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Phytochemical screening, metal concentration determination, antioxidant activity, and antibacterial evaluation of *Drymaria diandra* plant



Ashish Phuyal¹, Pawan Kumar Ojha², Biswash Guragain¹ and Narendra Kumar Chaudhary^{1*}

Abstract

Background: Several phytochemical constitutes possess natural antioxidant activity and are in fact advantageous in reducing many oxidative stress related diseases. *Drymaria diandra* of Caryophyllaceae family is one such plant which exhibits various medicinal properties. The aim of the current study is to provide detailed exploration of phytochemical constitutes, metal concentration determination, antioxidant, and antibacterial activity of *Drymaria diandra*.

Results: Qualitative phytochemical analysis showed the presence of maximum number of metabolites in methanolwater (1:1) extract. The heavy and toxic metals such as As, Cd, Cr, and Pb were almost near to the detection limit. The concentration of Fe (19.64 mg/l) was highest followed by Mn (2.35 mg/l). The (IC_{50}) value for methanol extract was found 195.61 which is greater than the standard Ascorbic acid. Antibacterial activity of methanol extract was found higher for *Staphylococcus aureus* and *Escherichia coli* with 22-mm and 14-mm sized diameter of zone of inhibition respectively and methanol-water extract for *Proteus vulgaris* with 17-mm diameter of zone of inhibition.

Conclusion: The findings of the present study showed the presence of various valuable phytochemical constitutes responsible to give antibacterial and antioxidant potency. The presence of bio-metals and the absence of toxic metals further highlight the importance of plant as the source of food that bears medicinal properties.

Keywords: Antibacterial, Antioxidant, Drymaria diandra, Phytochemical screening

1 Background

The emerging medical problems in commonly employed treatment processes are due to the antimicrobial toxicity and undesirable side effects and are considered the root cause of chemotherapeutic failure which needs extensive investigation to reshuffle drug candidates [1]. Besides their synthetic procurement, several modern drugs with potential activity have been derived from natural resources and so, medicinal plants are focused in research to obtain new chemotherapeutic agents [2]. Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of

years. The report of WHO has claimed, 80% world population relies on traditional medicine for their primary health care needs [3]. The tribes of Nepal have long history of the use of natural products for the successful treatment of chronic diseases [4]. Nepal is a small landlocked country situated in the lap of the great Himalayas in South Asia, bordered by China to the north and India to other three sides. Due to wide variation in biodiversity and geographical distribution, Nepal has considered as one of the richest habitat of medicinal flora and fauna in the world [5]. In more than 6000 species of plants, of which, more than 1600 species with medicinal value are distributed in various climatic zones of Nepal and they offer great possibilities of novel and bioactive compounds.



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Human body requires several biometals for the proper functioning of the systems, either to activate the bioenzymes or to foster immune power in the body [6]. Plants are one such source that can fulfill the requirements in right amount. So the investigation regarding the types of biometals present and their specific concentration in the plants is very essential to highlight their importance in medicinal chemistry [7]. A number of Drymaria species are mentioned as food and for medicinal properties in Avurvedic and Traditional Chinese Medicine (TCM) [8]. In Nepal, most of the species of *drymaria* are found in tropical and subtropical parts, extending from southern plane region to northern Himalayan region up to an elevation of 2100 m height [9]. With insufficient knowledge, this plant has various traditional medicinal uses such as in treating cold, headache, coryza, bronchitis, as poultice on sore (to treat aching, inflamed, or painful parts), leprosy, tumors, as fumigant for eye troubles, as cerebral stimulant and antifebrile agent [10]. But detail investigation on its indigenous use is still a subject of research interest. The present study has aimed to evaluate the phytochemical constitutes and metal concentration determination of Drymaria diandra plant. As its application, we have extended our research to screen antibacterial and antioxidant activity of plant extracts.

2 Materials

2.1 Plant materials

The whole plant of *Drymaria diandra* was collected from the wetlands of *Sundarharaicha morang*, during March 2018. Healthy and mature plants were selected for the study. The taxonomic identification of plant was authenticated by Dr. T. P. Gautam of Department of Botany, Mahendra Morang Adarsh Multiple Campus, Biratnagar and deposited to the department as a sample herbarium. The plants were thoroughly washed with tap water followed by second distilled water to remove the dirt and specks of dust.

