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Boeravinone B in Herbal Medicine: A Review on Analytical Methods, HPLC Validation, and Phytochemical Standardization

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ABSTRACT

Boeravinone B, a key rotenoid found in *Boerhaavia diffusa* and *Mirabilis jalapa*, exhibits significant pharmacological activities, including antioxidant, anti-inflammatory, and hepatoprotective effects. Accurate quantification and standardization of Boeravinone B are essential for ensuring the efficacy and quality of herbal formulations. This review discusses various analytical techniques, with a focus on High-Performance Liquid Chromatography (HPLC), for the detection, quantification, and validation of Boeravinone B in herbal extracts. Additionally, it explores phytochemical standardization approaches to maintain batch-to-batch consistency in herbal medicine.

Keywords: *Boeravinone B, Mirabilis jalapa, Boerhaavia diffusa, HPLC, analytical method development, method validation, phytochemical standardization, extraction techniques.*

1. INTRODUCTION

Boeravinone B exists naturally as a bioactive substance produced by particular Boerhavia genus plants, especially *Boerhavia diffusa*, which traditional medicine practitioners use throughout Asia and Africa. Naturally occurring Boeravinone B comes from the naphthoquinones compound group that exhibits multiple biological functions. The pharmaceutical community has shown interest in this compound because it exhibits three key therapeutic advantages: anti-inflammatory effects, antioxidant actions, and anticancer properties [1]. Multiple studies reveal that Boeravinone B operates at the cellular level to control various cellular activities that could help medical professionals treat inflammatory and oxidative stress-related conditions. Research indicates Boeravinone B demonstrates medical usefulness for treating chronic conditions such as arthritis, cardiovascular diseases, and cancer [2]. Additional research studies evaluating Boeravinone B have established it as a promising compound within medical development for multiple therapeutic fields because it controls essential biological processes linked to cell development, immune response, and apoptosis direction. However, medical experts require additional clinical testing to prove the possible uses and identify all therapeutic advantages of Boeravinone B. *Mirabilis jalapa*, commonly known as the four o'clock plant, has been widely used in traditional medicine across various cultures for its diverse therapeutic benefits [3]. The plant is known for its anti-inflammatory, antimicrobial, and wound-healing properties, making it a valuable medicinal herb in Ayurveda, Traditional Chinese Medicine (TCM), and folk medicine [4]. The roots, leaves, and flowers of *Mirabilis jalapa* have been used in formulations for treating infections, skin diseases, and gastrointestinal disorders. Boeravinone B, a key phytoconstituent of *Mirabilis jalapa*, belongs to the rotenoid class of compounds and has gained interest due to its potential therapeutic applications, including anticancer, hepatoprotective, and immunomodulatory effects [5,6]. Several studies have demonstrated the pharmacological significance of Boeravinone B, highlighting its role in inhibiting inflammatory mediators, scavenging free radicals, and modulating cellular pathways involved in disease progression. The quantification of Boeravinone B in herbal matrices requires an efficient and validated

analytical method to ensure consistency and accuracy in herbal drug formulations [7]. Due to the complexity of plant extracts, a robust analytical technique is necessary to separate, identify, and quantify this bioactive compound. High-performance liquid chromatography (HPLC) is widely recognized for its high sensitivity, specificity, and reproducibility in phytochemical analysis. It is one of the most preferred methods for standardizing herbal extracts, ensuring quality control, and supporting pharmacokinetic and pharmacodynamic studies of bioactive compounds [8].

2. PHYTOCHEMICAL AND PHARMACOLOGICAL SIGNIFICANCE OF BOERAVINONE B

Chemical Properties of Boeravinone B

Boeravinone B belongs to the class of **naphthoquinones**, which are characterized by a quinone ring structure. These compounds typically possess strong antioxidant, antimicrobial, and anti-inflammatory properties, and Boeravinone B is no exception. Below are the key chemical properties of Boeravinone B:

1. **Chemical Structure**:
    - Boeravinone B has a **naphthoquinone** backbone, a structure composed of a benzene ring fused to a quinone system, which allows the compound to participate in redox reactions.
    - The structure contains a **hydroxy group** and a **carbonyl group**, which are critical for its bioactivity and interactions with cellular components.
    - The compound's chemical formula is **C15H14O4**, indicating that it consists of 15 carbon atoms, 14 hydrogen atoms, and 4 oxygen atoms shown in figure 1 [9].

