

Evaluating the In Vitro Antioxidant and Antimicrobial Properties of *Hybanthus enneaspermus* with GC-MS Profiling

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Abstract

Phytochemicals, the chemical substances that occur naturally in plants, are known to demonstrate several biological activities. The current study was performed to assess the phytochemical profile and the antimicrobial and in vitro antioxidant effects of the *Hybanthus enneaspermus* plant. The leaves and root powder of *H. enneaspermus* were extracted using an ethanol solvent. The phytochemical profile of the plant was assessed using GC-MS analysis. The occurrence of the diverse functional groups and metabolites in the *H. enneaspermus* leaves and roots was assessed using FT-IR and ¹H NMR analyses, respectively. The capacity for antioxidant activity of the *H. enneaspermus* leaves was studied by several analytical techniques, including DPPH scavenging, FRAP scavenging, TRAP capacity, ABTS scavenging, NO scavenging, and superoxide radical scavenging assays. The antimicrobial properties of the *H. enneaspermus* leaves and roots were tested by the disc diffusion technique. The findings of the GC-MS assessment have exhibited the occurrence of various bioactive compounds in the *H. enneaspermus* leaves and root extracts. The findings of the FT-IR and ¹H NMR analyses proved the existence of numerous functional groups and metabolites in the *H. enneaspermus* leaves and root extracts. The findings of this study also demonstrated potential antimicrobial properties against *B. cereus*, *S. marcescens*, and *S. aureus*. The findings of the antioxidant studies highlighted that the *H. enneaspermus* leaf extract successfully scavenged DPPH, FRAP, ABTS, NO, and superoxide radicals. The current study proved that the *H. enneaspermus* extracts exhibited the occurrence of numerous phytochemicals and demonstrated potential antimicrobial and free radical scavenging activities. Hence, it was clear that *H. enneaspermus* could be beneficial in combating infectious and oxidative stress-mediated diseases.

Keywords: Infectious diseases, Oxidative stress, Free radicals, *Hybanthus enneaspermus*, Antimicrobial activity.

INTRODUCTION

The world of herbal plants is a veritable treasure trove of medicinal marvels, offering a rich tapestry of remedies and healing properties that have captivated the interest of researchers and healthcare practitioners alike. Across the globe, herbal plants have been the backbone of conventional medicine systems, over 80% of individuals in developing countries depend on these natural resources for their main healthcare requirements [1]. Delving deeper into the history, we find that the utilization of plant extracts for their therapeutic potential can date back to the earliest of traditions. The progression of these plant-based medicine systems, rooted in the flora of local regions, has given rise to the well-known conventional medical systems, including Ayurvedic and Unani in India, China, and Tibet [2,3].

The importance of medicinal plants cannot be overstated. Over 35,000 to 70,000 plant species are used as therapeutics, representing 14–28% of identified species[4]. The medicinal properties of these plants are multifaceted, ranging from their ability to ease pain and treat a wide array of ailments to their potential in the development of modern drugs. In fact, more than 50 major drugs on the global market have their origins in tropical plants, underscoring the immense potential of herbal medicines [5]. Conventional medical systems have long recognized the healing powers of these plants, employing them for their curative properties, easy availability, and minimal side effects, especially in regions where modern pharmaceuticals are scarce or unaffordable. The continued reliance on herbal remedies by the majority of the population worldwide highlights the enduring importance of medicinal plants in healthcare [6].

Plants serve as a very advantageous and renewable reservoir of chemical compounds that possess diverse qualities that are advantageous to humans. Nevertheless, plants generate a diverse range of chemicals, posing a challenge in pinpointing the individual molecules linked to a certain trait. In order to address this challenge, scientists have devised several chromatographic methods to effectively isolate and detect the constituents of a complex sample. Gas chromatography-mass spectrometry (GC-MS) identifies compounds with antimicrobial properties in various studies. These compounds are typically extracted from different parts of plants using various solvents [7-9]. Throughout history, natural products have been widely recognized as essential resources for medicinal substances. Scientific progress has led to the examination and analysis of the distinct characteristics of these natural substances in terms of their chemical and structural variety and their effects on therapeutic and other biological functions. Moreover, it is well-established that medicinal plant species exhibit changes in their chemical contents based on the diverse settings in which they develop [10]. Therefore, it is crucial to examine these therapeutic plants and also imperative to conserve and sustain wild indigenous plants that have economic and medicinal value.

Hybanthus enneaspermus is a small perennial medicinal plant from the Violaceae family[11]. This plant has a lengthy history in traditional medicine for treating various ailments like infections and seizures[12-14]. Apart from these medicinal properties, the further validation of its phytochemical profile and biological properties needs to be clarified. Therefore, the present study was performed to assess the phytochemical profile and the antimicrobial and free radical scavenging effects of the *H. enneaspermus* plant.

MATERIALS AND METHODS

Collection and authentication of plant samples

The fresh leaves and root samples of *H. enneaspermus* were gathered from a specific region in Tiruchirappalli district, Tamil Nadu, during the March–April month of 2022. The sample was presented to the Rapinet Herbarium Center, Tiruchirappalli, for the purpose of identification and confirmation of the plant species and the assignment of a voucher number. Dr. John Britto, a well known botanist, authenticated the specimen.

Preparation of *H. enneaspermus* extract

Leaves and roots of *H. enneaspermus* were washed, dried in the shade for a week, ground into powder, mixed with ethanol, extracted in a Soxhlet apparatus for 30 min, and stored at 4 °C for testing.

