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Exploring the Antioxidant Power of Bauhinia Variegata, Boerhavia Diffusa, and Limonia Acidissima: Natural Defenders against Oxidative Stress

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The rising interest in natural antioxidants has led to the exploration of medicinal plants for their ability to combat oxidative stress, a factor associated with various chronic diseases including cancer, cardiovascular, and neurodegenerative disorders. This study investigates the antioxidant potential of Bauhinia variegata, Boerhavia diffusa, and Limonia acidissima, three plants traditionally known for their medicinal properties. The bark, whole plant, and leaves of these species, respectively, were collected, authenticated, and subjected to sequential solvent extraction to obtain bioactive compounds. Phytochemical screening identified the presence of flavonoids, saponins, phenols, and other compounds that may contribute to antioxidant activity. To evaluate antioxidant capacity, DPPH radical scavenging and nitric oxide scavenging assays were conducted. The extracts demonstrated significant free radical scavenging and metal ion chelation abilities, indicating their potential to mitigate oxidative damage. The results suggest that these plant extracts are promising sources of natural antioxidants, which may have applications in developing therapeutic agents for oxidative stress-related conditions. This research reinforces the potential of Bauhinia variegata, Boerhavia diffusa, and Limonia acidissima as natural alternatives to synthetic antioxidants and encourages further investigation into their pharmaceutical applications.

Keywords: Bauhinia Variegata, Boerhavia Diffusa, And Limonia Acidissima, Anti-Oxidant Activity.

1. Introduction

The search for natural sources of antioxidants has become increasingly vital in the medical and pharmaceutical fields due to their potential role in combating oxidative stress and preventing related diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. Antioxidants are compounds that inhibit or delay cellular damage by neutralizing free radicals, which are highly reactive molecules generated by metabolic processes and external factors like pollution, UV radiation, and certain chemicals. An imbalance between free radicals and antioxidants in the body leads to oxidative stress, which can damage DNA, lipids, proteins, and other cellular components. This has spurred considerable interest in identifying plant-based antioxidants that could serve as safer, effective alternatives to synthetic antioxidants commonly used in food and medicine.[1]

Bauhinia variegata, commonly known as the mountain ebony or orchid tree, is well-known for its vibrant flowers and medicinal bark. The bark of this tree has been traditionally used to treat digestive issues, skin disorders, and diabetes. Phytochemical studies on Bauhinia variegata have identified various compounds, including flavonoids, saponins, and phenols, which contribute to its antioxidant and anti-inflammatory activities. The presence of these compounds suggests that Bauhinia variegata bark may be effective in neutralizing free radicals and thus reducing oxidative damage in cells. Investigating its antioxidant properties not only adds value to its traditional uses but also paves the way for its potential application in pharmaceutical formulations targeting oxidative stress-related conditions.[2]

Boerhavia diffusa, commonly known as punarnava, is a perennial herb that has been used extensively in Ayurvedic medicine. Known for its whole plant use, Boerhavia diffusa is traditionally employed in managing liver disorders, kidney problems, inflammation, and as an adaptogen to enhance overall health. Its bioactive profile includes alkaloids, flavonoids, and steroids, each contributing to its antioxidant, anti-inflammatory, and hepatoprotective properties. The herb's antioxidant potential is particularly notable due to the high concentration of flavonoids and other polyphenolic compounds, which are effective free radical scavengers. By neutralizing reactive oxygen species (ROS), Boerhavia diffusa can potentially prevent oxidative damage and support cellular health, making it a promising candidate for therapeutic development.[3]

Limonia acidissima, commonly known as wood apple or kaitha, is a small tropical tree found in the Indian subcontinent. The leaves of Limonia acidissima are traditionally used for their antimicrobial, anti-inflammatory, and antioxidant effects. Known for their rich content of flavonoids, tannins, and phenolic compounds, the leaves possess potent antioxidant activity. These bioactive constituents are believed to play a significant role in protecting cells from oxidative stress, thereby potentially reducing the risk of chronic diseases. Additionally, the antioxidant potential of Limonia acidissima leaves may contribute to its traditional use in treating infections and wounds by minimizing inflammation and promoting healing. Given these therapeutic properties, studying its antioxidant capacity could offer new insights into its applicability in modern medicine.[4]

This study aims to explore and quantify the antioxidant potential of these plant extracts through various in vitro assays, assessing their capacity to scavenge free radicals and chelate metal ions. By employing a sequential solvent extraction process, bioactive compounds from

these plants were isolated, and their antioxidant efficacy was tested using DPPH radical scavenging, nitric oxide scavenging. These methods provide insight into the plants' ability to combat oxidative stress, highlighting their potential as natural sources of antioxidants and their possible applications in pharmaceutical formulations for disease prevention and health enhancement.

