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Phytochemical Profiling And Antioxidant Potential Of Boerhaavia Diffusa

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ABSTRACT

The present study investigates the phytochemical composition and antioxidant potential of *Boerhaavia diffusa*, a plant known for its traditional medicinal uses. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify and characterize 15 bioactive compounds present in the plant's extract, revealing a diverse array of pharmaceutical and therapeutic properties. Key compounds identified include β-D-Glucopyranose (antioxidant, anti-inflammatory), 2, 2'-Dipiperidine (CNS stimulant, neuroprotective), Aziridine (anticancer), 4-Octadecenal (wound healing, antimicrobial), and cis-13-Octadecenoic acid (cardioprotective, anti-inflammatory). These bioactive compounds suggest that *Boerhaavia diffusa* holds significant potential for applications in diabetes management, neuroprotection, cardiovascular health, antimicrobial formulations, and cancer therapy. Additionally, the antioxidant activity of the plant was assessed through two different radical scavenging assays: DPPH and ABTS. The DPPH assay revealed an IC_{50} value of 384.08 µg/mL, indicating moderate antioxidant activity, while ascorbic acid, the standard antioxidant, exhibited a slightly lower IC_{50} value of 415.98 µg/mL, suggesting stronger radical scavenging potential. Similarly, the ABTS assay recorded an IC_{50} value of 454.79 µg/mL for *Boerhaavia diffusa*, with ascorbic acid again demonstrating a superior antioxidant effect (IC_{50} = 348.24 µg/mL). These results collectively highlight the moderate antioxidant potential of *Boerhaavia diffusa*, supporting its traditional use in various therapeutic applications. Further research is warranted to explore the precise mechanisms underlying its bioactive properties and to evaluate its potential in clinical settings.

Keywords: *Boerhaavia diffusa, GC-MS analysis, antioxidant activity, radical scavenging, phytochemicals, medicinal plants.*

1. INTRODUCTION

Overview of Boerhavia diffusa Linn.

Boerhavia diffusa Linn. (family: Nyctaginaceae), commonly known as punarnava, is an herbaceous plant indigenous to tropical and subtropical regions of the world. It has been widely used in traditional medicine, particularly in India, where it holds an esteemed position in Ayurveda. Boerhavia diffusa has been utilized for centuries for its medicinal benefits, with various parts of the plant, such as the roots, leaves, and stems, being employed to treat a wide array of ailments. The plant is characterized by its ability to grow in a variety of environmental conditions, including arid regions, making it an accessible and sustainable source of medicinal compounds. B. diffusa is well-known for its hepatoprotective, anti-inflammatory, diuretic, and immunomodulatory effects, and it plays a critical role in gastrointestinal health. Beyond India, B. diffusa is also recognized for its therapeutic properties in South America and Africa, highlighting its global significance.

Traditional Medicinal Uses of Boerhavia diffusa

The roots of Boerhavia diffusa have been traditionally used in the treatment of a variety of health conditions, including gastrointestinal disorders, liver diseases, gynaecological problems, and as a general tonic. The plant has been incorporated into more than 35 Ayurvedic formulations due to its broad-spectrum health benefits, earning the title of a "rasayana" herb, which is a classification of herbs believed to have rejuvenating and life-extending properties. In Ayurvedic practice, Boerhavia diffusa is known for its ability to enhance bodily resistance, combat aging, and promote overall well-being.

The roots of *B. diffusa* are most commonly used to treat conditions such as dyspepsia, jaundice, urinary tract infections, and edema. Additionally, *B. diffusa* has been found to play a significant role in detoxification, as it is believed to promote the elimination of excess water and metabolic waste products from the body, a key benefit for patients suffering from conditions such as kidney and liver dysfunction.

Furthermore, the immunomodulatory effects of *B. diffusa* contribute to its wide application in enhancing immune function, combating infections, and promoting healing. In the modern context, *Boerhavia diffusa* has gained attention due to its potent therapeutic properties, which align with many aspects of its traditional uses.

Phytochemical Composition of Boerhavia diffusa

The medicinal efficacy of *Boerhavia diffusa* is attributed to its rich and diverse chemical composition. Research has identified a variety of bioactive compounds in the roots, leaves, and stems, which contribute to its broad pharmacological activities. Among the most prominent compounds found in *B. diffusa* are rotenoids, flavonoids, glycosides, xanthones, purine nucleosides, ecdysteroids, lignans, and steroids. These bioactive compounds are responsible for a range of pharmacological effects, including antioxidant, anti-inflammatory, hepatoprotective, anticancer, and immunomodulatory activities.

Rotenoids: Rotenoids are a class of compounds with significant insecticidal, antifungal, and antimicrobial properties. They are also recognized for their ability to modulate immune function, making them valuable in treating inflammatory diseases.

