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Review Article

Optimizing Bioactive Compounds Extraction: Advanced Food Safety Protocols for Enhanced Bioavailability and Processing Stability

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Abstract | Ensuring food safety is paramount in the extraction of bioactive compounds, aiming for optimal bioavailability and enhanced stability throughout the processing stages. This review aims to explore the critical relationship between food safety measures and the extraction of bioactive compounds, to ensure optimal bioavailability and enhanced stability throughout processing stages. Ensuring food safety is paramount in the extraction of bioactive compounds, aiming for optimal bioavailability and enhanced stability throughout the processing stages. This abstract delves into the crucial nexus between food safety measures and the extraction process, recognising its pivotal role in delivering bioactive compounds with maximum efficacy. Rigorous adherence to stringent hygiene practices, cutting-edge technologies, and robust quality control mechanisms emerges as a fundamental approach. The personnel involved in the extraction process must undergo comprehensive training to uphold the highest cleanliness and procedural adherence standards. Facility design and adherence to regulatory standards are crucial in minimising risks associated with microbial contamination, chemical residues, and allergens. Continuous monitoring of environmental conditions, documentation, and traceability systems is are essential component of a holistic safety framework. The dynamic relationship between food safety and bioavailability is explored, emphasising the need for a comprehensive approach throughout the supply chain, from raw material cultivation to final processing. The integration of advanced technologies, such as supercritical fluid extraction and innovative filtration methods, is pivotal in elevating safety and efficacy. The pursuit of food safety in bioactive compound extraction is positioned as more than a regulatory requirement; it is an integral aspect of delivering products that align with evolving consumer expectations for safety, efficacy, and overall well-being. The recommendations presented serve as a guide for industry stakeholders, researchers, and policymakers in establishing and maintaining the highest standards of food safety in the quest for optimal bioavailability and improved stability in bioactive compound processing.

Keywords | Extraction, Bioavailability, Food safety, Stability, Solvents, Allergens

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INTRODUCTION

Bioactive compounds are naturally occurring molecules that exert specific physiological effects on living organisms, including humans. Derived from plants, animals, or microorganisms, these compounds contribute to health and well-being. Notable types include phytochemicals, such as flavonoids and alkaloids, found in plants and offering diverse benefits. Antioxidants, like vitamin C and polyphenols, combat oxidative stress (Ye *et al*., 2025a). With categories like terpenoids, sterols, and saponins, bioactive compounds showcase a rich diversity, each type uniquely supporting various aspects of human health. Bioactive compounds are pivotal in promoting health through their diverse physiological effects. Found in various foods, they contribute to the prevention of chronic diseases, bolster the immune system, and support overall well-being. Incorporating bioactive-rich diets is essential for harnessing the nutritional benefits that positively impact human health. Natural sources like fruits, vegetables, and herbs are rich in bioactive compounds. For instance, citrus fruits contain flavonoids, broccoli provides glucosinolates, and turmeric contains curcumin (Kusmayadi *et al*., 2021).

Bioactive chemicals are extracted from natural sources using a variety of extraction procedures, such as solvent extraction and supercritical fluid extraction. In solvent extraction, the target chemicals are dissolved and separated using organic solvents such as ethanol or hexane. Supercritical fluid extraction, on the other hand, makes use of supercritical fluids, frequently carbon dioxide, which combine the characteristics of both gas and liquid, increasing the extraction efficiency. Efficiency and safety are the two most important factors to compare extraction techniques. Despite its effectiveness, solvent extraction can be dangerous since some solvents are combustible. Supercritical fluid extraction is frequently seen as safer since it is a cleaner and more ecologically friendly procedure (Geada *et al*., 2021).

Various factors influence the bioavailability of extracted compounds, including their chemical form, formulation, and interactions with other components during digestion. The compound's solubility, stability, and molecular size also play crucial roles in determining its bioavailability. Methods to enhance bioavailability involve optimizing formulations, employing nanotechnology for improved delivery systems, and considering the co-administration of certain compounds to enhance absorption. Emphasizing these strategies ensures that bioactive compounds are effectively absorbed and utilized by the body, maximizing their therapeutic potential (Rai *et al*., 2019).

