RESEARCH PAPER

Integrated FTIR and GC–MS Profiling Reveals Bioactive Metabolites and Antioxidant Potential in *Bulbophyllum rosemarianum* Leaf Extracts

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Abstract
Bulbophyllum rosemarianum is an orchid species known for its richness in plant diversity with considerable ornamental, ecological, and ethnomedicinal value. The current study sought to determine the chemical composition and antioxidant activities of ethanolic, methanolic, and aqueous extracts of B. rosemarianum leaf. The results of the GC-MS analyses of the extracts indicated the presence of various bioactive components. While the ethanolic extract was predominantly made up of 9,12,15-octadecatrienoic acid (Z,Z,Z) (23.25%), 10E, 12Z-octadecadienoic acid (20.99%), and n-hexadecanoic acid (10.43%), the methanolic extract was predominantly made up of 9,12,15-octadecatrienoic acid (Z,Z,Z) (18.75%) and 10E, 12Z-octadecadienoic acid (17.44%). On the other hand, the aqueous extract was composed of cyclopentane, 1-acetyl-1,2-epoxy- (17.30%). As far as FTIR analysis is concerned, there were different functional groups in the extracts. While the ethanolic and methanolic extracts were made up of 11 different types of functional groups, the aqueous extract had 23 types of functional groups. The results of DPPH and ABTS assays demonstrated that antioxidant activities varied according to the extract type. While the DPPH assay indicated higher levels of free-radical scavenging activity in methanolic and ethanolic extracts than in aqueous extracts, the ABTS assay showed higher free radical scavenging activity in aqueous extracts than in methanolic and ethanolic extracts. It means that antioxidant activities vary according to the composition of extracts and the radical system involved. Overall, the results indicate the diverse nature of chemical constituents of *B.* rosemarianum and its antioxidant activity.

Keywords: Antioxidant Activity; *Bulbophyllum rosemarianum*; FTIR Spectroscopy; GC–MS; Phytochemical Profiling; Radical Scavenging Assays.
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Introduction

Orchids are one of the most ecologically diverse families of flowering plants and are known for their distinctive floral variety and beauty. Not only because of their ornamental value but

also due to their medical importance, many types of orchids have found wide use in traditional healthcare (Bazzicalupo et al., 2023; Choudhary et al., 2023). There are several types of preparations made using orchids that were traditionally used by herbalists to treat various diseases, thereby confirming the pharmaceutical significance of this group of plants. The family Orchidaceae is the largest family of angiosperms and includes around 28,000 species belonging to 736 genera and varies greatly in terms of morphology, ecological preferences, and phytochemistry (de Vasconcelos et al., 2023, 2025). The historical significance of orchids in traditional medicine has prompted scientists to undertake thorough research aimed at proving the pharmacological value of orchids and studying their medicinal active substances. In conjunction with modern developments in phytochemistry, biotechnology, and pharmaceutical science, there is an increasing tendency of studying secondary metabolites isolated from crude plant extracts as potential sources for new medicines (Choudhary et al., 2023).

The medicinal use of orchids has been linked to their rich collection of phytochemicals with various physiological roles. The phytochemicals found in orchids have been described as possessing several biological activities such as antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and anticancer properties (Ghai et al., 2021; Kumar & Singh, 2023; Sliwinski et al., 2022; Yatung et al., 2025). Traditional medicine, which refers to the accumulated knowledge, practice, and experience in relation to health and illnesses within a culture, offers useful clues in the discovery of pharmacologically significant natural products (Pomini et al., 2023). In this regard, natural products are still a major pillar in modern drug discovery owing to their structural variety and the potential of providing novel chemical molecules with therapeutic value. Phytochemicals from plants are especially appealing owing to their biological strength and possibility of providing safer therapeutic agents with minimum adverse effects (Ahmed et al., 2022).

