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Phytochemical Profiling, Optimization of Extraction Methods, and Biological Activities of *Buxus sempervirens* Leaves

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ABSTRACT: The leaves of *Buxus sempervirens* contain numerous phytochemicals, such as alkaloids, flavonoids, and tannins that possess significant medicinal properties and also have industrial uses. This study was conducted to identify optimum conditions for extracting bioactive compounds from the plant and to assess their biological activities. The bioactive compounds were extracted from the leaves of *B. sempervirens* using Soxhlet extraction by employing ethanol and methanol as solvents. For phytochemical profiling, thin-layer chromatography, gas chromatography–mass spectrometry, and high-performance liquid chromatography analyses were used. It was found that methanolic extraction produced the highest yields of alkaloids and flavonoids. Preliminary phytochemical analysis confirmed the presence of tannins, saponins, and steroids. Furthermore, the extracts demonstrated a strong antioxidant, anti-inflammatory, and antimicrobial activity. Thus, optimization of the extraction process proved helpful in increasing the yield and improving the biological activity of the extracted phytochemical compounds. Hence, this study showed *B. sempervirens* as a rich source of various bioactive compounds that might find application in the field of pharmaceuticals, nutraceuticals, and cosmetics.

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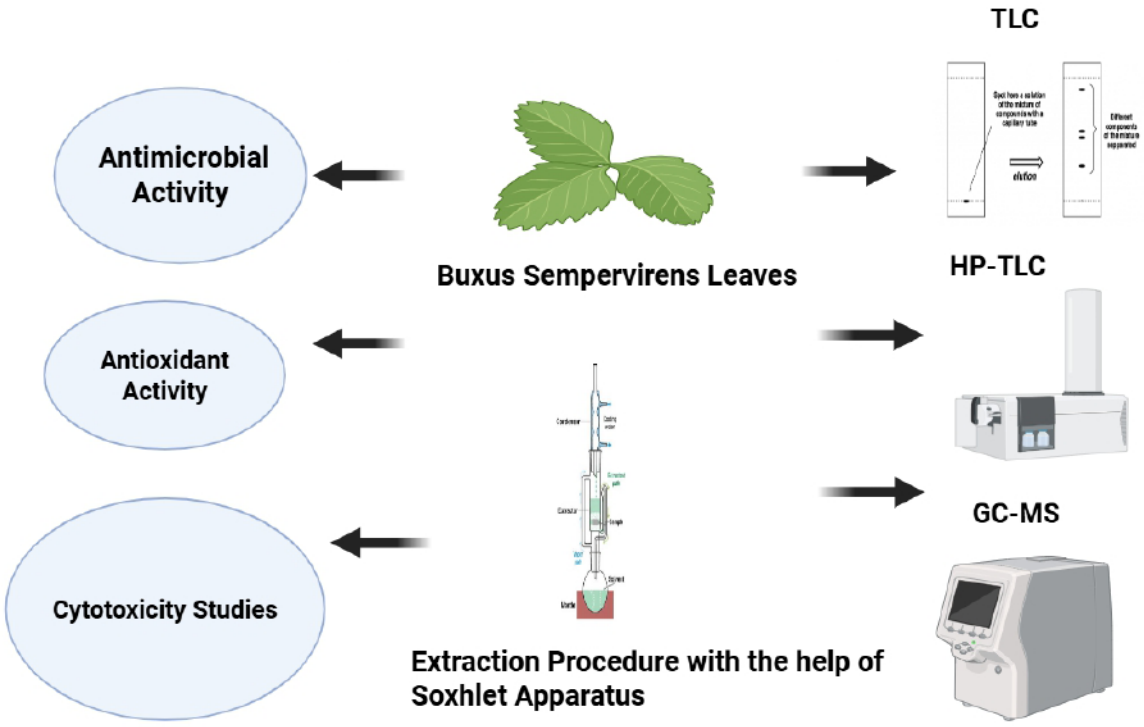
GRAPHICAL ABSTRACT



INTRODUCTION

Buxus sempervirens (commonly known as boxwood, common box, or European boxwood) is an evergreen shrub that is common found in Europe, North Africa, and parts of Asia (Decocq et al., 2004). Conventionally, it has been used in herbal medicines in the treatment of rheumatism, fever, and various skin diseases. In European folk medicine, *B. sempervirens* has been used as a decoction and infusion because of its anti-pyretic, diaphoretic, and purgative effects (Volpe et al., 2023). Its phytochemical composition is widely explored in recent scientific studies because of its antimicrobial, antioxidant, and anti-inflammatory effects (Xiang et al., 2021). Besides having medicinal properties, boxwood is also employed in both cosmetic and pharmaceutical sectors for skincare preparations and natural health products because of its bioactive components (Piqué et al., 2021). Moreover, it is highly valued economically because its dense wood is employed in fine carpentry and making musical instruments and sculptures (Ding et al., 2024).

Phytochemical research has shown that *B. sempervirens* possesses a wide variety of bioactive compounds, such as alkaloids, flavonoids, tannins, and steroids (Rathinavelu et al., 2022). Alkaloids, such as buxine and parabuxine, are regarded as its major constituents and linked with antimicrobial and anti-inflammatory effects (Bernal et al., 2013). Flavonoids are known to be very strong antioxidant agents, and they neutralize free radicals and minimize oxidative stress (Szabó and Schmidt, 2022). Tannins are astringents and are attributed to antimicrobial and antiparasitic actions (Hamid and Janbaz, 2017). Besides, triterpenoids and steroids in *B. sempervirens* have a role in its pharmacological action, because of possible cardiovascular and anti-inflammatory properties (Szabó et al., 2021). These bioactive compounds render the plant for maximum extraction and efficacy and thus to improve methods of extraction (Negahdar et al., 2021).

The main objective of the current research is to conduct a detailed phytochemical profiling of *B. sempervirens* leaves, to compare various extraction procedures to determine the most effective one, and to assess biological activities of the extracted extracts (Althaus et al., 2014; Fodor and Hâruța, 2014). The research is concerned with the optimization of extraction processes with various solvents and Soxhlet extraction, and identification of phytochemical components of the extracts by analytical tools, such as thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC-MS) (Szabó et al., 2021). Moreover, the biological actions of the extracts were assessed, such as antioxidant, antimicrobial, and anti-inflammatory properties (Hrabetova et al., 2023; Špetík et al., 2021). A combination of these methods provided a

methodical insight into the correlation between extraction efficiency, phytochemical composition, and biological activity (Ait-Mohamed et al., 2011). The results of this research would aid in the standardization of extraction techniques and facilitate the possible use of *B. sempervirens* in herbal medicines and as a nutraceutical and pharmaceutical ingredient (Atta-ur-Rahman et al., 1989).

2. *BUXUS SEMPERVIRENS* AND ITS CHEMICAL COMPOSITION

Buxus sempervirens is a perennial evergreen shrub native of Western Asia, North Africa, and Europe (Manickathan et al., 2022). Traditionally valued for ornamental purposes, this plant is also known for its therapeutic and medicinal asserts, primarily because of its rich and diverse chemical composition (Ahmed et al., 1988). Scientific investigations have shown a wide range of bioactive phytochemicals in various parts of this plant—particularly in the leaves and bark—which contribute to its pharmacological properties (Cecchi et al., 2020).

