

Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING, TOTAL PHENOL AND FLAVONOID CONTENT OF MIMOSA RUBICAU LIS AND REINWARDITA INDICA

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ABSTRACT

Objective: Phytochemicals as phenol and flavonoid have a powerful biological activity. So, this study aimed to carry out phytochemical screening, total phenol and flavonoid content in two plant species i.e. *M. rubicaulis* and *R. indica*.

Methods: The extraction of different parts of two plant species was done by maceration using ethanol. Phytochemical screening was done to confirm the presence of phytochemicals. Total phenol content was done by Folin ciocalteu method and total flavonoid content was done by Aluminium chloride colorimetric method.

Results: Phytochemical analysis revealed the presence of flavonoid, phenol, terpenoids in both plant species. The highest concentration of phenol content was observed in the root and stem of an extract of *M. rubicaulis* i.e. 281.83±1.98 mg GAE/g dry extract weight and 225.37±0.60 mg GAE/g dry extract weight. The highest concentration of flavonoid contents was observed in the leaves of *R. indica* i.e. 462.21±4.67 mg QE/g dry extract weight followed by stem and root of *M. rubicaulis* i.e. 381.06±5.23 mg QE/g dry extract weight and 337.43±1.39 mg QE/g dry extract weight.

Conclusion: Phytochemical analysis concluded the presence of biologically important phytoconstituents like flavonoid and phenol in both plant species. Further studies, should be carried out to isolate specific chemical constituents and should be used in different studies to explore their biological effects.

Keywords: *Mimosa rubicaulis*, *Reinwardita indica*, Phytochemical Screening, Total Phenol Content, Total Flavonoid Content, Quercetin, Gallic acid

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INTRODUCTION

Medicinal plants are the greatest source of all kinds of medicines including a traditional system of medicines, modern medicines, nutraceuticals and compounds for new chemical entities because it contains different types of phytochemicals or secondary metabolites [1]. Alkaloids, flavonoids, phenols, tannins, glycosides, terpenoids, etc are mostly found secondary metabolites or phytochemicals in plant sources like fruits, vegetables, beans, cereals, and plant-based beverages such as tea, wine, etc [2] and are synthesized by primary or rather secondary metabolism of living organisms [3]. Phytochemicals are important because it gives protection to plant and also possesses several pharmacological activities, so they can be used for the treatment of various kinds of diseases [4].

Phenol consists of an OH (Hydroxyl group) bonded directly to aromatic hydrocarbon groups. They are secondary metabolites in plants and exhibit health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor and antimicrobial [5]. Phenols have potent antioxidant properties due to the presence of hydroxyl and carboxyl group, they can bind to the free radicals and deactivate them along with the ability to donate electron or the hydrogen atom to the unpaired free radicals [6].

Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection [7]. Flavonoid is derived from the Latin word *flavus*, which means yellow, their color in, and structurally flavonoids are classified as flavones (Myricetin, Quercetin, Rutin, Kaempferol), flavanones (Naringenin, Fisetin), catechins (Catechin, Epicatechin), and anthocyanins (Cyanidin, Peonidin, Malvidin)[8]. A different study suggested that flavonoids possess different biological activity as antioxidants, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic activity, antihepatotoxic, antitumoral, and antimicrobial [9]. Flavonoids may also act as chemical messengers, physiological regulators and cell cycle inhibitors, protection of certain cell types, for example, red blood cells, reduce capillary hyperpermeability by protecting the micro-

circulation from inflammatory processes [6, 10-12]. But the specific function of many phytochemicals is still unclear. However, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases [13].

Due to the safe and effective results of plant source as medicine and strong evidence of biological activities of phenolic compounds, the study was focused on the determination of total phenolics and flavonoids content in two plant species i.e. *Mimosa rubicaulis* (Leguminosae) and *Reinwardita indica* (Linaceae). Traditionally these two plant species are used in peptic ulcer, dislocated bone, sprains, backache, hemorrhoids, wound, fever, boils, headaches, scabies [14] but the literature related to the phytochemical screening, total phenol, and flavonoid content in this two species is lacking till the date.

MATERIALS AND METHODS

Chemicals

Aluminium Chloride (Qualigens Fine Chemicals, India), Folin-Ciocalteu Reagent (Qualigens Fine Chemicals, India), Quercetin (Qualigens Fine Chemicals, India), Gallic Acid (Wako Pure Chemical Industries, Ltd., Japan) and all the other required chemicals were obtained from the laboratory of School of Health and Allied Sciences, Pokhara University, Lekhnath-12, Kaski, Nepal. All chemicals and reagents used were of analytical grade.

