JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/34179
RESEARCH ARTICLE

Phytochemical Profiling and *In Silico* Approach for Hepato-protective Activity of Various Extracts of *Boerhavia diffusa* Linn

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer. Approximately 30-40 percent of HCC patients are eligible for curative therapy, primarily surgical resection, percutaneous ablation, and liver transplantation. **Aim:** To development of state-of-the-art therapies for HCC is highly needed. *Boerhavia diffusa* Linn leaves have been reported to have medicinal benefits, including antioxidant and anti-inflammatory properties. **Methods:** Chemical constituents of various solvent extract from *B. diffusa* Linn. and were studied and found that the methanol extract contains more phyto-constituents. **Results:** Compounds with the highest composition in methanol extracts, namely inositol, tridecanoic acid, 11,14,17-eicosatrienoic acid and phytol, were docked against various targets such as human cyclin-dependent kinase 2 (CDK2), capase 3, and NRF2 involved in HCC were studied. **Conclusion:** Phytol shown negative binding energies values higher than others, suggesting that it may have anti-hepato carcinoma potential.

Major Findings: Phytochemicals present in the methanol extract showed anticancer activity, suggesting *Boerhavia diffusa* as a therapeutic potential for hepatocellular carcinoma.

Keywords: *B. diffusa* Linn, Capase 3, Human Cyclin Dependent Kinase 2 (CDK2), NRF2 – R34G Codon

1. Introduction

One of the main causes of cancer-related death is HCC. The effectiveness of the many treatments that have been suggested is still very much in debate[1]. Approximately thirty to forty percent of those suffering from HCC are eligible for curative therapies, that include surgical excision as priority, percutaneous ablations, and liver transplantation[2]. However, Asia and Africa had a high incidence rate of HCC. It was reported as the 3rd rank in cancer death around the world[3]. Hepatocellular carcinoma staging must be accurate in order to determine each patient's prognosis and the most effective course of treatment[4].

Different parts of the plant extracts have versatile therapeutic properties and have been widely recognized[5,6]. Over 200 medicinal plant species have been incorporated throughout ancient Ayurvedic literature, in addition to descriptions regarding how plants are employed in healing[7,8]. Among these, the entire plant of *B. diffusa* Linn was traditionally utilised to treat syphilitic ulcers and wounds[9]. In accordance with numerous ethnopharmacological studies, several Indian tribes rely on both the leaves and roots of *B. diffusa* for the treatment of a wide range of ailments. *B. diffusa* L. has been referred to by the name Punarnava in the conventional medical system of India[10,11]. It is a periodic crawling herb that spreads in profusion throughout India. *B. diffusa* has

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Article Received on: 22.06.2023 Revised on: 13.02.2024 Accepted on: 19.02.2025

recently been found to have a wide range of traditional pharmacological uses[12]. Based on cross-sectional imaging studies and serum alpha-fetoprotein (AFP) analysis in the recent years HCC identified at the early[1,13]. It is expected that the HCC threat will continue to increase[14,15].

In the current work, we utilised GCMS, qualitative and quantitative analysis of multiple extracts of *B. diffusa* Linn to characterize the phytoconstituents. The extract containing the higher composition of the phytochemicals were evaluated further evaluated through molecular docking and their binding affinities were predicted against the NRF2-R34G, Human cyclin dependent kinase 2, and Caspase 3 targets of HCC.

2. Materials and Methods

2.1 Extraction

Boerhavia diffusa Linn was evaluated and extracted with distilled water, methanol, ethanol, benzene, and hexane using Soxhlet's equipment. The solvents were subsequently extracted from the extracts utilizing a rotating vacuum evaporator, and the filtrate was then stored in a desiccator for use later.

2.2 Phytochemical Analysis

Preliminary phytochemical studies were carried out as per standard procedures[16].

2.2.2 Quantitative Determination of Phytochemicals

2.2.2.1 Determination of Total Alkaloids

About 1 mg/mL equivalent of extract had been dispersed in DMSO and I mL of 2 N HCl and filtered. The filtrate was added with bromo-cresol green solution and phosphate buffer each 5 mL was added, shaken and made up to 10 mL with chloroform. The procedure was followed for both samples and the standard atropine. Finally, the absorbance was measured at 470 nm[17].