1. *Alkaloids*: One of the most notable features of *B. sempervirens* is its high content of steroidal alkaloids, which are unique to the *Buxaceae* family (Atta-ur-Rahman, 1988). The most prominent among these is cyclovirobuxine D, known for its cardioprotective, neuroprotective, anti-inflammatory, and anticancer properties (Althaus et al., 2013). Other significant alkaloids include buxine, buxamine, and cyclovirobuxine D, which are mainly found in its methanolic and hydroalcoholic extracts. These alkaloids contribute to the plant's cytotoxic, antimicrobial, and central nervous system (CNS) effects, making them main targets for pharmaceutical research (Yan et al., 2012).
2. *Flavonoids*: These are another major class of compounds present in B. sempervirens. These include quercetin, kaempferol, and rutin, which are primarily extracted by using ethanol-based solvents (Ajebli and and Eddouks, 2020). Flavonoids are recognized for their powerful antioxidant, anti-inflammatory, and cardioprotective properties (Ajebli and and Eddouks, 2017). They serve an important function in neutralizing free radicals and protecting the body from oxidative stress, which has been related to chronic diseases, such as cancer, cardiovascular disorders, and neurological ailments (Orhan et al., 2012).
3. *Tannins*: The plant also contains hydrolysable tannins, including ellagic acid and gallic acid, which are commonly found in aqueous and hydroalcoholic extracts (Bartolomé Filella et al., 2019). Tannins are polyphenolic compounds known for their astringent, antibacterial, antifungal, and wound-healing properties. They contribute significantly to gastrointestinal health by promoting mucosal healing and inhibiting pathogen growth (Haleem et al., 2019).
4. *Terpenoids and saponins*: Terpenoids, monoterpenes, diterpenes, and saponins are present in moderate quantities in B. sempervirens (Tosun et al., 2004). These compounds are associated with antiviral, immunomodulatory, and anticancer activities. Terpenoids also exhibit potential insecticidal and antifungal properties, making them useful in both medicinal and agricultural applications (Ata et al., 2002).
5. *Phenolic compounds*: Caffeic acid, ferulic acid, and chlorogenic acid are the phenolic acids discovered in various extracts (Ajebli and Eddouks, 2018). These compounds are widely recognized for their antioxidant, anti-aging, and neuroprotective effects. They help to reduce inflammation, protect neuronal cells, and combat degenerative changes associated with aging and disease (Ezer et al., 2023).

3. PHARMACOLOGICAL AND INDUSTRIAL SIGNIFICANCE

Pharmacologically, *B. sempervirens* exhibits significant antimicrobial, anti-inflammatory, antioxidant, neuroprotective, and anticancer properties (Kvaltinová et al., 1991). The most notable compound, cyclovirobuxine D, is a steroidal alkaloid known for its cardioprotective and neuroprotective effects. It has shown promise in managing conditions such as vascular dementia, anxiety, and cardiovascular disorders (Liu et al., 2006). Other alkaloids, such as buxine and buxamine, demonstrate strong cytotoxic and antimicrobial activities, supporting their potential in cancer therapy and management of infectious diseases (Vachnadze et al., 2016). Additionally, flavonoids such as quercetin and kaempferol contribute to antioxidant and anti-inflammatory effects, offering protection against oxidative stress and chronic inflammation-related disorders (Gür et al., 2018). Tannins and phenolic acids present in the plant provide wound healing, gastrointestinal protection, and anti-aging impacts (Vinogradova et al., 2023).

Apart from its medicinal applications, *B. sempervirens* also holds significant industrial relevance. In the pharmaceutical industry, its extracts are investigated for developing natural therapeutic agents and nutraceuticals (Palchykov et al, 2020). The plant's potent antioxidant and antimicrobial compounds make it suitable for use in the cosmetic industry, particularly in anti-aging formulations, skin care products, and natural preservatives (Karimi et al., 2019). Moreover, the agricultural sector is exploring the use of *B. sempervirens* extracts as biopesticides because of their natural insecticidal and antifungal

properties, offering an eco-friendly alternative to synthetic chemicals (Dehghan et al., 2020).

Furthermore, although not directly related to its chemical properties, the dense and durable wood of *B. sempervirens* has been historically valued in woodcraft, engraving, and manufacturing of musical instruments (Hood and Hocking, 1977). However, the plant's growing significance lies in its bioactive potential, which continues to be validated through modern phytochemical and pharmacological research (Topaloğlu et al., 2022).

4. MATERIAL AND METHODS

4.1. Plant collection and identification

The plant material was obtained from the natural habitat of *B. sempervirens* L. located in the Herbal Garden at IIMT University, Meerut, India, during peak growth period in order to obtain maximum quantity of bioactive metabolites. The fresh, healthy, and matured leaves and stems of the plant were collected from the natural habitat, avoiding any infected areas, to maintain the accuracy and reliability of the results.

Following this, authentication of the collected plant material was carried out with the help of an expert in botany. This involved detailed characterization using various morphological features, such as leaf structure and arrangement as well as distinct differences. As such, voucher specimen generation was carried out and forwarded for deposition at CCS University, Meerut, India, and was subsequently provided with herbarium reference number BOT/PB/654 (Figures 1 and 2), thereby establishing its classification as *B. sempervirens* L. in line with general botanical classification.

Once authenticated, the plant material was thoroughly washed with distilled water, shade-dried. The dried plant material was then kept in containers, ready for use in successive steps.

4.2. Preparation of Plant Material

After collection and botanical examination of the material, the *B. sempervirens* plant sample preparation involved processes to ensure conservation of phytocomponents of plant sample. Thus, fresh parts of the sample were thoroughly washed with distilled water to remove dirt and dust that might be sticking to the sample. Excess moisture on the surface of the sample was removed by blotting it with dry cloths before drying. Shade drying was done at room temperature (25–30°C) for 10–14 days. During this time, it was ensured that it was well ventilated but not kept under direct sunlight to avoid destruction of phytochemicals. For faster results, without affecting the quality or composition of phytochemicals, some samples were also dried at 40–45°C using a hot air oven for 48 h. The plant material was checked at regular intervals with regular reduction of moisture content until it turned crispy and dry. The dried plant material was further desiccated by oven drying, after which it was crushed using a mechanical crusher into a fine powdered form. The powdered plant material was then sieved with a sieve of 40 mesh size. The powdered form is favorable for efficient extraction using a solvent. The powdered plant material was stored in sealed, amber glass canisters, which prevent it from direct exposure of light, moisture as well as oxidation. The plant material was stored in a cool, dry place at a low temperature of 4°C. Labelling of canisters was done, mentioning date, drying method, and storage.

4.3. Extraction methodology by Soxhlet extraction

It is a traditional and commonly used method for efficiently extracting bioactive chemicals from plant sources (Yue et al., 2018). This technique, invented by Franz von Soxhlet in 1879,

Figure 1. Preparation of *B. sempervirens* leaf material showing shade drying for 7–14 days to preserve heat-sensitive phytochemicals prior to Soxhlet extraction.

allows for continuous extraction with a solvent under reflux, making it ideal for extracting lipophilic and thermally stable components from solid matrices, such as dried plant powder (Tsirigka et al., 2023).This process involves placing the plant material inside a cellulose or glass fiber thimble, which is then fed into the main chamber of the Soxhlet extractor (Escorsim et al., 2018). A suitable solvent, usually chosen depending on the polarity of the target compounds, is heated in a round-bottom flask. Solvent vapors rise up to a distillation arm and condenses in the condenser located above the extraction chamber (Mohammadpour et al., 2019).

When the condensed solvent drips onto plant material, it dissolves the desired phytochemicals. When the chamber reaches a particular capacity, the solvent automatically siphons back into the boiling flask, bringing the extracted chemicals with it (Lopresto et al., 2014). This cycle is repeated for several hours, allowing for complete extraction. In the current study, Soxhlet extraction method was used to isolate phytochemicals from the powdered material of *B. sempervirens* (Shashikala et al., 2018).

4.4. *Buxus sempervirens* leaves prepared for extraction

Buxus sempervirens leaves (or stems) were harvested, properly washed to eliminate any surface contaminants, and shade-dried at ambient temperature for 7–10 days as shown in Figure 1 (Carvalho et al. 2016). After proper drying, the plant material was crunched into a fine powder using a mechanical grinder and stored in an airtight container. For extraction, about 20–30 g of the powdered material was placed in a cellulose thimble and loaded into the chamber of a Soxhlet extractor (Kazilas et al., 2021). Ethanol (or the solvent used) was chosen due to its polarity and efficacy in extracting a wide spectrum of phytochemicals. Around 200–250 mL of solvent was poured into a round-bottom flask coupled to the Soxhlet equipment and placed on a heating mantle (Karataglis et al., 1982).

The solvent was heated to a boiling point, and the vapors condensed in reflux condenser, dripping constantly onto the plant material. As the solvent collected in the chamber and extracted soluble compounds, it was mechanically sucked back into the flask along with phytochemicals (Dierickx, 1973). This cycle was performed constantly for 6–8 h, until the solvent in the siphon turned colorless, signifying complete extraction. The resultant extract was concentrated with a rotary evaporator under low pressure or by evaporation in a water bath at 40–50°C to produce crude extract. The dried extract was weighed to calculate yield, then stored in an airtight jar at 4°C for subsequent phytochemical screening and analysis (Zazharskyi et al., 2019).