Challenges in maintaining stability during processing and storage include exposure to factors like heat, light, and oxygen, leading to the degradation of bioactive compounds. Strategies for improving stability involve the use of protective packaging, controlled storage conditions, and the incorporation of stabilizing agents, ensuring the preservation of the bioactive properties throughout the production and shelf life of the product (Bessada *et al*., 2019).

The pursuit of optimal bioavailability and improved stability necessitates a thorough understanding of food safety measures throughout the extraction journey. This introduction explores the critical interplay between bioactive compound extraction, ensuring their availability for absorption, and the implementation of measures to guarantee product stability, laying the foundation for a comprehensive exploration of these intricate dynamics (Raza *et al*., 2024b). By navigating the delicate balance between extraction efficiency and safety, this study aims to unravel the complexities associated with achieving optimal bioavailability and stability in the processing of bioactive compounds. Addressing a crucial nexus in the intersection of nutrition, health, and food science, the study also highlights current challenges in the industry. These challenges include regulatory hurdles that necessitate strict adherence to evolving standards and technological limitations that impact the scalability and efficiency of extraction processes. By addressing these challenges, the study seeks to provide actionable insights for overcoming barriers and advancing the field (Puligundla *et al*., 2020).

Food safety measures in extraction

Toxicity in extracting bioactive compounds can arise from various sources and poses a significant concern for the safety of the final product. For instance, the use of solvents like hexane in the extraction process has been linked to neurotoxic effects. Hexane, commonly used for its efficiency in extracting lipophilic compounds, can accumulate in the nervous system, leading to symptoms such as numbness, muscle weakness, and impaired coordination if not properly managed. Additionally, improper handling of raw materials can lead to contamination with heavy metals. Contamination with lead, for example, has been observed in herbal extracts, often due to the use of contaminated soil or water during cultivation. Lead contamination can have severe health implications, including neurological damage, particularly in children and pregnant women. Moreover, microbial contamination is another critical hazard. For example, the presence of *Salmonella* or *E. coli* in raw materials can lead to foodborne illnesses, which not only pose immediate health risks but can also compromise the bioavailability of bioactive compounds by disrupting the gut microbiota and causing inflammation. These incidents highlight the potential hazards that warrant careful consideration to ensure the safety and efficacy of the final product. Implementing rigorous quality control measures, such as solvent purification, raw material testing, and microbial screening, is essential to mitigate these risks and produce safe, effective bioactive compounds (Figure 1).

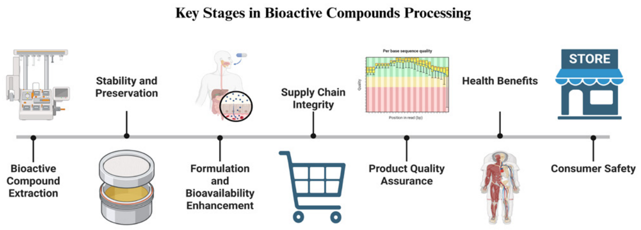

Figure 1: Key stages in bioactive compounds processing.

Potential hazards in extracting bioactive compound

Toxic solvents

The use of toxic solvents, such as certain organic solvents (e.g., dichloromethane), can introduce harmful residues into the extracted bioactive compounds, posing health risks if not properly removed. Considering the chronic toxicity, the typical effects of high exposure to solvents are often of a neurobehavioral and cardiotoxicological nature. It should be considered that dichloromethane can potentially be absorbed via the skin in addition to being inhaled. Dichloromethane was categorized as a hazardous chemical with limited industrial use. Additionally, dichloromethane was recently reclassified as probably carcinogenic to humans (classification 2A) from possibly carcinogenic to humans (classification 2B) (Schiavone *et al.*, 2023).

Contaminant presence

Contaminants such as pesticides, heavy metals, or microbiological pathogens may be present in raw materials and might concentrate in the extracted molecules, potentially causing toxicity (Raza *et al.*, 2024a). For example, seaweeds may be eaten raw or added to food items as components and are a great source of macronutrients, micronutrients, and bioactive substances (Maqbool *et al.*, 2024). It is heavily polluted with microbial diseases and heavy metals, according to researchers. As a result, seaweeds have the potential to collect and become a source of several chemicals, including arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) (Tofalo *et al.*, 2020). Additionally, the authors used the microbiological challenge test to determine if *B. cereus* might develop in commercial *Undaria pinnatifida* and *Palmaria palmate* RTE meals while they were being stored in a refrigerator. The findings demonstrated that the RTE dehydrated seaweed-based food items included coliforms as well as marine bacteria *Listeria* spp. and *B. cereus* (Fasolin *et al.*, 2019).

