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Phytochemical Analysis and Bioactivity of Nepalese Medicinal Plants, with Compound Isolation from *Dischidia bengalensis* Colebr.

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ABSTRACT: Numerous medicinal plants have been used traditionally to treat, relieve, and prevent various diseases since ancient time, yet many of them are to be scientifically investigated. The present study was carried out with the aim of phytochemical screening, determination of 1,1–diphenyl–2–picrylhydrazyl (DPPH) free radical scavenging activity and antibacterial activity of 70% methanolic extracts of six medicinal plants of Nepal used by different ethnic tribes, and to isolate chemical compounds from one of them. Agar well diffusion method was used to assess the *in vitro* antibacterial activity, whereas the chemical isolation was carried out using different chromatographic techniques. Then, the structure of the isolated compounds was determined by using NMR (Nuclear Magnetic Resonance) spectroscopic method, followed by spectral analysis in comparison with published data. Of the six extracts, the extracts of *Angiopteris helferiana* and *Drynaria coronans* showed moderate antibacterial activities. Moreover, the extract of *Dischidia bengalensis* showed the most potent antioxidant activity and was thus subjected to chromatographic separations utilizing different chromatographic principles to isolate 2"-*O*-rhamnosylvitexin **(1)** and isovitexin **(2)**. These secondary metabolites are reported for the first time from this plant whilst, their pharmacological activity is yet to be screened.

1. INTRODUCTION

Around 80% of the developing world is thought to rely on traditional medicine to treat a variety of diseases, and 85% of these traditional medicines contain plants or their extract (WHO, 2022). Ancient medical systems like Ayurveda from the Indus civilization, Arabian medicine from Mesopotamia, Chinese and Tibetan medicine from yellow river culture of China, and Kampo from Japan are primarily based on plants. These medicinal plants are employed to relive, prevent, or treat disease as well as alter pathological and physiological processes (Gewali, 2008). However, most of them are still not fully investigated for their potential medicinal values and chemical constituents responsible for their biological activity (Sai et al., 2019).

Antioxidants are substances that inhibit oxidative processes by delaying or preventing oxidative damage (Halliwell, 1995). Antioxidants are crucial in preventing a variety of medical conditions like cancer, diabetes, a neurodegenerative disorder, atherosclerosis, aging and so on (Kurnia et al., 2021; Tailor & Goyal, 2014). Although synthetic antioxidants like butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) are accessible globally, they have harmful and carcinogenic effects when tested on animals, resulting in the need for novel natural antioxidants (Coulombier et al., 2021).

Anti-bacterial agents are those agents that are obtained from natural, synthetic, or semi-synthetic sources and are used to kill or inhibit microorganisms growth (Ansari et al., 2021). Antibiotic resistance is a serious public health concern that raises mortality rates and increases the cost of healthcare. Haphazard consumption and exploitation of antibiotics is the culprit behind increasing antimicrobial resistance. This has made it difficult to treat microbial infections, thus creating necessities for new antimicrobial medication (Nath et al., 2018). Moreover, the search for alternatives in medicine

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has been emphasized by the increasing multidrug resistant disease and the well-documented side effects of some prevalent medications (Pokharel et al., 2008).

The indigenous people of Tanahun, Nepal have been using the rhizome of *Angiopteris helferiana* (Marattiacea), leaf of *Dischidia bengalensis* Colebr. (Apocynaceae), rhizome of *Drynaria coronans* (Polypodiaceae), leaf of *Gynura nepalensis* (Asteraceae), and stem of *Mikania spp*. (Asteraceae) to treat bone fracture and muscle sprain. Similarly, the rhizome of *Curcuma caesia* (Zingiberaceae) is used by the locals of Kaski, Nepal to treat bone fractures. Thus, based on these traditional uses these six plants were selected to explore their phytochemicals, antioxidant, and antibacterial activity. Compound isolation was performed on *Dischidia bengalensis* Colebr. whole plant because much is not known about this plant's constituents. *Dischidia bengalensis* is an epiphytic, tiny tropical succulent herb with a trailing stem, generally seen at altitudes of 900–1100 meters in Nepal, Malaysia, India, Burma, Thailand, Sumatra, Borneo, Java, New Guinea, and Bismarck Arch (Rintz, 1980). This species generally hangs down from trees like *Ficus bengalensis*, *F. glaberrima*, *F. religiosa*, or *Castanopsis indica* and grows on the support of tree trunks or branches (Bhandari & Shrestha, 2016).

2. MATERIALS AND METHODS

2.1. Plant Materials

Based on the ethnomedicinal uses, six plants chosen for this study were collected from different areas of Tanahu and Kaski district of Nepal. The dried specimens were mounted on authentic standard herbarium sheets, labelled correctly, and stored in the Laboratory of Pharmacognosy, School of Health and Allied Sciences, Pokhara University, Nepal. The herbarium was then sent to National Herbarium and Botanical Laboratories, Nepal for identification purpose. Table 1 contains information about the plant samples that were collected for this study.

2.2. Extraction of Plant Materials

The plants were extracted by using double maceration method with 70% methanol in the ratio of 1:10 w/v (dried plant material: solvent). Then the obtained extracts were filtered through cotton and were dried in a rotatory evaporator (40°C, 70-80 rpm). The obtained concentrated extracts were then kept in a vacuum desiccator (50-60 mbar pressure) until complete drying. The weight of each extract was measured daily until a constant weight was obtained.

2.3. Phytochemical Screening

All six plant extracts were screened for different classes of secondary metabolites using analytical test methods as mentioned in different literatures (Chen et al., 2022; Shaikh & Patil, 2020). The stock solution of concentration 1 mg/ml was prepared by dissolving the plant extract in methanol and water to prepare methanolic and aqueous extract solutions, respectively. Table 2 illustrates the methods employed for phytochemical screening of plant extracts.

2.4. Antioxidant Activity

2.4.1 DPPH Radical Scavenging Assay

The antioxidant activity of different plant extracts was determined by using a 1,1–diphenyl–2–picrylhydrazyl (DPPH) assay (More et al., 1920). In this study, ascorbic acid served as a standard positive control. 10 ml methanol was used to dissolve 10 mg of each dried plant extract and ascorbic acid to obtain 1 mg/ml stock solution. Then, from that stock solution, test solutions of four different concentrations (100 μg/ml, 10 μg/ml, 1 μg/ml, 0.1 μg/ml) were prepared by serial dilution. All diluted samples were prepared in triplicate, and their findings were present as the mean of three tests. Each triplicated 2 ml sample received 2 ml of 60 μm strength DPPH solution, which was then left in the dark for 30 minutes. Absorbance was recorded at 517 nm by using a UV-Visible Spectrophotometer (Tailor & Goyal, 2014). To calculate the percentage of DPPH free radical scavenging activity, the following equation was used (More et al., 1920).

$$\%\ DPPH\ RSA = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} X\ 100$$

Where, control is solution without sample extract. The graph was plotted as % DPPH radical scavenging activity versus concentration in Excel 2019 form where IC_{50} value was calculated.