2.2.2.2 Determination of Total Flavonoids

Utilizing a colorimetric technique applying aluminium chloride, total flavonoids were identified. A mixture of 1 mL extract with 3 mL distilled water was mixed with 0.3 mL of a 5% $NaNO_2$, 0.3 mL of a 10% $AlCl_3$, and 2 mL of IM NaOH solutions, and the final volume was made up to 10 mL with distilled water. The procedure was followed for both samples and the standard quercetin. Finally, the absorbance was measured at 510 nm[17].

2.2.2.3 Determination of Total Saponins

About 15g of the extract was mixed with 100 mL of 20 % ethanol and heated at 45° C for 3-4hr with steady stirring. The same procedure was repeated twice, and finally 20 mL of diethyl ether and the aqueous layer was obtained while the ether layer was eliminated. Then n-butanol was added to the aqueous phase, and rinsed with 5% NaCl solution. The procedure was followed for both samples and the standard GinsenosideRbl. Finally, the absorbance was measured at 550 nm[17].

2.2.2.4. Determination of Total Tannins

The technique developed by Folin-Ciocalteu was employed to determine the total tannins. A small amount over 1 mL of each extract was diluted with 5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, and 1.5 mL of a 35% sodium carbonate solution, and total volume was made upto 10 mL. The procedure was followed for both samples and the standard gallic acid. Finally, the absorbance was measured at 725 nm[17].

2.2.2.5 Determination of Total Phenolics

The Folin-Ciocalteu test procedure was employed for estimating total phenolics. The extract was diluted with 8 mL of distilled water and 1 mL of the Folin-Ciocalteu phenol reagent and mixed well. Then 10 mL of a 7% Na_2CO_3 solution was added and the total volume was made upto 25 ml, and incubated for 60 minutes. The procedure was followed for both samples and the standard gallic acid. Finally, the absorbance was measured at 550 nm[17].

2.3 GC-MS Analysis of the Extract

Gas chromatography paired with Mass spectrometry (GCMS) QP2010 plus, Shimadzu, Japan, combined to utilise a RTX-5 MS GC capillary of 0.5 m diameter and 30 m length, was used. Working conditions for GC: The value of the temperature was progressively increased by 8°C/min and maintained within 40°C and 280°C. Temperatures for the column oven and injection were established at 110°C and 260°C respectively. Helium was utilized as the gas carrier (mobile phase) using a flow rate of 1 mL/min. The ion source and interface temperatures for the MS were 210°C and 250°C. The detector voltage was set at 0.1 kV, and the solvent cut-off period was 3 min, injection temperature was 240°C; 20–300 m/z mass range. The analytes were compared in NIST and Wiley libraries[18].

2.4 Molecular Docking

To predict the binding affinities of the hit compounds from the GCMS, they were docked against the various targets involved in HCC[19,20]. Docking against the crucial targets is a potential was to inhibit the cancer and overcome its resistance[21]. The 3D structure of the HCC receptor were obtained from the Protein Data Bank similarly ligand from the PubChem and covert as PDBQT files sutible for docking process in AutoDock 4.2. The default parameter was set to point out the receptor pockets[21]. And the binding affinity and energies were identified[22].

3. Result and Discussion

3.1 Qualitative Phytochemical Determination

Tables 1 and 2 represents the qualitative determination of ethanol, water, methanol, hexane and benzene extracts of *B. diffusa* Linn. The result reveals the presence of flavonoids, carbohydrates and steroids in all the extracts. Protein, amino acids, fats and oils, phenolics and saponins were absent in both hexane and benzene extracts. From the above results, its confirm that the methanolic extract had higher amount of phyto-contituents compared to other extracts.

