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Review Article

A Comprehensive Review of Solvent-Induced Variability in Antioxidant Profiling of Plants Extract: *Justicia secunda*

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ABSTRACT

Variability in antioxidant profiles, particularly within plant extracts like *Justicia secunda* and other plant extracts, presents a significant challenge for researchers striving to attain consistent and dependable outcomes in the realm of antioxidant profiling. This variability is predominantly attributed to the choice of solvents used during the extraction process. Our study delves deeply into the impact of solvent selection in the extraction process, revealing notable discrepancies in the antioxidant profiles of these plant extracts. This variation becomes evident through the diverse antioxidant potential observed across different solvents, emphasizing the pressing need for standardized methodologies to ensure research uniformity and reliability. Our review further explores the intricate interplay of diverse solvents employed during extraction procedures and their potential to induce variations in antioxidant profiles. It meticulously highlights the discernible fluctuations in antioxidant potential resulting from the use of different solvents, underscoring the imperative need for a systematic approach to research methodologies to guarantee consistent outcomes. The review concludes by presenting a forward-looking research agenda, including a comprehensive effort to identify and analyze specific antioxidant compounds within these extracts under various solvent conditions. Ultimately, our research seeks to enrich our understanding of the antioxidant properties and potential benefits associated with *Justicia secunda*. This review serves as an invaluable resource for researchers operating within this domain, spotlighting the pivotal role of solvent selection in antioxidant profiling and championing the cause of standardized methodologies to propel our knowledge forward in this area.

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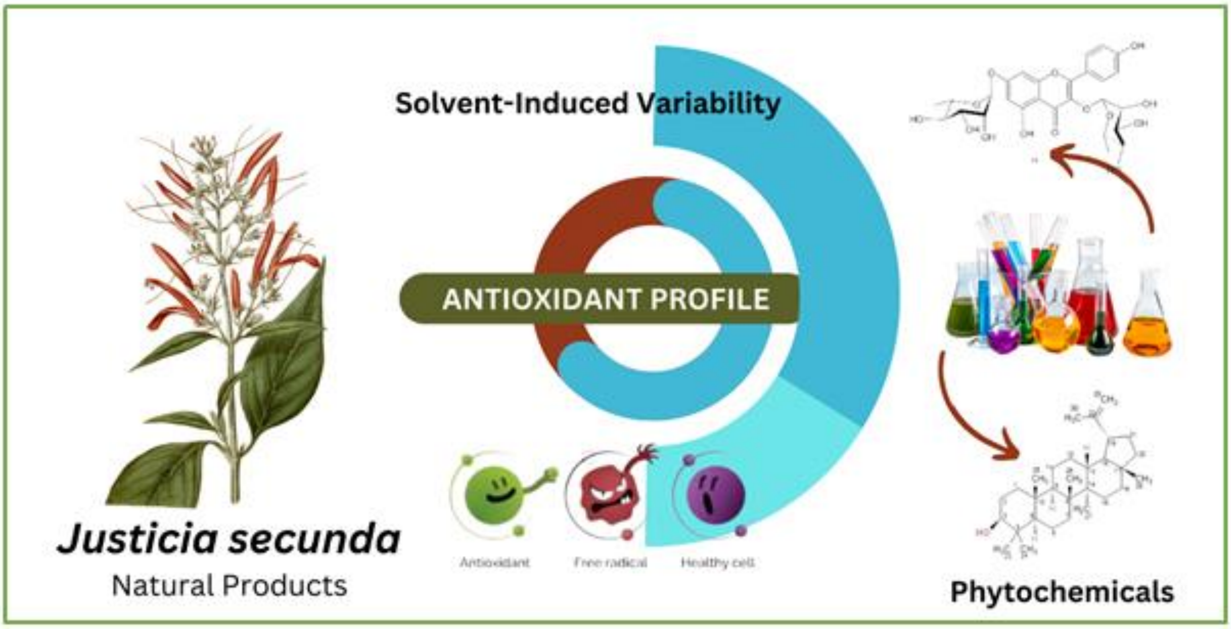
GRAPHICAL ABSTRACT



Introduction

In recent years, the exploration of natural sources for bioactive compounds with potential health benefits has gained considerable attention in the scientific community. Plants, with their diverse array of phytochemicals, have become a primary focus of research, particularly in the pursuit of antioxidant compounds [1]. Antioxidants play a critical role in safeguarding the body against oxidative stress, a phenomenon linked to various chronic diseases, including cancer, cardiovascular disorders, and aging [3]. The study of antioxidants derived from plant sources, therefore, holds immense promise for the development of therapeutic and preventive strategies.

