

## COMPARATIVE ANTIOXIDANT POTENTIAL OF SELECTED MEDICINAL PLANTS THROUGH FREE RADICALS SCAVENGING ACTIVITY

Hiwase K.D.<sup>1</sup>, Wadhai V.S.<sup>2</sup>

<sup>1</sup>Department of Microbiology, Mohsinbhai Zaveri Mahavidyalaya, Desaijanj (Wadsa), Dist. Gadchiroli (MS), India.

<sup>2</sup>Professor and Head, Department of Microbiology, Sardar Patel Mahavidyalaya, Chandrapur (MS), India.

\*Corresponding Author:

email id :[krunalhiwase49@gmail.com](mailto:krunalhiwase49@gmail.com)

### Abstract

*This research investigates the free radical scavenging properties of five medicinal plant species—Adhatoda vasica, Holarrhena antidysenterica, Gloriosa superba, Vitex negundo, and Vinca rosea—and examines the relationship between their phytochemical profiles and antioxidant potential. The antioxidant activities of plant extracts were quantitatively assessed using the DPPH assay, and major bioactive constituents were identified. Among the evaluated species, Adhatoda vasica and Vinca rosea demonstrated the strongest radical scavenging effects, markedly surpassing the others. The outcomes indicate that these plants could be promising natural sources of antioxidants, with potential roles in combating oxidative stress—a contributing factor in many chronic diseases. This highlights their relevance for the development of innovative antioxidant-based interventions and emphasizes the need for continued exploration of their bioactive compounds for use in integrative and preventive health practices.*

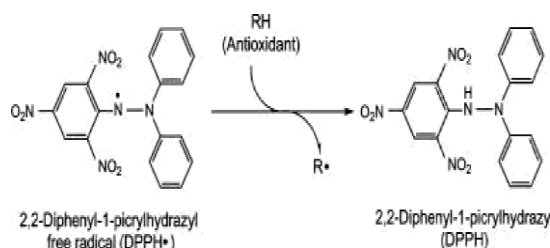
**Keywords:** scavenging, phytochemical, bioactive, antioxidant, pathogenesis

The rising incidence of chronic illnesses, often linked to oxidative stress—an imbalance between free radicals and the body's antioxidant defenses—has fueled growing scientific interest in exploring natural antioxidants for therapeutic use. This pursuit has directed attention toward plant species traditionally valued in folk medicine for their health-promoting properties. Notable examples include *Adhatoda vasica*, *Holarrhena antidysenterica*, *Gloriosa superba*, *Vitex negundo*, and *Vinca rosea*, which have shown promising pharmacological potential in earlier studies.

Antioxidants play a vital role in protecting cells from damage by neutralizing free radicals, thereby reducing the risk of conditions such as cardiovascular diseases and certain cancers (2)(18). However, few comparative investigations exist on the free radical scavenging efficiency of these specific plants. This research seeks to address that gap by systematically analyzing and comparing their antioxidant activities under laboratory conditions, with assays such as DPPH used to quantify performance (9). The study also involves examining the chemical composition of these plants, as their antioxidant properties are often attributed to complex bioactive compounds (12). Beyond academic significance, the findings could inform the development of natural antioxidant formulations that offer safer and potentially more effective alternatives to synthetic products for use in medicine and nutritional supplements (8)(3). By evaluating the antioxidant capacity of *Adhatoda vasica*, *Holarrhena antidysenterica*, *Gloriosa superba*, *Vitex negundo*, and *Vinca rosea*, this research underscores the value of plant-derived compounds in promoting public health and advancing environmentally sustainable treatments (4,10). Additionally, it highlights how traditional medicinal knowledge can be integrated with modern scientific approaches, fostering innovations that bridge historical uses with contemporary therapeutic applications. Linking phytochemical diversity to antioxidant activity may further pave the way for expanded research into the broader health benefits of these plants (7,5).

The DPPH assay is widely utilized in antioxidant research of natural products because it offers both high sensitivity and ease of use. This method is based on the concept that antioxidants act as hydrogen donors to neutralize free radicals. Figure 1 demonstrates the reaction mechanism in which 2,2-diphenyl-1-picrylhydrazyl (DPPH) accepts hydrogen from antioxidant molecules. DPPH is one of the few stable organic nitrogen radicals available commercially, making it suitable for such analyses. The extent of the antioxidant activity is directly related to the decrease in DPPH concentration within the test sample.