3.2 Qualitative Phytochemical Determination

Table 3 represents the quantitative determination of all the extracts of *B. diffusa* Linn. and all the data were mentioned in SD. Total alkaloid content was higher (12.35+0.05) in methanolic extract. Saponin (0.65±0.05) and tannin (0.35+0.05) content was lesser in benzene extract. Tannin (0.25±0.05) and phenol (0.55+0.05) content was lesser in hexane extract. Comparatively, methanolic extract contains higher amount of alkaloid, flavonoid, and phenolic, and low tannin content in contrast to the other extracts, thus the methanolic extract has been taken into the future study.

3.3. GCMS Analysis

To identify the phytoconstituents that were present, GC-MS analysis was performed on the methanolic extract of *B. diffusa* Linn. GC-MS chromatograms were used to identify nearly 20 substances based on differences in peak area% and retention time (RT). Numerous

Table 1. Qualitative analysis of phytochemicals from *B. diffusa* leaf extracts

SL. No.	Photochemical	Analysing Method	Water Extract	Methanol Extract	Ethanol Extract	Benzene Extract	Hexane Extract
1	Phenolic	Ferric Chloride test	P	HP	P	N	N
2	Terpenoid	Copper acetate test	N	P	P	P	P
3	Tannin	Ferric Chloride test	P	N	P	N	P
4	Alkaloid	Mayer's test	P	HP	P	N	P
5	Saponin	Foam test	P	HP	N	N	N
6	Flavonoid	Sodium Hydroxide test	P	HP	HP	P	P
7	Steroid	Salkowski test	P	P	P	P	P
8	Amino acid	Ninhydrin test	N	P	P	N	N
9	Cardiac glycoside	Kellar Killani'stest	P	N	P	N	P

Table 2. Qualitative determination of nutrition of *B. diffusa* leaf extracts

SL. No.	Nutrition	Analysing Method	Water Extract	Methanol Extract	Ethanol Extract	Benzene Extract	Hexane Extract
1	Carbohydrate	Molisch's test	P	P	P	P	P
2	Protein	Millon's test	P	P	P	N	N
3	Fats and Oil (Fixed)	Saponification	P	P	P	N	N

(HP) denotes - Strong present, (P) denotes – Present, (N) denotes – Not detected.

Table 3. Quantitative analysis of phytochemicals from *B. diffusa* leaf extracts

S. No.	Extracts	Quantitative estimation (%)				
		Alkaloid /Atropine equivalent	Flavonoid / Quercetin equivalent	Saponin / Diosgenin equivalent	Tannin / Tannic acid equivalent	Phenol / Gallic acid equivalent
1	**Water extract**	5.65±0.25	10.47±0.25	8.24±0.05	2.35±0.05	3.25±0.25
2	**Methanol extract**	12.35±0.05	20.60±0.05	16.30±0.05	2.26±0.05	10.29±0.05
3	**Ethanol extract**	06.45±0.05	11.0±0.25	2.22±0.05	4.75±0.05	3.65±0.5
4	**Hexane extract**	2.35±0.05	1.65±0.05	1.15±0.15	0.55±0.05	0.25±0.05
5	**Benzene extract**	1.05±0.05	2.10±0.05	0.65±0.05	0.35±0.05	3.29 ±0.05

The mean value and standard deviation (SD) for each experiment were calculated.

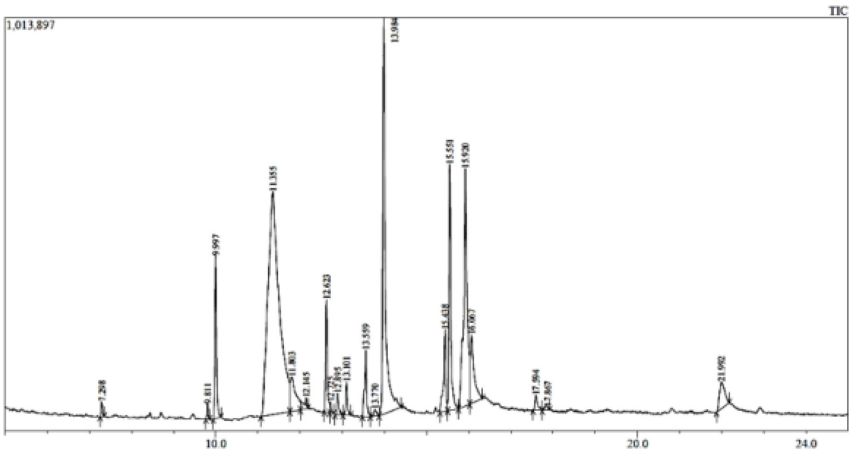


Figure 1. GCMS chromatogram.