In the realm of natural compounds with potential health benefits, *Justicia secunda* stands as an intriguing subject of study. Its historical use in treating various ailments underscores its potential as a valuable resource in modern healthcare [1]. *Justicia secunda* is a medicinal plant widely distributed in tropical regions, and has been traditionally used for its therapeutic properties. Previous research on *Justicia secunda* has reported its various pharmacological activities, including antioxidant, anti-inflammatory, and analgesic effects [2]. The focus of this study was on the antioxidant profiles derived from its leaf and stem extracts, specifically exploring how different solvent extraction methods influence these profiles. Understanding the distribution of antioxidants in different parts of plants, such as leaves and stems, is essential. These plant components often possess unique phytochemical profiles, leading to varied antioxidant potential. In addition, the choice of solvents for extraction can significantly influence the types and quantities of antioxidants obtained [3].

The extraction of bioactive compounds from plant materials necessitates the use of solvents to separate and concentrate these compounds. It is this crucial stage of solvent selection that has profound implications on the antioxidant potential of the resulting extracts. Our comprehensive review delves deep into this aspect, illuminating the remarkable influence that solvent choice wields over the antioxidant profiles of *Justicia secunda* extracts. We aimed to underline the urgency of implementing standardized methodologies to mitigate this variability, thus ensuring the uniformity and reliability of research outcomes.

This review serves as an invaluable resource, not only for researchers specifically interested in *Justicia secunda*, but also for the broader scientific community engaged in plant-based antioxidant profiling. Our research delves into the intricate interplay of diverse solvents used during the extraction procedures and the subsequent variations in antioxidant potential that they induce. By examining and comparing the diverse antioxidant potential observed across different solvents, we emphasize the paramount need for a systematic approach to research methodologies.

Furthermore, the implications of solvent-induced variability on the bioactivity of *Justicia secunda* extracts are discussed, shedding light on the potential consequences for applications within the realms of medicine and health. As these applications hold the promise of addressing pressing health issues, the need for reliable and consistent data becomes even more pronounced.

This exploration enhances our comprehension of the plant's overall antioxidant capacity. This review aims to provide a comprehensive comparative analysis of the antioxidant profiles in leaf and stem extracts of *Justicia secunda*. By employing various solvents for extraction, we seek to elucidate the influence of solvent variability on the observed antioxidant activity. This knowledge is pivotal for harnessing the full potential of *Justicia secunda* as a source of natural antioxidants with potential implications for human health.

Antioxidants and Their Significance

In our quest to unravel the intricacies of solvent-induced variability in antioxidant profiling, we should initially acknowledge what antioxidants are and their profound importance in our review. These bioactive compounds (antioxidants) are the unsung heroes of our biological systems, silently combating the insidious effects of oxidative stress that assail our cells daily [22]. Oxidative stress, at its core, emerges from an imbalance between the production of harmful free radicals and the body's innate defense mechanisms [23-24]. Antioxidants emerge as the champions in this battle, swooping in to neutralize these free radicals and reactive oxygen species (ROS), preventing them from wreaking havoc on our cellular structures [25-27].

Relevance of Antioxidant Profiling

Antioxidants play a crucial role in protecting cells from oxidative damage caused by free radicals and reactive oxygen species (ROS) [28]. Antioxidant profiling is a valuable analytical tool in nutrition, medicine, and food science [29]. It involves the identification and quantification of various antioxidants present in a substance, such as food, plant extracts, or biological samples [30]. Profiling antioxidants in foods and dietary supplements helps assess their potential health benefits. This information aids consumers and healthcare professionals in making informed decisions about incorporating antioxidant-rich foods or supplements into their diets. Antioxidant profiling of foods can guide individuals in making dietary choices that promote health [31]. For example, people seeking to increase their intake of antioxidants can select foods known to be rich in specific antioxidants, like vitamin C, vitamin E, or flavonoids, based on profiling data.

Researchers use antioxidant profiling to study the antioxidant capacity of different foods, botanical extracts, and dietary patterns [32-33]. This information contributes to the understanding of the role antioxidants play in preventing chronic diseases, such as heart disease, cancer, neurodegenerative disorders, and so on. In food and supplement industries, antioxidant profiling helps ensure product quality and consistency. Manufacturers can use these profiles to verify the presence and concentration of antioxidants claimed on product labels. This quality control process enhances consumer trust and safety [34]. In pharmaceutical research and development, antioxidant profiling can be vital in identifying potential drug candidates. Compounds with strong antioxidant properties may be explored for their therapeutic potential in treating diseases associated with oxidative stress [35-36].

