

## Original Research Paper

### *In vitro* Clot Lysis Activity of *Boerhavia diffusa* L. Leaves

Bhavika Kunwar<sup>1</sup>, Vartika Jain<sup>2\*</sup>, S. K. Verma<sup>3</sup>

<sup>1</sup>Research Scholar, <sup>2</sup>Assistant Professor,  
Department of Botany, Government Meera Girls' College, Udaipur-313001, Rajasthan, India

<sup>3</sup>Professor Emeritus and Director  
Department of Medicine, Pacific Medical College & Hospitals, Udaipur-313001, Rajasthan, India

Corresponding author Email: vartikabotany@gmail.com

#### ABSTRACT

*Boerhavia diffusa* L. (Family – Nyctaginaceae), is commonly known as Horse Purslane, Hogweed, Punarnava, Raktapunarnavaa etc. in various languages. It is a perennial creeping weed having small pinkish flowers and distributed throughout the India, tropical and subtropical Asia, Africa and America. Leaves and roots of *B. diffusa* are edible in many regions of India. It is widely used for the treatment of several human ailments for example, anemia, asthma, blood impurity, body pain, cough, rheumatism, gall bladder stone, joint pain, heart disease, enlargement of liver and spleen etc. in both codified and non-codified traditional systems of medicine. The plant has also shown to possess antioxidant, anti-inflammatory, antidiabetic, adaptogenic, hypolipidemic, antimicrobial, hepatoprotective, antiproliferative, anticonvulsant, nootropic, immunomodulatory activities in various *in vitro* and *in vivo* studies.

Abnormal thrombus formation is one of the major reasons behind ischemic heart disease and treatment with synthetic thrombolytic agents has many side effects. As *B. diffusa* is recommended in traditional medicine for treatment of vascular diseases, the leaves of the plant were assessed for preliminary qualitative phytochemical analysis and *in vitro* thrombolytic potential. Preliminary phytochemical screening of leaves has shown presence of flavonoids, terpenoids, cardiac glycosides, saponins, tannins, steroids, phenol, phlobatannin, carbohydrates and amino acids. A significant percent clot lysis activity of  $38.42 \pm 1.28$  was exhibited by methanolic extract of leaves of *B. diffusa* in comparison with the standard drug streptokinase as positive control ( $44.72 \pm 0.87$  %) and sterile distilled water as a negative control ( $3.74 \pm 0.37$  %). This is the first report of *in vitro* clot lysis potential of *B. diffusa* leaves and it could be recommended to use this plant as a dietary nutraceutical. Further studies to find out the bioactive molecules and molecular mechanism responsible for its thrombolytic potential are warranted.

**KEYWORDS:** Cardiovascular diseases, Nutraceutical, Thrombolysis, Punarnavine, Boeravinone

#### INTRODUCTION

Abnormal thrombus formation due to the imbalance or failure in homeostasis in the blood vessels may cause serious events such as heart attack, stroke, ischemic heart disease, deep vein thrombosis, pulmonary embolism etc.<sup>1</sup>. Due to injury of blood vessels in arterial system, recruitment of platelets to the vascular endothelial membrane occurs and their hyper-aggregation leads to formation of thrombus resulting in cardio-vascular diseases; the leading cause of morbidity and mortality in world<sup>2,3</sup>. There is need for the lysis of the dangerous intravascular clots in order to improve

blood flow to prevent ischemic damage<sup>4</sup>. For this purpose, there are some widely used synthetic thrombolytic agents such as streptokinase (SK), tissue plasminogen activator (t-PA), urokinase (UK), alteplase etc. These synthetic thrombolytic drugs are used in acute catastrophic situation only and possess serious side effects of bleeding, anaphylactic reactions and shock<sup>5</sup>.

Plants are thought to be less harmful and free of adverse effects than synthetic thrombolytic drugs<sup>6</sup>. Many plants have shown thrombolytic and antiplatelet potential<sup>7</sup>.