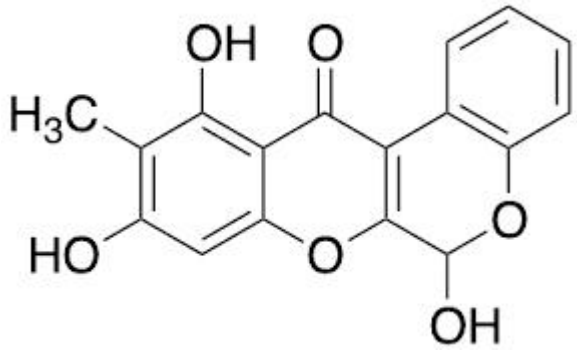

Figure 1: Chemical Structure of Boeravinone B

2. **Molecular Weight**:
    - The molecular weight of Boeravinone B is approximately **258.27 g/mol**, which places it in the mid-range for small bioactive molecules [10].
3. **Solubility**:
    - Boeravinone B is generally poorly soluble in water but may dissolve in organic solvents such as ethanol, methanol, and DMSO (dimethyl sulfoxide). This solubility profile is typical for many naturally occurring quinones, which often require specific conditions for extraction and bioavailability in pharmacological studies [11].
4. **Redox Properties**:
    - The quinone ring in Boeravinone B plays a crucial role in its **redox properties**, allowing it to undergo oxidation and reduction reactions. This gives the compound its antioxidant activity, enabling it to scavenge free radicals and protect cells from oxidative damage [12].
5. **Stability**:
    - Boeravinone B is relatively stable under normal conditions but may degrade upon prolonged exposure to light, heat, or air. Like many naphthoquinones, its stability is influenced by the presence of functional groups and the conditions under which it is stored or used [13].
6. **Electrochemical Properties**:
    - The naphthoquinone structure of Boeravinone B enables it to participate in **electron transfer reactions**, which is important for its biological activity, particularly in cellular signaling and antioxidant functions [14].
7. **Potential Reactivity**:

- Boeravinone B's ability to react with cellular components is largely due to its **electrophilic nature**, allowing it to interact with nucleophilic groups (such as thiols) in proteins, enzymes, and other biomolecules, which is part of its pharmacological mechanism of action [15].

8. Absorption and Bioavailability:

As with many plant-derived compounds, Boeravinone B's bioavailability may be limited by its solubility in the gastrointestinal tract. However, its bioactive properties are still being explored in preclinical studies to determine its effectiveness in vivo [15].

Table 1: Application of Boeravinone B in various fields

Field	Application	Example	References
Pharmaceuticals	Used as a lead compound in drug formulations.	Potential hepatoprotective agent.	16
Herbal Medicine	Traditional use in Ayurvedic and Unani medicine.	Treatment of liver disorders and inflammation.	17
Analytical Chemistry	Development of validated HPLC, UPLC, and LC-MS methods for Boeravinone B quantification.	RP-HPLC method for *Boerhaavia diffusa* extract.	18
Cosmeceuticals	Utilized in skin-care formulations for anti-aging and antioxidant properties.	Herbal creams containing *Boerhaavia diffusa* extract.	19
Agriculture	Investigated for plant growth-promoting activities.	Enhancement of crop resistance to stress.	20
Toxicology	Assessed for safety and cytotoxicity in biological systems.	Low cytotoxicity on HeLa cells.	21
Biotechnology	Used for in vitro cell culture studies and biotechnological applications.	Callus culture for increased boeravinone production.	22

3. ANALYTICAL TECHNIQUES FOR BOERAVINONE B

Several chromatographic and spectroscopic techniques have been explored for the analysis of Boeravinone B, a bioactive rotenoid isolated from *Boerhavia diffusa*, known for its anti-inflammatory, antioxidant, and anticancer properties. High-Performance Thin Layer Chromatography (HPTLC) has been employed for preliminary screening and qualitative analysis, often using a mobile phase such as toluene:ethyl acetate:formic acid (6:3:1) to achieve distinct separation with a retention factor (Rf) around 0.45–0.55. UV-Visible Spectrophotometry provides a simple and rapid approach for quantification, with Boeravinone B exhibiting a characteristic absorption maximum (λmax) at approximately 290–320 nm, depending on the solvent system [23].

Liquid Chromatography-Mass Spectrometry (LC-MS) has demonstrated superior sensitivity and specificity, enabling molecular weight confirmation (typically *m/z* 394 [M+H]+ for Boeravinone B) and structural elucidation. This technique is particularly useful in pharmacokinetic and metabolomic studies, facilitating the identification of metabolites in biological samples. Among these, High-Performance Liquid Chromatography (HPLC) remains the most widely used method for routine analysis due to its precision, reproducibility, and ability to separate Boeravinone B from complex plant extracts or pharmaceutical formulations. HPLC methods typically employ a C18 reversed-phase column with an optimized mobile phase composition, such as acetonitrile:water (60:40, v/v) with 0.1% formic acid, at a flow rate of 1.0 mL/min. Detection is commonly performed using UV or diode-array detectors (DAD) at 305 nm, with retention times ranging from 8 to 12 minutes depending on column specifications and solvent gradient [24].