Formation of by-products

The extraction process can generate by-products or degradation products, which may be harmful. Conditions such as high temperature or extended extraction times can contribute to the formation of undesirable compounds. The extracted polyphenols may also be impacted by the creation of new substances known as Maillard reaction products at high extraction temperatures. Maillard and Berset explained how polyphenols behaved at high temperatures using three different processes. First, when the lignin links to phenolic acids are broken, the insoluble phenolic compounds may be liberated. It has been demonstrated that the amount of bound phenolic acids is double that of free phenolic compounds when measured following the hydrolysis of plant tissue (Ishaq *et al.*, 2024). Secondly, lignin itself may be degraded at high temperatures, giving rise to more phenolic acids. This could explain the increase in phenolic yield with increasing temperature in PLE extractions. Lastly, at high temperatures, thermal degradation of the polyphenols may occur. Thermal degradation is the most common mechanism used to explain the fall in polyphenol yield during high-temperature extractions (Amara and El-Baky, 2023).

Enzyme

Plant tissue contains various enzymes that play significant roles in the breakdown of bioactive compounds. For instance, glycosidases, polyphenol oxidases (PPO), and peroxidases are key enzymes that can break down polyphenols. Specifically, anthocyanins can be quickly broken down by glycosidases into chemically unstable anthocyanidins and sugars. Polyphenol oxidase (PPO) can catalyze the conversion of o-dihydrophenols (such as chlorogenic acid) into o-quinones (such as chlorogenoquinone) in the presence of oxygen. Similarly, peroxidase can catalyze the same process when hydrogen peroxide (H_2O_2) is present. In addition to these enzymes, cellulases and proteases are also widely used in the extraction of bioactive compounds. Cellulases, for example, are enzymes that break down cellulose into glucose. They are particularly effective in breaking down plant cell walls, facilitating the release of bioactive compounds. The ideal conditions for cellulase activity typically include a pH range of 4.5 to 5.5 and a temperature of around 50°C. Proteases, on the other hand, are enzymes that break down proteins into smaller peptides and amino acids. They are commonly used to enhance the extraction of bioactive peptides from plant and animal sources. The optimal conditions for protease activity can vary depending on the specific enzyme. For example, pepsin, a protease found in the stomach, operates optimally at a pH of around 2 and a temperature of 37°C, while trypsin, another protease, functions best at a pH of 7.5 to 8.5 and a temperature of 37°C. By understanding and optimizing the conditions for these enzymes, the extraction process can be significantly enhanced, leading to higher yields and improved bioavailability of bioactive compounds (Amin *et al.*, 2021).

Allergen cross-contamination

Cross-contamination with allergens during the extraction process, especially in facilities handling multiple ingredients, can pose risks to individuals with allergies, affecting the safety of the bioactive compounds (Koukoumaki *et al*., 2024).

Inadequate purification

Incomplete removal of impurities or residual solvents during purification processes can result in a final product with compromised purity, impacting both safety and efficacy. Inadequate purification of bioactive compounds in food can lead to various food safety issues, as impurities may pose health risks or compromise the quality of the final product (Shaheen *et al*., 2024). Inadequate purification may result in the presence of toxic impurities or contaminants from the plant material, soil, or processing equipment. Some plants, including St. John's Wort, can accumulate heavy metals, pesticides, or other harmful substances. These contaminants could pose health risks to consumers (Zhang *et al*., 2024).