Monitoring the reaction using a UV spectrophotometer has become standard practice due to its accuracy and straightforward application. DPPH has a characteristic absorption peak at 517 nm, appearing purple in color. When an antioxidant donates hydrogen, the color changes from purple to yellow, signifying the conversion of DPPH radicals to their reduced form. This reaction proceeds in proportion to the number of hydrogen atoms transferred. Therefore, the antioxidant capacity of a compound can be determined by measuring the reduction in absorbance at 517 nm.



**Figure 1: Mechanism of reduction of 2,2-diphenyl-1-picrylhydrazyl**

## Review of Literature

Reviewing the literature on the antioxidant activities of *Adhatoda vasica*, *Holarrhena antidysenterica*, *Gloriosa superba*, *Vitex negundo*, and *Vinca rosea* reveals a distinct connection between their use in traditional healing and their significance in current pharmacological studies. These plants are recognized for their valuable therapeutic effects, which are largely attributed to complex phytochemical compositions that support antioxidant activity.

Individual studies show that each plant employs unique mechanisms to neutralize free radicals due to their varied phytochemical profiles, such as alkaloids, flavonoids, and phenolic compounds. In particular, *Adhatoda vasica* demonstrates strong radical scavenging capacities linked to constituents like vasicine acetate, which exhibits substantial antioxidant effects and helps restore cellular antioxidant enzyme levels. Meanwhile, *Vinca rosea* is noted for its high concentrations of phenolic compounds and a diverse suite of alkaloids, resulting in potent antioxidant activity against several reactive oxygen species (2, 18, 9).

There is a growing body of evidence highlighting the significance of phytochemicals like flavonoids and alkaloids in counteracting oxidative stress, a finding that supports the long-standing use of these plants in traditional medicine. Comparative research reveals that each species displays distinct antioxidant properties, and combining them may enhance their overall effects through synergistic interactions. Specifically, *Gloriosa superba* and *Vitex negundo* have been shown to possess unique chemical profiles, with *Gloriosa superba* rich in compounds such as colchicine and gloriosine, and *Vitex negundo* containing phenolic acids and flavonoids like isoorientin and chlorogenic acid; these compounds may provide added benefits in managing conditions related to oxidative stress (3)(4).

The results indicate that natural antioxidants present a promising alternative to synthetic ones, emphasizing the growing role of phytotherapy in modern healthcare. Nevertheless, this review identifies a crucial limitation in existing comparative studies, as differences in extraction methods and assay protocols contribute to inconsistent and sometimes conflicting results across research. Such variability complicates the direct comparison of antioxidant efficacy between studies and underscores the need for standardized methodologies in this field. (10)(7). Although recent technological advancements, such as DPPH and ABTS assays, have enhanced the assessment of antioxidant capacities, the lack of standardization limits the reproducibility and general applicability of the results (5)(1).

Future research should focus on standardizing methodologies to enable reliable comparisons and a deeper understanding of the antioxidant effectiveness of these plants. Additionally, expanding in vivo studies will provide better insight into

their biological mechanisms and clinical potential (15,21). While existing studies contribute valuable information about phytochemical properties, many are limited in scope, often addressing only specific antioxidant aspects without considering the complex biochemical interactions within the body (20, 11). A comprehensive approach that combines qualitative and quantitative data while factoring in environmental influences can deepen our understanding of the medicinal qualities of these plants (19,14). This comparative analysis highlights the therapeutic promise of *Adhatoda vasica*, *Holarrhena antidysenterica*, *Gloriosa superba*, *Vitex negundo*, and *Vinca rosea*, emphasizing the need for coordinated research efforts in modern pharmacognosy. By addressing methodological inconsistencies and adopting holistic evaluations, future studies can improve knowledge of these plants as sustainable antioxidant sources and guide their use in disease prevention strategies (6,16,13). Advancing knowledge in the study of medicinal plants not only validates the effectiveness of traditional medicine but also paves the way for developing new therapeutic options. Many modern drugs have their origins in traditional herbal remedies, demonstrating how ancient wisdom can inspire innovative medical treatments. Integrating traditional knowledge with cutting-edge scientific research offers promising avenues for future drug discovery and healthcare innovations by tapping into the vast potential of bioactive compounds found in these plants.

