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Exploration of Bioactive Compounds and Health Benefits of *Boerhavia diffusa*

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ABSTRACT

Objective: *The present study assessed the different biological activities of Boerhavia diffusa leaf extracts from the Vidarbha region of Maharashtra in both methanolic and aqueous form.*

Methods: *Liquid chromatography-mass spectrometry was used to determine the phytochemical composition of methanolic and aqueous extracts of Boerhavia diffusa leaves. The total phenolic content and total flavonoid content were quantified using standard spectroscopic methods. The antioxidant potential was assessed with the DPPH assay. Additionally, in vitro assays evaluated the antidiabetic properties through alpha-amylase and alpha-glucosidase inhibition assays, along with the anti-inflammatory effects assessed via Bovine Serum Albumin (BSA) denaturation and trypsin inhibition assays. The cytotoxic effects were studied on HepG-2 cancer cells.*

Results*: The LCMS analysis revealed the occurrence of several phytochemicals in both the methanolic and aqueous extracts. The methanolic and aqueous extract demonstrated potent antioxidant activity (IC50 = 71.21 ± 0.002 µg/ml & IC50 = 66.46 ± 0.001 µg/ml respectively) than that of standard ascorbic acid (IC50=79.44 ± 0.002). Methanolic and aqueous extracts showed α-amylase (IC50 = 56.29±0.003 µg/ml and IC50 = 71.85±0.003 µg/ml) and α-glucosidase (IC50 = 58.59±0.004 µg/ml and IC50 = 79.34±0.001 µg/ml) inhibitory activity compared to acarbose. Additionally, both the extract methanolic and aqueous exhibited moderate anti-inflammatory activity via BSA denaturation (IC50 = 58.89±0.011 µg/ml and 75.25±0.003µg/ml) and trypsin (IC50 = 57.60 ± 0.003 µg/ml and 80.93 ± 0.003 µg/ml) inhibition assays compared to indomethacin and cytotoxic activity on HepG-2 cell line with IC50 = 351.1 ± 0.1258 µg/ml.*

Conclusion: *The results demonstrate the medicinal potential of B. diffusa leaves as a natural basis of bioactive chemicals with cytotoxic, anti-inflammatory, antidiabetic, and antioxidant qualities.*

Keywords: *B. diffusa, Phenolic, flavonoids, antioxidant, antidiabetic, anti-inflammatory and cytotoxic assay.*

1. Introduction

The liver is an essential organ that normalizes metabolic functions and detoxifies many harmful substances. It is susceptible to external toxic substances and organic compounds[1]. Exposure to these toxicants, including drugs, alcohol, and environmental pollutants, can lead to liver damage through metabolic activation, this leads to the creation of highly reactive substances. D-galactosamine is a proven hepatotoxin; it produces diffuse-type liver injury that closely mimics human viral hepatitis (viral hepatitis type) and acute self-limited hepatitis (injury with necrosis, inflammation, and regeneration), similar to human drug-induced liver diseases[2]. The toxic effects of D-galactosamine are primarily due to uridine pool depletion, which results in inhibited RNA and protein synthesis in the liver, ultimately affecting hepatocellular activity[3]. Despite advances in modern medicine, pharmacotherapeutic treatment with synthetic drugs for liver protection has not yet been achieved. Therefore, alternative therapeutic strategies, such as *Boerhavia diffusa* leaf extract, are of great interest for liver protection[4].

Diabetes is a common long-term metabolic disorder marked by higher levels of sugar in the blood. It is due to insufficient or ineffective insulin production and is associated with abnormalities in the metabolism of carbohydrate, protein, and fat[5-6]. Recent findings have shown that high blood sugar can cause non-enzymic glycosylation of several macromolecules. The build up of harmful oxygen-containing molecules and the weakening of the build's natural antioxidant scheme can performance a part in the long term health problems linked to diabetes[7]. The concept that oxidative stress significantly contributes to diabetes onset, alongside postprandial hyperglycemia, creates valuable opportunities for developing treatment and management strategies to decrease the risk of long-term vascular complications. One practical way to lower post-meal blood sugar spikes is to prevent carbohydrate absorption after eating. Complex polysaccharides are initially broken down by alpha-amylase into oligosaccharides, which are then further cleaved into glucose by intestinal α-glucosidase. The glucose molecules are absorbed through the lining of the intestine and then enter the bloodstream[8].Constraints of α-amylase and α-glucosidase can help lower post-meal hyperglycemia by blocking the breakdown of carbohydrates, which slows glucose absorption[9].Various synthetic drugs, including acarbose, voglibose, and miglitol, are commonly prescribed as enzyme inhibitors for managing type 2 diabetes. However, these medicines can often cause side effects like gas, stomach pain, bloating, and sometimes diarrhea[10].Research is focused on exploring natural and safer alternatives for inhibiting alpha-amylase and alpha-glucosidase. Phytochemicals, especially phenolics with strong antioxidant potential, have been reported as effective enzyme inhibitors.

This constitutes a complete strategy in the supervision of hyperglycaemia and the anticipation of oxidative stress-related sequelae in diabetes[11].

Medicinal plants (MPs) have proven to be effective in regulating metabolic functions, hyperglycemia, and metabolic syndrome. This effectiveness is primarily due to their high content of therapeutic phytochemicals, which include saponins, tannins, flavonoids, phenolics and glycosides. These compounds enhance various bioactivities[12], such as antioxidant and anti-inflammatory properties. Additionally, medicinal plants are often readily available, generally more affordable than commercial synthetic drugs, and tend to have fewer side effects. A classic example of such a medicinal plant is *Boerhavia diffusa*.[10] *Boerhavia diffusa*, generally known as Punarnava in the Indian system of medicine, associated to the family Nyctaginaceae. It is a perennial creeping herb widely initiate in wastelands throughout India.[13] The genus *Boerhavia* comprises 40 species, which are circulated across tropical and subtropical regions with warm climates. In India, six species of *Boerhavia* are present: *B. diffusa, B. erecta*, *B. rependa*, *B. chinensis*, *B. hirsuta* and *B. rubicunda*. *Boerhavia diffusa* is typically initiate in the furnace parts of the country and can grow at altitudes equipped 2,000 meters in the Himalayan region.[14] This plant is a perennial, creeping weed characterized by its spreading branches and tough, tapering roots. The stem is arboreal, sometimes purplish, and covered with hairs, becoming thicker at the nodes. The plant is fleshy and hairy, with leaves that grow in unequal pairs and produce small pinkish-red flowers. *Boerhavia diffusa* is commonly found in abundance in unused areas, waterways, and muddy spaces, particularly throughout the rainy season. Additionally, it is sophisticated to some range in West Bengal.

In India, diverse parts of *B. diffusa*, such as the leaves, roots, and aerial parts, are commonly used to treat kidney issues, rheumatism, and liver disorders, among other health problems[15]. Leaf extracts from the plant have been shown to have a diversity of beneficial properties, comprising hepatoprotective, anti-diabetic, anti-inflammatory, and antioxidant effects.[16-17] The leaves of *Boerhavia diffusa* are rich in phytochemicals, such as essential oils, rotenoids, alkaloids, flavonoids, alkamides, terpenoids, xanthones, and phenolic compounds.[18]The chemical profile of plant-derived extracts can differ significantly built on aspects such as species origin, extraction method (e.g., methanolic, aqueous), temperature, and solvent concentration. Methanol has excellent solubility properties, allowing it to dissolve a varied range of polar and semi-polar compounds found in plants. Many bioactive substances in plants, such as phenolic acids, fall into these categories. Methanol's ability to effectively dissolve these compounds enhances the extraction yield. For instance, when extracting flavonoids from plant leaves, methanol can penetrate the cell walls and dissolve the flavonoid molecules, making it easier to recover them from the extraction solution.[19]Similarly, water effectively extracts highly glacial compounds such as sugars, amino acids and some phenolics.[20] The current study aims to explore the biological potential of the leaf extract of *Boerhavia diffusa*. The study includes quantitative analysis to determine the phenolic and flavonoid content, as well as the evaluation of its hepatoprotective activity[21]on the HepG-2 cell line, antidiabetic potential (through α-amylase and α-glucosidase inhibition assays), anti-inflammatory potential[22] (via protein denaturation(BSA) and proteinase inhibition (Trypsin) assays), and antioxidant activity[23] (using the DPPH assay) of the above ground parts of *B. diffusa*.

This study search for to explore the likely of this plant as a capable source of innovative therapeutic candidates for managing contemporary health problems, especially those arising from oxidative stress, metabolic imbalance, and infectious conditions.

2. Materials and Methods

2.1 Collection of *Boerhavia diffusa* leaves

In the months of July and October, new Boerhavia diffusa samples were unruffled from the Hudkeshwar region of Nagpur in Maharashtra. A taxonomist of Rashtrasant Tukadoji Maharaj Nagpur University's Department of Botany identified the plant, and a herbarium specimen bearing voucher number 10923 was sent to the department. The leaves were splashed with distilled water to take away any surface impurities and then desiccated in the shadow at a temperature of 20-25°C for two weeks. The desiccated leaves were then powdered using a crusher and kept in hermetically sealed pliable containers at room temperature[24].

2.2 Preparation of Plant Extracts

The leaf powder (10 g) was extracted using methanol and distilled water in a Soxhlet apparatus for a period of 12 to 14 hours. After being filtered, the methanol and aqueous extracts were kept for additional examination at 4°C. Whatman filter paper No. 1 was used to filter the extracts.

$$\text{Yield}(\%) = \frac{Weight\ of\ crude\ extract\ (g)}{Weight\ of\ the\ dry\ leaf\ powder\ (g)} \text{ x } 100$$

2.3 Phytochemical analysis

To identify the livelyelements in the methanolic and aqueous extracts of *Boerhavia diffusa*, phytochemical screening was done. The extracts, namely the aqueous and the methanolic of *Boerhavia diffusa* leaves were screened for Alkaloids (Dragendorff's reagent, Mayer's reagent), Terpenoids (Salkowski test), Flavonoids (Shinoda's test, Lead Acetate test),Carbohydrates (Molisch's test, Fehling's test and Benedict's test), Anthraquinones (Borntrager's test), Glycosides (Keller-Kiliani test).[25]

Determination of Total Phenolic Content (TPC)

A partial of 12.5 µl of plant extract was cooperative with 12.5 µl of Folin-Ciocalteu reagent in a 300 µl reaction container. The combination was allowed to stand at room temperature for ten minutes. Subsequently, 125 µl of 7% sodium carbonate was added to the solution. The reaction mixture was nurtured in the dark at 37°C for 90 minutes. Following incubation, the volume was adjusted to 300 µl using distilled water. Finally, the optical density was sedate at 760 nm using a spectrophotometer. The phenolic concentration was determined using a standard calibration curve for gallic acid ranging from 20 to 100 µg/ml.[26]

Determination of Total Flavonoid Content (TFC)