2.2 Drying

The cleaned plants were cut into small pieces and were left in shade to dry for 10 days. Afterwards, they were dried in hot air oven at 40 $^{\circ}$ C for 2 h to remove the equilibrium moisture before the extraction process.

2.3 Extraction

Extraction was performed by Soxhelt extraction technique [11]. Whole plants were pulverized by using a mechanical grinder, which was later sieved through mesh size 80 to get the powder of uniform size. Around 8 g of the powder was packed in a thimble of filter paper. The apparatus was then assembled and the extractions were carried out using 250 ml each of methanol, hexane, and methanol-water (1:1) as solvent (Fig. 1). The temperature was maintained at 35 °C for methanol and



hexane, and 60 °C for methanol-water (1:1). The extraction was continued for 10 h for methanol, 5 h for hexane, and 16 h for methanol-water (1:1) following the color of the solvent collected in the thimble chamber. Ten milliliters each of concentrated extract was kept for phytochemical screening and remaining extracts were dried. The dried extract was used for antioxidant and antibacterial analysis.

2.4 Phytochemical screening

Phytochemical screenings were carried out for hexane, methanol-water, and methanol soluble fractions as per the standard methods [12–15], and following tests were performed:

- Test for alkaloids
 - a. Mayer's test

To 2 ml of each extract, few ml of 2N HCl along with few drops of Mayer's reagent was added. Gelatinous white precipitation confirms the presence of alkaloids. b. Wagner test

To 2 ml of extract few drops of Wagner reagent was added, a reddish brown precipitation observed confirms the presence of alkaloids.

- Test for coumarins Few drops of FeCl₃ were added in 2 ml of sample; yellow coloration confirms the presence of coumarins.
- Test for saponin

Froth flotation test: 2 ml of sample was added in a test tube with few ml of water, a froth observed and persisted on constant shaking confirms the presence of saponin.

- Test for tannins and phenolic compound To a 2 ml of extract few drop of 5% FeCl₃ solution was added, blue black precipitation confirms the presence of tannins and phenolic compound.
- Test for flavonoids To 2 ml of extract, 1 ml of lead acetate solution was added. An intense yellow color was appeared which confirms the presence of flavonoids.
- Test of cardiac glycoside
 - To 2 ml of plant extract, 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution and 1 ml of concentrated H_2SO_4 was added. Appearance of a brown ring indicates the presence of cardiac glycoside.
- Test for reducing sugars

Fehling test: 1 ml each of Fehling's A and Fehling's B solutions was mixed and boiled for 1 min and equal volume of test solution was added. The whole solution is heated in a boiling water bath for 5–10 min. Formation of brick red ppt. confirmed the presence of carbohydrate.

- Test of glucoside To 2 ml of plants extract, few drop of concentrated sulfuric acid was added. The presence of black coloration confirms the presence of glucoside.
- Test for anthraquinone

In 2ml plant extract, 3 ml of benzene and 5 ml of 10% NH_3 were added. Appearance of pink, violet, or red coloration in ammonical layer indicates the presence of anthraquinones.

- Test for glycoside (killer killiani test) To 2 ml of extract, 1 ml of glacial acetic acid, few drop of FeCl₃, and few drops of concentrated H₂SO₄ were added. Green/blue precipitation indicates the presence of glycoside.
- Test for emodins To 2 ml of extract, 2 ml of NH₄OH and 3 ml of benzene were added. Red coloration indicates the presence of emodins.
- Test for phlobatannins To 2 ml of extract, 2 ml of 1% HCl was added and heated. Red precipitate indicates the presence of phlobatannins.
- Test for terpenoids To 2 ml of extract, 2 ml of chloroform and 2 ml of concentrated H₂SO₄ was added. A reddish brown coloration indicates the presence of terpenoids.
- Test for protein To 2 ml of extract, few drops of concentrated H₂SO₄ was added on it. White precipitate indicates the presence of protein.
- Test for steroid (Salkowski test) To 2 ml of extract, 2 ml of $CHCl_3$ and 2 ml of conc. H_2SO_4 were added on it. A reddish brown ring at the junction indicates the presence of steroid.

2.5 Heavy metal concentration test

The quantification of metals in the plant extract was done by flame AAS technique equipped in ICE 3000 series atomic absorption spectrometer. For this, 1.0 g of dried sample was taken in a 250-ml conical flask, and 5 ml of conc. HNO_3 (GFS Chemicals Inc., Columbus, 69%) was added slowly. The mixture was heated on the hot plate till the brown fumes disappeared yielding the white fumes. Water was added to make the solution, and it was then filtered in a 50-ml volumetric flask. Finally, the volume was adjusted to 50 ml by adding triple distilled water up to the mark [16, 17]. This filtrate was then introduced in flame AAS for the detection of metals.