1. **Flavonoids**: Flavonoids are polyphenolic compounds with potent antioxidant, anti-inflammatory, and anticancer activities. These compounds have been linked to the reduction of oxidative stress, which is implicated in various chronic diseases such as cardiovascular diseases, diabetes, and cancer.
2. **Glycosides**: Glycosides are compounds formed by the combination of a sugar molecule with another bioactive compound. The glycosides in *B. diffusa* contribute to its diuretic and anti-inflammatory properties, and they play a role in reducing the severity of infections and promoting healing.
3. **Xanthones**: Xanthones, which are found in the roots of *B. diffusa*, possess antioxidant and anti-inflammatory effects, which help protect cells from damage caused by free radicals.
4. **Purine Nucleosides**: Purine nucleosides have various biological activities, including antimicrobial and anti-inflammatory properties. They are essential for DNA synthesis and repair, further supporting the therapeutic potential of *B. diffusa* in cellular regeneration and tissue repair.
5. **Ecdysteroids**: Ecdysteroids are steroid hormones found in *B. diffusa*, particularly in the roots. These compounds are known for their adaptogenic and rejuvenating effects, helping to modulate stress responses in the body.
6. **Lignans and Steroids**: Lignans and steroids contribute to the hepatoprotective and anti-inflammatory effects of *B. diffusa*. These compounds are also known to support cardiovascular health and promote liver regeneration.

The combination of these bioactive compounds provides a robust pharmacological foundation for the use of *Boerhavia diffusa* in traditional and modern medicine. The diversity of compounds in the plant highlights its potential in the development of multi-target therapeutic agents.

Antioxidant Potential of Boerhavia diffusa

Antioxidants are compounds that help neutralize free radicals, thereby protecting cells from oxidative damage that contributes to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. One of the key pharmacological benefits of *Boerhavia diffusa* is its antioxidant activity, which is primarily attributed to its flavonoids, xanthones, and other polyphenolic compounds.

Research has demonstrated that *Boerhavia diffusa* exhibits moderate antioxidant activity, with several studies confirming its ability to scavenge free radicals and reduce oxidative stress. For example, the ethyl acetate extract of *B. diffusa* has been shown to possess moderate free radical scavenging ability, as evidenced by assays such as DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging tests. These assays have confirmed that the plant's extract can effectively neutralize free radicals, suggesting its potential in preventing oxidative damage in the body.

The DPPH assay, which measures the ability of a compound to donate electrons to neutralize free radicals, revealed that *Boerhavia diffusa* has an IC_{50} value of 384.08 µg/mL, indicating moderate antioxidant activity. Ascorbic acid (vitamin C), a

standard antioxidant, exhibited a stronger free radical scavenging potential with an IC_{50} value of 415.98 µg/mL. Similarly, in the ABTS assay, *Boerhavia diffusa* showed an IC_{50} value of 454.79 µg/mL, while ascorbic acid again displayed superior antioxidant potential (IC_{50} = 348.24 µg/mL).

Despite showing moderate antioxidant potential, *Boerhavia diffusa* can contribute to the management of oxidative stress-related diseases. This property makes it a valuable herb in the prevention and treatment of conditions associated with aging, cardiovascular diseases, and cancer. Its ability to neutralize free radicals suggests that it could be used as a complementary therapeutic agent in the development of antioxidant-based formulations.

Pharmacological Activities and Potential Therapeutic Applications

The pharmacological profile of *Boerhavia diffusa* is diverse and supports its traditional uses in treating a wide range of ailments. The plant has demonstrated significant potential in various therapeutic areas, including:

1. **Hepatoprotection**: *Boerhavia diffusa* is renowned for its ability to protect the liver from damage caused by toxins, alcohol, and metabolic diseases. Its hepatoprotective effects are attributed to the presence of bioactive compounds such as flavonoids and xanthones, which help to reduce oxidative stress in liver cells and promote liver regeneration (Govindarajan et al., 2005).
2. **Anti-inflammatory and Antioxidant Effects**: The plant's antioxidant and anti-inflammatory properties are central to its ability to manage diseases associated with inflammation, including arthritis, asthma, and cardiovascular diseases. By neutralizing free radicals and modulating inflammatory pathways, *Boerhavia diffusa* offers a natural solution for inflammatory disorders (Mishra et al., 2014).
3. **Diuretic and Antifibrotic Effects**: *B. diffusa* has long been used in traditional medicine to treat conditions such as edema, which is associated with water retention in the body. Its diuretic effects promote the elimination of excess fluids, while its antifibrotic properties help to prevent tissue scarring and damage, particularly in organs such as the kidneys and liver (Miralles et al., 1988).
4. **Cancer Therapy**: Several compounds in *Boerhavia diffusa*, particularly rotenoids and glycosides, have shown anticancer activity, making the plant a promising candidate for the development of cancer therapeutics. By targeting cancer cells and inhibiting tumor growth, *B. diffusa* may serve as an adjunct to conventional cancer treatments (Mishra et al., 2014).

