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Crude extracts and essential oil of *Platycladus orientalis* (L.) Franco: a source of phenolics with antioxidant and antibacterial potential as assessed through a chemometric approach

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Abstract: The chemical composition, antioxidant properties and antibacterial activities of different extracts (water, ethanol, acetate ethyl and hexane) and essential oil from *Platycladus orientalis* leaves were analysed in this study. The essential oil was extracted by hydrodistillation, and its chemical composition was determined by gas chromatography coupled to mass spectrometry (GC-MS). The major components of the essential oil were α-pinene (40.2%), β-phellandrene (9.3), α-cedrol (9.2%) and β-caryophyllene (6.3%). Phytochemical analysis of crude extracts and essential oil revealed the richness of ethyl acetate and ethanolic extracts in total phenolic content (62.69 and 61.44 mg GAE/g DW, respectively). The antioxidant activities of all samples were evaluated by four different methods including total antioxidant activity (TAC), 1,1-diphenyl-2-picrylhydrazyl radical scavenging (DPPH), ABTS free radical scavenging activity (ABTS) and reducing power assay (RPA). Results showed that ethanol extract exhibited the highest antioxidant activity with all assays. The antimicrobial activity was evaluated by the disc diffusion method against five bacterial strains: *Staphylococcus aureus* CIP 53156, *Bacillus subtilis* CIP 5262, *Micrococcus luteus* CIP 5345, *Salmonella enterica* CIP 8039, and *Escherichia coli* CIP 53126. Results demonstrated that essential oil of *P. orientalis* leaves is more effective against the tested strains than extracts, especially against *Micrococcus luteus* and *Staphylococcus aureus*. This study argues the use of *P. orientalis* essential oil and ethanol extracts as a potential source of natural antioxidants and antimicrobial agents and supports the valorization of these natural substances in further application domains.

Key words: *Biota orientalis*, essential oil, crude extracts, antioxidants, antimicrobials, chemometric approaches

1. Introduction

Located on the shores of the Mediterranean Sea, Tunisia is a rich repository of medicinal plants that are commonly used in traditional food and folk medicine as well as for drug formulation and biotechnological applications (Aidi Wannes and Marzouk, 2016; Najjaa et al., 2017). The interest in plant extract is still increasing since their bioactive compounds have shown beneficial effects on health and the human body (Zhao et al., 2015; Ozkan et al., 2016; Altemimi et al., 2017; Li et al., 2021; Yu et al., 2021). Essential oils, phenolics, anthocyanins, carotenoids and vitamins of the medicinal plants are well known to have potent biological activities (Komes et al., 2011; Tungmunnithum et al., 2018; Golkar and Moattar, 2019; León-Méndez et al., 2019; Mancianti and Ebani, 2020; Yener et al., 2020) as anticancer (Andrade et al., 2018; Ishfaq et al., 2018; Fitsiou and Pappa, 2019), antimicrobial (Valdivieso-Ugarte et al., 2019; Reyes-Jurado et al., 2020), antiviral (Abou Baker et al., 2021; Javed et al., 2021; Mani et al., 2021), antibacterial (Bhardwaj et al., 2021), antioxidant (Xu et al., 2017; Farouk et al., 2021; Tafrihi et al., 2021) and antiinflammatory (Darwish et al., 2020; Spisni et al., 2020). *Platycladus orientalis* (L.) Franco, also known as *Biota orientalis* (L.) or *Thuja orientalis* (L.), is an evergreen coniferous tree that belongs to the Cupressaceae family, native to East Asia but introduced...
elsewhere in temperate climates, and characterized by broad or flattened shoots (Morgan, 1999; Shan et al., 2014). According to the IUCN red list of threatened species, *P. orientalis* is considered as near threatened (Group, 2011). It was commonly used as traditional Chinese medicine for thousands of years for the treatment of hemorrhages, hypertension, tuberculosis, cough, bronchitis, rheumatoid arthritis, and chronic tracheitis (Shan et al., 2014). In China, leaves have been used for their antibacterial properties and hair restoration; this property has been clinically demonstrated on mice with hot water extract of *P. orientalis* leaves (Zhang et al., 2013). According to ancient Chinese and Korean herbal textbooks, *Platycladus* seeds, considered to be very nutritious and fattening, have long been used to treat pruritus, dry and itchy skin. They have been also applied in the dermatological disorder of skin and hair, such as the haggard face, scabies, and alopecia (Shizhen, 2006). Furthermore, clinical studies and reports revealed a wide range of pharmacological properties and medicinal applications, namely anticancer, neuro-protective, antimicrobial, antiviral, antioxidant, and antiinflammatory (Emami et al., 2005; Shan et al., 2014). Indeed, *P. orientalis* leaves and needles extracts have been reported to have diuretic, antioxidant, antidiabetic, fungitoxic, antibacterial and neuroprotective effects (Nizam and Mushfiq, 2007; Guleria et al., 2008; Pearson et al., 2009; Jasuja et al., 2013; Mohadjerani et al., 2016; Seo et al., 2017) and could even be used in cosmetics (Zeng et al., 2017). Phytochemistry analyses of *P. orientalis* shed the light on many chemical constituents such as diterpenes and flavonoids from essentials oils (Lee et al., 2009; Ismail et al., 2013; Kim et al., 2013; Shan et al., 2014) or fatty acids from seeds oil (Lie et al., 1991). These constituents may be effective agents and have pharmacological bioactivity. Indeed, it has been reported that *P. orientalis* seeds have an inhibitory effect on 5α-reductase of mouse and could be used against male pattern baldness (Park et al., 2003). Also, in vitro assays performed by Kim et al. (Kim et al., 2010) showed that *Platycladus* extract inhibits inflammatory biomarkers, most likely through the antiinflammatory activity of a new labdane diterpenoid (Kim et al., 2013). Moreover, flavonoids of fruits displayed free radical scavenging and antielastase activities (Xu et al., 2009). Furthermore, essential oils and flavonoids from this tree showed cytotoxicity against cancer cells (Lee et al., 2010). In the course of our search for biological activities from medicinal plants, we found many reports on *Platycladus orientalis* in many countries but little is known about this species in Tunisia. Therefore, our study aimed to analyse the chemical composition of essential oil and to determine the phenolic content, antioxidant and antimicrobial capacities of *P. orientalis* leaves extracts and essential oil which may provide data and insights for its exploitation in several domains.