Phytochemical analysis and characterization of the compounds obtained from plant extracts are of paramount importance in understanding the properties of plant metabolites and possible medicinal applications. Modern techniques have significantly increased the efficiency of phytochemical detection and characterization in comparison to traditional approaches (Bylappa et

al., 2024). Various chromatography and spectroscopy methods are especially useful for the initial screening and structural characterization of bioactive phytochemicals. Among such methods, GC–MS and FTIR spectroscopy are widely used in studying the chemical composition of medicinal plant extracts. Gas chromatography–mass

spectrometry is a powerful method of separation and identification of volatile and semi-volatile compounds on the basis of their chromatographic and mass spectral properties (Bylappa et al., 2024). On the other hand, FTIR spectroscopy allows for fast and easy characterization of the chemical bonds and functional groups in complex biological substances, thus providing useful information on the types of phytoconstituents contained in plant extracts (Pasieczna-Patkowska et al., 2025). Thus, the complementary application of these techniques may be of great help in understanding the chemical structure and functional groups of the phytochemicals responsible for the biological activity of medicinal plants. These investigations have greatly contributed to the discovery of biologically active phytoconstituents, which can be used as the lead molecules in developing medicinal agents based on plant extractives (Najmi et al., 2022).

Oxidative stress is one more field that deserves close attention during studies on natural products since the excessive production of reactive oxygen species can result in cell damage and the development of various diseases (Chaudhary et al., 2023). Plants can be considered promising sources of natural antioxidants that can neutralize free radicals and prevent oxidative stress (Akbari et al., 2022). Medicinal orchids have become an object of growing interest as possible sources of antioxidants and have been studied for the ability to prevent free-radical-induced cell damage (Sharma, 2023). It is suggested that phenolics and other secondary metabolites can act as antioxidants due to the capacity to donate hydrogen or electrons to reactive species (Bylappa & Nag, 2024). The study of the antioxidative potential of plants can help to determine whether they can be used as sources of natural antioxidants.

Although medicinal orchids have received considerable attention from the scientific community, the phytochemistry and the antioxidant potential of *B. rosemarianum* are relatively less known. Hence, the current investigation was carried out to determine the chemical composition of ethanolic, methanolic, and aqueous leaf extracts of *B. rosemarianum* by GC-MS and FTIR spectroscopy. Besides, the in vitro antioxidant activity of these extracts was determined through DPPH and ABTS radical scavenging experiments. The objective of the current investigation is to give preliminary knowledge on the chemical composition and antioxidant activity of *B. rosemarianum* and, consequently, to help understand its ethnomedicinal value and possible role as a source of naturally occurring bioactive compounds.

Material and Methodology

• **Preparation of samples and extraction**

Leaves of *Bulbophyllum rosemarianum* were collected from various habitats within Ponnampete Taluk, Kodagu District, Karnataka, and washed under sterile water to remove any soil particles present on the plant leaves. The leaves were gently patted dry, shade-dried, and then cut into fine pieces before grinding to obtain coarse powder with the help of a mechanical grinder. The powder thus obtained was stored in clean and airtight bottles at 4 °C for future extraction. Extraction of powdered leaf material with methanol, ethanol, and aqueous as solvents was done by soaking 25 g of the powdered material in 300 mL of the solvent each in a Soxhlet apparatus for six cycles of 3 hours in each cycle, maintaining the temperature below the boiling point of the respective solvent. The temperature was set at 60 °C for methanol, 75 °C for ethanol, and 95 °C for water. The solvents were evaporated, and the extracts were stored in airtight bottles kept at 4 °C for further experimentation (Singh and V, 2024).

Fourier Transform Infrared analysis:

The Fourier Transform Infrared (FTIR) spectroscopy technique was applied to determine the presence of main functional groups in ethanolic, methanolic, and aqueous extracts of *Bulbophyllum rosemarianum* leaves by virtue of their distinct infrared absorption bands. Spectroscopic analysis was done using an FTIR spectrometer (Cary 630, Agilent Technologies) in the wavenumber region 400–4000 cm^{-1} with resolution 2 cm^{-1}. The absorption spectra of each of the three extracts were analyzed on the basis of specific vibration frequencies that correspond to definite chemical bonds/functional groups. The acquired FTIR spectra were informative about the functional-group composition of extracts and their initial phytochemical characterization (Madurapperumage et al., 2022).