2.6 Antioxidant activity study

The antioxidant activity of methanol extract was measured by DPPH free radical scavenging assay and it was carried out by Asadujjaman et al. method [18]. The sample solution was prepared in methanol at five different concentrations (25, 50, 75, 100, and 150 μ g/ml). Onemilliliter sample solution of each concentration was thoroughly mixed with 2-ml DPPH solution and shaken well for complete interfusion. The solution was

S. N.	Phytochemical tests	Methanol extract	Hexane extract	Methanol-water (1;1) extract
1	Test for alkaloid			
	Mayer's test	+	+	+ +
	Wagner test	+ + +	+ +	+ + +
2	Test for coumarins	+	+	+
3	Test for saponin	+	+	+
4	Test for phenol	_	_	-
5	Test for flavonoids	+	_	+
6	Test for glycoside	+ +	_	+ + +
7	Test for glucoside	+ +	_	+ + +
8	Test for reducing sugar	_	+	+ + +
9	Test for steroid	+	+	+ +
10	Test for tannins	_	_	-
11	Test for protein	_	+	-
12	Test for tarpenoids	+	+	+
13	Test for emodins	_	_	-
14	Test for anthraquinines	_	_	+
15	Test for cardiac glycoside	-	_	+ +

Table 1 Phytochemical composition of *D. diandra* in methanol, methanol-water (1:1) and hexane extract

+ + + high concentration, + + medium concentration, + low concentration, and - absent

incubated in dark for 30 min at room temperature for complete reaction with DPPH and the absorbance was measured at 517 nm by a UV/Vis. spectrophotometer against blank. The free radical scavenging activity of sample was calibrated in terms of % age inhibition of DPPH radical and compared with ascorbic acid as standard. The % age inhibition of DPPH radical is

$$I\% = \frac{A_o - A}{A_o} \times 100$$

where *I* represents DPPH inhibition (%), A_o is the absorbance of control sample, and *A* is the absorbance of a tested sample at the end of the reaction. The antioxidant

Table 2 Concentration of different heavy metal (mg/l) in a plant sample

S. N.	Metals	Concentration (mg/l)
1	Iron (Fe)	19.64
2	Nickel (Ni)	0.09
3	Zinc (Zn)	1.44
4	Arsenic (As)	< 0.005
5	Cadmium (Cd)	< 0.003
6	Chromium (Cr)	< 0.05
7	Lead (Pb)	< 0.01
8	Manganese (Mn)	2.35
9	Cobalt (Co)	0.23

activity of the sample was also expressed in terms of IC_{50} value, which represents the concentration of sample required to inhibit 50% formation of DPPH radical [19–21].

2.7 Antibacterial activity study

Antibacterial activity study was used to examine the antibacterial potency of the extracted plant sample. The antibacterial study was performed in the laboratory of the Department of Microbiology at Mahendra Morang Adarsh Multiple Campus, Biratnagar. The pathogenic bacteria used in the study were collected from the biochemistry laboratory of Suraksha Hospital, Biratnagar, Nepal.

The agar diffusion test (Kirby-Bauer (KB) antibiotic testing, also called disc diffusion antibiotic sensitivity testing) is a well-known standard method for testing the antibacterial sensitivity of bacteria. The samples (methanol, hexane, and methanol-water (1:1) extract) were tested in vitro against some gram positive and gram negative human pathogenic bacteria; namely Escherichia coli, Proteus vulgaris, and Staphylococcus aureus. Wellsterilized Whatman paper discs of 5-mm size were used as antibiotic assay discs. The discs were made by punching machine and well dried. Firstly, all the glassware, paper disc, and the Mueller-Hinton agar (MHA) media were sterilized. Twenty-five milliliters of MHA was carefully poured into each petri discs and allowed to rest for an hour in the sterilized zone for solidification. Freshly revived bacterial culture was wiped all over the media

S.N.	Concentration (µg/ml)	Absorbance	% inhibition	IC ₅₀ (µg/ml) of sample	IC ₅₀ (µg/ml) of ascorbic acid
1	25	0.528	4.86		
2	50	0.413	25.53		
3	75	0.386	30.45	195.61	39.25
4	100	0.375	32.3		
5	150	0.358	35.45		