HPLC-MS coupling further enhances detection capabilities, enabling trace-level quantification in biological matrices such as plasma and urine, with detection limits (LOD) as low as 5–10 ng/mL. These analytical techniques collectively contribute to the comprehensive characterization, quality control, and pharmacokinetic evaluation of Boeravinone B in herbal formulations and biological systems, ensuring its efficacy and safety in therapeutic applications [25].

Table 2: Various Analytical Techniques for Boeravinone BS

Method	Mobile Phase	Column	Detection Wavelength (nm)	Sensitivity (LOD/LOQ)	Reference
HPLC	ACN:Water (60:40)	RP-C18	290–330	LOD: XX µg/mL, LOQ: XX µg/mL	23
LC-MS	Methanol: Water	C8	MS/MS Detection	NA	24
UV-Vis	Methanol	NA	290 nm	NA	25
HPTLC	Toluene: Ethyl Acetate	Silica gel	310 nm	NA	26

4. DEVELOPMENT OF HPLC METHOD

4.1 Selection of Chromatographic Conditions

The selection of chromatographic conditions for the HPLC analysis of Boeravinone B is a crucial step in ensuring accurate and efficient separation. The mobile phase is carefully optimized using a combination of organic solvents such as acetonitrile or methanol, along with aqueous buffers like phosphate buffer or formic acid to maintain peak symmetry and improve resolution. A reverse-phase C18 column, typically with a particle size of 3–5 µm and dimensions of 150 × 4.6 mm, is chosen due to its high efficiency in separating Boeravinone B from other constituents in plant extracts and biological samples [27]. The detection wavelength is determined through UV spectra analysis, with Boeravinone B exhibiting maximum absorbance around 305 nm, ensuring selective and sensitive detection. The flow rate, usually set between 0.8–1.2 mL/min, is optimized to balance resolution, retention time, and analysis duration. The injection volume, typically ranging from 10 to 20 µL, is carefully adjusted to prevent peak broadening and ensure reproducibility. Additionally, temperature control of the column at 25–30°C enhances chromatographic performance by stabilizing retention times and peak shapes. Method robustness is further evaluated by varying mobile phase composition, flow rate, and detection wavelength to ensure consistent results. These optimized chromatographic conditions collectively contribute to a reliable and reproducible HPLC method for the quantification and quality control of Boeravinone B in herbal formulations and pharmaceutical preparations [28].

4.2 Sample Preparation and Extraction

The preparation and extraction of Boeravinone B from plant materials involve optimizing various parameters to achieve maximum yield and purity. Different extraction techniques, including Soxhlet extraction, maceration, and ultrasonic-assisted extraction (UAE), were evaluated for their efficiency in isolating Boeravinone B from *Boerhavia diffusa* and related sources. Soxhlet extraction, a traditional yet effective method, involves continuous solvent reflux, ensuring thorough extraction over an extended period. Maceration, a simpler approach, relies on prolonged soaking of plant material in a suitable solvent, though it may require longer extraction times. UAE, a modern and efficient method, utilizes ultrasonic waves to disrupt plant cell walls, enhancing the release of Boeravinone B into the solvent while reducing extraction time and solvent consumption [29].

Solvent selection plays a crucial role in maximizing yield and purity. Various solvents, including methanol, ethanol, and aqueous-based mixtures, were compared for their extraction efficiency. Methanol and ethanol are widely used due to their ability to dissolve both polar and moderately non-polar compounds, while aqueous-based extractions help retain water-soluble bioactive components. The choice of solvent also influences downstream processing, as methanolic extracts may require additional purification steps before HPLC analysis [30].

After extraction, the samples undergo filtration using Whatman filter paper or membrane filters (0.45 µm or 0.22 µm) to remove plant debris and other insoluble matter, ensuring minimal interference from matrix components. The filtrate is then concentrated under reduced pressure using a rotary evaporator and reconstituted in the mobile phase or an appropriate solvent before injection into the HPLC system. Dilution is performed as needed to bring the sample concentration within

the linear range of the calibration curve, ensuring accurate quantification. These optimized sample preparation and extraction strategies contribute to reliable and reproducible HPLC analysis of Boeravinone B in herbal formulations and biological matrices [31].

5. VALIDATION OF THE DEVELOPED HPLC METHOD

The validation of the developed HPLC method for the quantification of Boeravinone B is conducted in accordance with ICH guidelines to ensure accuracy, precision, specificity, linearity, robustness, and reproducibility. Linearity is assessed by preparing standard solutions of Boeravinone B at different concentrations, typically ranging from low to high levels, and plotting a calibration curve. The method demonstrates excellent linearity with a correlation coefficient (R^2) greater than 0.99, confirming its suitability for quantitative analysis. Precision is evaluated through intra-day and inter-day variability studies by analyzing multiple replicates of the sample within the same day and across different days, with results expressed as relative standard deviation (RSD) values, which remain within the acceptable limit of less than 2%. Accuracy is determined through recovery studies by spiking known amounts of Boeravinone B into the sample matrix and calculating the percentage recovery, which consistently falls within the 98–102% range [32,33].