**Figure 1:** *Boerhavia diffusa* L. Plant

*Boerhavia diffusa* L. (Figure 1); member of family – *Nyctaginaceae* is a perennial diffusely branched, creeping herb with stout rootstock. It is known as Horse Purslane, Pigweed, Hogweed, *Punarnava*, *Raktapunarnavaa*, *Shophaghni*, *Kathila*, *Varshabhu*, *Shilatika*, *Kuthillaka* etc. in different languages. The name *Punarnava* is derived from two words *Punar* (once again) and *Nava* (new, renew or young), meaning, one which becomes new or young again. This name indicates about rejuvenating property of *B. diffusa*<sup>8</sup>. It is distributed in India, tropical and subtropical Asia, Africa and America. Leaves, shoot and roots of the plant are considered edible in various ethnic communities of India<sup>9-11</sup>. It is important to note that *Boerhaavia* Miller is an orthographic variant which was described in 1754 after Linnaeus proposed the scientific name *Boerhavia* in 1753; hence the *Boerhavia diffusa* L. is the accepted nomenclature of the plant<sup>12</sup>.

*B. diffusa* is recommended in both codified and non-codified systems of medicine for the treatment of various human ailments namely anemia, asthma, blood impurity, body pain, cough, diabetes, dropsy, gall bladder stone, heart disease, joint pain, oedema, kidney problem, night blindness, rheumatism, skin disease, snake bite, stomachache, urinary trouble, menorrhagia, malaria, insomnia, enlargement of liver and spleen etc.<sup>11,13,14</sup>.

*B. diffusa* is classified as *rasayana* in Ayurveda and possesses ethnopharmacological and therapeutic importance. Some Ayurvedic formulations such as *Punarnavasava*, *Sukumara ghruta*, *Punarnavadyarishta*, *Punarnavadi mandura*, *Sothaghna Lepa*, *Maha Narayan Taila*, *Punarnavastaka kvathaurna*, *Punarnava guggulu*, *Punarnavadi kvathaurna* and *Varuni* have the major ingredient of *B. diffusa*. All these ingredients are used for the treatment of various human ailments like heart disease, sciatica, inflammation, diseases of abdomen, spleen, liver disorders, arthritis etc.<sup>15-16</sup> The root of *B. diffusa* has laxative and diuretic properties which are due to the presence of an active constituent, *Punarnavine*<sup>10</sup>.

Besides, various pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, anticonvulsant, antidiabetic, hypolipidemic, cytotoxic, diuretic, nootropic etc. have been reported from *B. diffusa* in various scientific studies<sup>8,17</sup>. Looking to its immense pharmacological potential and uses in traditional medicine, the present study has been carried out to evaluate *in vitro* thrombolytic potential of leaves of *B. diffusa*.

## MATERIALS AND METHODS

### *Collection, Identification and Preparation of Plant Material*

Leaves of *B. diffusa* were collected from an open land in Arvind Nagar, Sunderwas, Udaipur, Rajasthan, India. Plant was identified by Dr. Vartika Jain and a voucher specimen was preserved at Herbarium, Department of Botany, Government Meera Girls College, Udaipur. Further authentication of the plant was done at Botanical Survey of India (BSI), Arid Zone Regional Centre, Jodhpur, Rajasthan (BSI/AZRC/I.12012/Tech./2020-21- (Pl.Id.)/424 dated 08/02/2021, Sl. No. 1). Leaves were dried under the shade and ground to make a fine powder to prepare suitable plant extracts.

### *Preparation of Plant Extracts*

#### 1. Methanolic Extracts:

- a. *Methanolic Extract-I* (ME-I) – Five gram dried powder of leaves of *B. diffusa* was soaked in 50 ml methanol for 24 hours at room temperature with occasional stirring and filtered. This process was repeated for three times each with 50 ml of methanol. This extract was used for qualitative phytochemical analysis of leaves.
- b. *Methanolic Extract-II* (ME-II) – This was prepared using cold maceration technique in which 100 g dried powder of leaves of *B. diffusa* was soaked in 500 ml of methanol for eight days. It was then filtered with Whatman's filter paper

no. 1 and solvent was evaporated on boiling water bath at 40°C. The crude methanolic extract, thus obtained was named as ME-II and stored at 4°C in refrigerator. ME-II was used for evaluation of *in vitro* clot lysis activity.

## 2. Aqueous Extract:

This extract was freshly prepared for preliminary qualitative phytochemical analysis. To prepare this, 400 mg dried powder of leaves *B. diffusa* was soaked in 20 ml distilled water and then boiled for 20 minutes. After boiling, it was filtered with Whatman's filter paper no. 1 and aqueous extract was ready to be used.