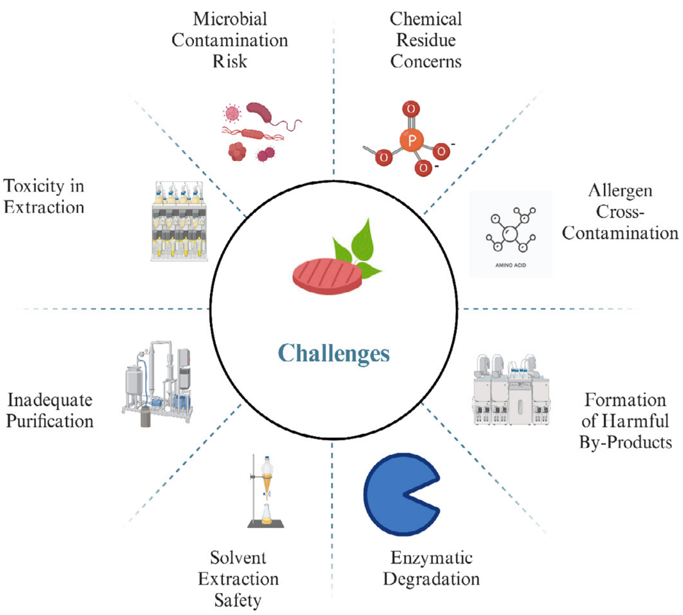

Figure 2: Key challenges in ensuring food safety during bioactive compound extraction.

Toxicity in extraction

Toxicity concerns in the extraction of bioactive compounds are significant considerations for both the extraction process and the safety of the final product. To protect public health, when a novel food or ingredient is proposed, it is necessary to ensure consumer health, and for that, safety assessments and procedures are proposed in regulations (Kanwal *et al*., 2024). The toxicological evaluation is fundamental in all safety assessments, whatever the country and the laws that govern them. In EU countries, the toxicological evaluation should be followed by the EFSA guidelines. These guidelines were made to perform the safety assessment of the food additives, vitamins and minerals, novel foods, food supplements, and botanicals and follow the tier toxicity testing approach proposed for food additives in 2012 (Figure 2) (Su *et al*., 2023).

The FDA's toxicological principles for the safety assessment of direct food additives and color additives used in food, often known as the redbook, should be observed in the United States. The EFSA standards, which are separated into three categories, employ a tiered approach, or sequential testing technique, across EU nations. Tier 1 comprises the bare minimum of information needed for every chemical. At this level, a case-by-case strategy that takes into account all of the relevant data must be developed. So, all compounds must be analyzed for the minimum tests required (Tier 1) and depending on the results they may need further testing. For example, in vitro positive results in genotoxicity tests (Tier 1) obligate a follow-up for in vivo genotoxicity tests (Tier 2) (Saleem *et al*., 2024). Moreover, the tiered approach is designed to evaluate four core areas: Toxicokinetics, genotoxicity, toxicity, and reproductive and developmental toxicity. Accordingly the guidelines provided by EFSA, there are several general issues that should be considered in the design, conduct, and interpretation of toxicological studies for submission to approval, the most important are: (1) The studies should be performed with the additive according to the proposed specifications and should be produced according to the application; (2) studies with animals and humans should comply the EU standards and regulations for ethical approval and welfare standards; (3) studies in animals should follow the internationally agreed test guidelines (Directive 2010/63/EU); (4) toxicokinetics and toxicity of food additives in animals should be conducted using internationally agreed test guidelines described in OECD test guidelines (OECD TG) or in Council Regulation (EC) No. 440/2008; (5) non-clinical studies should follow the principles of Good Laboratory Practice (GLP) described in Directive 2004/10/EC19; (6) the oral route should be selected for testing substances and the bioactive ingredients should be added to solid food, or to both solid and liquid, and normally via the diet. For substances applied in beverages, administration via drinking water may be the best option; (7) botanical food additives derived from conventional food sources with a long-term history of food use, may benefit from a "presumption of safety" when adequate information exists (Sillman *et al*., 2019).

Foodborne pathogen on bioavailability

Foodborne pathogens can significantly impact the bioavailability of bioactive ingredients in food. Infections caused by these pathogens may trigger inflammatory responses and disrupt the balance of gut microbiota, influencing the absorption and metabolism of bioactive compounds (Shah *et al*., 2021). Foodborne pathogens such as *Salmonella* and *E. coli* can significantly impact the bioavailability of bioactive ingredients in food. These pathogens may trigger inflammatory responses and disrupt the balance of gut microbiota, which in turn influences the absorption and metabolism of bioactive compounds. For example, *Salmonella* infection can lead to gastrointestinal inflammation, impairing the absorption of essential nutrients and bioactive compounds. Similarly, E. coli can produce toxins that affect gut health, further compromising the bioavailability of these compounds. Addressing these interactions is crucial for ensuring food safety and maximizing the health benefits of bioactive compounds (Langyan *et al*., 2022).