GC–MS analysis of ethanolic extract of *H. enneaspermus*

GC-MS analysis of *H. enneaspermus* leaves and roots evaluated phytoconstituents at 300 °C, with transfer and ion source temperatures at 200 °C. The unrefined solution mixed with hexane was filtered through nylon. The 0.7 aliquot was injected into the chromatogram using split-less mode, and the chromatogram was equipped with a MS (Agilent, USA). The fused silica capillary column was employed for separation. The sector mass analyzer was set to scan within the range of 40 to 650 atomic mass units (amu). The volatile components of the extract were identified by analyzing the spectrum data and comparing it with the NIST library using computer-assisted matching.

In vitro antioxidant assays

DPPH assay

The DPPH radical-lowering ability of *H. enneaspermus* leaf extract was assessed by mixing 2 ml of extract with 2 ml of 0.1 mM DPPH reagent at 37 °C for 30 minutes, measuring absorbance at 517 nm, with ethanol as reference [15]. The DPPH radical reduction percentage was detected using an equation:

$$\text{Percentage (\%)} \text{ of DPPH scavenging} = [(A_0 - A_1) / A_0] \times 100.$$

where A_0 was the control absorbance and A_1 was the extract-treated absorbance.

Ferric-reducing antioxidant potential (FRAP) assay

The ethanolic extract of *H. enneaspermus* leaves was evaluated for its ability to reduce ferric ion generation using a method from the literature[16]. Various dosages of the extract were mixed with FRAP solution and incubated at 37 °C for 30 minutes. Absorbance was measured at 593 nm, indicating antioxidant capacity through ferric ion reduction.

Total reactive antioxidant potential (TRAP) assay

The 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was prepared by mixing the AAPH with a 100 mM glycine buffer with a pH of 8.6. The final concentration of AAPH was 120 mM, and the final volume of the suspension was 20 mL. Next, 4 μ L of luminol was mixed with the solution, resulting in a final concentration of 0.001 mM. This step was performed in a dark place. Various concentrations of *H. enneaspermus* extract were introduced, and the luminescence resulting from the reactivity of free radicals was measured using a liquid scintillator counter. The system exclusively focused on the chemoluminescence emitted by AAPH thermolysis [17].

Analysis of ABTS scavenging activity

The ABTS analysis was conducted using the previous technique [18]. A solution containing ABTS (0.075 mmol/L) and AAPH (2 mmol/L) was combined with the acetic acid buffer. Subsequently, the combination was subjected to a reaction in a water bath maintained at 45 °C for 60 min. In addition, 2 mL of the suspension was combined with 1 mL of the *H. enneaspermus* leaf extract, and the absorbance was taken at 734 nm. The standard curve was generated using the equation:

$$\text{ABTS+ Scavenging rate(\%)} = (1 - A_0/A_1) \times 100\%$$

where A_0 was sample solution (1 mL) mixed with reaction solution (2 mL) and A_1 was distilled water (1 mL) mixed with 2 mL of the reaction solution.

NO scavenging assay

The NO radical reduction capacity of *H. enneaspermus* leaf extract was assessed using the previous method [19]. To perform the assay, 2 ml of 10 mM sodium nitroprusside solution and 0.5 ml of PBS were mixed with 0.5 ml of extract and incubated at 25 °C for 15 min. A 0.5 ml nitrite sample was added to 1 ml of sulfanilic acid for 5 min, followed by 1 ml of NEDD (1%) for 30 min before measuring absorbance at 540 nm.

Superoxide radical scavenging assay

The scavenging effect of *H. enneaspermus* leaves' ethanolic extract on superoxide radicals was evaluated using a solution of NBT, extract, and alkaline DMSO. Absorbance was measured at 560 nm, following a specified previous method for analysis [20].

Analysis of antimicrobial activity

The antibacterial effectiveness of the *H. enneaspermus* leaves and root extracts against *Bacillus cereus*, *Serratia marcescens*, and *Staphylococcus aureus* was evaluated using the disk diffusion technique. Following the introduction of pathogens into the agar plates, the discs containing diverse dosages (25, 50, 75, and 100 μ g) of the *H. enneaspermus* leaves and root extracts were added to the agar medium surface and then incubated at 37°C for 24 h. The inhibitory zone diameters were observed after a period of incubation.

FT-IR spectroscopy analysis

The FT-IR spectrum was acquired using the PerkinElmer FT-IR machine, employing the KBr disc method for sample preparation, with the maximum wavenumber (ν_{max}) measured in cm^{-1} . The *H. enneaspermus* leaves and root extracts were mixed with KBr in a ratio of 1:10 and then compressed into pellets using a hydraulic press. Subsequently, the pellet was placed into the sample slit, and the transmittance was quantified.

¹H NMR spectroscopy analysis

The plant extracts were studied using ¹H NMR spectroscopy. 50 mg of sample was mixed with CD₃OD, agitated for 2 min at 37 °C, and then ultrasonicated for 15 min. An NMR spectrometer with a frequency of 500 MHz (JEOL NMR Spectrometer, USA) was utilized to examine 800 μ L of samples. The NMR spectrum was obtained at a frequency of 500.16 MHz. Every ¹H NMR spectrum necessitated 128 scans, with an acquisition time of 10 minutes and 26 seconds and a relaxation delay time of 1.5 seconds. The temperature was maintained at a constant 25 °C. The NMR spectra were manually subjected to phasing, baseline, and reference adjustments using MNOVA

version 13.0. The identification of the metabolites was achieved through a comparison of the ¹H NMR spectra of the sample with those documented in the published literature.