Gas Chromatography Mass Spectrometry Characterization

The chemical components found in the three different extracts of *Bulbophyllum rosemarianum* were studied using gas chromatography–mass spectrometry (GC–MS) through a Shimadzu GC–MS system (Model 2030 TQ 8040NX, Shimadzu, Japan). Chromatographic analysis was done using a Perkin Elmer Elite-5 capillary column with a 30 m length, 0.25 mm inner diameter, and 0.25 µm film thickness. The stationary phase used in the capillary column consists of 95% dimethyl polysiloxane. Helium gas was used as the carrier gas, and the column flow rate was held constant at 0.95 mL/min. Each extract was analyzed by

injecting a 1 µL volume sample. The temperature of the column oven is 100°C, while the injection temperature was 280°C. The pre-column pressure is 68.3 kPa (Bylappa & Nag, 2024).

Mass spectrometric analysis of the extracts was performed using the electron ionization (EI) method at 70 eV. The temperature of the ion source is 250 °C while that of the interface is 280 °C. The mass spectra are obtained in the m/z 50-550

range. Chemical components found in the extracts were determined based on the comparison of the retention time (RT), retention index (RI) values and mass spectrometric fragmentations with those from the GC–MS spectral library. Operating conditions for chromatographic and mass spectrometric analysis are presented below. In GC analysis, the temperature of the column oven is 100°C, injection volume is 1 µL, injection temperature is 280°C, column flow rate is 0.95 mL/min, column length is 30 m, column thickness is 0.25 mm, pre-column pressure is 68.3 kPa, and the carrier gas used is helium. In MS analysis, electron ionization at 70 eV was utilized, ion source temperature is 250°C, interface temperature is 280°C, and the acquisition range is m/z 50-550.

Antioxidant activity

• **DPPH Assay**

The antioxidant property of the three extracts (ethanol, methanol, and aqueous) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity according to the procedure described by Bylappa and Nag, 2023 (Bylappa & Nag, 2023). A stock solution of the plant extracts at the concentration of 1 mg/mL and DPPH was made in 95% methanol. In order to perform the experiment, 1 mL of the fresh DPPH solution was mixed with 1 mL of each plant extract separately into individual test tubes. The samples were incubated in the dark room at room temperature for 30 minutes after mixing. Absorbance reduction was recorded at a 517 nm wavelength through a spectrophotometer. Quercetin was used as the standard, and the absence of the plant extract in DPPH solution was taken as the control. Percentage DPPH free radical scavenging activity was calculated using the equation below:

DPPH scavenging activity (%) = (AC – AS)/AC × 100

Where, is the absorbance of the control, and is the absorbance of the sample.

ABTS Assay

The in vitro antioxidant properties of the three extracts were determined using the ABTS radical cation decolorization test according to the procedure described by Bylappa and Nag, 2023 (Bylappa & Nag, 2023). The ABTS radical cation ($ABTS^{+}$) was prepared by mixing an aqueous solution of ABTS containing a 7 mM concentration and $K_2S_2O_8$ having a 2.45 mM concentration. This mixture was left to react in darkness for 12-16 h in order to allow $ABTS^{+}$ radical cation formation. Before conducting the assay, the stock solution of $ABTS^{+}$ was diluted using the respective solvents so that the working solution gave an absorbance of 0.70

± 0.02 at 734 nm. Quercetin was used as a reference standard, and its various concentrations were prepared for analysis. In the experiment, the reaction mixture of the extracts or the standard solution was allowed to react in darkness for 5 minutes, and then the absorbance at 734 nm was measured. The percentage of ABTS radical cation decolorization achieved by the extracts and the reference standard was calculated using the formula:

ABTS scavenging activity (%) = (AC – AS)/AC × 100 where is the absorbance of the control and is the absorbance of the sample.