Antioxidant profiling is useful in plant breeding and agriculture to develop crops with enhanced antioxidant content [37]. This can lead to the production of healthier foods with increased nutritional value. Antioxidant profiling of biological samples, such as blood or urine, can aid in identifying biomarkers of oxidative stress and related diseases [38]. These biomarkers can be used for diagnostic purposes and monitoring disease progression or treatment efficacy [39]. Antioxidant profiling can contribute to the emerging field of personalized nutrition. By assessing an individual's antioxidant status through profiling, tailored dietary and supplement recommendations can be made to address specific health needs [40]. Profiling antioxidants can also help monitor the effects of environmental pollutants, toxins, or contaminants on antioxidant levels in foods and ecosystems [41]. This information is essential for ensuring food safety and environmental health.

Health Benefits Associated with Antioxidants

Antioxidants are a cornerstone of holistic health, offering a profound array of benefits in combating chronic diseases and preserving well-being. From safeguarding cardiovascular health by reducing oxidative stress and inflammation to potentially thwarting cancer by protecting DNA integrity, their role is pivotal [42]. They hold promise in staving off neurodegenerative disorders, supporting the immune system, and nurturing skin health. By incorporating a diverse array of antioxidant-rich foods and embracing a healthy lifestyle, we harness the transformative potential of these compounds, equipping ourselves with a powerful defense against a range of health challenges and embracing the profound impact antioxidants have on our overall health and longevity.

Antioxidants play a crucial role in combating oxidative stress, a factor in various chronic diseases [16]. *Justicia secunda*, a plant with a rich history in traditional medicine, could hold valuable antioxidant compounds. Our research aims to uncover these compounds, paving the way for potential applications in health and wellness. Antioxidants, whether occurring naturally or synthesized, possess the potential to hinder or postpone specific forms of cellular harm [43]. These molecules combat free radicals within the body, these being substances that may inflict damage when their concentrations rise excessively [17]. Free radicals are perpetually generated within the body and can result in oxidative stress, a state that can impair cells and tissues, thereby contributing to chronic illnesses including cardiovascular diseases, diabetes, dementia, and cancer [18]. Antioxidants play a pivotal role in maintaining human health by counteracting oxidative stress, a process implicated in aging and various chronic diseases [19]. Natural antioxidants derived from plants, like those found in *Justicia secunda*, offer a promising avenue for promoting wellness and preventing oxidative damage [20].

Types of Antioxidants

Antioxidants are pivotal in protecting biological systems from the detrimental effects of oxidative stress by neutralizing harmful free radicals and reactive oxygen species (ROS). Their diverse forms constitute a multifaceted defense network against the relentless assault of free radicals and reactive oxygen species (ROS) [44]. This section delves into the various types of antioxidants, highlighting their distinct roles and significance in maintaining cellular health and overall well-being.

Enzymatic Antioxidants

Enzymatic antioxidants serve as the vanguard against oxidative stress. Superoxide dismutase (SOD) and catalase are exemplary defenders operating within cells [45]. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, while catalase subsequently breaks down hydrogen peroxide into harmless water and oxygen [46]. Together, these enzymes form the primary line of defense against superoxide and hydrogen peroxide, two highly reactive and damaging ROS. Their presence within cellular organelles ensures immediate neutralization of harmful radicals, preventing cellular damage and dysfunction [46].

Non-Enzymatic Antioxidants

Non-enzymatic antioxidants complement the enzymatic defense system, extending protection beyond the confines of cells [47,48]. Prominent among them are vitamins C and E, along with the versatile glutathione. Vitamin C, which is an aqueous-phase antioxidant, scavenges free radicals in body fluids and cellular cytoplasm, regenerating vitamin E in the process. Vitamin E, which is a lipid-soluble antioxidant, specializes in shielding cell membranes from lipid peroxidation [50]. Glutathione, often referred to as the "master antioxidant," orchestrates a cascade of reactions that detoxify harmful compounds and recycle other antioxidants, reinforcing the overall antioxidant defense system [51].

