

INVITRO ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITIES OF BOERHAVIA
ELEGANS

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ABSTRACT

Background: Boerhavia elegans leaves extract contains alkaloids, tannins, saponins, triterpenoids, flavonoids, volatile oils, phenolic and glycosides. Pharmacological screening provided more proof of its antioxidant and antihyperlipidemic properties. The antioxidant and antihyperlipidemic capacity are evaluated using DPPH and Nitric oxide scavenging activity. The HMG COA reductase enzyme blocks the conversion of HMG COA and NADPH. This interference in the biosynthesis pathway results in lower cholesterol levels. Meanwhile, both the aqueous and ethanolic extracts of Boerhavia elegans have been found to exhibit anti hyperlipidemic effects, helping reduce elevated lipid levels in the body. **Objective:** To evaluate in vitro both anti-oxidant and lipid-reducing properties of Boerhavia elegans. To carry out the preliminary screening, statistical studies, antioxidant and Antihyperlipidemic effectiveness of the leaf extract of plant Boerhavia elegans. **Materials and Procedures:** The leaf extract of Boerhavia elegans contains multiple components such as alkaloids, tannins, saponins, triterpenoids, flavonoids, volatile oils, phenolic substances, and glycosides. To investigate its antioxidant capabilities, tests involve the DPPH and nitric oxide scavenging methods. Furthermore the anti cholesterol potential is examined through HMGCR inhibition assays. **Results:** After that pharmacological screening of Antihyperlipidemic studies, the effect of Boerhavia elegans is HMGR levels was considerably lowered to almost normal by taking atorvastatin.

KEYWORDS: Hyperlipidemia, Boerhavia elegans, DPPH, Dyslipidemia, Antioxidant, phytochemical, Nitric oxide, Soxhlet.

INTRODUCTION

Cardiovascular diseases and related disorders are highly prevalent worldwide. They are often connected to high levels of total cholesterol and LDL, along with low HDL levels. Hyperlipidaemia involves increased lipid levels in the blood. Traditional lipid lowering drugs, including fibrates, statins, and bile acid sequestrants, are known for their side effects, making the development of alternatives from natural sources increasingly appealing. As a result there is a renewed focus on traditional medicine globally thanks to intensive research on plant-based therapeutic agents. Herbal drugs are gaining acceptance due to their safety profile, reduced side effects, and economic benefits.

Based on the literature, Boerhavia diffusa has hepatoprotective, antidiabetic and activity.

Boerhavia diffusa root, plant used in Indian traditional medicine, has significant immunomodulatory potential. The trial outcomes demonstrated the powerful antioxidant properties of Boerhavia elegans extract. Although additional research is advised to identify the active components, it is possible that the plant's high

phenolic content is related to its strong Antioxidant activity.

HYPERLIPIDEMIA OVERVIEW– Hyperlipidemia is characterised by excessive lipids, mainly cholesterol and triglycerides, circulating in the blood. This condition is also identified as hyperlipoproteinemia due to the presence of substances in the bloodstream. The American Heart Association describes it as a condition involving high levels of the fat in the blood.

ETIOLOGY

The causes of hyperlipidemia are divided into primary and secondary factors.

Primary causes: these include polygenic family and hypercholesterolemia where genetic defects, often combined with environmental inferences, lead to increased serum cholesterol.

Secondary causes – Factors such as excessive alcohol consumption, obesity, high carbohydrate intake, diabetes, kidney failure, and pancreatitis contribute to the development of hyperlipidemia. Conditions like familial

combined hypertriglyceridemia, familial endogenous hypertriglyceridemia, or hyperchylomicronemia can lead to primary hypertriglyceridemia.

MATERIALS AND METHODS

Collection and Verification of Plant material

In May 2023, the necessary *Boerhavia elegans* leaves for this study were collected from around Hyderabad Telangana. Dr. A Vijaya Bhaskar Rey, Head of Botany department at a Hyderabad university, confirmed the authenticity of the plant samples. A voucher specimen, numbered OUAS- 125, was retained by the department for future reference. Extraction Process: The collected leaves were thoroughly cleaned and dried under the shade for approximately 7 days. They were then ground into a coarse powder using a mechanical grinder. The powder the leaves and the went extraction via soxhlet apparatus using solvents of increasing polarity: water, ethanol, and petroleum ether. The marc obtained after extraction was air-dried. The filtrate was concentrated by steam distillation; the recovered solvent was reused in subsequent extractions. Further concentration over a water bath resulted in a dark green residue. are dried extract was stored in a desiccator for further phytochemical and pharmacological studies.