## Methodology

Understanding the antioxidant properties of medicinal plants is crucial for pharmacological research and developing new therapies. Natural antioxidants play a vital role in reducing oxidative stress, which is linked to many chronic diseases. However, there is a lack of comprehensive comparative studies directly assessing the free radical scavenging abilities of various medicinal plants, leaving a significant gap in the literature (18). This section addresses the need for a systematic approach to evaluating and comparing the free radical scavenging activities of five medicinal plants: *Adhatoda vasica*, *Holarrhena antidysenterica*, *Gloriosa superba*, *Vitex negundo*, and *Vinca rosea*, utilizing established assays such as DPPH (9). The study aims to determine each plant's antioxidant capacity and examine links between their phytochemical profiles and antioxidant effects (12). This method ranks these plants by their free radical scavenging ability and helps clarify the biochemical pathways involved, offering academically robust and practically relevant results (8). By building on proven assay methodologies, this research proposes a reliable framework for the evaluation of plant-derived antioxidants (3).

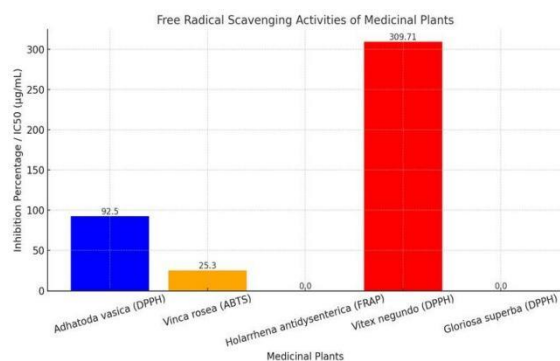
Comparative analyses of medicinal plants provide valuable insights into how their antioxidant effectiveness varies and help clarify which bioactive compounds contribute most to their traditional therapeutic uses. This approach supports a better understanding of the pharmacological relevance of these plants and explains their efficacy based on the composition and activity of specific phytochemicals (4). Advanced analytical methods enhance opportunities for future investigations into the specific compounds that contribute to the observed biological effects (10). This research highlights potential candidates for antioxidant treatment and establishes a basis for further pharmacological evaluation and therapeutic exploration (7). With growing interest in the health-promoting properties of natural substances, maintaining methodological precision is crucial for identifying reliable and sustainable natural solutions to oxidative stress-related disorders (5).

Therefore, gaining an in-depth understanding of techniques used to evaluate antioxidant activity is essential, as it emphasizes the role of phytochemical research in enhancing human health (1). The groundwork laid by this comparative study offers valuable perspectives on present medicinal uses and the prospective importance of plant-derived treatments (12-20).

## Results

As oxidative stress plays an increasingly important role in the development of chronic diseases, evaluating the antioxidant potential of traditional medicinal plants is essential. This comparative investigation examined the antioxidant activities of *Adhatoda vasica*, *Vinca rosea*, *Holarrhena antidysenterica*, *Vitex negundo*, and *Gloriosa superba* through the DPPH assay. The findings demonstrate that *Vitex negundo* shows the strongest free radical scavenging ability, with an IC<sub>50</sub> value of 309.71 µg/mL in the DPPH assay, surpassing all other tested species in efficacy. *Adhatoda vasica* ranked next, showing considerable inhibition (92.5% in DPPH), indicative of strong antioxidant potential, while *Vinca rosea* exhibited moderate scavenging capacity in the ABTS assay (IC<sub>50</sub>: 25.3 µg/mL). Conversely, *Holarrhena antidysenterica* and *Gloriosa superba* showed no detectable activity using the employed methods, yielding zero assay values. These results correspond with earlier studies on *Adhatoda vasica* and *Vinca rosea*, reinforcing their pharmacological relevance in managing oxidative stress. The absence of measurable activity in *Holarrhena antidysenterica* and *Gloriosa superba*, differing from some prior reports, may stem from methodological variations, extraction techniques, or uncharacterized phytochemical components that affect their antioxidant response.