Phytochemical Antioxidants

Phytochemical antioxidants, which are found in abundance within plant extracts, comprise a diverse range of compounds that make a significant contribution to our dietary intake of antioxidants. [52]. Flavonoids, carotenoids, and polyphenols are among the most studied phytochemical antioxidants. Flavonoids, found in fruits, vegetables, and beverages such as tea and red wine, possess diverse antioxidant properties, offering protection against oxidative damage [53]. Carotenoids which are responsible for the vibrant colors of many fruits and vegetables, are renowned for their ability to quench singlet oxygen and protect against photooxidative processes [53, 55]. Polyphenols found in foods like berries, nuts, and dark chocolates, exhibit antioxidant, anti-inflammatory, and potential anti-cancer properties [54].

Overview of Methods and Solvents Used for Antioxidant Profile from J. secunda Extracts and other plants

Assessing the antioxidant properties of plant extracts is crucial for understanding their potential health benefits. However, the use of different extraction methods and solvents in various studies can make it challenging to compare and draw meaningful conclusions about the antioxidant profiles of "Justicia secunda" (J. secunda) and other plants. Many researchers have caried similar studies on the antioxidant profile using different solvents and methods, as presented in Table 1.

Overview of Phytochemicals Composition of Justicia secunda and Its Potential Bioactive (Antioxidant) Compounds

Phytochemicals are bioactive compounds that occur naturally in plants. They contribute to the plants' color, taste, and smell, while also serving essential functions in defending the plant and interacting with its environment [4]. *Justicia secunda*, a plant of interest in botanical and pharmaceutical research, has been a subject of investigation due to its potential therapeutic properties attributed to its phytochemical content

[102]. *Justicia secunda* is rich in a diverse array of bioactive compounds, including flavonoids, alkaloids, terpenoids, phenolics, and glycosides. These compounds, abundant in both its leaves and stems, are responsible for the plant's medicinal properties. Flavonoids, for instance, exhibit antioxidant, anti-inflammatory, and anticancer activities. Alkaloids may possess analgesic and antimicrobial properties, while terpenoids contribute to various therapeutic effects [5]. This botanical species offers a captivating case study of how distinct plant parts can harbor unique assortments of bioactive compounds, reflecting their specialized functions within the plant's lifecycle. These distinctive phytochemical profiles are orchestrated by a harmonious interplay of intrinsic physiological roles and extrinsic environmental cues. In this species, the leaves and stems exhibit noteworthy variations in their phytochemical profiles, with each part containing a unique array of compounds that serve diverse biological functions. These variations are the result of intricate biochemical processes influenced by both intrinsic factors related to plant physiology and extrinsic factors like environmental conditions [5].

Table 1. Comparison of Extraction Methods and Solvent Used for Antioxidant Profile from *J.* secunda Extracts and other plants

Plants	Extraction Method	Solvent Used	Antioxidant Activity	Ref.
Justicia secunda Leaf and Stem		Hexane	Highest	[96]
		Ethyl acetate	Significant	
		Acetone	Significant	
	Cold Maceration	Methanol	Significant	
Justicia secunda		Dichloromethane	Significant	[21]
		Ethyl acetate	Significant	
	Maceration and Infusion	Methanol	Highest	
		Water	Highest	
Dipsacus asperoides		Acetone	Significant	[100]
	Ultrasonic-assisted Extraction with Filtration and Concentration.	Methanol,	Highest	
		Water	Significant	
Justicia secunda Vahl Leaf	Maceration	Methanol	High	[8]
Justicia secunda Vahl	Maceration	Methanol	High	[99]

Significant variations in phytochemical composition exist between the leaves and stems of *Justicia secunda*. Studies have shown that leaves may contain higher concentrations of certain flavonoids, such as quercetin, whereas stems may exhibit elevated levels of alkaloids like vincamine [56]. These differences in specific compounds could be attributed to distinct physiological functions of leaves and stems within the plant, as well as environmental factors [6].

The distinct phytochemical profiles of *Justicia secunda*'s leaves and stems hold promising implications for potential health benefits [97]. Utilizing different parts of the plant in traditional medicine or herbal remedies may target specific health conditions [7,8].

For example, the higher flavonoid content in the leaves may make them more suitable for antioxidant and anti-inflammatory applications, while the stem extracts, rich in alkaloids, could be valuable for their analgesic or antimicrobial effects.

Understanding these differences allows for targeted utilization of *Justicia secunda*'s various parts to maximize its therapeutic potential and contribute to the development of natural health products.

