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Phytochemical Compound Profile and the Estimation of the Ferruginol Compound in Different Parts (Roots, Leaves, and Seeds) of *Juniperus procera*

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Abstract: Secondary plant metabolites and their derivatives play a significant role in human health. Ferruginol is a diterpene phenol that has recently received attention for its pharmacological properties, including antibacterial, antitumor, antimalarial, and cardioprotective effects. Recently, we detected the ferruginol compound in the leaf and seed extracts of *Juniperus procera* using different analytical approaches. The present work aims at detecting phytochemical compounds in a root extract of *J. procera* and estimating the amount of ferruginol compound in different parts of *Juniperus procera*. To screen the phytochemical compounds present in the root extract of *J. procera*, Gas chromatography/mass spectrometry (GC/MS) was performed. For ferruginol identification and estimation, high-performance liquid chromatography (HPLC) with the ferruginol reference standard and high-resolution direct analysis in real-time (DART) time-of-flight mass spectrometry (TOFMS) (DART-TOF-MS) analysis were used. GC/MS analysis revealed more than 20 bioactive compounds related to secondary plant metabolites in the root extract of *J. procera* with biological activity. The DART-TOF-MS result showed the typical positive ion spectra of ferruginol, and the HPLC result confirmed that the root extract of *J. procera* contains the ferruginol compound. In contrast, the root extract of *J. procera* contained a significant amount of ferruginol compared to that in the leaf and seed extracts. All parts of the *J. procera* contained the ferruginol compound and proved that ferruginol might be accumulated in the roots, leaves, and seeds of *J. procera*.

1. Introduction

Juniperus procera Hoechst. ex Endl. is indigenous to the mountains of East Africa from East Sudan to Zimbabwe, and the southern region of the Arab Peninsula across the Red Sea; it is widely spread out throughout the southern part of Saudi Arabia [1,2]. In Saudi Arabia, *J. procera* is traditionally used for treating jaundice, gastrointestinal disturbances, hepatic diseases, and pharyngitis as an antirheumatic for gout and several inflammatory conditions [3]. Additionally, *J. procera* is a natural source of photochemical components that may have antioxidant, insecticidal, antibacterial, and anticancer properties [4–7].

Bioactive components such as phenolics, flavonoids, terpenoids, alkaloids, and their derivatives are very important compounds due to their applications in different fields such as the medical, cosmetic, pharmacological, agricultural, and food industries [8–11]. Abietane diterpenoids are a type of these bioactive compounds [12,13] that exhibit several interesting biological activities, such as antitumor, antioxidant, antimicrobial, antifungal, antiviral, cytotoxic, and anti-inflammatory activities [14,15]. In this regard, abietane-type diterpenoids are significant phytochemical compounds exhibiting a wide range of pharmacological properties [16]. The pharmacological properties of ferruginol, which include

antibacterial, anticancer, antimalarial, and cardioprotective effects, have recently received attention [17,18]. Cancer cell growth is inhibited by the ferruginol compound [18]. Moreover, ferruginol showed a strong protective impact in animal gastric ulcer models [19]. In the literature, the ferruginol compound was widely investigated and showed many important biological activities [16,20–22]. A previous study detected ferruginol in the berries of *J. procera* [23], while we recently detected ferruginol in the leaves and seeds of *J. procera* through different analytical approaches [24,25], and it is the dominant compound there. In this context, ferruginol is widely distributed in the *Juniperus* genus [26,27]. Phytochemical components are ubiquitous secondary metabolites in the plant kingdom, and are not nutritional, but vital ingredients for human health maintenance [28]. For better understanding the biological activities that plant species exert, it is important to explore and to evaluate their phytochemical constituents [29]. Different plant parts dominate a pool of bioactive compounds containing potential chemical groups and may contain different amounts of these compounds [30]. New sources of bioactive compounds such as ferruginol are really needed due to their pharmacological properties. Therefore, besides the identification of phytochemical compounds in root extracts of *J. procera*, this research evaluates the ferruginol compound in different parts (roots, leaves, and seeds) of *J. procera*. Hence, GC/MS was used for bioactive compound identification from the root extracts, while DART-ToF-MS and HPLC with a ferruginol reference standard were used for the quantification of the ferruginol compound in the different parts (seeds, leaves, and roots) of *J. procera*.