Statistical analysis

The data was analyzed through IBM SPSS Statistics (Version 26.0). All experiments were conducted in triplicate, and the data have been reported as mean ± standard deviation (SD). The descriptive statistics have been computed, and one-way analysis of variance (ANOVA) has been utilized to determine the variations between the concentration of the ethanolic, methanolic, and aqueous extracts. A significance level of $p<0.05$ was considered for statistical analysis. Error bars in the graph denote the standard deviation of the mean value.

Result and Discussion

The current study provides a comprehensive assessment of the phytochemical constituents and antioxidant activity of the leaf extracts of *Bulbophyllum rosemarianum* through the use of ethyl alcohol, methanol, and aqueous extracts via FTIR and GC-MS, and scavenging tests for DPPH and ABTS. It was observed that the choice of the extraction solvent had a significant impact on the chemical composition of the produced extracts based on the differences in the FTIR and GC-MS spectra of the produced extracts. This was because of the variation in the

polarity and chemical affinity that influenced the selective extraction of phytochemicals from the plant matrices. As the isolated phytochemicals from the plant matrices differ greatly in polarity and chemical structure, then the various solvents used would selectively extract the phytochemicals.

Fourier Transform Infrared analysis

The IR spectra of the leaf extracts of the B. rosemarianum provided further fingerprints with respect to the variations in the nature of the functional groups present. The IR spectrum of the ethanolic extract revealed well-resolved peaks for N-H, C-H, C=C, C-C, NO_2, and C-O, which are found at the wave number of 3361, 2977, 2899, 2933, 1648, 1454, 1421, 1383, 1089, 884, and 1048 cm^{-1}, indicating the presence of alcohol, amine, alkane, alkene, aromatic, and oxygen-containing functional groups, respectively (**Figure 1A**). Several FTIR studies have detected the specific absorption peaks of hydroxyl, amine, hydrocarbon, aromatic, carbonyl, and oxygen-containing functional groups (De et al., 2023; Mohammed et al., 2023).

Figure 1: FTIR Spectral Analysis and Interpretation of Compounds in A: Ethanolic, B: Methanolic, and C: Aqueous Leaf Extracts of *Bulbophyllum rosemarianum*

The methanolic extract contained absorption peaks of 11 peaks representing functional groups O–H, C–H, C≡N, C≡C, C–C, C–N, and N–H. Specifically, there is an extremely

significant peak observed at 3324 cm^{-1} due to the stretching of the O–H group, while the absorptions at 2948 and 2836 cm^{-1} are attributed to the stretching of the C–H group (**Figure 1B**). It should be noted that the attribution of the broad peak at ~3200–3400 cm^{-1} as the OH groups has been supported by FTIR studies of methanolic extracts of plants where the broad band in this region has been identified for the stretching of the O–H group of alcohols and phenols (Chaithong et al., 2025). Moreover, the FTIR spectrum of the Citrullus colocynthis methanolic extract contains the hydroxyl absorption peaks at the range of 3400-3200 cm^{-1} and C-H peaks in the range of 2855-2975 cm^{-1}, demonstrating the similarity of the spectral region with the one of the current methanolic extract (Marzouk et al., 2011). Additionally, there are absorptions at 2221, 2251, 2136, and 2180 cm^{-1} representing nitriles and alkynes.

The FTIR spectrum of the aqueous extract was rather broad and diverse in nature, as it had vibrational absorption peaks of O-H, C-H, C=O, and C=C bonds, as well as of carboxylic acids, conjugated ketones, alkenes, and aromatics (**Figure 1C**). It is a fairly usual phenomenon to obtain broad FTIR spectra for aqueous and hydroalcoholic extracts of plants, since multiple absorption peaks correspond to various kinds of phytochemicals present in the extract (Padi et al., 2022). Notably, comparative extraction procedures showed that the use of a different solvent for extraction yielded different FTIR spectra, hence different functional chemistry of the fractions obtained.