Extraction

Collection, authentication, and sequential solvent extraction of the bark of Bauhinia variegata, the whole plant of Boerhavia diffusa, and the leaves of Limonia acidissima.

Collection of plant materials

The bark of Bauhinia variegata (Leguminosae), the entire plant of Boerhavia diffusa (Nyctaginaceae), and the leaves of Limonia acidissima (Rutaceae) were sourced from the Seshachalam Hills in Tirumala, located in the Chittoor district of Andhra Pradesh, India.

Authentication of plant materials

The identity of the bark from Bauhinia variegata (Leguminosae), the entire Boerhavia diffusa plant (Nyctaginaceae), and the leaves of Limonia acidissima (Rutaceae) was confirmed by Dr. Madhava Chetty, Assistant Professor in the Department of Botany at S.V. University, Tirupati. The corresponding voucher specimens are archived in the herbarium collection of the Pharmacology Department at S.V.U. College of Pharmaceutical Sciences, Tirupati.

Powdered of plant materials for the extraction

The plant materials—500 g of Bauhinia variegata bark (Leguminosae), 1 kg of the whole Boerhavia diffusa plant (Nyctaginaceae), and 1 kg of Limonia acidissima leaves (Rutaceae)—were gathered and thoroughly rinsed with distilled water to remove any dirt and soil. These materials were then air-dried in the shade at room temperature for 10 to 14 days. Once fully dried, they were ground into a coarse powder using a milling machine and subsequently passed through a 40-mesh sieve.

Successive solvent extraction

The powdered plant material underwent extraction with chloroform, ethyl acetate, and 70% ethanol in a sequential solvent extraction process based on increasing solvent polarity. Soxhlet extraction was utilized for compounds with limited solubility in the solvent, allowing impurities to remain undissolved. However, this technique is not ideal for thermolabile compounds, as prolonged heating may lead to degradation.

To initiate the process, 100 g of coarse powder was subjected to successive extraction for 6 hours using a Soxhlet apparatus with solvents of increasing polarity—chloroform, ethyl acetate, and 70% ethanol. The aqueous fraction was prepared through hot percolation. The extracts were then filtered with Whatman No.1 filter paper, and the filtrates were concentrated to dryness with a rotary evaporator at 40°C. The crude extracts were stored at 4°C in a refrigerator until further use.

Alkaloid Tests

Dragendorff's Test:

Add Dragendorff's reagent (potassium bismuth iodide) to the extract. The formation of an orange-red precipitate may suggest the presence of alkaloids.

Mayer's Test:

To 1 ml of the extract, add 1 ml of Mayer's reagent (potassium mercuric iodide). A cream-colored precipitate may indicate alkaloids.

Hager's Test:

Add 1 ml of Hager's reagent (picric acid solution) to 1 ml of the extract. A yellow precipitate may confirm alkaloids.

Wagner's Test:

Mix 1 ml of the extract with 2 ml of Wagner's reagent (iodine in potassium iodide). The presence of a reddish-brown precipitate may indicate alkaloids.

Glycoside Tests

Legal's Test:

Dissolve the extract in pyridine and make alkaline with sodium nitroprusside solution. A pink or red coloration may indicate glycosides.

Baljet's Test:

Add 1 ml of sodium picrate solution to the extract. A change in color from yellow to orange may suggest glycosides.

Keller-Killani Test:

To powdered extract, add 10 ml of 70% ethanol and filter. Add 10 ml of water, 0.5 ml lead acetate solution, and filter again. Shake the filtrate with 5 ml chloroform, separate the chloroform layer, and evaporate. Add 3 ml of glacial acetic acid, cool, then add 2 drops of 5% ferric chloride. Transfer into a test tube with 2 ml of concentrated sulfuric acid. A reddish-brown layer at the junction and a bluish-green color upon standing may indicate glycosides.

Carbohydrate Tests

Molisch's Test:

To 2-3 ml of the extract, add 1 ml of α-naphthol and carefully add concentrated sulfuric acid along the test tube sides. A violet ring at the interface may indicate carbohydrates.

Benedict's Test:

Mix the test solution with Benedict's reagent and heat in a water bath. A brick-red color may indicate carbohydrates.

Fehling's Test:

Combine 1 ml of the extract with equal parts of Fehling's solution A and B, then heat in a water bath. A brick-red precipitate may confirm carbohydrates.

Barfoed's Test:

Mix 1 ml of test solution with 1 ml of Barfoed's reagent and heat in a water bath. A red precipitate may indicate carbohydrates.

Protein & Amino Acid Tests

Xanthoprotein Test:

Add a few drops of concentrated nitric acid to the extract. A yellow color may indicate proteins.