2. Materials and Methods

2.1. Chemical Reagents

Solvents such as HPLC-grade methanol, acetonitrile, and water were purchased from Sigma-Aldrich, St. Louis, MO, USA), while the ferruginol standard (95%) was purchased from WuXi App Tec- LabNetwork (Shanghai, China).

2.2. Plant Materials and Extraction

Different parts (roots, leaves, and seeds) of *J. procera* were collected from the alpha region, Saudi Arabia accepting the terms and conditions of national and international standards. A voucher specimen (13,497) was deposited in the herbarium of the center. The collected plant materials were washed carefully using distilled water to remove unwanted particles and dust. Then, plant materials were dried at room temperature. One gram from each part of plant was ground using an electronic blender and placed into 20 mL of methanol. Next, the extraction was carried out using an Innova 44 Inc incubator at 120 rpm, and the temperature was maintained at 28 °C for 5 days. The organic and aqueous phases were separated via centrifugation at 5000 rpm for 15 min. The collected supernatant was filtered through a 0.45 mm nylon syringe and stored at 4 °C for further use.

2.3. GC/MS Analysis of Root Extract

The root extract of *J. procera* was subjected to GC/MS analysis as follows. Gas chromatography/mass spectrometry (GC/MS) was performed with an Agilent 6890 gas chromatograph (Agilent Inc., Palo Alto, CA, USA) coupled to a 5973MSD operated in electron impact mode at 70 eV ion source energy. The gas chromatograph (GC) was fitted with a 30 m × 0.25 mm fused capillary column coated with 0.25 mm film thick DB-5MS (Agilent), and helium was used as the carrier gas with flow rate 1 mL/min and a split flow of 25 mL/min. The oven temperature was adjusted from 60 °C (1 min initial hold) to 280 °C (isothermal for 10 min final time), with injector temperature of 250 °C and total run time of 50 min. The transfer line temperature was set at 250 °C. The MS was operated in electron impact mode at 70 eV ion source energy. Mass spectrometric data were acquired and processed using the GC-MS ChemStation data system (WILEY 9th edition, NIST-08 MS library, Gaithersburg, MD, USA).

2.4. High-Resolution DART-ToF-MS Analysis

Direct real-time time-of-flight mass spectrometry (DART-ToF-MS) analysis (conditions: ionization ES+ − He; temperature, 250 °C; peak voltage, 500V). AccuTOF LC-plus from JEOL (Tokyo, Japan) was used for extracted sample characterization. Volatile plant extract compounds were evaporated in a stream of helium that had been heated to 250 °C, and ionized with the excited metastable helium atoms before entering the ion source of the time-of-flight mass spectrometer. In positive ionization mode, the molecules were mainly protonated without any fragmentation.

2.5. Quantification of Ferruginol with HPLC

The ferruginol compound was identified and quantified using acetonitrile and mobile-phase methanol (40:60) (*v/v*). Ferruginol was quantified through HPLC analysis with an Agilent Technologies 1290 Infinity system (Agilent Inc., Palo Alto, CA, USA). The separations were conducted using ZOBRAX RX-C18 column (4.6 × 150 mm) in which the mobile phases were pumped at a flow rate of 1.000 mL/min with a 5 min run time and injection volume of 1 μL. The column temperature was maintained at 27 °C. The chromatogram was recorded at 220 nm. Ferruginol in the sample was identified through its retention time spiked with the ferruginol standard under similar conditions. Ferruginol was estimated by using linear equation $y = 967.6x - 44.425$ with $R^2 = 0.9997$ on the basis of the reference standard curve prepared with different concentrations of ferruginol (0.2, 0.4, 0.6, and 0.8 μg/mL).