Table 3 Antioxidant activity data of methanol extract

using sterile swab and the discs were stuck over it. Consequently, these discs were supplied with test compounds at three different concentrations (150, 75, and 37.5 μ g/ μ l in DMSO). One of the disc was soaked in DMSO and used as the solvent control, while amikacin (30 μ g/disc) was used as positive control. The whole process was carried out under UV laminar flow to eliminate the bacterial contamination, and the loaded discs were dried within the laminar flow chamber. The inoculated plates were incubated at 37 °C for 24 h. Also, the diameter of the zone of inhibition was measured by using anti-biogram zone measuring scale. The results were interpreted using the chart of NCCLS.

3 Results

3.1 Phytochemical screening

The phytochemical screening revealed the presence of alkaloids, saponins, coumarins, steroids, and terpenoids, in all three hexane, methanol, and methanol-water (1:1) extracts [22]. Flavonoids, glycosides, and glucosides were present in methanol and methanol-water extract but were absent in hexane extract. Protein was found to be present only in hexane extract. Anthraquinones and cardiac glycoside were present only in methanol-water extract whereas tannins, phenols, and emodins were absent in all three extracts.

The concentration of alkaloid by Mayer's test was medium in the methanol-water extract and low in remaining other two extracts. Similarly, in Wagner's test, it was high in methanol and methanol-water (1:1) and low in hexane extract. Coumarins, saponins, and terpenoids were present in low concentration in all three extracts. The concentration of Flavonoids was low in methanol and methanol-water (1:1) extract, whereas it was absent in hexane extract. Glycoside and glucosides were found to be in the high concentration in methanol and methanol-water (1:1) extracts and were absent in hexane extract. The reducing sugar was at high concentration in methanol-water (1:1) extract, low in hexane, and absent in methanol extract. Proteins were present only in hexane extract with low concentration. Anthraquinones were found present in low concentration and cardiac glycoside in medium concentration in methanolwater (1:1) extract and were absent in remaining two extracts. The report of phytochemical screening is presented in Table 1.

3.2 Heavy metal concentration test

Table 2 reports metal concentration data in mg/l of the plant sample. The inspection of data revealed higher concentration of Fe (19.64 mg/l) followed by Mn, Zn, Co, and Ni. The remaining 4 metals As, Cd, Cr, and Pb



Plant extract	Diameter of zone of inhibition in mm								
	E. coli			S. aureus			P. vulgaris		
Conc ⁿ . (µg/µl)	150	75	37.5	150	75	37.5	150	75	37.5
Methanol	14 ± 0.577	13 ± 0.577	11 ± 1.0	22 ± 1.0	15 ± 1.0	12 ± 0.577	12 ± 0.577	11 ± 0	10 ± 0.577
Hexane	12 ± 0.577	11 ± 1.0	10 ± 1.0	15 ± 0.577	14 ± 0.577	13 ± 0.577	11 ± 0.577	11 ± 0.577	10 ± 0.577
Methanol-water (1:1)	13 ± 0	11 ± 1.0	10 ± 0.577	13 ± 0.577	12 ± 0.577	11 ± 0.577	17 ± 1.0	11 ± 0.577	10 ± 0
Amikacin	18 ± 0.577			18 ± 0.577			15 ± 0.577		

Table 4 Antibacterial data of methanol, hexane, and methanol water (1:1) extracts of D. diandra in DMSO

were found in very little amount which is nearly below the detection limit.

3.3 Antioxidant activity study

The methanol extract of *Drymaria diandra* was assessed for DPPH free radical scavenging activity and the results are presented in Table 3. Figure 2 shows graphical plot of concentration verses % antioxidant activity inhibition of DPPH radical and from this plot, IC_{50} value was calculated. Lower IC_{50} value signifies higher antioxidant activity [23]. IC_{50} value of control (i.e., ascorbic acid) was found to be 39.25 µg/ml. In the present study, the IC_{50} value of the plant extract was found approx. 5 times greater than that of ascorbic acid.