Phytochemical Screening

Various methods documented in literature were employed to screen for the presence of several phytochemicals, including alkaloids, tannins, saponins, triterpenoids, flavonoids, volatile oils, phenolic compounds, and glycosides.

Alkaloids (Test of Dragendorff)

0.5 g of filtered ammoniacal alcohol mixed with 20 ml of it. After the filtrate was evaporated, the residue was shaken with 1% H₂SO₄ and filtered. Chloroform was added to the filtrate to make it alkaline. The chloroform layer was then separated and evaporated until it was completely dry. After dissolving the residue in 1% H₂SO₄ and one There was a drop of Dragendorff's reagent added.

Presence of alkaloid is indicated by an orange-red precipitate.

Saponins (Frothing Test)

Distilled 1g of *Boerhavia elegans* extract (SNE) was dissolved in distilled water, filtered, and vigorously shaken. The extract was left undisturbed for 5 minutes. The persistence of froth after settling indicated the presence of saponins.

Phytosterols (Lieberman-Burchard Test)

Half a gram of SNE was extracted with 5 ml of chloroform in a test tube. After adding. A few drops of Acetic anhydride and mixing well, concentrate H₂SO₄ was carefully added along the side of the tube. The formation of a Violet to blue ring at the junction of the liquids confirmed the presence of phytosterols.

Terpenoids (Salkowski Test)

0.5 g of SNE Dissolved and filtered into a test using 5 ml of chloroform. Terpenoids were detected by adding concentrated H₂SO₄ along D test tube wall, which resulted in violet colouration.

Glycosides (General Test)

0.5 g of SNE was warmed with 5 ml of diluted H₂SO₄ for 2 minutes on a water bath. The mixture was then made alkaline with 2-5 drops of 20% After adding 1ml each of Fehling's solutions A and B, the mixture was heated for two minutes. The presence of glycosides was confirmed by the formation of a brick-red precipitate.

Tannins (Ferric Chloride Test)

0.5 g of SNE was boiled in distilled water for 5 minute, allowed to cool, and then filtered. The extract was diluted with 10ml of distilled water and 2 to 10 drops of 1% for ferric chloride was added. A blue- green or blue-black colour indicated the presence of tannins.

Phenolic Substances (Acetic Acid Test)

0.5 g of SNE was boiled in distilled, cooled, filtered into a 25 ml measure. After adding 10 ml of distilled water and 3-4 drops of acetic acid, a colour change to red signified the presence of phenolic compounds.

Flavonoids (Kumar test)

0.5 g of SNE was dissolved and filtered into water. Adding 2 ml of 10% NaOH resulted in a yellow coloration. When diluted HCl was added, the colour changed from yellow to colourless, indicating the presence of flavonoids.

Values extracted

The extractive, or sample yield, to a particular solvent is frequently an approximate constituent or set of related constituents present in the sample. In some situations can be determined by how much of it dissolves in a particular solvent. The extra to be able to dissolve significant *amounts of the targeted substances.*

Antioxidant activity

Free radicals are highly reactive molecular fragments, typically formed naturally within the body during metabolic processes or through exposure to environmental pollutants like pesticides, air pollutants, and cigarette smoke. These unstable molecules are continually produced as a part of normal bodily functions and can be exacerbated by external factors. The body naturally maintains a balance between the generation of free radicals and the presence of antioxidants that neutralises them. However, an increase in free radicals from either internal or external sources can disrupt this equilibrium, leading to oxidative stress, tissue damage, and potentially a variety of diseases.

Diseases search as cancer, inflammation, atherosclerosis, Hypertension, ischemic diseases, Alzheimer's and Parkinson's are increasingly linked to imbalances

between pro-oxidants and antioxidants. Antioxidants derived from natural sources offer a broad range of actions and potential benefits for correcting these imbalances. Historically recognised for causing

imitations, recent research suggests that free radicals also play a role in initiating genetic and phenogenetic changes that contribute to cancer development.

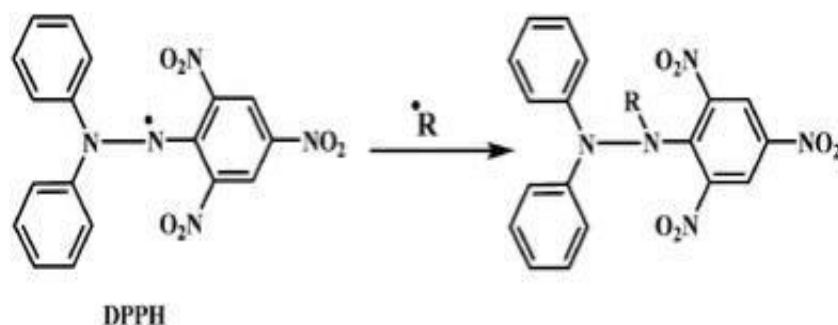


Fig: Reduction of DPPH free radical.