2.6. Statistical Analysis

SPSS one-way analysis of variance (ANOVA)–Duncan's test was performed for evaluating statistical significance at $p < 0.05$. The described results represent the mean of three replicates ± standard deviation (SD).

3. Results and Discussion

3.1. GC/MS Analysis of Different Parts of Juniperus procera

J. procera is a natural source of photochemical components that may have antioxidant, insecticidal, antibacterial, and anticancer properties. The methanolic extracts of the leaves and seeds of *J. procera* were subjected to GC/MS analysis, and the obtained results were published recently [24,31]. In this study, the root extract of *J. procera* was examined with GC-MS analysis for phytochemical component detection. The bioactive compounds in the root extract were identified using commercial libraries and a comparison of the mass spectra, match percentages, and retention times of the reference compounds. The recorded results of GC analysis reveal that the root extracts of *J. procera* are rich in phytochemical compounds. In this regard, *J. procera* is a natural source of phytochemical constituents with potential antimicrobial, insecticidal, anticancer, and antioxidant activities [4–6,32]. For accurate results, the methanol that was used as a solvent in the extraction process and the methanolic extract of the root of *J. procera* were examined with GC/MS analysis (Figures S2 and 1). Then, the detected compounds in the plant sample were compared with the bank (methanol solvent) for bioactive compound identification. GC/MS analysis of the root extract showed about 20 bioactive components related to secondary plant metabolites with biological activity (Figure 1 and Table 1). These detected phytochemical compounds in the root extract of *J. procera* have antimicrobial, antioxidant, anticancer, and anti-inflammatory biological activities. For example, octanoic acid shows antimicrobial activity [33], while longifolene and undecanoic acid act as antifungal agents [34,35]. The extracted bioactive compounds of *J. procera* were effective and potent against SCC-9 cancer cells [36]. The phytochemical compounds highlighted above, other phytochemical constituents detected in the root extract of *J. procera*, and their biological activity are presented in Table 1. These results may support and justify the use of *J. procera* in traditional medicine.
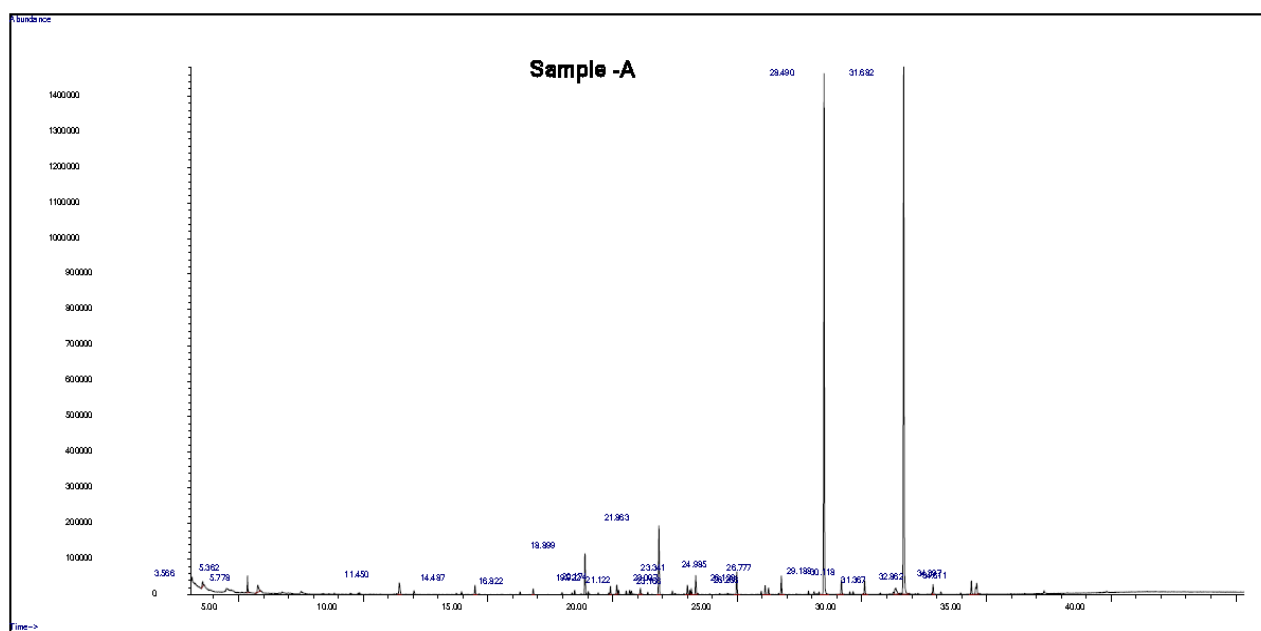
**Figure 1.** GC-MS chromatogram of the root extract of *J. procera*.