2.5. Antibacterial Activity

2.5.1 Agar Well Diffusion Method

The bacterial susceptibility of all 70% methanolic plant extracts to *Staphylococcus aureus* and *Escherichia coli* was assessed using the agar well diffusion method. The standard antibiotic discs of azithromycin (30 mcg) and gentamicin (10 mcg) were used as positive controls, with distilled water serving as a negative control. The clinical isolates of *S. aureus* and *E. coli* were bought from Western Regional Hospital, Kaski, Nepal. The bacteria were then sub cultured in Muller Hilton Agar (MHA) plate and incubated at 37 °C for 24hr (Ansari et al., 2021; Balouiri et al., 2016; Timilsina et al., 2020).

Muller Hilton Agar was used to prepare culture media, which was inoculated with subculture bacterial strains using cotton swab. After this, a well of 4 mm depth and 6 mm width was made with the help of sterile micropipette tips. Next, the 70% methanolic extract of all six plants were dissolved in distilled water to prepare 1 g/ml stock solution. From the stock solution, three different concentrations of test solution (100 mg/ml, 50 mg/ml, 25 mg/ml) were prepared. By using micropipette, 20 μl of each test solutions and distilled water were then poured into respective wells. Simultaneously, the standard azithromycin and gentamicin disc were inserted into the petri dish with the help of sterile tweezer. The test solutions were allowed to diffuse into the MHA media for 20 minutes at room temperature and was

Table 1
Scientific and local name of ethnomedicinal plants selected for study along with its traditional uses

Name of the Plant	Local name	Parts Used	Place of Collection	Traditional Uses	Crude Drug Voucher No.
Angiopteris helferiana (Marattiacea)	Gaikhurey	Rhizome	Shuklagandaki-12, Tanahu	Bone fracture, Muscular pain, Sprain	PUCD-2022-34
Curcuma caesia (Zingiberaceae)	Kalo haledo	Rhizome	Pokhara-33, Kaski	Bone fracture, Muscular pain	PUCD-2022-35
Dischidia bengalensis (Apocynaceae)	Thirjo	Whole plant	Shuklagandaki-12, Tanahu	Bone fracture, Sprain, Muscular pain	PUCD-2022-36
Drynaria coronans (Polypodiaceae)	Kammaru	Rhizome	Shuklagandaki-12, Tanahu	Bone fracture, Sprain	PUCD-2022-37
Gynura nepalensis (Asteraceae)	Kholey harchula	Leaves	Shuklagandaki-12, Tanahu	Bone fracture	PUCD-2022-38
Mikania spp. (Asteraceae)	Shikari lahara	Stem	Shuklagandaki-03, Tanahu	Bone fracture, Sprain, Muscular pain	PUCD-2022-39

Table 2
Different analysis techniques used in phytochemical screening of ethnomedicinal plants

S.No.	Phytochemical Constituents	Type of Test	Method	Result
1.	Alkaloids	Dragendorff's reagent test	1 ml of methanolic extract solution + 1 ml of Dragendorff's reagent	Formation of light yellow or reddish-brown precipitate indicated presence of alkaloids.
		Mayer's reagent test	1 ml of methanolic extract solution + 1 ml of Mayer's reagent	Formation of a white or yellow precipitate indicated the presence of alkaloids.
2.	Tannin	Gelatin test	1 ml of aqueous extract solution + 0.5% of gelatin solution + 10% NaCl solution	Turbidity or formation of white precipitate indicated the presence of tannins.
3.	Saponin	Foam test	1 ml of aqueous extract solution + 5 ml of distilled water, followed by vigorous shaking	The generation of a stable foam suggested the presence of saponins.
4.	Phenol	$FeCl_3$ test	1 ml of aqueous extract solution + 1 ml of 2% $FeCl_3$ solution	Formation of a blue-green or black color indicated the presence of phenols.
5.	Carbohydrate	Molisch's reagent test	1 ml of aqueous extract solution + 1 ml of Molisch's reagent, followed by pouring into another test tube containing 1 ml of H_2SO_4	Formation of purple ring at the aqueous phase/organic phase interface indicated the presence of carbohydrates.
		Fehling's reagent test (For reducing sugars)	1 ml of aqueous extract solution + 1 ml of Fehling's reagent, followed by gentle boiling	Formation of a brick-red precipitate indicated the presence of reducing sugars
6.	Flavonoid	Shinoda test	1 ml of methanolic extract solution + magnesium ribbon, followed by 2 drops of conc. HCl	Formation of red to the red-purple color indicated the presence of flavonoids
		Alkaline reagent test	1 ml of methanolic extract solution + 1 ml of 2% NaOH solution	Formation of an intense yellow color followed by a change to colorless on the addition of a few drops of oil. HCl indicated the presence of flavonoids.
7.	Terpenoid	Salkowski test	1 ml of methanolic extract solution + 2 ml of $CHCl_3$ + 2 ml of H_2SO_4	The formation of reddish-brown color indicated the presence of terpenoids.
8.	Quinone	Test for quinones	1 ml of methanolic extract solution + few drops of conc. H_2SO_4	The formation of red color indicated the presence of quinones.

incubated at 37 °C for 24 hr. After this, the diameter of the clear zone of inhibition (mm) were measured with the help of scale. The experiment was performed in triplicate, and the mean value was calculated. All the apparatus and equipment were sterilized prior to their use in autoclave (121 °C temp. for 15 mins). Also, the working area was sterilized by using spirit.

2.6. Chemical Isolation

Dried extract of *Dischidia bengalensis* (whole plant) (550 g) was used for chemical isolation. Extraction in 70% methanol yielded 76.2 g of the crude extract, which was dissolved in sufficient amounts of distilled water and then the water-soluble extract (DNW) (66.19 g) was introduced into MCI (Mitsubishi Chemical Corporation) GEL^{TM} column. The column was eluted with water (1500 ml), 40% MeOH (1500 ml), 60% MeOH (1500 ml), 80% MeOH (1000 ml), MeOH (1000 ml) and chloroform (500 ml) respectively. TLC (Thin layer chromatography) patterns of alternate fractions were observed on solvent system $CHCl_3$: MeOH: H_2O (6:4:1) for fractions 1-35 and with $CHCl_3$: MeOH: H_2O (7:3:0.5) for fractions 36-74. The developed TLC chromatograms were then visualized under UV (long UV-365 and short UV-254) followed by H_2SO_4 (10% v/v) spray with heat for 2 min and $FeCl_3$ (10% w/v) spray. Fractions with similar spots on TLC were mixed to give 12 sub-fractions (DNW-1 to DNW-12) and were evaporated in rotary evaporator. Of the 12 subfractions, DNW-6 and DNW-9 were further subjected to column chromatography to obtain compound **1** (DNW-6-4-2-4) and compound **2** (DNW-9-4-2-3).

