



Article

Phytochemical Analysis and Specific Activities of Bark and Flower Extracts from Four Magnolia Plant Species

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Abstract: This study rigorously investigates the bioactive properties and characteristics of extracts derived from the flowers and bark of four distinct Magnolia species: *Magnolia champaca*, *Magnolia denudata*, *Magnolia grandiflora* and *Magnolia officinalis*. The primary objective is to evaluate the potential application of these extracts in cosmetics and other relevant industries. We used ethanol to extract compounds from these plants and conducted various tests, including spectrophotometry, HPLC, GC-MS, and microbiological analyses. The extracts, particularly rich in polyphenols (55.18 mg GAE/g), displayed significant antioxidant capabilities, with IC₅₀ values ranging between 9.99 mg/mL and 23.23 mg/mL. We quantified different compounds: phenolic acids (6.259 to 27.883 mg/g dry weight), aglycone flavonoids (61.224 to 135.788 mg/g dw), glycosidic flavonoids (17.265 to 57.961 mg/g dw), and lignans (150.071 to 374.902 mg/g dw). We identified 76 volatile compounds, predominantly oxygenated monoterpenes and sesquiterpene hydrocarbons, which contribute to the antibacterial effectiveness of the extracts. These extracts showed greater inhibitory potential against Gram-negative bacteria than Gram-positive bacteria. The diverse chemical compounds and their demonstrated activities suggest these extracts could be valuable in the cosmetics industry, pharmaceutical industry, or other industries.



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1. Introduction

Currently, there is an upward trend in the use of biological and ecological products in the cosmetics industry to obtain valuable, biodegradable, and environmentally friendly compounds. Many of these products are derived from biological sources, extracts from microorganisms, or plants. A series of plant extracts can provide protection against pathogenic or facultative pathogenic microorganisms, and as a result, studies have been conducted, especially in the cosmetics field, where products come into direct contact with consumers. Bactericidal effects are exhibited by skincare products or disinfectants for the skin, soaps, or creams, many of which straddle the line between cosmetic treatment and pharmaceuticals themselves. In these cases, a careful evaluation of the active principles that define the product and its scientific certification is necessary [1]. Currently, there is a great interest for researchers to study biologically active compounds in different plant species so that they can be used for medicinal, pharmaceutical, or cosmetic purposes. The identification and quantification of these compounds are associated with studies on their effects and mechanisms of action, with multiple economic benefits. Plants rich in polyphenols are recommended for their antioxidant and antimicrobial effects, with there being a correlation between compounds (phenolic acids, flavonoids, tannins, lignans, and stilbenes) and their biological qualities [2]. One of the plants appreciated for its complex compounds and biological activity is Magnolia.

Magnolias are ornamental and medicinal plants that have been known of and used since ancient times, with the variety of species exceeding two hundred. They are native to America (Central and South America) and Asia, and they have been acclimatized in most warmer regions of Europe, including Romania.

The objective of this research was to meticulously identify, quantify, and contrast the phytochemical properties and the capacity to elicit bacterial inhibitory effects of extracts derived from four *Magnolia* species: *Magnolia champaca*, *Magnolia denudata*, *Magnolia grandiflora* and *Magnolia officinalis*. The findings of this study offer a foundational platform for the cosmetics, pharmaceutical, and other relevant industries to select the most suitable species or extract tailored to their specific application needs.

M. champaca (yellow-orange flowers) has spasmolytic properties, including relaxation of respiratory pathways and vasodilator, thus validating its therapeutic use for diarrhea, asthma, and hypertension. Phytochemical compounds, including sesquiterpene hydrocarbons, sesquiterpene alcohols, and β -caryophyllene, have been identified, leading to their use in preventing or treating various ailments [3,4]. Valuable compounds, including phenolic compounds, were identified in *Magnolia* flower extracts (*M. denudata*), with this study focusing on the variation of bioactive elements depending on their processing parameters. Studies on the leaves and flowers of this pale pink plant have demonstrated the existence of phytochemicals, including lignans, and primary and secondary metabolites, leading to antioxidant and anti-inflammatory properties [5].

Magnolia grandiflora L. is abundant in magnolol and honokiol. Phytochemical studies demonstrate that extracts from *Magnolia grandiflora* contain flavonoids, terpenes, tannins, and alkaloids. Importantly, the extracts demonstrated antioxidant properties and were non-cytotoxic, suggesting their suitability for both medicinal and cosmetic purposes [6–8].

Furthermore, *Magnolia officinalis* has a complex phytochemical composition, and it is recommended as a tonic during the convalescent period, with its buds known to alleviate discomfort and intestinal issues while also exhibiting antiviral effects. Magnolol, a natural compound isolated from *M. officinalis*, exhibits a range of biological activities and is regarded as a promising candidate for clinical research despite its challenges related to low water solubility and rapid metabolism. Its biological activity is evident in various aspects, including anti-inflammatory, anticancer, neuroprotective, and antiepileptic aspects, as well as cardiovascular protection and mediation of ion activity [9–12]. The antimicrobial activity of *Magnolia* extracts was examined against various types of microorganisms, including bacteria, yeasts, and molds, and their effectiveness was observed across all three major categories of microorganisms [13–15].

2. Materials and Methods

2.1. Materials: *Magnolia* Samples, Reagents, Microbial Strains, and Culture Media

In May–June 2023, flowers and bark from four *Magnolia* species—*Magnolia champaca*, *Magnolia denudata*, *Magnolia grandiflora* and *Magnolia officinalis*—were collected from the Nursery of Ornamental Shrubs and Plants. These samples were then identified by experts at the CCBIA Research Center, part of the Faculty of SAIAPM/Lucian Blaga University of Sibiu. Each sample was assigned a unique registration voucher number, falling within the range of 390/1 to 390/4. The reagents used include 96% ethanol, Folin–Ciocâlteu reagent, sodium carbonate, gallic acid, DPPH 2,2-diphenyl-1-picrylhydrazyl, ABTS (2,2'-azinobis-diammonium salt), TPTZ (2,4,6-tripyridyl-s-triazine) Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid), acetonitrile, and acetic acid, the standard phenolic suitable for HPLC analysis supplied by Sigma-Aldrich GmbH, Steinheim, Germany.

To assess antibacterial effectiveness, we utilized specific reference bacterial strains. For Gram-positive bacteria, these included *Staphylococcus aureus* ATCC 43300 (MRSA), *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 19443, *Streptococcus pyogenes* ATCC 12347, *Streptococcus sanguinis* ATCC 10556, *Actinomyces israelii* ATCC 12102, and *Propionibacterium acnes* ATCC 6921/4311. For Gram-negative bacteria, we used *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Pre-*

vatella intermedia ATCC 25611, *Porphyromonas gingivalis* ATCC 33277, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 27853. We also employed standard antibiotics such as ampicillin, gentamicin, and tetracycline for comparison. The bacterial strains were grown and activated on Mueller–Hinton agar and Mueller–Hinton broth, both sourced from Sigma-Aldrich GmbH, Steinheim, Germany.

2.2. Methods

The bark and flowers of the selected plants were dried over a period of 10 days, maintaining a temperature of $40\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$. This process continued until the materials consistently weighed the same. The dried material, weighing 100 g, was then finely ground to a size between 300 and 500 microns and stored at a cool temperature of $4\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

The dried material was then macerated for four days in a solvent mixture consisting of 500 mL of an ethanol: water solution (70:30 *v/v*), with the temperature maintained steadily at $18\text{ }^{\circ}\text{C}$. Following the maceration period, the samples were filtered and concentrated using a rotary evaporator. This extraction process was carried out three times. Finally, the concentrated extracts were re-dissolved in distilled water at a 1:1 ratio (mg/mL) for further analysis or application.

2.2.1. Identification of Total Polyphenols and Determination of Antioxidant Activity

To measure total polyphenol concentration, we adapted the Folin–Ciocâlteu method. This involved mixing 0.20 mL of the plant extract with 0.80 mL Folin–Ciocâlteu reagent (10% *v/v*) and 1 mL of a sodium carbonate solution (7.5% *w/v*). These samples were incubated for an hour at room temperature, ensuring they were shielded from light [16]. The polyphenol levels were measured using a UV-1900 SHIMADZU spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 750 nm. Results were expressed as milligrams of gallic acid equivalent per gram of the dried matter (mg GAE/g).

Antioxidant activity was determined using three methods: Determination of the DPPH free radical scavenging method, slightly modified by Popescu et al. [16]. This method involves preparing a stock methanolic solution of DPPH (25:100) and samples composed of dried extract and methanol in a 1:1 ratio. The working solution for the DPPH assay was prepared by mixing 10 mL of the stock solution with 90 mL of methanol. Subsequently, 25 μL of the sample was reacted with 975 μL of this DPPH working solution for 30 min at $20\text{ }^{\circ}\text{C}$ in the dark, followed by measuring the absorbance at 517 nm using a UV-1900 SHIMADZU spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The control sample will be conducted following the same procedure, with the extract replaced by methanol. The calibration curve is made using Trolox, with the results expressed in milligrams of Trolox equivalent per gram of dried extract (mg TE/g DE).

The inhibition percentage (Formula (1)) is calculated according to the following equation:

$$\% I = \frac{Ab - Aa}{Ab} \times 100 \quad (1)$$

where *Ab* is the absorbance of the control, and *Aa* is the absorbance of the reaction between the sample and radicals.

Determination of the FRAP (Ferric-Reducing Antioxidant Power)

The ferric-reducing antioxidant power (FRAP) assay involves the preparation of a FRAP reagent, constituted by homogenizing 50 mL of a 300 mM acetate buffer solution with 5 mL of a 20 mM FeCl_3 solution and 5 mL of a 10 mM TPTZ solution, acidified using 150 μL of HCl. For the evaluation of the antioxidant capacity of the extracts, a mixture comprising 2 mL of bidistilled water, 0.1 mL of the extract, and 0.5 mL of the FRAP solution is prepared. This mixture is then incubated in darkness for one hour, followed by absorbance measurement at a wavelength of $\lambda = 595\text{ nm}$ using a spectrophotometer. Ascorbic acid is employed as a positive control. The outcomes are benchmarked against a Trolox calibration curve and expressed in μmol Trolox equivalents (TE)/g dry weight (d.w) [17].

The ABTS radical cation scavenging activity [17] is determined by preparing a stock solution consisting of 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (7 mM) and diammonium potassium persulfate (2.45 mM). This solution is stored in the dark for 16 h and subsequently diluted to achieve an absorbance of 0.02 to 0.7 at 734 nm. For the assay, 2 mL of the diluted stock solution is vortexed with 20 μ L of the extract for 30 s, and the absorbance is measured at 734 nm using a spectrophotometer after 60 s. Ascorbic acid is again used as a positive control. The results are compared with the calibration curve with Trolox and expressed in μ mol TE/g d.w.

2.2.2. Identification and Quantification of Phenolic Compounds

Phenolic compounds were identified and quantified using a modified version of the LC-ESI-QTOF-MS method [18], using Agilent 1200 HPLC equipment (Agilent Technologies, Santa Clara, CA, USA). Mobile phase A was a mixture of water and acetic acid (95/5 *v/v*), and mobile phase B consisted of acetonitrile, water, and acetic acid (100/95/5 *v/v/v*), previously degassed at 20 °C for 20 min. The chromatographic column used was Zorbax SB-Aq; 250 mm \times 4.6 mm i.d., 5.0 μ m particle size. The gradient elution program was established following a mix between A and B, with the program also being used in the quantification of phenolic compounds. Using the ESI ionization system, positive and negative modes of droplets were established, with mass spectra identified in the *m/z* 50–1300 range.