In vitro Antioxidant Studies

a. The DPPH method (diphenyl-1-picrylhydrazyl)

Based on the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical's capacity to scavenge radicals, the antioxidant activity of several leaf and seed extracts from the two plants was evaluated. When the DPPH free radical interacts with a hydrogen donor, it is reduced to a corresponding hydrazine.

The DPPH radical is purple in colour; it turns yellow when it reacts with a hydrogen donor. This discoloration assay measures the amount of absorbance that decreases when an antioxidant is added to a DPPH solution in ethanol or methanol.

Preparation of the DPPH solution

To create the DPPH solution, 22 mg of 2,2-diphenyl-1-picrylhydrazyl was dissolved. From this stock solution, 18 ml was taken and diluted with 100 ml of methanol to achieve a 100 μ M concentration.

Preparation of test solutions

Leaf extracts, each weighing 105 mg, were separately dissolved in dimethyl sulfoxide (DMSO). Serial dilutions were performed to obtain concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 μ g/ml for each.

Preparation of standard solution

A 1 mg/ml ascorbic acid solution was prepared in DMSO. This solution was serially diluted to achieve concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 μ g/ml.

Procedure

The assay was conducted in a 96-well microtiter plate. For each well, 10 μ l of the standard solution or each test sample was added to 200 μ l of DPPH solution. After incubating at 37° C for 30 minutes, the absorbance of each solution was measured at 490 nm using an ELISA microtiter plate reader, with appropriate blanks for reference.

b. Nitric oxide radical inhibition Activity

Nitric oxide is highly unstable under aerobic conditions and readily reacts with oxygen to form stable products such as nitrates and nitrites through the intermediates like NO₂, N₂O₂, and N₃O₄. The Griess reagent is used to measure this reaction. When a scavenger compound is present, it reduces the amount of nitrous acid formed. The degree of scavenging is quantified at 546 nm and reflects the decrease in nitrous acid levels.

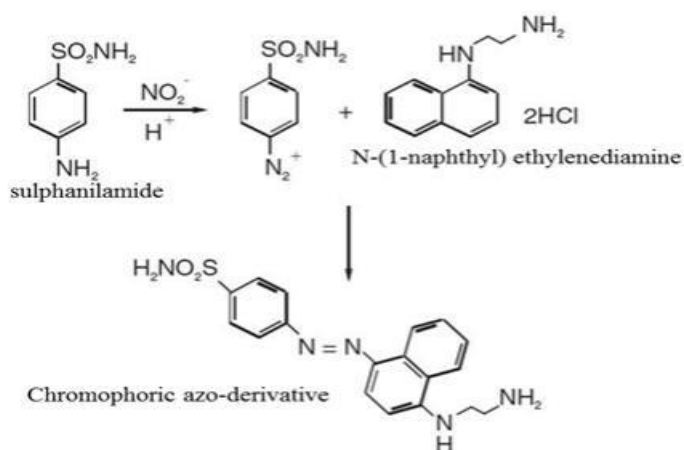


Fig: Principle involved in NO* scavenging activity.

Preparation of Reagents

Sodium nitroprusside solution: Dissolved 0.2998 grams of sodium nitroprusside in 100 millilitres of distilled water.

Naphthyl Ethylene Diamine Dihydrochloride (NEDD) solution: Heated 0.1 gram of NEDD in 60 millilitres of 50% glacial acetic acid and then diluted 100 millilitres in a volumetric flask using distilled water. Sulphanalic Acid solution: Dissolved in 0.33 grams of Sulphanalic acid in 100 millilitres of 20% glacial acetic acid by heating.

Preparation of Test Solutions

84 milligrams of leaf extract from each batch were dissolved in dimethyl sulfoxide (DMSO) and Serially diluted to achieve concentrations of 100, 50, 25, and 12.5 µg/ml.

Preparation of standard solution

Ascorbic acid was prepared at a concentration of 1 mg/ml DMSO. Serial dilutions were performed to obtain concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml.

Relative Organ Weight:

Following a 14- day acute toxicity study, all organs were collected using standardized surgical procedures to determine absolute and relative organ weights.