2.6.1 For DNW-6-4-2-4 (Compound 1)

DNW-6 (3.72 g) fraction obtained from MCI GEL^{TM} column was dissolved in 40% MeOH and eluted through Sephadex® LH20 column with 500 ml MeOH. From this, 48 sub-fractions each of 10 ml were collected, where fractions with similar spots in TLC were combined to give 5 major sub-fractions (DNW-6-1 to DNW-6-5) and evaporated. DNW-6-4 (1760 mg) obtained from Sephadex® LH20 column was dissolved in 40% MeOH and eluted through Octadecylsilyl groups (ODS) column with 500 ml of 40% MeOH. 36 sub-fractions each of 10 ml were collected in test tubes, where TLC with similar spots were combined to get 4 major sub-fractions (DNW-6-4-1 to DNW-6-4-4). DNW-6-4-2 (1550 mg) fraction obtained from ODS column was then eluted through the Silica column (containing activated silica gel) with 8:2.5:0.2 and 7:3:0.5 ($CHCl_3$: MeOH: H_2O). Fractions showing similar spots in TLC were mixed to give 14 sub-fractions (DNW-6-4-2-1 to DNW-6-4-2-14) and were evaporated. TLC pattern of all the fraction was observed on solvent system $CHCl_3$: MeOH: H_2O (7:3:0.5) under UV (long UV-365 and short UV-254) followed by H_2SO_4 (10% v/v) spray with heat for 2 min and $FeCl_3$ (10% w/v) spray.

2.6.2 For DNW-9-4-2-3 (Compound 2)

DNW-9 (2.03 g) fraction obtained from MCI GEL^{TM} column was dissolved in 70% MeOH and eluted through Sephadex® LH20 column with 500 ml of 70% MeOH. 45 sub-fractions each of 10ml were collected, where fractions with similar spots in TLC were combined to give 5 major sub-fractions (DNW-9-1 to DNW-9-5) and evaporated. DNW-9-4 (60 mg) obtained from Sephadex® LH20 column was dissolved in 35% MeOH and eluted in ODS column with 500 ml of 35% MeOH to obtain 49 sub-fractions, of which the fractions with similar spots were combined to get 3 major sub-fractions (DNW-9-4-1 to DNW-9-4-3). DNW-9-4-2 (9.4 mg) and DNW-9-4-3 (15.6 mg) fraction obtained from ODS column were mixed and was eluted through the Silica column with 9:3:0.1 ($CHCl_3$: MeOH: H_2O) solvent. 43 sub-fractions each of about 5ml were collected in test tubes, where fractions with similar spots were mixed to give 3 sub-fractions (DNW-9-4-2-1 to DNW-9-4-2-3) and were evaporated. The TLC patterns of all the fractions was observed on solvent system $CHCl_3$: MeOH: H_2O (7:3:0.5) under UV (long UV-365 and short UV-254) followed by H_2SO_4 (10% v/v) spray with heat for 2 min and $FeCl_3$ (10% w/v) spray.

Chromatograms of DNW-6-4-2-4 (60 mg) and DNW-9-4-2-3 (2.6 mg) at different mobile phase ratios gave single spots on TLC plates when visualized using afore mentioned visualization techniques. Therefore, these were sent to Kumamoto University, Japan for NMR spectral analysis.

2.6.3 2”-O-rhamnosylvitexin (Compound 1)

Pale yellow amorphous powder. ^{1}H-NMR (600 MHz, CD_3OD) 8.03 (2H, d, J=8.82Hz, H-2’, H-6’), 6.95 (2H, d, J=8.75Hz, H-3’, H-5’), 6.75 (1H, s, H-3), 6.23 (1H, s, H-6), 4.97 (1H, s, H-1”’), 4.77 (1H, brd, J=9.96Hz, H-1”), 3.76 (2H, dd, J=11.52 Hz, H-6”), 0.63 (3H, d, J=6.12Hz, H-6”’). ^{13}C-NMR (150 MHz, CD_3OD) 181.9 (C-4), 163.8 (C-2, C-7), 161.3 (C-4’), 160.6 (C-5), 155.9 (C-9), 128.9 (C-2’, C-6’), 121.5 (C-1’), 115.9 (C-3’, C-5’), 104.5 (C-8), 103.9 (C-10), 102.3 (C-3), 102.2 (C-1”’), 98.5 (C-6), 81.7(C-5”), 79.9 (C-3”), 75.1 (C-2”), 71.7 (C-1”, C-4”’), 70.7 (C-4”, C-2”’), 70.4 (C-3”’), 68.2 (C-5”’), 17.67 (C-6”’).

2.6.4 Isovitexin (Compound 2)

Pale yellow amorphous powder. ^{1}H-NMR (600 MHz, CD_3OD) 7.90 (2H, d, J=8.82Hz, H-2’, H-6’), 6.92 (2H, d, J=8.72Hz, H-3’, H-5’), 6.76 (1H, s, H-3), 6.48 (1H, s, H-8), 4.58 (1H, brd, J=9.78Hz, H-1”), 3.68 (2H, dd, J=10.26 Hz, H-6”), 3.50-4.04 (Remaining Sugar Protons). ^{13}C-NMR (150 MHz, CD_3OD) 181.6 (C-4), 163.4 (C-2, C-7), 161.2 (C-4’), 160.6 (C-5), 156.3 (C-9), 128.4 (C-2’, C-6’), 121.1 (C-1’), 115.9 (C-3’, C-5’), 108.9 (C-6), 102.9 (C-3), 102.7 (C-10), 98.5 (C-6), 93.7(C-8’), 81.5(C-5”), 78.9 (C-3”), 73.1 (C-1”’), 70.6 (C-2”), 70.3 (C-4”’), 61.5 (C-6”).