Quantification of phenolic compounds was performed using the Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with a PDA detector, an automatic injection system, and quaternary pump. A C18 chromatographic column was used (Zorbax SB-Aq; 250 mm \times 4.6 mm i.d., 5.0 μ m particle size). Mobile phase A was a mixture of water and acetic acid solution (95/5 *v/v*), and mobile phase B consisted of acetonitrile/water/acetic acid (100/95/5 *v/v/v*). The sample injection volume was set at 20 μ L, with a flow rate of 0.8 mL/minute. Both mobile phases A and B were degassed at 20 °C for 20 min. The gradient elution program mixed mobile phases A and B in varying proportions: initially, 15% B for the first quarter-hour; 15–25 min, 25% B; 40% B from 25–40 min, 45% B during 40–65 min, 60% B for 65–70 min, 80% B from 70–75 min, and finally 100% B for 75–80 min, before returning to 15% B for the last 5 min. Phenolic compounds were detected in the 190 nm to 400 nm range. Standard calibration curves were used, and the results were expressed in mg/g dry matter. Data analysis was conducted using Agilent LC-MS-QTOF/HPLC MassHunter software version B.03.01.

2.2.3. Identification of Volatile Compounds

To analyze and identify the volatile compounds in Magnolia extracts, we used GC-MS (gas chromatography–mass spectrometry) technology, specifically a slightly adapted version of the method outlined by Popescu et al. [19]. The primary analysis of these volatile compounds was carried out using a Varian CP-3800/Saturn 2000 gas chromatograph manufactured by Varian in California, USA, equipped with a Zebron ZB-5 MSI capillary. The column measures 30 m \times 0.25 mm \times 0.25 μ m (Phenomenex, Torrance, CA, USA). This setup allows for the precise separation and identification of the various volatile compounds present in the Magnolia extracts.

In the GC-MS analysis of Magnolia extracts, we adhered to specific temperature settings for different components: the ion source was maintained at 230 °C, the quadrupole at 150 °C, and the injector at 220 °C. We injected a 1 μ L sample using a splitter. The temperature program for the run was methodically set: initially, the temperature was held at 30 °C for 10 min, then increased at a rate of 5 °C per minute up to 160 °C. It was held at 160 °C for 15 min, after which it was raised at a rate of 15 °C per minute to 250 °C. Subsequently, the temperature was increased at a rate of 5 °C per minute to 270 °C and maintained at 270 °C for a final duration of 10 min. The ionization energy used in the process was 70 eV. Helium served as the carrier gas, flowing at a rate of 0.5 mL/min. To accurately identify the volatile compounds, we compared the resulting mass spectra with

those in the Wiley 275 library and the NIST 17 Mass Spectral and Retention Index Libraries (NIST17), as well as the NIST WebBook and our laboratory's own database.

2.2.4. Determination of Minimum Inhibitory Concentration (MIC)

To establish the minimum inhibitory concentration (MIC) of Magnolia samples against selected microorganisms, a series of MHB dilutions was prepared, ranging from 0.625 µg/mL to 30 µg/mL of extract (0.625, 1.25, 2.5, 5, 7.5, 10, 12.5, 15, 30 µg/mL). Dilutions were made in Mueller–Hinton broth (MHB) following a slightly adapted version of the method by Ibrahim et al. [20]. We tested the Magnolia extract dilutions on various bacterial strains, including *Staphylococcus aureus* ATCC 43300 (MRSA), *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 19443, *Streptococcus pyogenes* ATCC 12347, *Streptococcus sanguinis* ATCC 10556, *Actinomyces israelii* ATCC 12102, *Propionibacterium acnes* ATCC 6921/4311, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Prevotella intermedia* ATCC 25611, *Porphyromonas gingivalis* ATCC 33277, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 27853. These bacterial strains were activated by growing them for 24 h in the MHB culture medium.

To conduct the MIC test, each test tube was inoculated with 10 µL of a bacterial strain suspension (density 0.5 McF = 1.5×10^8 CFU/mL). The concentrated extracts were dissolved in distilled water at a 1:1 ratio (mg/mL). 1 mL of diluted extract was added in the established order of dilutions. The tubes were thoroughly mixed to ensure uniform distribution of the bacterial suspension and extract. Following this, the tubes were placed in a Memmert incubator set at 36 °C. After a 24 h incubation period, each set of tubes was examined to assess the growth of bacteria. The MIC was determined by observing the level of turbidity in each tube, which correlates with bacterial growth. The presence or absence of turbidity in the different dilutions helped to establish the lowest concentration of extract that effectively inhibited bacterial growth. To ensure accuracy and reproducibility, all these determinations were performed in triplicate.

2.2.5. Multivariate Statistical Analyses

Multivariate statistical analyses were performed using Addinsoft XLSTAT software, version 2014.5.03 (Addinsoft Inc., New York, NY, USA). The primary goal of this analysis was to uncover significant associations between the quality parameters of the Magnolia extracts and the identified volatile compounds. For this purpose, Pearson correlation analysis was used, allowing us to determine the strength and direction of the relationships between all the variables we had measured. We considered correlations statistically significant if they had a *p*-value less than 0.05 and highly significant if the *p*-value was less than 0.01. By doing this, we could understand which volatile compounds were most closely related to the quality parameters of the extracts, providing valuable insights into their characteristics and potential applications.

3. Results

The identification of total polyphenols and their associated antioxidant activity in this study revealed significant insights into the properties of hydro-alcoholic extracts. Polyphenols, known for their antioxidant capabilities, also contribute to inhibiting the activity of various microorganisms. According to the data presented in Table 1, the concentration of polyphenols in the hydro-alcoholic extracts was found to be directly proportional to both the source of extraction and the specific species of plant from which the samples were derived. The findings from this research, which focused on assessing the total polyphenol content and antioxidant activity in different parts of Magnolia species, demonstrate that these naturally occurring chemical compounds are found in both the bark and flowers of these plants. Particularly noteworthy is the bark of *Magnolia champaca*, which was found to contain a significant number of polyphenols, approximately 73.12 mg of gallic acid equivalent (GAE) per gram. Additionally, this part of the plant exhibited notable antioxidant properties. This was evidenced by its performance in the DPPH antioxidant test, where it

showed a relatively strong ability to neutralize free radicals, as indicated by its IC₅₀ value of 19.50 mg/mL. This study's results also indicate a variation in the polyphenol content and antioxidant activity between different parts of the Magnolia species. In Magnolia flowers, the polyphenol concentration is slightly lower, at around 68.62 mg GAE/g, compared to the bark. However, the antioxidant activity of the flowers is like that of the bark, with an IC₅₀ value of 18.66 mg/mL in the DPPH test. In the case of *Magnolia denudata*, the hydro-alcoholic bark extracts contain 65.18 mg GAE/g of polyphenols and show moderate antioxidant activity, with an IC₅₀ value of 17.01 mg/mL. The flowers of this species have a lower polyphenol content, approximately 55.23 mg GAE/g, and exhibit reduced antioxidant activity, reflected in a lower IC₅₀ value of 9.99 mg/mL. For *Magnolia grandiflora*, the bark has an average polyphenol content of 72.52 mg GAE/g, demonstrating relatively good antioxidant activity, with an IC₅₀ value of 19.07 mg/mL in the DPPH test.

Table 1. Total polyphenols and antioxidant activity of hydro-ethanolic Magnolia extracts (*M. champaca*, *M. denudata*, *M. grandiflora*, *M. officinalis*).

Magnolia Species	Source of Extracts	TPC mg GAE/g d.w.	DPPH IC ₅₀ mg/mL	FRAP μmol TE/g d.w.	ABTS μmol TE/g d.w.
<i>M. champaca</i>	Bark	73.12 ± 0.25	19.50 ± 0.25	38.56 ± 0.27	44.21 ± 0.41
	Flowers	68.62 ± 0.21	18.66 ± 0.21	32.23 ± 0.19	40.36 ± 0.32
<i>M. denudata</i>	Bark	65.18 ± 0.28	17.01 ± 0.24	30.48 ± 0.17	39.25 ± 0.27
	Flowers	55.23 ± 0.34	9.99 ± 0.25	28.75 ± 0.19	34.66 ± 0.21
<i>M. grandiflora</i>	Bark	72.52 ± 0.45	19.07 ± 0.32	37.11 ± 0.21	43.99 ± 0.39
	Flowers	55.18 ± 0.38	10.55 ± 0.18	19.66 ± 0.23	34.11 ± 0.22
<i>M. officinalis</i>	Bark	98.44 ± 0.49	23.23 ± 0.48	45.39 ± 0.25	49.01 ± 0.55
	Flowers	66.02 ± 0.28	18.78 ± 0.27	30.82 ± 0.18	40.14 ± 0.58
Ascorbic acid	-	-	-	16.21 ± 1.01 mmol TE/g d.w.	17.51 ± 1.11 mmol TE/g d.w.

TPC: total polyphenol content; GAE: gallic acid equivalents; DPPH free radical scavenging IC₅₀; FRAP: ferric-reducing antioxidant power; TE: Trolox equivalents; ABTS: radical cation scavenging assay. The values represent the average of the three determinations with the corresponding standard deviation.

The flowers, in contrast, contain fewer polyphenols, about 55.18 mg GAE/g, and exhibit lower antioxidant activity, with an IC₅₀ value of 10.55 mg/mL. Among the Magnolia species studied, *M. officinalis* stood out for having the highest polyphenol content in its hydro-alcoholic bark extracts, approximately 98.44 mg GAE/g. This was the greatest concentration observed among all the species examined. Additionally, it demonstrated the most potent antioxidant activity, with an IC₅₀ value of 23.23 mg/mL in the DPPH test.

In contrast, the flowers of *M. officinalis* had a lower polyphenol content compared to its bark, about 66.02 mg GAE/g. Despite this, they still exhibited significant antioxidant activity, with an IC₅₀ value of 18.78 mg/mL. Further supporting these observations, other researchers have also identified polyphenols in Magnolia flowers.

The FRAP results obtained are for bark extracts between 30.48 ± 0.17 μmol Trolox equivalents (TE)/g d.w. and 45.39 ± 0.25 μmol Trolox equivalents (TE)/g d.w., and for flower extracts, they are between 19.66 ± 0.23 μmol Trolox equivalents (TE)/g d.w. and 32.23 ± 0.19 μmol Trolox equivalents (TE)/g d.w. The maximum values characterize the extracts from the bark of *M. officinalis*, respectively, and those from the flowers of *M. champaca*.

The radical scavenging ABTS test shows maximum values in the case of extracts from the bark of *M. officinalis* of 49.01 ± 0.55 μmol Trolox equivalents (TE)/g d.w. and from the flowers of *M. champaca* of 40.36 ± 0.32 μmol Trolox equivalents (TE)/g d.w. The analyzed extracts have a much lower antioxidant activity than that of ascorbic acid (16.21 ± 1.01 mmol TE/g, respectively, and 17.51 ± 1.11 mmol TE/g d.w.)

3.1. Identification and Quantification of Phenolic Compounds in Magnolia Extracts

Table 2 presents detailed data on the content of various types of compounds—namely phenolic acids, aglycone flavonoids, glycosidic flavonoids, and lignans—in different Mag-

nia species. This table specifically focuses on hydro-ethanolic extracts obtained from both the bark and flowers of these plants. The table helps in identifying which Magnolia species might be more suitable for specific uses based on their chemical profiles.

In the analysis of the four Magnolia species, gallic acid was found to be a common phenolic compound across all of them, with its highest abundance observed in *M. champaca*, reaching 5.021 mg/g.

Additionally, 4-hydroxybenzoic acid, another phenolic compound, was identified in all the Magnolia species. Notably, its concentration was highest in the bark extracts. In *M. grandiflora*, the concentration of 4-hydroxybenzoic acid in the bark was measured at 5.667 mg/g, while in *M. denudata*, it reached up to 11.097 mg/g. On the other hand, the flower extracts of these species also contained this compound, but in lower amounts. For instance, *M. grandiflora* flowers had 1.224 mg/g, and *M. officinalis* flowers had a higher concentration of up to 6.127 mg/g.

p-Coumaric acid was found in significant amounts in *M. champaca*, particularly in its flowers, at a concentration of 2.671 mg/g. In *M. denudata*, this acid was notably prevalent in the bark extracts, with a concentration of 3.108 mg/g. Salicylic acid, another phenolic compound with well-known benefits, was detected in the bark extracts of *M. champaca*, *M. grandiflora*, and *M. officinalis*. However, it was absent in the flower extracts of these species. This suggests a variation in the distribution of this compound within the plants. Caffeic acid, often praised for its anti-inflammatory and antioxidant effects, showed higher concentrations in flower extracts. It was most abundant in the flowers of *M. grandiflora*, with a level of 1.044 mg/g. In addition, caftaric acid was quantified in all the studied Magnolia species. Interestingly, there were negative values reported for this acid in the bark extracts of *M. champaca* and *M. grandiflora*, as well as in the flower extracts of *M. denudata*.