Processing stability of bioactive compounds

The processing of bioactive compounds can significantly affect their composition, stability, and bioavailability. Key factors such as temperature and pH play crucial roles in determining the stability of these compounds. High temperatures during processing, such as cooking or pasteurization, can degrade heat-sensitive bioactive compounds, reducing their concentration and potential health benefits. Similarly, extreme pH levels, whether acidic or alkaline, can alter the structure and stability of bioactive compounds, impacting their bioavailability. Recent research highlights the importance of optimizing processing conditions to maintain the integrity of bioactive compounds. For instance, studies have shown that using gentle heating methods and maintaining a neutral pH can help preserve the stability and bioavailability of these compounds. Additionally, innovative techniques like supercritical fluid extraction and cold pasteurization have been found to be effective in minimizing degradation and preserving the health benefits of bioactive compounds (Amin *et al*., 2021). When exposed to unfavorable circumstances, such as high temperatures and the presence of oxygen and light, the volatile organosulfur compounds found in the produced garlic products are thermally unstable and prone to oxidation, volatilization, and degradation (Ahmed *et al*., 2021). During manufacturing and storage, bioactive compounds are frequently exposed to light and oxygen, which can oxidize them and reduce their antioxidant capacity and overall effectiveness. Key factors such as temperature and pH significantly impact the stability of bioactive compounds. For instance, high temperatures can accelerate degradation, while changes in pH, such as acidic or alkaline treatments, can alter their structure and bioavailability. Recent studies highlight the importance of optimizing processing conditions to maintain stability. For example, maintaining a neutral pH and using gentle heating methods can help preserve the integrity of bioactive compounds. Additionally, enzymes present in raw materials or added during processing can break down bioactive substances, potentially reducing their beneficial effects. Recent research suggests that enzyme inhibitors or controlled enzyme activity can mitigate this issue, ensuring better retention of bioactive compounds (Fasolin *et al*., 2019).

Food waste bioactive compounds regulatory and legislative issues

The food industry is becoming more interested in creating functional and nutraceutical products, and the bioactive substances that are extracted from food can be utilized again in the human food supply chain as functional foods, supplements, additives, fortification, and other small ingredients (Bourassi *et al*., 2024).

The absence of an effective policy and the growing need to enhance the legislative regulations, codes of behavior, and guidelines are two issues about food valorization. Due to a lack of waste utilization regulations, the use of bioactive chemicals recovered from food waste is currently restricted to scientific research and patents (Lv *et al*., 2022).

The State Administration for Market Regulation (SAMR) in China released regulations about the labeling of health foods. The National Health Commission (NHC), which creates food safety standards and registers new food additives, and the State Administration for Market Regulation (SAMR) are the primary authorities responsible for overseeing the production and distribution of food additives as well as inspecting and quarantining import and export food additives (Ahmed *et al*., Year). Any intention to produce, operate, use, or import a new food additive must first register with the NHC. Any license request must include the following information: name, function category, dose level, application, certificates proving technical necessity and use effect, safety assessment materials, raw materials or sources, chemical structure and physical properties, production methods, toxicology safety assessment documents, and testing reports on quality specifications (Wang *et al*., 2020).