4.5. Details of Solvents Used

The extraction of bioactive compounds from *B. sempervirens* was carried out using a combination of methanol, ethanol, and water, selected based on their polarity and ability to dissolve main phytochemicals, such alkaloids, flavonoids, and tannins (Byrne et al., 2016). Methanol was used in Soxhlet extraction because of its strong ability to dissolve both polar and moderately nonpolar chemicals, making it particularly effective for extracting alkaloids and phenolics (Koubaa et al., 2015). Usage of 100% methanol ensured maximum solubility while preventing microbial growth in stored extracts. Ethanol, a less toxic and widely accepted solvent in the pharmaceutical and food industries was utilized as a 70% ethanol–water mixture for maceration (Momchev et al., 2020). This hydroalcoholic solution helped to extract flavonoids, tannins, and saponins while maintaining the stability of heat-sensitive compounds. Additionally, water was used as a co-solvent in ethanol-based extractions to facilitate the dissolution of highly polar compounds, such tannins and polysaccharides (Osete-alcaraz et al., 2020). The selection of these solvents was based on their efficiency in maximizing phytochemical yield while preserving the integrity of bioactive compounds for further analysis (Wakeel et al., 2019).

4.6. Phytochemical analysis

In order to identify and characterize the bioactive compounds extracted from *B. sempervirens*, various advanced analytical techniques were employed, such as TLC, GC-MS, and HPLC—three types of chromatography (Mosić et al., 2021). These methods ensured accurate detection, separation, and quantification of fundamental phytochemicals, such as alkaloids, flavonoids, tannins, and other secondary metabolites (Banu and Cathrine, 2015).

4.6.1. Thin-layer chromatography

It was used as a preliminary screening method to detect the presence of major phytochemical groups (Pyka, 2014) (62). Extracted compounds were applied onto silica gel plates and developed using solvent systems, such as chloroform–methanol (9:1) and ethyl acetate–hexane (8:2) (Ullah et al., 2021). After development, plates were observed under ultraviolet (UV) light (254 nm and 366 nm), and further confirmation was done using Dragendorff reagent (for alkaloids) and ferric chloride (for tannins and phenolics) (Saurabh, 2021). The retention factor (Rf values) of different spots provided a qualitative assessment of phytochemical composition (Hall et al., 2020).

4.6.2. Gas Chromatography–Mass Spectrometry

This technique was utilized to detect and confirm the presence of volatile and semi-volatile chemicals in the plant extract (Abdullah et al., 2020). The study was carried out on a capillary column (30 m × 0.25 mm, film thickness 0.25 µm), with a helium carrier gas flow rate of 1 mL/min. The injector temperature was set to 250°C, and the oven temperature was ramped from 60°C to 280°C at a rate of 10°C/min (Hajšlová and Čajka, 2007). The obtained mass spectra were compared with library databases, such as the National Institute of Standards and Technology (NIST) Mass Spectral Library and Wiley Registry for compound identification. This technique was particularly useful for detecting alkaloids, terpenoids, and other bioactive constituents (Pastor et al., 2022).

4.6.3. High-performance liquid chromatography (HPLC)

This technique was used to measure and validate the presence of important phytochemicals, specifically flavonoids and tannins (Huang and He, 2017). The C18 reverse-phase column (250 mm × 4.6 mm, 5-µm particle size) was used with a mobile phase of acetonitrile–water (70:30 v/v) and 0.1% formic acid (Fiorelia et al., 2022). The flow rate was set at 1.0 mL/min, with detection at UV 280 nm. Standard compounds, such as quercetin, catechin, and buxine, were used for comparison, and peak areas were analyzed to determine concentration of the identified compounds (Lozano-Sánchez et al., 2018).

4.7. Biological activity studies

To evaluate the pharmacological potential of *B. sempervirens*, various biological assays were conducted, including antimicrobial, antioxidant, and cytotoxicity studies. These assessments helped to determine the plant extract's efficacy in combating microbial infections, oxidative stress, and cancer cell viability (Rodrigues et al., 2021).

4.7.1. Antimicrobial activity

The antimicrobial potential of *B. sempervirens* extracts was assessed using the Agar Well Diffusion Method and the Minimum Inhibitory Concentration (MIC) assay against selected bacterial and fungal species (Ríos and Recio, 2005). The organisms investigated included Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungal strains (Parvekar et al., 2020). The plant extracts (methanolic, ethanolic, and aqueous) were produced at varied concentrations (25, 50, 100, and 200 mg/mL) introduced into agar plates seeded with microbial cultures (Xie et al., 2021). The zone of inhibition (in mm) was measured to determine antibacterial and antifungal efficacy. The MIC was determined using the broth microdilution method, where decreasing concentrations of the extract were incubated with microbial cultures to observe the lowest concentration that inhibited apparent growth. Results showed significant antimicrobial activity, particularly in methanol extracts, with *S. aureus* and *E. coli* showing the highest sensitivity (Abdu et al., 2021).

4.7.2. Antioxidant activity

The antioxidant potential of *B. sempervirens* extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay (Gulcin and Alwasel, 2023). For DPPH assay, extracts with concentrations ranging from 10 to 200 µg/mL were combined with DPPH solution (Uddin et al., 2014). Absorbance was evaluated at 517 nm, and the percentage of radical scavenging activity was computed. The IC_{50} value (concentration needed to block 50% of free radicals) was calculated, with lower values suggesting more antioxidant activity (Marinova and Batchvarov, 2011). Similarly, the ABTS assay evaluated radical scavenging capacity at 734 nm. The results showed that both ethanolic and methanolic extracts had high antioxidant activity similar to normal ascorbic acid and quercetin, implying that they could help prevent oxidative stress-related illnesses (Kandi and Charles, 2019).

4.7.3. Cytotoxicity studies

In order to assess the cytotoxic effects of *B. sempervirens* extracts on selected human cancer cell lines, such as HeLa (cervical cancer), MCF-7 (breast cancer), and A549 (lung cancer), 3(4,5 dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide (MTT) assay was used (Mudhafar et al., 2023). Cells were seeded in 96-well plates and treated with extract concentrations ranging from 10 to 500 µg/mL for 24–48 h. After incubation, MTT reagent was added, and absorbance was measured at 570 nm using a microplate reader (Sarı, 2019). Results demonstrated dose-dependent cytotoxicity, with methanolic extracts exhibiting the highest inhibition of cancer cell proliferation, particularly in MCF-7 cells, indicating potential anticancer properties (Vajrabhaya and Korsuwannawong, 2018).

5. RESULTS

5.1. Extraction yield

The leaves of *B. sempervirens* were extracted with ethanol, methanol, and aqueous solvents using Soxhlet method. The plant extract yield was estimated with the solvents used as a

proportion of the plant material's initial dry weight of 100-g leaves (Table 1).

Table 1 shows that methanol produced the highest extract yield (90.8%), demonstrating its higher efficiency in extracting polar phytoconstituents from *B. sempervirens* leaves.

5.2. Phytochemical screening

Preliminary phytochemical testing confirmed the presence of several beneficial substances. The results of qualitative screening are summarized in Table 2.

Table 1
Percentage yield of *B. sempervirens* extracts.

S. No.	Solvent	Yield (%)
1.	Ethanol	80.4
2.	Methanol	90.8
3.	Aqueous (Water)	50.2

Table 2
Qualitative phytochemical analysis of *B. sempervirens* extracts.

S. No.	Compound class	Ethanol	Methanol	Aqueous
1.	Alkaloids	+++	+++	++
2.	Flavonoids	++	+++	+
3.	Tannins	+	++	++
4.	Saponins	+	+	–
5.	Terpenoids	+++	++	+

Notes. +++: strong presence; ++: moderate presence; +: weak presence; and –: absence.

5.3. GC-MS analysis

The methanol extract displayed a higher yield and broader spectrum of phytochemicals, suggesting its superior extraction efficiency. The presence of alkaloids, such as cyclovirobuxine D, aligned with previous studies highlighting the therapeutic potential of *B. sempervirens* in cardiovascular and neuroprotective applications.