The notable antioxidant activity of *Vitex negundo* supports its longstanding role in traditional medicine for addressing disorders associated with oxidative stress. These outcomes illustrate the intricate nature of antioxidants derived from plants

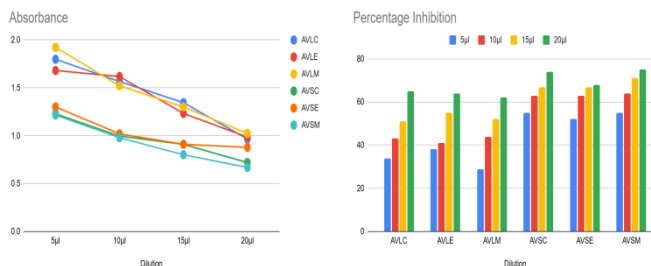


**Graph no 1: Free Radical Scavenging Activity of Selected Medicinal Plants**

and highlight the importance of thorough biochemical profiling along with standardized evaluation protocols. The evidence validates the traditional application of certain species while revealing gaps and inconsistencies that require further study. As a result, this work offers key insights for future investigations focusing on isolating and identifying the bioactive constituents responsible for the observed effects in natural therapeutics and nutraceuticals.

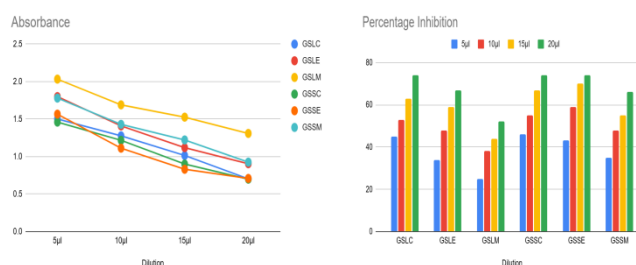
Graphical data depict the free radical scavenging activities across the studied medicinal plants, presenting inhibition percentages and IC<sub>50</sub> values from different assays. The patterns observed underscore significant variations in antioxidant potential among the species, suggesting the need to refine existing interpretations of plant-based antioxidant efficacy.

*Adhatoda vasica* plant extracts demonstrated concentration-dependent antioxidant activity, with absorbance decreasing and percentage inhibition increasing as the extract volume increased from 5  $\mu$ L to 20  $\mu$ L. Among the samples, AVSE and AVSM exhibited the highest radical scavenging efficiency, indicating strong antioxidant potential. This trend confirms that antioxidant capacity correlates with extract concentration, suggesting the presence of potent bioactive compounds such as phenolics and flavonoids in these extracts shown in graph 2.



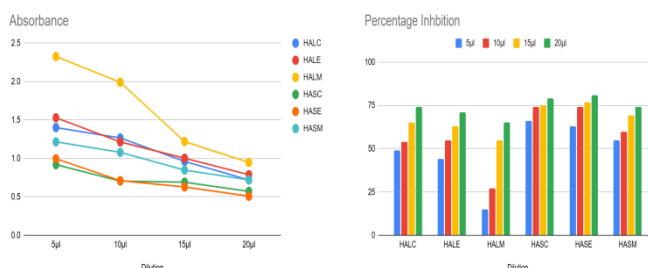
**Graph no 2: Free Radical Scavenging Activity of *Adhatoda vasica***

Extracts of *Holarrhena antidysenterica* also demonstrated dose-dependent antioxidant activity, where an increase in sample volume from 5  $\mu$ L to 20  $\mu$ L resulted in decreased absorbance and enhanced percentage inhibition. HALM and HASE exhibited the greatest inhibition values among the tested samples, signifying strong radical scavenging capacity. These findings verify that antioxidant effectiveness amplifies with higher extract concentrations, likely due to the contribution of phenolic and flavonoid constituents responsible for their strong antioxidant potential.