One hundred microliters of plant extract were mixed with one hundred microliters of 2% aluminum chloride and protected at room temperature for ten minutes. After gestation, a spectrophotometer was used to quantity the optical density at 367 nm. To determine the flavonoid content, a standard calibration curve for quercetin, stretching from 20 to 100 µg/ml, was utilized.[27]

2.4 UPLC-QTOF-MS metabolite profiling of the extracts

The occurrence of numerous bioactive combinations in the aqueous and methanolic leaf extracts of *Boerhavia diffusa* leaves

was recognized using Liquid Chromatography Mass Spectrometry (LC-MS) of these extracts. The extract's metabolites were profiled using quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) in both positive (ESI+) and negative (ESI-) modes using ultra-performance liquid chromatography.[28]The aqueous and methanolic leaf extracts were clarified using 0.25 mm polyvinylidene fluoridemembrane nozzle sieves and then relocated straight into 2mL vials for analysis. Analytes were chromatographically separated using a 5 µL sample injection volume on an RPC-18 column that had proportions of 50 mm (length), 2.1 mm (internal diameter), and 2.7 µm (particle size). A 45 minute analytical run was planned, during which the movementcontour of the movable phase was determined. Covering gas temperature was 350°C, covering gas flow was 11, vaporizer pressure was 35 psig, and the scan rate was 2 spectra per minute in Acquisition approach with a range of 60-1700 (m/z).The movable phase followed a binary gradient system, using solvent A as 0.1% formic acid in deionized water and solvent B as acetonitrile. The elution was carried out at room temperature with a flow rate of 0.4 mL/min. From 0 to 18 minutes, the movable phase consisted of 95% solvent A and 5% solvent B. Between 18 and 25 minutes, the composition gradually shifted to 5% solvent A and 95% solvent B. At 25 minutes, the ratio briefly returned to 95% solvent A and 5% solvent B until 25.10 minutes. Finally, from 25.10 to 30 minutes, the gradient was changed back to 5% solvent A and 95% solvent B.

2.5 Antioxidant Activity

The rummaging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was evaluated with slight modifications to the protocols described by[29]. In this protocol, 10 µL of the plant extract was mixed with 290 µL of the DPPH solution in a 96-well plate. The plate was protected in the dark for 20 minutes. After the evolution period, the absorbance was measured at 517 nm. A blank solution was used as the control, and the absorbance of the samples was compared to that of the blank solution to measure their free radical scavenging activity. Ascorbic acid was used as the standard. The IC50 value (µg/mL) was the concentration of the extract required to scavenge 50% of the DPPH free radicals.

$$\text{DPPH Scavenging Activity \%} = \left[\frac{OD1-OD2}{OD1}\right] X100$$

Where, OD1 and OD2 are the Optical density of control and test sample respectively.

2.5 Antidiabetic Activity

Alpha-amylase inhibition assay

Methanolic and water extracts were diluted to appropriate concentrations (0–200 µL). Each dilution was then supplemented with 0.4 mg/mL (800 µL) of porcine pancreatic alpha-amylase and incubated in a 0.02 M sodium phosphate buffer for ten minutes at 25°C (pH 6.9; 0.006 M NaCl). Following this, 600 µL of a 0.5% starch solution in the same sodium phosphate buffer was added to the tubes. After nursing the reaction mixtures for ten more minutes at 25°C, 0.5 mL of dinitrosalicylic acid color reagent was added to stop the reaction. After 10 minutes of boiling in a water bath, the combinations were allowed to cool to room temperature. After cooling, distilled water was added to dilute the reaction solutions to a total volume of 20 mL.

The alpha-amylase inhibitory activity was measured by calculating the percentage of inhibition based on the absorbance readings obtained at 540 nm.[30]

Alpha-glucosidase inhibition assay

Both the methanolic and aqueous extracts underwent repeated dilution. The mixture was then nurtured for 10 minutes at 25°C after 100 µL of a 0.1 M phosphate buffer (pH 6.9) containing an alpha-glucosidase solution (1.0 U/mL) was added. The solutions were then mixed with 50 µL of a 5 mM solution of p-nitrophenyl-alpha-d-glucopyranoside in 0.2 M phosphate buffer (pH 6.7). After that, the samples were once more nurtured at 25°C for ten minutes. A spectrophotometer was used to detect the absorbance at 405 nm following the incubation period. Based on these findings, the alpha-glucosidase inhibition percentage was computed[30].

$$\text{\% inhibition} = \left(1 - \frac{As}{Ac}\ X\ 100\right)$$

Where As and Ac is the absorbance in the presence of the sample and the control respectively.

2.5 Anti-inflammatory activity

Protein denaturation (BSA) inhibition assay

Bovine serum albumin was utilized as the test protein to evaluate the inhibition of protein denaturation. A 0.2% BSA solution was prepared in phosphate-buffered saline at pH 6.4, consisting of 10 mM Na_2HPO_4, NH_4PO_4, and 150 mM NaCl. Indomethacin was used as the standard drug at various concentrations. The mixed reaction volume contained 900 µL of BSA and 100 µL of plant extract. The solution was excited to 70°C for 15 minutes and then allowed to cool. All measurements were taken at 280 nm, except for rare instances where turbidity was used as a control. In these cases, only BSA was used as the control. The percentage inhibition of protein denaturation was calculated using a conventional formula.[31]

$$\text{\% Protein denaturation inhibition} = \left[\frac{OD2}{OD1} - 1\right] \text{x } 100$$

Where, OD1 and OD2 are the Optical density of the control and test sample, respectively.

Proteinase Inhibition(Trypsin) Assay

This work investigated the potential of plant extracts as trypsin inhibitors. The reaction mixture contained 60 mL of Tris-HCl buffer (20 mM, pH 6.4), 40 mL of 0.2% w/v casein (substrate) and 50 µL of each plant sample or a standard Indomethacin as the inhibitor. The Room temperature initial reaction was for 10 min. Subsequently, 50 µL of trypsin solution (concentration: 60 mg/mL) was introduced into the reaction mixture. This preparation was then kept at 38°C for 20 minutes to allow the enzymatic activity to proceed. After the incubation period, the solution's absorbance at 280 nm was measured. For reference purposes, a blank sample containing only trypsin and casein was also prepared and analyzed under identical conditions.[31]

$$\text{Inhibition of Proteinase activity} = \frac{OD1-OD2}{OD1} \text{ x } 100$$

Where, OD_1 and OD2 are the optical density of Control and test sample, respectively.

2.5 Cell Cytotoxicity

The National Cell Centre (NCCS) in Pune, India, provided the HepG-2 cell line.

The tumorous cells were placed in a flask full of 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781), 2-10% (fetal bovine serum) FBS, and DMEM (Dulbecco's Modified EagleMedium-AT149-1L) media. The flask was then incubated at 35°C with 5% CO2. These adherent cells were trypsinized for 3–5 minutes, nurtured at 37°C for 24 hours and then centrifuged (1,400 rpm for 5 min). Ten thousand cells were counted per 96-well ELISA plate. The plate was protected at 37°C with 5% CO_2 for 24 h to permit cells to be attached[32].

MTT Assay

The MTT colorimetric test was used to assess cell viability with a few modest adjustments[32]. In short, 200 µl of MTT [3-(4, 5-dimethylthiazol-2)-2, 5-diphenyl tetrazolium bromide] without phenol red (yellowish-coloured solution; 5 mg/ml in PBS) was added per well at a volume of 20 µl and the plates were nurtured for 3 hours under a standard atmosphere containing 5% CO_2 to enable metabolically active cells reduce MTT by dehydrogenase enzymes which produces reducing equivalents (NADH and NADPH). The corresponding purple insoluble formazan crystals were then solubilized and supernatants removed prior to spectrophometric measurement.

100 µL of DMSO was added to each well to dissolve the MTT crystals. After shuddering for 15 minutes, the optical densities were measured at 580 nm using a Thermo Scientific Multiskan Sky Plate Reader spectrophotometer. The absorbance levels correlate with the number of viable cells. Each experiment was performed in duplicate. Percentage cell viability was calculated by dividing the test sample absorbance by the control absorbance (medium without sample) and multiplying by 100.

$$\% \text{ Viable cells} = \frac{A(test)}{A(Control)} \times 100$$

Where, A(test) and A(Control) is Absorbance of the test sample and the Absorbance of the control.

2.5 Statistical Analysis

The mean ± standard deviation (SD) of two separate experiments, each carried out in triplicate, are used to display the data. Using GraphPad Prism 8 Statistical Software (San Diego, California, USA), one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests were used to evaluate statistical differences between the treatments and the control group.

A statistically significant P-value was defined as < 0.05. The primary results were also calculated using Microsoft Excel formulae.

3. Results

3.1. Phytochemical Screening

Several phytochemicals listed below were found in the aqueous and methanolic sources of *Boerhavia diffusa* leaves after qualitative screening. Both of the mines contained the same phytochemicals[33]. But the methanolic extract only failed the anthraquinones test (Table 1).

Table 1: List of different phytochemicals present in aqueous and methanolic extracts of theBoerhavia diffusaleaves

Test	Aqueous Extract	Methanolic Extract
Alkaloids	+	+
Carbohydrates	+	+
Glycoside	+	+
Phenol	+	+
Amino Acid	+	+
Flavonoids	+	+
Anthraquinones	+	-
Terpenoids	+	+
Saponins	+	+

Determination of Total Phenolic and Flavonoid Content

The B. diffusa methanolic and aqueous extracts had total phenolic values of 93.34 ± 0.024 and 73.34 ± 0.035 mg of gallic acid equivalents per gram of extract (mg GAE/g), respectively. The B. diffusa aqueous and methanolic extracts were found to have total flavonoid concentrations of 43.03 ± 0.023 and 52.30 ± 0.009 mg quercetin equivalents per gram of extract (mg QE/g), respectively. These values were determined using calibration curves constructed using gallic acid and quercetin as standard references. The detailed results are presented in Table 2.

