- Short communication

Assessment of Phytochemical Constituents, Antimicrobial Activity and Heavy Metal Contents of *Gynura Procumbens*

Rifah Noor Chowdhury[1], Md. Rafat Tahsin[2], Jakir Ahmed Chowdhury[3], Ishrat Jahan[4], Mohammad Shahriar[4], Fahima Aktar[5], Abu Asad Chowdhury[5], Shaila Kabir[5] and Md. Shah Amran[5]

[1]Department of Pharmacy, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
[2]Department of Pharmaceutical Sciences, North South University, Plot # 15, Block # B Bashundhara R/A, Dhaka 1229, Bangladesh
[3]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka Dhaka 1000, Bangladesh
[4]Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh
[5]Molecular Pharmacology and Herbal Drug Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000

(Received: August 14, 2023; Accepted: May 2, 2024; Published (web): June 13, 2024)

Gynura procumbens (GP) is an edible vine. The plant's leaves are broadly consumed and scientifically substantiated to be safe for eating.[1] Flavonoids, saponins, tannins, terpenoids and steroidal glycosides are among the active chemical elements found in GP leaves.[2,3]

Qualitative investigation is indispensable for determining phytochemical components in this species. The microbial load test determines the number of bacteria present in a sample. A sulfate ash test is done for the determination of the impurities present in the plant. The ash's contents alter with time and from organ to organ. Typically, ash represents the plant's inorganic component.[4]

Lead (Pb), copper (Cu) and cadmium (Cd) are examples of non-essential heavy metals that can damage bodily cells in even minute doses. These metals can be absorbed by plants, endangering therapeutic herbs and their extracts. They can also enter the environment through a variety of causes, including tires, motor oil and brake wear. The analysis of plant phytochemicals, microbial load, and heavy metal concentrations were the main goals of our work. Additional investigation may reveal novel bioactive substances.[5]

For the phytochemical investigation, the presence of alkaloids, glycosides, saponins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, phenols and tannins, flavonoids, proteins, carbohydrates, steroids, fats and fixed oils, resin and terpenoids were evaluated through different chemical tests.[6-11] Phytochemical constituent analysis and observations are listed in table 1 where '+' and '-' signs indicated the presence and absence of that particular compound, respectively.

For testing the antibacterial activity, 30 ml of agar solution was poured into each petri dish. A cotton bud was used to swab dirty floor surfaces to inoculate the media. Subsequently, 1 ml of blank ethanol and three different doses of extract dissolved in ethanol (100, 300, and 500 mg) were added to separate plates. An ethanol control group was also included for comparison, as ethanol possesses antibacterial activity and served as the solvent for the extracts. All plates were kept in a laminar airflow cabinet for further analysis.

Correspondence to: Md. Shah Amran
Email: amranms@du.ac.bd

Dhaka Univ. J. Pharm. Sci. **23**(2): 195-199, 2024 (December)
DOI: https://doi.org/10.3329/dujps.v23i2.74101

Table 1. Phytochemical constituent of *Gynura procumbens*.

Phytochemicals	Observation	Result
Alkaloids	Mayer test: White color precipitation.	+
Glycoside	Legal test: The color appeared to be blood crimson.	+
Saponin	Froth test: A stable froth (foam) raised on standing.	+
Anthraquinone glycoside	Bontrager's test: Rose pink, red color was produced in the ammoniacal layer.	+
Cardiac glycoside	Killer Killian test: A brown ring formed between the layers.	+
Cyanogenetic glycoside	The reaction produced reddish-purple color.	+
Phenol and tannin	Ferric chloride test: A greenish-blue color precipitation was observed.	+
Flavonoid	Shinoda test: Pink color was observed.	+
Protein and amino acid	Ninhydrin test: The color of solution was changed to purple or blue.	+
Carbohydrate	Molischs's test: A purple-to-violet color appeared at the interface.	+
Steroid	Lubermann - Bunchard test: Formation of the violet color ring at the junction.	+
Fat and fixed oils	Saponification test: Soap formation with partial neutralization of alkali.	+
Resin	Acetic anhydride test.	-
Terpenoid	No change of extract color.	-

Because ethanol has antibacterial qualities, the ethanol control group's microorganism growth was much lower ($p<0.05$) than the positive controls. The medium was the sole thing (absent of microorganisms) in the negative controls. Peak colony development was observed in the positive control group, whereas all groups treated with extract demonstrated a decrease in microbial growth that were dose-dependent. The strongest antibacterial action was shown by the highest extract dose, which also had the lowest microbial growth.

For microbial optical density test, λ_{max} was measured by scanning in a UV spectrophotometer (500-700 nm range), calibrated to 600 nm, using liquid nutrient media as a blank. After incubation, all the agar medium plates containing extract were taken and weighed carefully. Then, it was diluted ten folds using the same liquid media and dissolved by putting it in a water bath if required. Absorbance was taken for every solution and optical density was measured.