Table 3
Result of phytochemical screening of selected plant extracts

S.No.	Phytochemical Constituents	Type of Test	AHR	CSR	DBWP	DCR	GSL	MSS
1.	Alkaloids	Dragendorff's reagent test	+	+	+	+	+	+
		Mayer's reagent test	-	+	+	-	+	-
2.	Tannin	Gelatin test	+	+	-	+	+	+
3.	Saponin	Foam test	+	+	+	+	-	+
4.	Phenol	$FeCl_3$ test	-	+	+	+	+	-
5.	Carbohydrate	Molisch's reagent test	+	+	+	+	+	+
		Fehling's reagent test	-	-	-	+	-	+
6.	Flavonoid	Shinoda test	+	-	+	+	-	-
		Alkaline reagent test	-	+	+	-	-	+
7.	Terpenoid	Salkowski test	+	-	+	-	+	+
8.	Quinones		+	+	+	+	+	-

(+) sign indicates the presence ofphytochemical constituents; (-) sign indicates the absence of phytochemicalconstituents; AHR- *Angiopteris helferiana* rhizome; CCR- *Curcuma caesia* rhizome; DBWP- *Dischidia bengalensis* wholeplant; DCR- *Drynaria coronans* rhizome; GSL- *Gynura nepalensis* leaves; MSS- *Mikania spp.* Stem

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Screening

Around the world, there has been a lot of interest in studying the chemical constituents of medicinal plant as they possess variety of biological properties, including medicinal properties, and are often used in traditional medicines (Chhetri & Khatri, 2017). The phytochemical screening of 70% methanolic extract of six plant samples revealed either presence or absence of the different secondary metabolites i.e., alkaloids, tannins, saponins, phenols, carbohydrates, flavonoids, terpenoids, and quinones. The presence or absence of phytochemicals was confirmed by visual observation of the change in color or turbidity. In the present study, *Gynura nepalensis* (GSL) leaves lacked flavonoids but the study conducted by Chakrabarty et al. (2022) revealed the presence of flavonoids. Similarly, the extract of *Angiopteris helferiana* and *Mikania spp.* showed no response to $FeCl_3$ phenol test indicating the absence of phenol. However, a study reported high phenol content in rhizome of *A. helferiana* (Lamichhane et al., 2019). The extract of *Curcuma caesia* revealed the presence of alkaloid, saponin, quinone, phenol, flavonoid, carbohydrate, and tannin, but lacked terpenoid. Interestingly, a study reported the presence of terpenoids in this plant (Pakkirisamy et al., 2017). This variation in phytochemicals may be due to difference in plant collection time, difference in solvent system used for extraction, parts of the plant used, age and various ecological and climatic conditions. Moreover, the number of phytochemical substances varies greatly from species to species and even from plant to plant (Shaikh & Patil, 2020). The result of the phytochemical screening has been shown in Table 3.

3.2. Antioxidant Activity

Antioxidant activity was measured using DPPH free radicals scavenging assay with ascorbic acid as the standard. A graph of concentration versus percentage of free radical scavenging activity was generated, as well as the IC_{50} value of each plant was calculated. The antioxidants in the DPPH method react with the stable free radical, 2,2-diphenyl-1-picrylhydrazyl (deep violet color), and convert it to 2,2-diphenyl-1-picrylhydrazine with discoloration, indicating the sample's scavenging potential (Gurung et al., 2019). It is a pink-colored nitrogen-containing radical that loses its pink color when it accepts an electron or a hydroxyl group from antioxidants. Color loss is proportional to the number of electrons accepted and can be quantified using light absorption changes at 517 nm. The IC_{50} value in DPPH free radical scavenging method is the sample concentration that can scavenge 50% of the DPPH free radical, evaluated by recording the change in absorbance due to DPPH reduction, which is inversely related to the sample's antioxidant property (Giri et al., 2020). In the current study, all plant extract showed a dose-dependent increase in activity from concentrations of 0.1 μg/ml to 100 μg/ml. Comparatively, the extract of *Dischidia bengalensis* and *Curcuma caesia* showed potent

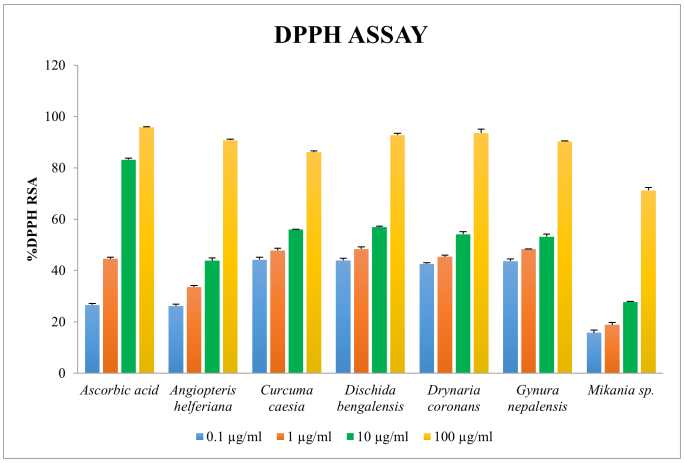

Figure 1. Percentage DPPH free radical scavenging activity of selected plant samples with reference to standard ascorbic acid at 517nm

Table 4
DPPH-radical scavenging activity of selected plant samples and standard ascorbic acid in four different concentrations (0.1 μg/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml) along with IC_{50} Value

Sample	Concentrations % Radical Scavenging Activity				IC_{50} Value (μg/ml)
	0.1 μg/ml	**1 μg/ml**	**10 μg/ml**	**100 μg/ml**	
Ascorbic Acid	26.55±0.54	44.55±0.57	83.20±0.60	95.75±0.19	2.76
Angiopteris helferiana	26.17±0.67	33.54±0.64	43.81±1.08	90.75±0.39	30.19
Curcuma caesia	44.17±1.05	47.82±0.77	55.98±0.05	86.07±0.45	5.81
Dischidia bengalensis	43.94±0.87	48.36±0.79	56.91±0.30	92.72±0.65	4.60
Drynaria coronans	42.57±0.50	45.32±0.68	54.12±1.02	93.51±1.56	9.46
Gynura nepalensis	43.64±0.81	48.28±0.14	53.16±1.02	90.26±0.27	7.61
Mikania spp.	15.72±1.11	18.81±0.86	27.74±0.23	71.24±1.02	59.17

The value of each plant standard was expressed as a mean ± standard deviation (n=3)

Table 5
Zone of inhibition of standard drugs against selected microorganisms

S.N.	Standard drugs	Microorganisms	Zone of inhibition
1.	Azithromycin (30 mcg)	*Staphylococcus aureus*	11.33±4.13
		Escherichia coli	6.33±2.07
2.	Gentamicin (10 mcg)	*Staphylococcus aureus*	15.33±1.63
		Escherichia coli	14.17±2.32

Table 6
Zone of inhibition of selected plant samples against test microorganisms *S. aureus* and *E. coli* at three different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml)

S. No (Plant Code)	Plants Used	Parts Used	Concentration (mg/ml)	Microorganisms	
				Staphylococcus aureus	*Escherichia coli*
1	*Gynura nepalensis*	Leaf	100	-	-
			50	-	-
			25	-	-
2	*Angiopteris helferiana*	Rhizome	100	6.75 ± 3.89	5.50 ± 2.12
			50	8.00 ± 1.42	3.00 ± 1.41
			25	3.50 ± 0.71	-
3	*Curcuma caesia*	Rhizome	100	-	-
			50	-	-
			25	-	-
4	*Dischidia bengalensis*	Whole plant	100	-	5.00 ± 0.00
			50	-	-
			25	-	-
5	*Drynaria coronans*	Rhizome	100	4.50 ± 2.12	8.00 ± 1.42
			50	4.00 ± 0.00	5.00 ± 0.00
			25	-	3.50 ± 0.00
6	*Mikania spp.*	Stem	100	-	4.00 ± 0.00
			50	-	3.50 ± 0.00
			25	-	3.00 ± 0.00

Values are expressed as the mean of zone of inhibition (mm) ± SD. The (-) sign indicates the absence of zone of inhibition.