Cinnamic acid was found in the bark of *M. champaca*, *M. denudata*, and *M. officinalis*, ranging from 0.002 mg/g to 0.092 mg/g. Chlorogenic acid was detected in all species, both in bark and flower extracts, being most abundant in the bark of *M. officinalis*, with a concentration of 5.619 mg/g. Ellagic acid, another compound with potential health benefits, was notably absent in the flower extracts of *M. champaca* and *M. officinalis*. Similarly, ferulic acid was not found in the flower extracts of *M. grandiflora* and *M. officinalis*.

From Table 2, it is evident that the highest concentration of phenolic acids was observed in the bark extracts of *M. denudata*, reaching up to 27.883 mg/g. This is closely followed by the bark of *M. champaca*, which showed a significant phenolic acid content of 26.296 mg/g.

In contrast, when looking at the flower extracts, *M. officinalis* stood out with the highest number of phenolic acids, measuring 12.582 mg/g. This suggests that while the bark of certain Magnolia species may have higher overall concentrations of phenolic acids, the flowers of other species, like *M. officinalis*, also contain substantial amounts of these compounds.

Catechin, an aglycone flavonoid known for its potent antioxidant properties, was found to be the most abundant of its kind in all the Magnolia extracts studied. The concentrations of catechin were notably high, particularly in both the bark and flower extracts of certain species. For instance, in *M. officinalis*, catechin levels reached 81.034 mg/g in the bark and 32.333 mg/g in the flowers. In *M. denudata*, these values were even higher, with 91.227 mg/g in the bark and 78.936 mg/g in the flowers. Additionally, this study found that myricetin and luteolin, two other types of aglycone flavonoids, were present in the bark extracts of all four Magnolia species. However, these compounds were absent in the flower extracts of *M. grandiflora*. The presence of taxifolin was observed specifically in the flower extracts of the Magnolia species studied. However, it was notably absent in the bark extracts of both *M. denudata* and *M. grandiflora*. Additionally, eriodictyol was found in very low quantities in certain parts of the Magnolia species. The concentrations ranged between 0.001 mg/g and 0.021 mg/g. Eriodictyol was detected in the flower extracts of *M. champaca* and *M. grandiflora*, as well as in the bark of *M. grandiflora* and *M. officinalis*.

Table 2. Phenolic compounds identified in the four Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora* and *M. officinalis*).

Compounds	<i>M. champaca</i>		<i>M. denudata</i>		<i>M. grandiflora</i>		<i>M. officinalis</i>	
	Bark	Flowers	Bark	Flowers	Bark	Flowers	Bark	Flowers
Phenolic Acids (mg/g dry matter)								
Gallic	5.021 ± 0.106	1.191 ± 0.051	1.278 ± 0.079	1.782 ± 0.092	2.371 ± 0.098	0.984 ± 0.041	1.999 ± 0.097	2.035 ± 0.089
4-hydroxybenzoic	9.289 ± 0.123	4.133 ± 0.104	11.097 ± 0.133	3.191 ± 0.101	5.667 ± 0.106	1.224 ± 0.069	10.012 ± 0.126	6.127 ± 0.108
p-coumaric	0.111 ± 0.006	2.671 ± 0.067	3.108 ± 0.088	0.154 ± 0.005	n.d.	1.793 ± 0.092	0.010 ± 0.001	1.119 ± 0.052
Salicylic	7.789 ± 0.111	n.d.	4.578 ± 0.102	n.d.	9.123 ± 0.122	n.d.	2.333 ± 0.088	n.d.
Caffeic	0.019 ± 0.002	0.662 ± 0.008	0.027 ± 0.001	0.276 ± 0.002	0.113 ± 0.006	1.044 ± 0.051	0.022 ± 0.002	0.993 ± 0.041
Caftaric	n.d.	0.011 ± 0.001	0.001 ± 0.000	n.d.	n.d.	0.015 ± 0.001	0.017 ± 0.001	0.024 ± 0.001
Cinnamic	0.092 ± 0.007	0.023 ± 0.001	0.002 ± 0.000	0.064 ± 0.005	n.d.	0.048 ± 0.004	0.076 ± 0.007	n.d.
Chlorogenic	1.357 ± 0.062	2.543 ± 0.138	4.504 ± 0.116	3.934 ± 0.111	4.441 ± 0.111	1.147 ± 0.016	5.619 ± 0.119	2.281 ± 0.096
Ellagic	0.487 ± 0.028	n.d.	0.983 ± 0.051	0.001 ± 0.000	0.578 ± 0.026	0.001 ± 0.000	0.568 ± 0.025	n.d.
Ferulic	1.129 ± 0.052	0.111 ± 0.006	1.222 ± 0.067	0.127 ± 0.062	1.341 ± 0.068	n.d.	0.991 ± 0.035	n.d.
Syringic	1.002 ± 0.044	n.d.	1.083 ± 0.045	n.d.	1.321 ± 0.067	0.001 ± 0.000	1.242 ± 0.066	0.001 ± 0.000
Vanillic	n.d.	0.005 ± 0.001	n.d.	0.003 ± 0.000	0.001 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.002 ± 0.000
Total	26.296	11.350	27.883	9.532	24.956	6.259	22.890	12.582
Aglycone Flavonoids (mg/g dry matter)								
Catechin	85.333 ± 8.161	52.744 ± 6.554	91.227 ± 9.609	78.936 ± 7.231	71.772 ± 7.163	66.033 ± 6.222	81.034 ± 8.137	32.333 ± 4.112
Myricetin	1.024 ± 0.064	0.221 ± 0.012	0.023 ± 0.001	2.003 ± 0.101	0.992 ± 0.056	n.d.	0.835 ± 0.045	n.d.
Luteolin	2.001 ± 0.101	0.001 ± 0.000	0.779 ± 0.069	0.287 ± 0.021	1.429 ± 0.076	n.d.	1.002 ± 0.063	0.110 ± 0.011
Taxifolin	0.004 ± 0.001	1.983 ± 0.102	n.d.	5.661 ± 0.118	n.d.	1.111 ± 0.060	0.002 ± 0.000	2.003 ± 0.108
Eriodictyol	n.d.	0.001 ± 0.000	n.d.	n.d.	0.001 ± 0.000	0.021 ± 0.006	0.001 ± 0.000	n.d.
Apigenin	0.772 ± 0.006	4.229 ± 0.096	0.189 ± 0.016	12.002 ± 1.216	0.991 ± 0.015	7.456 ± 1.015	1.283 ± 0.072	2.342 ± 0.093
Quercetin	0.615 ± 0.005	0.357 ± 0.002	0.995 ± 0.008	1.911 ± 0.102	0.937 ± 0.012	0.999 ± 0.016	0.691 ± 0.007	1.001 ± 0.053
Epicatechin	22.418 ± 2.592	29.919 ± 3.056	20.361 ± 2.444	34.988 ± 4.100	31.912 ± 3.605	39.397 ± 5.121	34.562 ± 4.103	23.435 ± 2.789
Total	112.167	89.455	113.574	135.788	108.034	115.017	119.410	61.224
Glycosidic flavonoids (mg/g dry matter)								
Rutin	27.035 ± 3.319	23.923 ± 3.003	45.327 ± 5.778	15.075 ± 2.199	56.782 ± 5.109	17.249 ± 2.016	51.103 ± 5.112	20.200 ± 2.121
Luteolin-7-O-glucoside	1.002 ± 0.061	5.552 ± 0.135	2.033 ± 0.964	2.003 ± 0.961	1.023 ± 0.669	1.279 ± 0.075	0.435 ± 0.044	6.771 ± 5.168
Isoquercetin	0.004 ± 0.001	0.213 ± 0.017	n.d.	0.016 ± 0.001	n.d.	0.111 ± 0.010	n.d.	0.098 ± 0.010
Hyperoside	n.d.	0.111 ± 0.008	n.d.	0.032 ± 0.001	n.d.	n.d.	n.d.	0.011 ± 0.006
Kaempferol-3-O-rutinoside	0.012 ± 0.002	0.241 ± 0.016	0.023 ± 0.001	0.104 ± 0.011	0.122 ± 0.010	0.286 ± 0.014	0.192 ± 0.013	0.197 ± 0.014
Apigenin-7-O-glucoside	0.003 ± 0.000	0.044 ± 0.004	0.115 ± 0.009	0.011 ± 0.003	0.012 ± 0.004	0.101 ± 0.006	0.011 ± 0.001	0.099 ± 0.009
Isorhamnetin-3-O-glucoside	0.002 ± 0.000	n.d.	0.001 ± 0.000	n.d.	0.001 ± 0.000	0.001 ± 0.000	n.d.	n.d.
Naringenin-7-O-glucoside	0.001 ± 0.000	n.d.	n.d.	0.002 ± 0.000	n.d.	n.d.	n.d.	n.d.
Astragalín	n.d.	n.d.	n.d.	0.001 ± 0.000	n.d.	n.d.	n.d.	n.d.
Quercitrin	n.d.	0.001 ± 0.000	n.d.	n.d.	n.d.	n.d.	0.001 ± 0.000	n.d.

Table 2. Cont.

Compounds	<i>M. champaca</i>		<i>M. denudata</i>		<i>M. grandiflora</i>		<i>M. officinalis</i>	
	Bark	Flowers	Bark	Flowers	Bark	Flowers	Bark	Flowers
Isorhamnetin-3-O-rutinoside	0.003 ± 0.000	0.004 ± 0.001	n.d.	0.021 ± 0.003	0.021 ± 0.003	0.018 ± 0.002	0.114 ± 0.009	0.007 ± 0.001
Total	28.062	30.089	47.499	17.265	57.961	19.045	51.856	27.383
Lignans								
4'-O-methylhonokiol	2.011 ± 0.106	7.012 ± 1.122	5.561 ± 0.777	1.021 ± 0.099	19.092 ± 2.105	18.098 ± 2.161	21.021 ± 3.444	16.546 ± 0.996
Magnolol	9.319 ± 1.155	5.666 ± 0.703	9.999 ± 1.235	2.001 ± 0.142	7.021 ± 0.819	19.021 ± 2.453	97.093 ± 10.787	23.021 ± 3.133
Honokiol	5.092 ± 0.611	4.090 ± 0.607	5.892 ± 0.801	2.098 ± 0.011	6.607 ± 0.801	18.917 ± 2.134	56.785 ± 6.193	13.195 ± 1.288
3-methoxymagnolol	2.367 ± 0.175	2.311 ± 0.033	1.285 ± 0.101	0.227 ± 0.005	7.000 ± 0.763	5.662 ± 0.126	2.368 ± 0.068	4.432 ± 0.222
Isomagnolol	1.025 ± 0.099	0.098 ± 0.005	1.374 ± 0.112	0.112 ± 0.004	6.789 ± 0.566	2.023 ± 0.095	3.479 ± 0.094	7.776 ± 0.767
Total	19.814	19.177	24.111	5.459	46.509	63.721	180.746	64.970
Total Phenolic Compounds	186.339	150.071	213.067	168.044	237.46	204.042	374.902	166.159

The values represent the average of the three determinations with the corresponding standard deviation (S.D.); n.d.: not detected.

Apigenin and quercetin are present in all species, with values ranging from 0.991 to 1.283 mg/g in bark and 2.342 to 12.002 mg/g in flowers, respectively, and 0.615 to 0.995 mg/g and 0.357 to 1.911 mg/g, with the most significant quantities being found in *M. denudata*. Epicatechin was identified in all species, with values in bark extracts ranging from 20.361 mg/g for *M. denudata* to 34.562 mg/g for *M. officinalis*. In the flower extracts of the studied Magnolia species, epicatechin was found in particularly high concentrations in *M. grandiflora*. The maximum value of epicatechin in *M. grandiflora*'s flower extracts was remarkably high, at 39.397 mg/g.