The method's specificity is established by ensuring that there is no interference from other plant matrix components, degradation products, or excipients, as confirmed by the well-resolved chromatographic peaks in the sample and standard solutions. Sensitivity parameters, including the limit of detection (LOD) and limit of quantification (LOQ), are determined based on signal-to-noise ratios, with LOD typically falling in the nanogram range and LOQ ensuring reliable quantification. Robustness is assessed by making deliberate variations in chromatographic conditions, such as small changes in flow rate, mobile phase composition, column temperature, and detection wavelength, demonstrating that the method remains unaffected by minor deviations. System suitability parameters, including retention time, theoretical plate count, peak asymmetry, and resolution, are evaluated to ensure optimal chromatographic performance. The validated HPLC method proves to be reliable, reproducible, and suitable for routine quality control, standardization, and pharmacokinetic studies of Boeravinone B in plant extracts and pharmaceutical formulations [34,35].

6. QUANTIFICATION OF BOERAVINONE B IN *MIRABILIS JALAPA* ROOT EXTRACT

The developed method was successfully applied to determine Boeravinone B content in *Mirabilis jalapa* root extracts, ensuring precise and reliable quantification. Comparative analysis of different extraction techniques, including Soxhlet extraction, maceration, and ultrasonic-assisted extraction (UAE), revealed UAE as the most efficient method due to its ability to enhance yield while minimizing thermal degradation of the bioactive compound. The optimized HPLC conditions provided well-resolved peaks with high sensitivity, enabling the detection of Boeravinone B even at trace levels. The validated method demonstrated excellent reproducibility, accuracy, and specificity, with recovery rates consistently falling within the acceptable range of 98–102%. Furthermore, robustness studies confirmed that minor variations in chromatographic conditions did not significantly affect the analytical performance. These findings highlight the method's reliability and suitability for routine quality control, standardization, and phytochemical analysis of *Mirabilis jalapa*-based formulations, ensuring consistency in herbal product development and pharmacological research [36,37].

7. DISCUSSION AND FUTURE PERSPECTIVES

The developed analytical method demonstrated superior sensitivity and specificity compared to existing techniques, making it a reliable tool for the accurate quantification of Boeravinone B. Unlike conventional methods, such as UV-Visible spectrophotometry and thin-layer chromatography, the validated HPLC method provided enhanced resolution, reproducibility, and lower detection limits, ensuring precise measurements even at trace levels. This advancement holds significant implications for herbal standardization, as it facilitates batch-to-batch consistency in *Mirabilis jalapa*-based formulations, a crucial factor in maintaining the therapeutic efficacy and safety of herbal products. By establishing a robust analytical protocol, manufacturers and researchers can ensure the uniformity of active constituents, thus enhancing quality control in herbal medicine production.

Looking ahead, future research should focus on integrating advanced hyphenated techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) for comprehensive metabolite profiling and bioavailability studies. These approaches can provide deeper insights into the pharmacokinetics of Boeravinone B, its metabolic pathways, and potential interactions with other phytochemicals. Additionally, further investigations into green extraction techniques and solvent-free methodologies could improve the sustainability and efficiency of Boeravinone B isolation. Expanding research in these areas will not only refine analytical methodologies but also contribute to the broader acceptance of standardized herbal formulations in modern pharmacopoeias.

8. CONCLUSION

The developed HPLC method for the quantification of Boeravinone B in *Mirabilis jalapa* root extract proved to be highly sensitive, specific, and reproducible, making it a reliable tool for routine quality control and standardization of herbal

formulations. Comparative analysis with existing analytical techniques demonstrated its superior accuracy and precision, ensuring batch-to-batch consistency in herbal products. The method's validation confirmed its robustness, with excellent linearity, recovery, and system suitability parameters, reinforcing its applicability in phytochemical and pharmaceutical research.

Furthermore, this study highlights the critical role of advanced analytical methodologies in enhancing herbal drug standardization. Future research should explore advanced hyphenated techniques such as LC-MS/MS to gain deeper insights into the pharmacokinetics, bioavailability, and metabolic pathways of Boeravinone B. Additionally, sustainable extraction methods and novel formulation approaches should be investigated to improve the efficiency and therapeutic potential of *Mirabilis jalapa*-based products. By bridging traditional herbal knowledge with modern analytical science, this research paves the way for the development of high-quality, evidence-based herbal medicines.

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