Ninhydrin Test:

To a small amount of the extract, add 2 drops of 0.2% Ninhydrin reagent. A blue color may indicate amino acids.

Flavonoid Tests

Shinoda Test:

To the ethanolic extract, add magnesium foil and concentrated HCl. The formation of an intense cherry-red or orange-red color may confirm flavonoids.

Alkaline Reagent Test:

Add a few drops of sodium hydroxide solution to the extract. A yellow color, which turns colorless with dilute acid, may indicate flavonoids.

Steroid & Triterpenoid Tests

Libermann-Burchard Test:

Add a few drops of acetic anhydride to the extract, heat briefly, and cool. Add concentrated sulfuric acid along the test tube side. A brown ring at the interface and a green upper layer suggest steroids, while a deep red color suggests triterpenoids.

Salkowski Test:

Dissolve the extract in chloroform and add concentrated sulfuric acid. A red color in the lower layer may indicate steroids, while a yellow color suggests triterpenoids.

Tannin Test

Gelatin Test:

Add 1% gelatin solution with sodium chloride to the extract. A white precipitate may indicate tannins.

Phenol Test

Ferric Chloride Test:

Add 3-4 drops of ferric chloride to the extract. A bluish-black color may suggest phenols.

Saponin Tests

Foam Test:

Shake the ethanolic extract with 20 ml of distilled water in a cylinder for 15 minutes. If a 1 cm foam layer persists for 10 minutes, saponins may be present.

Hemolytic Test:

Place a drop of blood on the extract on a glass slide. A hemolytic zone may suggest saponins.

In Vitro Antioxidant Studies

DPPH radical scavenging assay

The leaf extract, in concentrations of 50, 100, 150, 200, and 250 µg/ml, was each added to 0.5 ml of methanolic DPPH solution along with 0.48 ml of methanol. The mixture was allowed to stand at room temperature for 30 minutes. Methanol was used as the blank, while DPPH in methanol, without leaf extract, served as the positive control. After the 30-minute incubation, the decrease in purple color intensity was measured at 517 nm using a spectrophotometer. Ascorbic acid and BHT (butylated hydroxy toluene) were included as reference standards. Blank readings were taken, and the DPPH radical scavenging activity was then calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

IC_{50} value is the concentration of the sample required to scavenge the 50% DPPH free radical.[5,6,7]

Assay of nitric oxide scavenging activity

The reaction was initiated by combining 2.0 ml of sodium nitroprusside, 0.5 ml of PBS, and 0.5 ml of leaf extract (50 mg), followed by incubation at 25°C for 30 minutes. Afterward, 0.5 ml of Griess reagent was added, and the mixture was incubated for an additional 30 minutes. Control samples were prepared without the leaf extract. Absorbance was measured at 546 nm against a reagent blank using a spectrophotometer. Ascorbic acid and BHT (butylated hydroxy toluene) were used as reference standards.[8,9]

The percentage inhibition was calculated according to the following equation:

$$\text{(\%) of inhibition} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

2. Results and Discussion

Results of Extraction

Percentage yield of ethanolic extracts of Bauhinia variegata (bark), Boerhavia diffusa (whole Plant) and Limonia acidissima(leaves) result were given in the table 5.

A. Percentage yield of 70% ethanolic extracts of Bauhinia variegata

Part used : Bark ,

Weight of dried bark : 1 kg

Extracted with : Chloroform, ethyl acetate, 70% ethanol & water

% yield of 70% BOEE : 10.60%

B. Percentage yield of 70% ethanolic extracts of Boerhavia diffusa

Part used : Whole plant

Weight of dried leaves : 1kg

Extracted with : Chloroform, ethyl acetate, 70% ethanol & water.

% yield of 70% IBEE : 9.20%

C. Percentage yield of ethanolic extract of Limonia acidissima

Part used : Leaves

Weight of dried leaves : 1 kg

Extracted with : Chloroform, ethyl acetate, 70% ethanol & water.

% yield of 70% RBEE : 12.50%

Table 1: Percentage yield of different extracts of Bauhinia variegata (bark) A, Boerhavia diffusa (whole plant) B & Limonia acidissima (leaves) C, with different solvents and results were given in Table 5.