Method for Nitric Oxide Scavenging

A 6 ml reaction mixture consisting of extracts in DMSO, 1 ml of phosphate buffer saline, and 4 ml of sodium nitroprusside was incubated at 25°C for 15 minutes. After incubation, 1 ml of Sulphanalic acid reagent was added, mixed well, and allowed to stand 5 minutes. Then 1ml of NEDD solution was added, mixed thoroughly, and left to stand for 30 minutes under diffused light. 0.5 ml of the reaction mixture was taken, forming a pink chromophore. the absorbance of these solutions was measured at 540nm compared to blank solution.

Statistical Analysis

Statistical analysis involved performing Turkey's multiple comparison test after one-way analysis of variance (ANOVA) Using GraphPad Prism software. Results are presented as the mean ± Standard deviation for six rats in each group. P values below 1.05 were considered as significant.

In vitro HMG-CoA Reductase Inhibition Assay

HMG-CoA reductase kinetic studies and in vitro cell-free assays. Utilising the manufacturer's instructions, the

HMG-CoA reductase assay kit from Sigma-Aldrich was utilised. It is based on the catalytic domain of the human enzyme. All of the fractions that were separated from plant extract were quickly screened using the kit.

The HMG-CoA reductase assay kit from Sigma -Aldrich was used according to the manufacturer's instructions. This kit is based on the catalytic domain of the human enzyme, facilitating studies of HMG-CoA reductase kinetics and cell-free assays. All extracted fractions were rapidly screened using the kit. Extract samples were prepared in a 5% DMSO solution at a concentration of 10 mg/ml. Pravastatin at 50 mg/ml served as a positive control. The reaction mixture, consisted of samples were added to 250 µm of NADPH, 250 µm of HMG-CoA, and 100 mm of potassium phosphate buffer (400 mm of KCl, 100 mg/ml of bovine serum albumin, and 3.5 mm of EDTA) (final volume of 557 ml). The initiation of reactions involved adding 3 ml of HMGCOA reductase inhibitors (50 ug/ml). Absorbance was measured at regular intervals while shaking the mixture. The enzyme activity was calculated using a specific formula.

Investigation of HMG-CoA reductase inhibitors

The anti- cholesterol effect of extracts was evaluated based on their HMG-CoA reductase inhibitory activity, which is the primary mechanism of action for cholesterol-lowering drugs. Utilising established protocols the inhibitory activity of all fractions was assessed in this study.

RESULTS

Percentage yield

The percentage yield of extractive values of *Boerhavia elegans* were documented in a table.

Table No. 1: Percentage Yield of Plant Extracts in Different Solvents.

S.No	Plant Name	Parts used	Petroleum Ether	Ethanollic Extract	Aqueous Extract
1.	<i>Boerhavia elegans</i>	Leaves	17.23%	9.14%	11.38%

Table No-1 Displays The Percentage Yield *Boerhavia Elegans* Leaf Extracts, With Results Showing 17.23% For Petroleum Ether, 9.14% For Ethanollic Extraction & 11.38% For Aqueous Extraction.

Qualitative Assessment of Phytochemical**Table No. 2: Qualitative phytochemical analysis for indicates the presence of the various secondary metabolites in leaves of Boerhavia elegans.**

Phytochemical constituent	Petroleum Ether extract	Ethanol extract	Aqueous extract
Carbohydrates	-	-	+
Flavonoids	+	+	+
a.Shinoda's test	-	+	+
b.Lead acetate test	-	+	+
c.Alkaline reagent test	-	+	+
Alkaloids			
Dragendorff's test	-	+	+
Wagner's test	-	+	+
Mayer's test	+	+	+
Hager's test	-	+	+
Tannins			
a.Gold Beater's skin test	-	+	+
b.Geltain's test	+	+	+
Terpenoids			
Salkowski's test	-	+	+
Phenols	-	+	+
Far & oils	-	-	-
Saponins			
Foam test	-	-	-
Glycoside			
a. Bontrager's test	-	+	+
b.Legalstest	-	+	+
c.keller-killiani test	-	+	+
Amino acids& proteins			
a.Biuret test	-	+	+
b.Ninhydrin test	-	+	+
Resin	-	-	-
Sterols	+	+	+