### 2. Material and methods

#### 2.1. Plant material

*Platycladus orientalis* leaves (Figure 1) were harvested from the region of Ariana (36.845707 latitude/10.195843 longitude) in March 2017. Five samples were collected from ten trees at distances exceeding 15 m to avoid sampling closely related individuals. Leaves were air-dried, ground powdered and then stored at room temperature in dark until analyses. After botanical identification, a voucher specimen (BO17) was submitted to the herbarium division of the Institute.

#### 2.2. Essential oil extraction and analysis

Following the European Pharmacopoeia method (Council of Europe et al., 2004) and with a Clevenger-type apparatus, the essential oil was obtained by hydrodistillation of 100 g of powdered leaves. Then, anhydrous sodium sulphate was used for drying over the essential oil, and the oil yield (%) was calculated based on the dried weight. Essential oil analysis was performed using a Hewlett Packard 5890 II GC equipped with a HP 5972 masse selective detector and an HP- (5%-phenyl)-methylpolysiloxane capillary column (L × ID : 30 m × 0.25 mm, film thickness: 0.25 μm). Helium was used as a carrier gas at a flow rate of 1.2 mL/min. The temperatures of the injector and transfer line were respectively set at 250 and 280 °C. The ionization mode was electron impact at 70eV and the mass range was recorded from m/z of 40–300. One microliter of diluted sample was injected following the splitless mode. Compounds identification was based on mass spectra (compared with Wiley 275. L, 6th edition mass spectral library) or with authentic compounds and then confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature as described by Adams (2007).

#### 2.3. Extract preparation

Polar and nonpolar solvents were used in this study. Fifty grams of dry powdered leaves were extracted with 500 mL of water, ethanol, ethyl acetate or hexane by maceration for 24 h. The extracts were vacuum filtered through Whatman No. 4 filter paper and then rotary evaporated to remove solvents. The dried extracts were stored at 4 °C in a refrigerator until analysis. The extraction yields (%) were calculated and given in Table 1.