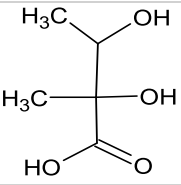
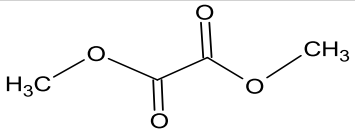
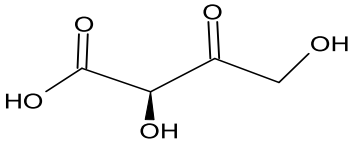
Table 2: Phenolic and Flavonoid Contents of B. diffusa extract (n=3)

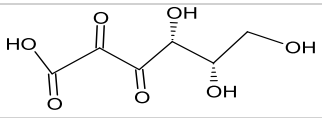
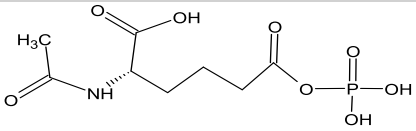
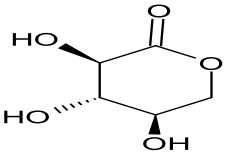
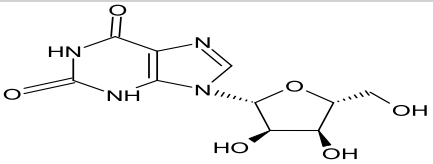
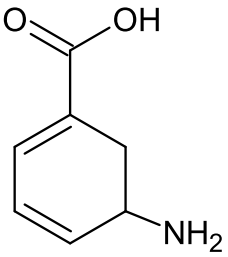
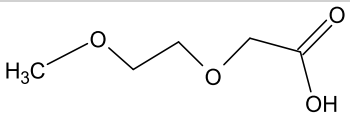
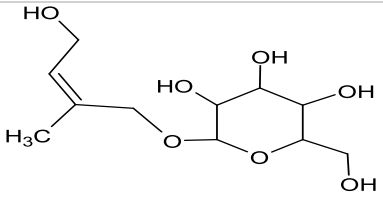
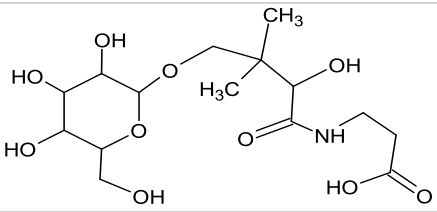
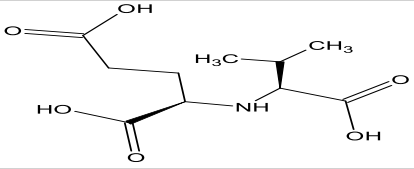
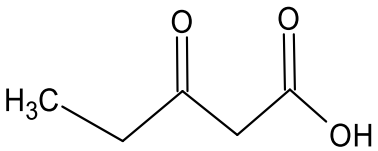
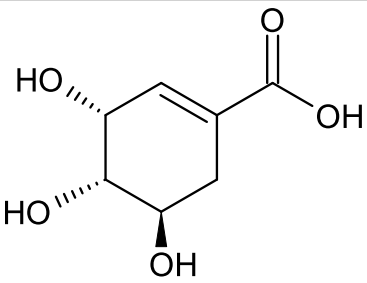
B. diffusa extract	Phenolic Content mg GAE/g	Flavonoid Content mg QE/g
Aqueous extract	73.34 ± 0.035	43.03 ± 0.023
Methanolic extract	93.34 ± 0.024	52.30 ± 0.009

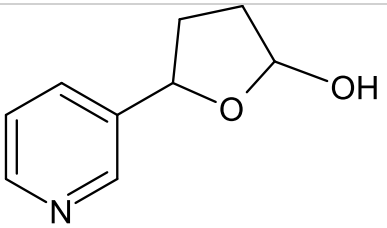
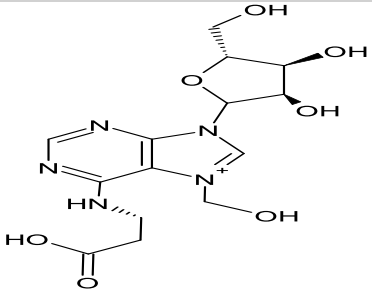
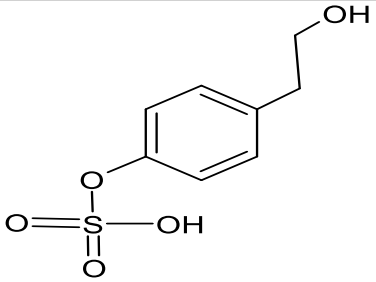
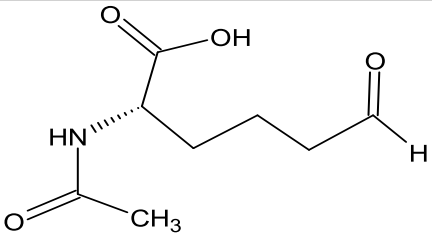
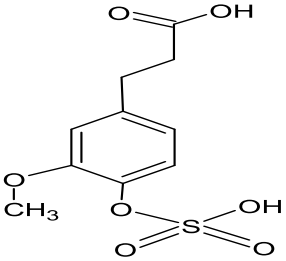
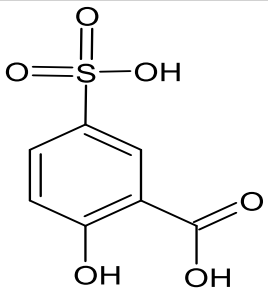
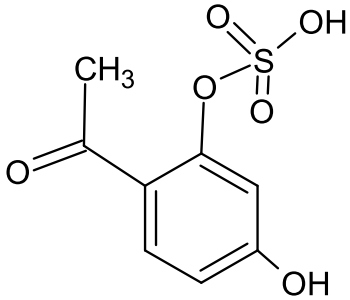
3.2 Metabolite profiling of the extracts by UPLC-QTOF-MS

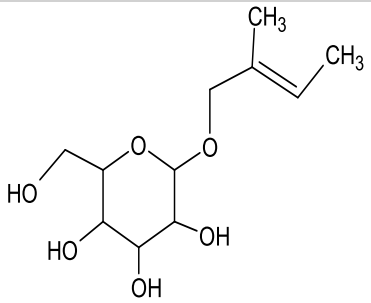
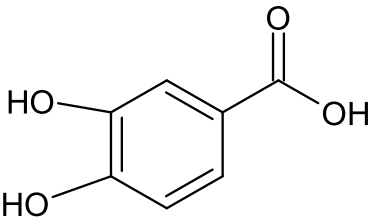
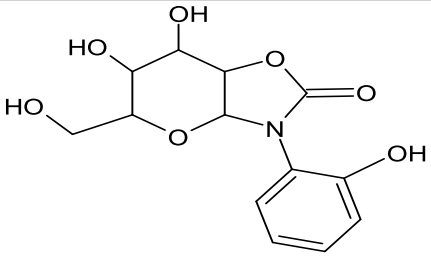
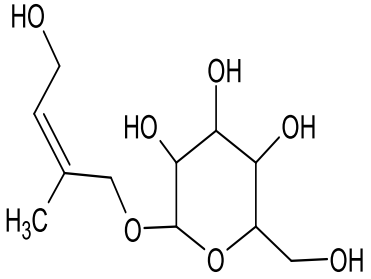
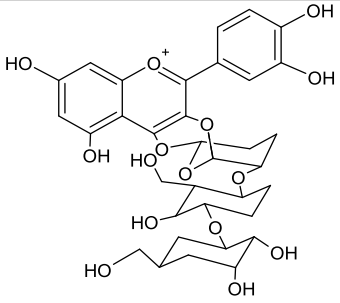
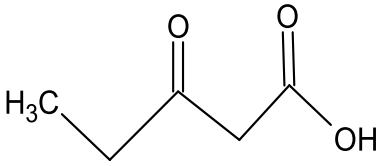
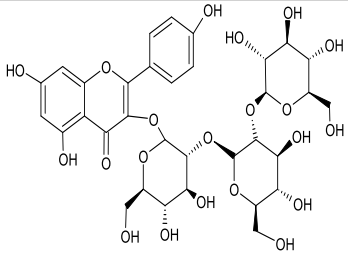
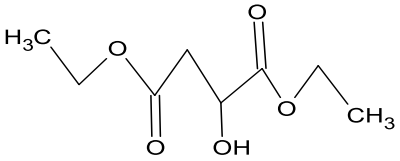
LCMS analysis was conducted on both plant extracts, revealing the occurrence of the compounds listed in Table 3 and Table 4.

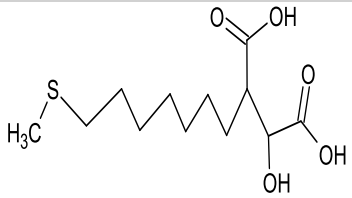
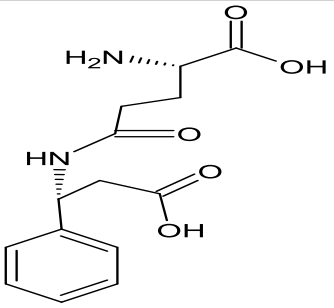
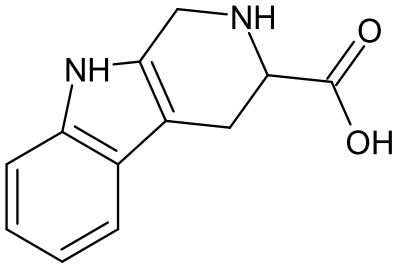
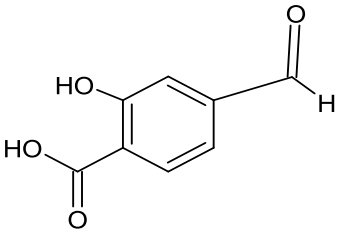
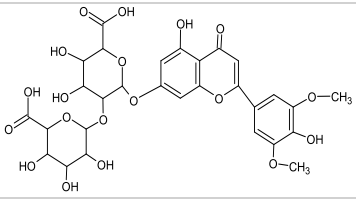
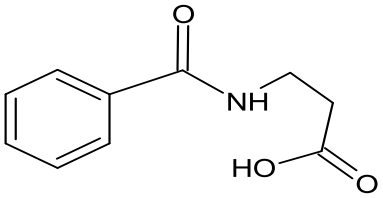
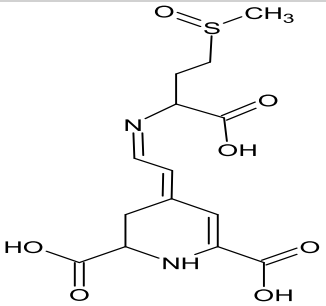
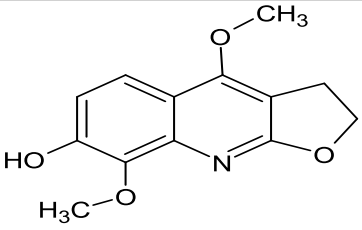
Table 3: Phytochemical compounds identified from the aqueous extracts of the Boerhavia diffusa leavesoid Contents of B. diffusa extract (n=3)