Table 1. Secondary gas-chromatography/mass-spectrometry metabolites profile and biological activity of the root extract of *J. procera*.

Compound Name	RT	Area	Mol. Wt.	Biological Activity
Octanoic acid	12.042	283.354	158.131	Antimicrobial [33].
Dodecane	13.947	188.425	170.203	-
1,2,4-Metheno-1H-indene	17.981	149.130	204.188	-
1H-Cycloprop[e]azulene	18.485	233.181	204.188	Analgesic and anti-inflammatory activities [37].
Longifolene	18.897	2.433	204.188	Antifungal [34].
Undecanoic acid	19.028	164.046	200.178	Antifungal [35].
Thunbergol	19.429	104.044	290.261	Antimicrobial activity [38].
Humulene	19.852	84.054	204.188	Anti-inflammatory [39].
(E,Z)-.alpha.-Farnesene	20.173	648.868	204.188	Antibacterial activity [40].
(1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl)spiro	20.247	242.138	204.188	-
beta-Humulene	20.55	214.483	204.188	Antibacterial activity [41].
2,4-Di-tert-butylphenol	20.762	160.545	206.167	Antifungal [42].
Cycloisolongifolene, 8,9-dehydro-	21.42	125.370	202.172	Antibacterial and antifungal activities [43].
2,5-di-tert-Butyl-1,4-benzoquinone	21.861	4.120	220.146	-
Tridecanoic acid	23.102	22.647	228.209	Anthelminthic, anti-inflammatory, antimicrobial, and anticancerous [44].
2-Tridecanone	28.092	201.866	198.198	Insecticidal activity [45].
cis-10-Heptadecenoic acid	28.286	147.813	282.256	Antioxidant [46].
Hexadecanoic acid	29.654	167.380	284.272	Anti-inflammatory [47].
9-Octadecenoic acid	31.365	980.013	296.272	Anti-inflammatory [48].
Nonadecanoic acid	33.173	178.676	312.303	-
2-Phenanthrenol	34.398	840.324	286.23	Antimicrobial [48].
Docosanoic acid	37.316	196.258	354.35	Antioxidant [49].

3.2. Ferruginol Identification and Estimation

Ferruginol is a diterpene phenol that has recently received attention for its pharmacological properties, including antibacterial, antitumor, antimalarial, and cardioprotective effects [17]. In addition, ferruginol inhibits the growth rate of cancer cells [50]. We re-