#### 2.4. Determining phenolic compounds

*Total phenolic content (TPC)*

TPC was evaluated by the Folin-Ciocalteu method according to the protocol described by Tili et al. (2015) with slight modifications. One hundred twenty-five microliters of each diluted sample were mixed with 125 μL of Folin-Ciocalteu reagent. After a rest of 3 min, 1250 μL of sodium carbonate (Na₂CO₃, 7%) were added. Samples were then incubated in the dark for 90 min and the optical
density (OD) was read at 760 nm. Total phenolic contents were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW), and gallic acid was used for the calibration of the curve. Three repetitions were considered for all samples.

Total flavonoids content (TFC)

The aluminium chloride colorimetric method was used to determine the TFC in the extracts following the protocol of Nguyen and Eun (Nguyen and Eun, 2011) with minor modifications. Seventy-five microliters of NaNO$_2$ (7%) were mixed with 0.25 mL of extract. Then, 0.5 mL of NaOH (1 M) and 0.15 mL of AlCl$_3$ (10%) were added to the mixture. The total flavonoids content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

Condensed tannins content (CTC)

CTC was determined according to the method described by Oueslati et al. (2012). Five hundred microliters of each extract were added to a mixture of 2 mL of methanol-vanillin solution (4%) and 2 mL of sulphuric acid (25% v/v sulphuric acid solution in methanol). The mixture was incubated for 15 min in the dark and the absorbance was measured at 500 nm. CTC was expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

### 2.5. Antioxidant activity assays

Each sample was dissolved and diluted in order to prepare the series of concentrations for antioxidant assays. Chemicals references were used for comparative purposes in all assays.

**Total antioxidant activity (TAC)**

For the determination of TAC, a spectrophotometric method was used based on the reaction of reduction of Mo (VI) to Mo (V) by the extract, leading to the green phosphate/Mo (V) complex at acid pH (Prieto et al., 1999). One-tenth of a milliliter of each extract was added to 1 mL.

---

### Table 1. Total polyphenols, flavonoids and tannins contents in *Platycladus orientalis* crude extracts and essential oil.

<table>
<thead>
<tr>
<th></th>
<th>Yield (%)</th>
<th>TPC (mg GAE/g DW)</th>
<th>FC (mg QE/g DW)</th>
<th>TC (mg CE/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>0.33 ± 0.01$^c$</td>
<td>34.73 ± 1.3$^d$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>2.5 ± 0.4$^d$</td>
<td>37.93 ± 0.13$^c$</td>
<td>14.65 ± 1.43$^a$</td>
<td>7.48 ± 1.09$^b$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.6 ± 0.6$^a$</td>
<td>61.44 ± 4.01$^a$</td>
<td>28.1 ± 2.16$^a$</td>
<td>9.89 ± 1.65$^a$</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>2.9 ± 0.4$^d$</td>
<td>62.69 ± 3.93$^a$</td>
<td>10.23 ± 1.54$^a$</td>
<td>4.12 ± 0.7$^c$</td>
</tr>
<tr>
<td>Hexane</td>
<td>1.8 ± 0.1$^b$</td>
<td>42.16 ± 1.56$^a$</td>
<td>12.33 ± 1.26$^c$</td>
<td>2.15 ± 0.9$^d$</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM; SEM: standard errors of mean; means followed by different letters within the same column are significantly different (p ≤ 0.05).
of reagent solution (28 mM of sodium phosphate, 0.6 M of sulphuric acid and 4 mM of ammonium molybdate). Then, samples were incubated at 95 °C in boiling water. OD was measured after 90 min at 695 nm. The antioxidant capacity was expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW).

**DPPH assay**

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method was used to determine the free radical scavenging activity of the extracts, following the protocol of Hatano et al. (1988) with some modifications. For each sample, 250 µL were added to 1500 µL of DPPH solution (dissolved in methanol 10⁻⁴ M) as the free radical source. Then, mixtures were incubated for 60 min at room temperature in the dark. Afterward, the absorbance was measured at 517 nm. Inhibition (I) of DPPH free radical, expressed as a percentage, was calculated using the following equation: I (%) = 100 × (Ab–As)/As, where Ab is the absorbance of the control reaction and As the absorbance of the sample. The IC₅₀ is defined as the concentration of the sample required to scavenge 50% of the free radicals. All samples were analysed in triplicate. BHT (butylated hydroxytoluene) was used as a positive control.