Role of Specific Bioactive Compounds in Contributing to Antioxidant Activity Differences among Solvents

The presence of unique bioactive compounds in *Justicia secunda* plays a crucial role in the observed differences in antioxidant activity among solvents. Some solvents may be more efficient at extracting specific compounds due to their chemical nature [96]. For instance, certain solvents might excel at extracting flavonoids, while others are better suited for alkaloids. Understanding the distribution of these compounds in different solvents provides insights into the targeted extraction of specific antioxidants and the potential modulation of *Justicia secunda*'s bioactivity based on solvent selection.

The interplay between solvent properties, extraction conditions, and the specific bioactive compounds present in *Justicia secunda* contributes to the variability in antioxidant profiles [98].

This knowledge enables researchers to strategically select solvents for extracting antioxidants of interest, ensuring that the unique health-promoting properties of different bioactive compounds are effectively harnessed for potential therapeutic applications.

Solvent Extraction Methods

Solvent extraction is a commonly utilized method for extracting bioactive compounds from plant materials and involves using various techniques to isolate these compounds, including maceration, Soxhlet extraction, and ultrasound-assisted extraction [57].

Maceration

Maceration is a conventional approach to solvent extraction, employed for the isolation of bioactive substances from botanical materials [58-60]. In this method, plant materials are submerged or soaked in a suitable solvent like hexane, ethanol, or acetone for an extended period, often spanning from several days to weeks. During this period, the solvent gradually permeates the plant material, causing the dissolution of the desired bioactive components. Maceration is known for its simplicity and cost-effectiveness, making it a favored choice for extracting compounds such as phytochemicals, phenolics, and alkaloids. However, it is important to note that it may necessitate a longer extraction duration when compared to more modern methodologies [57].

Soxhlet Extraction

Soxhlet extraction is another widely embraced technique for the extraction of bioactive compounds from plant sources. This method is particularly effective for extracting compounds with limited solubility in conventional solvents. It entails a continuous cycle of extraction, reflux, and condensation [61]. The plant material is typically placed within a porous thimble, which is then inserted into a Soxhlet extractor. A solvent, often a high-boiling-point solvent such as hexane, is heated, vaporized, and subsequently condensed back into the extraction chamber, effectively cycling the solvent through the plant material. This process continues until the extracted compounds are concentrated within the solvent. Soxhlet extraction is renowned for its efficiency and its capability to extract a diverse array of compounds [62].

Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction is a contemporary and highly efficient method that harnesses ultrasound waves to augment the extraction process. In this technique, the plant material is mixed with a suitable solvent within an ultrasound bath [63]. The application of ultrasound energy generates cavitation bubbles within the solvent, which implode in proximity to the plant material's surface. This phenomenon creates localized microenvironments characterized by elevated temperatures and pressures, thereby facilitating the extraction of bioactive substances. UAE is applauded for its rapid extraction speed, reduced solvent consumption, and enhanced yield of target compounds. It is often the preferred choice for extracting heat-sensitive substances such as specific polyphenols and essential oils [64].

Each of these solvent extraction methods offers unique advantages, with the selection contingent upon the specific characteristics of the plant material and the targeted bioactive compounds [65]. Researchers judiciously opt for the most appropriate technique to optimize the extraction process and maximize the yield of bioactive constituents for subsequent analysis and potential applications in diverse fields, including pharmaceuticals, food production, and cosmetics. The choice of technique depends on the type of plant material, the desired compounds, and the equipment available. Maceration involves soaking the plant material in a solvent to allow for compound dissolution, while Soxhlet extraction employs continuous solvent cycling. The ultrasound-assisted extraction uses ultrasonic waves to enhance compound release from the plant matrix, facilitating higher extraction efficiency [9].

Among the methods mentioned for isolating bioactive compounds from plant materials, the ultrasound-assisted extraction (UAE) has gained significant attention and popularity among researchers in recent years. This technique has shown promising results in terms of higher extraction efficiency and reduced extraction time compared to traditional methods like maceration and Soxhlet extraction [66]. Researchers have utilized ultrasound-assisted extraction in various studies to extract bioactive compounds from plant materials. [10] investigated the use of ultrasound-assisted extraction for obtaining bioactive compounds from plants. They found that the UAE significantly improved the extraction efficiency and reduced the extraction time compared to traditional methods. In a study by [11] reviewed the use of ultrasound-assisted extraction for plant bioactive compounds, including phenolics, flavonoids, thymols, saponins, and proteins. The review concluded that ultrasound-assisted extraction is a green extraction technique that offers high performance with less solvent and time consumption. The study also found that ultrasound-assisted extraction can extract bioactive components in less time, at low temperature, with lesser energy and solvent consumption. The study suggests that ultrasound-assisted extraction is an effective method for extracting bioactive compounds from plants. Another study by [12] focused on extracting polyphenols from *Lonicera japonica* (Japanese honeysuckle) flowers using ultrasound-assisted extraction. They found that this method significantly improved the extraction efficiency and enabled the recovery of a higher amount of polyphenolic compounds.