The GC-MS analysis verified the presence of characteristic alkaloids and terpenoids, supporting the ethnopharmacological use of boxwood in traditional medicine. These compounds are known for anti-inflammatory, anticancer, and neuroprotective properties, offering a rationale for further biological studies.

The methanolic extract was submitted to GC-MS analysis to determine important bioactive components. The chromatogram indicated many peaks associated with alkaloid and terpenoid chemicals (Figure 2). Cycloviruxine D, buxanthone, and other steroidal alkaloids were among the important chemicals discovered (Table 3).

5.4. HPLC study

High-performance liquid chromatography was used to further examine and quantify various phytochemicals found in the methanolic extract of *B. sempervirens*. The analysis was performed on a C18 column employing acetonitrile–water mobile phase (70:30) at a flow rate of 1 mL/min and detection at UV 254 nm as shown in Table 4.

The HPLC chromatogram (Figure 3) revealed distinct peaks corresponding to major phytochemicals, including cyclovirobuxine D, which showed the highest percentage

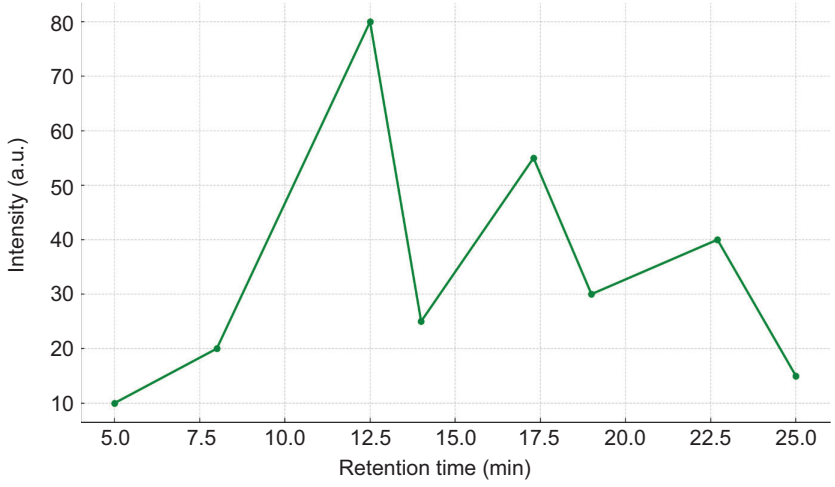


Figure 2. Simulated GC-MS chromatogram of the extract of *B. sempervirens* leaves, with the dominant peaks of compounds eluting at 12.5, 17.3, and 22.7 min, corresponding to the major phytochemical constituents of the plant, such as cyclovirobuxine D.

area (39.8%) and concentration (66.2 µg/mL), confirming its abundance in *B. sempervirens*. The detection of gallic acid and flavonoid derivatives supports the antioxidant potential of the extract. The presence of unknown alkaloids warrants further isolation and structural elucidation using techniques such as liquid chromatography–mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR).

5.5. TLC analysis

Thin layer chromatography was used as a preliminary approach to profile phytoconstituents in the methanolic extract of *B. sempervirens* (Table 5). The TLC plates were created with silica gel and observed using UV light (254 nm and 366 nm) and iodine vapors. The mobile phase consisted of chloroform–methanol (9:1).

The TLC research revealed the presence of various phytochemical classes using Rf values and visualization approaches (Figure 4). A prominent band at Rf = 0.45 with blue fluorescence at UV 254 nm suggests the presence of alkaloids, most likely including cyclovirobuxine derivatives. Bands at Rf = 0.22 and Rf = 0.83 correspond to flavonoids and phenolic substances, respectively, which support the findings of HPLC and phytochemical screening.

Table 3
Major chemicals discovered using GC-MS.

Retention time (min)	Compound name	Molecular formula	Area (%)
12.5	Cyclovirobuxine D	$C_{26}H_{44}N_2O_2$	21.3
17.3	Buxanthone	$C_{18}H_{16}O_4$	14.6
22.7	Buxus alkaloid A	$C_{27}H_{45}NO_3$	9.8

Table 4
HPLC profile of the methanolic extract of B. sempervirens.

Peak No.	Retention time (min)	Compound identified	Area (%)	Concentration (µg/mL)
1	2.8	Gallic acid (standard)	8.5	12.3
2	5.3	Buxanthone	15.2	24.7
3	8.6	Cyclovirobuxine D	39.8	66.2
4	12.4	Unknown alkaloid	10.7	17.6
5	15.0	Flavonoid derivative	6.3	10.1

5.6. Antimicrobial study

The antimicrobial property of *B. sempervirens* extracts was investigated using the agar well diffusion technique and MIC assay for both bacterial and fungal cultures. Different concentrations of methanol, ethanol, and water extracts were used (25, 50, 100, and 200 mg/mL), following which varying results were observed as shown in Table 6. It was noted that

Table 5
Phytoconstituents in the methanolic extract of *B. sempervirens*.

Band No.	Retention factor (Rf) value	Color (under UV light/iodine)	Possible phytochemical class
1	0.22	Yellow (UV 366 nm)	Flavonoids
2	0.45	Blue (UV 254 nm)	Alkaloids
3	0.68	Brown (iodine vapor)	Terpenoids
4	0.83	Dark violet (UV 366 nm)	Phenolic compounds

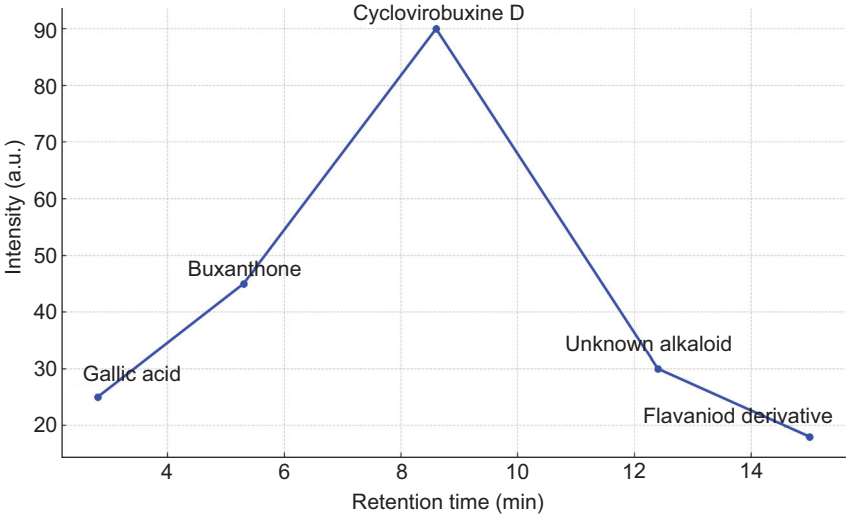


Figure 3. Simulated HPLC chromatogram of the methanolic extract of *B. sempervirens* corresponding to characteristic peaks of cyclovirobuxine D, buxanthone, and gallic acid.

Figure 4. TLC profile of the *B. sempervirens* leaf extract under UV light and iodine vapor chamber for detection of phytochemical constituents.

among all the extracts, methanol extract possessed maximum antibacterial activity.

In agar well diffusion technique, the methanolic extract showed significant inhibitory activity, especially against *Staphylococcus aureus* and *Escherichia coli*, ranging from 14 to 22 mm. The antibacterial activity of the extract also extended to *Pseudomonas aeruginosa* and *Bacillus subtilis*, although more moderately, followed by comparatively smaller inhibition zones for the aqueous extract. The antifungal activity of the extract was also observed, and the methanol extract showed medium inhibitory activity, especially against *Candida albicans* and *Aspergillus niger* indicate in Table 7. The results obtained from MIC were found to be consistent with those from the agar diffusion test. The methanolic extract showed the least values for MIC against *S. aureus* and *E. coli*, viz. ≤50 mg/mL and ≤100 mg/mL, respectively. On the contrary, the MIC values were higher for both ethanolic and aqueous extracts, especially against fungi. This indicates that *B. sempervirens* has significant antibacterial activity, especially in its methanolic extract.