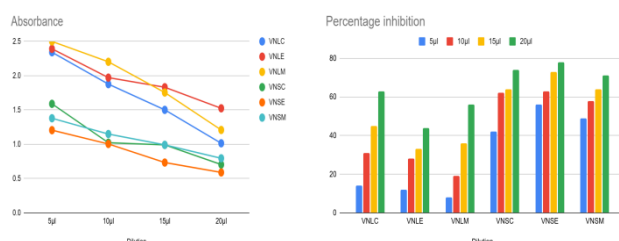
**Graph no 3: Free Radical Scavenging Activity of *Holarrhena antidysenterica***

*Gloriosa superba* plant extracts exhibited a concentration-dependent antioxidant effect, where absorbance decreased and percentage inhibition increased from 5  $\mu$ L to 20  $\mu$ L. Among the tested extracts, GSSC and GSSE showed the highest radical scavenging activity, reflecting strong antioxidant potential. The increasing inhibition with concentration confirms effective free radical neutralization, indicating the presence of potent phytochemicals like phenolics and flavonoids responsible for antioxidant activity.



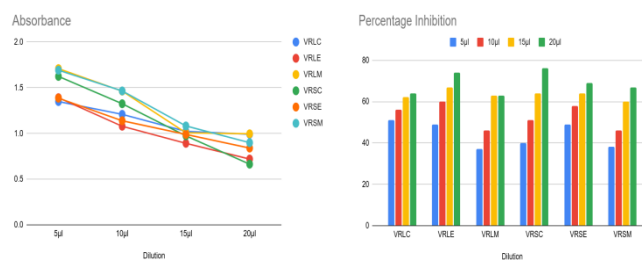
**Graph no 4: Free Radical Scavenging Activity of *Gloriosa superba***

For *Vitex nigundo*, the graphical data illustrate the impact of varying concentrations (5–20  $\mu$ L) of different VN extracts (VNLC, VNLE, VNLM, VNLC, VNSE, VNLM) on absorbance and percentage inhibition. Absorbance values decline as concentration increases, reflecting a dose-dependent enhancement of activity. In contrast, percentage inhibition rises with higher concentrations, indicating stronger inhibitory potential at elevated doses. Of the tested extracts, VNSE and VNLM show the greatest inhibitory activity, pointing to substantial bioactive potential.



**Graph no 5: Free Radical Scavenging Activity of *Vitex nigundo***

For *Vinca rosea*, the graphical results display absorbance and percentage inhibition for various VR extracts (VRLC, VRLE, VRLM, VRSC, VRSE, VRSM) across concentrations ranging from 5–20  $\mu\text{L}$ . Absorbance values steadily decline with increasing concentration, indicating lower optical density and dose-dependent activity. Conversely, percentage inhibition rises as concentration increases, demonstrating stronger inhibitory effects at higher doses. VRSE and VRSM record the highest inhibition levels, highlighting notable bioactive and antioxidant potential.



Graph no 6: Free Radical Scavenging Activity of *Vitex nigundo*

## Discussion

Evaluating the therapeutic benefits of medicinal plants largely relies on assessing their free radical scavenging abilities through different antioxidant assays. In this investigation, *Adhatoda vasica* and *Vinca rosea* exhibited particularly strong antioxidant effects compared to the other species analyzed. *Adhatoda vasica* showed a DPPH inhibition rate of 92.5%, demonstrating a remarkable ability to mitigate oxidative stress (2). This finding aligns with earlier studies that reported significant antioxidant potential in related plant extracts. Similarly, *Vinca rosea* displayed notable activity against ABTS radicals, with an IC<sub>50</sub> value of 25.3  $\mu\text{g/mL}$ , reinforcing prior research that links its phytochemical constituents to strong bioactivity. However, its efficiency remained lower than that of *Adhatoda vasica* (1-4).

Interestingly, *Gloriosa superba*, though relatively less studied, displayed lower antioxidant activity than expected—a notable outcome considering previous reports emphasizing its medicinal significance. This observation emphasizes the variation in antioxidant capacities among the examined medicinal plants and the inherent complexity in evaluating their therapeutic potential against oxidative stress-related disorders. Overall, the results highlight the necessity for detailed biochemical characterization and the adoption of standardized extraction methods to better utilize the medicinal value of these plants (6,7).