Overall, ultrasound-assisted extraction has demonstrated its effectiveness in enhancing the extraction of bioactive compounds from plant materials. Researchers have reported improved extraction efficiency, shorter extraction times, and higher yields of target compounds. However, the success of the technique can also depend on factors such as the type of plant material, the solvent used, and the specific bioactive compounds of interest. It is important to note that while the UAE has shown promise, the choice of extraction method may still vary based on the research goals and the characteristics of the plant material being studied.

Factors Influencing Solvent Selection for Antioxidant Extraction

The choice of solvent in antioxidant profiling plays a pivotal role in the effectiveness of the extraction process and the quality of obtained results [67]. Researchers should carefully consider several factors when selecting the most suitable solvent for their specific study. These factors need to be carefully considered to ensure accurate and effective extraction of bioactive compounds. According to [3], factors such as solvent polarity, selectivity, and compatibility with the target compounds influence the choice. The nature of the bioactive compounds in *Justicia secunda*, as well as their desired application, dictates the suitable solvent. The solvent polarity impacts the extraction of specific phytochemicals, with polar solvents being more effective for extracting polar compounds like flavonoids, while non-polar solvents may better extract lipophilic compounds [13]. Here are the key factors that influence solvent selection in antioxidant profiling:

Solubility of Target Compounds

The solubility of antioxidants in a chosen solvent is a fundamental factor. Different antioxidants exhibit varying degrees of solubility based on their chemical properties [68]. For example, hydrophilic antioxidants, such as ascorbic acid or polyphenols, are more soluble in polar solvents like water, methanol, or ethanol. In contrast, lipophilic antioxidants, including carotenoids or tocopherols, tend to dissolve better in nonpolar solvents like hexane or ethyl acetate [69]. Therefore, carefully assessment of the chemical characteristics of their target antioxidants to select a solvent that facilitates efficient extraction.

Nature of Plant Material

The composition of the plant material under investigation significantly influences solvent selection [96]. Different plant tissues contain a wide range of phytochemicals, and the choice of solvent should align with the constituents' present [98]. For example, extracting antioxidants from fruit peels, which often contain lipophilic compounds, may necessitate the use of nonpolar solvents. Conversely, extracting antioxidants from leaves or stems, which may contain hydrophilic compounds, may require polar solvents [70].

Heat Sensitivity

Some antioxidants are sensitive to heat, and exposure to elevated temperatures during the extraction process can lead to degradation and loss of bioactivity. To preserve the integrity of these compounds, researchers opt for solvents that allow for extraction at lower temperatures. Cold solvents, such as cold methanol or ethanol, are often chosen to prevent heat-induced degradation while still achieving effective extraction [71].

Safety and Toxicity

Safety considerations are paramount in solvent selection. Some solvents, such as chloroform or dichloromethane, may pose health risks or have adverse environmental impacts, and thus should be used with caution or avoided [72]. The safety data sheets for solvents provide essential information about their toxicity, flammability, and safe handling procedures [73].

Cost and Availability

It is noteworthy that practical factors like cost and availability influence solvent selection. Certain solvents may be expensive, particularly those of high purity grades, and could significantly impact research budgets. In addition, the availability of solvents in the required quantities and grades can be a logistical consideration, particularly for large-scale studies [74].

Extraction Efficiency

The efficiency of a solvent in extracting antioxidants is a crucial factor. Researchers aim to achieve high extraction yields while minimizing solvent usage. Solvents that efficiently extract target compounds without excessive waste are preferred to optimize resource utilization and minimize environmental impact [75].

Compatibility with Analytical Techniques

Solvent compatibility with the chosen analytical techniques is essential for obtaining accurate and reliable results. Different analytical methods, such as spectrophotometry, high-performance liquid chromatography (HPLC), or gas chromatography (GC), may require specific solvents to ensure proper sample preparation and reliable measurements. Compatibility considerations include the solvent's chemical compatibility with the analytical instrument and its ability to provide clean and interference-free extracts [76].