Plant materials

Whole plants of *M. rubicaulis* and *R. indica* were collected from different regions of Pokhara valley, Nepal by the proper identification under the supervision of local traditional healers. Then the root, stem, and leaves of *M. rubicaulis*, flower, and leaves of *R. indica* were separated from unnecessary parts of plants.

Identification of crude drug

A specimen of the plant was used to prepare herbarium and properly identified by the botanist Prof. Dr. Radhe Shyam Kayastha. Then, a sample of crude drugs were preserved in Pharmacognosy Laboratory of School of Health and Allied Sciences, Pokhara University, Nepal.

Drying of the crude drug

The collected crude samples were dedusted and cleaned properly, cut into small pieces and shade dried.

Extraction

Different parts of plants were extracted by maceration process using ethanol as solvent. Shade dried samples were first ground into fine powders. Then, 25 g of each sample was weighed and macerated in 175 ml ethanol (ethanol: crude drug= 7:1) for 24 h. After 24 h, filtration was done by using filter paper and the obtained residue was again macerated with 175 ml i.e. the same volume of ethanol for 24 h. Then, obtained filtrates were mixed and dried by using a rotatory vacuum evaporator and collected in the Petri dish. Again that, samples were kept in a vacuum desiccator for the complete removal of solvent present in the extract. The obtained dried extracts were collected in a sample vial and preserved in the refrigerator. Extractive yield for each sample was determined by using formula;

$$\% \text{Yield} = \text{Weight of extract} / \text{Weight of crude sample taken} \times 100 \% \dots (1)$$

Qualitative phytochemical screening

Phytochemical screening of ethanolic extract (root, stem, and leaves) of *M. rubicaulis* and (flower and leaves) of *R. indica* were carried out to reveal the presence of secondary metabolites according to the method described previously with some modification [15].

Quantitative phytochemical screening

Determination of total phenol contents

Folin ciocalteu method was used for the determination of total phenol content according to the method of [16] with some modifications. In brief, 1 ml of sample was mixed with 5 ml of

distilled water and 1 ml of Folin reagent. After standing for 5 min, 1 ml of 10% sodium carbonate was added and stirred. The mixture was incubated for 1 h at room temperature and the absorbance was measured at 725 nm against a blank. The total phenol content expressed as mg of GAE (Gallic Acid Equivalent) per g dry extract weight using the calibration curve of Gallic acid (50 mg/l-500 mg/l) standards.

Determination of total flavonoid contents

The aluminium chloride colorimetric method was used for flavonoids determination according to the method of [17] with some modifications. In brief, 1 ml of the sample solution was mixed with 4 ml of distilled water. Then, 300 µl of sodium nitrite was added. After 5 min, 300 µl aluminium chlorides were added and allowed to stand for 6 min. Then, 2 ml of sodium hydroxide was added. The mixture was stirred and the absorbance was measured at 510 nm using UV spectrophotometer and compared with standard. The total flavonoids content expressed as mg QE (Quercetin Equivalent) per g dry extract weight using a calibration curve of Quercetin (50 mg/l-500 mg/l) standards.

Data analysis

The experimental data obtained were expressed as mean±standard deviation.

RESULTS

Extraction yield value

The extract yield percentage of the plant sample was given in table 1. The extract yield percentage was relatively higher in root and leaves of *M. rubicaulis*, and flower of *R. indica* while relatively lower in the stem of *M. rubicaulis* and leaves of *R. indica*.

Table 1: List of sample for the study with their extraction yield value in ethanol

Scientific name	Parts used	Sample code	% Yield value (Ethanol)
<i>Mimosa rubicaulis</i>	Root	A	8
	Stem	B	3.2
	Leaves	C	11.52
<i>Reinwardita indica</i>	Flower	D	7.95
	Leaves	E	3.52