The choice of the solvent was highly influential on the functional chemistry of the leaf extracts, causing diversefingerprints and compounds (Bharathi & Prakash, 2024; De et al., 2023). The existence of the absorption peak of a hydroxyl group suggests a high capacity of the sample to neutralize radicals, which correlates with the well-known dependence of the antioxidant properties on oxygen-containing functional groups (Padi et al., 2022). Nevertheless, the FTIR analysis only provides the data on the functional groups but not on the compounds. The combination of the FTIR and the GC-MS analysis eliminates this disadvantage, as the latter method yields far more information than each of these methods separately. Thus, the FTIR analysis is employed to determine the functional groups, whereas the GC-MS identifies the compounds (Bharathi & Prakash, 2024; De et al., 2023).

Gas Chromatography Mass Spectrometry Characterization

In addition to that, results obtained in the GC-MS analysis demonstrate that the solvent dependence was rather high in terms of chemical composition of the leaf extract of *B. rosemarianum*. Up to 44 compounds were detected in the ethanolic extract, whereas the

number of components in the methanolic and aqueous extracts reached 61 and 63, respectively. Even though it might seem that the aqueous fraction contained more components than the others, the high number of compounds did not correlate with the presence of high amounts of antioxidants. This proves that the number of components identified using chromatography techniques does not reflect the biological activity (Nicforovic et al., 2010). Other researches comparing various extraction techniques in terms of their influence on plant phytochemistry and biological activity have proven the same thing (Zreen et al., 2022; Akter et al., 2022).

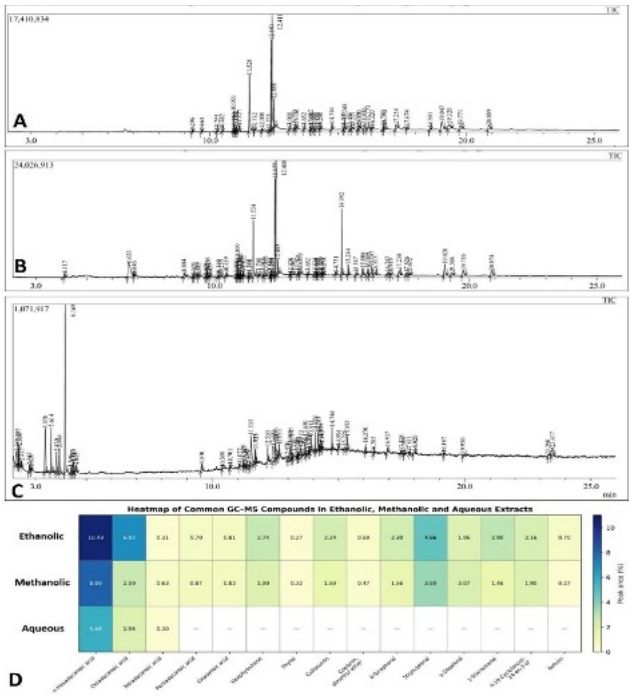


Figure 2: GCMS analysis data in A: Ethanolic, B: Methanolic, C: Aqueous, and D: a heatmap of common compounds in all the extracts.