Regulatory Compliance

Regulatory compliance is vital, especially in applications related to food, pharmaceuticals, or cosmetics [77]. Researchers should adhere to regulatory guidelines and standards when working with solvents to ensure the safety and quality of the final products. Compliance includes following good laboratory practices, handling hazardous materials in accordance with safety regulations, and ensuring that solvents used meet regulatory purity standards.

Impact of Solvent Choice on Antioxidant Profiling

Several solvents are commonly employed in plant extraction, each with its advantages and limitations. Several studies have explored the variability in antioxidant profiling of *Justicia Secunda* extracts resulting from various solvent choices. Notably, ethanol, methanol, acetone, and water are among the commonly employed solvents [15,98].
1. Ethanol Extracts: Ethanol is frequently used for extracting antioxidants from *Justicia secunda* [78]. Research shows that ethanol-based extractions yield a diverse range of antioxidants, including phenolic compounds and flavonoids. These extracts have demonstrated strong free radical scavenging activity and significant potential for health-promoting properties [79].
2. Methanol Extracts: Methanol is another widely used solvent for plants [81]. Studies suggest that methanol extractions are effective at capturing a broad spectrum of antioxidants, particularly polyphenols [80]. Methanol-based extracts exhibit substantial antioxidant activity and are valued for their potential in combating oxidative stress-related diseases [82].
3. Acetone Extracts: Acetone is chosen in some studies for its ability to extract antioxidants efficiently. Acetone-based extracts of *Justicia secunda* have been found to contain various polyphenols and flavonoids. These extracts exhibit antioxidant potential, contributing to the plant's overall therapeutic value [82].
4. Water Extracts: Water extraction, while milder than organic solvents, remains a valuable method for obtaining antioxidants from *Justicia secunda* [83]. Water-based extracts are rich in water-

soluble antioxidants, such as vitamin C, and exhibit antioxidant activity, albeit to a lesser extent than organic solvent extracts [84].

Polar solvents like methanol and ethanol offer high extraction efficiency for a broad range of compounds, but might also co-extract undesired substances. Non-polar solvents such as hexane are effective for lipophilic compounds but may miss some polar antioxidants. Ethyl acetate combines characteristics of both polar and non-polar solvents, making it suitable for diverse phytochemicals, yet its use requires careful consideration due to potential toxicity [14].

Methodologies in Antioxidant Profiling

Antioxidant profiling relies on a range of analytical techniques, each with its strengths and applications [20]. The choice of technique should align with research objectives, sample type, and the specific antioxidants of interest. Furthermore, sample preparation techniques play a crucial role in minimizing variability and ensuring the accuracy of antioxidant analysis. This review discusses the diverse methodologies and analytical techniques commonly employed in antioxidant profiling, emphasizing their importance in elucidating the antioxidant capacity of various substances. In addition, we explore the relevance of sample preparation techniques and their impact on solvent-induced variability.

1. Spectrophotometry: Spectrophotometry is a widely used technique for assessing antioxidant activity based on the absorbance measurement at specific wavelengths. The most common assays include the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays. Spectrophotometric assays are rapid and cost-effective, providing a preliminary assessment of antioxidant capacity [85].

2. HPLC (High-Performance Liquid Chromatography): HPLC is a powerful tool for identifying and quantifying specific antioxidants within complex mixtures. It enables the separation of compounds based on their chemical properties, such as polarity and molecular weight. HPLC coupled with various detectors, such as UV-Vis, fluorescence, or mass spectrometry, allows for precise quantification of antioxidants, including vitamins (e.g., vitamin C and E) and polyphenols [86].

3. Gas Chromatography (GC): GC is primarily used for analyzing volatile antioxidants and lipid-soluble antioxidants. It involves the vaporization of compounds and their separation based on their volatility. GC is especially valuable for studying antioxidants like carotenoids and tocopherols, commonly found in lipids [87].

4. Liquid Chromatography-Mass Spectrometry (LC-MS): LC-MS combines the separation capabilities of liquid chromatography with mass spectrometry's specificity, making it suitable for identifying and quantifying antioxidants. LC-MS can handle complex samples and is essential for characterizing the structure of novel antioxidants [88].