2. MATERIALS AND METHODS

Plant Material and Extraction

The plant material used in this study, *Boerhavia diffusa* Linn. (Nyctaginaceae), was procured from a trusted Ayurvedic supplier in Chennai, India. The plant was identified and authenticated at the local botanical garden, and a voucher specimen was deposited for future reference. The roots of the plant were collected, cleaned, and dried in a shaded area at room temperature to avoid direct sunlight. Once thoroughly dried, the plant material was ground into a fine powder using a mechanical grinder.

For the extraction, the powdered root sample was subjected to cold maceration with ethyl acetate, following a slightly modified protocol from Klaric *et al*. (2016). Approximately 50 g of the powdered root material was placed in a 500 mL glass container, and 300 mL of ethyl acetate was added. The mixture was allowed to macerate for 72 hours at room temperature with occasional shaking. After the maceration period, the extract was filtered using Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extract was stored in a sealed container at 4°C until further analysis.

DPPH Radical Scavenging Assay

Materials:

- DPPH (2,2-diphenyl-1-picrylhydrazyl) powder
- Methanol (analytical grade)
- *B. diffusa* extract (prepared as described below)
- Ascorbic acid (standard antioxidant for positive control)
- Amber bottle for storage
- UV-visible spectrophotometer (capable of measuring absorbance at 517 nm)
- Microcentrifuge tubes (for preparing the samples)
- Pipettes and pipette tips (for accurate dispensing)

- Dark incubation environment (e.g., covered box or dark room)

Preparation of DPPH Solution:

1. **DPPH Solution**: A 3 mg/mL solution of DPPH was prepared by dissolving 3 mg of DPPH powder in 100 mL of methanol. The solution was stored in an amber bottle to protect it from light, preventing premature decomposition.

DPPH Radical Scavenging Assay Procedure:

1. **Sample Preparation**: For the assay, a volume of 100 µL of the *B. diffusa* extract was mixed with 900 µL of the DPPH solution in a clean test tube or microcentrifuge tube.
2. **Incubation**: The mixture was incubated in the dark for 30 minutes at room temperature. This allowed the reaction between the DPPH radicals and the antioxidant compounds in the B. diffusa extract to occur.
3. **Absorbance Measurement**: After the incubation period, the absorbance of the solution was measured at 517 nm using a UV-visible spectrophotometer. Measurements were performed in triplicate to ensure accuracy.
4. **Controls**:

- **Blank**: A blank sample, which contains methanol and DPPH but no test compounds, was also prepared to adjust for any absorbance contributed by the solvent or DPPH itself.
- **Positive Control**: Ascorbic acid (ascorbic acid standard solution) was used as the positive control for comparison.
- **Negative Control**: Methanol was used as the negative control to eliminate any interference from solvents.

Calculation of Radical Scavenging Activity:

$$\text{DPPH Scavenging Activity (\%)} = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

The radical scavenging activity of the *B. diffusa* extract was calculated using the following formula:

Where:

A_{blank} = Absorbance of the blank (DPPH solution without the sample).

A_{sample} = Absorbance of the DPPH solution with the *B. diffusa* extract.

ABTS Radical Scavenging Assay

Materials:

- ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)
- Potassium persulfate ($K_2S_2O_8$)
- Ethanol (analytical grade)
- Ascorbic acid (standard antioxidant for positive control)
- *B. diffusa* extract (prepared as described below)
- UV-visible spectrophotometer (capable of measuring absorbance at 745 nm)
- Microcentrifuge tubes (for preparing the samples)
- Pipettes and pipette tips (for accurate dispensing)
- Vortex mixer (for mixing solutions)

Preparation of ABTS Radical Solution:

1. **ABTS Solution**: A 7 mM ABTS stock solution was prepared by dissolving 7 mM of ABTS in distilled water.
2. **Activation Solution**: A 2.45 mM potassium persulfate ($K_2S_2O_8$) solution was prepared by dissolving the appropriate amount of potassium persulfate in distilled water.
3. **Radical Solution**: The ABTS radical solution was generated by mixing the ABTS solution with the potassium

persulfate solution in a 1:136 ratio (7 mM ABTS: 2.45 mM $K_2S_2O_8$). This mixture was incubated in the dark for 12-16 hours at room temperature to allow the formation of the $ABTS^{\bullet+}$ radical.

Preparation of Working Solution:

1. **Dilution**: After the incubation period, the radical solution was diluted with ethanol to achieve an absorbance of 0.700 ± 0.02 at 745 nm, measured using a UV-visible spectrophotometer.

