PHYTOCHEMICAL AND IN VITRO ANTIOXIDANT ACTIVITY OF *Boerhavia diffusa* LEAVES

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ABSTRACT

The current research assessed the phytochemical and antioxidant properties of different extracts from Boerhavia diffusa leaves using in vitro models. The extracts were prepared using various solvents, including ethanol, water, and hexane. The different extracts contained various phytoconstituents, particularly the ethanolic extracts, which had higher amounts of phytocompounds such as tannins, phenols, flavonoids, and carbohydrates. Based on the findings, the ethanolic extract was selected for further examination. This extract underwent several assays, including the DPPH assay, reducing power assay, nitric oxide assay, H2O2 assay, and SOD assay. All methods indicated that the ethanolic extract of Boerhavia diffusa exhibits antioxidant potential in a concentration-dependent manner. The antioxidant activity of the extract was compared to a standard. The study concluded that the ethanolic extract of Boerhavia diffusa leaves is a significant source of antioxidants, containing approximately 142 phytocompounds as identified in its GC-MS report. Additional research is recommended.

Keywords: *Boerhavia diffusa* , Phytochemical Analysis, Medicinal Plants, In vitro antioxidant activity

Introduction

The liver is essential in the human body, as it performs crucial roles in metabolizing biomolecules, synthesizing proteins, producing various biochemical compounds, detoxifying substances, and regulating homeostasis (Rose and Wilson, 2001). Furthermore, it is involved in bile secretion and vitamin storage (Ahsan et al., 2009).

The liver faces exposure to substances from external sources, such as environmental toxins, medications, and alcohol, which can lead to liver-related complications, manifesting as various diseases, including cirrhosis, hepatitis, haemochromatosis, and cholestatic or mixed liver

disorders (Nadkarni, 1976). Compounds like paracetamol, anti-tubercular drugs, and toxic agents such as carbon tetrachloride (CCl4), thioacetamide, dimethyl nitrosamine, and D-galactosamine/lipopolysaccharide can harm the liver and cause associated diseases (Deshwal et al., 2011). Any disturbances in liver function or composition can lead to alterations in its structure, potentially resulting in complications like portal hypertension, jaundice, and increased bleeding, affecting other organs as well (Ibrahim et al., 2008). While numerous synthetic drugs are available to treat liver damage, they may also have adverse effects. Researchers are focusing on medicinal plant-based drugs that are free from side effects. Antioxidant compounds derived from these plants can aid in preventing oxidative harm caused by free radicals to cellular components (Amat et al., 2010). Numerous medicinal plants demonstrate hepatoprotective effects by boosting antioxidant levels (Kamisan et al., 2014), making them vital for decreasing the occurrence of various liver diseases and conditions related to oxidative stress (Vladimir-Knežević et al., 2015). This study focuses on the plant Boerhavia diffusa (B. diffusa) Linn. (Nyctaginaceae), a widely recognized medicinal plant in traditional Indian medicine as well as in other regions like South America and Africa. Different parts of the plant, especially the roots, have been used for gastrointestinal, liver-protecting, and gynecological applications in these areas and across India. B. diffusa has been thoroughly studied for its chemical constituents and therapeutic benefits. The roots contain a distinct group of isoflavonoids known as rotenoids, in addition to flavonoids, flavonoid glycosides, xanthones, purine nucleosides, lignans, ecdysteroids, and steroids. Therefore, this research intends to explore the antioxidant, hepatoprotective, and other potentially beneficial properties of B. diffusa.

Materials and Methods

Fresh leaves of B. diffusa were gathered locally in Trichy. The leaves were shade-dried and then mechanically ground into powder, which was stored in an airtight container. The extraction process was performed using a Soxhlet apparatus through a successive solvent extraction method. The powdered leaves underwent a systematic phytochemical analysis using various solvents to test for the presence of bioactive compounds. The extracts obtained through successive solvent extraction were then analyzed with various phytochemical tests to identify the phytoconstituents present. Subsequently, the samples were evaluated for antioxidant activity against hydrogen peroxide (Ruch et al., 1989), assessed for reducing power (Oyaizu, 1986),

nitric oxide assay, superoxide anion scavenging ability (Nishimiki et al., 1972), and DPPH activity was quantified by measuring the decrease in absorbance at 517 nm.

Result and Discussion

Medicinal plants are utilized to enhance individual health and treat various ailments, including diabetes, heart diseases, and different forms of cancer, without leading to side effects. In this context, the current study highlights the phytochemical and antioxidative properties of B. diffusa leaves.

PHYTOCHEMICAL ANALYSIS OF THE LEAVES OF B. diffusa

The biological efficacy of medicinal plants primarily depends on the compounds they contain. These compounds are produced by plants as a defense against herbivores, but recent research has also demonstrated their protective effects against numerous human diseases.