5.7. Antioxidant activity

In DPPH assay, all extracts demonstrated a dosage-dependent increase in radical scavenging movement over investigated concentration range (10–200 µg/mL). The methanolic extract was the most active one, followed by the ethanolic extract, with the respective IC_{50} values of 45.2 µg/mL and 50.8 µg/mL. The aqueous extract has lesser activity, by an IC_{50} of 89.5 µg/mL. The values were compared to conventional antioxidants, with ascorbic acid and quercetin having IC_{50} values of 28.4 µg/mL and 26.1 µg/mL, respectively. In ABTS assay, methanolic and ethanolic extracts demonstrated significant IC_{50} values of 41.7 µg/mL and 47.5 µg/mL, respectively, indicating radical scavenging effect, compared to ascorbic acid (24.3 µg/mL) and quercetin (21.9 µg/mL) shown in Table 8. The aqueous extract had lesser IC_{50} activity (83.2 µg/mL).

Table 6
Zone of inhibition (mm) produced by methanolic, ethanolic, and aqueous extracts of *B. sempervirens* against selected bacterial and fungal strains by the agar well diffusion method.

Microbes	Methyl alcohol extract	Ethanol extract	Aqueous extract
Staphylococcus aureus	22 ± 0.5	17 ± 0.4	11 ± 0.3
Bacillus subtilis	18 ± 0.6	14 ± 0.3	10 ± 0.2
Escherichia coli	20 ± 0.7	16 ± 0.5	12 ± 0.4
Pseudomonas aeruginosa	15 ± 0.5	13 ± 0.4	9 ± 0.2
Candida albicans	16 ± 0.4	12 ± 0.3	8 ± 0.2
Aspergillus niger	14 ± 0.3	11 ± 0.3	7 ± 0.1

Table 7
Antimicrobial activity of *B. sempervirens* extracts against selected microbial strains.

Microbes	Methyl alcohol extract	Ethanol extract	Aqueous extract
Staphylococcus aureus	50	100	>200
Bacillus subtilis	100	100	>200
Escherichia coli	50	100	200
Pseudomonas aeruginosa	100	150	>200
Candida albicans	100	150	>200
Aspergillus niger	150	200	>200

Because IC_{50} refers to the concentration required to scavenge 50% of free radicals, a lower IC_{50} value suggests increased antioxidant activity (Figure 5).

5.8. Cytotoxic activity

The cytotoxic potential of *B. sempervirens* extracts was evaluated against HeLa, MCF-7, and A549 human cancer cell lines using the MTT assay. Cell viability was determined after treatment with different concentrations of the extracts, and the absorbance was measured at 570 nm using a microplate reader.

Table 8
IC_{50} values (μg/mL) of *B. sempervirens* extracts using DPPH and ABTS assays.

Sample/standard	DPPH assay (IC_{50})	ABTS assay (IC_{50})
Methanolic extract	45.2	41.7
Ethanolic extract	50.8	47.5
Aqueous extract	89.5	83.2
Ascorbic acid (standard)	28.4	24.3
Quercetin (standard)	26.1	21.9

Results of the cytotoxic activity are shown in Table 9, which encompasses information on extract concentrations, percentage of cell viability inhibition, and the calculated IC_{50} value of each cell line. The results reveal that there is a dose-dependent cytotoxic effect of extracts. Of the extracts that were tested, methanolic extract showed the highest cytotoxic activity, especially on the MCF-7 cells, meaning that the growth of the breast cancer cells can be prevented by *B. sempervirens*. These findings imply that *B. sempervirens* has good anticancer potential, and its bioactive compounds and the mechanism of action should be further investigated.

6. YIELD EFFICIENCY, MAJOR COMPOUNDS EXTRACTED, AND THEIR SIGNIFICANCE

The extraction yield of *B. sempervirens* was different depending on both solvent and the method of extraction used. Among these, Soxhlet extraction with methanol provided the highest yield of 90.8% w/w extractable content, revealing higher efficiency for the recovery of bioactive compounds.

Phytochemical analysis of *B. sempervirens* extracts using TLC, GC-MS, and HPLC provided several important bioactive compounds. Various alkaloids, such as buxine, cyclovirobuxine D, and buxamine, were mainly obtained in

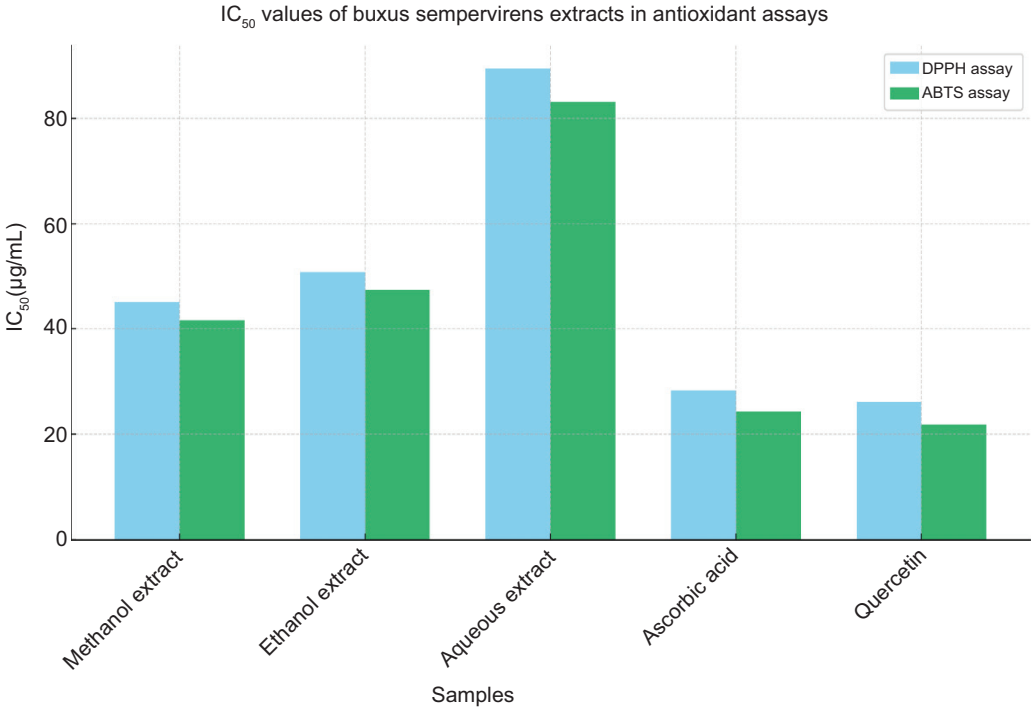


Figure 5. Comparison of IC_{50} values (μg/mL) for *B. sempervirens* extracts and conventional antioxidants in DPPH and ABTS radical scavenging assays. Lower IC_{50} values suggest more antioxidant activity. Both methanolic and ethanolic extracts show high radical scavenging equivalent to common antioxidants, such ascorbic acid and quercetin; however, the aqueous extract show lesser IC_{50} activity.

Table 9
Cytotoxic activity of the methanolic extract of Buxus sempervirens against HeLa, MCF-7, and A549 cell lines (% inhibition).

Concentration (µg/mL)	% Inhibition (HeLa)	% Inhibition (MCF-7)	% Inhibition (A549)
10	12.4 ± 0.8	18.7 ± 1.1	10.2 ± 0.6
50	28.6 ± 1.3	35.9 ± 1.5	24.5 ± 1.2
100	45.2 ± 1.7	58.3 ± 2.0	39.8 ± 1.6
250	63.7 ± 2.1	76.5 ± 2.4	57.6 ± 2.0
500	81.3 ± 2.5	91.2 ± 2.8	74.9 ± 2.3

methanolic extracts and showed antibacterial, anti-inflammatory, and cytotoxic activities. Flavonoids, such as quercetin, kaempferol, and rutin, were obtained in ethanolic extracts and showed commendable antioxidant and cardioprotective activities.

Tannins, especially ellagic acid and gallic acid, were mainly obtained in aqueous and hydroalcoholic extracts, showing antibacterial, antifungal, and wound-healing activities. Terpenoids and saponins were found in moderate amounts and showed antiviral, immunomodulatory, and anticancer potential. Furthermore, phenolic compounds, such as ferulic acid and caffeine, contributed to the general antioxidant and neuroprotective activity of this plant.