A. Bauhinia variegata (Bark)					
S. No	Parameters	Chloroform	Ethyl acetate	70% Ethanol	water
1	Consistency	Oily	Oily	Viscous	Viscous
2	Color	Light green	Brownish	Radish black	Cream
3	% of yield	3.50%	4.96%	10.60%	5.40%
B. Boerhavia diffusa (Whole plant)					
S No	Parameters	Chloroform	Ethyl acetate	70% Ethanol	water
1	Consistency	Oily	Oily	Viscous	Viscous
2	Color	Green	Brownish green	Radish black	Cream
3	% of yield	4.75%	3.50%	9.20%	4.20%
C. Limonia acidissima (Leaves)					
S .No	Parameters	Chloroform	Ethyl acetate	70% Ethanol	water
1	Consistency	Oily	Oily	Viscous	Viscous
2	Color	Light green	Brownish green	Reddish black	Cream
3	% of yield	3.36%	5.90%	12.50%	4.75%

Result of Preliminary Phytochemical Screening

In this study, color forming and precipitating chemical reagents were used to screen extracts of bark from Bauhinia variegata, leaves from Limonia acidissima, and the whole plant from Boerhavia diffusa. As a result of chemical tests, major secondary metabolites such as alkaloids, glycosides, carbohydrates, proteins, amino acids, flavonoids, steroids, tri-terpenoids, tannins, phenols and saponins were detected in different extracts (chloroform,

ethyl acetate, 70% ethanol, and aqueous extract) of Bauhinia variegata bark, Boerhavia diffusa leaves, and Limonia acidissima leaves. Further, in Table 6, we summarize the findings of preliminary phytochemical investigations conducted on bark from Bauhinia variegata, the whole plant from Boerhavia diffusa, and the leaves from Limonia acidissima leaves.

Table 2:Preliminary phytochemical screening of the different extracts of Bauhinia variegata (A), Boerhavia diffusa (B) & Limonia acidissima (C).

A: Bauhinia variegata ethanolic extract (BVEE)

S. No	Phytoconstituents	Chloroform	Ethyl acetate	70% Ethanol	water
1	Alkaloids	-	-	-	-
2	Glycosides	+	-	+	+
3	Carbohydrates	-	-	+	-
4	Proteins	-	-	-	-
5	Amino acids	-	-	-	-
6	Flavonoids	+	+	+	+
7	Steroids	+	+	+	-
8	Triterpenoids	+	+	+	-
9	Tannins	+	+	+	+
10	Phenols	+	+	+	+
11	Saponins	-	-	-	-

Where, (+) = Presence, (−)s = Absence

B: Boerhavia diffusa ethanolic extract (BDEE)

S. No	Phyto constituents	Chloroform	Ethyl acetate	70% Ethanol	Water
1	Alkaloids	-	-	-	-
2	Glycosides	+	+	+	-
3	Carbohydrates	+	+	+	-
4	Proteins	-	-	-	-
5	Amino acids	-	-	-	-
6	Flavonoids	+	+	+	+
7	Steroids	-	+	+	-
8	Triterpenoids	-	+	+	-
9	Tannins	+	+	+	+
10	Phenols	+	+	+	+
11	Saponins	-	-	-	-

C: Limonia acidissima ethanolic extract (LAEE)

S. No	Phyto constituents	Chloroform	Ethyl acetate	Ethanol	Water
1	Alkaloids	-	-	+	-
2	Glycosides	+	-	+	+
3	Carbohydrates	-	+	+	+
4	Proteins	-	-	-	-
5	Amino acids	-	-	-	-
6	Flavonoids	+	+	+	+
7	Steroids	+	+	+	-
8	Triterpenoids	+	+	+	-
9	Tannins	+	+	+	+
10	Phenols	+	+	+	+
11	Saponins	-	-	-	-

Different extracts of the bark of Bauhinia variegata, the plant as a whole, and the leaves of Limonia acidissima were examined for phytochemical properties in a preliminary study. In comparison to chloroform, ethyl acetate, and aqueous extracts, 70% of ethanolic extracts

contained high amounts of desired phytochemical constituents. To investigate the potential antioxidant, hepatoprotective, antidiagnostic, and antiarthritic activities of 70% ethanolic extracts of Bauhinia variegate bark (BVEE), Boerhavia diffusa leaves (BDEE), and Limonia acidissima leaves (LAEE), 70% ethanolic extracts of boerhavia diffusa leaves have been selected for future studies.

Results of in Vitro Antioxidant Activity

As shown in Table 8 & Figure 1, the antioxidant activity of the 70% BVEE, BDEE, and LAEE on the DPPH and NO assays, H2O2 and metal chelating activities was determined.

DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity.

A direct correlation was found between DPPH disappearance and the amount of antioxidant present in the reaction mixture (antioxidants react with stable free radicals such as diphenyl-1,2-dimethylaminobutoethanol (deep violet) to produce discolored hydrazine). A higher concentration of 70% BVEE, BDEE & LAEE led to a higher scavenging effect on DPPH.