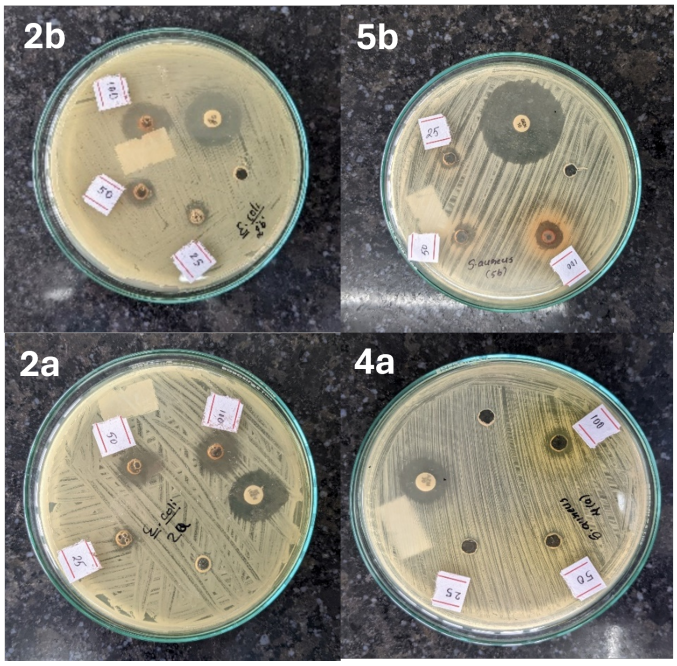

Figure 2. Zoneof inhibition of *A. helferiana* (2), *D. bengalensis* (4), and *D. coronans* (5) along with standard Azithromycin (a) and Gentamycin (b) against *S. aureus* and *E. coli* at three different concentrations, i.e. 100 mg/ml, 50 mg/ml, and 25 mg/ml.

antioxidant activity (IC_{50}= 4.60 μg/ml and 5.81 μg/mL respectively), as compared to the positive control, ascorbic acid (IC_{50} = 2.76 μg/mL), and this may be due to presence of phytochemicals like flavonoids and phenols. According to studies, phenolic compounds with higher concentrations have increased antioxidant properties. The antioxidant property of phenolic compounds is mainly due to their ability to scavenge injurious free radicals such as superoxide and hydroxyl radicals (Meng et al., 2021). Also, the current investigation found the IC_{50} value of *Angiopteris helferiana* extract to be 30.19 μg/ml, which is lower than the value reported by (Lamichhane et al., 2019). Comparatively, *Mikania spp*. stem extract showed less scavenging activity (IC_{50}= 59.17 μg/ml) than other plant extracts, which may be correlated with the absence of phenols in it (Ekram et al., 2017). The IC_{50} value of *Drynaria coronans* and *Gynura nepalensis* were found to be 9.46 μg/ml and 7.61 μg/ml, respectively. The results of DPPH free radical scavenging assay are depicted in Table 4 and Figure 1.

3.3. Antibacterial Activity

Antibacterial activity of 70% methanolic extract of *Angiopteris helferiana*, *Curcuma caesia*, *Dischidia bengalensis*, *Drynaria coronans*, *Gynura nepalensis*, and *Mikania spp*. were screened by the agar well diffusion method. The diameter of the zone of inhibition of plant samples indicated sensitivity or resistance against *Escherichia coli* and *Staphylococcus aureus* as shown in Table 5 and Figure 1.

In the present study, *A. helferiana* extract modestly inhibit the growth of *E. coli* at concentrations of 50 and 100 mg/ml (zone of inhibition = 3.00±1.41 and 5.50±2.12 mm, respectively) but at the conc. of 50 mg/ml, *A. helferiana* extract exhibited the maximum zone of inhibition (8.00±1.42 mm) against *S. aureus*. However, in the study conducted by Bhattacharjee et al. (2017). *A. helferiana* ethyl acetate extract exhibited antibacterial activity against *S. aureus* only but not against *E. coli* and *Pseudomonas aureus*. Although the extract of *Curcuma caesia* rhizome and *Gynura nepalensis* leaves in the current investigation failed to demonstrate any antibacterial activity against *E. coli* and *S. aureus*. *G. nepalensis* essential oil and methanolic extract exhibited antibacterial against *S. aureus, P. aeruginosa*, and *E. coli* in the study performed by Meng et al. (2021). Also, (Rajkumari & Sanatombi, 2017) reported that *C. caesia* has potential antibacterial properties. This variance in antimicrobial activities may be brought on by the different concentration of extract used throughout the study, difference in the solvent used for extraction, variations in geographic location, climatic conditions, and plant growing season (Rasheed et al., 2010). In the current investigation, the methanolic extract of *Dischidia bengalensis* exerted antibacterial activity against *E. coli* (zone of inhibition 5.00±0.00 mm) at a concentration of 100 mg/ml but failed to do so against *S. aureus*. The methanolic extract of *Drynaria coronans* was found to inhibit the growth of both *S. aureus* and *E. coli*, where the highest zone of inhibition was measured against *E. coli* at a concentration of 100 mg/ml (zone of inhibition 8.00±1.42 mm). The value of zone of inhibition discovered in the present study, however, is significantly lower than what the study reported (Khanal et al., 2020). Similarly, *Mikania spp*. methanolic stem extract exhibited antibacterial action against *E. coli* only. This may be due to the presence of flavonoids and tannins, as these phytochemicals are responsible for antibacterial action against many of the bacterial strains that humans encounter (Havsteen, 2002). Furthermore, the antibacterial activity of *Angiopteris helferiana*, *Dischidia bengalensis*, and *Drynaria coronans* may be due to presence of these phytochemicals as well.