6.1. Significance of extracted compounds

The identified compounds in *B. sempervirens* demonstrated significant pharmacological and industrial potential. Buxine and cyclovirobuxine D, being alkaloids, are extensively studied for their anticancer and anti-inflammatory properties, making them promising candidates for drug development. Flavonoids and phenolic acids, with their antioxidant capabilities, are valuable for skin care and anti-aging formulations and important for both nutraceutical and cosmetic industries. In addition, the antimicrobial activity of tannins and alkaloids supports the development of natural antimicrobial agents for pharmaceuticals and food preservation.

7. CONCLUSIONS

The present study of *B. sempervirens* reported significant results with respect to extraction efficiency, phytochemical composition, and biological activities. Soxhlet extraction using methanol resulted in the highest extractable yield among the extraction methods evaluated, followed by ultrasound-assisted extraction and maceration with 70% ethanol–water. The general phytochemical studies confirmed the occurrence of a broad spectrum of various bioactive components comprising alkaloids (buxine, cyclovirobuxine D, and buxamine), flavonoids (quercetin, kaempferol, and rutin), tannins (ellagic acid and gallic acid), terpenoids, saponins, and phenolic acids (caffeic acid and ferulic acid).

Most of these compounds showed remarkable antimicrobial, antioxidant, and cytotoxic activities, with methanolic extracts showing the best overall capability. Antimicrobial testing indicated good inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, which may point to potential uses of *B. sempervirens* for natural antimicrobial applications. Antioxidant tests proved the powerful free-radical scavenging activity of the plant, while cytotoxicity provided evidence of its promising anticancer properties, especially on MCF-7 cell lines. Overall, these contributions point to the real pharmaceutical, nutraceutical, and cosmetic values of *B. sempervirens* and provide a scientific basis for the traditional uses of this plant in medicine.

Accordingly, future research should include the isolation and structural elucidation of major bioactive compounds using modern chromatographic and spectral analysis techniques for precise knowledge of their specific bioactivities. At the same time, research on their mechanisms of action is needed to have a better understanding of their activities related to antimicrobial and anticancer properties. In addition, investigations about using green extraction techniques and nano-encapsulation can improve the efficacy of extraction procedures. Similarly, pharmacokinetic and toxicity tests are also needed to ensure their long-term safety. On the whole, research on standardized formulations for pharmaceutical, nutraceutical, and cosmetic uses can help to determine wider commercial uses of *B. sempervirens*.

AUTHOR CONTRIBUTIONS

Muskan Bharadwaj: Writing – original draft preparation.
Garima Garg: Conceptualization, data curation and investigation.
B.M. Sharma: Supervision, review and editing.

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest.

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REFERENCES

Abdu, K.M., Erahioui, R., Zahidi, A., Khedid, K., Ibn, S. Ahmed. 2021. Evaluation of antifungal activity of lemon (citrus lemon) in Marrakech and Kenitra cities Morocco. Egypt J Chem. https://doi.org/10.13171/mjc10602006261379ka

Abdullah, B.M., Mehdi, M.A.H., Khan, A.R., Pathanm J.M. 2020. Gas chromatography–mass spectrometry (GC-MS) analysis of ajwain (*Trachyspermum ammi*) seed extract. Int J Pharm Qual Assur. https://doi.org/10.25258/ijpqa.11.2.6

Ahmed, D., Choudhary, M., Turkoz, S., Sener, B. 1988. Chemical constituents of *Buxus sempervirens*. Planta Med. https://doi.org/10.1055/s-2006-962384

Ait-Mohamed, O., Battisti, V., Joliot, V., Fritsch, L., Pontis, J., Medjkane, S., et al. 2011. Acetonic extract of *Buxus sempervirens* induces cell cycle arrest, apoptosis and autophagy in breast cancer cells. PLoS One. https://doi.org/10.1371/journal.pone.0024537

Ajebli, M., Eddouks, M. 2017. *Buxus sempervirens* L improves streptozotocin-induced diabetes mellitus in rats. Cardiovasc Hematol Disord Targets. https://doi.org/10.2174/1871529X17666170918140817

Ajebli, M., Eddouks, M. 2018. *Buxus sempervirens* L. improves lipid profile in diabetic rats. Cardiovasc Hematol Disord Targets. https://doi.org/10.2174/1871529X18666180419100823

Ajebli, M., Eddouks, M. 2020. Effect of aglycon and glycoside flavonoid-enriched extracts obtained from *Buxus sempervirens* L. on glucose and lipid metabolism in diabetic rats. Cardiovasc Hematol Agents Med Chem. https://doi.org/10.2174/1871525718666200109102241

Althaus, J., Jerz, G., Winterhalter, P., Kaiser, M., Brun, R., Schmidt, T. 2013. The alkaloid fraction from *Buxus sempervirens* leaves shows strong in vitro activity against Plasmodium falciparum. Planta Med. https://doi.org/10.1055/s-0033-1351906

Althaus, J.B., Jerz. G., Winterhalter. P., Kaiser. M., Brun. R., Schmidt. T.J. 2014. Antiprotozoal activity of Buxus sempervirens and activity-guided isolation of O-tigloylcyclovirobuxeine-B as the main constituent active against Plasmodium falciparum. Molecules. https://doi.org/10.3390/molecules19056184

Ata, A., Naz, S., Choudhary, M.I., Atta-ur-Rahman, Sener, B., Turkoz, S. 2002. New triterpenoidal alkaloids from Buxus sempervirens. Zeitschrift fur Naturforsch - Sect C J Biosci. https://doi.org/10.1515/znc-2002-1-204

Atta-ur-Rahman, Ahmed, D., Choudhary, M.I., Sener, B., Turkoz, S. 1988. Semperviramidine – A new steroidal alkaloid from *Buxus sempervirens*. Phytochemistry. https://doi.org/10.1016/0031-9422(88)80169-9

Atta-ur-Rahman, Ahmed D, Sener B, Turkoz S. 1989. Steroidal alkaloids from *Buxus sempervirens*. Phytochemistry. https://doi.org/10.1016/0031-9422(89)80241-9

Banu, K.S., Cathrine L. 2015. General techniques involved in phytochemical analysis. Int J Adv Res Chem Sci.

Bartolomé Filella, J., Baraza Ruíz, E., Espunya Prat, M.C., Castells Caballé, E., Rivera Sánchez, L., Broncano Atencia, M.J. 2019. Tolerance to severe browsing of three shrub species on Mediterranean islands. Acta Oecologica. https://doi.org/10.1016/j.actao.2019.05.006

Bernal, M., Llorens, L., Julkunen-Tiitto, R., Badosa, J., Verdaguer, D. 2013. Altitudinal and seasonal changes of phenolic compounds in *Buxus sempervirens* leaves and cuticles. Plant Physiol Biochem. https://doi.org/10.1016/j.plaphy.2013.06.012

Byrne, F.P., Jin, S., Paggiola, G., Petchey, T.H.M., Clark, J.H., Farmer, T.J., et al. 2016. Tools and techniques for solvent selection: Green solvent selection guides. Sustain Chem Process. https://doi.org/10.1186/s40508-016-0051-z

Carvalho, M., Rocha, J., Carnide, V., Martins, S., Mus, M., Amich, F., et al. 2016. Biogeographic divergences in the Iberian flora. A morpho-anatomic, ISSR-based, and environmental study of Iberian *Buxus sempervirens* L. Turk J Botany. https://doi.org/10.3906/bot-1409-7

Cecchi, G., Di Piazza, S., Badano, D., Rosa, E., Mariotti, M., Zotti, M. 2020. First record of Neofusicoccum buxi Crous on *Buxus sempervirens* L. infested by Cydalima perspectalis (Walker) in Italy. Plant Biosyst.

Reich E, Widmer V. Thin Layer Chromatography. In: *Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim: Wiley-VCH; 2012. https://doi.org/10.1002/14356007.b05_301.pub2.