+ = Present

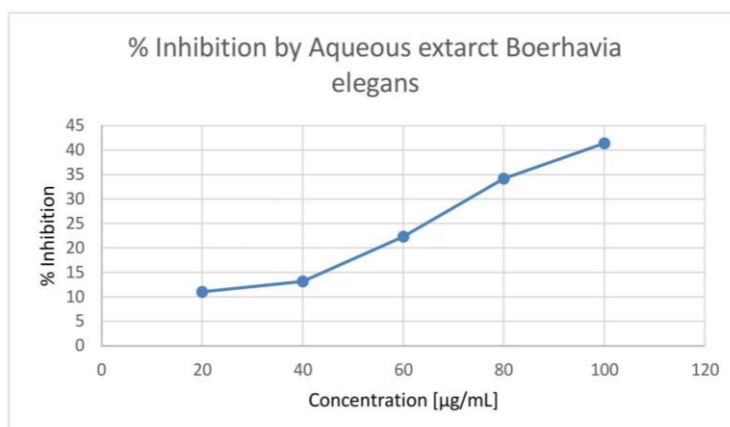
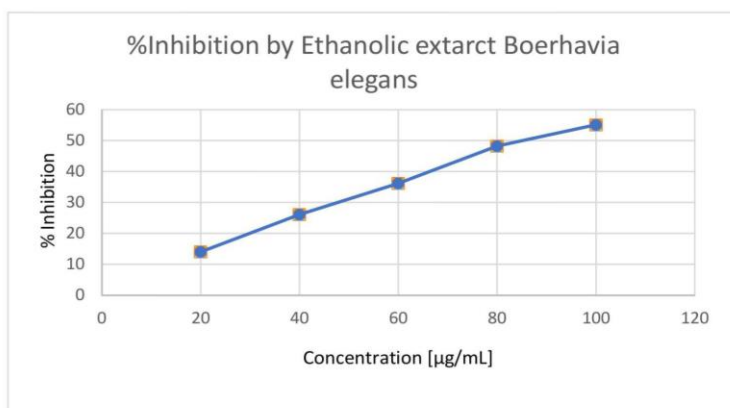
- = Absent

IN-VITRO ASSESSMENT ANTIOXIDANT EFFECTS**DPPH Antioxidant Assay Technique**

S.No	Treatment	Concentration (µg/mL)	%Inhibition	IC ₅₀ Value
1.	Ethanolic extract of Boerhavia elegans	20	14.02±0.13	88.51
		40	26.11±0.17	
		60	36.18±0.25	
		80	48.28±0.53	
		100	55.13±0.42	
2.	Aqueous extract of Boerhavia elegans	20	11.03±0.28	70.94
		40	13.14±0.15	
		60	22.33±0.65	
		80	34.18±0.93	
		100	41.41±0.83	
3.	Ascorbic acid	20	19.11±0.22	77.10
		40	30.07±0.26	
		60	42.20±0.32	
		80	52.16±0.46	
		100	60.13±0.29	

Table 3**IC₅₀ of DPPH Radical Neutralization Assay**

Extract	Concentration
Ethanolic extract of Boerhavia elegans	88.51
Aqueous extract of Boerhavia elegans	70.94

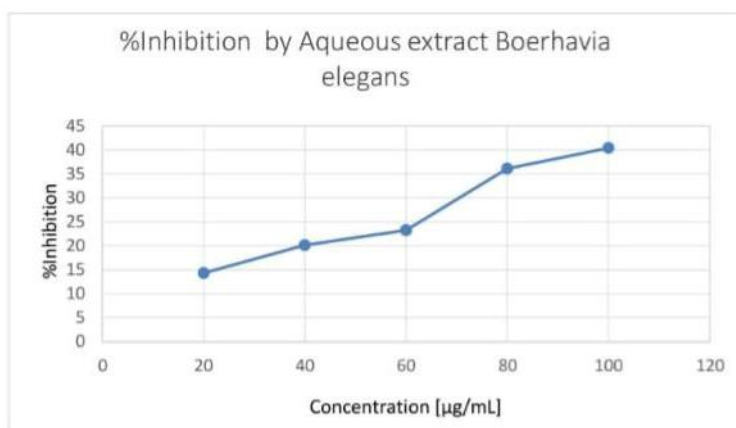
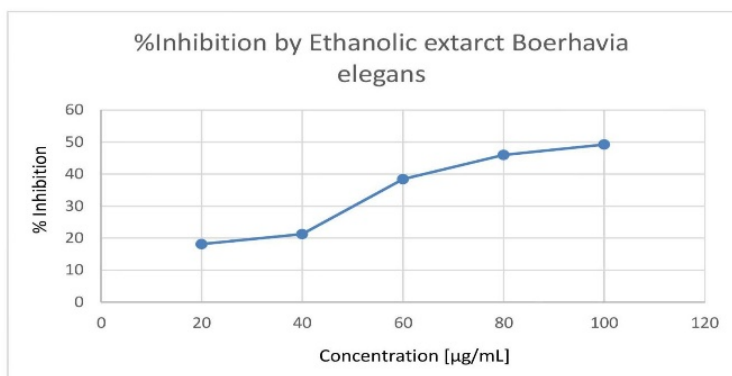
Table 4