Recommendations for ensuring food safety

To ensure the highest standards of food safety during the extraction of bioactive compounds for optimal bioavailability and improved stability in processing, the following key recommendations are proposed: Implement thorough training programs for personnel involved in the extraction process, emphasizing the importance of hygiene, adherence to safety protocols, and the proper use of personal protective equipment (PPE) (Ye *et al*., 2025b). Invest in modern extraction equipment and technology that meet or exceed industry standards for safety and efficiency. Regularly maintain and calibrate equipment to ensure accurate and reliable results (Latif *et al*., 2019). Establish a HACCP system to systematically identify, evaluate, and control potential hazards at critical points in the extraction process. This proactive approach enhances the overall safety of bioactive compound production (Khalid *et al*., 2025a). Maintain strict control over environmental factors such as temperature and humidity, creating conditions that discourage microbial growth and ensure the stability of bioactive compounds. Conduct regular testing of raw materials for contaminants, allergens, and other potential hazards. Additionally, implement robust testing protocols for finished products to verify the safety and quality of the extracted bioactive compounds (Latif *et al*., 2019). Collaborate closely with suppliers and implement stringent quality assurance programs, ensuring that raw materials meet specified safety standards and are sourced from reputable and compliant producers. Document all extraction processes, creating standardized operating procedures that include safety checkpoints. Regularly review and update these documents to reflect the latest safety standards and best practices. Implement traceability systems that allow for the tracking of raw materials from their source through the extraction process (Khalid *et al*., 2025b). To ensure food safety in the extraction of bioactive compounds, it is crucial to adopt a multifaceted approach. Transparency in the supply chain, as seen in the implementation by major food companies like Nestlé, aids in quickly identifying and addressing any safety concerns. Investing in ongoing research and development, similar to the efforts by Unilever, helps explore and adopt the latest technologies and methodologies that enhance safety. Embracing advanced technologies, such as sensor-based monitoring and real-time data analytics, as demonstrated by the success of companies like PepsiCo, can significantly enhance the precision and efficiency of the extraction process while ensuring safety. Fostering a workplace culture that prioritizes safety at every level, as exemplified by the practices of Danone, encourages employees to actively participate in safety initiatives, report any potential issues promptly, and take ownership of maintaining a safe working environment. Regularly reviewing and assessing safety measures, as seen in the continuous improvement strategies of General Mills, ensures that best practices are maintained and updated (Manzoor *et al*., 2019). Stay informed about emerging technologies and advancements in food safety to enhance the efficiency and effectiveness of the extraction process. Maintain open lines of communication with relevant regulatory bodies to stay informed about evolving food safety standards and ensure compliance with all applicable regulations. Establish clear communication channels with stakeholders, including consumers, regulatory bodies, and industry partners, to convey a commitment to food safety, share relevant information, and address concerns proactively. Develop and communicate clear emergency response protocols to address any unexpected safety incidents swiftly and effectively, minimizing potential risks and ensuring the well-being of consumers and employees alike (LaFountain and Yuan, 2021).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the meticulous implementation of food safety measures in the extraction of bioactive compounds is essential for ensuring optimal bioavailability and enhanced stability during processing. The integration of advanced technologies, such as supercritical fluid extraction and novel filtration techniques, significantly elevates the safety profile and efficacy of bioactive compounds. Key findings highlight the importance of rigorous adherence to stringent hygiene protocols, quality control procedures, and regulatory standards to mitigate risks associated with microbial contamination, chemical residues, and other hazards. Continuous monitoring of environmental conditions, comprehensive training of personnel, and facility design also play crucial roles in maintaining high food safety standards. Future research opportunities include exploring the impact of emerging technologies on the stability and bioavailability of bioactive compounds, as well as investigating the long-term effects of foodborne pathogens on gut health and nutrient absorption. Additionally, there is a need for further studies on the optimisation of extraction conditions, such as temperature and ph, to maximise the retention of bioactive compounds. Research into the development of enzyme inhibitors or controlled enzyme activity could also provide valuable insights into preserving the integrity of bioactive substances during processing. By prioritising food safety measures and pursuing continuous improvement through research and development, we can unlock the full potential of bioactive compounds and contribute to the creation of products that meet consumer expectations for safety and efficacy. The commitment to excellence in food safety not only safeguards public health but also fosters a healthier, more resilient future for the global food industry.

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NOVELTY STATEMENT

This review article provides a comprehensive exploration of the critical relationship between food safety measures and the extraction of bioactive compounds, highlighting the importance of advanced food safety protocols in enhancing bioavailability and processing stability. The study integrates insights from recent advancements in extraction technologies and regulatory standards, offering novel perspectives on optimizing extraction processes to meet evolving consumer expectations for safety and efficacy.

AUTHOR'S CONTRIBUTION

Md. Shabudden Ahamed contributed to the conceptualization and writing of the original draft, as well as review and editing. Ayesha Asghar, Nimra Hayat, Muqaddas, Lubna Bashir, Sumaira Saif, Rabiya Riaz, Muhammad Atiq Ashraf, Burhan Khalid, Ariba Sadia, and Muhammad Moeid Khan participated in the investigation, data curation, and review and editing of the manuscript. Sha Md. Shahan Shahriar provided supervision and contributed to the review and editing process. Talha Riaz was responsible for conceptualization, writing the original draft, and overall project supervision.

Conflict of interest

The authors have declared no conflict of interest.

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