Absorbance at 600 nm was measured. The negative control showed no growth, while the ethanol control group had an optical density of 1.288 and the positive control 2.334, indicating vigorous bacterial growth. Ethanol significantly reduced bacterial growth ($p<0.05$). Despite decreased optical density for all doses of *Gynura procumbens* ($p<0.05$), the extracts showed dose-dependent antimicrobial activity. The results are shown in table 2.

For determining the total ash value, 100 g of *G. procumbens* entire plant powder was weighed correctly and placed in a previously fired (350°C for 1 hour) and tarred crucible. The dried material was distributed in an even layer in the crucible and ignited by congruously elevating the heat to 600°C in a muffle furnace for 5 hours until it was white, demonstrating the absence of carbon. It was cooled in a desiccator and weighed. The operation was performed multiple times to get a steady weight.[12]

Table 2. Data of optical density study of extract of *Gynura procumbens*.

Group	Absorbance (nm)	Optical density (nm)	Average optical density (nm)
Negative control	0	0	0
	0	0	
	0	0	
	0	0	
	0	0	
Positive control	0.267	2.67	2.334
	0.173	1.73	
	0.279	2.79	
	0.199	1.99	
	0.249	2.49	
Ethanol control	0.179	1.79	1.288
	0.183	1.83	
	0.068	0.680	
	0.130	1.30	
	0.084	0.840	
Gynura procumbens 100 mg	0.066	0.660	0.808
	0.094	0.940	
	0.079	0.790	
	0.088	0.880	
	0.077	0.770	
Gynura procumbens 300 mg	0.066	0.660	0.288
	0.022	0.220	
	0.015	0.150	
	0.017	0.170	
	0.024	0.240	
Gynura procumbens 500 mg	0.011	0.110	0.098
	0.006	0.060	
	0.011	0.110	
	0.007	0.070	
	0.014	0.140	

The total amount of ash was calculated in percentage by the following method:

$$\% \text{ of sulfate ash} = \frac{\text{wt of crucible+sample(after dry)} - \text{wt of crucible}}{\text{wt of crucible+sample(before dry)} - \text{wt of crucible}}$$

The percent calculation of total sulfate ash is demonstrated below - Sample to be taken: 1g, Weight of the crucible= 31.48936 g, Weight of the crucible + sample (before dry) = 32.48937 g, Weight of the crucible + sample (after dry) = 31.53960 g

$$\% \text{ of sulfate ash} = \frac{\text{wt of crucible+sample(after dry)} - \text{wt of crucible}}{\text{wt of crucible+sample(before dry)} - \text{wt of crucible}}$$

$$= \frac{31.53960 - 31.48936}{32.48937 - 32.48936} \times 100$$

$$= \frac{0.05024}{1.00001} \times 100 = 5.02\%$$

Sulfate ash content is used to determine plant contaminants and assure the purity and quality of the plants. Our plant had a sulfate ash content of 5.02%.

With an atomic absorption spectrophotometer (AAS), elements such as Hg, Cd, Ni, Cu, As, Pb, Mn, Cr and Co were measured in plant samples. The prescribed protocols were followed in the processing of leaves. In a porcelain or platinum crucible, 1677.7 g of dry plant powder was burned at 600℃ till 14.29 g of ash was produced. After being progressively heated and dissolved in 25% HNO_3, the residue was moved to a 25 ml volumetric flask.

The presence of Hg, Cd, Ni, Cu, As, Pb, Mn and Co were detected. However, no traces were detected in the case of Cr. The WHO/FDA has established permissible levels in herbal medicines for arsenic, mercury, lead and cadmium which are 10, 1, 10, and 0.3 ppm, respectively.[13,14]

The amount of the ten heavy metals was determined and listed in table 3 where 'BDL' indicates below the detection limit. The accepted limit is also included in the Table.

Table 3. Heavy metal contents present in *Gynura procumbens*.

Heavy metal	Amount (ppm)	Acceptable limit (ppm)[40]
Hg	0.238	0.50
Cd	0.001	0.40
Ni	0.075	67.9
Cu	0.042	5
As	0.0.152	0.2
Pb	0.005	0.2
Mn	0.212	200
Cr	BDL	0.05
Co	0.091	0.48

A thorough analysis of the research results revealed that the leaves of *G. procumbens* contain diverse phytochemical constituents having antimicrobial activity. The amount of impurities in the sulfated ash test is low indicating that the plants are safe to use. The amount of heavy metals in the plant does not exceed the acceptable limit showing the possibility of the plant causing no heavy metal poisoning. Detailed research on the *Gaynura procumbens* plant is warranted to open up new horizons of the various versatile uses of the plant.

ACKNOWLEDGEMENTS

This work was supported by a financial grant from the Grant for Advanced Research in Education (GARE) from Bangladesh Bureau of Educational Information and Statistics (BANBEIS) under the Ministry of Education, Government of the People's Republic of Bangladesh, Project ID: LS 20191133 (no. 37.20.0000.004.033.020.2016.1053, dated: 13- 10-2019).