Unsaturated fatty acids, especially 9,12,15-octadecatrienoic acid (Z,Z,Z) (23.25%), 10E,12Z-octadecadienoic acid (20.99%) and n-hexadecanoic acid (10.43%), were the most abundant among the compounds identified in the ethanolic extract (**Figure A**). Among the other constituents were octadecanoic acid, stigmasterol, tocopherols, phytol, long chain alcohols, hydrocarbons, and phenolic/aromatic compounds. Presence of large amounts of unsaturated fatty acids along with the sterols and tocopherol related metabolites suggests that the ethanolic fraction contains a significant amount of lipids. Fatty acids, phytol, sterols and tocopherol related compounds have been reported in the GC–MS analysis of medicinal plants' extracts (Akter et al., 2022; Ali et al., 2022). Comparing with the ethanolic extract, methanolic and aqueous extracts contain more diverse peaks in their phytochemical profile (**Figure B and C**), consisting of 61 and 63 identified constituents (for detailed information see Table 1 in the supplementary file). Major compounds in methanolic extract include 9,12,15-octadecatrienoic acid (Z,Z,Z) (18.75%), 10E,12Z-octadecadienoic acid (17.44%) and n-hexadecanoic acid (8.09%), in addition to phenolic/aromatic compounds, sterols, tocopherols, long chain alcohols, hydrocarbons, and aldehydes. In aqueous extract, in addition to fatty acids, hydrocarbons, siloxanes, esters/phthalate related compounds, and nitrogen/sulfur containing constituents, ketones and other oxygenated compounds were observed. Cyclopentane, 1-acetyl-1,2-epoxy- (17.30%) was the most abundant constituent, followed by n-hexadecanoic acid (5.68%), 2-hexanone, 3,3-dimethyl- (5.38%) and 2-pentanone, 3-methyl- (4.91%). The presence of both lipid derived and phenolic/aromatic metabolites suggest chemical diversity of the methanol extract which may serve as an appropriate chemical background for understanding the antioxidant activity of this fraction. Metabolite diversity similar to that of methanolic fraction has been found in GC–MS analysis of methanolic plant extracts (Akter et al., 2022; Ali et al., 2022). Further analysis of the chromatograms of all three revealed the existence of various metabolites common to all three (**Figure D**). Some of the metabolites that occurred in all three methods were the n-hexadecanoic acid, octadecanoic acid, and tetradecanoic acid (Otari et al., 2022).

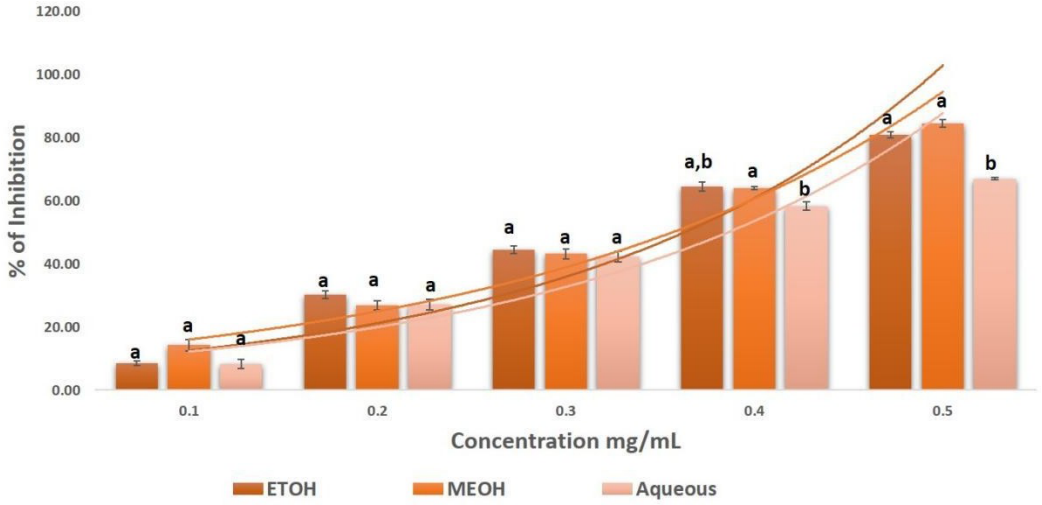
Antioxidant Assays:

In addition to these different phytochemical compositions, each extract also exhibited different antioxidant activities in relation to its composition. Both DPPH and ABTS showed

an overall increase in radical-scavenging activity with increasing concentrations of the extracts, which means that with an increase in the concentration of the extract, the quantity of antioxidants in the extract increased and was able to interact and scavenge the respective free radicals (Otari et al., 2022). The above observation shows that the effect of the antioxidant capacity of the extracts of the leaves of *B. rosemarianum* is dependent on the concentration of the extract in the reaction system.

DPPH Assay

Antioxidant activity of all three extracts of *B. rosemarianum* leaves was in accordance with the concentration-dependent nature of the results of the DPPH analysis, as an increase in concentration led to a continuous increase in radical-scavenging activity of the extracts. The extract of methanol was the most active in the DPPH analysis, showing an inhibition of 81.70% at 500 µg/mL concentration, while the ethanolic extract showed an inhibition of 77.69%, with the aqueous extract showing a



significantly lower level of inhibition (47.86%).