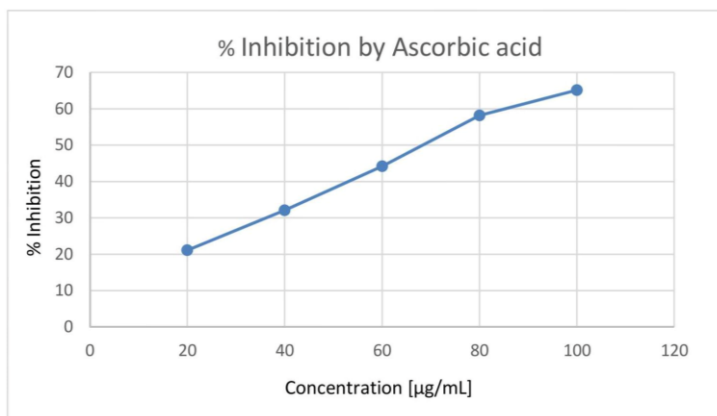
Inference

The free radical scavenging properties of *Boerhavia* were evaluated using the DPPH and Nitric Oxide methods, with the findings summarized in tables 3 and 4.

The results indicate that the aqueous extract of *Boerhavia elegans* exhibited significant IC₅₀ potential when compared to ascorbic acid. Notably, the Ethanolic ethanolic extract showed a more favorable IC₅₀ value in relative to aqueous extract.

S.No	Treatment	Concentration (µg/mL)	% Inhibition	IC50 Value
1.	Ethanolic extract of <i>Boerhavia elegans</i>	20	18.13±0.4	84.77
		40	4	
		60	21.26±0.4	
		80	5	
		100	38.42±0.5	
2.	Aqueous extract of <i>Boerhavia elegans</i>	20	14.31±0.4	75.43
		40	5	
		60	20.15±0.2	
		80	8	
		100	23.25±0.9	
3.	Ascorbic acid	20	21.11±0.2	78
		40	2	
		60	32.07±0.2	
		80	6	
		100	44.20±0.3	
			2	
			58.16±0.4	
			6	
			65.13±0.2	
			9	





CONCLUSION

According to reports, The extract of *Boerhavia elegans* is abundant in antioxidant compounds and has shown potential in lowering cholesterol levels by inhibiting the activity of cholesterol esterase and HMG-CoA reductase enzymes. Further studies using in vivo models are necessary to validate its efficacy as a supplementary treatment for hypercholesterolemia.

Hyperlipidemia is one of the main risk factors for diseases linked to a lifestyle, such as atherosclerosis, and for associated cardiovascular problems, such as myocardial infarction and cerebral paralysis.

Through dietary changes and/or medication administration, the underlying hyperlipidemia can be targeted in order to prevent or treat such disorders.