Table 2: Phytochemical analysis of the selected parts of plants

S. No.	Phytochemical test	Test performed	Inferences	<i>Mimosa rubicaulis</i>			<i>Reinwardita indica</i>	
				Root extract	Stem extract	Leaves extract	Flower extract	Leaves extract
1	Alkaloid	Mayer's Reagent	Appearance of yellow cream ppt.	-	-	-	-	-
		Wagner's Reagent	Formation of brown/reddish brown ppt.	-	-	-	-	-
2	Glycosides	Hager's Reagent	Formation of yellow color ppt.	-	-	-	++	+
		Modified Borntrager's test	Formation of rose pink color	--	-	-	-	-
		Legal's test	Formation of pink to blood-red color	-	-	-	+	-
3	Flavonoids	Alkaline Reagent Test	No intense yellow color obtained	+	+	-	+	+
4	Carbohydrates	Molish's Reagent	Formation of violet ring at the junction	+	+	+	+++	++
		Benedict's Reagent	Formation of orange red ppt.	++	+++	+	+	+
		Fehling's Reagent	Formation of red ppt.	++	+++	+	+	+
		Foam Test	Produced foam lasts for more than 10 min	++	-	-	-	-
6	Tannins	Gelatin Test	Formation of white ppt.	+	+	-	-	-
7	Resin Test	Acetone water Test	Appearance of turbidity	+	+	-	+	-
8	Phenol test	Ferric chloride Test	Appearance of bluish black ppt.	++	++	+	+	+
9	Terpenoids	Salkowski's Test	Appearance reddish brown precipitates	++	++	+	-	+
10.	Proteins and aminoacid test	Xanthoproteic Test	Formation of yellow color	+	+	-	+	+
11.	Fixed oils and Fats test	Filter paper press Test	Appearance of oily stain on filter paper	+	+	+	++	++

Note: +++: Strong intensity reaction, ++: Medium intensity reaction, +: Weak intensity reaction, -: Non detected

Qualitative phytochemical analysis

The qualitative phytochemical analysis revealed the presence of flavonoid, phenol, carbohydrate, resin, protein, fixed oil, and terpenoids in the ethanolic extract of both plant species i. e. *M. rubicaulis* and *R. indica* but alkaloid was absent in *M. rubicaulis* extract and glycoside was absent in both *M. rubicaulis* and *R. indica* extract which had been tabulated in table 2.

Total phenol contents

The calibration curve of Gallic acid as standard at concentrations 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, and 500 mg/l, as shown in fig. 1. The total phenolic content of the different extract was expressed as mg of GAE per g dry extract. The highest concentration of phenol content was observed in root and stem of extract of *M. rubicaulis* i.e. 281.83±1.98 mg GAE/g dry extract weight and 225.37±0.60 mg GAE/g dry extract weight and the

lowest concentration of phenol content was observed in flower extract of *R. indica* i.e. 24.33±0.26 mg GAE/g dry extract weight as shown in table 3.

Total flavonoid contents

The total flavonoid content was performed by precipitating with aluminium chloride in an alkalized medium. Quercetin was taken as standard and the calibration curve of Quercetin as at concentrations 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, and 500 mg/l as shown in fig. 2. The highest concentration of flavonoid contents were observed in the leaves of *R. indica* i.e. 462.21±4.67 mg QE/g dry extract weight followed by stem and root of *M. rubicaulis* i.e. 381.06±5.23 mg QE/g dry extract weight and 337.43±1.39 mg QE/g dry extract weight The lowest concentration of flavonoid content was observed in the leaves of *M. rubicaulis* i.e. 159.85±5.23 mg QE/g dry extract weight. The total flavonoid contents of plant extract were listed in table 4.

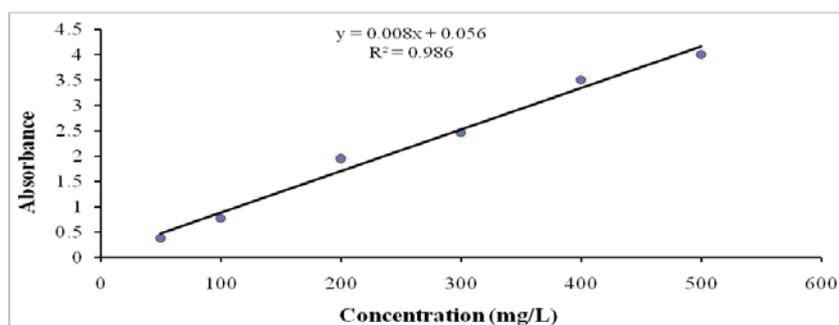


Fig. 1: Calibration curve of Gallic acid for total phenol calculation

Table 3: Total phenol content expressed as mg GAE per g dry extract

S. No.	Sample	Total phenol content (mg GAE/g dry weight of extract)
1.	A	281.83±1.98
2.	B	225.37±0.60
3.	C	130.86±0.09
4.	D	24.33±0.26
5.	E	79.33±0.17

Data expressed as a mean±standard deviation for three experiments. Total Phenol content was calculated with the help of the calibration curve of Gallic acid as standard.