3.4. Structure Elucidation

Compound separation from the various fractions of *Dischidia bengalensis* entire plant was performed using adsorption chromatography based on TLC profiling in the current study, and structural elucidation of the isolated compounds was done using ^{1}H NMR, ^{13}C NMR, and DEPT 135° (Distortion less Enhancement by Polarization Transfer) spectra. To get pure chemicals, a 70% MeOH extract of *Dischidia bengalensis* was fractionated using MCI GEL^{TM}, Sephadex® LH-20, ODS gel column, and Silica gel column chromatography, in a sequential manner. TLC profiling of each fraction and sub-fractions were performed based on the solvent system ratios $CHCl_3$: MeOH: H_2O (7:3:0.5 and 6:4:1) followed by detection using 10% aq. $FeCl_3$ and 10% H_2SO_4 as spraying agent. Comparable solvent ratios and spraying agents in similar concentrations were employed in reference with various literature addressing chemical isolation and TLC profiling (Joshi et al., 2014; Sai et al., 2020).

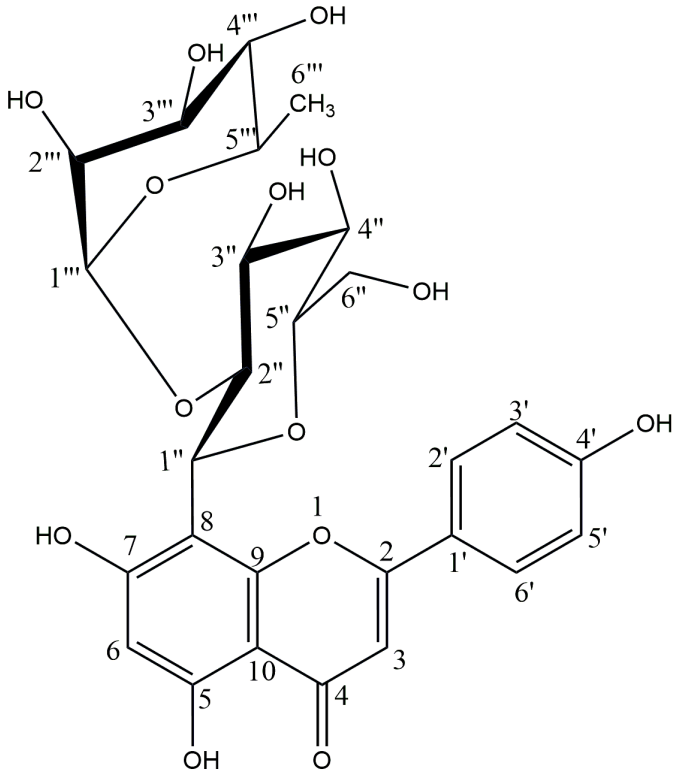

Figure 3. Structure of 2"-*O*-rhamnosylvitexin (copmpound-**1**) isolated from *Dischidia bengalensis.*

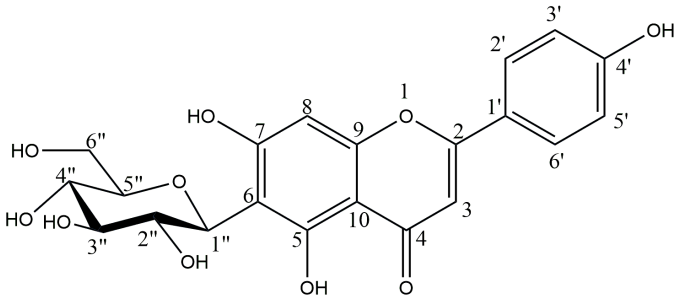

Figure 4. Structure of isovitexin (compound-**2**) isolated from *Dischidia bengalensis.*

The entire assignment of the proton and carbon atoms, their location and linkage with anomeric protons were determined and compared with chemical shift values of related similar compounds on the literature (Joshi et al., 2013; Thoa et al., 2019; Wen et al., 2017). Two compounds were isolated; compound **1** as 2"-*O*-rhamnosylvitexin (Figure 3) and compound **2** as isovitexin (Figure 4). The ^{13}C NMR spectrum of compound **1** showed signals equivalent to total 27 carbons, in with 12 carbon signals were assignable to a 2"-O- substituted rhamnosyl moiety. The detection of 15 carbon signal through ^{13}C NMR spectra for Kaempferol moiety confirmed the compound carbon skeleton structure of flavonoid. Similarly for compound **2**, ^{13}C NMR spectrum showed signals equivalent to total 21 carbons, in which 15 carbon signals were assignable to kaempferol moiety.

Similarly, in DEPT-135° ^{13}C NMR spectroscopy, negative signals at around 61.0 ppm indicates the presence of CH_2 group in both the isolated compounds. Likewise, CH_3 group present in the rhamnose moiety was confirmed by the appearance of signals at 17.67 ppm in compound **1** (Joshi et al., 2013). All quaternary carbon signals were absent in the given spectrum.

In the study conducted by Wei et al. (2014), 2"-*O*-rhamnosylvitexin significantly reduced H_2O_2-induced late and early apoptosis/necrosis in human adipose-derived stem cells (hADSCs) compared to the corresponding H_2O_2-treated group, indicating that 2"-O-rhamnosylvitexin protects hADSCs by inhibiting apoptosis. Similarly, isovitexin had effective free radical scavenging capacities of 91.48%, indicating that it has anti-oxidant properties, possibly because it can act as both proton donor or metal ion chelators (Azubuike-Osu et al., 2021; Lee et al., 2019). Prevention in reduction of antioxidant defense i.e. free radical scavenging activity, ferric reducing ability, and Glutathione levels in inflammatory foci resulted in analgesic and anti-inflammatory response. Thus, indicating that potent free-radical scavenging activity leads to increased anti-inflammatory response and pain relief (Babaei et al., 2020; Borghi et al., 2013).

A study found that isovitexin, when pretreated with acute lung injury (ALI) mice, can have anti-inflammatory properties. It showed a dramatical decline in tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6) production, reactive oxygen species (ROS) generation, myeloperoxidase (MPO) and malondialdehyde (MDA) content. This in turn, effectively inhibited the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins, which protects against LPS (Lipopolysaccharide) induced inflammation and oxidative stress in ALI mice (Lv et al., 2016). Since, this plant is traditionally used to treat bone fracture and muscle pain, and have potent antioxidant activity, these compounds might be responsible for this activity. However, a detail investigation is needed to fully understand the role of these compounds on traditional uses of this plant.