### A. Qualitative Phytochemical Analysis

Plant extracts (Aqueous/ME-I) or dried leaves powder of *B. diffusa* were used for preliminary qualitative phytochemical screening of amino acids, carbohydrates, terpenoids, steroids, cardiac glycosides, phlobatannins, flavanoids, phenols, tannins, and saponins as per standard methodology<sup>18-22</sup>.

### B. Evaluation of *in vitro* Thrombolytic Activity

Assessment of *in vitro* thrombolytic activity of ME-II was carried out as per the methodology demonstrated by Prasad *et al.*<sup>23</sup>. For this purpose, institutional ethical approval was sought (Ref.PMU/PMCH/IEC/2019, dated 26.12.2019) and informed consent was obtained from ten healthy volunteers who were not taking any medication, or oral contraceptives or anticoagulant therapy.

#### Preparation of Plant Extract

For evaluation of *in vitro* thrombolytic activity, 100 mg crude methanolic extract (ME-II) was suspended in 10 ml of sterile distilled water to prepare a concentration of 10mg/ml. This was shaken vigorously on a vortex mixer and kept overnight at room temperature. The next day, using a syringe filter of 0.22µ pore size, it was filtered and 100 µl volume (1 mg/ml) of this extract was used for evaluation of thrombolytic activity.

#### Preparation of Streptokinase as Positive Control

As a well-known standard thrombolytic agent, Streptokinase was used as a positive control. STPase (commercially available lyophilized SK, manufactured by Cadila Pharmaceuticals, Ahmedabad, India) of 15,00,000 IU was

dissolved in 5 ml of sterile distilled water and mixed thoroughly. This was always prepared fresh and 100 µl (30,000 IU) was used for determination of *in vitro* thrombolytic activity.

### Evaluation of *in vitro* Clot Lysis Activity

Ten ml. fasting blood samples were drawn from the volunteers and 500 µl was poured in previously weighed sterile micro-centrifuge tubes. For clot formation, the tubes were incubated at 37°C for 45 min. To separate the serum from blood clot, tubes were then centrifuged at 2000 rpm for 10 min and serum was removed carefully with the help of micropipette. Clot weight was then determined by reweighing the tubes as: Weight of micro-centrifuge tube having clot – Weight of micro-centrifuge tube. Addition of either 100 µl of plant extract, sterile distilled water (negative control) or streptokinase (positive control) was performed to the tubes having the pre-weighed clot and again incubated at 37°C for 90 min to observe the clot lysis. The fluid obtained after clot lysis was removed carefully. Weight of clot, after lysis was determined by again weighing the tubes as: Weight of micro-centrifuge tube having clot – Weight of micro-centrifuge tube after clot lysis. Percent clot lysis was determined as: Weight of clot after lysis / Weight of clot × 100.

### Statistical Analysis

Results of *in vitro* clot lysis activity are expressed as Mean ± SEM (standard error of the mean) for three replicates and statistical comparisons were performed using Student's Paired t-test using Microsoft Excel (2010). Differences between means were considered to be significant when p value was < 0.01.