A. Effect of BVEE

As the concentration of the extract increased from 50 to 250 µg/ml, the percentage of DPPH radical scavenging effect increased. Further the DPPH radical inhibition was measured at different doses (50 µg/ml, 250 µg/ml of extract) was found to be varying from 16.44±0.65 to 56.23± 1.64. According to the results, the IC50 of BVEE, ascorbic acid, and BHT is 223.94, 142.14, and 158.26 µg/ml, respectively.

B. Effect of BDEE

The maximum % inhibition of BDEE, ascorbic acid and BHT was found to be 45.28±1.84, 79.16±1.98 & 72.38±1.81 were identified at a concentration of 250 µg/ml. Based on the results of the experiments, the IC50 value for BDEE was determined to be 274.44 µg/ml. At all concentrations, the extracts showed a dose-dependent inhibition of free radicals.

C. Effect of LAEE

As LAEE concentrations increased from 50 g/ml to 250 µg/ml, the effect of LAEE on DPPH radicals increased. There were varying levels of inhibition for LAEE, ascorbic acid, and BHT ranging from 18.33±0.70 to 62.25±1.64, 35.34 ± 0.78 to 89.47±2.11 & 31.25 ±0.39 to 74.12±2.12. IC50 values for LAEE, ascorbic acid, and BHT were determined to be 209, 101.42, and 139.50. It was found that extracts had dose-dependent effects at all concentrations

Table 3: The absorbance and scavenging activity or % inhibition of DPPH by 70% BVEE (A)/ BDEE (B) /LAEE (C).

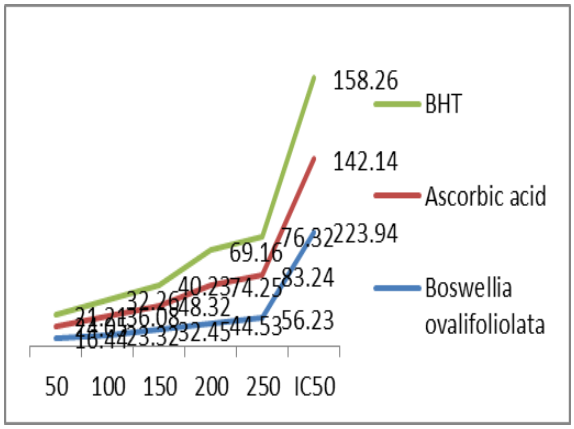
A. Bauhinia variegata ethanolic extract (BVEE - A)			
Conc. µg/ml	Bauhinia variegata	Ascorbic acid	BHT
50	16.44±0.65	24.05±0.76	21.21±0.98
100	23.32±0.96	36.08±0.79	32.26±0.74
150	32.45±0.72	48.32±0.98	40.23±1.11
200	44.53±1.32	74.25±1.26	69.16±1.36
250	56.23±1.64	83.24±1.51	76.32±1.62
IC_{50}	223.94	142.14	158.26
B. Boerhavia diffusa ethanoic extract (BDEE – B)			

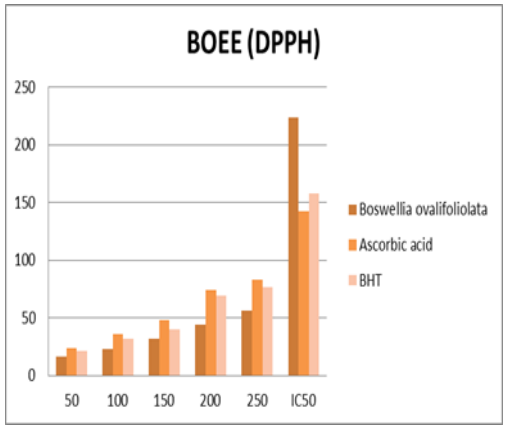
Conc. µg/ml	Boerhavia diffusa	Ascorbic acid	BHT
50	15.38±0.65	20.12±0.51	18.16±0.99
100	19.25±0.96	28.12±0.89	21.36±1.11
150	28.36±1.21	39.46±1.16	33.16±1.32
200	37.34±1.75	56.23±1.52	47.19±1.58
250	45.28±1.84	79.16±1.98	72.38±1.81
IC_{50}	274.44	168.62	193.32
C. Limonia acidissima ethanolic extract (LAEE –C)			
Conc. µg/ml	Limonia acidissima	Ascorbic acid	BHT
50	18.33±0.70	33.34±0.78	31.25±0.39
100	24.61±0.44	49.56±0.81	42.05±0.79
150	32.75±0.94	64.74±1.12	58.74±1.72
200	46.12±1.36	76.58±1.64	68.89±1.89
250	62.25±1.64	89.47±2.11	74.12±2.12
IC_{50}	209	101.42	139.5

Data expressed as mean ± SEM. Each sample was analyzed in triplicate.