4. CONCLUSION

All the plant species used in this study have demonstrated to be a great source of secondary metabolites from which pure compounds can be isolated. The antioxidant and antibacterial activity were determined using DPPH radical scavenging activity and agar well diffusion method respectively. Among six plants studied, *Dischidia bengalensis* (IC_{50} value = 4.60 μg/ml) and *Curcuma caesia* (IC_{50} value = 5.81 μg/ml) showed the most potent antioxidant activity while, *Drynaria coronans* (zone of inhibition = 8.00±1.42 mm at 50 mg/ml concentration against *Staphylococcus aureus*) and *Angiopteris helferiana* (zone of inhibition = 8.00±1.42 mm at 100 mg/ml concentration against *Escherichia coli*) showed greater antibacterial activity. For the first time, two flavonoids i.e. 2"-O-rhamnosylvitexin and isovitexin were isolated from *Dischidia bengalensis*, which might be responsible for the potent free radical scavenging activity of this plant. Similarly, the traditional bone healing and pain relief property of this plant may be related to the presence

of these compounds as well. In conclusion, there is need of further research to establish the bone healing property as well as other health benefits of these ethnomedical plants and isolated fractions.

5. SUPPLEMENTARY INFORMATION

Supplementary information to this article can be found online at https://doi.org/10.53365/nrfhh/188221.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

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AUTHOR CONTRIBUTIONS

KRJ, NG, MS, SG, AG- Layout and Concept of the research; NG, MS, SG, AG, KRJ, SP- Isolation; NG, MS, SG, AG- Antibacterial and Antioxidant Activity; NG, MS, SG, AG, KRJ, HPD- Data and Spectra Analysis; HRP- Plant Identification; NG, MS, SG, AG- Article writing; HPD, KRJ, NG, MS, SG, AG, AK- Final Review.

REFERENCES

Ansari, J., Kushwaha, S.P., Ansari, V.A., Singh, K., Hasan, S.M., 2021. Agar Well Diffusion: A Prominent Method for In Vitro Screening of Antimicrobials. International Journal of Botany Studies. 6(5), 836–839.

Azubuike-Osu, S., Ohanenye, I., Jacob, C., Ejike, C., Udenigwe, C., 2021. Beneficial Role of Vitexin and Isovitexin Flavonoids in the Vascular Endothelium and Cardiovascular System. Current Nutraceuticals. 2(2), 127–134. https://doi.org/10.2174/2665978601999201105160405

Babaei, F., Moafizad, A., Darvishvand, Z., Mirzababaei, M., Hosseinzadeh, H., Nassiri-Asl, M., 2020. Review of the effects of vitexin in oxidative stress-related diseases. Food Science & Nutrition. 8(6), 2569–2580. https://doi.org/10.1002/fsn3.1567

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for In Vitro Evaluating Antimicrobial Activity: A Review. Journal of Pharmaceutical Analysis. 6(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bhandari, P., Shrestha, K., 2016. *Dischidia bengalensis* (Apocynaceae), a New Record for the Flora of Nepal. Journal of Japanese Botany. 91, 310–313.

Bhattacharjee, A., Mitra, A., Choudhury, D., Chetri, S., Bhowmik, D., Bhattacharya, M.K., Talukdar, A.D., Nath, K., 2017. Antibacterial Activity of Certain Ferns Against Multi Drug Resistant Organisms. Journal of Natural Remedies. 17(4), 1–10.

Borghi, S.M., Carvalho, T.T., Staurengo-Ferrari, L., Hohmann, M.S.N., Pinge-Filho, P., Casagrande, R., Verri, W.A., 2013. Vitexin Inhibits Inflammatory Pain in Mice by Targeting TRPV1, Oxidative Stress, and Cytokines. Journal of Natural Products. 76(6), 1141–1149. https://doi.org/10.1021/np400222v

Chakrabarty, N., Chung, H.J., Alam, R., Emon, N.U., Alam, S., Kabir, M.F., Islam, M.M., Hong, S.T., Sarkar, T., Sarker, M.M.R., Rahman, M.M., 2022. Chemico-Pharmacological Screening of the Methanol Extract of *Gynura nepalensis* D.C. Deciphered Promising Antioxidant and Hepatoprotective Potentials: Evidenced from in vitro, in vivo, and Computer-Aided Studies. Molecules. 27(11), 3474. https://doi.org/10.3390/molecules27113474

Chen, M., He, X., Sun, H., Sun, Y., Li, L., Zhu, J., Xia, G., Guo, X., Zang, H., 2022. Phytochemical Analysis, UPLC-ESI-Orbitrap-MS Analysis, Biological Activity, and Toxicity of Extracts from *Tripleurospermum limosum* (Maxim.) Pobed. Arabian Journal of Chemistry. 15, 103797. https://doi.org/10.1016/j.arabjc.2022.103797

Chhetri, S.B., Khatri, D., 2017. Phytochemical Screening, Total Phenolic and Flavonoid Content and Antioxidant Activity of Selected Nepalese Plants. World Journal of Pharmacy and Pharmaceutical Sciences. 6.

Coulombier, N., Jauffrais, T., Lebouvier, N., 2021. Antioxidant Compounds from Microalgae: A Review. Marine Drugs. 19(10), 549. https://doi.org/10.3390/md19100549

Ekram, M.A.E., Sarker, M.E.H., Talukder, A.P., Sarker, B., Uddin, M.S., Zaman, S., 2017. Biological Activities of the Methanolic Extracts of *Coccinia indica* and Mikania scandens Leaves Available in Bangladesh. Dhaka University Journal of Pharmaceutical Sciences. 16(1), 87–93. https://doi.org/10.3329/dujps.v16i1.33386

Gewali, M.B., 2008. Aspects of Traditional Medicine in Nepal, and others, (Eds.). Institute of Natural Medicine, University of Toyama, Toyama, Japan, p. 72.

Giri, S., Giri, U., Subedi, K., Magar, K., Pant, S., Joshi, K.R., 2020. Thin Layer Chromatography (TLC) Based Chemical Profiling and Antioxidant Activity of Selected Nepalese Medicinal Plants. Journal of Health and Allied Sciences. 10(2), 15–22. https://doi.org/10.37107/jhas.158

Gurung, T.M., Kaundinnyayanna, A., Parajuli, K., 2019. Evaluation of antioxidant, anti-inflammatory and analgesic activities of traditional polyherbal combination used in western nepal. World Journal of Pharmacy And Pharmaceutical Sciences. 8(3), 757–775.