**ABTS free radical scavenging activity**

ABTS⁺ assay was assessed following the methods described by Elfalleh et al. (2009). ABTS⁺ radical cation was produced by mixing 2.45 mM of potassium persulphate with 7 mM of ABTS solution. The mixture was conserved at room temperature in the dark for 16 h before use. Then, the ABTS⁺ solution was adjusted to an absorbance of 0.70 ± 0.02 at 734 nm after dilution with ethanol. Twenty-five microliters of each diluted sample or Trolox standard were added to 2 mL of ABTS⁺ solution. The absorbance was read at 734 nm.

Free radical scavenging activity of ABTS (%) = [(Ac–As)/Ac] × 100,

where Ac is the absorbance of the control and As the absorbance of the sample solution. ABTS scavenging activity is expressed as an IC₅₀ value (µg/mL).

**Reducing power assay**

The method of Oyaizu (Oyaizu, 1986) was followed to assess the reducing power. Samples at different concentrations were mixed (2.5/2.5/2.5 mL) with 1% potassium ferricyanide and sodium phosphate buffer (0.2 M; pH 6.6). The mixture was incubated for 20 min at 50 °C. Afterward, 2.5 mL of 10% trichloroacetic acid were added and the mixture was centrifuged at 3000 rpm for 10 min. Two and five-tenths milliliters of supernatant were mixed with 0.5 mL of 0.1% of ferric chloride and 2.5 mL deionised water, and the absorbance was measured at 700 nm. The EC₅₀ value (µg/mL) was the effective concentration at which the absorbance was 0.5% for the reducing capacity. BHT was used as a standard.

**2.6. Antimicrobial activity**

Disc diffusion assay was conducted for antibacterial activity of the extracts and essential oil according to the method described by Khammassi et al. (Khammassi et al., 2018). The reference strains used in this study included three gram-positive bacteria: Staphylococcus aureus CIP 53156, Bacillus subtilis CIP 5262, and Micrococcus luteus CIP 5345; and two gram-negative strains: Salmonella enterica CIP 8039 and Escherichia coli CIP 53126. Bacterial species were cultured on tryptone soy agar BK047HA (TSA) purchased from BIOKAR diagnostics (France). One hundred microliters of microorganisms' suspension adjusted to 10⁷ CFU/mL were spread on the appropriate agar medium plates. Sterile filter paper discs (6 mm diameter) were placed in the inoculated Petri dishes and were impregnated with 10 µL of essential oil or extract. Ampicillin (10 µg/disc) were used as a standard for bacteria. After incubation of bacteria at 37 °C for 24 h, antimicrobial activity was assessed by measuring the growth inhibition zone diameter (mm). For each treatment three replications were performed.

**2.7. Statistical analysis**

Data were subjected to one-way analysis of variance (ANOVA) using the SPSS 18.0 software package. Differences between means were tested through Student–Newman–Keuls and values of p ≤ 0.05 were considered significantly different (Ben Ayed et al., 2018). Principal component analysis (PCA) is one of the factor analysis methods that reduce data by defining the main axes (or principal components) from the monitored parameters. The observations are represented in relation to the main axes. In this study, PCA analysis was applied by using the “AMAP” package of R language (Lucas, 2019) in order to study interactions between physicochemical compounds of P. orientalis crude extracts and essential oil and the antioxidant and antimicrobial activities. Moreover, these relationships between chemical compounds and antioxidants, and antibacterial activities were studied by the Spearman correlation test using “pspearman” package (Savicky, 2014).

**3. Results and discussion**

**3.1. Extraction yield**

Four solvents with different polarities were chosen for the extraction of P. orientalis leaves. Upon comparison between the different extracts (Table 1), ethanol presented the highest extraction yield (4.6%) followed by ethyl acetate, water and hexane extracts (2.9%, 2.5%, and 1.8%, respectively), while essential oil was found to yield the lowest level (0.33%).