STATISTICAL ANALYSIS

Statistical studies employing SPSS provided outcomes as mean±SD from triplicates; one-way ANOVA and DMRT evaluated data with p<0.05 as significant.

RESULTS

GC-MS analysis of the *H. enneaspermus* leaves and root extracts

GC-MS analysis of *H. enneaspermus* leaves and root extracts revealed numerous phytochemical constituents, evidenced by various peaks in the chromatogram, confirming their rich phytoconstituent content.(Fig.1 and Fig.2).

Fig. (1). GC-MS chromatogram of the ethanolic extract of *H. enneaspermus* leaves

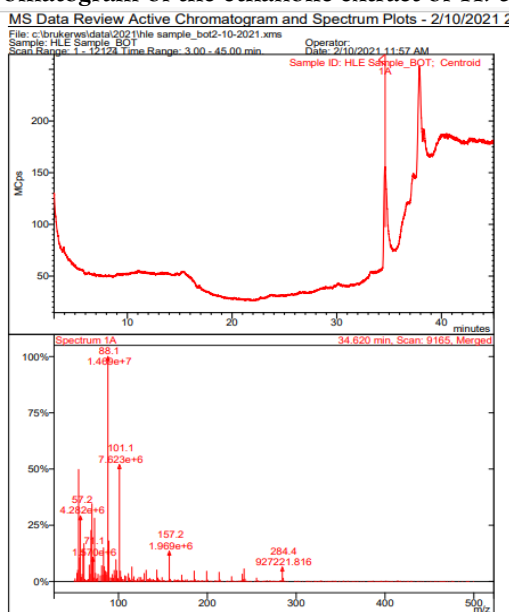
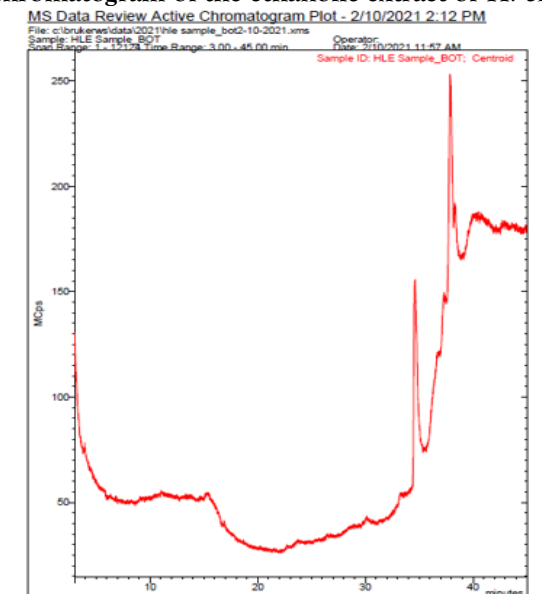


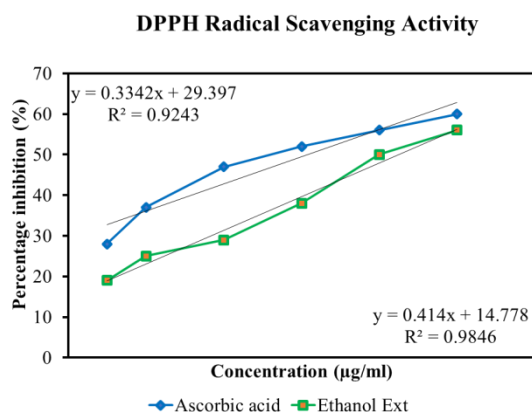
Fig. (2). GC-MS chromatogram of the ethanolic extract of *H. enneaspermus* roots



Effect of *H. enneaspermus* leaves extract on the DPPH scavenging assay

Figure 3 illustrates the DPPH radical-scavenging ability of the *H. enneaspermus* leaf extract. The DPPH scavenging capacity of various dosages (10 to 100 µg/ml) of the ethanolic extract of *H. enneaspermus* leaves was investigated. The findings clearly illustrated that the *H. enneaspermus* leaf extract effectively scavenged the DPPH radicals in vitro. The inhibitory percentage of DPPH was significantly enhanced with the higher doses of *H. enneaspermus* leaf extract. These results demonstrate that the *H. enneaspermus* leaves effectively scavenged the DPPH radicals. Vitamin C, a standard drug, has also scavenged the DPPH radical, which supports the activity of the *H. enneaspermus* leaves.