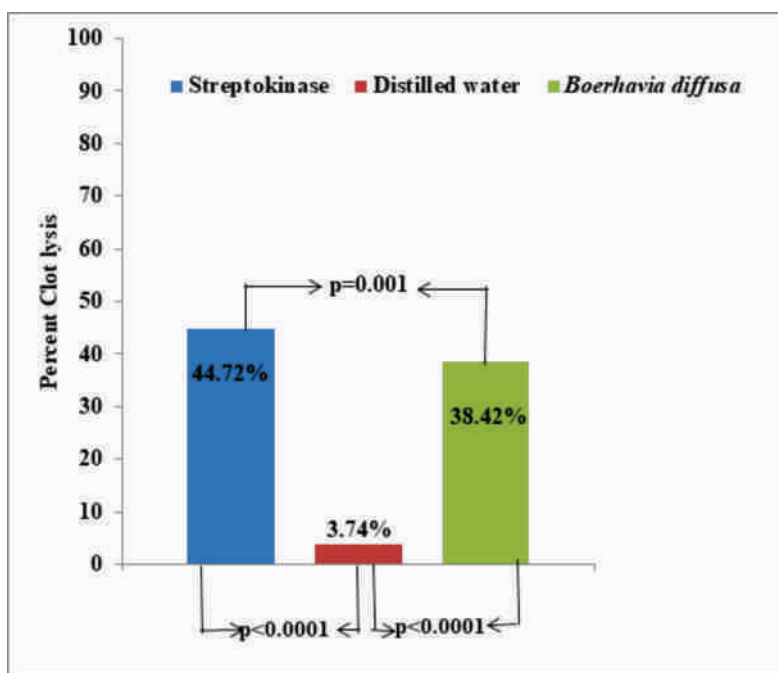
## RESULTS AND DISCUSSION

Preliminary qualitative phytochemical screening revealed the presence of primary metabolites such as carbohydrates and amino acids along with presence of secondary metabolites such as flavonoids, tannins, phlobatannins, phenols, saponins, cardiac glycosides, steroids and terpenoids in the leaves of *B. diffusa* collected from Udaipur, Rajasthan (Table 1). Interestingly, methanolic extract of aerial parts of *B. diffusa* prepared by continuous hot extraction method has shown absence of cardiac glycosides, saponins and steroids<sup>24</sup>.

**Table 1.** Preliminary Qualitative Phytochemical Analysis of Leaves of *B. diffusa*

S. No.	Phytochemical Test	<i>B. diffusa</i> leaves
01.	Carbohydrate	+
02.	Amino acid	+
03.	Saponin	+
04.	Flavanoid	+
05.	Phenol	+
06.	Tannin	+
07.	Phlobatannin	+
08.	Terpenoid	+
09.	Cardiac glycoside	+
10.	Steroid	+

+ = Present



**Figure 2.** *In vitro* Thrombolytic Activity of *Boerhavia diffusa* Leaves

A statistically significant *in vitro* clot lysis activity of methanolic extract of *B. diffusa* leaves as compared to SK as positive control and distilled water as a negative control was observed in the present study for the first time (Figure 2).

Methanolic extract of leaves of *B. diffusa* in a concentration of 1 mg/ml (100 µl) exhibited 38.42±1.28 % *in vitro* clot lysis whereas streptokinase demonstrated 44.72±0.87 % clot lysis.

10.26±2.06% *in vitro* thrombolytic potential of methanolic extract of aerial parts of *B. diffusa*. In this regard, current results obtained from leaves of *B. diffusa* are more specific.

There are many plant species which have shown similar *in vitro* thrombolytic activity for example, hydro-alcoholic extracts of bulbs of *Allium cepa* and seeds of *Linum usitatissimum* have demonstrated 38.93 % and 35.65 % clot

**Table 2.** Percent Clot Lysis Activity of *B. diffusa* leaves, Streptokinase (30000 IU) as Positive Control and Distilled Water as Negative Control

S. No	Plant extract / SK / DW	Percent clot lysis (Mean±SEM)
1.	Methanolic extract of <i>B. diffusa</i> leaves	38.42±1.28 <sup>a,b</sup>
2.	Streptokinase (SK)	44.72±0.87 <sup>c</sup>
3.	Distilled water (DW)	3.74±0.37

Values are expressed as Mean ± SEM

**p value:**

a- 1 v/s 2 p=0.001

b- 1 v/s 3 p<0.0001

c- 2 v/s 3 p<0.0001

Negative control of sterile distilled water exhibited negligible clot lysis of 3.74±0.37 % indicating that water had no role in the *in vitro* clot lysis (Table 2). Apu et al.<sup>24</sup> have shown

lysis activity respectively<sup>6</sup>. 39.30 ± 0.96% clot lysis by leaves of *Lea indica*<sup>25</sup>, 38.86 ± 2.05 % clot lysis by fruits of *Tribulus terrestris*<sup>26</sup>, 36.89 ± 1.94% clot lysis by chloroform soluble

fraction of *Brassica nigra* flowers<sup>27</sup> etc. and therefore, the present results obtained with leaves of *B. diffusa* are quite promising.

Plants of *Nyctaginaceae* family are rich in many bioactivities. Interestingly, the ornamental species of this family, *Bougainvillea spectabilis* were evaluated for *in vitro* thrombolytic potential and its leaves have shown 17.73% clot lysis at a concentration of 800 µg/ml after 24 hours of incubation<sup>28</sup>. In this regard, 38.42±1.28 % clot lysis of *B. diffusa* leaves (from the same family) after incubation of 90 minutes is a very important finding.