Medicinal plants are known to contain various phytoconstituents, particularly flavonoids, phenols, alkaloids, terpenoids, and pro-anthocyanins. Among these, flavonoids exhibit anti-inflammatory and anti-carcinogenic properties (Kuhnau et al., 1976; Cody et al., 1986). Phenols possess antioxidant and anticancer effects (Lekse et al., 2001), while terpenoids are recognized for their antioxidant and anti-diabetic actions. Pro-anthocyanins are associated with anticancer (Ray et al., 2005) and cardioprotective benefits, whereas alkaloids demonstrate antimicrobial activity (Ahmed et al., 1986). Therefore, the primary aim of the current study was to identify the phytoconstituents present in the different extracts of B. diffusa leaves.

In this investigation, the leaves of B. diffusa were collected, dried, and subsequently extracted using various solvents such as ethanol, aqueous solution, and hexane. All three extracts of B. diffusa leaves were then analyzed for the presence of carbohydrates, proteins, alkaloids, flavonoids, steroids, tannins, phenols, glycosides, terpenoids, and saponins. The findings are summarized in Table 1.

The present study revealed that terpenoids are found in all extracts of B. diffusa leaves. Phenols were detected in both the ethanolic and aqueous extracts of the leaves. Tannins were identified in the ethanolic and aqueous extracts as well. Flavonoids were present in the ethanolic, aqueous, and hexane extracts of B. diffusa leaves. All extracts contained carbohydrates as a component. Proteins were only found in the aqueous and hexane extracts of B. diffusa. The ethanolic extract

was noted to contain glycosides. A significant quantity of phytoconstituents was identified in the ethanolic extract of B. diffusa leaves. Hence, subsequent studies were focused exclusively on the ethanolic extract of B. diffusa.

IN VITRO ANTI-OXIDANT ACTIVITY

Free radicals play a significant role in various pathological conditions. Antioxidants counteract free radicals and protect against numerous diseases. Several methods (DPPH, reducing power, nitric oxide, superoxide radical, and H2O2) are utilized to evaluate the antioxidant capacity of B. diffusa.

In this investigation, three different extracts (ethanol, aqueous, and hexane) of B. diffusa at various concentrations (25, 50, 100, 150, 200, and 250 μg/ml) were applied to determine the antioxidant potential of these extracts in comparison to standard drugs (ascorbic acid, BHT, and α-tocopherol). Ascorbic acid serves as a standard for the DPPH, reducing power, and NO assays, while BHT is used for the superoxide radical and α-tocopherol for the H2O2 assays.

DPPH assay

Table 2 presents the antioxidant capacity of B. diffusa in the DPPH reducing assay using its ethanolic extract. In the DPPH assay, the ethanol extract of B. diffusa exhibited an antioxidant activity of 82.12%, while the standard ascorbic acid demonstrated a higher value of 91.98% at the maximum inhibitory concentration of 250 μg/ml. The IC50 values were determined to be 150 μg/ml for the ethanol extract of B. diffusa and 140 μg/ml for ascorbic acid.

The DPPH activity evaluated in this research is significant as it measures the electron donating capacity of natural products. DPPH serves as a stable free radical, and the donation of free electrons by a natural product leads to the reduction of DPPH, changing its violet color. The degree of color change is inversely related to the antioxidant capacity of the natural product.

In this study, the ethanolic extract of the leaves shows notable antioxidant potential. A high percentage of scavenging activity against DPPH was observed in the ethanolic extract of B. diffusa. The scavenging activity of the leaves is compared to the standard substance ascorbic acid. Similar findings were reported in a previous study by Raquibul Hasan et al. (2009), which highlighted the effects of methanolic leaf and root extracts of Hypochaeris radicata on DPPH.

REDUCING POWER ASSAY

Table 3 presents the results of the reducing power assay for the leaves of B. diffusa. In the reducing power assay, the ethanol extract of B. diffusa showed values of 82.65%, while the standard ascorbic acid reached 93.98% at maximum inhibitory activity observed at 250 µg/ml. The IC50 values for the ethanol extract of B. diffusa were 100 µg/ml, compared to 95 µg/ml for ascorbic acid.

The reducing power of a substance primarily depends on the reductants found in natural products. Antioxidants disrupt the free radical chain by donating hydrogen atoms. In this assay, the antioxidant compounds reduce ferric ions to ferrous ions and mitigate free radical formation.

In the current study, the reducing power correlates directly with the antioxidant capacity of the plant. A prior study by Luximon Ramma et al. (2005) demonstrated that the reducing power is directly proportional to total phenolic content. In this investigation, all three extracts from the plant leaves exhibited reducing ability, likely due to the presence of phenolic compounds in B. diffusa leaves. The results were compared with ascorbic acid as a standard.

NO ASSAY

The maximum inhibition percentage observed in the NO assay (Table 4) for all extracts of B. diffusa was 88.54%, whereas ascorbic acid achieved a level of 93.78%. The IC50 values were found to be 110 µg/ml for B. diffusa and 120 µg/ml for ascorbic acid. Nitric oxide (NO) is essential for the regulation of numerous physiological functions in humans. However, when NO is present in excess, it can react with oxygen, resulting in the production of stable peroxy nitrite and nitrite ions. This ion functions as a free radical and can cause considerable damage to cell membranes. As such, inhibiting elevated levels of NO is a vital approach for evaluating the antioxidant potential of plant extracts. In this research, all extracts of B. diffusa exhibited antioxidant activity in a manner that depended on the dosage. The inhibition percentage at higher concentrations of the ethanolic extracts of B. diffusa was similar to that of the standard ascorbic acid.