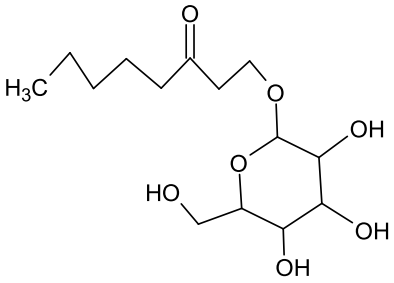
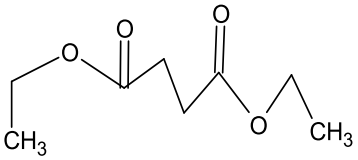
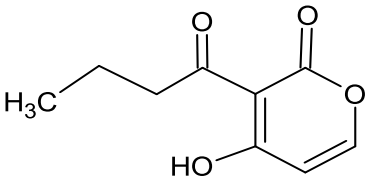
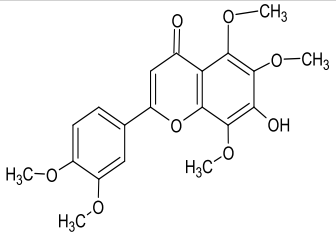
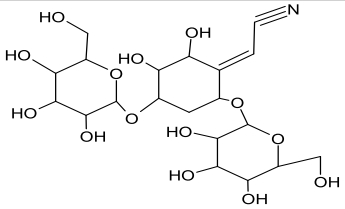
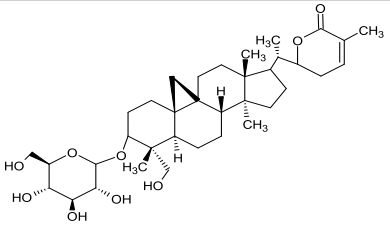
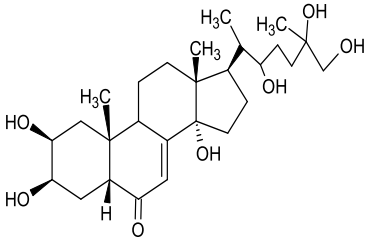
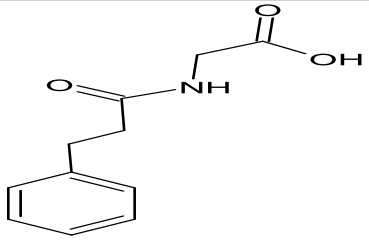
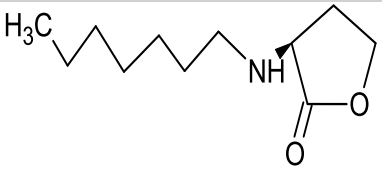
Sr. No.	Compounds	Retention time(min)	Molecular Mass	Structure	Group
1.	2,3-Dihydroxy-2 methylbutanoic acid	2.459	134.0575		Fatty acid
2.	Methyl oxalate	2.579	118.0262		Carboxylic acid
3.	3-Dehydro-L-threonate	2.725	134.0213		Carboxylic acid

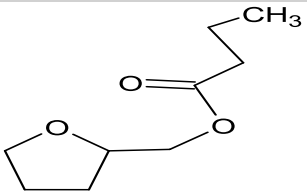
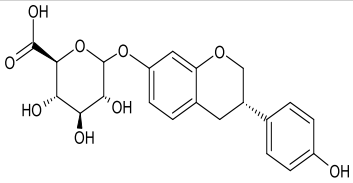
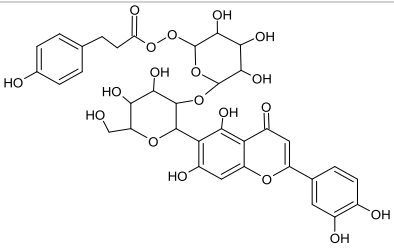
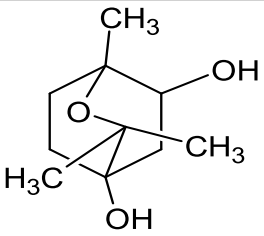
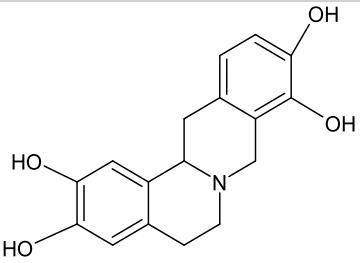
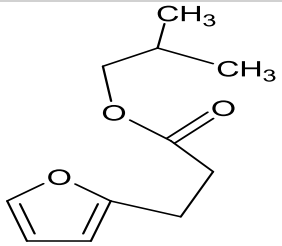
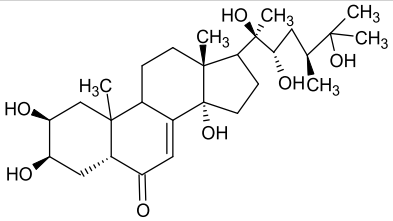
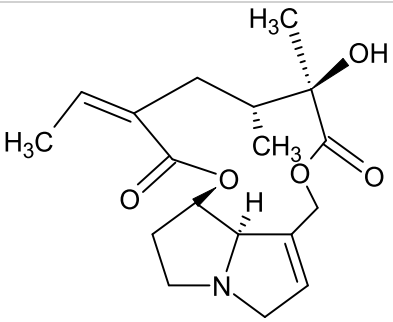
4.	2,3-Diketo-L-gulonate	2.926	192.0266		Carboxylic acid
5.	N2-Acetyl-L-aminoadipylδ-phosphate	2.939	283.0460		Amino Acid
6.	D-Xylono-1,5-lactone	3.131	148.0370		Lactone
7.	Xanthosine	3.362	284.0750		Alkaloid
8.	Gabaculine	3.397	139.0632		Amino Acid
9.	(2-Methoxyethoxy)propanoic acid	3.696	148.0731		Carboxylic Acid
10.	Ilicifolinoside A	3.764	264.1203		Carbohydrate
11.	(R)-Pantothenic acid 4'-O-b-D-glucosid	3.812	381.1627		Carbohydrate
12.	Valinopine	3.973	247.1046		Alkaloid
13.	3-keto valeric acid	4.194	116.0470		Carboxylic acid
14.	Shikimic acid	4.339	174.0525		Amino acid

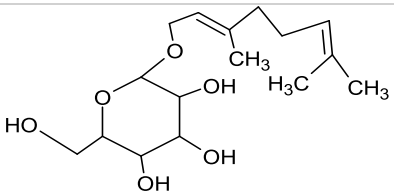
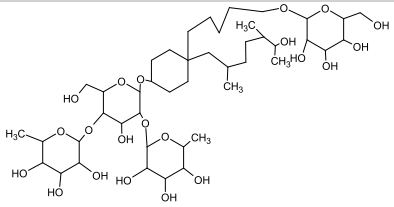
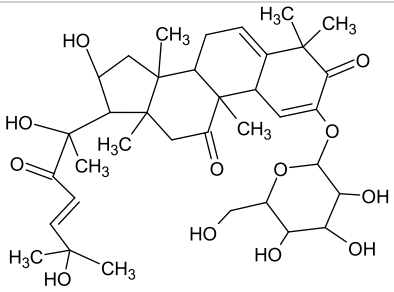
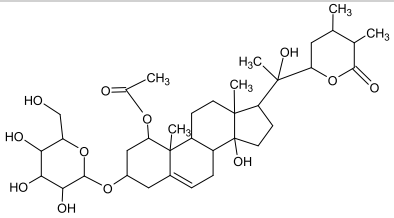
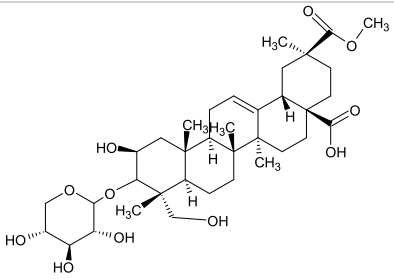
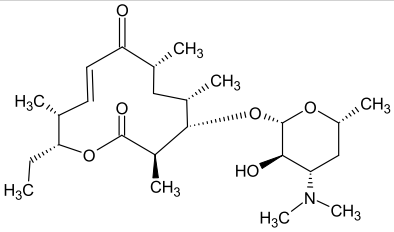
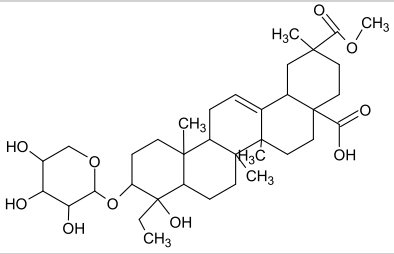
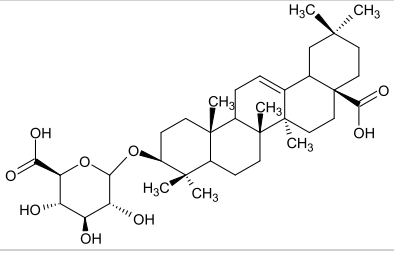
15.	5-(3-Pyridyl)-2-hydroxytetrahydrofuran	4.461	165.0789		Lactol
16.	Succinoadenosine	5.420	383.1071		Aspartic Acid
17.	Tyrosol 4-sulfate	5.479	218.0246		Phenolic
18.	N2-Acetyl-L-aminoadipate semialdehyde	5.481	187.0838		Amino Acid
19.	Dihydroferulic acid 4-sulfate	5.542	276.0296		Phenolic
20.	5-Sulfosalicylic acid	5.918	217.9879		Phenolic
21.	2,4-Dihydroxyacetophenone 5 sulfate	6.174	232.0033		Phenolic

22.	(E)-2-Methyl-2-buten-1-ol-O-beta-D-Glucopyranoside	6.234	248.1253		Carbohydrate
23.	3,4-Dihydroxybenzoic acid	6.663	154.0262		Phenolic acid
24.	Hexahydro-6,7-dihydroxy-5-(hydroxymethyl)-3-(2-hydroxyphenyl)-2H-pyrano[2,3-d]oxazol-2-one	7.063	297.0840		Phenol
25.	Ilicifolinoside A	8.403	264.1205		Cabohydrate
26.	Quercetin3-glucosyl-(1 >2)-galactosyl-(1->2) glucoside	9.695	788.2037		Flavonoid
27.	3-keto valeric acid	9.707	116.0473		Carboxylic acid
28.	Kaempferol3-O-β-D glucosyl	9.711	132.0785		Flavonoid
29.	Diethyl L-malate	10.082	190.0837		Carboxylic Acid

30.	3-(7' Methylthio)heptylmalic acid	10.136	278.1185		Carboxylic Acid
31.	Glutamylphenylalanine	10.137	294.1216		Carboxylic Acid
32.	L-1,2,3,4-Tetrahydro beta-carboline-3 carboxylic acid	10.502	216.0895		Carboxylic Acid
33.	4-Formylsalicylic acid	10.613	166.0263		Phenol
34.	Tricin 7-diglucuronoside	10.619	682.1395		Flavonoids
35.	Betamipron	10.832	193.0738		Amino Acid
36.	Miraxanthin-I	11.182	358.0830		Amino acid
37.	Haplopine	11.607	245.0683		Alkaloid

38.	1-(beta-D Glucopyranosyloxy)-3 octanone	12.409	306.1668		Glycosides
39.	Diethyl succinate	12.590	174.0888		Fatty acid
40.	4-Hydroxy-6-methyl-3-(1oxobutyl)-2H-pyran-2-one	12.706	196.0733		Lactone
41.	7-Hydroxy-3',4',5,6,8 .pentamethoxyflavone	12.762	388.1152		Flavonoid
42.	Dide-O-methyl-4-O alpha-D glucopyranosylsimmondsin	12.763	509.1729		Glycoside
43.	abrusoside A	12.823	646.3717		Saponin
44.	26-Hydroxyecdysone	12.862	480.3094		Steroid
45.	Phenylpropionylglycine	12.933	207.0892		Glycine
46.	N-heptanoyl-homoserine lactone	13.040	213.1357		Lactone