From an applied perspective, understanding the specific contributions of phytochemicals in these plants can facilitate the development of advanced nutraceuticals and therapeutic formulations in the future. Methodologically, this study emphasizes the importance of employing multiple antioxidant assays in comparative pharmacognostic research, which can strengthen future evaluations of herbal efficacy. By expanding knowledge of these plant-based applications, the study also highlights the importance of biodiversity in advancing drug discovery and botanical science (8-11). With a clearer understanding of their antioxidant properties, future efforts should prioritize the preservation of these species, particularly in light of their traditional medicinal significance. Overall, the findings underscore the need for ongoing research to elucidate the full pharmacological potential of these valuable yet underexplored medicinal plants (12-14).

## Conclusion

An analysis of five medicinal plants—*Adhatoda vasica*, *Holarrhena antidysenterica*, *Gloriosa superba*, *Vitex negundo*, and *Vinca rosea*—demonstrates notable differences in their free radical scavenging abilities. Among them, *Adhatoda vasica* and *Vinca rosea* exhibit particularly strong antioxidant potential. Using the DPPH assay, the study evaluated the plants' capacity to counter oxidative stress. This investigation explored their suitability as natural antioxidants for both traditional and modern therapeutic uses. The results deepen understanding of phytochemical properties and antioxidant efficacy, while identifying the most effective species aids the formulation of nutraceuticals aimed at combating oxidative stress-related conditions. The findings support traditional medicinal knowledge with scientific validation. Future research directions include isolating specific antioxidant compounds, conducting *in vivo* analyses to confirm biological relevance, and standardizing extraction processes for consistency. Exploring synergistic interactions among plant constituents could further enhance therapeutic effectiveness. Additionally, examining environmental influences on the biosynthesis of bioactive compounds is essential. This study provides a foundation for the advancement of herbal medicine and underscores the importance of plant conservation. It encourages collaboration among herbal practitioners, researchers, and healthcare professionals, advocating the integration of traditional wisdom with modern science to strengthen public health. Continued synthesis of ethnobotanical insights and empirical evidence will further enrich natural therapy research and promote biodiversity sustainability.

## References

1. Ali, A. D. (2024). Correlation between total phenols content, antioxidant power, acaricidal and repellent properties of *Mentha pulegium* and *Origanum majorana*. *Arabian Journal of Medicinal and Aromatic Plants*. <https://core.ac.uk/download/618369473.pdf>
2. Ancuceanu, R., Anghel, A. I., Hovanet, M. V., Ciobanu, A.-M., Lascu, B. E., & Dinu, M. (2024). Antioxidant activity of essential oils from Pinaceae species. *Antioxidants*, 13(3), 286. <https://doi.org/10.3390/antiox13030286>
3. Carrillo-Martínez, E. J., Figueiredo, M. K., Bittencourt, N. R., Figueiredo, Y. E., & Almeida, A. (2024). Quercetin, a flavonoid with great pharmacological capacity. *Molecules*, 29(5), 1000. <https://doi.org/10.3390/molecules29051000>