In the context of *Justicia secunda*, various antioxidant assays have been employed to assess the antioxidant activity of its leaf and stem extracts. These assays were chosen based on their relevance to the specific bioactive compounds present in *Justicia secunda* and their ability to provide comprehensive information on antioxidant potential. The rationale for selecting these assays involves their sensitivity, reproducibility, and established use in similar plant studies [21]. The influence of solvent variability on the observed antioxidant activity is discussed, with a focus on the unique antioxidant capacities of each plant part. The combination of selected antioxidant assays, the unique phytochemical composition of *Justicia secunda*, and the use of different solvents allows for a comprehensive evaluation of the antioxidant potential of the plant's extracts. This information is valuable for understanding the role of *Justicia secunda* in promoting health and its potential applications in the development of natural antioxidant-based products.

Implications of Solvent-Induced Variability

Solvent choice in antioxidant profiling significantly impacts the composition and potency of plant extracts. This variability has several implications, as follow [89]:

Health Applications

Given the potential health benefits of *Justicia secunda* antioxidants, such as cardiovascular protection and neuroprotection, solvent-induced variability can influence the efficacy of herbal remedies and dietary supplements. Inconsistent antioxidant profiles may lead to variations in therapeutic outcomes, posing challenges for healthcare practitioners and consumers seeking standardized treatments [90].

Pharmaceutical Industry

The pharmaceutical sector is increasingly exploring the therapeutic potential of natural antioxidants. Solvent-induced variability raises concerns about the consistency and efficacy of pharmaceutical products derived from *Justicia secunda*. Standardization becomes crucial to ensure reliable and reproducible formulations [91].

Food Industry

Justicia secunda is utilized in traditional foods and functional beverages [103]. Variability in antioxidant content can impact product quality and labeling accuracy. Food manufacturers need to address this variability to make credible health claims and meet consumer expectations [92].

Biomedical Research

Researchers studying plant's antioxidant properties should consider solvent-induced variability when designing experiments and interpreting results because the choice of solvent can significantly impact the extraction efficiency and, consequently, the antioxidant activity of phytochemicals from the plant material. Inaccurate or inconsistent antioxidant profiling can lead to erroneous conclusions about its health benefits and potential mechanisms of action [93].

Challenges and Opportunities for Standardization

Standardization of antioxidant profiling methodologies is essential to address solvent-induced variability and unlock the full potential of plant's extracts [94]. Some of the challenges and opportunities include:

Methodological Diversity

The plethora of analytical techniques and solvents used in antioxidant profiling poses a challenge to standardization [94]. Collaborative efforts among researchers, institutions, and regulatory bodies are needed to develop standardized protocols.

Reference Materials

The availability of reference materials, including certified antioxidant standards, can aid in method validation and comparison [94]. Developing a comprehensive database of reference materials specific to *Justicia secunda* can enhance standardization efforts.

Regulatory Guidelines

Regulatory bodies should establish clear guidelines for antioxidant profiling in herbal products and supplements. These guidelines can define acceptable methods, reporting standards, and acceptable levels of variability [95].

Data Sharing

Encouraging researchers to openly share their data and methodologies can foster collaboration and drive standardization efforts. Transparent reporting of extraction protocols, used solvents, and antioxidant quantification methods is critical.

Solvent-induced variability in antioxidant profiling of plant extracts has far-reaching implications for health and industrial applications. Addressing these challenges through standardization efforts is paramount to ensure the reliability and reproducibility of results, ultimately benefiting consumers, the pharmaceutical industry, food manufacturers, and biomedical research. Standardization will not only enhance the credibility of plants therapeutic potential, but also contribute to the broader field of antioxidant research, fostering trust in natural remedies and products.

Conclusion

This comprehensive review entitled: "*A Comprehensive Review of Solvent-Induced Variability in Antioxidant Profiling of Plants Extract: Justicia secunda*" emphasizes the critical role of solvent selection in antioxidant profiling, particularly concerning Justicia secunda. A key takeaway is the significant impact that different solvents can have on the assessment of antioxidant activity in Justicia secunda extracts. It underscores the need for researchers to exercise careful consideration when choosing solvents, as this choice profoundly influences the reliability and outcomes of their studies. Moreover, the review highlights the substantial variability in antioxidant potential observed across various solvents, with some yielding higher concentrations while others yield lower ones. This inherent variability necessitates a nuanced approach to study Justicia secunda's antioxidant properties. To address these challenges, future research should focus on identifying specific antioxidant compounds within Justicia secunda extracts under different solvent conditions and standardizing extraction and analysis methods to enhance consistency and reliability in antioxidant profiling, ultimately advancing our understanding of this plant's antioxidant potential and its broader implications.

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