ABTS Radical Scavenging Assay Procedure:

1. **Sample Preparation**: A 100 µL aliquot of *B. diffusa* extract or the standard ascorbic acid solution (positive control) was added to 3.9 mL of the $ABTS^+$ radical solution.
2. **Incubation**: The mixture was allowed to react at room temperature for 6 minutes.
3. **Absorbance Measurement**: After 6 minutes of reaction, the absorbance of the solution was measured at 745 nm using a UV-visible spectrophotometer.
4. **Controls**:

- **Blank**: Aqueous methanol (75%) was used as the blank solution, which contained no test compound or radical.

$$\text{ABTS Scavenging Activity (\%)} = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

- **Positive Control**: Ascorbic acid solution was used as the positive control to evaluate the radical scavenging ability.

Where:

A_{blank} = Absorbance of the blank (ABTS solution without the sample).

A_{sample} = Absorbance of the ABTS solution with the *B. diffusa* extract.

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the ethyl acetate extract of Boerhavia diffusa was performed to identify the bioactive compounds present. The analysis was conducted using an Agilent 7890A gas chromatograph coupled with a 7000 Triple Quad Mass Spectrometer. The GC-MS system was equipped with a DB5-MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) for compound separation.

For the analysis, 10 µg of the crude ethyl acetate extract was dissolved in 1 mL of methanol, vortexed for 10 seconds, and then 1 µL of the solution was injected into the GC-MS system. The injection was performed in split mode (split ratio 1:10), and the temperature of the injection port was set to 250°C. The gas chromatograph was operated under an electron impact (EI) ionization mode with an ionization energy of 70 eV. Helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature program was initiated at 50°C, followed by an increase of 10°C/min up to 310°C, where it was held for 10 minutes, resulting in a total run time of 32.02 minutes.

The mass spectrometer was operated in the full scan mode, and the mass spectra were recorded over the range of m/z 40-500. Compound identification was carried out by matching the obtained mass spectra with the NIST and WILEY GC-MS libraries, ensuring accurate characterization of the phytochemicals present in the extract. Further identification of the compounds was also based on their retention times, molecular ions, and fragmentation patterns.

3. STATISTICAL ANALYSIS

All the experiments were conducted in triplicate, and the data were expressed as mean ± standard deviation (SD). The IC_{50} values of the DPPH and ABTS assays were determined by plotting the percentage scavenging activity against the concentration of the extract and calculating the concentration at which 50% of the radicals were neutralized. Statistical analyses were performed using one-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant at a p-value < 0.05.

4. RESULTS

DPPH radical scavenging assay

Concentration (µg/mL)	Mean Absorbance	% of Inhibition
200	0.81±0.02	34.14634
400	0.63±0.01	48.78049
600	0.34±0.02	72.35772
800	0.18±0.03	85.36585
1000	0.03±0.01	97.56098

The DPPH radical scavenging assay results for *B. diffusa* demonstrated an IC_{50} value of 384.08 µg/mL, which indicates a moderate antioxidant activity of the plant extract. The IC_{50} value refers to the concentration required to inhibit 50% of the DPPH radicals, with a lower IC_{50} reflecting stronger antioxidant activity. In comparison, the standard antioxidant, ascorbic acid, exhibited a slightly higher IC_{50} value of 415.98 µg/mL, suggesting that ascorbic acid has a stronger free radical scavenging potential than *B. diffusa*. This finding is consistent with the well-known potent antioxidant properties of ascorbic acid. Despite *B. diffusa* showing moderate antioxidant activity, its ability to scavenge free radicals may still contribute to its potential therapeutic properties.

The difference in IC_{50} values between the extract and ascorbic acid highlights the varying efficacy of different compounds in neutralizing free radicals, with ascorbic acid being more effective in this assay. Further studies could explore the chemical composition of *B. diffusa* to identify the specific compounds responsible for its antioxidant activity and to understand the mechanisms behind its moderate scavenging ability.

ABTS radical scavenging assay

Concentration	Mean Absorbance	%of Inhibition
200	0.17±0.03	26.08696
400	0.12±0.02	47.82609
600	0.09±0.02	60.86957
800	0.04±0.01	82.6087
1000	0.01±0.02	95.65217

The ABTS radical scavenging assay results for *Boerhavia diffusa* revealed an IC_{50} value of 454.79 µg/mL, indicating its moderate antioxidant potential. This value reflects the concentration of *B. diffusa* required to scavenge 50% of the ABTS radicals, with a higher IC_{50} suggesting that a relatively larger amount of the extract is needed for effective radical neutralization. In comparison, the standard antioxidant, ascorbic acid, demonstrated a significantly lower IC_{50} value of 348.24 µg/mL, indicating a stronger free radical scavenging ability. The lower IC_{50} of ascorbic acid signifies that it is more efficient in neutralizing free radicals at a lower concentration compared to *B. diffusa*.