**3.2. Total phenolic, flavonoid and tannins contents**

The total phenolics content of essential oil and crude extracts of P. orientalis leaves varied significantly among
samples and ranged between 34.73 and 62.69 mg GAE/g DW (Table 1). Statistical analysis showed that ethyl acetate and ethanol extract were characterized by their higher content in total phenols (62.69 and 61.44 mg GAE/g DW, respectively). However, the lower content was noted for essential oil, hexane and water leaves extracts (34.73, 42.16 and 37.93 mg GAE/g DW, respectively). Ethanol extract exhibited also the highest flavonoid content (28.1 mg QE/g DW), followed by water and hexane extracts, whereas ethyl acetate extract possesses the lowest content (10.23 mg QE/g DW). Condensed tannins content of\textit{P. orientalis} extracts ranged from 2.15 to 9.89 mg CE/g DW. The highest content was obtained with ethanol, which was significantly different from the other extracts, while ethyl acetate and hexane extract have the lowest tannins (4.12 and 2.15 CE/g DW, respectively).

### 3.3. Essential oil composition
The essential oil composition of \textit{P. orientalis} leaves is reported in Table 2. The chemical analysis led to the identification of 21 compounds belonging to four subclasses of terpenic compounds and accounting for 98.6% of the total oil. Hydrocarbonated monoterpenes and hydrocarbonated sesquiterpenes were the two main subclasses (63.8% and 20%, respectively). The main constituents are α-pinene (40.2%) and β-phellandrene (9.3%) as hydrocarbonated monoterpenes, and β-caryophyllene (6.3%) and α-cedrene (4.6%) as hydrocarbonated sesquiterpenes, and α-cedrol (9.2%) as the only compound on the subclass of oxygenated sesquiterpenes. Similar results were obtained in previous studies (Ismail et al., 2013, 2015) reporting that \textit{P. orientalis} essential oils were characterized by their richness in α-pinene. However, a different result was

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Compounds</th>
<th>Subclass</th>
<th>Formula</th>
<th>RI</th>
<th>Area (%)</th>
<th>Identification methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-pinene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>939</td>
<td>40.2</td>
<td>RI, MS, Co-in</td>
</tr>
<tr>
<td>2</td>
<td>Sabinene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>968</td>
<td>1.2</td>
<td>RI, MS</td>
</tr>
<tr>
<td>3</td>
<td>β-pinene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>976</td>
<td>2.4</td>
<td>RI, MS</td>
</tr>
<tr>
<td>4</td>
<td>Myrcene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>991</td>
<td>2.3</td>
<td>RI, MS, Co-in</td>
</tr>
<tr>
<td>5</td>
<td>α-phellandrene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>1007</td>
<td>2.2</td>
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</tr>
<tr>
<td>6</td>
<td>δ-3-carene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>1011</td>
<td>2.6</td>
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<tr>
<td>7</td>
<td>β-phellandrene</td>
<td>HM</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>1032</td>
<td>9.3</td>
<td>RI, MS</td>
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<tr>
<td>8</td>
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<td>1088</td>
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<tr>
<td>10</td>
<td>α-terpin-4-ol</td>
<td>OM</td>
<td>C\textsubscript{10}H\textsubscript{16}O</td>
<td>1163</td>
<td>2.3</td>
<td>RI, MS</td>
</tr>
<tr>
<td>11</td>
<td>\textit{Iso} bornyl acetate</td>
<td>OM</td>
<td>C\textsubscript{10}H\textsubscript{16}O</td>
<td>1278</td>
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<tr>
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<tr>
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<tr>
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<td>HS</td>
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<td>1420</td>
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<tr>
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<td>1432</td>
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<tr>
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<tr>
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<td>Germacrene D</td>
<td>HS</td>
<td>C\textsubscript{15}H\textsubscript{12}</td>
<td>1478</td>
<td>2.9</td>
<td>RI, MS</td>
</tr>
<tr>
<td>19</td>
<td>β-selinene</td>
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<td>C\textsubscript{15}H\textsubscript{12}</td>
<td>1486</td>
<td>1.3</td>
<td>RI, MS</td>
</tr>
<tr>
<td>20</td>
<td>α-murrolene</td>
<td>HS</td>
<td>C\textsubscript{15}H\textsubscript{12}</td>
<td>1499</td>
<td>1.1</td>
<td>RI, MS</td>
</tr>
<tr>
<td>21</td>
<td>α-cedrol</td>
<td>OS</td>
<td>C\textsubscript{15}H\textsubscript{12}O</td>
<td>1596</td>
<td>9.2</td>
<td>RI, MS</td>
</tr>
</tbody>
</table>