Also, we would like to thank *Incepta Pharmaceuticals Limited* for permitting us to perform the heavy metal tests in their laboratory.

REFERENCES

1. Tahsin, M.R., Tithi, T.I., Mim, S.R., Haque, E., Sultana, A., Bahar, N.B., Ahmed, R., Chowdhury, J.A., Chowdhury, A.A., Kabir, S., Akter, F., Uddin, M.S. and Amran, M.S. 2022. *In vivo* and *in silico* assessment of diabetes ameliorating potentiality and safety profile of *Gynura procumbens* leaves. *Evid. Based Complement. Alternat. Med.* 1-18. Article ID 9095504
2. Mou, K. M. and Dash, P. R. 2016. A comprehensive review on *Gynura procumbens* leaves. *Int. J. Pharmacogn*. **3**, 167-174.
3. Tahsin, M.R., Tithi, T.I., Mim, S.R., Haque, E., Sultana, A., Bahar, N.B., Ahmed, R., Chowdhury J.A., Chowdhury, A.A., Kabir S., Aktar F., Uddin, M.S. and Amran, M.S. 2022. In vivo and In silico assessment of diabetes ameliorating potentiality and safety profile of *Gynura procumbens* leaves. Evidence-Based Complementary and Alternative Medicine. **2022**, 9095504.
4. Edoga, H.O., Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemicals constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* **4**, 685-688
5. Mishra, S., Bharagava, R. N., More, N., Yadav, A., Zainith, S., Mani, S. and amp; Chowdhary, P. 2019. Heavy metal contamination: an alarming threat to environment and human health. In Environmental biotechnology: For sustainable future (pp. 103-125). Springer, Singapore.

6. Efiong, E. E., Akumba, L. P., Chukwu, E. C., Olusesan, A. I. and amp; Obochi, G. 2020. Comparative qualitative phytochemical analysis of oil, juice and dry forms of garlic (*Allium sativum*) and different varieties of onions (*Allium cepa*) consumed in Makurdi metropolis. *Int. J. Plant Physiol. Biochem*. **12**, 9-16.
7. Yadav, R., Khare, R. K. and amp; Singhal, A. 2017. Qualitative phytochemical screening of some selected medicinal plants of *shivpuri district* (mp). *Int. J. Life. Sci. Scienti. Res*. **3**, 844-847
8. Rupak, M.A.H.B., Chowdhury, M.M., Shurovi, F.S., Ferdous, J., Tahsan, M.R., Sarif, S., Hasan, M.M., Chowdhury, J.A., Kabir, S., Chowdhury, A.A., Akter, F., Amran, M.S., Akter and Tahmina. 2022. An evaluation of analgesic and anti-inflammatory activity of ethanolic extract of *Cynodon dactylon* on stressed rodent model. Biomed. *J. Sci. and Tech. Res.* **42**, 33550-33557.
9. Hossainey, M.R.H., Sazed, S.A., Nima, M.K., Rahman, M.S., Ashraf, T., Chowdhury, A.A., Rashid, M.A., Haque R. and Alam, M.S. 2020. Investigation of antimalarial activity and cytotoxicity profiling of a Bangladeshi plant *Syzygium cymosum*. *J. Infect. Dev. Ctries***. 14**, 924-928.
10. De Silva, G. O., Abeysundara, A. T. and Aponso, M. M. W. 2017. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *Am. J. Essent Oil*. **5**, 29-32
11. Teja, M., Ramya, B., Reddy, S. M., Sudha, G. J., Janaki, B., Rama, S. and amp; Kumar, S. A. R. 2016. Preliminary phytochemical evaluation of *Caesalpinia sappan* and *saraca indica*. *Int. J. Res. Pharm Chem*. **6**, 107-112.
12. Dida Bulbula, D. and Urga, K. 2018. Study on the effect of traditional processing methods on nutritional composition and antinutritional factors in chickpea (*Cicer arietinum*). *Cogent Food Agricul*. **4**, 1422370
13. Rao, M. M. and Kumar Meena, A. 2011. Detection of toxic heavy metals and pesticide residue in herbal plants which are commonly used in the herbal formulations. *Environ. Monit. Assess*. **18**, 267-271.
14. Islam, Z., Tahsin, M.R., Faisal, A.U., Tithi, T.I., Nova, T.T., Nila, T.S., Gorapi, H., Zobayer, M., Nadvi, F.A., Mridula, T.N., Choudhury, A.A., Chowdhury, J.A., Kabir, S. and Amran, M.S. 2019. *In vivo* assessment of antidiabetic potential and mapping of pharmacological properties of ethanolic extract of leaves of coccinia grandis on alloxan-induced diabetic rats. *Asian J. Adv. Res.* **7,** 1-9.