47.	Tetrahydrofurfuryl butyrate	13.097	172.1097		Fatty acid
48.	Equol 7-O-glucuronide	13.130	418.1261		Flavonoid
49.	Isoorientin	13.147	756.1923		Flavonoid
50.	(1R,2R,4R)-1,8-Epoxy-p menthane-2,4-diol	14.548	186.1250		Terpenoid
51.	2,3,9,10-Tetrahydroxyberbine	14.642	299.1149		Alkaloid
52.	Isobutyl 2-furanpropionate	15.556	196.1096		Fatty acid
53.	Makisterone A	16.337	494.3242		Steroid
54.	Senecionine	16.456	335.1728		Alkaloid

55.	Neryl glucoside	17.180	316.1882		Glycosoide
56.	Pseudoprotodioscin	18.956	1030.5373		Saponin
57.	Cucurbitacin I 2-glucoside	19.086	676.3477		Terpenoid
58.	1-Acetyl-3,14,20 trihydroxywitha-5,24 dienolide 3-glucoside	19.376	678.3637		Glycoside
59.	Phytolaccoside B	19.473	664.3834		Terpenoid
60.	10-Deoxymethymycin	19.781	453.3086		Glycoside
61.	Phytolaccoside A	22.145	648.3886		Saponin
62.	Oleanolic acid 3-O-beta-D glucosiduronic acid	22.923	632.3934		Terpenoid

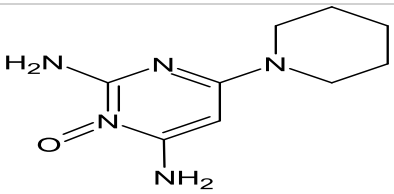
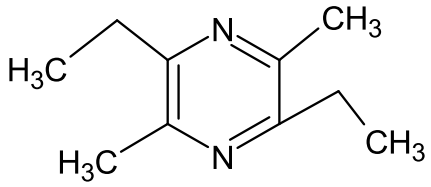
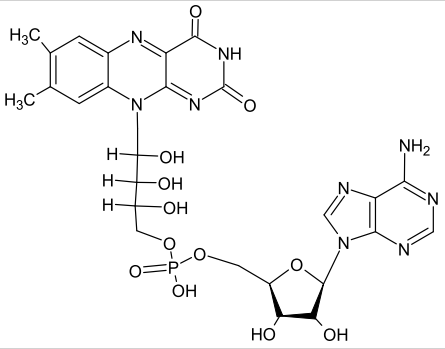
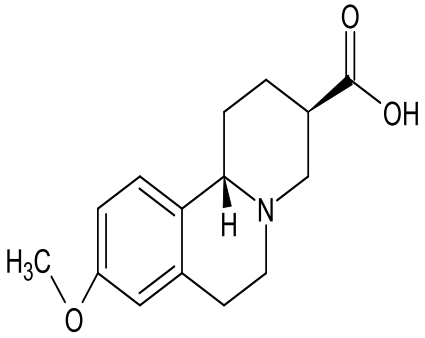
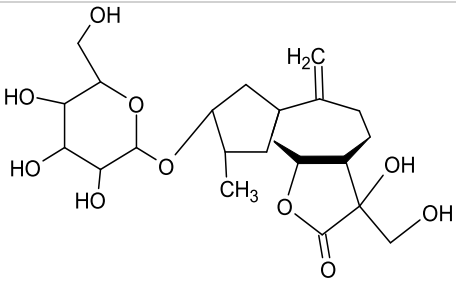
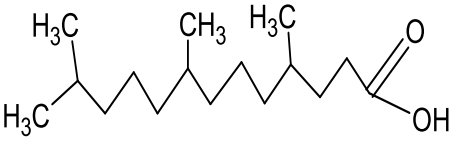
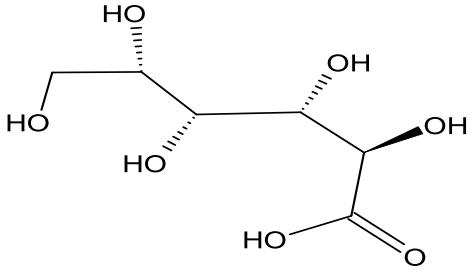
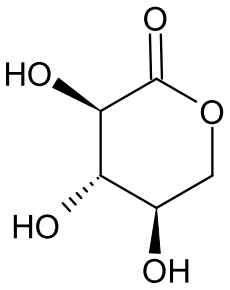
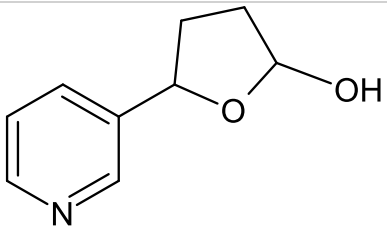
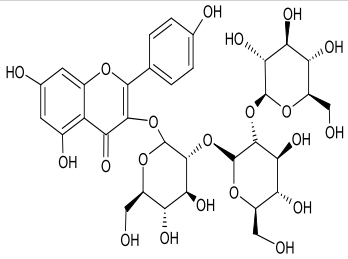
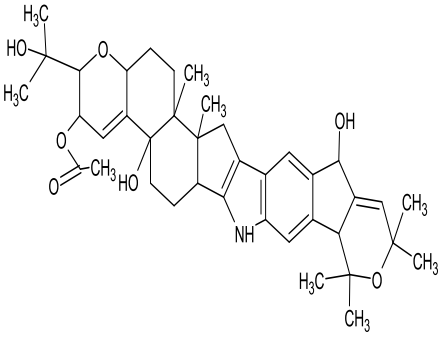
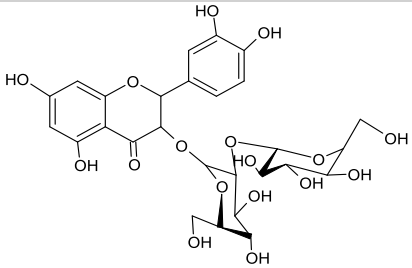
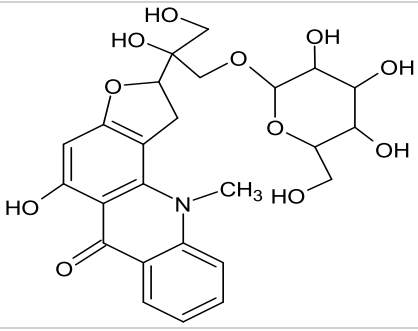
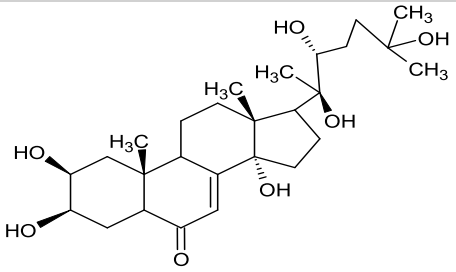
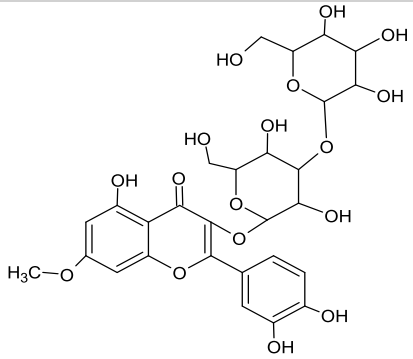
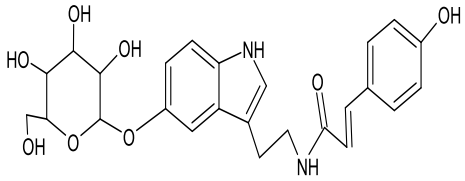
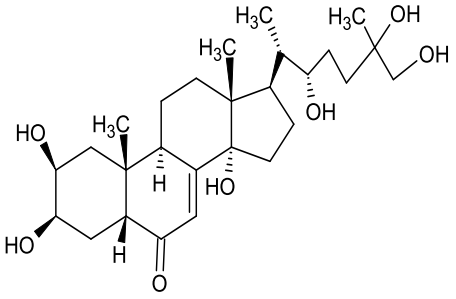
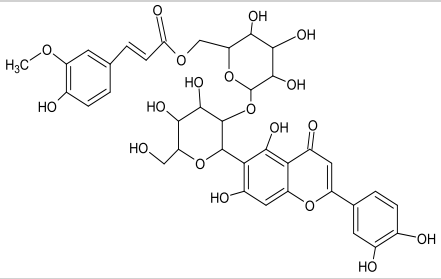
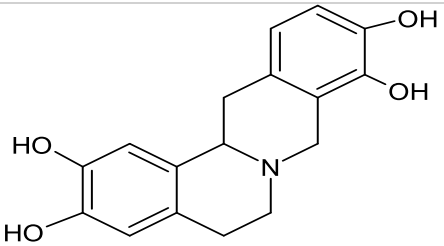
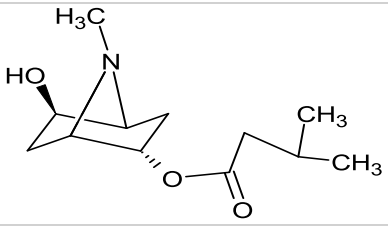
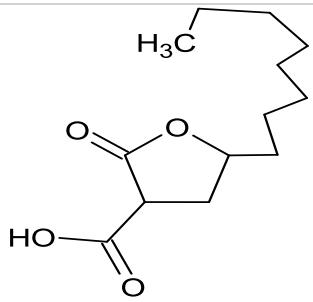
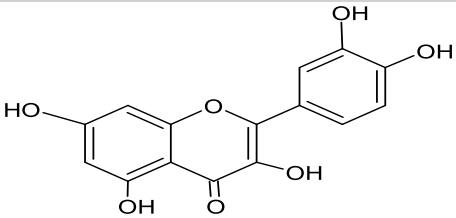
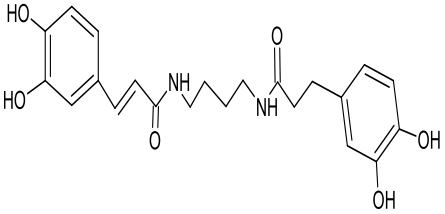
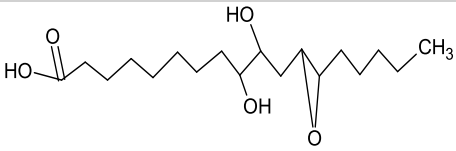
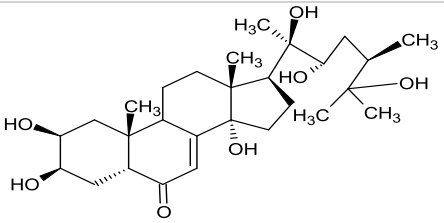
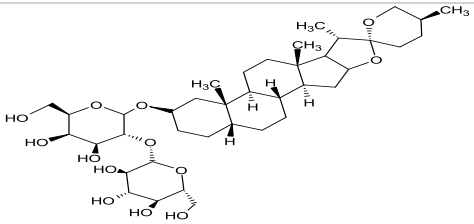
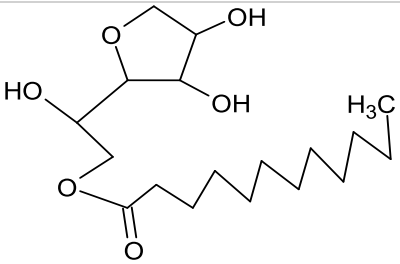
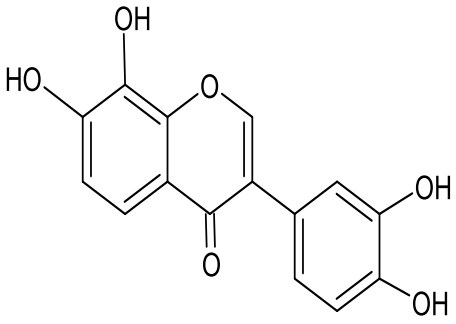
63.	Minoxidil	6.415	209.1277		Amines