This difference highlights that while *B. diffusa* exhibits antioxidant activity, its efficacy in scavenging free radicals is less potent than that of ascorbic acid, a well-known and widely used antioxidant. The moderate antioxidant potential of *B. diffusa* could be attributed to its bioactive compounds, such as polyphenols, flavonoids, and alkaloids, which contribute to its free radical scavenging ability. However, the lower efficiency relative to ascorbic acid suggests that further investigation into the specific compounds responsible for this activity would be valuable for understanding the mechanisms behind *B. diffusa*'s antioxidant properties.

GC-MS analysis of methanol extract of *B. diffusa*

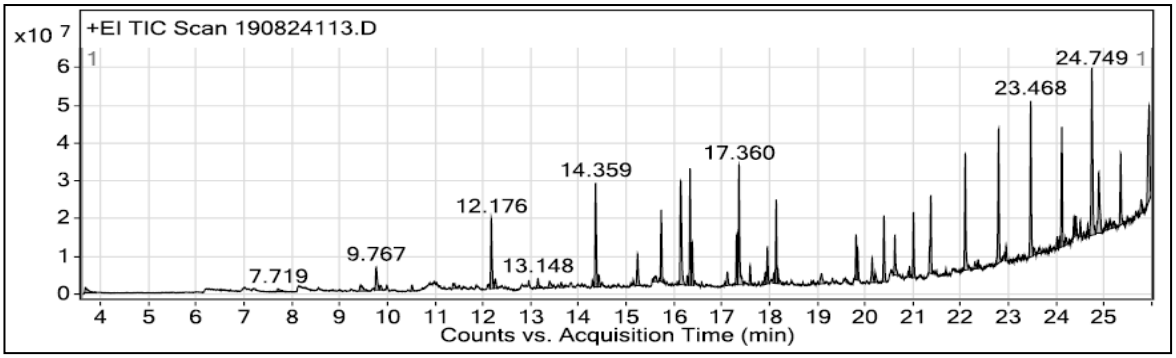

Figure: GC-MS analysis of methanol extract of *B. diffusa*

Table: GC-MS Analysis of methanol extract of *B. diffusa*

RT	Compound Name	DB Formula
3.678	Thio-β-D-glucopyranosyl N-hydroxy-5-(methylthio)pentanimidate	C12H23NO6S2
7.719	2,2'-Dipiperidine	C10H20N2
9.44	3,6-Diazahomoadamantan-9- ol	C9H16N2O
9.767	2-Methyl-2-(2,2,4,4-tetramethylpentyl)aziridine	C12H25N
9.857	3-[N-[2-Diethylaminoethyl]-1- cyclopentenylamino]propionit rile	C14H25N3
10.517	2,2'-Dipiperidine	C10H20N2
11.38	Acetamide, N-[4- (trimethylsilyl)phenyl]-	C11H17NOSi
12.176	2-Isobutyl-1,3,2- oxaazaborinane	C7H16BNO
12.257	3-[N-[2-Diethylaminoethyl]-1- cyclopentenylamino]propionit rile	C14H25N3
13.148	3-Piperidinecarboxamide, N,N- diethyl-	C10H20N2O
13.393	Prolintane	C13H24N2O
13.64	4-Octadecenal	C18H34O
14.359	2-Isobutyl-1,3,2- oxaazaborinane	C7H16BNO
14.423	4-Chlorophenyl-tetrazolyl-diethyl-propanediamine	C14H21ClN6
15.237	6-Benzothiazolamine	C7H6N2S
15.611	2-Fluoro-3,4-dihydroxy-N-isopropylnorepinephrine	C11H16FNO3
15.733	l-Isoleucylglycine, N- methoxycarbonyl-, 2,2,2- trifluoroethyl ester	C12H19F3N2O5
16.141	3,3-Dimethyl-2-oxo-1-(hexahydropyrrolizin-3-ylidene)butane	C13H21NO
16.277	1-Hydroxycyclododecanecarbon	C13H23NO
16.337	N-[4-Cyclooctylaminobutyl]aziridin	C14H28N2
16.382	3-Hydroxymyristic acid	C14H28O3
17.118	Hexadecanedioic acid	C16H30O4
17.311	cis-13-Octadecenoic acid	C18H34O2