Total identification (%): 98.6

Hydrocarbonated monoterpenes (HM, %): 63.8

Oxygenated monoterpenes (OM, %): 5.6

Hydrocarbonated sesquiterpenes (HS, %): 20

Oxygenated sesquiterpenes (OS, %): 9.2

obtained in Iran and Himalaya for whom Platycladus oils from Iran contained only 20.3% of α-pinene and a high level of δ-3-carene (14.2%), and those from Himalaya contained 21.8% of α-pinene and a high level of benzyl benzoate (19.1%) and δ-3-carene (10.5%) (Nickavar et al., 2003; Guleria et al., 2008). According to these studies, there are still some differences in the relative amounts of major and minor compounds found in the present study when compared with previous reports. Various factors could influence the levels of plant metabolites, including biotic and abiotic factors, which may lead to differences in secondary metabolite production, and differences in the quality and quantity of the secondary products isolated from the plant. Moreover, genetic background, soil types, the season of collection, and methods of extraction from plants could lead to differences in quantity and quality and may be attributed to the different results observed between the present study and other studies (Nickavar et al., 2003; Guleria et al., 2008; Saoud et al., 2013).

3.4. Antioxidant capacities

The antioxidant potential of plant extracts and their different modes of action is very complex. Thus, it is desirable to establish convenient methods for the evaluation of antioxidant activities of plant extracts (Dai Mumper, 2010). Therefore, the antioxidant activities of P. orientalis oil and extracts were evaluated by four methods. Table 3 details the results gathered in this study. The ethanol extract exhibited the lowest antioxidant activity with all methods compared to the other extracts derived from low polarity solvents, while the lowest activity was noted for essential oil (IC_{50} = 172 µg/mL) and then the highest activity was found in ethanol extract (42.33 μg/mL) and then the lowest activity was found in essential oil (78.13 mg EAG/g DW). The observed results could be attributed to the presence of phytochemicals present in the extract. Previous studies explain that the TAC is essentially due to the presence of phenolic compounds in the sample (Lu, 2001; Oueslati et al., 2012; Tili et al., 2015). Ethanol extract differed considerably and showed the highest antiradical activity with both DPPH and ABTS assay (IC_{50} = 45.23 and 63.2 µg/mL, respectively). However, the lowest activity was noted for essential oil (IC_{50} = 172 and 93.162 µg/mL, respectively). The antiradical activity could be due to the phenolic compounds such as polyphenols, flavonoids and tannins (Kumaran and Karunakaran, 2007). Statistical analysis showed that the ethanolic sample displayed the lowest EC_{50} (42.33 μg/mL) and then the highest iron-reducing potential. However, it displayed the lowest activity as compared to BHT (EC_{50} = 21.5 µg/mL). The reducing power of an extract might be due to its hydrogen-donating ability. In fact, extract might contain higher amounts of reductone that reacts with free radicals to stabilize and block radical chain reactions (Shimada et al., 1992). The results of this study showed the high variation of antioxidant activities between the extracts and essential oil of P. orientalis leaves. The reason is that the content and type of secondary metabolites involved in the evaluated bioactivities varied according to the extraction solvent. In fact, the polarities of secondary metabolites in plants varied greatly and therefore, it is necessary to select an adequate solvent for efficient extraction in quantity and quality of the molecules of interest. As well as, secondary metabolites have diverse nature, concentration ranges and physicochemical properties. Thus, each solvent is able to recover specific classes of secondary metabolites from a plant (Uysal et al., 2021).

3.5. Antimicrobial activity

The in vitro antibacterial activity of Platycladus orientalis crude extracts and essential oil was evaluated against five selected bacteria. The results are presented in Table 4. The

Table 3. Antioxidant capacities of crude extracts and essential oil of Platycladus orientalis leaves.