Figure 3: DPPH radical scavenging activity of ethanolic (ETOH), methanolic (MEOH), and aqueous leaf extracts of *Bulbophyllum rosemarianum* at different

concentrations. Values are expressed as mean ± SD (n = 3). Different lowercase superscript letters within the same concentration indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD post-hoc test ($p \leq 0.05$). Means sharing at least one common letter are not significantly different.

Possibly, the relatively high DPPH activity of the methanolic fraction may be due to its chemically diverse GC–MS profile consisting of phenolics, aromatics, as well as fatty acids,

tocopherols, sterols, and long-chain alcohols (Ngivprom et al., 2026). Specifically, phenolics can have a neutralizing effect on free radicals either via hydrogen-atom transfer or electron transfer (Leopoldini et al., 2004). Similarly, the relatively high DPPH activity of the ethanolic fraction may be linked with its high content of unsaturated fatty acids and presence of tocopherols, sterols, and other bioactive compounds. Nevertheless, the activity of crude extracts in terms of their antioxidant properties cannot be attributed to any single component detected by GC–MS because their activity is the result of complex interactions of various phytochemicals (e.g., additive or antagonistic) (Chen et al., 2022; Wink, 2022).

Notwithstanding the high content of various chemical compounds found in the aqueous fraction, it shows significantly lower activity in DPPH than the fractions obtained using methanol and ethanol. In general, results of the DPPH assay indicate the order of efficiency of antioxidant activity as methanol > ethanol > aqueous extract.

ABTS Assay

The ABTS test uses a water system and shows a concentration-dependent increase in the radical scavenging activity of the leaf extracts of *B. rosemarianum* (**Figure 4**). There was a gradual increase in the inhibition from 0.1 to 0.5 mg/mL, showing an increase in the ABTS radical scavenging activity as the concentrations of the extracts were increased. The inhibition recorded at 0.5 mg/mL was 80% for the aqueous extract, followed by ~64% and

~62% for the methanolic and ethanolic extracts respectively. In contrast to the results obtained from the DPPH assay, the order of ABTS activity of the extracts at 0.5 mg/mL was aqueous > methanolic > ethanolic. It is significant that the aqueous extract shows comparatively high ABTS activity despite showing a significantly low activity in the DPPH assay. This is interesting considering that the aqueous extract shows a chemically distinct GC-MS profile comprising of oxygenated compounds, ketones, fatty acids and other constituents. Its comparatively higher ABTS activity is a clear indication of the presence of constituents that are able to interact with the ABTS radical cation. This shows that the number of GC-MS detectable constituents cannot determine the antioxidant activity alone (Chaves et al., 2020; Seleshe et al., 2022).