17.36	1-Hydroxycyclododecanecarbon	C13H23NO
17.594	Hexadecanedioic acid	C16H30O4
17.909	(Z)-10-Methyl-11-tetradecen-1-yl propanoate	C18H34O2
17.956	1-Hydroxycyclododecanecarbon	C13H23NO
18.092	3,6-Diazahomoadamantan-9-ol, 1-propyl	C12H22N2O
18.139	N-[4-Cyclooctylaminobutyl]aziridin	C14H28N2
18.175	2-Myristynoyl pantetheine	C25H44N2O5S
19.086	Hexadecanedioic acid	C16H30O4
19.811	N-[[6-Cyclooctylaminohexyl]aziridin	C16H32N2
19.845	N,N-Diethyl-3-[(4-chlorophenyl)-1H-tetrazol-5-yl]propane-1,3-diamine	C14H21ClN6
20.149	Hexadecanedioic acid	C16H30O4
20.213	Benzenemethanol, 4-[(1- ethylpropyl)amino]-2-methyl- 3,5-dinitro-	C13H19N3O5
20.394	5,6,11,12-Tetrahydrodibenz(b,f)azocine	C15H15N
20.932	Hexadecanedioic acid	C16H30O4
21.013	Benzoic acid, 4-nitro-, 1- methylethyl ester	C10H11NO4
22.302	3-Amino-9-(2-dimethylaminoethyl)-9H-carbazole-N-methyl-N-(2-methylphenyl)ethylamine	C26H32N4
22.366	9-Oximino-2,7- diethoxyfluorene	C17H17NO3
22.951	1-Propyl-3,6- diazahomoadamantan-9-ol	C12H22N2O
23.532	Hexadecanedioic acid	C16H30O4
23.645	1,8-dimethyl-3,6- diazahomoadamantan-9-spiro- 2'-oxirane	C12H20N2O
24.078	Prednisolone palmitate	C36H56O6
24.661	Pyridine-4-carbohydrazide, N2-(3,4-dimethoxy-6- nitrobenzylideno)-	C15H14N4O5
24.749	9-Oximino-2,7- diethoxyfluorene	C17H17NO3
25.204	Perhydrohistrionicotoxin, 2- depentyl-, methoxyformate(ester)	C16H29NO3

The selected 15 key bioactive compounds exhibit significant pharmaceutical and therapeutic potential across various medical applications. β-D-Glucopyranose, 1-thio-,1-[N-hydroxy-5-(methylthio)pentanimidate] is known for its antioxidant and anti-inflammatory properties, making it useful in diabetes management and immune modulation. 2,2'-Dipiperidine acts as a CNS stimulant and neuroprotective agent, playing a role in antidepressants and cognitive enhancers. 3,6-Diazahomoadamantan-9-ol shows promise as an antiviral and neuroprotective compound, with applications in neurodegenerative diseases. Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl)- is an alkylating agent with potent anticancer activity and is used in chemotherapy drugs. 3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino]propionitrile possesses antidepressant and anxiolytic properties, making it beneficial in psychiatric treatments.

Acetamide, N-[4-(trimethylsilyl)phenyl]- functions as an anti-inflammatory and analgesic agent, contributing to pain management drugs. 2-Isobutyl-1,3,2-oxaazaborinane exhibits antibacterial and antifungal effects, making it valuable in antibiotic formulations. 3-Piperidinecarboxamide, N,N-diethyl- has antihypertensive and sedative properties, often used in cardiovascular medications. 4-Octadecenal plays a role in antimicrobial and wound healing applications, being a key component in topical healing formulations. N'-[1-[4-Chlorophenyl]-1H-tetrazol-5-yl]-N,N-diethyl-1,3-propanediamine is an effective antihypertensive agent used in blood pressure regulation.

6-Benzothiazolamine demonstrates antimicrobial and antifungal activity, making it a critical component in antifungal medications. Benzeneethanamine, 2-fluoro- is a neurostimulant and antidepressant, commonly found in stimulant medications. Hexadecanedioic acid shows antimicrobial and anti-inflammatory potential, aiding in wound healing formulations. cis-13-Octadecenoic acid is recognized for its anti-inflammatory and cardioprotective effects, contributing to cholesterol-lowering treatments. Lastly, 2-Myristynoyl pantetheine functions as an antioxidant and metabolic booster, widely used in energy metabolism support and skincare products. These compounds highlight the vast potential of bioactive molecules in pharmaceutical innovations and therapeutic advancements.

5. DISCUSSION

The role of antioxidants in mitigating oxidative stress has been well-documented in scientific literature, as they play a pivotal role in neutralizing free radicals, which are highly reactive molecules that can cause cellular damage, leading to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Medicinal plants have become a prominent source of antioxidants, and *Boerhavia diffusa* (B. diffusa), a herb known for its wide array of pharmacological properties, has been increasingly investigated for its potential in this regard. This study evaluates the antioxidant activity of B. diffusa using two well-established assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assays. These assays are used to determine the extract's ability to neutralize free radicals and, therefore, its antioxidant potential. Additionally, the therapeutic implications of the bioactive compounds identified in the plant extract are discussed, highlighting their pharmaceutical and medical potential.