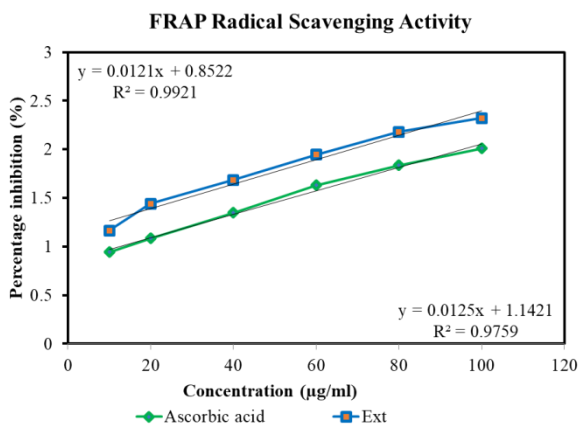
Fig. (3). Effect of ethanolic extract of *H. enneaspermus* leaves on the DPPH scavenging assay



Effect of *H. enneaspermus* leaves extract on the FRAP scavenging assay

The impact of *H. enneaspermus* leaf extract on ferric ion scavenging was evaluated, and the outcomes are presented in Figure 4. The *H. enneaspermus* leaf extract has the ability to scavenge ferric ion radicals. The study examined several doses (10–100 µg/ml) of an ethanolic extract of *H. enneaspermus* leaves. The findings clearly proved the ferric ion radical scavenging by the ethanolic extract of *H. enneaspermus* leaves. The higher doses of *H. enneaspermus* leaf extract effectively suppressed the ferric ions, which is similar to the results of the standard drug vitamin C.

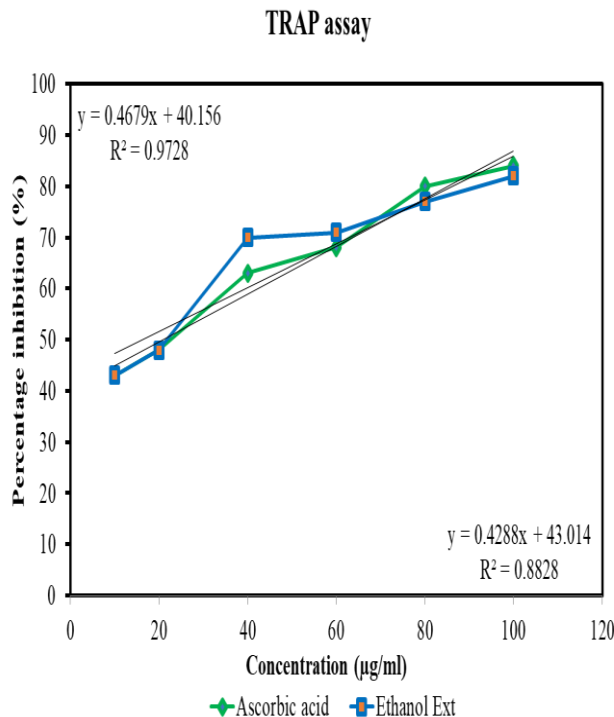
Fig. (4). Effect of ethanolic extract of *H. enneaspermus* leaves on the FRAP scavenging assay



TRAP capacity of the *H. enneaspermus* leaves extract

The overall antioxidant capacity of the *H. enneaspermus* leaf extract was investigated using TRAP assays. The TRAP experiment displayed a dose-dependent antioxidant capacity of the *H. enneaspermus* leaf extract at concentrations of 10 to 100 µg/ml (Fig.5). The ethanolic extract of *H. enneaspermus* leaves demonstrated superior results at increasing doses, which is comparable to the reference antioxidant vitamin C.

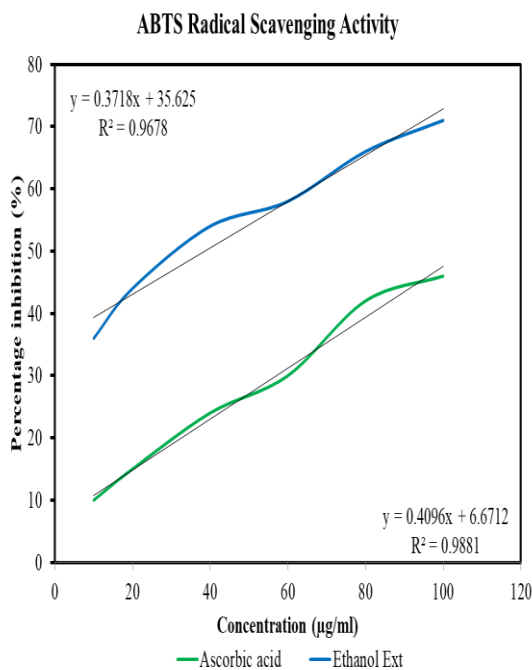
Fig. (5). TRAP capacity of the ethanolic extract of *H. enneaspermus* leaves



Effect of the *H. enneaspermus* leaves extract on the ABTS scavenging assay

The ABTS inhibitory activity of the *H. enneaspermus* leaf extract was investigated, and the outcomes are illustrated in Figure 6. *H. enneaspermus* leaf extract has the ability to scavenge ABTS radicals. The study examined various doses (10–100 µg/ml) of ethanolic extract of *H. enneaspermus* leaves to determine their effectiveness in scavenging ABTS radicals. The findings unequivocally proved that the scavenging of ABTS radicals by the ethanolic extract of *H. enneaspermus* leaves was dose-dependent. The higher concentrations of the *H. enneaspermus* leaf extract effectively suppressed the ABTS radicals, which is similar to the results of vitamin C.