REFERENCES

- Nasik SR. Antioxidants and their role in biological functions: An overview. *Indian Drugs*, 2003; 40: 501-15.
- Shetgiri PP, D'Mello PM. Antioxidant activity of flavanoids-A comparative study *Indian Drugs*, 2003; 40: 567-9.
- Gopinathan N, Srinivasan KK, Mathew JE. Free radical scavenging properties of ethanol extract of *Saccharum spontaneum*. *Indian Drugs*, 2004; 41: 633-5.
- Gurpreet Kaur, Saqrwar AM, Zoobi J, Kaleem J, Mohammad A. Evaluation of antioxidant activity of *Cassia Siamea* flowers. *J Ethnopharmacol*, 2006; 108: 340-8.
- Ambasta SP, editor. The wealth of India, Raw materials. Vol. 2. B. New Delhi: Publication and information directorate, CSIR, 1998; 56-7. Editor-in-chief.
- Ram PR, Mehrotra BN. Compendium of Indian medicinal plants. Vol. 3. New Delhi: Publication and information directorate, 1980; 84-91.
- Asima C, Satyesh CP. The treatise of Indian medicinal plants. Vol. 2. New Delhi: Publication and information directorate CSIR; 1992; 24-6.
- Col Herber D. Useful plants of India. 2nd ed. Dehradun: Allied Book Center, 1991; 75.
- Aksoy L, Kolay E, Agilolu Y, Aslan Z, Kargioglu M. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis*. *Turcica*, 2013; 20: 235-239.
- Bhatt P, Negi PS. Antioxidant and antibacterial activities in the leaf extracts of Indian Borage (*Plectranthusamboinicus*) *Food Nutr Sci*, 2012; 3: 146-152.
- Clarke G, Ting KN, Wiart Ch, Fry J. High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants*, 2013; 2: 1-10.
- Porcher, R.D., 1978. *Boerhavia diffusa* L. (B. Coccinea Mill.) (Nyctaginaceae) in the Carolinas. *Castanea*, Sep., 1978; 43(3): 172-174 59.
- Verma, H. N., Awasthi, L. P., and Saxena K. C., 1979. Isolation of the virus inhibitor from the root extract of *Boerhavia diffusa* inducing systemic resistance in plants. *Canadian Journal of Botany*, 1979; 57(11): 1214-1217. 10.1139/679-146
- Awasthi L.P., Menzel G, 1986. Effect of root extract from *Boerhavia diffusa* L. containing an antiviral principle upon plaque formation of RNA bacteriophages. *Zentralblatt für Mikrobiologie*, 1986; 141(5): 415-419 61.
- Kadota, S., Lami, N., Tezuka, Y., Kikuchi, T., 1988. *Boerhavia* A and B, new rotenoid analogues from *Boerhavia diffusa* Linn. *Chemical & pharmaceutical bulletin*, 1988-02-25; 36(2): 834-836.
- Chandan, B.K., Sharma, A.K., and Anand, K.K., 1991. *Boerhavia diffusa*: A study of its hepatoprotective activity. *Journal of Ethnopharmacology*, March 1991; 31(3): 299-307.63.
- Ahmed, B., and Yu Chung-Ping, 1992. *Boerhavia*, a dihydroisofuranoxanthone from *Boerhavia diffusa*. *Phytochemistry*, 1992; 31(12): 4382- 4384.
- Shukla, N., Sangwan, N.S., Misra, H.O., Sangwan, R.S., 2003. Genetic diversity analysis in *Boerhavia diffusa* L. Of different geographic locations in India