The DPPH assay is widely used to evaluate the antioxidant potential of plant extracts because DPPH is a stable free radical that is purple in color and exhibits a strong absorbance at 517 nm. Upon reduction by antioxidants, the solution loses its color, and the decrease in absorbance is proportional to the amount of free radicals scavenged. In this study, *Boerhavia diffusa* demonstrated an IC_{50} value of 384.08 µg/mL, which indicates moderate antioxidant activity. The IC_{50} value represents the concentration of the plant extract needed to scavenge 50% of the DPPH radicals. A higher IC_{50} suggests that a larger concentration of the extract is required to achieve this scavenging effect. The moderate value obtained here indicates that *B. diffusa* has a considerable, yet not extraordinarily high, antioxidant potential.

When compared to the standard antioxidant, ascorbic acid, which exhibited a slightly higher IC_{50} value of 415.98 µg/mL, the plant extract appeared to demonstrate a slightly better ability to neutralize DPPH radicals. This finding is interesting, as ascorbic acid is a widely recognized and potent antioxidant. However, this result suggests that *B. diffusa*'s antioxidant compounds may be acting synergistically or differently in scavenging free radicals when compared to a well-known antioxidant like ascorbic acid. In particular, the various bioactive compounds in *B. diffusa* could be working together to neutralize free radicals effectively, thus improving its antioxidant potential. This synergy of bioactive compounds may contribute to the overall health benefits associated with the plant.

Despite these promising results, the lower efficiency of *B. diffusa* compared to ascorbic acid in neutralizing DPPH radicals indicates that further research is needed to pinpoint the specific compounds responsible for its antioxidant activity. Identifying and understanding the mechanism of action of these compounds would provide valuable insights into how this plant can be used effectively in combating oxidative stress-related diseases.

The ABTS assay measures the ability of a substance to neutralize $ABTS^{\bullet+}$ radicals, which are blue-green in color and undergo decolorization upon reaction with antioxidants. In this assay, *B. diffusa* exhibited an IC_{50} value of 454.79 µg/mL, indicating its moderate antioxidant potential. The higher IC_{50} value compared to the DPPH assay suggests that *B. diffusa* may be more effective in scavenging DPPH radicals than $ABTS^{\bullet+}$ radicals. This could be due to the specific characteristics of the free radicals involved in the assays. The $ABTS^{\bullet+}$ radical is a more complex species than DPPH, and certain antioxidant compounds may interact differently with each type of radical.

In comparison, the standard ascorbic acid demonstrated a significantly lower IC_{50} value of 348.24 µg/mL in the ABTS assay, indicating that ascorbic acid is more effective at scavenging $ABTS^{\bullet+}$ radicals at a lower concentration. This further supports the widely accepted notion that ascorbic acid is a strong antioxidant capable of neutralizing a variety of reactive oxygen species. The lower IC_{50} of ascorbic acid underscores its potency as a free radical scavenger, reinforcing its effectiveness in neutralizing both $ABTS^{\bullet+}$ and DPPH radicals.

While the moderate IC_{50} value of *B. diffusa* in the ABTS assay suggests a weaker scavenging ability relative to ascorbic acid, the plant still demonstrates antioxidant potential that warrants further investigation. As with the DPPH assay, the moderate antioxidant activity observed in the ABTS assay suggests that *B. diffusa* contains bioactive compounds capable of contributing to its therapeutic effects, especially when used in combination with other antioxidants or in formulations where moderate scavenging activity is sufficient.

In addition to the antioxidant activity, *B. diffusa* is known for its rich array of bioactive compounds, which contribute to its diverse pharmacological properties, including anti-inflammatory, anti-diabetic, hepatoprotective, and neuroprotective effects. The antioxidant properties of *B. diffusa* may, in part, be attributed to the presence of various compounds, such as flavonoids, polyphenols, alkaloids, and terpenoids, all of which have demonstrated antioxidant activity in other studies.