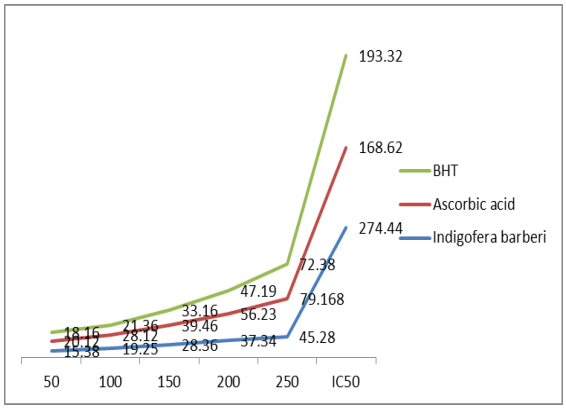
Fig. 1 Graphical representation of the absorbance and scavenging activity or % inhibition of DPPH by 70% BVEE (A) /BDEE (B) /LAEE (C).

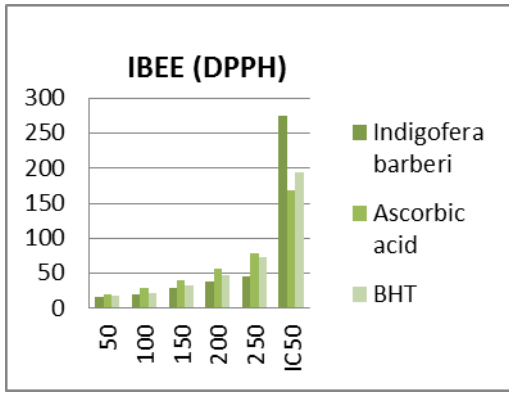
A. Effect of BVEE



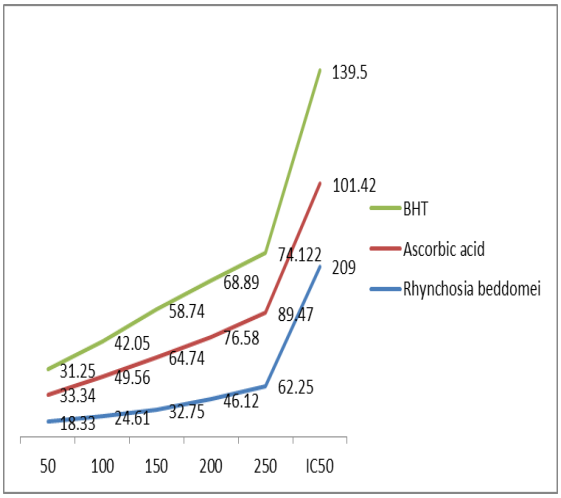


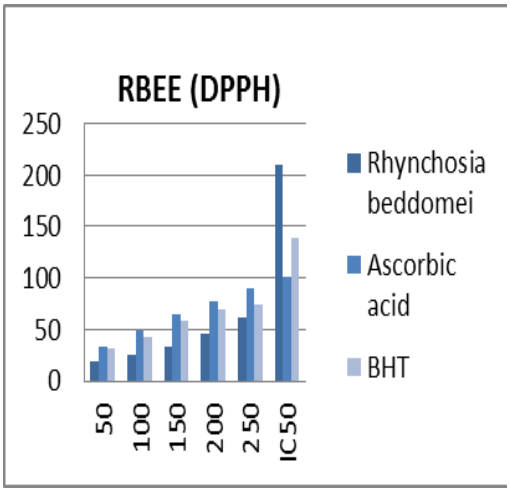
B. Effect of BDEE





C. Effect of LAEE





Data expressed as mean ± SEM. Each sample was analyzed in triplicate.

Nitric oxide radical scavenging assay

Nitric oxide is generated spontaneously by sodium nitroprusside in aqueous solution at physiological pH by interacting with oxygen and producing nitrite ions, which are measured by Griess reagent. The results of the study are presented in Table 9 and Fig. 1, where oxygen is competing with nitric oxide scavengers, reducing nitrite ion production.

A. Effect of BVEE

There was a significant difference in the percentage of inhibition between different concentrations of BVEE from 14.37± 0.85 to 54.27±1.22 (in 50µg/ml-300µg/ml of the extract). In the same study, BVEE, ascorbic acid, and BHT were found to have IC50 values of 259.24, 181.96 and 200.63, respectively.

B. Effect of BDEE

The BDEE scavenging effect increased with increasing concentrations of extract from 50-300 g/ml. There was a variation in the percentage of inhibition of NO radical by the BDEE from 16.18 ±0.46 to 49.06 ± 1.32 µg/ml (in 50 µg/ml to 300 µg/ml of extract). In this study, 283.24, 161, 33 and 190.55 µg/ml were determined to be the IC50 values for BDEE, ascorbic acid, and BHT, respectively. Further, the concentration-dependent inhibition of free radicals was observed in all concentrations of the extracts.