cently detected the ferruginol compound in leaf and seed extracts of African pencil cedar (*J. Procera*) through different analytical approaches [24,25,31]. We found that ferruginol is dominant compound in the leaves and seeds of *J. procera*. The DART-ToF-MS analysis of the extract showed a spectral range from 285.2 to 278.2 (Figure 2 and Table 2), indicating the presence of ferruginol in the extracts of *J. procera*. This result closely matched the ferruginol compound ($C_{20}H_{30}O$, mol. wt 286.5) according to the National Institute of Standards and Technology (NIST) Standard Reference Database [51]. Additionally, Kuroda K [52] reported that ferruginol normally generated peaks between 285 and 301. Thus, all these peaks (285.2, 286.2, 287.2) with chemical formulas $C_{20}H_{29}O$, $C_{20}H_{30}O$, and $C_{20}H_{31}O$, and 301. 2 are generated from *J. procera* tissue using DART-MS might originate from the ferruginol compound ($C_{20}H_{30}O$, mol. wt. 286.5) (Figure 2 and Table 2), which agreed with previous reports regarding the ferruginol compound [52–54]. DART-MS, with the characteristic of the rapid identification of bioactive compounds, is a new research tool for herbal medicine to very simply complete an experimental process [52,55,56]. As the presence of the ferruginol compound was confirmed in the different parts (roots, leaves, and seeds) of *J. procera*, we separated and evaluated the amount of ferruginol compound in the root, leaf and seed extracts of *J. Procera*. However, different factors, such as sample preparation, mobile phase, column types, and detectors should be considered for the chromatographic analysis of phenolic compounds [57]. Hence, for the separation of the ferruginol compound, HPLC with an authentic standard, and different mobile phases and conditions were tested. Among the tested mobile phases, the methanol and acetonitrile (60:40) (*v/v*) combination was suitable for ferruginol separation considering the conditions detailed in the methodology section (Figure 3a). In this regard, methanol and acetonitrile or their aqueous forms are the main mobile phases utilized in the HPLC quantification of phenolic compounds [58]. Ferruginol in the plant materials was identified via its retention time and by spiking with a reference standard under similar conditions, whereas ferruginol was quantified using a linear equation prepared with different concentrations of the standard (Figure S1).
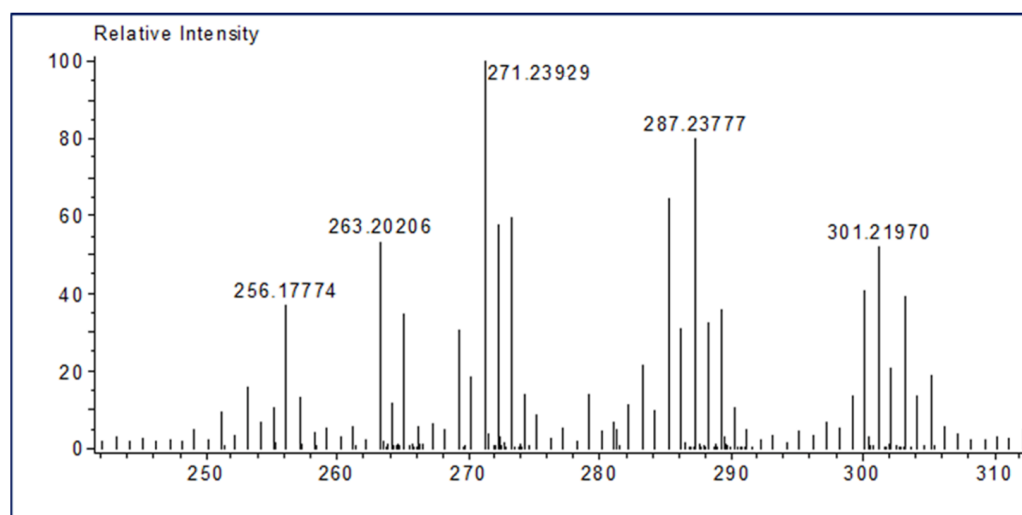
Figure 2. DART TOF-MS chromatogram of *J. procera* showing the ferruginol spectrum.

Table 2. Main phytochemical compound of the extracts of *J. procera* detected with DART-ToF-MS, indicating the presence of ferruginol in the extracts.