<table>
<thead>
<tr>
<th></th>
<th>TAC (mg EAG g⁻¹MS)</th>
<th>DPPH (IC_{50} µg mL⁻¹)</th>
<th>ABTS (IC_{50} µg mL⁻¹)</th>
<th>RPA (EC_{50} µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>78.13 ± 6.05c</td>
<td>172 ± 1.53a</td>
<td>93.16 ± 4.13a</td>
<td>135.6 ± 3.17a</td>
</tr>
<tr>
<td>Water</td>
<td>85.34 ± 12.69c</td>
<td>82.1 ± 2.90b</td>
<td>91.78 ± 5.2b</td>
<td>111.98 ± 2.8b</td>
</tr>
<tr>
<td>Ethanol</td>
<td>146.21 ± 7.11a</td>
<td>2345 ± 2.3e</td>
<td>63.2 ± 3.2c</td>
<td>52.3 ± 1.88e</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>138.42 ± 8.01b</td>
<td>76.33 ± 1.1c</td>
<td>85 ± 7.12c</td>
<td>96.1 ± 1.73d</td>
</tr>
<tr>
<td>Hexane</td>
<td>80.26 ± 5.9d</td>
<td>72.16 ± 2.18d</td>
<td>79.07 ± 4.21d</td>
<td>101 ± 2.64c</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>19.40 ± 0.10f</td>
<td>-</td>
<td>21.5 ± 0.85f</td>
</tr>
<tr>
<td>Trolox</td>
<td>-</td>
<td>-</td>
<td>10.3 ± 1.15f</td>
<td>-</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM; SEM: standard errors of mean; means followed by different letters within the same column are significantly different (p ≤ 0.05).
Table 4. Antibacterial activities of crude extracts and essential oil of Platycladus orientalis leaves (zone of inhibition in mm).

<table>
<thead>
<tr>
<th>Essential oil (10 µg/mL)</th>
<th>Crude extracts</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Ethanol</td>
</tr>
<tr>
<td>S. aureus</td>
<td>13.5 ± 0.33 b</td>
<td>9.5 ± 0.66cd</td>
</tr>
<tr>
<td>E. coli</td>
<td>11.5 ± 0.33b</td>
<td>10 ± 0.33b</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>10.33 ± 0.57b</td>
<td>9.33 ± 0.33b</td>
</tr>
<tr>
<td>M. luteus</td>
<td>15.5 ± 0.33b</td>
<td>10.5 ± 0.66c</td>
</tr>
<tr>
<td>S. enterica</td>
<td>11 ± 1.73b</td>
<td>10 ± 0.33bb</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM; SEM: standard errors of mean; means followed by different letters within the same line are significantly different (p ≤ 0.05).

essential oil of P. orientalis exhibited antibacterial activity against all the tested strains with a significant difference in the inhibitory activity (ranged from 10.33 to 15.5 mm). Staphylococcus aureus (13.5 mm) and Micrococcus luteus (15.5 mm) were the most sensitive strains. However, Escherichia coli, Bacillus subtilis, and Salmonella enterica showed less sensitivity. All the extracts were less efficient than the essential oil against the tested bacteria (9–10.5 mm). Statistical analysis showed significant difference between the effect of essential oil and extracts and the ampicillin against all strains. The higher sensitivity of gram-positive bacteria to P. orientalis essential oil can be explained by their outer peptidoglycan layer which was not an effective permeable barrier (Nostro et al., 2000). Leaves and cones essential oils of Platycladus orientalis have been reported to possess antifungal activity against phytopathogenic fungi. This activity was associated to their richness in monoterpens (Ismail et al., 2013). In fact, essential oils were characterized by their hydrophobicity rises bacterial cell permeability and membrane fluidity causing leakage (Sikkema et al., 1994). Previous studies strongly supported these changes in permeability and increase in membrane fluidity after treatment with terpenes which induce extensive leakage from bacterial cells or the exit of critical molecules and ions, and leading to death (Sikkema et al., 1994).