According to Table 2 the total content of aglycone flavonoid compounds in Magnolia species exhibits significant variation between bark and flower extracts. For the bark extracts, the total aglycone flavonoids ranged from 108.034 mg/g to 119.41 mg/g. In the case of flower extracts, the total aglycone flavonoid content varied between 61.224 mg/g and 135.788 mg/g. Again, *M. officinalis* and *M. denudata* stood out with the highest values in this category.

Glycoside flavonoids are most significantly represented by rutin, which was identified in bark extracts at a maximum value of 51.103 mg/g in *M. officinalis* and in flower extracts at a value of 56.782 mg/g in *M. grandiflora*. The minimum values are present in the bark of *M. champaca* (27.035 mg/g) and in the flowers of *M. denudata* (15.075 mg/g). Luteolin-7-O-glucoside is present in all species, with the highest quantities in the flower extracts of *M. officinalis*. Isoquercetin and hyperoside were present in relatively small quantities. Additionally, it was noted that these compounds were absent in the bark extracts of *M. denudata*, *M. grandiflora*, and *M. officinalis*. Kaempferol-3-O-rutinoside and Apigenin-7-O-glucoside were detected in all the Magnolia species studied, though in relatively small amounts. The highest concentrations of these compounds were found in the hydro-ethanolic extracts derived from the flowers of these species. Isorhamnetin-3-O-glucoside, on the other hand, was not found in any of the extracts from *M. officinalis*, neither in the bark nor in the flowers.

However, trace amounts of this compound, ranging from 0.001 mg/g to 0.002 mg/g, were identified in the bark of *M. champaca*, *M. denudata*, and *M. grandiflora*. Naringenin-7-O-glucoside was quantified in the Magnolia species, with a concentration of 0.001 mg/g in the bark extracts of *M. champaca*. In the flower extracts of *M. denudata*, this compound was found at a slightly higher concentration of 0.002 mg/g. Astragalin was detected exclusively in the flower extracts of *M. denudata*, present at a concentration of 0.001 mg/g. Quercitrin was also identified in these studies, with a concentration of 0.001 mg/g in both the bark of *M. officinalis* and the flowers of *M. champaca*. Isorhamnetin-3-O-rutinoside was found in various concentrations: in *M. champaca*, it was present in both bark and flowers, with concentrations of 0.003 mg/g and 0.004 mg/g, respectively; in *M. denudata*, this compound was found in the flowers at a concentration of 0.021 mg/g. For *M. grandiflora* and *M. officinalis*, the concentrations were 0.021 mg/g to 0.114 mg/g and 0.018 mg/g to 0.007 mg/g, respectively.

Lignans were found in both the bark and flower extracts of the studied Magnolia species. One such lignan, 4'-O-methylhonokiol, was identified across all species, displaying a range of concentrations. In the bark extracts, the concentration of 4'-O-methylhonokiol varied notably, with the lowest value being 2.011 mg/g in *M. champaca* and the highest reaching 21.021 mg/g in *M. officinalis*. Similarly, in the flower extracts, the levels of 4'-O-methylhonokiol also showed variability. The lowest concentration was found in *M. denudata* at 1.021 mg/g, while the highest was in *M. grandiflora*, with a concentration of 18.098 mg/g. Magnolol, a compound characteristic of Magnolia plants, was found in significant amounts, especially in *M. officinalis*.

In this species, the concentration of magnolol was remarkably high, with 97.093 mg/g in the bark and 23.021 mg/g in the flowers. Across all the studied species, magnolol was present in varying concentrations. In the bark extracts, its levels ranged from 7.021 mg/g to 9.999 mg/g.

For the hydro-ethanolic flower extracts, the range was between 2.001 mg/g and 19.021 mg/g. Honokiol, another lignan found in Magnolia species, was also identified in all the species included in this study.

The highest concentration of honokiol was in the bark extracts of *M. officinalis*, with a value of 56.785 mg/g. Conversely, the lowest concentration was observed in the flower extracts of *M. denudata*, at 2.001 mg/g. 3-Methoxymagnolol and isomagnolol, two lignan compounds, were detected in all the Magnolia species studied.

The most notable concentrations of these compounds were found in the bark and flower extracts of *M. grandiflora* and *M. officinalis*, indicating a higher accumulation of these lignans in these species.

According to Table 2, the total lignan content varied significantly between the bark and flower extracts. In the bark extracts, the total lignan concentration ranged from 19.814 mg/g to an impressive 180.746 mg/g. In the flower extracts, this range was slightly lower, spanning from 5.459 mg/g to 64.97 mg/g.

Additionally, the total phenolic compound values in the bark extracts were found to vary widely, ranging from 186.339 mg/g in *M. champaca* to as high as 374.902 mg/g in *M. officinalis*. In comparison, the flower extracts contained lower total phenolic values, ranging between 150.071 mg/g in *M. champaca* and 204.042 mg/g in *M. grandiflora*.

3.2. Identification and Quantification of Volatile Compounds in Magnolia Extracts

Table 3 provides a detailed account of the content of various chemical compounds found in the bark and flowers of four Magnolia species: *M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*. The table compares the concentrations of these compounds, highlighting significant variations not only between the species but also between the two plant parts (bark and flowers). This comparative analysis is essential for understanding the unique chemical makeup of each species and how it varies within the plant. Regarding the volatile compounds, this study focuses on comparing these compounds in the bark of the four Magnolia species. Common volatile compounds identified across all species include α -Thujene and α -Pinene. The concentrations of these compounds vary among the species: 0.1%, 0.1%, 0.4%, and 0.5%, respectively, and 0.4%, 1.1%, 0.2%, and 0.3%. Similar relative concentrations were presented by all bark extracts for the compound Camphene (1.1%, 1.1%, 1.1%, 1.3%).

Significant variations were observed in the case of Limonenes, where the percentages ranged between 0.5% in *M. grandiflora* and 2.2% in the bark extracts of *M. denudata*. 1,8-Cineole is present in all four species, with variable concentrations (0.5%, 2.1%, 0.5%, 1.7%).

Variable concentrations are noted for the compounds β -Pinene and Camphor, which can reach up to 6.6%, lower in the case of Phenylacetaldehyde and Borneol, where they are between 1.1% and 2.2%. Bornyl acetate is present in all four species, with concentrations of 25.6% (*M. champaca*), 14.9% (*M. denudata*), 14.2% (*M. grandiflora*), and 17.8% (*M. officinalis*). Significant results were obtained for the compound E-Caryophyllene, with obtained values in variable concentrations (11.1%, 22.4%, 21.1%, 15.6%). β -Caryophyllene was identified in all species with values ranging from 1.2%, 1.8%, 3.4%, to 5.6%, close to α -Selinene and Viridiflorene. 9-epi-(E)-Caryophyllene (3.7%, 2.9%, 3.8%, 3.1%) presents values close to n-Hexadecanol and n-Heneicosane. Germacrene D shows a maximum value in the bark extracts of *M. champaca* of 11.2%, followed by *M. officinalis* at 7.7%, *M. denudata* at 5.3%, and *M. grandiflora* at 4.6%. Present in all four species (0.9%, 0.5%, 1.5%, 1.7%) is also δ -Cadinene, n-Tricosene (7.7%, 5.5%, 1.7%, 4.2%), and n-Pentacosane (1.4%, 4.9%, 1.1%, 2.9%).

Table 3. Volatile compounds identified in the four Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*).

Compound	RI ^a	RI ^b	<i>M. champaca</i> %		<i>M. denudata</i> %		<i>M. grandiflora</i> %		<i>M. officinalis</i> %	
			Bark	Flowers	Bark	Flowers	Bark	Flowers	Bark	Flowers
Hexanal	801	800	-	0.1 ± 0.00	-	-	-	-	-	-
Heptanal	902	902	-	0.2 ± 0.00	-	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.3 ± 0.01	0.9 ± 0.01
α-Thujene	930	929	0.1 ± 0.00	1.2 ± 0.01	0.1 ± 0.00	1.5 ± 0.01	0.4 ± 0.01	0.7 ± 0.01	0.5 ± 0.01	2.1 ± 0.02
α-Pinene	939	932	0.4 ± 0.01	0.6 ± 0.01	1.1 ± 0.01	0.9 ± 0.01	0.2 ± 0.00	0.8 ± 0.01	0.3 ± 0.01	1.5 ± 0.01
Camphene	954	946	1.1 ± 0.01	-	1.1 ± 0.01	0.1 ± 0.00	1.1 ± 0.01	0.1 ± 0.00	1.3 ± 0.01	-
Sabinene	975	972	-	tr	-	tr	0.1 ± 0.00	tr	0.1 ± 0.00	tr
β-Pinene	979	980	3.6 ± 0.02	0.1 ± 0.00	2.9 ± 0.01	-	5.2 ± 0.03	tr	2.3 ± 0.02	tr
6-Methyl-5-hepten-2-one	986	985	-	1.1 ± 0.02	0.1 ± 0.00	-	0.1 ± 0.00	0.5 ± 0.01	-	-
α-Phellandrene	1002	1001	-	0.1 ± 0.00	-	-	-	0.2 ± 0.01	-	-
α-Terpinene	1016	1014	0.1 ± 0.00	0.5 ± 0.01	0.2 ± 0.00	0.5 ± 0.01	0.1 ± 0.00	0.7 ± 0.01	0.1 ± 0.00	1.2 ± 0.01
p-Cymene	1026	1024	-	0.2 ± 0.00	-	0.1 ± 0.00	-	0.2 ± 0.01	-	0.1 ± 0.00
Limonene	1029	1030	0.9 ± 0.01	4.4 ± 0.02	2.2 ± 0.01	7.1 ± 0.03	0.5 ± 0.01	2.9 ± 0.02	2.1 ± 0.02	1.8 ± 0.01
1,8-Cineol	1031	1031	0.5 ± 0.01	0.9 ± 0.01	2.1 ± 0.01	1.1 ± 0.01	0.5 ± 0.01	0.1 ± 0.00	1.7 ± 0.01	0.1 ± 0.00
β-Phellandrene	1034	1032	-	0.2 ± 0.00	-	0.4 ± 0.01	-	-	-	0.1 ± 0.00
(Z)-β-Ocimene	1038	1044	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.3 ± 0.01	0.1 ± 0.00	-
Phenylacetaldehyde	1042	1043	1.1 ± 0.01	1.8 ± 0.01	1.4 ± 0.01	3.9 ± 0.02	2.2 ± 0.02	1.6 ± 0.02	1.7 ± 0.02	-
(E)-β-Ocimene	1050	1052	0.1 ± 0.00	5.7 ± 0.02	0.1 ± 0.00	1.9 ± 0.01	0.1 ± 0.01	3.4 ± 0.02	0.1 ± 0.00	1.1 ± 0.02
γ-Terpinene	1060	1058	0.2 ± 0.00	3.2 ± 0.01	0.1 ± 0.00	2.1 ± 0.01	0.1 ± 0.01	0.8 ± 0.01	0.2 ± 0.00	0.3 ± 0.01
1-Octanol	1068	1066	0.4 ± 0.01	2.8 ± 0.01	2.0 ± 0.01	4.6 ± 0.02	2.4 ± 0.02	1.2 ± 0.01	1.6 ± 0.01	1.9 ± 0.02
cis-Sabinene hydrate	1070	1070	-	tr	-	0.1 ± 0.00	tr	-	-	0.1 ± 0.00
cis-Linalool oxide (furanoid)	1073	1074	0.2 ± 0.00	0.4 ± 0.01	0.1 ± 0.00	0.6 ± 0.01	0.2 ± 0.00	0.1 ± 0.00	-	0.3 ± 0.01
trans-Linalool oxide (furanoid)	1087	1085	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	-	0.2 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
Terpinolene	1092	1093	0.5 ± 0.01	0.2 ± 0.00	0.4 ± 0.01	0.3 ± 0.01	0.7 ± 0.01	0.3 ± 0.01	0.2 ± 0.00	0.8 ± 0.01
Linalool	1095	1100	1.4 ± 0.02	25.1 ± 0.06	2.7 ± 0.01	20.4 ± 0.07	1.8 ± 0.01	15.8 ± 0.06	2.0 ± 0.02	21.2 ± 0.07
ε-4,8-Dimethylnona-1,3,7-triene	1118	1120	0.1 ± 0.00	1.2 ± 0.01	1.0 ± 0.01	4.4 ± 0.02	1.1 ± 0.01	0.4 ± 0.01	1.3 ± 0.01	-
Perillene	1122	1123	-	0.1 ± 0.00	-	0.1 ± 0.00	-	0.1 ± 0.00	-	0.1 ± 0.00
Camphor	1141	1140	2.5 ± 0.02	1.1 ± 0.01	3.1 ± 0.01	1.3 ± 0.02	6.3 ± 0.03	0.8 ± 0.01	6.6 ± 0.03	3.4 ± 0.02
Borneol	1163	1165	1.1 ± 0.01	-	1.2 ± 0.01	0.1 ± 0.00	1.3 ± 0.01	0.1 ± 0.00	1.1 ± 0.01	-
Terpinen-4-ol	1186	1185	-	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
α-Terpineol	1189	1190	0.2 ± 0.00	2.2 ± 0.01	0.5 ± 0.01	0.8 ± 0.01	0.8 ± 0.01	1.6 ± 0.02	0.7 ± 0.01	2.1 ± 0.02
Myrtenol	1195	1193	0.1 ± 0.00	1.1 ± 0.01	0.1 ± 0.00	2.9 ± 0.02	0.1 ± 0.00	1.4 ± 0.01	0.1 ± 0.00	0.7 ± 0.01
Myrtenal	1196	1197	-	0.3 ± 0.01	tr	-	tr	0.5 ± 0.01	tr	-
β-Citronellol	1226	1223	0.4 ± 0.01	1.8 ± 0.01	0.9 ± 0.01	1.3 ± 0.02	0.7 ± 0.01	2.3 ± 0.02	0.5 ± 0.01	2.9 ± 0.02
Nerol	1230	1229	0.1 ± 0.00	1.6 ± 0.01	0.1 ± 0.00	1.1 ± 0.01	0.2 ± 0.00	4.9 ± 0.02	0.2 ± 0.00	2.2 ± 0.02
Neral	1238	1237	0.1 ± 0.00	0.1 ± 0.00	-	-	0.1 ± 0.00	-	-	-
Geraniol	1253	1255	1.1 ± 0.01	5.2 ± 0.02	0.5 ± 0.01	1.9 ± 0.01	0.9 ± 0.01	7.7 ± 0.03	0.8 ± 0.01	9.1 ± 0.03
Geranial	1257	1258	0.1 ± 0.00	0.3 ± 0.01	0.2 ± 0.00	0.1 ± 0.00	0.3 ± 0.01	0.3 ± 0.01	0.1 ± 0.00	0.9 ± 0.01
1-Decanol	1270	1273	0.2 ± 0.00	0.3 ± 0.01	0.1 ± 0.00	0.6 ± 0.01	0.1 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	0.7 ± 0.01
Bornyl acetate	1289	1290	25.6 ± 0.06	2.1 ± 0.01	14.9 ± 0.06	5.3 ± 0.03	14.2 ± 0.06	4.3 ± 0.03	17.8 ± 0.07	11.1 ± 0.04