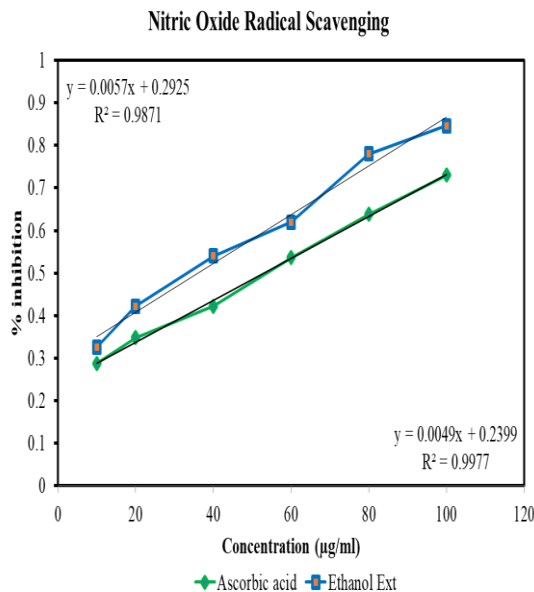
Fig. (6). Effect of ethanolic extract of *H. enneaspermus* leaves on the ABTS scavenging assay



Effect of *H. enneaspermus* leaves on the NO scavenging assay

As illustrated in Figure 7, the *H. enneaspermus* leaf extract effectively scavenged the NO free radicals. The NO scavenging ability was assessed at varied dosages (10–100 µg/ml) of the *H. enneaspermus* leaf extract. Figure 7 clearly showed that the *H. enneaspermus* leaves significantly suppressed the production of NO radicals dose-dependently. Vitamin C, a commonly used antioxidant, efficiently eliminated the NO radicals, which supports the NO radical scavenging ability of the *H. enneaspermus* leaf extract.

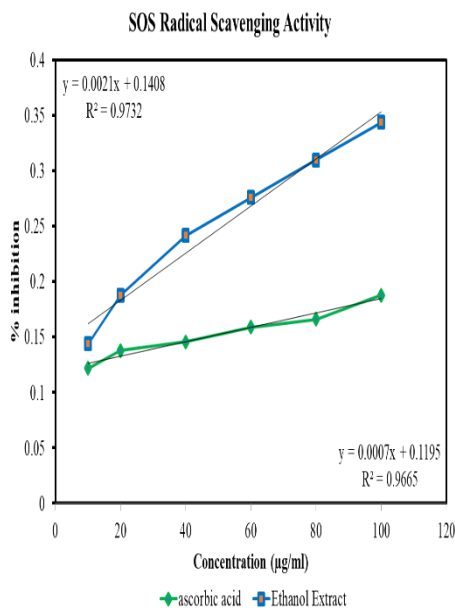
Fig. (7). Effect of ethanolic extract of *H. enneaspermus* leaves on the NO scavenging assay



Effect of *H. enneaspermus* leaves extract on the superoxide radical scavenging activity

The superoxide scavenging effects of the ethanolic extract of *H. enneaspermus* leaves were assessed, and the findings are presented in Figure 8. The *H. enneaspermus* leaf extract, at diverse dosages (10–100 µg/ml), effectively decreased the superoxide radical levels. The radical scavenging ability of the *H. enneaspermus* leaf extract was evidenced by a reduction in the superoxide radical levels (Fig. 8), which is similar to the results of vitamin C.

Fig. (8). Effect of ethanolic extract of *H. enneaspermus* leaves on the superoxide radical scavenging activity



Antimicrobial effects of the *H. enneaspermus* leaves and roots

The antimicrobial effectiveness of the *H. enneaspermus* leaves and root extracts against several pathogens, such as *B. cereus*, *S. marcescens*, and *S. aureus*, was evaluated using the disk diffusion technique, as depicted in Figures 9 and 10. The administration of various doses of *H. enneaspermus* leaves and root extracts revealed remarkable antibacterial efficacy against all the pathogens tested. The growth of these pathogens was considerably suppressed by the *H. enneaspermus* leaf and root extract treatment (Fig. 9 and Fig.10).

Fig. (9). Antimicrobial effects of the ethanolic extract of *H. enneaspermus* leaves

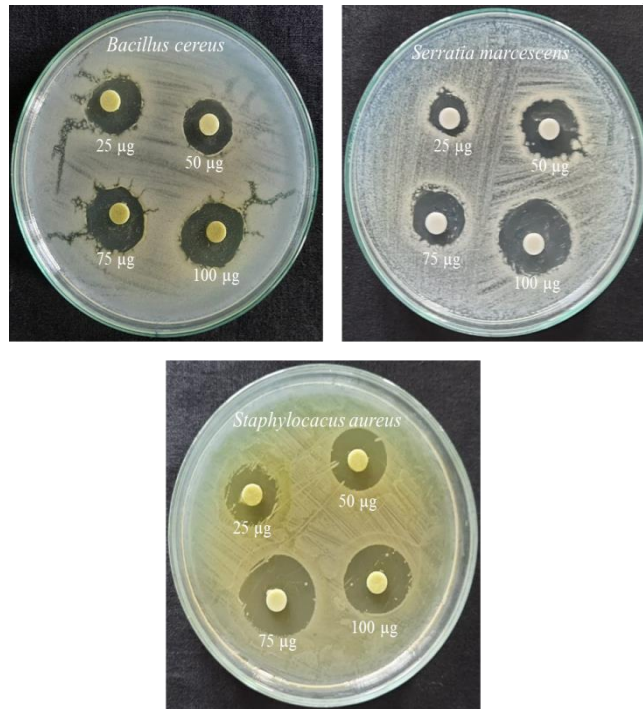
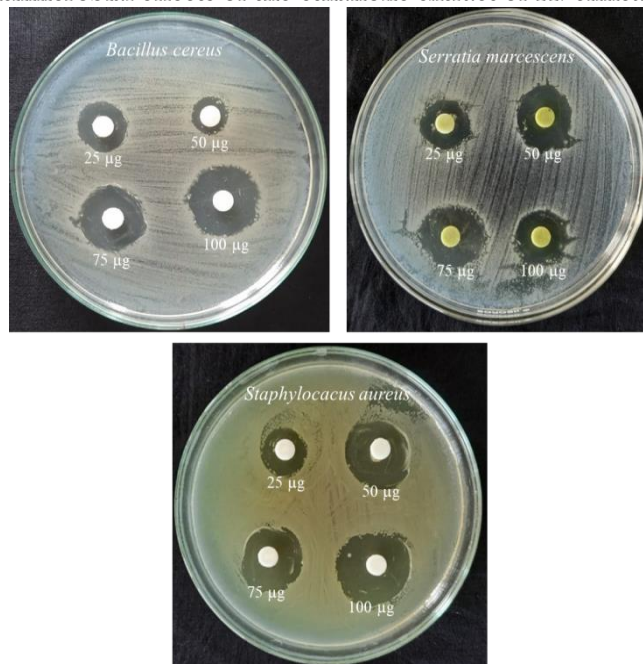