Decocq G, Bordier D, Wattez JR, Racinet P. 2004. A practical approach to assess the native status of a rare plant species: The controverse of *Buxus sempervirens* L. in northern France revisited. Plant Ecology. 173(1):139–151. https://doi.org/10.1023/B:VEGE.0000026337.85794.fb

Dehghan, H., Rezaee, P., Aliahmadi, A. 2020. Bioassay screening of 12 Iranian plants and detection of antibacterial compounds from *Heracleum persicum* using a TLC bioautography method. J Liq Chromatogr Relat Technol. https://doi.org/10.1080/10826076.2020.1725557

Dierickx, P.J. 1973. New β-diketones from *Buxus sempervirens*. Phytochemistry. https://doi.org/10.1016/0031-9422(73)80597-7

Ding, Z., Fu, F., Zheng, J., Yang, H., Zou, F., Linghua, K. 2024. Intelligent wood inspection approach utilizing enhanced swin transformer. IEEE Access. https://doi.org/10.1109/ACCESS.2024.3359048

Escorsim, A.M., da Rocha, G., Vargas, J.V.C., Mariano, A.B., Ramos, L.P., Corazza, M.L., et al. 2018. Extraction of acutodesmus obliquus lipids using a mixture of ethanol and hexane as solvent. Biomass and Bioenergy. https://doi.org/10.1016/j.biombioe.2017.10.035

Ezer, T., Alataş, M., Batan, N., Erata, H. 2023. The epiphytic bryophyte succession of Buxus sempervirens forests in the Fırtına Valley, Rize (North Türkiye). Acta Bot Croat. https://doi.org/10.5252/cryptogamie-bryologie2023v44a5

Fiorelia, N.E., Wibowo, A.D., Lae, N.L., Ang, A., Krisbianto, O. 2022. Types of high-performance liquid chromatography (HPLC) columns: A review. Food Tech J Teknol Pangan. https://doi.org/10.26418/jft.v5i1.57334

Fodor, E., Hâruța. O. 2014. Nestedness in bipartite networks of Thuja plicata, Prunus laurocerasus and Buxus sempervirens and their pathogens. Ann For Res. https://doi.org/10.15287/afr.2014.173

Gulcin, İ., Alwasel, S.H. 2023. DPPH radical scavenging assay. Processes. https://doi.org/10.3390/pr11082248

Gür, M., Güder, A., Verep, D., Güney, K., Özkan, O.E., Seki, N., et al. 2018. Some important plants for epilepsy treatment: antioxidant activity and flavonoid compositions. Iran J Sci Technol Trans A Sci. https://doi.org/10.1007/s40995-017-0361-3

Hajšlová, J., Čajka, T. 2007. Gas chromatography–mass spectrometry (GC-MS). n: Picó Y, editor. *Food Toxicants Analysis: Techniques, Strategies and Developments*. Amsterdam, The Netherlands: Elsevier; p. 161–197. https://doi.org/10.1016/B978-044452843-8/50013-4

Haleem, S., Niaz, S., Qureshi, N.A., Ullah, R., Mahmood, H.M., Shahat, A.A. 2019. Phytochemical analysis, antioxidant and antiprotoscolices potential of ethanol extracts of selected plants species against Echinococcus granulosus: *In vitro* study. Open Chem. https://doi.org/10.1515/chem-2019-0099

Hall, C. 2020. Thin layer chromatography: A complete guide to TLC. Chemistry Hall.

Hamid, I., Janbaz, K.H. 2017. Investigation of the laxative, spasmolytic and prokinetic properties of aqueous methanol extract of *Buxus sempervirens* Linn (Buxaceae). Trop J Pharm Res. https://doi.org/10.4314/tjpr.v16i8.16

Hood, S.C., Hocking, G.M. 1977. Box (*Buxus sempervirens* L.). Pharm Biol. https://doi.org/10.3109/13880207709081925

Hrabetova, M., Mrazkova ,M., Cerny, K. 2023. First report of *Phytophthora occultans* causing dieback of *Buxus sempervirens* in the Czech Republic. Plant Disease. https://doi.org/10.1094/PDIS-07-22-1537-PDN

Huang, T., He, J. 2017. Characterization of extracellular vesicles by size-exclusion high-performance liquid chromatography (HPLC). Methods Mol Biol. https://doi.org/10.1007/978-1-4939-7253-1_15

Kandi, S., Charles, A.L. 2019. Statistical comparative study between the conventional DPPH [rad] spectrophotometric and dropping DPPH [rad] analytical method without spectrophotometer: Evaluation for the advancement of antioxidant activity analysis. Food Chem. https://doi.org/10.1016/j.foodchem.2019.02.110

Karataglis, S., Babalonas, D., Kabasakalis, B. 1982. The ecology of plant populations growing on serpentine soils. Phyt.

Karimi, E., Mehrabanjoubani, P., Es-Haghi, A., Chamani, J. 2019. Phenolic compounds of endemic buxus plants in Caspian Hyrcanian forest (Buxus Hyrcana Pojark) and their biological activities. Pharm Chem J. https://doi.org/10.1007/s11094-019-02072-2

Kazilas, C., Kalaentzis, K., Demetriou, J., Koutsoukos, E., Strachinis, I., Andriopoulos, P. 2021. Utilization of citizen science data to monitor alien species: The box tree moth cydalima perspectalis (Walker, 1859) (lepidoptera: Crambidae) invades natural vegetation in greece. Bio Invasions Rec. https://doi.org/10.3391/bir.2021.10.4.28

Koubaa, M., Ktata, A., Bouaziz, F., Driss, D., Ghorbel, R.E., Chaabouni SE. 2015. Solvent extract from *Opuntia stricta* fruit peels: Chemical composition and biological activities. Free Radicals Antioxidants. https://doi.org/10.5530/fra.2015.2.2

Kvaltinová, Z., Ľukovič, L., Machov, J., Fatranska, M. 1991. Effect of the steroidal alkaloid buxaminol-E on blood pressure, acetylcholinesterase activity and (3H)quinuclidinyl benzilate binding in cerebral cortex. Pharmacology.

Liu, H.F., Ji, M., Cai, J. 2006. Short and efficient approach towards buxozine-C, an alkaloid from Buxus sempervirens. Arch Pharm (Weinheim). https://doi.org/10.1002/chin.200714207

Lopresto, C.G., Petrillo, F., Casazza, A.A., Aliakbarian, B., Perego, P., Calabrò, V. 2014. A non-conventional method to extract D-limonene from waste lemon peels and comparison with traditional Soxhlet extraction. Sep Purif Technol. https://doi.org/10.1016/j.seppur.2014.09.015

Lozano-Sánchez, J., Borrás-Linares, I., Sass-Kiss, A., Segura-Carretero, A. 2018. Chromatographic technique: High-performance liquid chromatography (HPLC). In: Modern Techniques for Food Authentication. https://doi.org/10.1016/B978-0-12-814264-6.00013-X

Manickathan, L., Defraeye, T., Carl, S., Richter, H., Allegrini, J., Derome, D., et al. 2022. A study on diurnal microclimate hysteresis and plant morphology of a Buxus sempervirens using PIV, infrared thermography, and X-ray imaging. Agric For Meteorol. https://doi.org/10.1016/j.agrformet.2021.108722

Marinova, G., Batchvarov, V. 2011. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulg J Agric Sci.

Mohammadpour, H., Sadrameli, S.M., Eslami, F., Asoodeh, A. 2019. Optimization of ultrasound-assisted extraction of *Moringa peregrina* oil with response surface methodology and comparison with Soxhlet method. Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2019.01.030

Momchev, P., Ciganović, P., Jug, M., Marguí, E., Jablan, J., Končić, M.Z. 2020. Comparison of maceration and ultrasonication for green extraction of phenolic acids from *Echinacea purpurea* aerial parts. Molecules. https://doi.org/10.3390/molecules25215142

Mosić, M., Dramićanin, A., Ristivojević, .P., Milojković-Opsenica, D. 2021. Extraction as a critical step in phytochemical analysis. Journal of AOAC International. https://doi.org/10.5740/jaoacint.19-0251

Mudhafar, M., Zainol, I., Alsailawi, H.A., Zorah, M., Karhib, M.M., Mahmood Mahdi, N. 2023. Preparation and characterization of FsHA/FsCol beads: Cell attachment and cytotoxicity studies. Heliyon. https://doi.org/10.1016/j.heliyon.2023.e15838

Negahdar, N., Hashemabadi, D., Kaviani, B. 2021. *In vitro* conservation and cryopreservation of Buxus sempervirens L., a critically endangered ornamental shrub. Russ J Plant Physiol. https://doi.org/10.1134/S1021443721040117