C. Effect of LAEE

There were 22.12 ± 0.49 to 70.01 ±2.11, 36.01 ± 0.42 to 88.09 ± 1.48 & 32.14 ± 1.12 to 79.24 ± 1.39 µg/ml inhibition (in 50 µg/ml to 300 µg/ml). In all concentrations, extracts, ascorbic acid, and BHT showed concentration-dependent inhibition of free radicals. IC50 values were determined for 70% LAEE, ascorbic acid, and BHT at 191.18, 135.55, and 151.48 µg/ml, respectively. Concentration-dependent effects were observed for all three BVEE/BDEE/LAEE extracts. In comparison to other concentrations of extracts, 300 µg/ml of BVEE, BDEE, and LAEE showed the strongest inhibitory effect on NO.

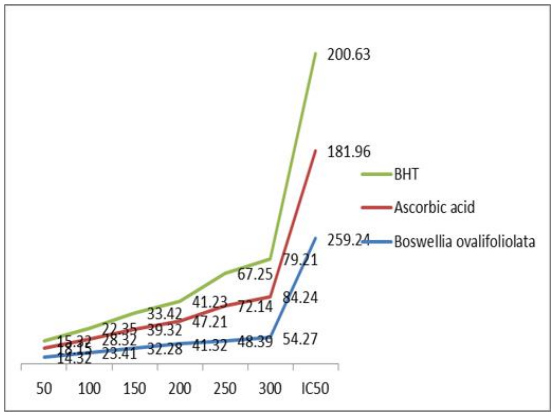
Table 4: The absorbance and scavenging activity or % inhibition of NO by 70% BVEE/BDEE/LAEE.

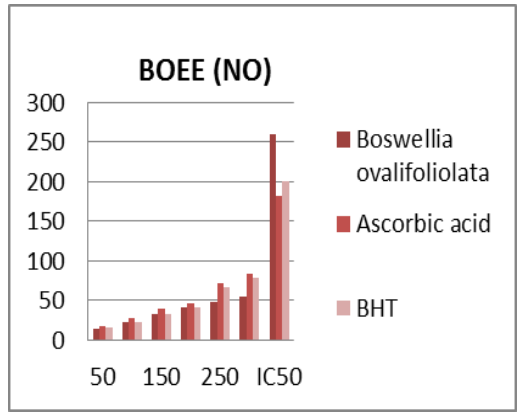
A. Bauhinia variegata ethanolic extract (BVEE - A)			
Conc. µg/ml	Bauhinia variegate	Ascorbic acid	BHT
50	14.32±0.85	18.15±0.78	15.32±0.89
100	23.41±0.83	28.32±0.58	22.35±0.59
150	32.28±1.54	39.32±1.90	33.42±1.62
200	41.32±1.87	47.21±1.53	41.23±1.29
250	48.39±1.95	72.14±2.05	67.25±1.98
300	54.27±1.22	84.24±1.43	79.21±1.06
IC_{50}	259.24	181.96	200.63
B. Boerhavia diffusa ethanolic extract (BDEE – B)			
Conc. µg/ml	Boerhavia diffusa	Ascorbic acid	BHT
50	16.18±0.46	28.36±0.76	20.17±0.75
100	22.39±0.94	39.38±0.89	31.25±0.91
150	32.25±1.98	48.16±1.13	42.36±1.27
200	39.38±1.56	59.24±1.09	53.19±1.65
250	44.16±1.71	70.36±1.89	64.29±1.17
300	49.06±1.32	84.29±1.87	72.39±1.69
IC_{50}	283.24	161.33	190.55
C. Limonia acidissima ethanolic extract (LAEE –C)			
Conc. µg/ml	Limonia acidissima	Ascorbic acid	BHT
50	22.12±0.49	36.01±0.42	32.14±1.12
100	33.41±0.84	49.22±0.98	43.15±0.91
150	42.15±1.06	58.32±1.14	55.24±1.15
200	54.26±1.94	68.35±1.58	64.32±1.38
250	63.5±1.36	76.32±1.32	73.33±1.62
300	70.01±2.11	88.09±1.48	79.24±1.39
IC_{50}	191.18	135.55	151.48

Data expressed as mean ± SEM. Each sample was analyzed in triplicate.