Table 4. GCMS chromatogram analysis

Peak number	RT	Area%	Height %	A/H	Name
1	7.298	0.30	0.84	2.09	Oxalicacid, isobutylpentylester
2	9.811	0.30	0.84	2.09	Tetradecane (CAS)n- Tetradecane
3	9.997	4.10	9.11	2.67	1,2-Benzoldicarbonsaeure, di-(hex-1-en-5-yl-ester)
4	11.355	38.59	12.44	18.43	Mome Inositol
5	11.803	2.87	1.91	8.92	Hexadecanoicacid(CAS)Palmiticacid
6	12.145	0.63	0.62	6.09	Decane 3-methyl-(CAS)3- Methyldecane
7	12.623	2.78	6.29	2.63	Neophytadiene
8	12.725	0.25	0.62	2.35	2-Nonanone,9-hydroxy
9	12.895	0.67	1.23	3.23	1-Octadecyne
10	13.101	0.92	1.87	2.93	1-Tetradecyne
11	13.559	2.18	3.71	3.49	Eicosanoicacidmethylester CAS)Arachidic
12	13.770	0.28	0.39	4.22	1-Decyne
13	13.984	15.96	22.16	4.28	Tridecanoicacid
14	15.438	2.89	4.55	3.78	9,12,15- Octadecatrienoicacidmethyleter (2
15	15.551	7.04	13.76	3.04	Phytol
16	15.920	13.12	13.28	5.87	11,14,17-Eicosatrienoicacid,methylester
17	16.067	4.03	3.83	6.25	Dodecane (CAS)n-Dodecane
18	17.594	0.66	0.84	4.67	2,6-Bis(2- (dimethylamino)ethoxy]pyridine
19	17.867	0.24	0.20	7.06	Cyclopentane,(3-methylbutyl)-(CAS) Cyclop
20	21.992	2.18	1.50	8.66	1-Eicosanol

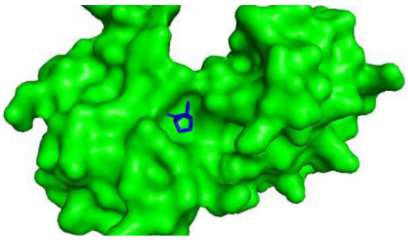


Figure 2. Surface model of NRF2-R34G codon with inositol interaction.

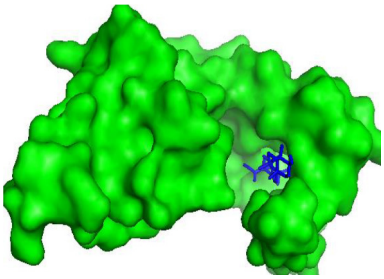


Figure 3. Surface model of NRF2-R34G codon with tridecanoic acid interaction.

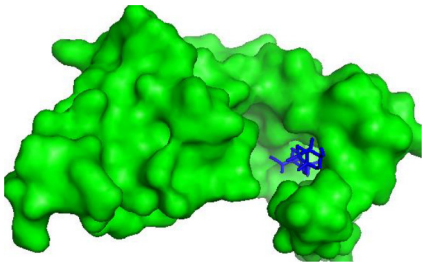


Figure 4. Surface model of NRF2-R34G codon with 11,14,17-eicosatrienoic acid interaction.