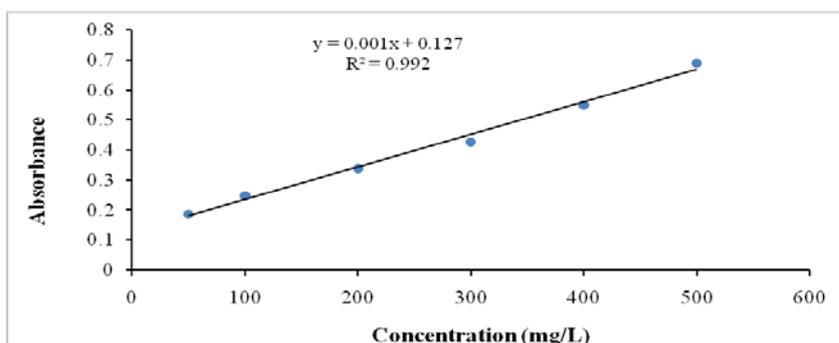


Fig. 1: Calibration curve of Quercetin for total flavonoid calculation

Table 4: Total flavonoid content expressed as QE per g dry extract

S. No.	Sample	Total flavonoid content (mg QE/g dry weight. extract)
1	A	337.43±1.39
2	B	381.06±5.23
3	C	159.85±5.23
4	D	194.09±0.91
5	E	462.21±4.67

Data expressed as mean±standard deviation for three experiments. Total flavonoid content was calculated with the help of the calibration curve of Quercetin as standard.

DISCUSSION

Phytochemical screening is done to reveal the presence of important phytoconstituent in plant samples, which helps in discovering of novel drugs having any pharmacological activity [18] and finding out the ingenuity of drug [19]. This study revealed the presence of flavonoid, phenol, tannin, carbohydrates, etc. in both species. It showed that most of the biologically active constituents are present in both plant species. This study showed a similar observation done in the previous in methanolic extract of *M. rubicaulis* as the presence of flavonoids like quercetin, lutein, and tannin [9].

Phenol is a secondary metabolite and has free radical scavenging activity due to the presence of the hydroxyl group. The phenolic compounds from the sample cause the reduction of heteropoly acids (Phosphomolybdate-phosphotungstate) contained in Folin-ciocalteu reagent that changes from yellow color to Prussian blue which can be measured in absorbance at 725 nm [20]. So, the intensity of the blue color is directly proportional to the phenolic content [5]. In this study, phenol content was found to be high in root and stem of *M. rubicaulis* i.e. 281.83±1.98 mg GAE/g dry extract weight and 225.37±0.60 mg GAE/g dry extract weight. Those parts of plant extracts which showed better antioxidant activity have higher phenol content. But *R. indica* contains lower phenol contents, This observation is comparatively lower than the previous study done on aqueous and methanolic extracts of *R. indica* leaves, which showed a significant total phenolic content DPPH (2,2-diphenyl-1-picrylhydrazyl) and anion scavenging activity [21].

Flavonoid is one of the most widespread and important secondary metabolites that possess various biological activities i.e. they are good scavengers of the most oxidizing molecule, including singlet oxygen, and various free radicals [22]. The total flavonoid content was determined by using the aluminium chloride colorimetric method. Flavonoid in the presence of aluminium chloride has intense yellow fluorescence which is observed in the UV spectrometer at 510 nm [17]. Among the plant extracts studied, total flavonoid contents were found to be high in leaves of *R. indica* followed by stem and root of *M. rubicaulis* i.e. 462.21±4.67 mg QE/g dry extract weight, 381.06±5.23 mg QE/g dry extract weight, and 337.43±1.39 mg QE/g dry extract weight. There is no correlation between phenolic content and flavonoid content of leaves extract of *R. indica*. This indicates that the leaves of *R. indica* may contain the flavonoids without the OH in aromatic benzene ring [23].

So, plants containing phenolic and flavonoids compound are of interest in the biological research for the discovery of novel leads molecules [24, 25].

CONCLUSION

Both qualitative and quantitative phytochemical analysis concluded the presence of biologically important phytoconstituents like flavonoid and phenol in the ethanolic extract of both plant species. So, these two plant species could be used as a natural antioxidant, anti-inflammatory, wound healing agent, etc. In further, a different study should be carried out to isolate specific chemical constituents as well as to explore their biological effects in the proper scientific ways which could be beneficial in the treatment and controlling of various diseases.

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AUTHORS CONTRIBUTIONS

Roshani Gurung: Literature search, done all the experiments, done data analysis, done designing of the draft for the manuscript, wrote the first draft, done review and editing of the draft.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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