Table 3. Cont.

Compound	RI ^a	RI ^b	<i>M. champaca</i> %		<i>M. denudata</i> %		<i>M. grandiflora</i> %		<i>M. officinalis</i> %	
			Bark	Flowers	Bark	Flowers	Bark	Flowers	Bark	Flowers
Myrtenyl acetate	1327	1325	0.5 ± 0.01	2.1 ± 0.01	1.0 ± 0.01	1.5 ± 0.01	1.1 ± 0.01	0.9 ± 0.01	1.3 ± 0.01	0.4 ± 0.01
Eugenol	1359	1360	0.1 ± 0.00	0.9 ± 0.01	0.2 ± 0.00	1.4 ± 0.01	0.2 ± 0.00	0.8 ± 0.01	0.1 ± 0.00	1.5 ± 0.01
α-Copaene	1388	1387	0.1 ± 0.00	0.4 ± 0.01	-	-	0.1 ± 0.00	-	0.1 ± 0.00	0.4 ± 0.01
β-Elemene	1404	1405	0.8 ± 0.01	1.2 ± 0.01	0.7 ± 0.01	2.0 ± 0.02	0.7 ± 0.01	1.9 ± 0.01	0.3 ± 0.01	1.0 ± 0.01
E-Caryophyllene	1417	1419	11.1 ± 0.05	1.2 ± 0.01	22.4 ± 0.07	0.9 ± 0.01	21.1 ± 0.07	0.7 ± 0.01	15.6 ± 0.06	1.2 ± 0.01
α-trans-Bergamotene	1435	1435	-	0.1 ± 0.00	-	0.1 ± 0.00	tr	0.1 ± 0.00	-	-
β-Caryophyllene	1437	1432	1.2 ± 0.01	0.2 ± 0.00	1.8 ± 0.01	1.3 ± 0.01	3.4 ± 0.02	2.9 ± 0.02	5.6 ± 0.03	1.1 ± 0.01
(Z)-β-Farnesene	1453	1447	-	0.1 ± 0.00	-	0.1 ± 0.00	0.1 ± 0.01	0.3 ± 0.01	0.7 ± 0.01	-
α-Humulene	1455	1455	0.1 ± 0.00	0.4 ± 0.01	0.3 ± 0.01	1.1 ± 0.01	0.2 ± 0.01	0.8 ± 0.01	0.5 ± 0.01	0.9 ± 0.01
9-epi-(E)-Caryophyllene	1470	1470	3.7 ± 0.02	0.1 ± 0.00	2.9 ± 0.01	1.8 ± 0.02	3.8 ± 0.02	0.9 ± 0.01	3.1 ± 0.02	0.3 ± 0.01
γ-Gurjunene	1477	1476	-	-	-	-	0.1 ± 0.00	0.1 ± 0.00	-	-
Germacrene D	1485	1481	11.2 ± 0.04	2.4 ± 0.01	5.3 ± 0.02	1.5 ± 0.01	4.6 ± 0.03	5.2 ± 0.03	7.7 ± 0.04	4.8 ± 0.02
α-Selinene	1488	1489	1.2 ± 0.01	0.1 ± 0.00	0.8 ± 0.01	0.5 ± 0.01	1.3 ± 0.01	0.7 ± 0.01	2.3 ± 0.02	0.1 ± 0.00
γ-Muurolene	1490	1494	0.1 ± 0.00	-	0.1 ± 0.00	0.8 ± 0.01	0.2 ± 0.00	0.5 ± 0.01	0.1 ± 0.00	0.3 ± 0.01
Viridiflorene	1496	1497	1.8 ± 0.02	2.1 ± 0.01	1.5 ± 0.01	1.8 ± 0.01	2.2 ± 0.02	2.6 ± 0.02	-	1.2 ± 0.01
Bicyclogermacrene	1500	1502	0.1 ± 0.00	0.4 ± 0.01	0.2 ± 0.00	0.4 ± 0.01	0.4 ± 0.01	1.1 ± 0.01	0.5 ± 0.01	-
δ-Cadinene	1523	1522	0.9 ± 0.01	2.0 ± 0.01	0.5 ± 0.01	0.8 ± 0.01	1.5 ± 0.01	0.3 ± 0.01	1.7 ± 0.01	3.5 ± 0.02
Hedycaryol	1548	1545	-	-	-	-	-	0.1 ± 0.00	-	-
Elemol	1550	1552	-	-	-	-	-	0.8 ± 0.01	-	0.5 ± 0.01
trans-Nerolidol	1556	1555	0.1 ± 0.00	0.1 ± 0.00	-	-	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	-
(E)-Nerolidol	1563	1563	0.2 ± 0.00	0.5 ± 0.01	-	0.1 ± 0.01	0.6 ± 0.01	0.2 ± 0.00	0.3 ± 0.01	0.1 ± 0.00
Palustrol	1567	1569	-	-	-	-	-	0.1 ± 0.00	-	-
Germacrene D-4-ol	1576	1577	0.1 ± 0.00	1.4 ± 0.01	0.1 ± 0.00	1.1 ± 0.01	0.2 ± 0.00	1.5 ± 0.02	0.2 ± 0.00	1.0 ± 0.01
Spathulenol	1578	1579	3.6 ± 0.02	0.1 ± 0.00	2.8 ± 0.01	0.5 ± 0.01	1.7 ± 0.01	0.1 ± 0.00	2.2 ± 0.02	0.1 ± 0.01
Globulol	1585	1588	0.1 ± 0.00	0.1 ± 0.00	-	-	-	0.8 ± 0.01	-	-
β-Eudesmol	1650	1651	-	-	-	-	-	0.1 ± 0.00	-	-
α-Cadinol	1654	1653	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	1.0 ± 0.01	0.3 ± 0.01	1.1 ± 0.01	0.1 ± 0.00	1.7 ± 0.01
Selin-11-en-4-α-ol	1659	1657	0.2 ± 0.00	0.1 ± 0.00	0.5 ± 0.01	1.9 ± 0.01	0.1 ± 0.00	-	0.4 ± 0.01	0.5 ± 0.01
Shyobunol	1688	1688	-	-	-	-	0.1 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	0.1 ± 0.01
(E)-Nerolidyl acetate	1717	1713	-	-	-	-	-	0.1 ± 0.00	-	-
(Z,E)-Farnesol	1725	1722	0.3 ± 0.01	0.4 ± 0.01	0.5 ± 0.01	-	0.9 ± 0.01	0.3 ± 0.01	0.6 ± 0.01	0.2 ± 0.01
14-Hydroxy-α-muurolene	1780	1777	0.2 ± 0.00	0.5 ± 0.01	0.2 ± 0.00	0.6 ± 0.01	0.5 ± 0.01	0.3 ± 0.01	0.4 ± 0.01	0.1 ± 0.01
14-Hydroxy-δ-cadinene	1802	1800	0.5 ± 0.01	0.5 ± 0.01	-	0.4 ± 0.01	0.1 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	0.3 ± 0.01
n-Hexadecanol	1875	1872	3.3 ± 0.02	7.4 ± 0.03	2.3 ± 0.01	2.2 ± 0.02	1.7 ± 0.01	2.7 ± 0.03	1.1 ± 0.01	3.3 ± 0.01
n-Heneicosane	2100	2100	2.1 ± 0.01	0.2 ± 0.00	2.7 ± 0.01	0.2 ± 0.01	1.6 ± 0.01	0.3 ± 0.01	0.5 ± 0.01	0.5 ± 0.01
Linoleic acid	2133	2130	0.1 ± 0.00	0.6 ± 0.01	0.1 ± 0.00	2.2 ± 0.02	0.1 ± 0.00	1.4 ± 0.01	0.4 ± 0.01	1.0 ± 0.01
n-Tricosene	2300	2300	7.7 ± 0.03	1.9 ± 0.01	5.5 ± 0.02	1.8 ± 0.01	1.7 ± 0.01	4.1 ± 0.03	4.2 ± 0.03	2.8 ± 0.01
n-Pentacosane	2500	2500	1.4 ± 0.02	1.9 ± 0.01	4.9 ± 0.02	1.3 ± 0.01	1.1 ± 0.01	2.1 ± 0.02	2.9 ± 0.02	1.9 ± 0.01
Total			95.3	96.0	96.9	95.1	94.1	91.1	97.5	97.7
Other compounds			4.7 ± 0.02	4.0 ± 0.01	3.1 ± 0.01	4.9 ± 0.01	5.9 ± 0.02	8.9 ± 0.02	2.5 ± 0.01	2.3 ± 0.01

RI^a—calculated retention index; RI^b—retention index from literature. Determinations were performed in triplicate.

Some of the volatile compounds identified are specific to each species, namely, *M. champaca* contains Heptanal (0.1%) and 1-Octanol (0.4%). *M. denudata* contains 6-Methyl-5-hepten-2-one (0.1%). *M. grandiflora* contains α -Phellandrene (0.1%) and p-Cymene (0.1%). *M. officinalis* contains (Z)- β -Farnesene (0.1%), γ -Gurjunene (0.1%), and γ -Muurolene (0.2%). To compare the volatile compounds in the four species (*M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*) in terms of their content in flowers, we will analyze the presence and relative concentrations of these compounds: α -Thujene and α -Pinene are present in all four species, with variations in concentration of 1.2%, 1.5%, 0.7%, and 2.1% and 0.6%, 0.9%, 0.8%, and 1.5%, respectively.