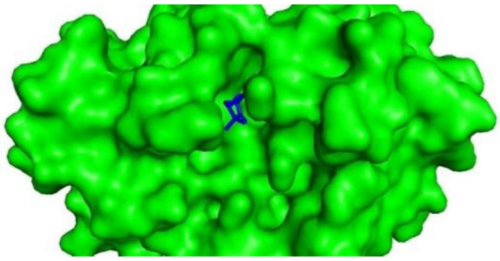


Figure 5. Surface model of NRF2-R34G codon with phytol interaction.

bioactive phytochemicals have been identified in the past, including phenolic acids, flavonoids, isoflavones, saponins and phytosterols. Oxalic acid was the first compound discovered with a retention time of 7.298 min, while 1-Eicosanol was the last with a retention time

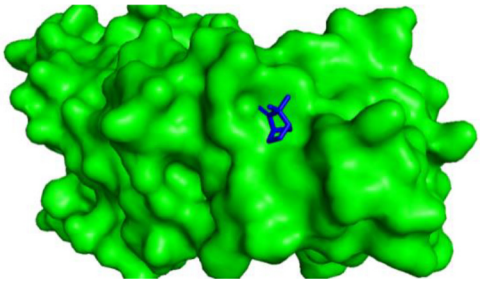


Figure 6. Surface model of human cyclin dependent kinase 2 (CDK2) with inositol interaction.

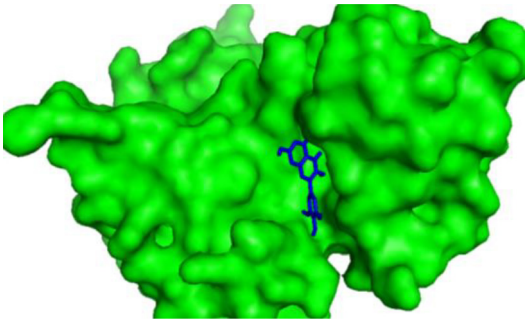


Figure 7. Surface model of human cyclin dependent kinase 2 (CDK2) with tridecanoic acid interaction.

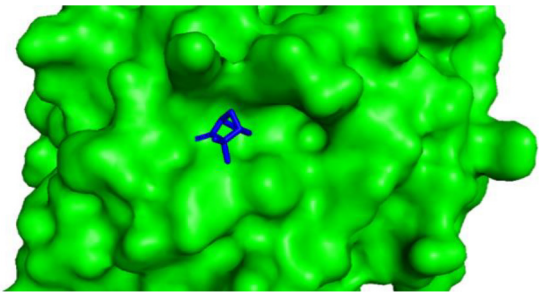


Figure 8. Surface model of human cyclin dependent kinase 2 (CDK2) with 11,14,17-eicosatrienoic acid interaction.

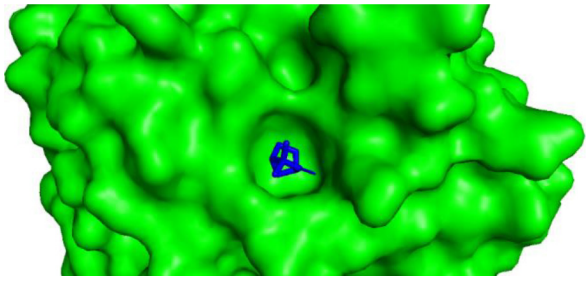


Figure 9. Surface model of human cyclin dependent kinase 2 (CDK2) with phytol interaction.

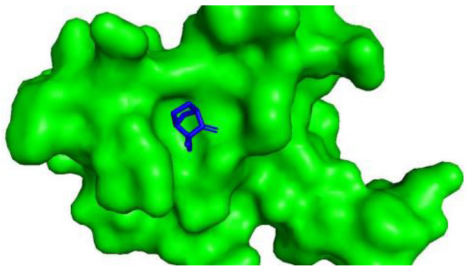


Figure 10. Surface model of caspase 3 with inositol interaction.