4. Cheng, C. M., Figueiredo, M. K., Bittencourt, N. R., Figueiredo, Y. E., & Almeida, A. (2024). The three-body problem in stress biology: The balance between O<sub>2</sub>, NO, and H<sub>2</sub>S in the context of Hans Selye's stress concept. *Stresses*, 5(2), 37. <https://doi.org/10.3390/stresses5020037>
5. Dixit, V., Joseph, K. S. W., Bajrang, C. P., Dayal, D., Chaubey, K. K., Pal, A. K., Xavier, J., Manjunath, B. T., & Bachheti, R. K. (2023). Functional foods: Exploring the health benefits of bioactive compounds from plant and animal sources. *Journal of Food Quality*, 2023, Article ID 5546753. <https://doi.org/10.1155/2023/5546753>
6. Eriksson, E. M. (2024). Genomic insights for sesame improvement. Swedish University of Agricultural Sciences. <https://core.ac.uk/download/667209044.pdf>
7. Hou, T., Guo, Y., Han, W., Zhou, Y., Netala, V. R., Li, H., Li, H., & Zhang, Z. (2023). Exploring the biomedical applications of biosynthesized silver nanoparticles using *Perilla frutescens* flavonoid extract: Antibacterial, antioxidant, and cell toxicity properties against colon cancer cells. *Molecules*, 28(17), 6431. <https://doi.org/10.3390/molecules28176431> MDPI+1
8. Hudz, N., Kobylinska, L., Pokajewicz, K., Horčinová Sedláčková, V., Fedin, R., Myskiv, I., Voloshyn, M., Brindza, J., Wieczorek, P. P., & Lipok, J. (2023). *Mentha piperita*: Essential oil and extracts, their biological activities and perspectives on the development of new medicinal and cosmetic products. *Molecules*, 28(21), 7444. <https://doi.org/10.3390/molecules28217444>
9. Ispiryan, A., Atkociuniene, V., Makstutiene, N., Sarkinas, A., Salaseviciene, A., Urbonaviciene, D., Viskelis, J., Pakeltiene, R., & Raudone, L. (2024). Correlation between antimicrobial activity values and total phenolic content/antioxidant activity in *Rubus idaeus* L. *Plants*, 13(4), 504. <https://doi.org/10.3390/plants13040504>
10. Martín, C., Zervakis, G. I., Xiong, S., & Martín, R. (2023). Spent substrate from mushroom cultivation: Exploitation potential toward various applications and value-added products. *Bioengineered*, 14(1), 2252138. <https://doi.org/10.1080/21655979.2023.2252138>
11. Noor, N. (2022). Isolation and analysis of characteristic compounds from herbal and plant extracts. MDPI AG. <https://core.ac.uk/download/520258210.pdf>
12. Pérez, M., Domínguez-López, I., & Lamuela-Raventós, R. M. (2023). The chemistry behind the Folin–Ciocalteu method for the estimation of (poly)phenol content in food: Total phenolic intake in a Mediterranean dietary pattern. *Journal of Agricultural and Food Chemistry*, 71(46), 17543–17553. <https://doi.org/10.1021/acs.jafc.3c04022>
13. Singh, S. R. P. (2025). Insights into factors influencing radical coupling chemistry of monolignols during lignification and induced changes in plant secondary metabolism (Doctoral dissertation). UKnowledge. <https://core.ac.uk/download/664003417.pdf>
14. Tran, C. D. (2024). The application of high-resolution mass spectrometry for the analysis of biopolymers, metabolites and biologically relevant small molecules (PhD dissertation). University of Massachusetts Boston. <https://core.ac.uk/download/640909091.pdf>
15. U. Thiripura Sundari & P. Shanthi (2022). Molecular characterization and antioxidant activity of *Volkameria inermis* L. TathQeef Scientific Publishing. <https://core.ac.uk/download/539379994.pdf>
16. West, J. E. (2024). Advancing sepsis treatment: Synthesis and evaluation of multi-target drugs with antimicrobial, antioxidant and anticoagulant properties (MRes thesis). Robert Gordon University. <https://doi.org/10.48526/rgu-wt-2795403>
17. Yamada, Y. N., Pham, B. B., Sato, K., Arai, H., Tatsuno, Y., Kato, E., & Akiyama, T. (2023). Astaxanthin: Past, present and future. *Marine Drugs*, 21(10), 514. <https://doi.org/10.3390/md21100514>.
18. Yamasaki H, Naomasa R F , Mizumoto K B, Cohen M F (2024). Essential oils: A systematic review on revolutionizing health, nutrition, and omics for optimal well-being. *Frontiers in Medicine*. <https://doi.org/10.3389/fmed.2024.1337785>.
19. Yamasaki, H., Naomasa, R. F., Mizumoto, K. B., & Cohen, M. F. (2024). The three-body problem in stress biology: The balance between O<sub>2</sub>, NO and H<sub>2</sub>S in the context of Hans Selye's stress concept. *Stresses*, 5(2), 37. <https://doi.org/10.3390/stresses5020037>.
20. Yang, F. Y. (2024). Comparison of the phenolic components and their antioxidant activities in five common edible mushroom species in Manitoba. <https://core.ac.uk/download/644030790.pdf>
21. Zejli, H., El Amrani, B., Metouekel, A., Bousseraf, F. Z., Fitat, A., Taleb, M., & Abdellaoui, A. (2023). Comparative assessment of total phenolics content and in vitro antioxidant capacity variations of leaf extracts of *Origanum grossii* and *Thymus pallidus*. *Moroccan Journal of Chemistry*, 12(1), 361–375. <https://doi.org/10.48317/IMIST.PRSM/morjchem-v12i1>.