3.4 Antibacterial activity study

The values of the diameter of the zone of inhibition of the tested bacterial pathogen for different extracts in three different concentrations are presented in Table 4 and are depicted in the bar graph below (Figs. 3, 4, and 5). These values of diameter of zone of inhibition correspond to the antibacterial strength of the extracts. The result shows interesting dose dependent antibacterial activity of the extract. All three extracts have shown activity against all bacterial pathogens but as compared with methanol and methanol-water extract, hexane extract showed little activity against all the bacterial pathogens. It is observed that hexane and methanol extracts were most active against *S. aureus*, and methanol-water extract (at the concentration 150 μ g/ μ l) was active against *P. vulgaris* even more than the control drug Amikacin. Similarly, all three extracts showed higher inhibition of *S. aureus* at the concentration 75 μ g/ μ l and 37.5 μ g/ μ l as compared with *P. vulgaris* and *E. coli*.

4 Discussion

The plant metabolites are essential chemicals that exhibit several noticeable effects in the human body. Flavonoids possess potent antioxidant activities and alkaloids show anticancer antimalarial activities. Steroids are very important class of alcohols with varying significance. The structures are so suited to be effectively transformed by the microbial actions to commercially valuable compounds which are otherwise difficult to synthesize [24]. The presence of steroids in all extracts posed an unlimited hope. The saponins are also known as the soap forming compounds and are economically important. The anthraquinones are naturally occurring coloring compounds used in food, drugs, cosmetics, etc. [25]. The Drymaria diandra plant inhabiting in Sundarharaicha region is found to accumulate much important class of compounds possessing therapeutic as well as commercial values. Furthermore, the area from where the plant was selected was free from metal pollution, and so





the plant is edible. Several metals were present in trace amount which is beneficial for several biological activities. The presence of iron suggests that the plants can be useful in the preparation of the drug for the treatment of anemia.

This low antioxidant activity of the plant extract may be due to absence of phenolic compounds and trace presence of flavonoids [26]. These are the chief components of plant extracts that are responsible to show the antioxidant activity in the living systems, acting as radical scavenger. The phenolic compounds do play the role of free radical scavenger and protect body from oxidative damage [27]. Over production of free radicals can cause oxidative stress in the living body which is associated to oxidative destruction of proteins, nucleic acids, and lipids. This destruction may lead to severe chronic degenerative diseases [28]. The current research result reveals less potency of antioxidant activity of *Drymaria diandra* plant.

The extracts showed a good spectrum of concentration dependent antibacterial actions against the tested pathogenic bacteria. So this plant may be useful for the preparation of broad spectrum antibacterial drugs. To sum up, this study is a good support for varied folklore uses of the *Drymaria diandra* plant.

5 Conclusion

Various phytochemicals, including alkaloids, steroids, glycosides, anthraquinones, etc., are present, while tannins and emodins were found absent in this plant. The presence of essential metals like Fe, Zn, and Mn in trace amount and the absence of toxic metals like Pb, Cd, and As provide biological significance of the studied plant. The paper disc diffusion test showed some good antibacterial potency, comparable to the standard antibiotics. Antioxidant potency shown by the plant is sure to maintain the free radicals in the body, and scavenge them if they are high and a very good defender to the DNA. Still, a large segment of the population in the world relies upon the traditional system of medicine. The folklore use though is very resourceful; the authenticity yet



needs serious research. The closely resembling species may sometimes create ambiguity that even leads to fatal disorders. Nepal, being a very rich source of vegetation, possesses an untapped reservoir with infinite possibility of medicinal species, and yet, we are poor. Therefore, there is an urgent need for improved management and investigation of such plants that bilaterally would upgrade her economy and promote the health worldwide. In the meantime, the bleak field of antibiotics is literally crying of resistance. Moreover, such natural antibiotics can have potential advantages over bacterial infections as resistance could somehow be detained. *D. diandra* may be one of the alternatives. With more resources and time, further investigation of chemical constituents of *D. diandra* and other poorly studied plants can be revealed.

Abbreviations

AAS: Atomic absorption spectroscopy; DMSO: Dimethyl sulfoxide; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; IC₅₀: Half maximal inhibitory concentration; KB: Kirby-Bauer; MHA: Mueller-Hinton agar; MMAMC: Mahendra Morang Adarsh Multiple Campus; NCCLS: National Committee for Clinical Laboratory Standard; TCM: Traditional Chinese Medicine

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Authors' contributions

AP and BG performed the study. NKC supervised the work and PKO managed antioxidant activity work. All authors have read and approved the manuscript.

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Availability of data and materials

All the data generated and analyzed during the study are included in the manuscript and are available for the readers.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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