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Assessment of Phytochemical Constituents, Antimicrobial Activity, Impurity and Heavy Metal Content of *Gynura nepalensis*

Sayma Akter Nirzana[1], Jannatun Noor Oishi[2], Jakir Ahmed Chowdhury[3], Tasnia Rahman[4], Tasnuva Sharmin Zaman[5], Rifat Ara Nisu[6] , Sabnam Roy Suma[6], Abu Asad Chowdhury[7], Shaila Kabir[7] and Md. Shah Amran[7]

[1]Department of Pharmacy, East West University, Dhaka 1212, Bangladesh
[2]School of Pharmacy, BRAC University, Dhaka, Bangladesh
[3]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka
[4]Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh
[5]Department of Pharmaceutical Sciences, North South University, Plot # 15, Block # B Bashundhara R/A, Dhaka 1229, Bangladesh
[6]Department of Pharmacy, Faculty of Science & Engineering, City University, Khagan, Birulia, Savar
[7]Molecular Pharmacology and herbal Drug Research Laboratory, Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka 1000, Bangladesh

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Abstract

This study explores the multifaceted area of herbal medicine, focusing on *Gynura nepalensis*, a medicinal plant. The qualitative analyses confirmed the presence of alkaloids, glycosides, phenols, tannins, flavonoids, protein-amino acids, saponins and carbohydrates. Terpenoids and resin were apparently missing. The antimicrobial activity of the plant extract was assessed and it revealed dose-dependent efficacy against microbial growth. Additionally, the study scrutinized the total ash content suggesting a moderate level of impurity and evaluated the heavy metal concentration, finding it well below acceptable limits. Overall, the findings establish the promising role of *Gynura nepalensis* in natural medicine, urging further investigations and potential applications in integrated healthcare.

Key words: *Gynura nepalensis*, natural medicine, phytochemistry, heavy metal, antimicrobial activity, impurity.

Introduction

Herbal medicine, a multi-component therapy, has been widely preferred over single target drugs in order to treat complicated multivariate conditions and aid bodily functions (Lee *et al*., 2022). The history of herbalism is strongly entwined with the development of medicine from ancient times till the establishment of the germ hypothesis of disease in the 19th century. Herbal medicine continues to be the notable approach of basic medical treatment for over 75% of the world's population, particularly in developing and underdeveloped nations (Sharma, 2019).

Plants have medical significance since they contain certain bioactive components that exert therapeutic effects on human physiology, behavior, and immunity. To date, plenty of bioactive compounds have been identified including tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. Moreover, the majority of bioactive molecules contain antibacterial, anti-inflammatory, anticarcinogenic and antioxidant activities (Hamzalioğlu and Gökmen, 2016)

Gynura nepalensis, a herb of Asteraceae family, contains more than 40 species. It is typically found in

Corresponding author: Jakir Ahmed Chowdhury, Email: jakir@du.ac.bd, Phone: +8801976333050
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India, the Himalayas, Bangladesh, China, Nepal, Thailand Philippines and sometimes even as far west as Kashmir. The roots of this perennial herb are fibrous and possess a lengthy, grey pubescence (Chakrabarty *et al.*, 2022).

Gynura nepalensis have so far become the focus of phytochemical research, leading to the discovery of approximately 338 different chemicals, notably phenolics, flavonoids, alkaloids, terpenoids, steroids, cerebrosides, aliphatics and others. This plant comprises of hydroxy-β-ionone, chlorogenic acid (3-O-caffeoylquinic acid), 3-p-coumaroylquinic acid, 3,4-dicaffeoylquinic acid, 9′-O- ethyl-dehydrodiconiferyl alcohol, 4,5-dicaffeoylquinic acid (isochlorogenic acid C), ethyl caffeate, loliolide, 4,5-dicaffeoylquinic acid methyl ester, dibutyl phthalate, 2-(1H-indol-3- yl)-2-oxoacetamide, (+)-medioresinol, 7S, 8R-9′-O-ethyl-dehydrodiconiferyl-9-acetate, 1H-indole-3-carbaldehyde, propiconazole, boscialin, 4-hydroxy-4,7-dimethyl-1-tetralone, dibenzothiophen-5-oxide, trans-4,5-dihydroxycorocalane (Meng *et al.*, 2021). This ethnomedicine is useful to treat plenty of human maladies, which involves hepatitis, coughing & asthma, diabetes, wounds, kidney as well as gall bladder stones, haemorrhoids and urinary tract bleeding. It effectively tackles septicemia, rheumatism, skin allergies and problems with reproduction. Additionally, it is utilized for the management of low blood pressure and high cholesterol (Afroz *et al.*, 2014, Aktar *et al.*, 2019, Peña *et al.*, 2018, Meng *et al.*, 2021 and Quattrocchi *et al.*, 2017).