3.6. Correlation analysis between phytochemical content, antioxidant and antimicrobial activities

Interaction between physicochemical content (TPC, TC and FC) and the bioactivities datasets from Platycladus orientalis crude extracts and essential oil were studied by the multivariate analysis principal component analysis (PCA). Regarding the PCA performed by considering 12 parameters, the first two components (PCA1 and PCA2) explained 91.2% of the total variation. The first component (PCA1) explained 72.9% of the variation, followed by 18.3% for the second component (PCA2) (Figure 2). Axis 1 was positively correlated with water (0.959) and essential oil (0.958). On the other hand, axis 2 was positively correlated with Ethanol (0.816). In addition, axis 1 (PCA1) is linked to the most of variables FC (–0.945), TC (–0.821), TPC (–0.793), RPA (0.974), E. coli (0.953), B. Subtilis (0.858), and M. luteus (0.842), whereas axis 2 (PCA2) is rather linked to the S. enterica (–0.846) and S. aureus (0.724). Based on the correlation matrix generated by factorial analysis, we showed several associations between studied parameters. In fact, the antioxidant and the antibacterial activities of Platycladus orientalis crude extracts and essential oil were clearly influenced by their TPC, TC and FC. Among them, TPC was positively correlated with the antioxidant activity: TAC (0.974), while negatively correlated with the RPA (–0.802) and the two antimicrobial activities E. coli (–0.740) and M. luteus (–0.67). TC was positively correlated with DPPH (0.704) and TAC (0.613) and negatively associated with RPA (–0.787) and E. coli (–0.695). On the other side, FC was positively correlated with antioxidant activities DPPH (0.810) and TAC (0.635), whereas negatively correlated with the antioxidant activities RPA (–0.939), ABTS (–0.855), E. coli (–0.846) and B. subtilis (–0.827). In order to illustrate correlations between TPC, FC and TC chemical compounds with the antioxidant and the antibacterial activities of Platycladus orientalis crude extracts and essential oil, we conducted the Spearman’s correlation coefficient analysis. Thus, in terms of relation between antioxidant activity and the physicochemical content, two high significant correlations were observed (TPC/RPA (p = 0.037, r = –0.9) and TC/TAC (p = 0.037, r = –0.9)). On the other hand, only TPC has shown a correlation with the antibacterial activity against M. luteus (p = 0.005, r = –0.975) (Table 5).

4. Conclusion

Our results showed that leaves of Platycladus orientalis are rich in phenolic compounds, tannins, and flavonoids. Ethanol and ethyl acetate extracts are the richest in phenolic compounds as compared to the other extracts.
Chemical analysis of essential oil revealed their richness in monoterpenes particularly α-pinene. Crude extracts exhibited an important antioxidant activity, and the highest activity was obtained with ethanol extract, and it can be therefore considered a promising source of natural antioxidants. Regarding the antibacterial activity, among the tested strains, essential oil exerted the highest antimicrobial potential against *S. aureus* and *M. luteus*. The data obtained from the present study suggest the use of *Platycladus orientalis* as a source of antioxidants and antimicrobials. Nevertheless, other in vivo investigations are needed to assess their safety and efficiency.

**Table 5.** Spearman correlation coefficient analysis of TPC, FC and TC with the antioxidant and antibacterial activities of *Platycladus orientalis* crude extracts and essential oil.

<table>
<thead>
<tr>
<th>Variables</th>
<th>TPC</th>
<th>FC</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td><strong>Antioxidant activities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>0.8</td>
<td>0.104</td>
<td>0.7</td>
</tr>
<tr>
<td>DPPH</td>
<td>–0.2</td>
<td>0.747</td>
<td>0.3</td>
</tr>
<tr>
<td>ABTS</td>
<td>–0.7</td>
<td>0.188</td>
<td>–0.7</td>
</tr>
<tr>
<td>RPA</td>
<td>–0.9</td>
<td>0.037</td>
<td>–0.6</td>
</tr>
<tr>
<td><strong>Antibacterial activities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>–0.2</td>
<td>0.747</td>
<td>–0.3</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>–0.821</td>
<td>0.089</td>
<td>–0.667</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>–0.359</td>
<td>0.553</td>
<td>–0.821</td>
</tr>
<tr>
<td><em>M. luteus</em></td>
<td>–0.975</td>
<td>0.005</td>
<td>–0.205</td>
</tr>
<tr>
<td><em>S. enterica</em></td>
<td>–0.667</td>
<td>0.219</td>
<td>–0.359</td>
</tr>
</tbody>
</table>

TPC: total phenolic content, FC: flavonoids content, TC: tannins content.
Conflict of interest
The authors declare that they have no conflict of interest.

References


Conflict of interest
This article does not contain any studies conducted on human or animal subjects.

Ethical approval
This article does not contain any studies conducted on human or animal subjects.

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