- using RAPD markers. Genetic Resources and Crop Evolution, September 2003; 50(6): 587-601.
19. Pari, L., and Amarnath, Satheesh M., 2004. Antidiabetic activity of *Boerhavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. *J Ethnopharmacol*, 2004 Mar; 91(1): 109-13.
 20. D. Pandey, R., Maurya, R., Singh, G., Sathiamoorthy, B., Naik, S., 2005. Munosuppressive properties of flavonoids isolated from *Boerhavia diffusa* Linn. *Ternational Immunopharmacology*, March 2005; 5(3): 541-553.
 21. Leyon, P.V., Lini, C.C., Kuttan, G., 2005. Inhibitory effect of *Boerhavia diffusa* on experimental metastasis by B16F10 melanoma in C57BL/6 mice. *Life Sciences*, 4 February 2005; 76(12): 1339-1349.
 22. Borrelli, F., Ascione, V., Capasso, R., Izzo, A. A., Fattorusso, E., and Taglialatela-Scafati O., 2006. Spasmolytic Effects of Nonprenylated Rotenoid Constituents of *Boerhavia diffusa* Roots. *J. Nat. Prod.*, 2006; 69(6): 903-906.
 23. Awasthi L. P., and Verma H. N., 2006. *Boerhavia diffusa* – A Wild Herb with Potent Biological and Antimicrobial Properties.
 24. Maurya R., Sathiamoorthy B., and Mundkinajeddu D., 2007. Flavonoids and phenol glycosides from *Boerhavia diffusa*. *Natural Product Research: Formerly Natural Product Letters*, 2007; 21(2): 126-134.
 25. Lohani, S., Jan, A., and Verma, H.N., In vivo and in vitro Resistance Induction in Tobacco by *Boerhavia diffusa* Systemic Resistance Inducing Protein and Transfer of Induced Resistance in in vitro Tobacco Plants. *Biotechnology*, 2007; 6(3): 389-392.
 26. Belkacem, A. A., Macalou, S., Borrelli, F., Capasso, R., Fattorusso, E., Taglialatela-Scafati, O., and Pietro, A. D., 2007. Nonprenylated Rotenoids, a New Class of Potent Breast Cancer Resistance Protein Inhibitors. *J. Med. Chem.*, 2007; 50(8): 1933-1938.
 27. Madhuri S, Kalasker V, Rambhimaiah, et al. Evaluation of diuretic activity of aqueous extract of *Boerhavia diffusa* roots in Rats. *Int J Pharm Bio Sci.*, 2013; 4(4): 843-848.
 28. Pareta SK, Patra KC, Mazumder PM, et al. *Boerhavia diffusa* Linn. Aqueous extract as curative agent in ethyl glycol induced urolithiasis. *Pharmacology online*, 2010; 3: 112- 120.
 29. Bhardwaj R, Yadav A, Sharma RA. Phytochemicals and Antioxidant Activity in *Boerhavia diffusa*. *Int J Pharm Pharm Sci.*, 2014; 6(1): 344-348.
 30. Dhakar P, Saini MR, Sharma J. Comparative evaluation of free radical scavenging activity of *Boerhavia diffusa* root extracts (BDRE) and determination of dose effectively against radiation induced damages in Swiss albino mice. *Int J Appl Res Nat Prod.*, 2013; 5(4): 9-18.
 31. Khalid M, Siddiqui HH, Fareed S. In vitro estimation of the antioxidant activity and phytochemical screening of *Boerhavia diffusa* root extract. *Asian J Trade Mod.*, 2011; 6(6): 259-266.
 32. Ammar AF, Zhang H, Siddeeg A. In Vitro Antioxidant Activity and Total Phenolic wood Flavonoid Contents of *Alhydwan* (*Boerhavia elegans* Choisy) Seeds. *J Food Nutr Res.*, 2014; 2: 215-220.
 33. Bokhari J, Khan MR, haq I. Assessment of Phytochemicals, antioxidant and ant inflammatory potential of *Boerhavia procumbens* Banks ex Roxb. *Toxicol Ind Health*, 2014; 32(8): 1456-1466.
 34. Mehrotra S. Singh VK, Agarwal SS, et al. Anti-lymphoproliferative activity of ethanolic extract of *Boerhavia diffusa* roots. *Exp Mol Pathol.*, 2002; 72(3): 236-242.
 35. Srivastava R, Saluja D, Dwarakanath BS, et al Inhibition of Human Cervical Cancer Cell Growth by Ethanolic Extract of *Boerhavia diffusa* Linn. (Punarnava) Root. *Evid Based Complement Alternat Med.*, 2011; 2011: 1-13.
 36. Ramazani A, Zakeri S, Sardari S, et al. In vitro and in vivo anti-malarial activity of *Boerhavia elegans* and *Solanum surattense*. *Malar J.*, 2010; 19: 119-124.
 37. Hilou A. Nacoulma OG, Guiguemde TR. In vivo antimalarial activities of extracts from *Amaranthus spinosus* L. And *Boerhavia erecta* L. In mice. *J Ethnopharmacol.*, 2006; 103(2): 236-240.
 38. Adefokun DI, Iwalewa EO, Omisore NO, et al. The Antimalarial Effect and Mechanism of Action of Methanolic Root Extract of *Boerhavia diffusa* in Mice. *Br J Pharm Res.*, 2015; 8: 1-14.
 39. Gharate M, Kasture V. Evaluation of anti-inflammatory, analgesic, antipyretic and antiulcer activity of *Punarnavasava*: an Ayurvedic formulation of *Boerhavia diffusa*. *Orient Pharm Exp Med.*, 2013; 13(2): 121-126.
 40. Yaowared S, Yukihiya M, Michihisa T, Opa V, Kinzo M, Hiroshi W. Evaluation of the nitric oxide radical scavenging acitivity of Manganese Complexes of curcumin and its Derivative. *Biol Pharm Bull.*, 2004; 27: 170-3.
 41. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, et al. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. Stem bark. *Braz J Med Biol Res.*, 2005; 38: 1015-24.
 42. Umesh KP, Saraf S, Dixit VK. Hypolipidemic activity of seeds of *Cassia tora* Linn. *J Ethnopharmacol.*, 2004; 90: 249-52.
 43. Ilhami G, Haci AA, Mehmet C. Determination of in vitro antioxidant and 15 radical scavenging activities of propofol. *Chem Pharm Bull*, 2005; 53: 281-5.
 44. Yaowared S, Yukihiya M, Michihisa T, Opa V, Kinzo M, Hiroshi W. Evaluation of the nitric oxide radical scavenging activity of Manganese Complexes of curcumin and its Derivative. *Biol Pharm Bull.*, 2004; 27: 170-3.
 45. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, et al. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. Stem bark. *Braz J Med Biol Res.*, 2005; 38: 1015-24.