Halliwell, B., 1995. How to characterize an antioxidant: an update. Biochemical Society Symposia. 61, 73–101. https://doi.org/10.1042/bss0610073

Havsteen, B., 2002. The Biochemistry and Medical Significance of the Flavonoids. Pharmacology & Therapeutics. 96, 67–202. https://doi.org/10.1016/S0163-7258(02)00298-X

Joshi, K.R., Devkota, H.P., Watanabe, T., Yahara, S., 2014. Thotneosides A, B and C: Potent Antioxidants from Nepalese Crude Drug, Leaves of *Aconogonon molle*. Chemical and Pharmaceutical Bulletin. 62(2), 191–195. https://doi.org/10.1248/cpb.c13-00748

Joshi, K.R., Devkota, H.P., Yahara, S., 2013. Chemical Analysis of Flowers of Bombax ceiba from Nepal. Natural Product Communications. 8(5), 934578X1300800508. https://doi.org/10.1177/1934578X1300800508

Khanal, L., Sharma, K., Pokharel, Y., Kalauni, S., 2020. Assessment of Phytochemical, Antioxidant and Antimicrobial Activities of Some Medicinal Plants from Kaski District of Nepal. American Journal of Plant Sciences. 11, 9. https://doi.org/10.4236/ajps.2020.119099

Kurnia, D., Ajiati, D., Heliawati, L., Sumiarsa, D., 2021. Antioxidant Properties and Structure-Antioxidant Activity Relationship of Allium Species Leaves. Molecules. 26(23), 7175. https://doi.org/10.3390/molecules26237175

Lamichhane, R., Pandeya, P., Lee, K.H., Kim, S.G., Kandel, D., Jung, H.J., 2019. Angiopteris helferiana, a Fern with Great Potential Medicinal Value: Antiadipogenic, Anti-Inflammatory, and Anti-Diabetic Activity. Pharmacognosy Magazine. 15(63), 423–432. https://doi.org/10.4103/pm.pm_430_18

Lee, J.S., Lee, A.Y., Quilantang, N.G., Geraldino, P.J.L., Cho, E.J., Lee, S., 2019. Anti-oxidant Activity of Avicularin and Isovitexin from *Lespedeza cuneata*. Journal of Applied Biological Chemistry. 62(2), 143–147. https://doi.org/10.3839/jabc.2019.020

Lv, H., Yu, Z., Zheng, Y., Wang, L., Qin, X., Cheng, G., Ci, X., 2016. Isovitexin Exerts Anti-Inflammatory and Anti-Oxidant Activities on Lipopolysaccharide-Induced Acute Lung Injury by Inhibiting MAPK and NF-κB and Activating HO-1/Nrf2 Pathways. International Journal of Biological Sciences. 12(1), 72–86. https://doi.org/10.7150/ijbs.13188

Meng, X., Li, J., Li, M., Wang, H., Ren, B., Chen, J., Li, W., 2021. Traditional uses, phytochemistry, pharmacology and toxicology of the genus Gynura (Compositae): A comprehensive review. Journal of Ethnopharmacology. 276, 114145–114145. https://doi.org/10.1016/j.jep.2021.114145

More, G.K., Meddows-Taylor, S., Prinsloo, G., 1920. Metabolomic Profiling of Antioxidant Compounds in Five *Vachellia* Species. Molecules. 26, 6214. https://doi.org/10.3390/molecules26206214

Nath, K., Talukdar, A.D., Bhattacharya, M., Bhowmik, D., Chetri, S., Choudhury, D., Mitra, A., Bhattacharjee, A., 2018. Antibacterial Activity of Certain Ferns Against Multi Drug Resistant Organisms. Journal of Natural Remedies. 17(4), 144–153. https://doi.org/10.18311/jnr/2017/15758

Pakkirisamy, M., Kalakandan, S.K., Ravichandran, K., 2017. Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacognosy Journal. 9(6), 952–956. ttps://doi.org/10.5530/pj.2017.6.149

Pokharel, K., Dhungana, B., Tiwari, K., Shahi, R., Yadav, D.B., Sharma, M., Shrestha, R., Shrestha, D., Poudel, B.H., 2008. Antibacterial Activities of Medicinal Plants of Nepal. Journal of the Institute of Medicine. 30, 28–32.

Rajkumari, S., Sanatombi, K., 2017. Nutritional Value, Phytochemical Composition, and Biological Activities of Edible Curcuma Species: A Review. International Journal of Food Properties. 20(sup3), 2668–2687. https://doi.org/10.1080/10942912.2017.1387556

Rasheed, E.M., Hamudi, M., Kreem, M.R., 2010.

Rintz, R.E., 1980. The Peninsular Malayan Species of Dischidia Asclepiadaceae. Blumea. 26(1), 46–46.

Sai, K., Devkota, H., Thapa, R., Poudel, P., Joshi, K., 2020. Free Radical Scavenging Activity and Chemical Constituents of the Unripe Fruits of *Spondias pinnata* (L.f.) Kurz. from Nepal. Current Perspectives on Medicinal and Aromatic Plants, 54–60. https://doi.org/10.38093/cupmap.730458

Sai, K., Thapa, R., Devkota, H.P., Joshi, K.R., 2019. Phytochemical Screening, Free Radical Scavenging and alpha-Amylase Inhibitory Activities of Selected Medicinal Plants from Western Nepal. Medicines (Basel). 6(2), 70. https://doi.org/10.3390/medicines6020070

Shaikh, J., Patil, M., 2020. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 8, 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Tailor, C.S., Goyal, A., 2014. Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves. American Journal of Ethnomedicine. 1, 244–249.

Thoa, N.T.K., Hien, N.T., Phuong, T., Thi, L., Hong, V.T., Dung, P.T., 2019. Isolation of Some Flavonoids from *Vitex Trifolia* In Bachma National Park, Thua Thien Hue, Vietnam. Hnue Journal of Science. 64(6), 130–135. https://doi.org/10.18173/2354-1059.2019-0040

Timilsina, H., Modi, B., Basnyat, R., 2020. Phytochemical, Antimicrobial and Ethnobotanical Study of *Calotropis gigantea*. Journal of Health and Allied Sciences. 10(2), 23–27. https://doi.org/10.37107/jhas.136

Wei, W., Ying, X., Zhang, W., Chen, Y., Leng, A., Jiang, C., Liu, J., 2014. Effects of Vitexin-2″- O -rhamnoside and Vitexin-4″- O -glucoside on Growth and Oxidative Stress-Induced Cell Apoptosis of Human Adipose-Derived Stem Cells. The Journal of Pharmacy and Pharmacology. 66, 988–997. https://doi.org/10.1111/jphp.12225

Wen, L., Lin, Y., Lv, R., Yan, H., Yu, J., Zhao, H., Wang, X., Wang, D., 2017. An Efficient Method for the Preparative Isolation and Purification of Flavonoids from Leaves of *Crataegus pinnatifida* by HSCCC and Pre-HPLC. Molecules. 22(5), 767. https://doi.org/10.3390/molecules22050767

WHO., 2022. Who Establishes the Global Centre for Traditional Medicine in India. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india