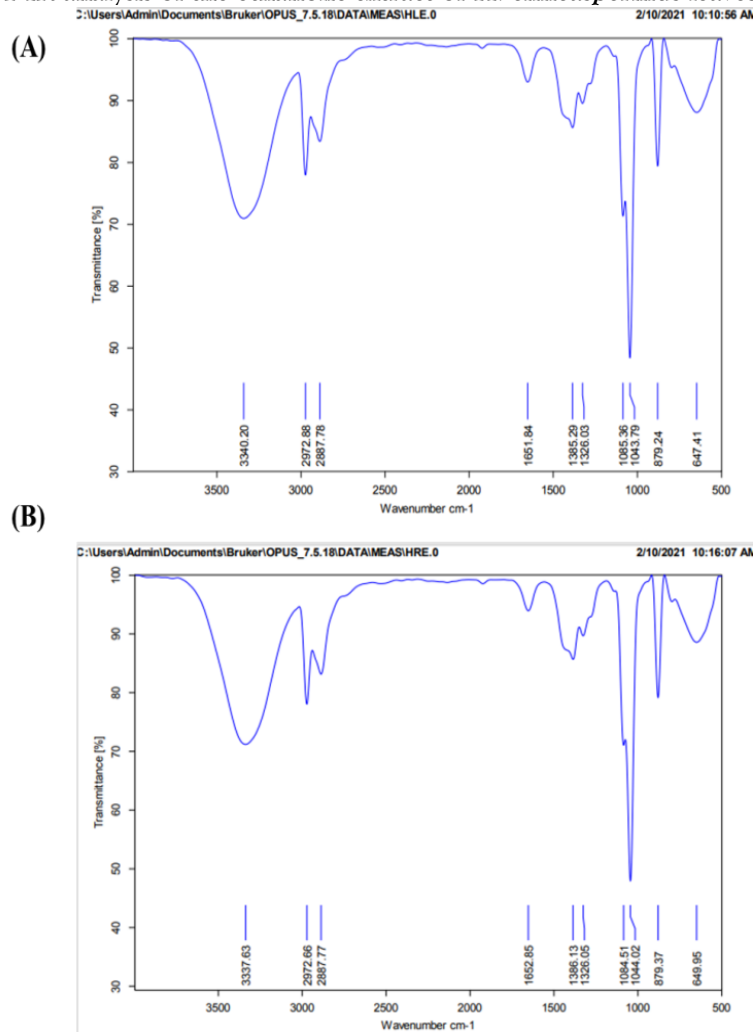
Fig. (10). Antimicrobial effects of the ethanolic extract of *H. enneaspermus* roots



FT-IR analysis of the *H. enneaspermus* leaves and root extracts

Figure 11 (A&B) illustrates the findings of the FT-IR analysis, which detected the functional groups of the *H. enneaspermus* leaves and root extracts. The FT-IR spectrum of the *H. enneaspermus* leaves and root extracts exhibited many peaks at various frequencies. The existence of numerous functional groups, including -OH, -COOH, -CH₂, and C=O, was revealed by FTIR spectroscopy results.

Fig. (11). FT-IR analysis of the ethanolic extract of *H. enneaspermus* leaves and roots



¹H NMR analysis of the *H. enneaspermus* leaves and root extracts

The ¹H NMR spectra of the *H. enneaspermus* leaves and root extracts (Fig. 12 and Fig.13) displayed strong signals, which can be accredited to saccharides and sugars. In particular, α -glucose and β -glucose were found in high amounts in all extracts. Additional strong signals can also be attributed to amino acids, such as alanine, glutamate, and threonine. The other signals seen in this spectrum can be associated with phenolic chemicals, which encompass flavonoids. The observed ¹H NMR spectrum also proved the existence of carbons and protons and their electronic states in several compounds.