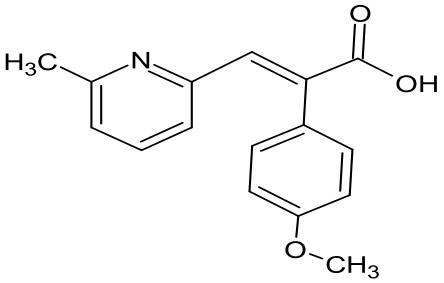
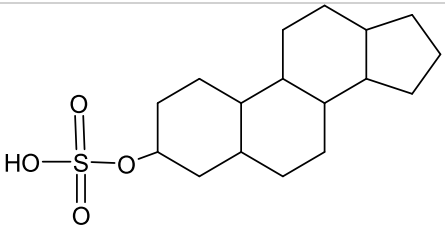
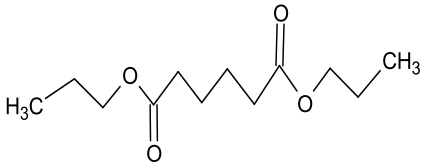
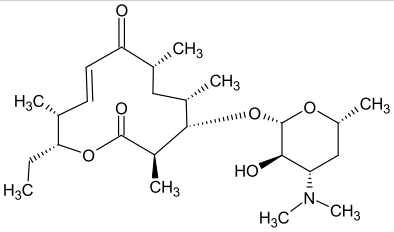
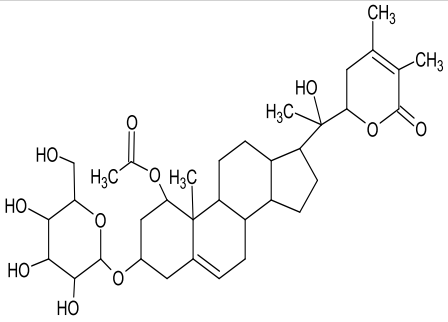
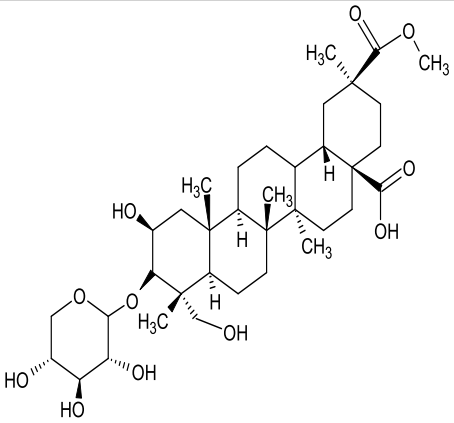
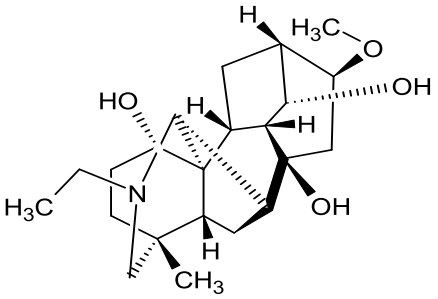
Table 4: Phytochemical compounds identified from the methanolicextracts of theBoerhavia diffusa leaves

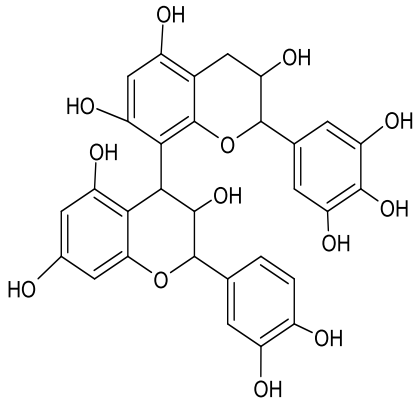
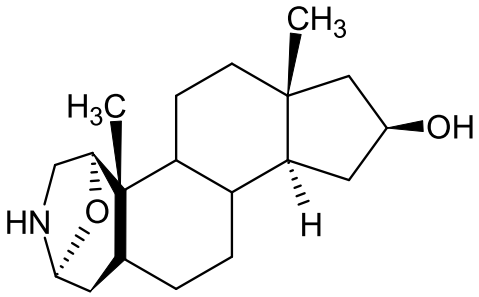
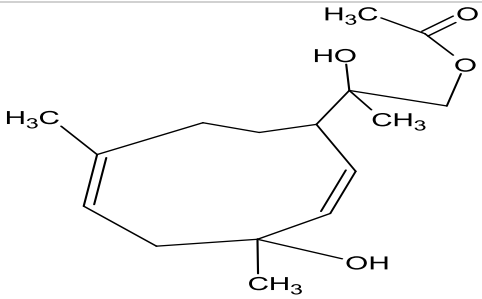
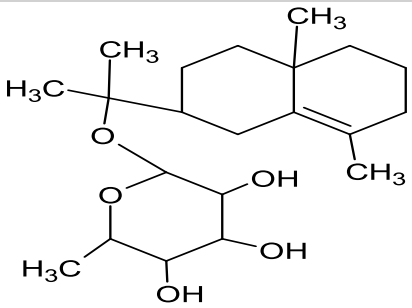
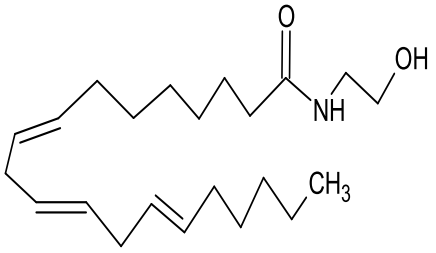
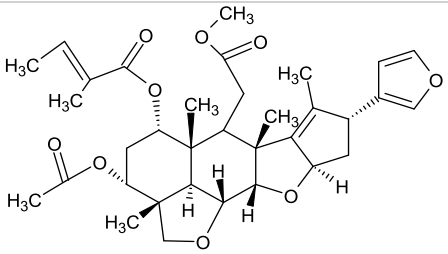
Sr. No.	Compounds	Retention time(min)	Mass	Structure	Group
1.	2,5-Diethyl-3,6 dimethylpyrazine	10.210	164.1320		Fatty acid
2.	Flavin adenine dinucleotide	13.162	785.1585		Amino acid
3.	16-Oxo-palmitate	16.862	270.2198		Fatty acid
4.	cis-1,3,4,6,7,11b Hexahydro-9-methoxy2H-benzo[a]quinolizine 3-carboxylic acid	17.781	261.1357		Carboxylic acid
5.	Cynaroside A	21.825	444.1991		Lactone
6.	Palmitic amide	22.995	255.2552		Fatty acid
7.	Tridecanoicacid, 4,8,12-trimethyl-; 4,8,12 Trimethyltridecanoic acid	24.974	256.2397		Fatty acid

8.	Gulonic acid	2.178	196.0577		Carbohydrate
9.	D-Xylono-1,5-lactone	3.272	148.0373		Lactone
10.	5-(3-Pyridyl)-2 hydroxytetrahydrofuran	4.453	165.0787		Lactol
11.	Kaempferol 3-O-β-D glucosyl	9.991	772.2087		Flavonoid
12.	Janthitrem F	11.468	645.3662		Terpene
13.	Quercetin-3-sophoroside	11.771	626.1486		Flavonoids
14.	Gravacridonetriol glucoside	12.122	519.1742		Glucoside

15.	Ecdysterone	12.251	480.3082		Steroid
16.	Rhamnetin-3-laminaribioside	12.337	640.1648		Flavonoid
17.	Nb-trans-p-Coumaroylserotonin glucoside	12.790	484.1844		Glycoside
18.	26-Hydroxyecdysone	12.849	480.3092		Steroid
19.	Isoorientin-2''-[feruloyl-(>6)-glucoside]	12.977	786.2031		Flavonoid
20.	2,3,9,10 Tetrahydroxyberbine	14.630	299.1149		Alkaloid
21.	Valeroidine	14.965	241.1673		Alkaloid

22.	2-Carboxy-4-dodecanolide	15.486	242.1510		Gamma lactone
23.	Quercetin	15.858	302.0421		Flavonoids
24.	Dicaffeoylputrescine	15.948	412.1630		Alkaloid
25.	9,10-Dihydroxy-12,13 epoxyoctadecanoate	16.303	330.2398		Fatty acid
26.	Makisterone A	16.316	494.3236		Steroid
27.	Timosaponin A-III	16.699	740.4363		Saponin
28.	Sorbitan laurate	17.035	346.2353		Fatty acid
29.	7,8,3',4' Tetrahydroxyisoflavone	17.299	286.0470		Flavonoid

30.	alpha-(p-Methoxyphenyl)-6 methyl-2-pyridineacrylic acid	17.388	269.1046		Carboxylic acid
31.	Steryl sulfate	17.712	328.1711		Steroid
32.	Dipropyl hexanedioate	18.260	230.1514		Fatty acid
33.	10-Deoxymethymycin	18.504	453.3081		Glycoside
34.	1-Acetyl-3,14,20 trihydroxywitha-5,24-dienolide 3-glucoside	19.359	678.3627		Glycoside
35.	Phytolaccoside B	19.448	664.3832		Triterpenoid
36.	Karakoline	20.462	377.2566		Alkaloid

37.	Epicatechin-(4beta->8) gallocatechin	20.913	594.1382		Flavonoid
38.	Samandarine	21.866	305.2350		Alkaloid
39.	(1(10)E,4a,5E)-1(10),5 Germacradiene-12-acetoxy 4,11-diol	22.159	296.1983		Terpenoid
40.	gamma-Eudesmol rhamnoside	22.282	368.2557		Terpenoid
41.	Dihomo-γ-linolenoyl-EA	24.454	349.2974		Lipid
42.	Salannin	26.465	596.2971		Terpene
43.	(+)-Prosopinine	29.195	313.2611		Alkaloid

44.	16-Hydroxy hexadecanoic acid	29.344	272.2350		Fatty acid

3.3 Antioxidant Assay

In this study, plant extracts were tested at deliberations of 20, 40, 60, 80, and 100 µg/ml for their free radical scavenging activity. Results (Table 5) showed that the highest concentration (100 µg/ml) exhibited strong antioxidant properties, effectively neutralizing free radicals. Both methanolic and aqueous extracts showed enhanced scavenging abilities compared to lower concentrations, as illustrated in Figure 1.