SUPEROXIDE RADICAL

Table 5 reflects the free radical scavenging ability of B. diffusa. The extracts of B. diffusa exhibited a superoxide radical scavenging percentage of 91.68%, while the standard BHT showed 96.42% at a higher concentration of 250 µg/ml. The IC50 values achieved were 105 µg/ml for B. diffusa and 100 µg/ml for BHT. Superoxide dismutase (SOD) is a vital enzyme that neutralizes superoxide radicals produced in the body. However, it exhibits low activity and lacks

the capability to penetrate lipid membranes. The superoxide anions are converted into H2O2 by the action of SOD. The percentage of inhibition of superoxide anion formation correlates directly with the antioxidant potential of plant extracts. In this study, the extracts of B. diffusa demonstrated strong inhibition of superoxide anion formation. A prior study by Manikandan et al. (2016) similarly showed this effect in the extracts of Psidium guajava leaves with regard to superoxide anion radical scavenging.

H2O2 ASSAY

Table 6 illustrates the reductive capability of B. diffusa in comparison to ascorbic acid. In the H2O2 assay, all extracts of B. diffusa achieved an inhibition percentage of 82.05%, while ascorbic acid reached 89.39% at a concentration of 250 μg/ml; their respective IC50 values were 150 μg/ml for B. diffusa and 140 μg/ml for α-tocopherol. Hydrogen peroxide (H2O2) is a weak reactive chemical, but it can become toxic when it forms hydroxyl radicals in cells (Gulein et al., 2003). Natural products can donate electrons to H2O2, converting it into water. Thus, inhibiting H2O2 is significant in evaluating antioxidant capacity. The current study indicates that extracts of B. diffusa effectively inhibit the H2O2 radical. Comparable effects were observed in the leaves of Pananginserg and Lagerstroemia speciosa (Saumya and Mahaboob Basha, 2011).

Conclusion

The ethanolic extract from the leaves of B. diffusa is abundant in various phytochemicals, including alkaloids, terpenoids, carbohydrates, tannins, flavonoids, and others. This extract shows a higher concentration of these phytochemicals. The study revealed the in vitro antioxidant capabilities of the crude ethanol extract derived from B. diffusa leaves. The plant demonstrated notable antioxidant properties in an in vitro setting, justifying further research to pinpoint the specific compounds that contribute to this activity.

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Table 1: Phytochemical constituents of various extracts of *B. diffusa* leaves

S.No	Phytoconstituents	Aqueous	Hexane	Ethanol
1	Alkaloids	+	+	+
2	Carbohydrates	-	+	+
3	Tannin	-	-	+
4	Terpenoids	-	-	+
5	Quinones	+	+	+
6	Total protein	-	+	+
7	Flavonoids	+	+	+
8	Phenols	+	-	+
9	Saponins	-	+	+

Table 2: DPPH radical scavenging activity of *B. diffusa*

S. No.	Concentration of plant extract (µg/ml)	% of inhibition of ethanolic extract of *B. diffusa*	Concentration of standard (µg/ml)
1	25	10.92	17.67
2	50	28.92	31.45
3	100	35.64	43.34
4	150	50.86	62.02
5	200	62.12	78.94
6	250	82.12	91.98
IC_{50}		150	140

Table 3: Reducing power assay of *B. diffusa*

S. No.	Concentration of plant extract (µg/ml)	% of inhibition of ethanolic extract of *B. diffusa*	Concentration of standard (µg/ml)
1	25	29.09	34.67
2	50	43.12	41.45
3	100	50.02	52.34
4	150	53.98	70.02
5	200	68.45	82.94
6	250	82.65	93.98

IC_{50}	100	95

Table 4: Nitric oxide assay of *B. diffusa*

S. No.	Concentration of plant extract (µg/ml)	% of inhibition of ethanolic extract of *B. diffusa*	Concentration of standard (µg/ml)
1	25	20.34	22.75
2	50	48.56	43.47
3	100	49.90	52.72
4	150	67.89	65.39
5	200	75.46	81.42
6	250	88.54	93.78
IC_{50}		110	120

Table 5: Superoxide radical scavenging assay of *B. diffusa*

S. No.	Concentration of plant extract (µg/ml)	% of inhibition of ethanolic extract of *B. diffusa*	Concentration of standard (µg/ml)
1	25	18.12	20.49
2	50	33.92	35.78
3	100	49.63	50.49

4	150	68.41	69.82
5	200	80.23	84.91
6	250	91.68	96.42
IC_{50}		105	100

Table 6: H_2O_2 Assay of extract of *B. diffusa*

S. No.	Concentration of plant extract (µg/ml)	% of inhibition of ethanolic extract of *B. diffusa*	Concentration of standard (µg/ml)
1	25	20.04	23.14
2	50	26.56	32.72
3	100	36.43	41.63
4	150	50.03	57.76
5	200	73.89	73.14
6	250	82.05	89.39
IC_{50}		150	140