No.	Experimental Mass	Calculated Mass	Mass Difference (mmu)	Formula	Unsaturation Degree	Possible Compound
1	285.22493	285.22184	3.09	$C_{20}H_{29}O$	6.5	
2	286.22961	286.22968	−0.05	$C_{20}H_{30}O$	6.0	Ferruginol
3	287.23777	287.23749	0.28	$C_{20}H_{31}O$	5.5	
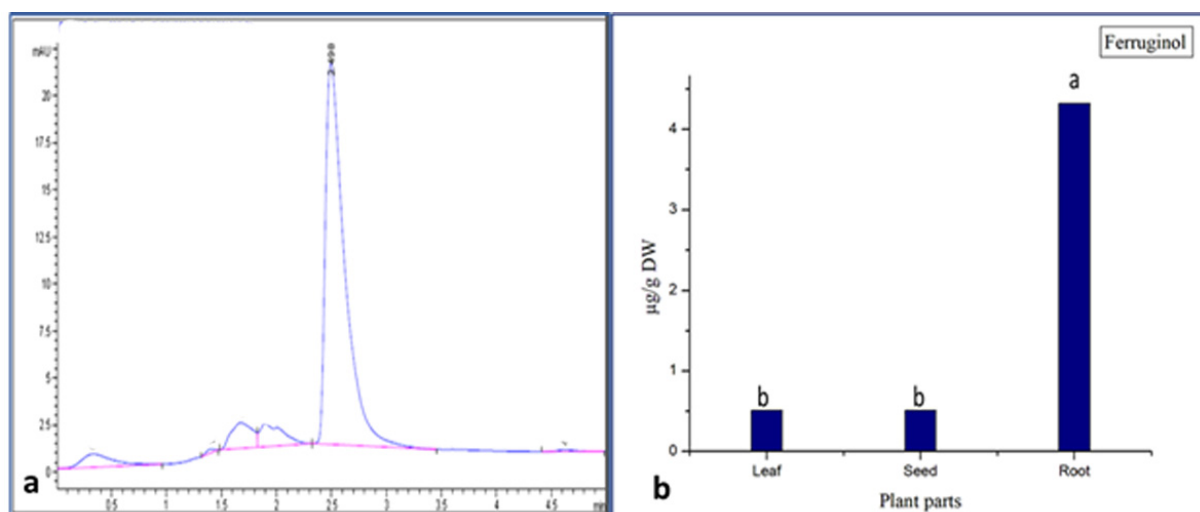
Figure 3. (**a**) Ferruginol standard (RT = 2.498); (**b**) concentration of ferruginol in different part of *Juniperus procera*. [a,b] different superscripts within the same column differ significantly ($p < 0.05$). Reported results represent the mean of three replicates ± standard deviation (SD).

The obtained results confirm that all parts (roots, leaves, and seeds) of *Juniperus procera* contain the ferruginol compound. These results also demonstrate that, among the different parts (leaves, seeds, and roots) of *J. procera*, the root extract contained a higher amount of the ferruginol compound (4.4 μg), followed by the leaf (0.43 μg) and seed (0.42 μg) extracts (Figure 3b). In the literature, the ferruginol compound was detected in the berries of *J. procera* [23], but no previous report examined the quantification of the ferruginol compound in the different parts (leaves, seeds, and roots) of *J. procera* or any related plant as a comparative study. However, the authors in [53] stated that ferruginol was distributed in heartwood tissue or accumulated in intermediate wood and heartwood [52]. This study proves that the ferruginol compound might be accumulated in different plant parts. Moreover, this research reveals that the roots of *J. procera* are a new source of ferruginol.

4. Conclusions

In conclusion, this study confirmed that *J. procera* is rich in bioactive compounds, and that all parts (roots, leaves, and seeds) of *J. procera* contain the ferruginol compound. This research also revealed that the root extracts contained a significantly greater amount of ferruginol compared to that in other parts of the plant. Moreover, this research demonstrated that the mobile phase consisting of methanol and acetonitrile is suitable for the chromatographic separation of the ferruginol compound. Thus, these results support and justify the utilization of *J. procera* in traditional medicine. Further, research should be conducted to investigate the medicinal value of the root extract of *J. procera*.

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