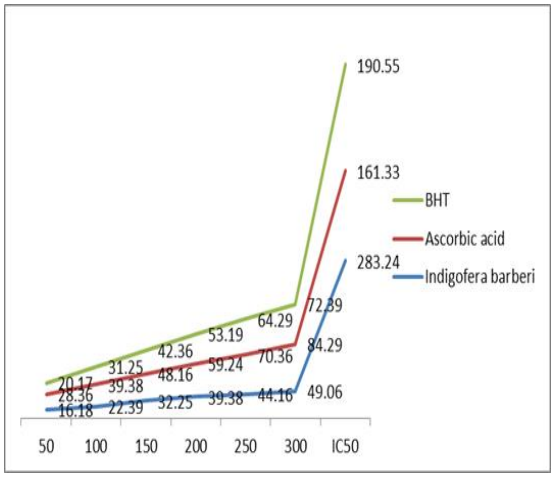
Fig. 2 Graphical representation of % inhibition of NO by 70% BVEE (A)/ BDEE (B) /LAEE (C).

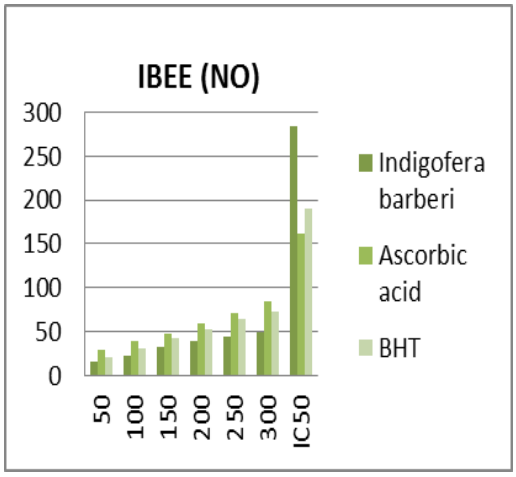
A. Effect of BVEE



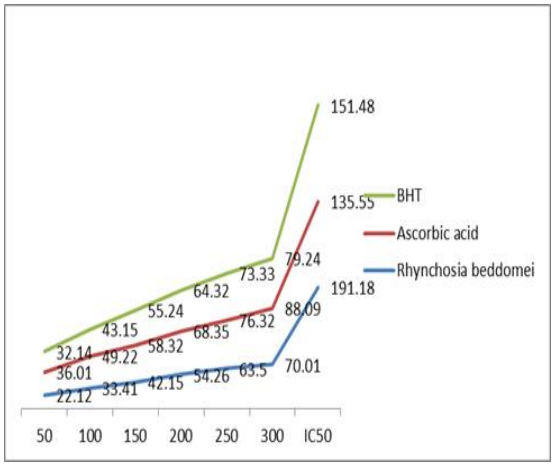


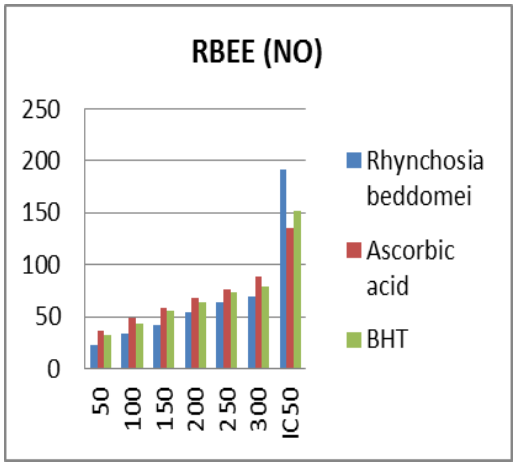
B. Effect of BDEE





C. Effect of LAEE





Data expressed as mean ± SEM. Each sample was analyzed in triplicate.

3. Conclusion

In conclusion, Bauhinia variegata, Boerhavia diffusa, and Limonia acidissima demonstrate significant antioxidant potential, supporting their traditional uses and suggesting their applicability in modern therapeutic contexts. The bioactive compounds present in these plants—particularly flavonoids, saponins, phenols, and other polyphenolics—contribute to their capacity to neutralize free radicals and reduce oxidative stress, highlighting their possible role in managing oxidative stress-related diseases, including cardiovascular disorders, diabetes, and neurodegenerative conditions. The use of in vitro assays, such as DPPH and nitric oxide scavenging, effectively illustrated the efficacy of these extracts in inhibiting reactive oxygen species and binding metal ions, reinforcing the value of these plants as potent, natural antioxidant sources.

The insights gained from this study lay the groundwork for further exploration of these extracts in clinical settings, particularly in pharmaceutical formulations aimed at preventive health and chronic disease management. In addition to validating the plants' traditional medicinal roles, these findings also open the door for the development of safer, plant-based alternatives to synthetic antioxidants. Thus, Bauhinia variegata, Boerhavia diffusa, and Limonia acidissima hold substantial promise as natural antioxidants, underscoring the importance of continued research into plant-based therapies for enhancing human health and well-being.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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