The volatile compound 1,8-Cineole exhibits its lowest values in *M. grandiflora* and *M. officinalis*, around 0.1%. In contrast, higher concentrations of this compound are found in the hydro-ethanolic flower extracts of *M. champaca* and *M. denudata*, at 0.9% and 1.1%, respectively. Phenylacetaldehyde and (E)- β -Ocimene were identified in all four Magnolia species, showing a range of concentrations from 1.1% to 5.7%. The concentration of 1-Octanol varies notably among these species. It is found to be 2.8% in *M. champaca*, and it reaches 4.6% in *M. denudata*. In *M. grandiflora*, the concentration is 1.2%, while *M. officinalis* has a slightly higher value at 1.9%. Other volatile compounds, such as cis-Linalool oxide (furanoid), are present in a range between 0.1% and 0.6%. Terpinolene, another volatile compound, is found in concentrations varying from 0.2% to 0.8% among these species.

Linalool is found in substantial amounts in the flower extracts of the studied Magnolia species. The identified concentrations are notably high, with 25.1% in *M. champaca*, 20.4% in *M. denudata*, 15.8% in *M. grandiflora*, and 21.2% in *M. officinalis*. Geraniol and α -Terpineol, two other volatile compounds, vary in concentration between 1.6% and 9.1% across these species.

Bornyl acetate is another compound present in all four Magnolia species, showing significant variations in its concentration: 2.1%, 5.3%, 4.3%, and 11.1%. Each Magnolia species also contains specific volatile compounds in their flower extracts: *M. champaca* is characterized by the presence of Hexanal (0.1%) and Myrtenal (0.3%); *M. denudata* includes trace amounts of cis-Sabinene hydrate and trans-Nerolidol (0.1%); *M. grandiflora* contains trace amounts of β -Pinene, as well as Perillene (0.1%), α -trans-Bergamotene (0.1%), and n-Eicosane (0.3%); and *M. officinalis* is distinguished by 6-Methyl-5-hepten-2-one (0.5%) and (E)- β -Farnesene (0.3%). When comparing the data across the species *M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis* and analyzing the content of volatile compounds in both bark and flowers, several observations emerge: In *M. champaca*, the flower extracts contain significantly higher amounts of volatile compounds compared to the bark extracts.

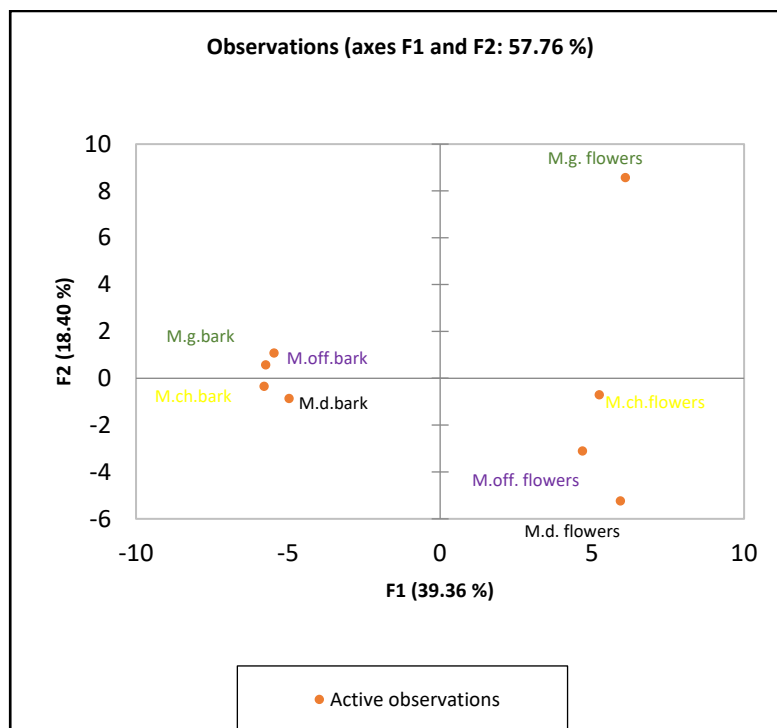
In the gas chromatography analyses conducted for this study, a total of 76 volatile components were identified across the Magnolia species. Using statistical models, the main components were grouped based on the aromatic profile of the extracts from the bark and flowers of the four Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*).

The concentration of oxygenated monoterpenes in the bark extracts ranged between 38.6% and 49.6%, while in the flower extracts, these values were significantly higher, ranging between 52.8% and 68.4%. This indicates a more pronounced presence of oxygenated monoterpenes in the flowers compared to the bark. Hydrocarbon monoterpenes varied between 7.2% and 19.6%. The concentration of oxygenated sesquiterpenes in the extracts ranged between 2.3% and 4.6%. In contrast, hydrocarbon sesquiterpenes varied more widely, comprising between 33.1% and 40.4% in the bark extracts and between 11.8% and 19.7% in the flower extracts.

Figure 1 in this study provides a graphical representation, a scatter plot or a similar type of chart showing the separation of extracts based on species (*M. grandiflora* and *M. officinalis*) and plant parts (bark or flowers). This separation is depicted along positive axes in the graph.

In Figure 1a, the flower extracts of *M. grandiflora* are particularly distinct, positioned in the second quadrant, and are characterized by a rich composition of various volatile

compounds. These include α -Thujene, α -Pinene, α -Terpinene, Limonene, (E)- β -Ocimene, γ -Terpinene, 1-Octanol, Linalool, α -Terpineol, Myrtenol, Nerol, and Geraniol. Their location in the second quadrant suggests a unique volatile profile that sets them apart from the other extracts studied. Bark extracts of *M. grandiflora* and *M. officinalis* are near the axis dividing the quadrants, being rich in volatile compounds such as α -Thujene, α -Pinene, β -Pinene, Limonene, 1-Octanol, Bornyl acetate, β -Caryophyllene, 9-epi-(E)-Caryophyllene, and Germacrene D. In quadrant one, on the negative semi-axis, the bark extracts of *M. champaca* (M.ch.) and *M. denudata* (M.d.) are shown, represented by compounds like β -Pinene, Limonene, Camphor, Bornyl acetate, E-Caryophyllene, Germacrene D, Viridiflorene, Spathulenol, etc. In quadrant two, on the negative semi-axis, the flower extracts of *M. champaca* (M.ch.) are shown, which are rich in α -Thujene, Limonene, (E)- β -Ocimene, γ -Terpinene, 1-Octanol, Linalool, and *M. denudata* (M.d.), along with *M. officinalis* (M.off.), with significant values in volatile compounds such as α -Thujene, α -Pinene, Limonene, 1-Octanol, Linalool, β -Citronellol, Geraniol, etc. (Figure 1b). The multivariate analysis was complemented by a cluster analysis that minimized variation within the group, revealing two distinct clusters for bark and flowers (Figure 2). In this study, a wide range of metabolites was observed, with the quantity of volatile compounds differing based on both the source and the species from which the extracts were derived. The vectors depicted in the graphical representations illustrate the contribution of each compound to the overall distribution of variables, highlighting how individual components influence the compositional makeup of the extracts. Additionally, the multivariate analysis was further enhanced by conducting a cluster analysis. This analysis focused on minimizing the variation within each group, leading to the identification of two distinct clusters corresponding to bark and flower extracts. This differentiation is clearly depicted in Figure 2, effectively showcasing the inherent compositional differences between the bark and flower extracts of the Magnolia species studied.



(a)

Figure 1. Cont.

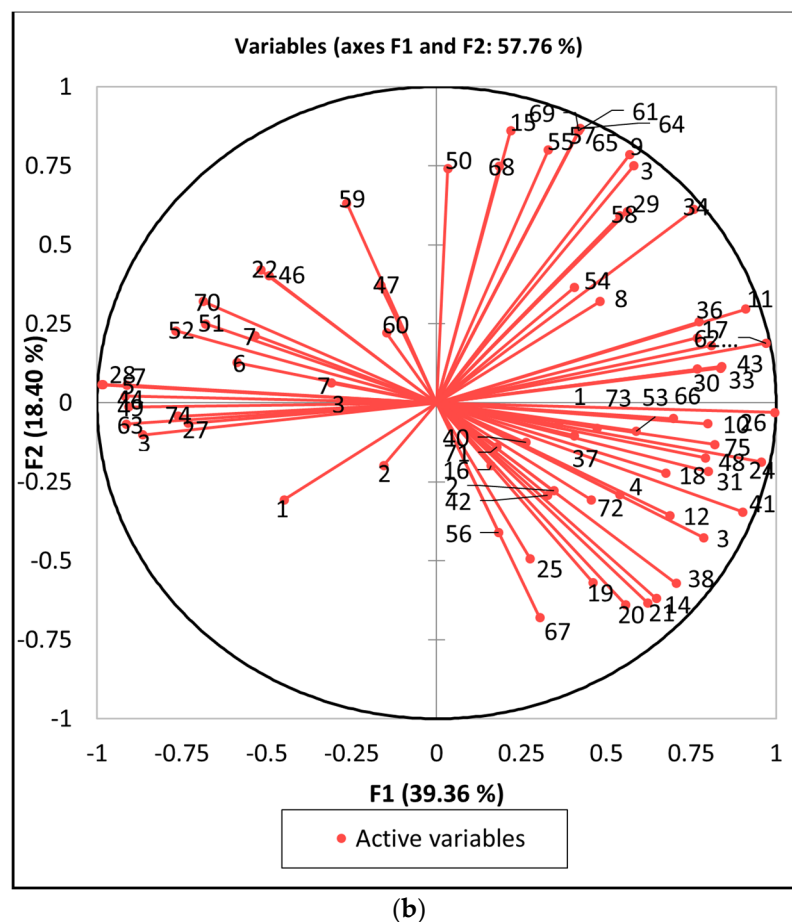


Figure 1. Differentiation based on species of bark and flower extracts against compositional profile: (a) the PCA diagram illustrates the differentiation of bark and flower extracts based on their compositional profiles. The principal axes represent 57.76% of the total variance in the data set. (b) displays the graphical representation of PCA variation according to the species studied, in relation to compositional variability. Legend: 1. Hexanal, 2. Heptanal, 3. α -Thujene, 4. α -Pinene, 5. Camphene, 6. Sabinene, 7. β -Pinene, 8. 6-Methyl-5-hepten-2-one, 9. α -Phellandrene, 10. α -Terpinene, 11. p-Cymene, 12. Limonene, 13. 1,8-Cineol, 14. β -Phellandrene, 15. (Z)- β -Ocimene, 16. Phenylacetaldehyde, 17. (E)- β -Ocimene, 18. γ -Terpinene, 19. 1-Octanol, 20. cis-Sabinene hydrate, 21. cis-Linalool oxide (furanoid), 22. trans-Linalool oxide (furanoid), 23. Terpinolene, 24. Linalool, 25. ϵ -4,8-Dimethylnona-1,3,7-triene, 26. Perillene, 27. Camphor, 28. Borneol, 29. Terpinen-4-ol, 30. α -Terpineol, 31. Myrtenol, 32. Myrtenal, 33. β -Citronellol, 34. Nerol, 35. Neral, 36. Geraniol, 37. Geranial, 38. 1-Decanol, 39. Bornyl acetate, 40. Myrtenyl acetate, 41. Eugenol, 42. α -Copaene, 43. β -Elemene, 44. E-Caryophyllene, 45. α -trans-Bergamotene, 46. β -Caryophyllene, 47. (Z)- β -Farnesene, 48. α -Humulene, 49. 9-epi-(E)-Caryophyllene, 50. γ -Gurjunene, 51. Germacrene D, 52. α -Selinene, 53. γ -Muurolole, 54. Viridiflorene, 55. Bicyclogermacrene, 56. δ -Cadinene, 57. Hedycaryol, 58. Elemol, 59. trans-Nerolidol, 60. (E)-Nerolidol, 61. Palustrol, 62. Germacrene D-4-ol, 63. Spathulenol, 64. Globulol, 65. β -Eudesmol, 66. α -Cadinol, 67. Selin-11-en-4- α -ol, 68. Shyobunol, 69. (E)-Nerolidyl acetate, 70. (Z,E)-Farnesol, 71. 14-Hydroxy- α -muurolole, 72. 14-Hydroxy- δ -cadinene, 73. n-Hexadecanol, 74. n-Heneicosane, 75. Linoleic acid, 76. n-Tricosene, 77. n-Pentacosane.