Fig. (12). ¹H NMR analysis of the ethanolic extract of *H. enneaspermus* leaves

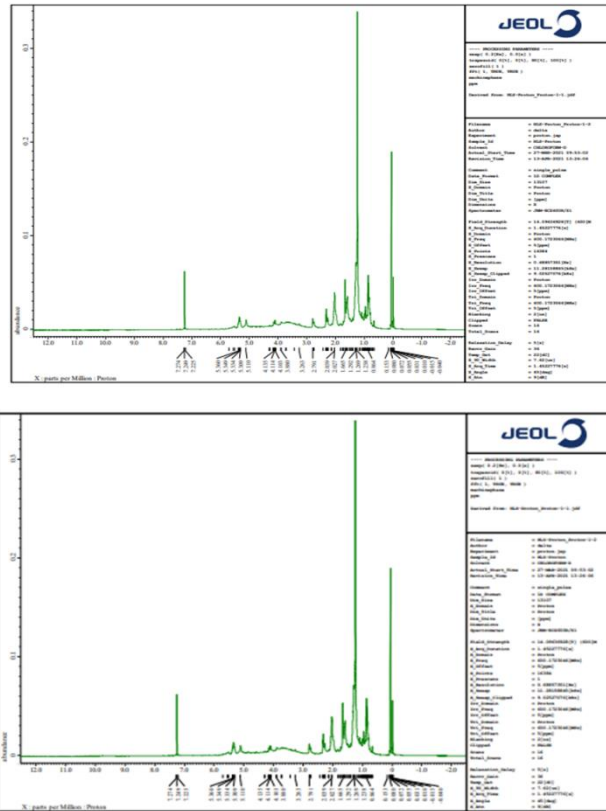
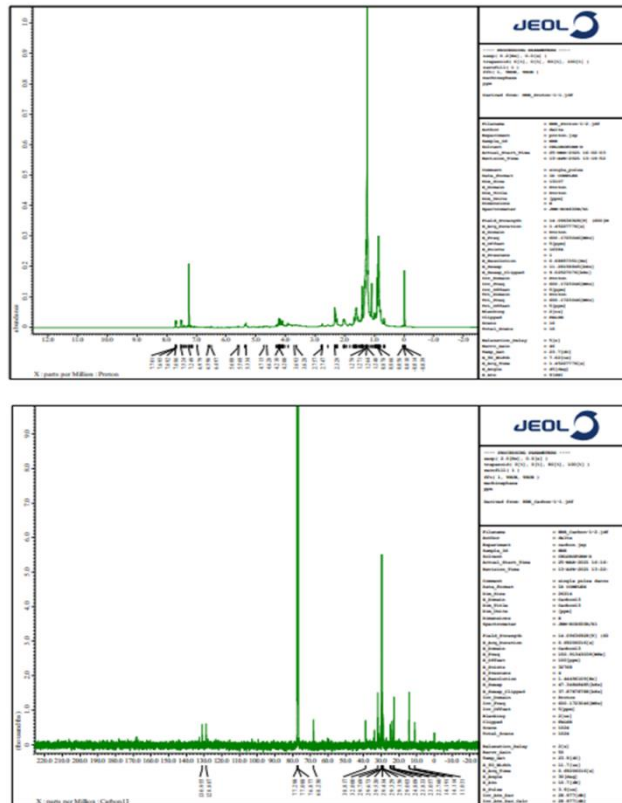


Fig. (13). ¹H NMR analysis of the ethanolic extract of *H. enneaspermus* roots



DISCUSSION

Phytochemicals, the naturally occurring chemical compounds found in plants, are known to demonstrate several biological activities that can be harnessed for various therapeutic applications [21]. Comprehensive phytochemical analysis is crucial for identifying and characterizing the active constituents within medicinal plants. This information can offer valuable insights into the pharmacological potential of the plant and can guide further research and development efforts [22]. The application of modern analytical techniques, such as GC-MS, has greatly advanced the field of phytochemistry. The chromatographic technologies enable the precise identification and quantification of individual phytochemicals, allowing researchers to attain a deep understanding of the plant's chemical profile and its therapeutic activities. The screening of the phytochemical profile of medicinal plants is a critical aspect of research, as it lays the foundation for understanding the plant's therapeutic potential and guides the development of novel plant-based pharmaceuticals and nutraceuticals [23]. The utilization of GC-MS analysis has become an invaluable tool in the field of plant science, providing researchers with a complete understanding of the chemical profile and phytochemical profiles of various plant extracts [24]. One of the primary advantages of GC-MS analysis of plant extracts is its capacity to separate, identify, and quantify a wide range of volatile and non-volatile compounds. This capability is particularly useful in the discovery and characterization of bioactive compounds, such as secondary metabolites, which are often of great interest in the fields of pharmacology and natural product chemistry [25]. Furthermore, GC-MS has been employed for the detection and confirmation of the structures of active constituents in plant-derived extracts, contributing to the development of new pharmaceutical products [26]. In this work, the *H. enneaspermus* leaves and root extracts were assessed using GC-MS analysis to identify the occurrence of bioactive compounds. The findings of the GC-MS analysis clearly showed that the *H. enneaspermus* extracts contain a diverse range of phytoconstituents.