The qualitative screening is crucial in terms of isolating different phytochemical elements prevalent in medicinal plants and evaluating their biological action. Alkaloid, tannin, flavonoids, carbohydrates, phenol, steroid, fat and other phytochemical assays were conducted. The microbial load test was performed to assess the microbiological presence of pharmaceutical raw materials, process samples and finished products. The sulfate ash test was carried out for the detection of the inorganic contaminants in the plants (Kadam *et al.*, 2013). To ensure that the product has the proper level of mineral content, this analysis procedure is certainly vital. Regardless of the fact that heavy metals are naturally present in soil, industrial and geologic processes have increased their concentration to levels that are detrimental to plants (Sperdouli, 2022). Significant physiological and biochemical modifications in plants are caused by prolonged heavy metal consumption (Singh *et al.*, 2016).

In the present study qualitative analysis of heavy metal concentration was also done. The purpose of this work is to gain updated information on the above mentioned factors.

Materials and Methods

Plant collection: The leaves that were used of this plant were obtained from the medicinal plant garden at the Department of Pharmacy, University of Dhaka. Following that, taxonomic verification and characterization were performed. The plant specimen was stored at Bangladesh's National Herbarium in compliance with their guidelines. The plant was provided with the accession number 47380, with the date 11 February, 2019, for further reference.

Extraction preparation: The leaves were crushed coarsely after being dried in the shadows for 7–10 days. After dipping in 70% ethanol, they were vigorously shaken for 96 hours. The extract was filtered and procured shortly after it had been soaked. Then, in order to increase the concentration, it was moved to a rotating evaporator machine. Subsequently, the dried extract was effectively collected and stored for future utilization (Tahsin *et al.*, 2022).

Alkaloids: The Mayer test was used to identify alkaloids. 1.36 g of mercury chloride and 5 g of potassium iodide were dissolved in 100 ml of water to create the reagent. 10 g of dry residue were combined with 20 ml of hydrochloric acid, and then the mixture was filtered. When 3 ml of filtrate and 1 ml of Mayer reagent were combined, a white precipitate was produced, which indicated of the alkaloids.

Glycosides: The Legal test, which uses two milliliters of extract, one milliliter of pyridine and

one milliliter of alkaline sodium nitroprusside, finds glycosides by turning the mixture red (Ugbaja *et al*., 2017).

Saponins: By employing the Froth method, saponins were identified. One milliliter of the filtrated extract was agitated with water in a semi-micro tube. The development of a stable froth was indicative of the presence of saponins (Khalil *et al.*, 2013).

Anthraquinones: The Bornträger's technique was utilized to identify anthraquinones. After heating 2 ml of plant extract and 2 ml of sulfuric acid for 5 minutes, the mixture was filtered and mixed with chloroform. Next, ammonia was added. The ammoniacal layer's rose-pink hue suggested the presence of anthraquinone glycosides.

Cardiac glycosides: It was examined using the Killer-Killian test. Two milliliters of aqueous plant extract, one milliliter of glacial acetic acid, and half a milliliter of concentrated sulfuric acid were combined with three drops of 5% ferric chloride. The presence of cardiac glycosides was suggested by a brown ring between the layers (Gul *et al.,* 2017).

Cyanogenetic glycosides: In order to ensure the presence of cyanogenetic glycoside, a few drops of water were added to the 200 mg extract in a conical flask. A cork was used to suspend a picric acid paper filled with a 5 percent sodium carbonate solution in the flask's neck, which was then gently warmed to 37 degrees (Jamshed *et al*., 2018,).

Phenols and tannins: To determine whether phenols and tannins were present, a ferric chloride test was carried out. In this experiment, 3 ml of extract was analyzed with 3 percent ferric chloride, and later approximately 2 ml of extract was tested with a few drops of 10 percent ferric chloride added slowly (Esienanwan *et al.,* 2020). The presence of phenols and tannins is specified by the formation of greenish-blue colored precipitate.

Flavonoids: The Shinoda test, according to which the presence of pink color indicates the presence of flavonoids, involves diluting roughly 2 gm of the dry extract in 95 percent ethanol, followed by the addition of 0.5 gm of magnesium and a few drops of HCl.

Proteins: The ninhydrin test was used to determine the amino acid and protein content. 3ml of extract were heated with a couple of drops of the ninhydrin reagent (Friedman, 2004, Oshadie *et al.*, 2017). Proteins and amino acids will produce a deep blue or purple color, which signifies their presence.

Carbohydrates: The Molischs's test was conducted to assess whether carbohydrate was present. Soon after two minutes of shaking, 2 ml of the extract was treated with a few drops of alcoholic alpha naphthol and 1 ml of sulfuric acid carefully added through the test tube wall (Rajasree *et al.*, 2021). If there are carbohydrates present, a purple-violet color can be noticed at the interface.