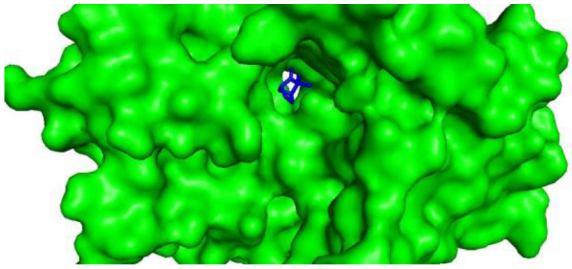


Figure 11. Surface model of caspase 3 with tridecanoic acid interaction

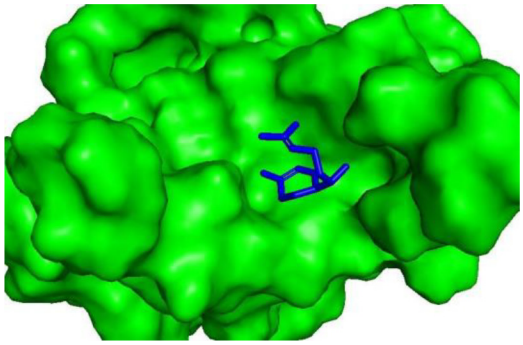


Figure 12. Surface model of caspase 3 with 11,14,17-eicosatrienoic acid interaction.

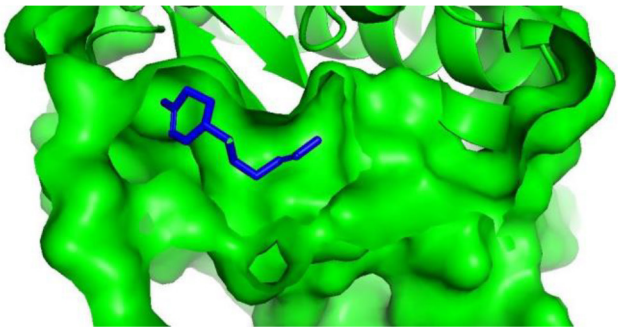


Figure 13. Surface model of caspase 3 with phytol interaction.

Table 5. Ligand and receptor interaction

Receptor	Ligand	Binding Energy (Kcal/mol)
NRF2-R34G Codon	Inositol	5.49
	Tridecanoic acid	6.96
	11,14,17-Eicosatrienoic acid	-2.21
	Phytol	-2.99
Human cyclin depen dent kinase 2 (CDK2)	Inositol	-4.36
	Tridecanoic acid	-5.33
	11,14,17-Eicosatrienoic acid	-4.65
	Phytol	-4.59
Caspase 3	Inositol	-4.12
	Tridecanoic acid	-2.34
	11,14,17-eicosatrienoic acid	-3.45
	Phytol	-4.89

of 21.992 min (Table 4 and Figure 1). When measured at 11.355 RT and 18.89 area%, MOMEINOSITOL was found. From the mentioned findings, we learned that the phytochemical compositions found in the methanolic extract of *B. diffusa* Linn. leaves varied greatly.

3.4. Molecular Docking

The top four highly present phytoconstituents in the methanolic extract of *B. diffusa* Linn were selected for the molecular modeling studies from the GCMS analysis. These substances were docked against the human cyclin dependent kinase 2 (CDK2), caspase 3, NRF2 - R34G codon, and four other hepato-protective receptors, and their PDB IDs were extracted. These four drugs' binding affinities to all the receptors were assessed, and their binding energy values in Kcal/mol are listed in Table 5 (Figures 2-13). In comparison, phytol exhibits strong affinities in all three receptors.

4. Conclusion

Boerhavia diffusa Linn has therapeutic potential due to its various phytoconstituents that possess versatile pharmacological activities. Herein this study, we evaluate the phytoconstituents of various extracts and particularly the methanolic extract reveals the presence of a significant number of phytoconstituents,

suggesting that methanol would be an appropriate solvent for extracting these phytoconstituents. In addition, the phytoconstituents were quantified through GCMS which showed unique retention times. Then the top 4 chemical molecules from the GCMS study, were docked against the HCC receptors which showed potent higher negative binding affinities. Comparatively, phytol showed negative binding energy values higher than others, suggesting that it may have anti-hepato carcinoma potential that will need to be investigated in the near future through *in vitro* and *in vivo* models.

5. Author Contribution

Sridhar: Conceptualization, methodology, interpretation and manuscript preparation. Kalaivannan: Conceptua lization, editing and supervision. Santhini: Methodology and investigation. Sangeetha: Interpretation of results and editing. All authors have read and agreed to the published version of the manuscript.

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