Ferreres et al.<sup>29</sup> reported that leaves of *B. diffusa* contains diverse classes of bioactive compounds including phenolics namely, quercetin, kaempferol, 3,4-dihydroxy-5-methoxycinnamoylrhamnoside, quercetin 3-O-robinobioside, quercetin 3-O-(2"-rhamnosyl)-robinobioside, kaempferol 3-O-(2"-rhamnosyl)-robinobioside, kaempferol 3-O-robinobioside, eupalitin 3-O-galactoside, 3,5,4'-trihydroxy-6,7-dimethoxyflavone 3-O-galactosyl (1 → 2) glucoside and caffeoyltartaric acid. Sharma and Sahai<sup>30</sup> have isolated various phytochemical constituents such as uridine triacetate, boeravinone B, eupalitin 3-O-β-Dgalactopyranoside, quercetin 3-O-α-D-rhamnoside, β-amyrin, β-amyrin acetate, 3-O-β-D-glucopyranosyl sitosterol, and β-sitosterol from leaves of *B. diffusa*. Boeravinone B is one of the important rotenoid of the plant which have shown significant *in vivo* anti-inflammatory activity at a dose of 50 mg/kg in carrageenan-induced rat paw model<sup>31</sup> as well as anticancer potential.<sup>32</sup> Several of these bioactive compounds have shown to possess antioxidant, anti-inflammatory, anti-platelet, and anti-coagulant potential and could be behind the thrombolytic action of the plant as observed in the present study.<sup>33</sup>

Besides, several minerals *viz.* sodium, potassium, calcium, manganese, iron, copper, zinc, magnesium, selenium etc. and vitamin C and E are also present in leaves of *B. diffusa*<sup>34,35</sup>. Recently, quantitative analysis of various phytochemicals such as flavonoid (14.14%), alkaloid (7.00%), oxalate (3.51 mg/g), saponin (14.00%), tannin (20.20 mg/100 g), total phenol (11.86 mg/100 g) and phytate (225.64 mg/100 g) contents have also been reported from ethanolic extract of *B. diffusa* leaves<sup>36</sup>.

Antioxidant and hepatoprotective property of leaves of *B. diffusa* has been reported<sup>34</sup>. Ethanolic extract of *B. diffusa* leaves (500mg/kg body weight) have also shown to reduce blood sugar and total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, phospholipids and increase in HDL-cholesterol in streptozotocin induced male wistar diabetic rats<sup>37</sup>. Juice of its leaves and lyophilized decoction has displayed anti-inflammatory activity by inhibiting abdominal writhing in mice<sup>38</sup>.

Many dietary plants and natural food resources are proven to be useful in alleviating cardiovascular diseases and comparatively safer than modern medicine<sup>33</sup>. *B. diffusa* is also edible and full of nutritive and therapeutic phytochemicals. Besides, it has also shown antioxidant, hypolipidemic,

hypoglycemic, and anti-inflammatory activities. In this regard, the present report of thrombolytic potential of *B. diffusa* is another feather in its cap and it could be recommended as a dietary supplement for prevention of cardiovascular disorders and associated symptoms.

## CONCLUSION

Due to shortcomings of standard thrombolytic drugs, herbal drugs could be employed for treatment of thrombosis induced abnormalities. The present study has demonstrated that methanolic extract of *B. diffusa* leaves possess a significant 38.42±1.28 % clot lysis activity for the first time. Natural plant based products have a number of medicinal properties because of presence of various phytochemical compounds. *B. diffusa* leaves have also shown to possess various primary and secondary metabolites in the qualitative phytochemical screening. However, studies are required to isolate the bioactive compound responsible for the thrombolytic action and large scale *in vivo* studies for evaluation of thrombolytic potential of *B. diffusa* leaves are also warranted.

**SOURCE OF FUNDING:** None

**CONFLICTS OF INTEREST:** None

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the help provided by Botanical Survey of India, Arid Zone Regional Centre, Jodhpur, Rajasthan for authentication of the plant and Dr. Nita Sahi, Prof. and Head, Dept. of Biochemistry, PMCH, Udaipur for providing laboratory facilities.