Table 5: Antioxidant (DPPH assay) activity of B. diffusa extract and its IC50 value compared to the standard drug. Data Represent Mean ± SEM (n=3)

Concentration (µg/ml)	% Scavenging activity of aqueous extract	% Scavenging activity of methanolic extract	% Scavenging activity of ascorbic acid (Standard)
20	9.36 ± 0.001	13.89 ± 0.004	11.41 ± 0.002
40	12.67 ± 0.006	39.37 ± 0.056	28.83 ± 0.001
60	32.19 ± 0.001	60.76 ± 0.003	38.81 ± 0.001
80	40.46 ± 0.001	66.81 ± 0.015	67.38 ± 0.003
100	66.46 ± 0.001	71.21 ± 0.002	79.44 ± 0.002
IC50(µg/ml)	**75.233 ± 0.001**	**49.374 ± 0.003**	**59.364 ± 0.003**

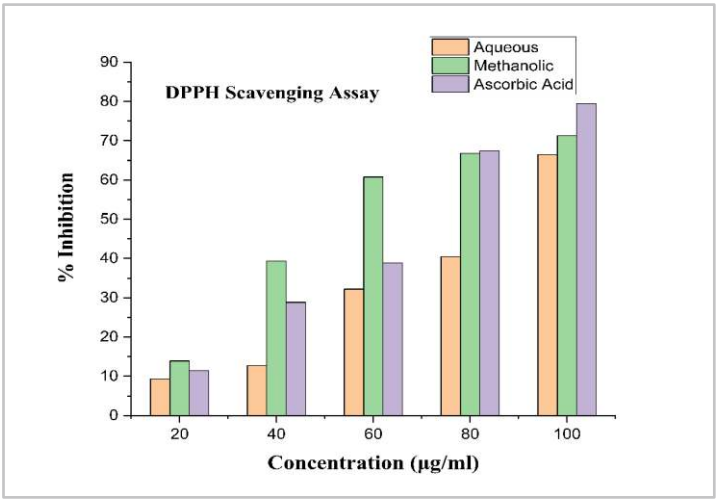


Figure 1: The antioxidant capacity of methanolic and aqueous extracts obtained from Boerhavia diffusa leaves

3.4 Antidiabetic Activity

Figure 2 illustrates the interaction of *Boerhavia diffusa* leaf extracts with α-amylase, specifically focusing on both aqueous and methanolic extracts.

Both extracts exhibit a dose-dependent inhibition of enzyme activity across the deliberation range of 20 to 100 µg/ml. The IC50 values, which indicate the potency of the inhibitory effect, are as follows: the aqueous extract has an IC50 of 71.85 ± 0.003 µg/ml, while the methanolic extract shows a lower IC50 of 56.29 ± 0.003 µg/ml. For comparison, the standard reference compound acarbose has an IC50 of 59.27 ± 0.001 µg/ml. Additionally, Figure 2 presents the effects of both aqueous and methanolic *Boerhavia diffusa* leaf extracts on α-glucosidase. As with α-amylase, both extracts inhibit the activity of α-glucosidase in a dose-dependent manner (20–100 µg/ml). The IC50 values for α-glucosidase inhibition are 79.34 ± 0.001 µg/ml for the aqueous extract and 58.59 ± 0.004 µg/ml for the methanolic extract.

The extract exhibited a strong, dose-dependent reticence of both α-amylase and α-glucosidase activity. In fact, it performed similarly to, and in some cases even exceeded, the efficacy of acarbose. These results clearly indicate that the *B. diffusa* extract contains potent constituents that inhibit both α-amylase and α-glucosidase. Complete data can be found in Tables 6 and Table 7.

Table 6 : In vitro Anti-diabetic (α-amylase) activity of B. diffusa extract and its IC50 value compared to the standard drug. Data Represent Mean ± SEM (n=3)

Concentration (µg/ml)	α-amylase inhibition % of aqueous extract	α-amylase inhibition % of methanolic extract	α-amylase inhibition % of acarbose(standard)
20	19.81 ± 0.009	16.69 ± 0.005	19.11 ± 0.007
40	32.68 ± 0.001	35.38 ± 0.002	34.32 ± 0.001
60	41.75 ± 0.003	53.29 ± 0.003	50.61 ± 0.001
80	59.95 ± 0.002	67.38 ± 0.001	66.19 ± 0.001
100	75.78 ± 0.007	81.05 ± 0.002	83.97 ± 0.001
IC_{50} (µg/ml)	**71.85 ± 0.003**	**56.29 ± 0.003**	**59.27 ± 0.001**

Table 7 : In vitro Anti-diabetic (α-glucosidase) activity of B. diffusa extract and its IC50 value compared to the standard drug Data Represent Mean ± SEM (n=3)

Concentration (µg/ml)	α-glucosidase inhibition % of aqueous extract	α-glucosidase inhibition % of methanolic extract	α-glucosidase inhibition % of acarbose(standard)
20	6.17 ± 0.002	18.66 ± 0.002	9.34 ± 0.001
40	22.35 ± 0.001	29.86 ± 0.004	22.01 ± 0.002
60	38.42 ± 0.003	51.20 ± 0.004	42.82 ± 0.002
80	50.41 ± 0.001	62.64 ± 0.002	56.12 ± 0.002
100	71.72 ± 0.002	76.70 ± 0.002	80.10 ± 0.001
IC_{50} (µg/ml)	**79.34 ± 0.001**	**58.59 ± 0.004**	**71.27 ± 0.002**

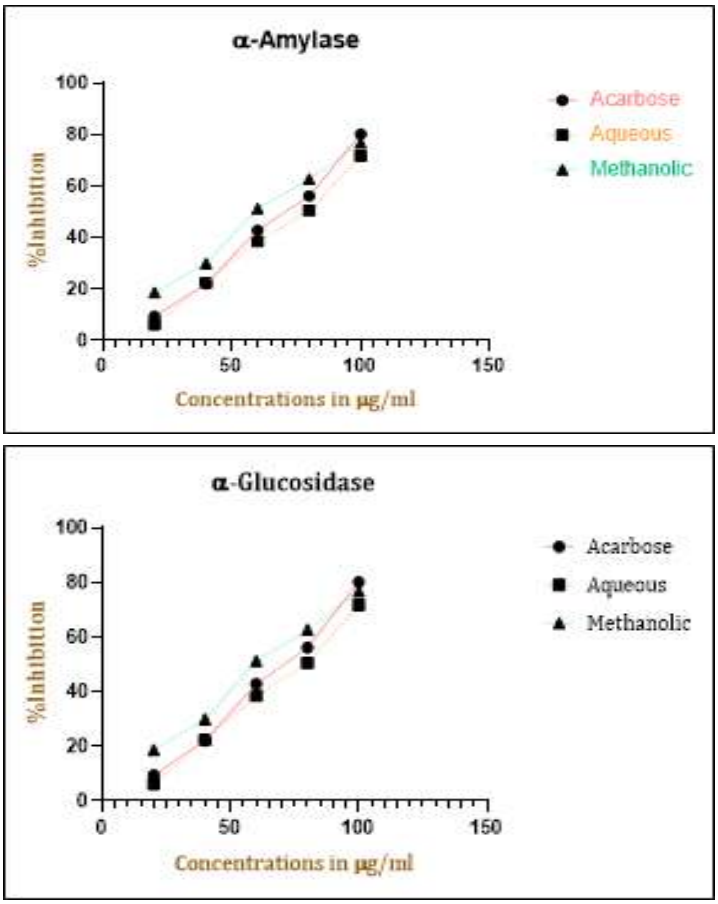


Figure 2: The inhibitory effects of Boerhavia diffusa aqueous and methanolic extracts on alpha-amylase and alpha-glucosidase

3.5 Anti-Inflammatory Activity

Protein denaturation inhibition assay(Bovine Serum Albumin)

Protein denaturation is a major source of inflammation. Ability of extracts to deter protein denaturation was determined as a further part of the investigation for the anti-inflammatory mechanism. Protein denaturation or its breakdown at higher temperature is enhanced and will become formation of peptides aggregates. At the various quantities shown in Figure 3, the plant demonstrated effectiveness against heat-induced albumin denaturation. With an IC50 value of 75.25 ± 0.003 µg/ml, the aqueous extract shows the maximum level of protein denaturation, whereas the methanolic extract has an IC50 value of 58.89 ± 0.011µg/ml.

Proteinase inhibition assay(Trypsin)

There is information linking proteinases to the arthritic response. Figure 3 shows the substantial antiprotease activity of the *B. difusa* plant parts in both aqueous and methanolic extracts at different doses.Aqueous and methanolic extracts exhibited their highest inhibitory effect at IC50 - 80.93 ± 0.003 µg/ml and IC50 -57.60 ± 0.003 µg/ml, respectively.

A dose-dependent increase in inhibition was observed for both the extract and the standard compound, as presented in Tables 8 and 9. These findings indicate that the *B. difusa* extract exhibits moderate inhibitory activity against BSA denaturation and trypsin inhibition assays compared to indomethacin. Complete data are presented in Table 8 and 9.

