flow of 1 μ L/min.