## REFERENCES

- Gholkar AA, Nikam YP, Zambare KK, Reddy KV et al.: Potential anticoagulant herbal plants: a review. *Asian J Res Phar Sci.* 2020; 10(1): 51-55. <https://doi.org/10.5958/2231-5659.2020.00010.7>
- Guguloth SK, Malothu N, Kulandaivelu U, Rao KGSN et al.: Phytochemical investigation and *In vitro* Thrombolytic activity of *Terminalia pallida Brandis* leaves. *Res J Pharm Technol.* 2021;14(2):879-882. <https://doi.org/10.5958/0974-360X.2021.00156.6>
- Mokdad AH, Ballestros K, Echko M, et al.: The State of US Health, 1990-2016: Burden of Diseases, Injuries, and Risk Factors Among US States. *JAMA.* 2018;319(14):1444-1472.
- Baig MU, Bodle J: Thrombolytic therapy. *StatPearls.* 2020; [Internet].

5. Collen D: Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator?. *Ann Intern Med.* 1990; 112(7):529-538. <https://doi.org/10.7326/0003-4819-112-7-529>
6. Muduli A, Rout SK, Prusty AK: *In-vitro* thrombolytic activity study of a polyherbal formulation developed by using extracts of different medicinal plants. *Indian J Pharm Pharmacol.* 2022; 9(1):53-57. <https://doi.org/10.18231/j.ijpp.2022.010>
7. Subramani B, Sathiyarajeswaran P: Current update on herbal sources of antithrombotic activity—a comprehensive review. *Egypt J Intern Med.* 2022; 34: 26. <https://doi.org/10.1186/s43162-021-00090-9>
8. Kumar R, Gautam S, Singh KD, Kumar, P et al.: Pharmacological properties of *Boerhavia diffusa*: A review. *Int J Chem Stud.* 2018; SP4: 72-80.
9. Tiagi YD, Aery NC: Flora of Rajasthan (South and South-east region). 2007; (Himanshu Publications, Udaipur).
10. The Wealth of India: A Dictionary of Indian Raw Materials & Industrial Products, Vol. V, p. 237, New Delhi: NISCAIR. 1948.
11. Jain V, Jain SK: Compendium of Indian Folk Medicine and Ethnobotany (1991-2015). New Delhi: Deep Publications. 2016.
12. Struwig M, Siebert SJ: A taxonomic revision of *Boerhavia* (Nyctaginaceae) in southern Africa. *South Afr J Bot.* 2013; 86: 116–134. <https://doi.org/10.1016/j.sajb.2013.02.172>
13. Sarkar PR: Yogic treatments and natural remedies. Kolkata: AMPS publications. 1983.
14. Bhutya RK: Ayurvedic Medicinal Plants of India, Vol. 1. Jodhpur, India: Scientific Publishers. 2011.
15. Mishra S, Aeri V, Gaur PV, Jachak SM: Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. *BioMed Res Int.* 2014; Article ID 808302:1-19. <https://doi.org/10.1155/2014/808302>
16. 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: 121–126 <https://doi.org/10.1007/s13596-012-0081-3>
17. Agrawal B, Das S, Pandey A: *Boerhaavia diffusa* Linn.: A review on its phytochemical and pharmacological profile. *Asian J Appl Sci.* 2011; 4(7): 663-684. <https://dx.doi.org/10.3923/ajaps.2011.663.684>
18. Edeoga HO, Okwu DE, Mbaebie BO: Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol.* 2005; 4(7): 685-688. <https://doi.org/10.5897/AJB2005.000-3127>
19. Bhalla S: Ethnomedicinal and ecophysiological studies in Fabaceae. Ph.D. Thesis submitted to Doctor Hari Singh Gour Vishwavidyalaya, Sagar (M.P.) 1992.
20. Anandjiwala S, Srinivas H, Kaloa J, Rajani M: Free radical scavenging activity of *Bergia suffruticosa* (Delile) Fenzl. *J Nat Med.* 2007; 61(1): 59-62. <https://doi.org/10.1007/s11418-006-0017-7>
21. Wall ME, Eddy CR, McClenna ML, Klump ME: Detection and estimation of steroid and sapogenins in plant tissue, *Anal Chem.* 1952; 24: 337-342 <https://doi.org/10.1021/ac60068a018>
22. Satyanarayana T: Phytochemical and pharmacognostic studies on some Indian medicinal plants. Ph.D. Thesis submitted to Andhra University, Waltair. 1986.
23. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, et al.: Effect of *Fagonia arabica* (Dhamasa) on *in vitro* thrombolysis. *BMC Complement Altern Med.* 2007; 7(1): 36. <https://doi.org/10.1186/1472-6882-7-36>
24. Apu AS, Liza MS, Jamaluddin AT, Howlader, MA et al.: Phytochemical screening and *in vitro* bioactivities of the extracts of aerial part of *Boerhavia diffusa* Linn. *Asian Pac J Trop Biomed.* 2012; 2(9): 673-678. [https://doi.org/10.1016/S2221-1691\(12\)60208-1](https://doi.org/10.1016/S2221-1691(12)60208-1)
25. Rahman MA, Sultana R, Emran TB, Islam MS, Rahman MA, Chakma JS, Hasan CMM: Effects of organic extracts of six Bangladeshi plants on *in vitro* thrombolysis and cytotoxicity. *BMC Complement Altern Med.* 2013; 13(1): 25.
26. Riaz M, Anjum F, Talpur MMA, Pirzada T, Mahmood Z, Yousaf F: Thrombolytic, cytotoxic and antitumor activities of selected medicinal plants. *Sylwan.* 2018; 161(9): 157-169.
27. Uddin MS, Millat MS, Islam MS, Hussainm MS et al.: Exploration of *in vitro* thrombolytic, anthelmintic, cytotoxic and *in vivo* anxiolytic potentials with phytochemical screening of flowers of *Brassica nigra*. *Future J Pharm Sci.* 2020; 6(1): 1-9.
28. Sherwani SK, Khan MM, Zubair A, Shah MA et al.: Evaluation of *in vitro* thrombolytic activity of *Bougainvillea spectabilis* leaf extract. *Int J Pharm Sci Rev Res.* 2013; 21: 6-9.
29. Ferreres F, Sousa C, Justin M, Valentão, P et al.: Characterisation of the phenolic profile of *Boerhaavia diffusa* L. by HPLC-PAD-MS/MS as a tool for quality control. *Phytochem Anal.* 2005; 16(6): 451-458. <https://doi.org/10.1002/pca.869>
30. Sharma K, Sahai M: Chemical constituents of *Boerhavia diffusa* leaves. *J Med Plants Stud.* 2017; 5(4):166-169.
31. Bairwa K, Singh IN, Roy SK, Grover J et al.: Rotenoids from *Boerhaavia diffusa* as potential anti-inflammatory agents. *J Nat Prod.* 2013; 76(8): 1393-1398. <https://doi.org/10.1021/np300899w>