Steroids: Steroid presence was examined utilizing the Lubermann-Bunchard test. After being treated with a few drops of acetic anhydride, the test sample's 2 mg extract was boiled, cooled, and 1 ml of sulfuric acid was gently added from the test tube's sidewalls (Rajasree *et al*., 2021, Sharma *et al.*, 2013). The development of the violet ring at the junction is evidence that there are steroids present.

Fats and fixed oils: The saponification method was applied to evaluate fats and fixed oils. 10 ml of plant extract were treated with 0.05 N of alcoholic potassium hydroxide and a drop of phenolphthalein, then heated in a water bath for one to two hours (Gupta *et al.*, 2013). The presence of fat and fixed oils is indicated by the partial neutralisation of alkali in soap production.

Resin: 1 ml of plant extract, 1 ml of concentrated H_2SO_4, and acetic anhydride solution were added to determine whether resin was present. The presence of resin will shift the color from a yellow to orange range (Kumar *et al.*, 2013, Singh and Kumar, 2017).

Terpenoids: 5 ml of plant extract, 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 were combined and then heated in a water bath to test for the existence of terpenoids. The interface would take on a reddish-brown color if the terpenoids were available (Gul *et al*., 2017).

Test of anti-microbial activity: Initially, a fresh 1-liter batch of agar-nutrient medium was made. After that, 30 petri dishes were used, 30 ml of this solution was placed into each one, and enough time was allowed for it to settle down. To confirm a broad range of organisms, a cotton bud was swabbed over grimy floor surfaces before it was utilized to swab the media. A 1000 ml micropipette was used to draw the appropriate solvent and evenly disperse it across the surface of the appropriate medium. Because of the bacteria, the plates were incubated at 37°C for 24 hours. Agar media and no microorganisms formed the negative control group. Particularly a change in the type of microorganisms in the nutritional medium takes place in the positive control group. The next four plates, which are kept in laminar airflow, were swapped with the cotton bud, and then 1 ml of blank ethanol, low, medium, and high doses of the extract diluted in 1 ml of ethanol, were added, accordingly. Since ethanol has antibacterial properties and our extracts were dissolved in it, we kept an ethanol control group in which 1 ml of ethanol was placed on each petri dish. The preparation of three distinct solutions in three separate doses (100, 300 and 500 mg) of extract are included in one ml of ethanol from three separate formulations. (Nawi *et al.,* 2019)

Microbial optical density test: When measuring optical density (OD), it is assumed that the OD value is proportional to the number of bacteria present in the liquid culture (Sutton, 2011, Sabiha *et.al,* 2015). By scanning in a UV-visible spectrophotometer between 500 and 700 nm, liquid nutritional media was used as the blank to determine the maximum value. At 600 nm after the calibration, the λmax value was found. All of the agar medium plates containing extract were removed after incubation and carefully weighed. The substance was then diluted 10 times using the same liquid medium and if necessary, dissolved by placing it in water bath. For each solution, an absorbance reading and an optical density measurement were done.

Determination of total ash : The whole plant powder weighing 100 g was accurately measured and added to the crucible that had been tarred ad burnt at 350°C for an hour. The dry material was placed evenly across the crucible and heated to 600 °C in a muffle furnace for five hours, at which point it was lit, turning white and indicating the absence of carbon. It was weighed after cooling in a desiccator. To get a stable weight, the technique was repeated several times (Dida Bulbula and Urga, 2018).

Determination of heavy metal: The elements Hg, Cd, Ni, Cu, As, Pb, Mn, Cr and Co were measured in plant samples using an atomic absorption spectrophotometer (AAS). Leaves of the plant were acquired and processed for tests in the manner specified in the literature for such materials. All the gathered leaves were scrupulously cleansed before being put in a ventilated chamber and oven dried at 80°C to a consistent weight. The dried samples were milled to a fine homogenous powder for metal analysis and kept in plastic bags. Weighed samples of 1500 g of dry plant powder were loaded into a platinum crucible or porcelain. Plant material was burnt in the flame of a gas burner. The burner temperature was coercively increased from room temperature to 400 °C in 1 hour. The samples were heated for around 4 hours until a grey or white ash deposit was formed. The ash content was 19.37. The residue was dissolved in 25% HNO_3 and the mixture was gradationally heated to dissolve the residue as necessary. The solution was transferred and made up to the mark into a 25 ml volumetric flask. The test samples were cloudy. In the same manner, a blank digest was performed. (Tüzen *et al.,* 2003)

Results and Discussion

Phytochemical constituent analysis and observations are listed in table 1. Here '+' and '-' signs indicated the presence and absence of that particular compound.