Orhan, I.E., Erdem, S.A., Senol, F.S., Kartal, M., Sener, B. 2012. Exploration of cholinesterase and tyrosinase inhibitory, antiprotozoal and antioxidant effects of *Buxus sempervirens* L. (boxwood). Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2012.03.004

Osete-alcaraz, A., Bautista-ortín, A.B., Gómez-plaza, E. 2020. The role of soluble polysaccharides in tannin-cell wall interactions in model solutions and in wines. Biomolecules. https://doi.org/10.3390/biom10010036

Palchykov, V.A., Zazharskyi, V.V., Brygadyrenko, V.V., Davydenko, P.O., Kulishenko, O.M., Borovik, I.V. 2020. Chemical composition and antibacterial effect of ethanolic extract of *Buxus sempervirens* on cryogenic strains of microorganisms *in vitro*. Chem Data Collect. https://doi.org/10.1016/j.cdc.2019.100323

Parvekar, P., Palaskar, J., Metgud, S., Maria, R., Dutta, S. 2020. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. Biomater Investig Dent. https://doi.org/10.1080/26415275.2020.1796674

Pastor, K., Ilić, M., Kojić, J., Ačanski, M., Vujić, D. 2022. Classification of cereal flour by gas chromatography–mass spectrometry (GC-MS) liposoluble fingerprints and automated machine learning. Anal Lett. https://doi.org/10.1080/00032719.2022.2050921

Piqué, R., Morera, N., Revelles, J., Castells, E., López-Bultó, O., Franch, A., et al. 2021. The distribution and use of box (*Buxus sempervirens* L.) in the Northeastern Iberian Peninsula during the holocene. Environ Archaeol.

Pyka A. 2014. Detection progress of selected drugs in TLC. BioMed Research International. 2014:732078. https://doi.org/10.1155/2014/732078

Rathinavelu, R., Arumugam, E., Paramathma, B.S. 2022. Extraction and characterization of natural cellulose fibers from the *Buxus sempervirens* for composite reinforcement. J Nat Fibers. https://doi.org/10.1080/15440478.2022.2101577

Reich E, Widmer V. 2012. Thin layer chromatography. In: Elvers B, editor. Ullmann's Encyclopedia of Industrial Chemistry. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; pp. 1–31. https://doi.org/10.1002/14356007.b05_301.pub2

Ríos, J.L., Recio, M.C. 2005. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. https://doi.org/10.1016/j.jep.2005.04.025

Rodrigues, L., Tilve, S.G., Majik, M.S. 2021. Synthetic access to thiolane-based therapeutics and biological activity studies. European Journal of Medicinal Chemistry. https://doi.org/10.1016/j.ejmech.2021.113659

Sarı, C. 2019. A comparative study of MTT and WST-1 assays in cytotoxicity analysis. Haydarpasa Numune Train Res Hosp Med J. https://doi.org/10.14744/hnhj.2019.16443

Shashikala, G.H., Shilpa, B.N., Shah MJ. 2018. Evaluation of anxiolytic activity of aqueous extract of Nerium oleander flowers in albino rats. Int J Basic Clin Pharmacol. https://doi.org/10.18203/2319-2003.ijbcp20183492

Špetík, M., Berraf-Tebbal, A., Pokluda, R., Eichmeier, A. 2021. Pyrenochaetopsis kuksensis (Pyrenochaetopsidaceae), a new species associated with an ornamental boxwood in the Czech Republic. Phytotaxa. https://doi.org/10.11646/phytotaxa.498.3.3

Szabó, L.U., Kaiser, M., Mäser, P., Schmidt, T.J. 2021a. Antiprotozoal nor-triterpene alkaloids from Buxus sempervirens L. Antibiotics. https://doi.org/10.3390/antibiotics10060696

Szabó, L.U., Kaiser, M., Mäser, P., Schmidt, T.J. 2021b. Identification of antiprotozoal compounds from Buxus sempervirens l. by pls-prediction. Molecules. https://doi.org/10.3390/molecules26206181

Szabó, L.U., Schmidt, T.J. 2022. Investigation of the variability of alkaloids in Buxus sempervirens l. using multivariate data analysis of LC/MS profiles. Molecules. https://doi.org/10.3390/molecules27010082

Topaloğlu, E., Ustaömer, D., Ozturk, M., Ay, N. 2022. Anadolu şimşiri (*Buxus sempervirens* L.) gövde odununun bazı teknolojik özelliklerinin araştırılması. Orman Araştırma Derg. https://doi.org/10.17568/ogmoad.1088765

Tosun, F., Kizilay, C.A., Şener, B., Vural, M., Palittapongarnpim, P. 2004. Antimycobacterial activity of some Turkish plants. Pharm Biol. https://doi.org/10.1080/13880200490505465

Tsirigka, A., Ntoula, M., Kontogiannopoulos, K.N., Karabelas, A.J., Patsios, S.I. 2023. Optimization of solvent extraction of lipids from Yarrowia lipolytica towards industrial applications. Fermentation. https://doi.org/10.3390/fermentation9010035

Uddin, R., Saha, M.R., Subhan, N., Hossain, H., Jahan, I.A., Akter, R., et al. 2014. HPLC-analysis of polyphenolic compounds in Gardenia jasminoides and determination of antioxidant activity by using free radical scavenging assays. Adv Pharm Bull.

Ullah, Q., Khan, S.A., Mohammad, A. 2021. Applications of green solvents in thin-layer chromatography (TLC) – An overview. Journal of Planar Chromatography – Modern TLC. https://doi.org/10.1007/s00764-021-00085-w

Vachnadze, N., Mchedlidze, Q., Novikova, J., Suladze, T., Vachnadze, V. 2016. Screening of wild spread and cultivated of Buxus species growing in Georgia on the content of alkaloids and biological activity. Georgian Medical News.

Vajrabhaya, La-ongthong, Korsuwannawong, S. 2018. Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays. J Anal Sci Technol. https://doi.org/10.1186/s40543-018-0146-0

Vinogradova, N., Vinogradova, E., Chaplygin, V., Mandzhieva, S., Kumar, P., Rajput, V.D., et al. 2023. Phenolic compounds of the medicinal plants in an anthropogenically transformed environment. Molecules. https://doi.org/10.3390/molecules28176322

Volpe, A.R., Carmignani, M., Cesare, P. 2023. Hydroalcoholic extract of Buxus sempervirens shows antiproliferative effect on melanoma, colorectal carcinoma and prostate cancer cells by affecting the autophagic flow. Front Pharmacol. https://doi.org/10.3389/fphar.2023.1073338

Wakeel, A., Jan, S.A., Ullah, I., Shinwari, Z.K., Xu, M. 2019. Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. Peer J. https://doi.org/10.7717/peerj.7857

Xiang, Z.N., Su, J.C., Liu, Y.H., Deng, B., Zhao, N., Pan, J., Hu, Z.F., Chen, F.H., Cheng, B.Y., Chen, J.C., Wan, L.S. 2021. Structurally diverse alkaloids from *Buxus sempervirens* with cardioprotective activity. Bioorg Chem. https://doi.org/10.1016/j.bioorg.2021.104753

Xie, J., Singh-Babak, S., Cowen, L. 2012. Minimum inhibitory concentration (MIC) assay for antifungal drugs. Bio-protocol. https://doi.org/10.21769/BioProtoc.252

Yan, Y., Sun, Y., Chen, J., Su, J., Li, Y., Qiu, M. 2012. ChemInform abstract: A new triterpenoid alkaloid from *Buxus sempervirens*. ChemInform. https://doi.org/10.1002/chin.201210213

Yue, Y., Qiu, Z.D., Qu, X.Y., Deng, A.P., Yuan, Y., Huang, L.Q., et al. 2018. Discoursing on Soxhlet extraction of ginseng using association analysis and scanning electron microscopy. J Pharm Anal. https://doi.org/10.1016/j.jpha.2018.08.003

Zazharskyi, V.V., Davydenko, P., Kulishenko, O., Borovik, I.V., Brygadyrenko, V.V. 2019. Antimicrobial activity of 50 plant extracts. Biosyst Divers. https://doi.org/10.15421/011922