Table 8: In vitro Protein denaturation inhibition assay (Bovine Serum Albumin) effect of B. diffusa aqueous and methanolic extract and IC50 value in Comparison to the standard. Data are presented as the Mean Value ± SEM (n = 3)

Concentration (µg/ml)	BSA inhibition % of aqueous extract	BSA inhibition % of methanolic extract	BSA inhibition % of indomethacin(standard)
20	17.12 ± 0.008	19.14 ± 0.008	22.77 ± 0.005
40	27.12 ± 0.006	35.58 ± 0.040	38.36 ± 0.006
60	46.91 ± 0.020	58.94 ± 0.011	55.99 ± 0.007
80	53.15 ± 0.003	67.62 ± 0.002	70.01 ± 0.003
100	66.55 ± 0.003	76.08 ± 0.004	83.42 ± 0.003
IC_{50} (µg/ml)	**75.25 ± 0.003**	**58.89 ± 0.011**	**53.58 ± 0.007**

Table 9: In vitro Proteinase inhibition assay (Trypsin) effect of B. diffusa aqueous and methanolic extract, and IC50 value in Comparison to the standard. Data are presented as the Mean Value ± SEM (n = 3)

Concentration (µg/ml)	Trypsin inhibition % of aqueous extract	Trypsin inhibition % of methanolic extract	Trypsin inhibition % of indomethacin(standard)
20	13.74 ± 0.015	16.69 ± 0.014	21.98 ± 0.004
40	16.27 ± 0.004	28.78 ± 0.002	39.88 ± 0.004
60	30.19 ± 0.005	52.08 ± 0.003	57.67 ± 0.007
80	44.46 ± 0.005	58.85 ± 0.003	67.45 ± 0.004
100	61.78 ± 0.003	76.05 ± 0.003	81.17 ± 0.005
IC_{50} (µg/ml)	**80.93 ± 0.003**	**57.60 ± 0.003**	**52.02 ± 0.007**

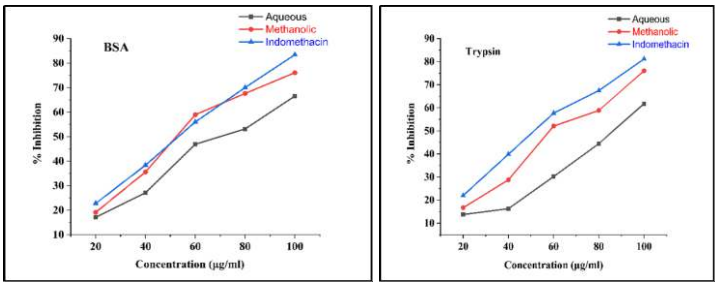


Figure 3: Assessment of the anti-inflammatory potential of Boerhavia diffusa leaf extracts was carried out through protein denaturation inhibition and proteinase inhibition assays, utilizing both methanolic and aqueous solvents for extraction

3.6 Cytotoxic Assay

To exclude that inhibitory effects of selected parts of *B. diffusa* extract are connected to cytotoxicity, we have assessed cell viability by the MTT test. Cell viability was measured using the MTT assay, based on conversion of yellow tetrazolium salt to a purple formazan product (Fig. 4). As seen in Table 10, the viability of HepG-2 cells was more than 60% for treatments relative to control (HepG-2 cells not treated), which demonstrated that the different parts of *B. diffusa* plant were not toxic to the cells at the concentrations used. IC50 of aqueous extract of *B. diffusa* leaves was 351.1 ± 0.1258 µg/ml.

Table 10: Cell cytotoxicity of B. diffusa leaf aqueous extract on HepG-2 cell line using MTT Assay. Data are presented as the Mean Value ± SEM (n = 3)

Concentration (µg/ml)	Cell viability (%) of B. diffusa leaf aqueous extract
0	100 ± 1.37
1	91.98 ± 1.76
10	80.14 ± 1.60
50	72.13 ± 1.07
100	66.12 ± 1.86
250	57.92 ± 1.53
500	46.81 ± 1.58
1000	41.34 ± 0.95

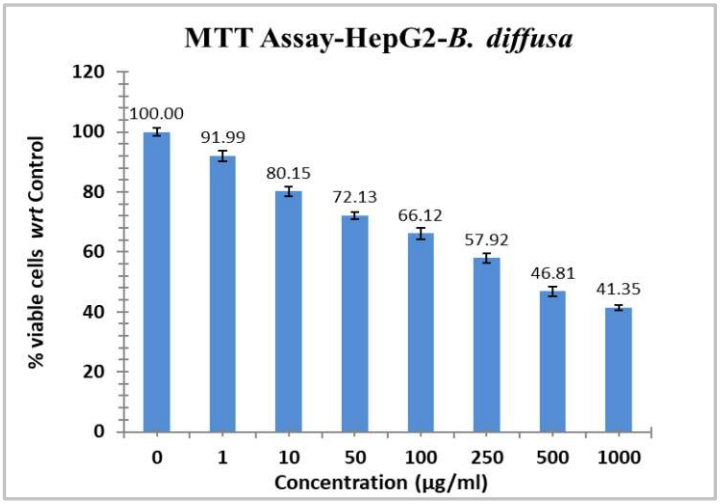


Figure 4: Anticancer probable of aqueous extract of theBoerhavia diffusa leaves extract on HepG-2 cell line

4. Discussion

The current study explored the in vitro antidiabetic, anti-inflammatory, and antioxidant potential of aqueous and methanolic extracts, as well as assessed cell cytotoxicity on the HepG-2 cell line using the aqueous leaf extract.Phytochemical studies depicted the presence of phytoconstituents. Quantitative phytochemical evaluation of *B. diffusa* reveals a higher concentration of phenolic compounds compared to flavonoids. The total phenolic content was measured at 93.34 ± 0.024mg/g in the methanolic extract and 73.34 ± 0.035mg/g in the aqueous extract. In contrast, the flavonoid content was found to be 43.03 ± 0.023mg/g in the methanolic extract and 52.30 ± 0.009mg/g in the aqueous extract. These findings suggest that phenolics are the predominant class of secondary metabolites in *B. diffusa*[34-37], with potential implications for its antioxidant and therapeutic properties.[23]Our study used a methanolic and aqueous extract on traditional spectroscopic methods,[38] or advanced techniques like LC-MS, or UPLC-QTOF-MS/MS.[39]

Among the various plant parts of *B. diffusa*, the leaves exhibit the maximum concentration of phenolic compounds, correlating with superior free radical scavenging activity[40]. The antioxidant efficacy of these compounds is attributed to their intrinsic redox properties, which assist them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. This activity is further enhanced by the presence of hydroxyl functional groups, which play a critical role in neutralizing reactive oxygen species[41-42].The IC50 value for the antioxidant potential of aqueous extract showing 75.233 ± 0.001 µg/ml and that of methanolic extract showing 49.374 ± 0.003 µg/ml. Therefore, the methanolic extractis more effective in scavenging free radicals and exhibits higher antioxidant activity than the aqueous extract.

The extract also displayed moderate anti-inflammatory activity, as evidenced by its inhibition of BSA denaturation and trypsin inhibition assay. This effect may be attributed to compounds Tyrosol 4-sulfate, Dihydroferulic acid 4-sulfate, 5-Sulfosalicylic acid, 2,4-Dihydroxyacetophenone 5 sulfate, Hexahydro-6,7-dihydroxy-5-(hydroxymethyl)-3-(2-hydroxyphenyl)-2H-pyrano[2,3-d]oxazol-2-one, Kaempferol 3-O-β-D glucosyl-(1->2)-β-D glucosyl-(1->2)-β-D glucoside, Quercetin-3-sophoroside, Rhamnetin-3-laminaribioside, Isoorientin-2''-[feruloyl-(>6)-glucoside] known for their anti-inflammatoryproperties.[43] However, while this assay is preliminarily acceptable for assessing anti-inflammatory activity.

The extract demonstrated notable inhibitory activity against key carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase.[44] The methanolic extract exhibited superior α-amylase inhibition (IC50 = 56.29 ± 0.003 µg/mL) compared to the pharmaceutical reference standard acarbose (IC50 = 59.27 ± 0.001 µg/mL), while the aqueous extract also showed appreciable activity (IC50 = 71.85 ± 0.003 µg/mL). Similarly, α-glucosidase inhibition was more pronounced in the methanolic extract (IC50 = 58.59 ± 0.004 µg/mL) relative to acarbose (IC50 = 71.27 ± 0.002 µg/mL), with the aqueous extract yielding an IC50 of 79.34 ± 0.001 µg/mL. These findings suggest potential antidiabetic properties of the extract, likely attributable to its bioactive constituents. However, the current evidence is confined to in vitro assays. Therefore, further investigations are warranted to evaluate in vivo efficacy and safety, isolate and characterize the active phytochemicals, and assess the therapeutic potential through clinical studies.[45]

The MTT assay was utilized to evaluate the cytotoxic effects of *B. diffusa* aqueous extract on HepG-2 cells[46]. Figure 4 illustrates the dose-dependent cytotoxic effect of the aqueous extract of *B. diffusa* on HepG-2 cells. Acquaintance to 1 µg/mL of the extract for 3 hours resulted in a modest reduction in cell viability to 91.99%. Increasing the concentration to 500 µg/mL and 1000 µg/mL led to a more pronounced decline in viability, reaching 46.81% and 41.35%, respectively. The half-maximal inhibitory concentration (IC50) of the aqueous extract was calculated to be 351.1 ± 0.1258 µg/mL, indicating significant antiproliferative activity against HepG-2 cells.[47]

The study demonstrates that *B. diffusa* leaves contain substantial levels of phenolics and flavonoids, which contribute to their notable antioxidant, antidiabetic and anti-inflammatory properties. The extracts also exhibited measurable cytotoxicity against the HepG-2 cell line, indicating additional therapeutic relevance. Collectively, the findings highlight *B. diffusa* as a promising candidate for developing plant-based therapeutics. Future research should focus on isolating the key active constituents, clarifying their mechanisms of action, and validating their safety and efficacy through comprehensive *in vivo* and clinical evaluations.

Conclusion

The current investigation aimed to assess the biological activities of *Boerhavia diffusa* leaf extract sourced from the arid region of Nagpur, Maharashtra. The results indicate that the extract possesses substantial antioxidant activity, demonstrated by its ability to scavenge free radicals, which may contribute to its overall pharmacological benefits. Additionally, a notable anti-inflammatory effect was observed, suggesting that the extract could influence inflammatory pathways associated with chronic metabolic and degenerative conditions. Furthermore, the study highlighted promising antidiabetic effects, indicating that the phytochemicals found in *B. diffusa* leaves may help regulate blood sugar levels and maintain

metabolic balance. The extract also demonstrated hepatoprotective properties, supporting its traditional use in medicine for liver-related issues. It appears to mitigate oxidative stress and biochemical changes linked to liver dysfunction. This protective effect may be accredited to the existence of various bioactive phytochemicals, including flavonoids and phenolics, which were characterized using UPLC-QTOF-MS analysis.

Despite these promising findings, the study has certain limitations. All biological evaluations were conducted in vitro, and the use of plant material from a single geographical location may not fully represent the chemotypic diversity of *B. diffusa*. Therefore, the results should be interpreted as preliminary.

Collectively, the present findings substantiate the potential of *B. diffusa* as a promising source of bioactive phytochemicals. Future investigations should emphasize the isolation and characterization of individual compounds, elucidation of underlying molecular mechanisms through target-based studies, and validation of pharmacological efficacy and safety using in vivo and clinical models.

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Ethical Approval

This study does not apply to human or animal studies that require ethical approval.

Credit author statements

Shivani R. Sharma (Conceptualization, designing the experiments, phytochemical analysis, biological activity assays, data collection, analysis and interpretation, writing-review and editing,Nilima M. Dhote (provided critical guidance in the experimental design and methodology, optimizing the phytochemical extraction and analysis technique), Mamta S. Wagh (Conceptualization, formal analysis, Data curation, visualization, Supervision, writing-original draft).

Conflict of interest statement

The authors declare that there are no conflicts of interest amongst them.

Data availability statement

The relevant authors can provide the data used to support the study's conclusions upon request.

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