32. Huang Y, Sun Y, Wang WW, Zhang L: Boeravinone B a natural rotenoid exerts anticancer activity via inducing internalization and degradation of inactivated EGFR and ErbB2 in human colon cancer cells. *Am J Transl Res.* 2018; 10(12): 4183-4192.
33. Mohiuddin AK.: Natural foods and Indian herbs of cardiovascular interest. *Pharm Pharmacol Int J.* 2019; 7(2): 60-84. <https://doi.org/10.15406/ppij.2019.07.00235>
34. Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA: Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* Linn against acetaminophen-induced liver damage in rats. *Food Chem Toxicol.* 2010; 48(8-9): 2200-2205. <https://doi.org/10.1016/j.fct.2010.05.047>
35. Jayachitra J, Janani B, Bharathi V, Manikandan R: Phytochemical analysis and mineral composition of methanolic extract of *Boerhavia diffusa* L. *Research J Pharm and Tech.* 2020; 13(10):4856-4860. doi: 10.5958/0974-360X.2020.00854.9
36. Adeku E, Osundahunsi OF, Malomo SA, Asasile II et al.: Phytochemical constituents and assessment of crude extracts from *Boerhavia diffusa* L. and *Lonchocarpus sericeus* (Poir.) Kunth ex DC. leaves for antioxidant and antibacterial activities. *Measurement: Food.* 2022; 5. <https://doi.org/10.1016/j.meaf.2021.100018>
37. Vasundhara CCS, Gayatri, DS: Antihyperlipidemic property of *Boerhavia diffusa* leaf extract in streptozotocin-induced diabetic rats. *Asian J Pharm Clin Res.* 2018; 11: 173-176.
38. Hiruma-Lima CA, Gracioso JS, Bighetti EJB, Germónsén Robineou L, et al.: The juice of fresh leaves of *Boerhaavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. *J Ethnopharmacol.* 2000;71(1-2):267-274.