The therapeutic potential of *B. diffusa* is also reflected in the key bioactive compounds identified in the plant, each of which holds significant pharmaceutical promise. Some of the notable compounds identified include: **β-D-Glucopyranose, 1-thio-**: This compound is known for its antioxidant and anti-inflammatory properties. It is particularly useful in the management of diabetes and for immune modulation. The anti-inflammatory effects of this compound may complement the antioxidant activity of the plant, making it beneficial in inflammatory diseases, where oxidative stress plays a role. **2,2'-Dipiperidine**: This compound acts as a central nervous system (CNS) stimulant and neuroprotective agent. It is useful in the development of antidepressants and cognitive enhancers, making it valuable in treating neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Its neuroprotective effects may also be enhanced by the antioxidant properties of the plant. **Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl)-**: Known for its potent anticancer activity, this compound is often used in chemotherapy drugs. Its ability to neutralize free radicals could contribute to its effectiveness in preventing cancer cell proliferation and oxidative DNA damage. **3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino]propionitrile**: This compound has antidepressant and anxiolytic properties. It may be beneficial in psychiatric treatments, particularly in managing anxiety and depression, conditions where oxidative stress is implicated. **Acetamide, N-[4-(trimethylsilyl)phenyl]-**: This compound exhibits anti-inflammatory and analgesic properties, contributing to its use in pain management drugs. By reducing oxidative stress and inflammation, it can help in the treatment of chronic pain and conditions like arthritis. **2-Isobutyl-1,3,2-oxaazaborinane**: Known for its antibacterial and antifungal effects, this compound makes it valuable in antibiotic formulations. It could potentially help in treating infections caused by resistant strains of bacteria and fungi. **3-Piperidinecarboxamide, N,N-diethyl-**: This compound has antihypertensive and sedative properties. It is used in cardiovascular medications to regulate blood pressure and alleviate stress-induced hypertension. **6-Benzothiazolamine**: This compound demonstrates antimicrobial and antifungal activity, essential for antifungal medications. It can be used in treating fungal infections, providing an additional therapeutic angle for *B. diffusa*. **Benzeneethanamine, 2-fluoro-**: Known for its neurostimulant and antidepressant properties, this compound can be used in stimulant medications. It can enhance cognitive function and mood, potentially improving the quality of life for individuals with cognitive disorders. **Hexadecanedioic acid**: With antimicrobial and anti-inflammatory properties, this compound is useful in wound healing formulations. It can be used to reduce inflammation in wounds, promoting faster healing and recovery. **Cis-13-Octadecenoic acid**: Recognized for its anti-inflammatory and cardioprotective effects, this compound contributes to cholesterol-lowering treatments. Its antioxidant properties can also help in the prevention of cardiovascular diseases. **2-Myristynoyl pantetheine**: This compound functions as an antioxidant and metabolic booster, widely used in energy metabolism support and skincare products. Its antioxidant properties may enhance cellular metabolism and promote skin health.

These bioactive compounds represent a broad spectrum of therapeutic activities, ranging from antioxidant, anti-inflammatory, and antimicrobial effects to anticancer, neuroprotective, and cardiovascular benefits. Their synergy within *B. diffusa* further enhances the plant's potential as a multifaceted therapeutic agent.

6. CONCLUSION

In conclusion, *Boerhavia diffusa* demonstrates moderate antioxidant potential, as evidenced by its performance in both the DPPH and ABTS radical scavenging assays. The plant extract's IC_{50} values of 384.08 µg/mL for DPPH and 454.79 µg/mL for ABTS indicate that while it exhibits antioxidant activity, its scavenging ability is less potent than that of the standard ascorbic acid, which is well-known for its strong antioxidant properties. Despite this, *B. diffusa*'s moderate antioxidant capacity suggests that it could still contribute to the prevention of oxidative stress-related diseases, such as cancer, cardiovascular diseases, and neurodegenerative disorders.

Moreover, the diverse array of bioactive compounds found in *B. diffusa*, including polyphenols, flavonoids, alkaloids, and other phytochemicals, adds to its therapeutic potential. These compounds have demonstrated significant pharmacological activities, including anti-inflammatory, anticancer, neuroprotective, antimicrobial, and anti-diabetic properties, further enhancing the plant's value in modern medicine. The presence of compounds such as β-D-glucopyranose, aziridine, and 2-isobutyl-1,3,2-oxaazaborinane highlights the broad therapeutic scope of *B. diffusa*, making it a promising candidate for the development of natural-based therapeutic agents.

While the current findings underscore the moderate antioxidant potential of *B. diffusa*, further research is needed to fully explore its bioactive compounds and their mechanisms of action. Identification of the specific compounds responsible for its antioxidant and therapeutic effects, along with clinical studies, will be essential to determine the plant's full potential as a medicinal resource. Overall, *Boerhavia diffusa* presents a valuable opportunity for the development of multifaceted therapeutic products aimed at combating oxidative stress and various chronic diseases, offering a natural alternative to synthetic antioxidants and pharmaceuticals.

7. FUTURE WORK SUGGESTIONS

Future research on *Boerhavia diffusa* should focus on the identification and isolation of its specific bioactive compounds responsible for its antioxidant and therapeutic activities. Investigating the mechanisms underlying its radical scavenging potential through molecular and cellular studies will provide deeper insights into its biological effects. In vivo studies using

animal models and clinical trials are essential to evaluate its efficacy, safety, and potential for human use. Additionally, exploring the synergistic effects of *B. diffusa* with other phytochemicals and developing optimized formulations, such as nanoencapsulation, could enhance its therapeutic outcomes. Long-term toxicity and safety assessments are also crucial to establish safe usage protocols. Sustainable cultivation practices and large-scale production methods should be developed to ensure the availability of this plant for medicinal purposes.

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