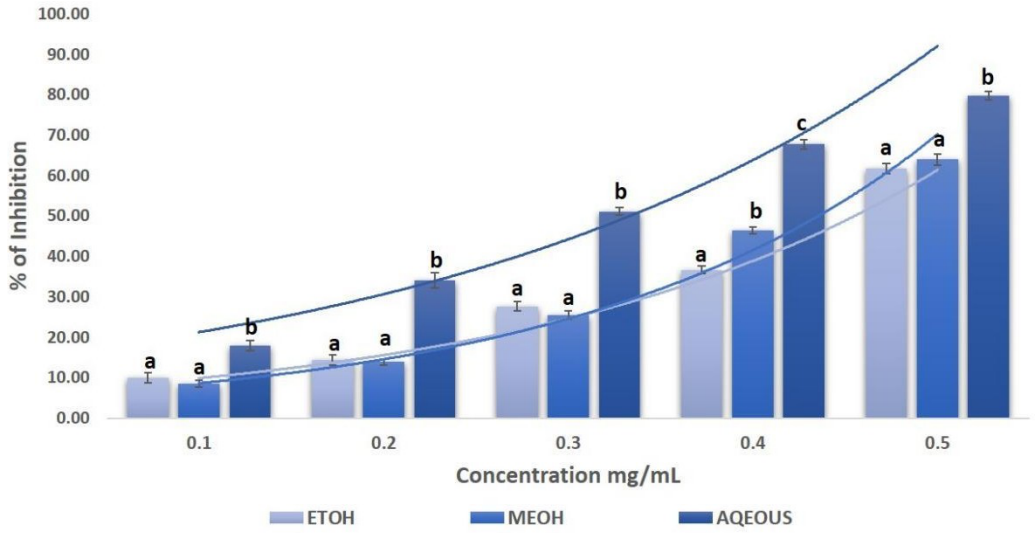


Figure 4: ABTS radical scavenging activity of ethanolic (ETOH), methanolic (MEOH), and aqueous extracts at different concentrations. Values are expressed as mean ± SD (n = 3).

Different lowercase letters above the bars within each concentration indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD post-hoc test ($p \leq 0.05$). Bars sharing the same letter are not significantly different.

The methanolic and ethanolic extracts exhibited notable concentration-based enhancement as well, exhibiting about 64% and 62% inhibition at 0.5 mg/mL. The activity can be linked to the combination of fatty acids, phenolic and aromatic compounds, sterols, tocopherols, and other metabolites revealed via GC–MS analysis. Metabolomic studies have shown correlations between phytochemical profile and antioxidant activity determined via DPPH and ABTS assays (Chaves et al., 2020; Zheng et al., 2022).

In addition, the statistical grouping shown in Figure 4 clearly indicates that distinctions among extracts were better seen at higher concentrations, especially at 0.4 mg/mL when the extracts exhibited different significance groups. Thus, it can be said that the effect of solvent on ABTS scavenging activity increased with the increase in concentration. Importantly, the contrasted DPPH and ABTS activities must be noted. While the methanolic extract showed the highest DPPH activity, the aqueous extract showed the highest ABTS activity. This is normal since DPPH and ABTS are based on different radicals and, thus, will show different activity depending on individual phytochemicals (Chaves et al., 2020; Zheng et al., 2022). Such variation in the ranking of antioxidants via various assays has been reported previously for the plant extracts (Seleshe et al., 2022). Overall, the ABTS assay shows concentration-

dependent activity and provides complementation for the DPPH assay in assessing the antioxidant capacity of *B. rosemarianum* leaf extracts.

Statistical analysis

Further analysis was carried out to establish whether the difference among the different concentration levels is statistically significant through one-way ANOVA with the post hoc Tukey's HSD multiple comparison test. The statistical test showed that there were variations in amounts, but these varied from one concentration level and extraction solvent used to another. It is clear that as the concentration increased, the separation among the treatment means was distinct, with antioxidant responses being high in methanolic and ethanolic extracts compared to aqueous extract in DPPH and vice versa in ABTS. Tukey's HSD test showed the differences among the concentration levels and the treatment levels that caused the significant difference. Graphically, this result can be illustrated through the use of lower-case superscripts, where treatment means with different letters are statistically significant while those with common letters are comparable.

Conclusion

In summary, there is a clear variation in the phytochemical composition of *B*. *rosemarianum* leaves depending on the type of solvent, with 44, 61, and 63 phytochemicals identified in the ethanolic, methanolic, and aqueous extracts, respectively, and the presence of diverse functional groups confirmed by FTIR. GC-MS analysis showed the presence of fatty acids, phenolics, sterols, tocopherols, alcohols, and other metabolites, with unsaturated fatty acids being predominant in organic extracts while oxygenated compounds dominated in the aqueous extract along with other chemically diverse compounds. DPPH showed the highest activity of methanolic (81.70%), ethanolic (77.69%) and aqueous (47.86%) extract at 500 µg/mL while ABTS showed 64%, 62% and 84%, respectively. The results indicate that there are significant variations in antioxidant activity depending on solvent used and concentration, which were statistically validated.

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