Medicinal plants have gained increasing attention for their potential as natural sources of antioxidants, which play a key role in inhibiting the deleterious effects of oxidative stress. The antioxidant effectiveness of medicinal plants is often accredited to the occurrence of various bioactive compounds, which can scavenge free radicals and modulate redox signaling pathways [27]. To better understand the antioxidant potential of medicinal plant extracts, researchers have employed various analytical techniques, including DPPH scavenging, FRAP scavenging, TRAP capacity, ABTS scavenging, NO scavenging, and superoxide radical scavenging assays [28]. The DPPH scavenging assay is a broadly utilized technique for evaluating the radical scavenging activity of plants. The results from this assay have been found to correlate well with the antioxidant potential of the plants. Similarly, the FRAP assay detects the capacity of plants to diminish ferric ions, which can serve as an indicator of their antioxidant capacity [29]. The TRAP assay is another technique used to study the overall antioxidant potential of plant samples, as it measures its effectiveness in trapping peroxy radical scavenging. The ABTS radical scavenging assay is another popular technique for assessing the antioxidant capability of the plant samples [30]. In addition to these assays, researchers have also studied the capability of medicinal plants to scavenge other types of free radicals, such as NO and superoxide radicals. The results from these various antioxidant assays have provided valuable insights into the antioxidant capacity of medicinal plants [31]. The outcomes of the current work proved that the *H. enneaspermus* leaf extract successfully scavenged various free radicals, including DPPH, ferric ions, ABTS, NO, and superoxide radicals. These findings highlighted the antioxidant potential of *H. enneaspermus* leaves.

Microbial infections pose a major threat to human life, and the increasing resistance of pathogens to antibiotics has driven the exploration of novel antimicrobial candidates. Herbal plants have long been known as a rich source of bioactive compounds with therapeutic potential, including antimicrobial activities [32]. In this work, we assessed the antimicrobial activity of the *H. enneaspermus* extracts against *B. cereus*, *S. marcescens*, and *S. aureus*. The utilization of higher plants and their preparations for the treatment of infections is a long-standing tradition. Currently, there is a keen interest in investigating plants that possess antimicrobial properties due to the increasing problems linked to the persistent use of antibiotics and the subsequent emergence of resistant pathogens. Historically, antibiotics have mostly been obtained from microbes. However, recent studies have demonstrated that higher plants also have the potential to be valuable sources of new antibiotic prototypes. The utilization of medicinal plant extracts shows significant promise in the development of novel drugs that can effectively combat infections that are now challenging to treat [33]. Plants comprise a diverse array of phytochemicals that have the ability to hinder the growth of harmful microbes. The indigenous people of India

have long utilized many plant species as traditional remedies, specifically for treating infectious ailments [34]. The current study investigated the antimicrobial effectiveness of *H. enneaspermus* leaves and root extracts against *B. cereus*, *S. marcescens*, and *S. aureus*. The results demonstrate the antimicrobial capacity of the *H. enneaspermus* extracts that could be utilized to treat drug-resistant infectious diseases.

Medicinal plants have long been a valuable source of natural compounds with therapeutic potential. FT-IR spectroscopy and ¹H NMR analysis have emerged as powerful analytical techniques for the characterization and identification of the functional groups and metabolites present in these plant extracts [35]. FT-IR spectroscopy is a broadly utilized technique for the metabolomic profiling and fingerprinting of medicinal plants. It allows for the identification of various functional groups such as phenolics, carbonyl, aldehyde, ether, aromatic, and alkyl groups [36]. Similarly, ¹H NMR analysis provides a comprehensive overview of the metabolite composition of medicinal plant samples. This method can be utilized to detect and quantify numerous compounds, including various types of alcohols, phenols, aldehydes, aromatic compounds, and carboxylic acids [37]. The combination of FT-IR and ¹H NMR analyses can offer a holistic understanding of the chemical composition and functional group profiles of medicinal plant extracts. By recognizing the main and effective compounds in these plants, researchers can more easily step towards the development of biomolecules and herbal medicines [38]. FT-IR spectroscopy provides a unique "fingerprint" of the chemical composition of plant samples, allowing for the detection of various functional groups, including phenolics, carbonyls, aldehydes, ethers, aromatics, and alkyl groups [39]. This method has been broadly utilized to analyze the phytochemical profiles of diverse medicinal plants [40]. The integration of FT-IR and ¹H NMR techniques provides a comprehensive understanding of the chemical profile and potential bioactive compounds present in medicinal plant extracts [41]. This knowledge can be valuable in the development of new herbal remedies as well as the standardization and quality control of existing natural products [42]. In the current study, the FT-IR spectrum of the *H. enneaspermus* leaves and root extracts exhibited many peaks at various frequencies, which confirms the existence of numerous functional groups, including -OH, -COOH, -CH₂, and C=O. Furthermore, the ¹H NMR spectra of the *H. enneaspermus* leaves and root extracts displayed strong signals, which can be attributed to the existence of numerous metabolites, including saccharides and sugars, amino acids, and phenolic chemicals.

CONCLUSION

The present study has supported the occurrence of numerous bioactive compounds in *H. enneaspermus* leaves and root extracts. The present results also proved that the bioactive compounds found in the *H. enneaspermus* leaves and root extracts have demonstrated potential antimicrobial and free radical scavenging activities. The current investigation illustrates that the *H. enneaspermus* leaves and root extracts exhibited a dose-dependent suppression of free radicals and enhanced antioxidant capacity. The *H. enneaspermus* leaves and roots displayed potent antimicrobial properties against the tested pathogens. Our investigation has shown that *H. enneaspermus* exhibits strong antioxidant and antimicrobial properties and may be beneficial in combating infectious and oxidative stress-mediated diseases.

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DECLARATIONS

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Radhakrishnan completed the research work plan and Manuscript writing and while Geethaharipriya completed the review of the literature collection Natarajan handled the editing and review and supervision. Every author has reviewed and approved the published version of the manuscript.

CONFLICTS OF INTERESTS: No conflicts of interest.

DATA AVAILABILITY

All the data in this study is completely incorporated in the manuscript.

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