3.3. Inhibitory Potential of Hydro-Ethanollic Magnolia Extracts

This study involved testing both bark and flower extracts of Magnolia species against selected bacterial strains to determine their minimum inhibitory concentration (MIC). The purpose of this testing was to evaluate the potential of these extracts for use in developing natural cosmetic products with antibacterial properties. As detailed in Table 4, a range of both Gram-positive and Gram-negative bacteria were chosen for this assessment. The

inclusion of different types of bacteria, both Gram-positive and Gram-negative, ensures a thorough assessment of the extracts' broad-spectrum antibacterial capabilities.

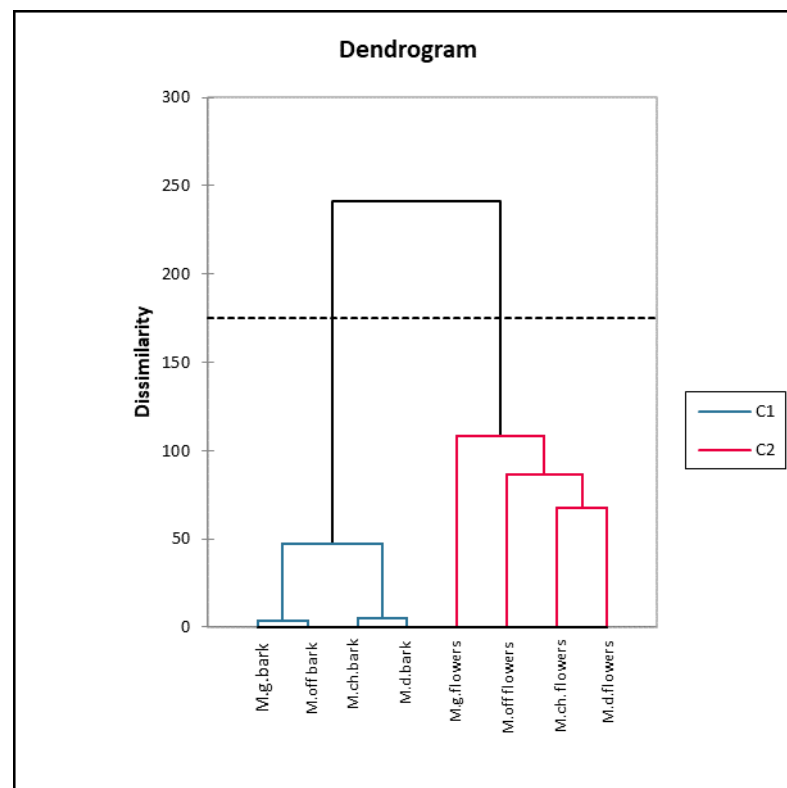


Figure 2. Cluster distribution of bark and flower extracts from four Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora*, *M. officinalis*).

The data from Table 4 in this study clearly demonstrate the varying sensitivity of different bacterial strains to extracts from the bark and flowers of four Magnolia species: *M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*. Specifically, this study focused on the sensitivity of these extracts to the *Staphylococcus aureus* ATCC 43300 (MRSA) strain, a commonly known resistant bacteria. The results indicate that the MRSA strain showed relative insensitivity to both conventional antibiotics (ampicillin, tetracycline) and the Magnolia extracts from both bark and flowers. Notably, this strain exhibited no sensitivity to the bark extracts of *M. champaca* and *M. grandiflora*. In contrast, the bark extracts from *M. denudata* and *M. officinalis* showed some effectiveness, with MIC values recorded at 30 µg/mL. Similarly, the flower extracts from *M. grandiflora* and *M. officinalis* also demonstrated MIC values of 30 µg/mL.

The results from Table 4 contribute to a broader understanding of the antibacterial properties of different Magnolia species and their potential applicability in addressing antibiotic-resistant bacterial strains. This study also examined the effectiveness of Magnolia extracts against *Staphylococcus epidermidis* ATCC 12228, a bacterium known to show moderate resistance to antibiotics. The bark extracts from the Magnolia species displayed varied effectiveness against this strain: *M. officinalis* bark extract showed the highest efficacy with an MIC value of 5 µg/mL, while *M. champaca* and *M. denudata* had MIC values of 15 µg/mL. On the other hand, the flower extracts of *M. champaca* and *M. denudata* were less effective, exhibiting weaker action with MIC values of 30 µg/mL.

However, flower extracts from *M. grandiflora* and *M. officinalis* were more potent, with MIC values of 7.5 µg/mL and 5 µg/mL, respectively. Furthermore, this study revealed that *Streptococcus faecalis* ATCC 19443 responded effectively to Magnolia extracts. The bark extracts from the Magnolia species had MIC values ranging from 5 µg/mL to 15 µg/mL, whereas the flower extracts showed a range of 2.5 µg/mL to 15 µg/mL.

Table 4. Inhibitory potential of hydro-ethanolic Magnolia extracts (*M. champaca*, *M. denudata*, *M. grandiflora*, *M. officinalis*) expressed in MIC ($\mu\text{g/mL}$).

Bacterial Strains	MIC ($\mu\text{g/mL}$)										
	Ampicillin	Gentamicin	Tetracycline	<i>M. champaca</i>		<i>M. denudata</i>		<i>M. grandiflora</i>		<i>M. officinalis</i>	
				Bark	Flo-wer	Bark	Flo-wer	Bark	Flo-wer	Bark	Flo-wer
<i>Staphylococcus aureus</i> ATCC 43300(MRSA)	n.a.	30	n.a.	n.a	n.a	30	n.a.	n.a.	30	30	30
<i>Staphylococcus epidermidis</i> ATCC 12228	10	5	5	15	30	15	30	7.5	7.5	5	5
<i>Streptococcus faecalis</i> ATCC 19443	2.5	2.5	2.5	15	2.5	7.5	15	7.5	7.5	5	2.5
<i>Streptococcus pyogenes</i> ATCC 12347	1.25	1.25	2.5	30	30	n.a.	30	15	n.a.	15	n.a.
<i>Streptococcus sanguinis</i> ATCC 10556	2.5	2.5	n.a.	n.a	n.a.	n.a.	n.a.	n.a.	30	30	30
<i>Actinomyces israelii</i> ATCC 12102	0.625	0.625	0.625	15	30	12.5	15	10	10	7.5	7.5
<i>Propionibacterium acnes</i> ATCC 6921/4311	1.25	1.25	0.625	7.5	2.5	10	12.5	5	7.5	5	2.5
<i>Enterobacter aerogenes</i> ATCC 13048	0.625	0.625	30	15	10	30	30	10	12.5	7.5	10
<i>Escherichia coli</i> ATCC 35218	0.625	0.625	30	n.a.	n.a.	15	30	15	15	10	12.5
<i>Klebsiella pneumoniae</i> ATCC 13883	0.625	0.625	30	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	30	n.a.
<i>Preootella intermedia</i> ATCC 25611	0.625	0.625	0.625	5	15	n.a.	n.a.	10	2.5	15	15
<i>Porphyromonas gingivalis</i> ATCC 33277	0.625	30	15	0.625	5	7.5	10	5	2.5	0.625	1.25
<i>Proteus vulgaris</i> ATCC 13315	0.625	0.625	10	10	10	15	15	7.5	12.5	5	10
<i>Pseudomonas aeruginosa</i> ATCC 27853	n.a.	0.625	n.a.	5	12.5	12.5	15	7.5	10	2.5	2.5

n.a.: no activity.

Notably, the extracts from *M. officinalis* demonstrated the best results, with the bark extract having an MIC of 5 $\mu\text{g/mL}$ and the flower extract even lower at 2.5 $\mu\text{g/mL}$. These findings highlight the potential of Magnolia extracts as effective antibacterial agents against specific bacterial strains, including those resistant to conventional antibiotics. *Streptococcus pyogenes* ATCC 12347, a bacterium sensitive to all three tested antibiotics, exhibited varying responses to Magnolia extracts. It showed no reaction to the bark extracts from *M. denudata* and the flower extracts from both *M. grandiflora* and *M. officinalis*. However, a slight sensitivity was observed with an MIC value of 30 $\mu\text{g/mL}$ for both the bark and flower extracts of *M. champaca*, as well as the flower extracts from *M. denudata*. In tests involving *Streptococcus sanguinis* ATCC 10556, no substantial inhibitory potential was observed with most of the Magnolia extracts. Only very low sensitivity was detected in the case of the bark and flower extracts from *M. officinalis* and the flower extracts from *M. grandiflora*, each with an MIC of 30 $\mu\text{g/mL}$.

These results indicate that while Magnolia extracts can be effective against certain bacterial strains, their efficacy varies significantly depending on the species of the extract and the bacterial strain. The Magnolia extracts tested in this study exhibited a range of effectiveness, from moderate to sensitive, against the bacterial strains *Actinomyces israelii* ATCC 12102 and *Propionibacterium acnes* ATCC 6921/4311. The minimum inhibitory concentration (MIC) values for these extracts against these strains varied, with the most notable values ranging between 2.5 $\mu\text{g/mL}$ and 15 $\mu\text{g/mL}$. However, an exception was noted in the case of the flower extracts from *M. champaca*, which displayed a higher MIC of 30 $\mu\text{g/mL}$ against the strain *Actinomyces israelii* ATCC 12102. In the assessment of Gram-negative bacteria, this study found varying levels of effectiveness of the Magnolia extracts against *Enterobacter aerogenes* ATCC 13048. Most extracts exhibited medium to low inhibitory activity, with minimum inhibitory concentration (MIC) values ranging between 10 $\mu\text{g/mL}$ and 30 $\mu\text{g/mL}$. However, an exception was noted in the case of the bark extracts from *M. officinalis*, which demonstrated intense inhibitory potential with a MIC of 7.5 $\mu\text{g/mL}$. In the case of *Escherichia coli* ATCC 35218, the extracts generally showed moderate to low inhibitory action, with MIC values again falling within the range of 10 $\mu\text{g/mL}$ to 30 $\mu\text{g/mL}$.

Notably, the extracts from both the bark and flowers of *M. champaca* did not inhibit this strain of bacteria.

This study revealed that the Magnolia extracts generally did not exhibit inhibitory activity against the *Klebsiella pneumoniae* ATCC 13883 strain, as no significant antibacterial effects were observed in most cases. However, there was a notable exception with the bark extract from *M. officinalis*, which demonstrated a mild effect on this strain, reflected in a minimum inhibitory concentration (MIC) of 30 µg/mL. In contrast, the bark and flower extracts from various Magnolia species showed medium to very good inhibitory potential against *Prevotella intermedia* ATCC 25611 and *Porphyromonas gingivalis* ATCC 33277. The MIC values for these strains ranged from as low as 0.625 µg/mL to 15 µg/mL. Particularly effective were the bark extracts from *M. champaca* and *M. officinalis*, which yielded the best results against these strains. The extracts also displayed medium inhibitory potential against *Proteus vulgaris* ATCC 13315, with MIC values ranging between 5 µg/mL and 15 µg/mL.

This study showed that *Magnolia officinalis*, including both bark and flower extracts, exhibited very good inhibitory potential against the *Pseudomonas aeruginosa* ATCC 27853 strains, with a minimum inhibitory concentration (MIC) of 2.5 µg/mL. This indicates a high level of effectiveness in inhibiting the growth of this bacterial strain. In contrast, the extracts from the other three Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora*) displayed moderate inhibitory potential against the same strain, with MIC values ranging between 7 µg/mL and 15 µg/mL.