Assessment of antimicrobial activity: Due to the fact that ethanol has antibacterial capabilities, the

multiplication of microorganisms is dramatically reduced ($p > 0.05$) in the ethanol control group compared to the positive control group. The negative control groups appeared to have only nutritional medium and no microbes. The positive control group's colony development peaked in the presence of a nutritious medium. In contrast, all groups that received extract treatment exhibited a substantial reduction in microbial proliferation. Additionally, colony development progressively and dose-dependently decreased. As a consequence, the highest extract dose indicated the highest degree of antibacterial efficacy because the rate of microbial growth was least.

Table 1. **Phytochemical constituent of *Gynura nepalensis.***

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 ammonical layer.	+
Cardiac glycoside	Killer Killian test: Between the layers, a brown ring was formed.	+
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: No change of extract color.	-
Terpenoid	Salkowski test: No change of color.	-

Table 2. Data of optical density study of *Gynura nepalensis.*

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	2.704±0.65
	0.239	2.39	
	0.199	1.99	
	0.392	3.92	
	0.255	2.55	
Ethanol control	0.098	0.980	0.982±0.14
	0.086	0.860	
	0.089	0.890	
	0.125	1.25	
	00.093	0.930	

Table 2 contd.

Gynura nepalensis	250 mg	0.094	0.940	
		0.139	1.39	
		0.084	0.840	1.084 ± 0.21
		0.099	0.99	
		0.126	1.26	
		0.114	1.14	
		0.094	0.940	0.932 ± 0.01
Gynura nepalensis	500 mg	0.082	0.820	
		0.085	0.850	
		0.091	0.91	
		0.086	0.860	
		0.097	0.970	
Gynura nepalensis	1000 mg	0.088	0.880	1.032 ± 0.02
		0.126	1.26	
		0.119	1.19	

Content of total ash: The percent calculation of total ash is demonstrated below.

Sample taken: 1 g

Weight of the crucible= 33.63946 g

Weight of the crucible + sample (before dry) = 34.63944 g

Weight of the crucible + sample (after dry) = 33.77988 g

$$\% \text{ of total ash } = \frac{wt\ of\ crucible+sample(after\ dry)-wt\ of\ crucible}{wt\ of\ crucible+sample(before\ dry)-wt\ of\ crucible} = \frac{33.77988-33.63946}{34.63944-33.63946} \times 100$$

$$= \frac{0.14042}{0.99998} \times 100 = 14.04\%$$

Optical density: At a wavelength of 600 nm, the agar media's absorbance was assessed. No bacteria was developed in the negative control group, therefore there was no absorbance. The optical density of the positive control group was 2.334, however, the optical density of the ethanol control group was 1.288 on average. On the petri dish, the bacteria grew rapidly in the positive control group. Nevertheless, the proliferation of bacteria was significantly ($p > 0.05$) reduced by ethanol. The antibacterial effect of extracts is dose-dependent, despite the fact that the OD of all dosages of *G. nepalensis* dramatically lowered ($p > 0.05$).

The total ash percentage of 14.04 suggests a moderate level of impurity in the analyzed material. This metric is crucial in assessing the inorganic content, as it represents the residue remaining after combustion and sulfation processes. In the context of plants, a total ash percentage ranging from 8% to 22% is generally considered acceptable. The variation in this range is indicative of the diverse mineral composition inherent to different plant species. In the context of plants, a total ash percentage ranging from 8% to 22% is generally considered acceptable. The variation in this range is indicative of the diverse mineral composition inherent to different plant species.

Table 3. Different amount of heavy metal content.

Heavy metal	Amount (ppm)	Acceptable Limit (ppm)[40]
Cu	0.062	5
Pb	BDL	0.2
Co	0.023	0.48
Cr	BDL	0.05
As	0.074	0.2
Cd	BDL	0.40
Mn	0.467	200
Ni	0.038	67.9
Hg	0.27	0.50

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

BDL: Below detection level

Based on the data listed in table 3, it is evident that the plant material's heavy metal level is considerably less than the acceptable limit, which generally indicates favorable for both human and environmental health. To provide additional details, specifically, the content limits for Pb, Cr and Cd among all heavy metals are below the detection threshold. This is advantageous for the physiological condition, as it ensures compatibility and mitigates the risk of potential toxicity.

Conclusions

In conclusion, the above comprehensive study provides valuable insight on the multifaceted aspects of this medicinal plant. The in-depth analysis of the phytochemical components and antimicrobial effects highlights the promising attributes of this herbal treatment. The findings on impurity and heavy metal content provide crucial insights into the plant's safety and quality. Overall, this research not only contributes to our understanding of *G. nepalensis* but also emphasizes its promising role as herbal remedy, paving the way for further exploration and utilization in the field of natural medicine.

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