4. Discussion

This study's identification of total polyphenols in hydro-ethanolic extracts and their antioxidant activities provides profound insights. These extracts revealed a significant variation in chemical composition between the bark and flowers of Magnolia species. Notably, while the bark generally exhibits higher phenolic acid concentrations, the antioxidant activities of flower extracts are comparably effective. Polyphenols, known for their antioxidant capabilities, also contribute to inhibiting the activity of various microorganisms.

Although flower extracts generally exhibit slightly lower polyphenol concentrations than bark extracts, their antioxidant activities are comparable. Further supporting these observations, other researchers have also identified polyphenols and antioxidant activity in Magnolia flowers. Studies reported polyphenols levels, ranging from 14 mg/g to 17 mg/g, between 86 mg GAE/g and 192 mg GAE/g, at 46.8 mg GAE/g, with variations depending on factors like moisture and temperature. The antioxidant activity in these studies recorded to range between 84 and 311 mg VCE/g MFE, or was 54.5 µg/mL (DPPH/IC50), 459.6 µmol TE/g (ABTS), thus demonstrating the significant antioxidant potential of Magnolia species [21–23].

It is observed that, in general, the bark of these species contains a greater concentration of various phenolic acids than the flowers. Specifically, *M. champaca* and *M. grandiflora* demonstrate a notably high content of these acids in both the bark and flowers, in contrast to *M. denudata* and *M. officinalis*. This finding indicates the potential high value of *M. champaca* and *M. grandiflora* in applications where elevated levels of phenolic compounds are advantageous, given their abundance in both essential parts of the plant. Comparative studies have identified phenolic acids ranging from 0.018 mg/g dry weight (D.W.) to 4.372 mg/g D.W. in these species [21,24,25]. In the analyzed Magnolia species, compounds such as catechin, myricetin, luteolin, taxifolin, apigenin, and epicatechin are predominantly concentrated in the bark relative to the flowers. However, *M. denudata* is distinguished by its significant flower-based content of catechin, luteolin, and apigenin, suggesting a unique flavonoid profile in this species. Conversely, *M. grandiflora* displays elevated levels of myricetin and epicatechin in both bark and flowers, indicative of a more homogeneous distribution of these compounds throughout the plant. Analysis of flavonoid glycosides in these species reveals that compounds like rutin, luteolin-7-O-glucoside, kaempferol-3-O-rutinoside, apigenin-7-O-glucoside, and isorhamnetin-3-O-rutinoside are generally more

abundant in the bark than in the flowers. However, *M. champaca* is an exception, showing higher rutin content in both bark and flowers, suggesting a uniform distribution of rutin within the plant. Additionally, in *M. grandiflora*, luteolin-7-O-glucoside is significantly present in both bark and flowers, denoting a consistent presence of this compound across different plant parts. Earlier research has identified the presence of rutin in the flower extracts of *Magnolia denudata* [22], chlorogenic acid and coumaric acid in *Magnolia sieboldii* foliage [26], and quercetin and rutin in *Magnolia obovata* leaves [27].

Furthermore, this comparative study of *Magnolia* species reveals that the bark, in general, has higher levels of lignans, such as 4'-O-methylhonokiol, magnolol, honokiol, 3-methoxymagnolol, and isomagnolol, relative to the flowers. Notably, *M. officinalis* is distinguished for its significantly high content of lignans in the bark, particularly magnolol and honokiol. The presence of lignans in both the bark and flowers of *Magnolia* species has been confirmed in several studies [28–31]. These studies underscore the notable presence and impact of these compounds in *Magnolia* extracts, attributing antibacterial, antioxidant, and antitumor properties to them [32–36].

The analysis of volatile compounds in *Magnolia* species reveals a complex picture. While there are overarching similarities, significant differences are evident in the concentration and types of these compounds in the flowers and bark of the four studied species. Particularly, the flowers were identified as primary reservoirs of these volatile compounds, which are essential contributors to the unique aromas and characteristics of each *Magnolia* species. This variation is critical for understanding the potential applications of these compounds in industries like cosmetics, perfumery, and medicine. Compounds such as α -Thujene, α -Pinene, β -Pinene, Limonene, Camphor, Bornyl acetate, E-Caryophyllene, and Germacrene D, as well as linalool, n-Tricosene, or linoleic acid, show varying concentrations. Terpenoids like linalool and limonene, which are commonly found in essential oils and known for their floral and citrus aromas, are present in significant quantities in all four *Magnolia* species. These compounds are typical for essential oils and have distinct floral and citrus aromas. Aldehydes, including heptanal and octanal, are found in modest amounts in the bark and flowers of *Magnolia* species. These aldehydes play a role in contributing to the specific aromas of the extracts. Alongside these, alcohols such as linalool and terpineol are significantly present in the flowers of *Magnolia* species, imparting fresh and floral scents to the extracts. Ketones are generally present in small quantities or are absent in most *Magnolia* species, with the notable exception of Camphor, which is abundant and falls under this chemical category. Esters, including bornyl acetate and myrtenyl acetate, are found in considerable quantities in both the bark and flowers of *Magnolia* species. These esters likely contribute to the fruity and sweet aromas of the extracts. Hydrocarbons, particularly α - and β -pinene, are variably present across all *Magnolia* species. These compounds are known for adding fresh and woody aromas to the extracts. The varying presence and concentration of these hydrocarbons across different species contribute to the diversity in scent profiles [37–41].

The identified volatile compounds show significant variations not only between species but also between the two parts of the plant (bark and flowers). This comparative analysis is essential for understanding the unique chemical makeup of each species and how it varies within the plant. When comparing the data across the species *M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis* and analyzing the content of volatile compounds in both bark and flowers, several observations emerge: In *M. champaca*, the flower extracts contain significantly higher amounts of volatile compounds compared to the bark extracts.

Linalool and Limonene are dominant in the flower extracts of this species, contributing to its distinct aromatic profile. For *M. denudata*, like in *M. champaca*, the flower extracts exhibit a notably higher concentration of volatile compounds compared to the bark. Linalool and Limonene are prominent in the flowers of *M. denudata*, contributing significantly to their characteristic aroma. This suggests that the flowers of *M. denudata* are particularly enriched with these compounds, which are known for their distinctive fragrances. In the case of *M. grandiflora*, there are significant differences in the volatile compound profiles between

the bark and flowers. The flowers of *M. grandiflora* are characterized by a high content of Linalool, whereas the bark is notable for its substantial amounts of Camphor. This indicates a distinct chemical composition in different parts of the plant, each contributing uniquely to the overall aromatic and phytochemical profile of *M. grandiflora*. Like *M. champaca* and *M. denudata*, the flowers of *M. officinalis* also contain a greater variety and concentration of volatile compounds than the bark. Notably, Linalool, Limonene, and Camphor are key compounds in the flowers of this species. This pattern reinforces the trend observed across these Magnolia species, where the flower extracts generally have a richer and more diverse array of volatile compounds compared to the bark. Using statistical models, the main components were grouped based on the aromatic profile of the extracts from the bark and flowers of the four Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*). The obtained values are notably lower than those reported in previous studies. Baez et al. [42] reported the most abundant compounds: β -Pinene (10.5%), geraniol (7.4%) and germacrene D (6.2%), (*E*)- β -ocimene (24.6%), geraniol (18.9%), and β -elemene (11.2%) and germacrene D (9.9%); Farag et al. [43] reported farnesol (18%), 2-phenylethanol (10%), germacrene D (17%), and β -bisabolene (17%). The observed differences in the data reflect the variability in aromatic profiles both between different Magnolia species and between various parts of the plant [44]. Moreover, there is a notable variation in both the concentration and composition of these volatile compounds across different species. It is imperative to acknowledge that these diverse volatile entities significantly contribute to the distinct aromas and characteristics inherent to each Magnolia species. The unique aromatic profiles emanating from these compounds present the potential for utilization in a variety of sectors, including but not limited to the cosmetic, perfumery, and medicinal industries. Their distinctive properties can be leveraged in the development of fragrances, therapeutic agents, or other specialized applications, highlighting the significance of comprehensively understanding and characterizing these volatile compounds in Magnolia species.

However, the extent of this antibacterial potential varies based on both the plant part from which the extract is derived (bark versus flowers) and the specific Magnolia species. Among the extracts tested, the strongest inhibitory potential was observed in the bark extracts. These extracts were particularly effective against a range of bacterial strains, including *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 19443, *Propionibacterium acnes* ATCC 6921/4311, *Prevotella intermedia* ATCC 25611, *Porphyromonas gingivalis* ATCC 33277, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 27853. These findings highlight the potential of Magnolia bark extracts as a source of natural antibacterial agents. The presence and concentration of these compounds are directly linked to the strength of the potential inhibitory effects, ranging from strong to moderate or weak. The lower MIC levels observed in *M. officinalis* extracts suggest a greater efficacy in inhibiting bacterial growth compared to the extracts from the other species. This effectiveness can be attributed to the specific volatile compounds identified in the extracts. The presence and concentration of these compounds are directly linked to the strength of the antibacterial effects, ranging from strong to moderate or weak.

These findings highlight the potential of *M. officinalis* as a particularly effective source for antimicrobial applications and emphasize the importance of understanding the composition of volatile compounds in Magnolia extracts for their targeted use in combating bacterial infections.

More studies reported antibacterial activity against *Staphylococcus aureus* and *E. coli* strains, according to their chemical composition [45–48].

The antimicrobial properties of magnolol and honokiol, compounds extracted from *M. officinalis*, were studied by Ho et al. [49]. Their research focused on testing these compounds against a range of bacterial strains, including *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Micrococcus luteus*, and *Bacillus subtilis*.

The results showed a minimum inhibitory concentration (MIC) of 25 $\mu\text{g}/\text{mL}$ for these strains, indicating effective inhibitory potential. However, the same study found that magnolol and honokiol were less effective against other bacteria, such as *Shigella*

flexneii, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with MIC values of 100 µg/mL or higher, indicating relatively lower efficacy against these strains. Subsequent studies recognized and confirmed the antimicrobial qualities of phytochemical compounds of Magnolias extracts [50,51].

5. Conclusions

Hydro-ethanolic extracts of Magnolia bark and flowers show significant concentrations of bioactive compounds. The extracts derived from both the bark and flowers of Magnolia species are rich in polyphenols, phenolic compounds, and volatile compounds, making them valuable for various applications in cosmetics, medicine, the food industry, and pharmaceuticals due to their antioxidant activity. Each Magnolia species possesses a unique olfactory profile due to the distinct composition of these aromatic compounds. This uniqueness in aroma profiles potentially influences not only the sensory attributes but also the specific properties of each species, making them suitable for different applications in areas like aromatherapy, perfumery, and natural product formulations. The four species studied are rich in flavonoids. They have a decisive role in the antioxidant and antimicrobial properties they have.

These studies establish the significance of these compounds as potential antimicrobial agents, although their effectiveness varies depending on the specific bacterial strains. The variation in efficacy between different species and plant parts underscores the importance of targeted selection and utilization of these extracts, depending on the specific bacterial strains to be targeted.

This study demonstrates that all four Magnolia species (*M. champaca*, *M. denudata*, *M. grandiflora*, and *M. officinalis*) possess notable potential to induce the inhibitory effect against a variety of bacterial strains.

These flower extracts showed considerable effectiveness against *Streptococcus faecalis* ATCC 19443, *Propionibacterium acnes* ATCC 6921/4311, *Prevotella intermedia* ATCC 25611, *Porphyromonas gingivalis* ATCC 33277, and *Pseudomonas aeruginosa* ATCC 27853. However, this study also highlighted instances where the Magnolia extracts displayed very low or even no inhibitory effect. This was especially evident against strains such as *Staphylococcus aureus* ATCC 43300 (MRSA), *Streptococcus pyogenes* ATCC 12347, *Escherichia coli* ATCC 35218, and *Klebsiella pneumoniae* ATCC 13883.

These observations underscore the variability of Magnolia extracts, which can be influenced by factors such as the specific bacterial strain and the part of the plant from which the extract is derived. While some extracts show promising results against certain pathogens, their effectiveness is not universal across all bacterial species. This highlights the need for selective and targeted use of Magnolia extracts in applications where their properties are desired, considering their specific range of activity.

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