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## Physicochemical analysis and phytochemical screening of some medicinal plants of Letang Municipality of Morang district, Nepal

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### ABSTRACT

Ethnobotany gives the basic idea about the medicinal properties of plants. Identification of active compounds of the medicinal plants and their standardization is essential for the production of new drugs. In the present work, different parts of the five medicinal plants (*Curcuma caesia*, *Cheilocostus speciosus*, *Drymaria cordata*, *Leea macrophylla*, *Plumbago zeylanica*) were washed, air dried and crushed. Three different extracts of each powdered material were prepared and standard phytochemical analysis procedure was followed for the analysis of physicochemical properties of plants and the identification of active chemical constituents. Among 5 plants, the highest moisture content (14.83%) was found in *Plumbago zeylanica*, higher total ash (9.22%) and acid insoluble ash (4.43%) were observed in *Curcuma caesia*. Phytochemical analysis revealed the presence of 12 varieties of bioactive chemicals in all 5 different plants. The plants of the area have great diversity of phytochemicals of numerous medicinal properties. In conclusions, these five important medicinal plants could be useful for the people of the locality to cure several diseases as well as to generate the source of income.

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## 1. Introduction

The existence of traditional remedies in the disease treatment procedure is considered an integral part of modern pharmaceutical science. Several bio-ingredients of natural products do play a significant role in the discovery of synthetic medicines [1]. Nepal has a varied ecosystem for the cultivation of medicinal plants which has been supported by climatic and geographical diversity [2]. It is a small

landlocked country situated in the lap of the great Himalayas, bordered by China from the north side and India by other three sides. The variation of geographical diversity extends from snow-capped Himalayas in the north to the tropical alluvial plains in the south [3]. The major land in Nepal is covered by dense forests with possibilities of several medicinal plants [4]. The Nepalese tribes still use medicinal herbs for the treatment of chronic diseases. The literature reported the

tremendous effect of crude extracts of medicinal herbs on the disease curing process than the isolated ingredients and this may be due to their synergistic actions [5]. The synthetic chemical-based drugs, besides their specific biological functions, also possess undesired side effects in the human body, so the phyto based drugs have utmost importance in medical science which has less toxic effects. The phytochemical ingredients of the medicinal plants produce defense mechanism and protect us from various diseases. In plants, the phytochemicals are produced by primary and secondary metabolism [6]. The primary metabolites are simple molecules involved in cellular process. The secondary metabolites are the active compounds which perform defense mechanism against the predator, insects, microorganisms, etc [7]. In recent years, the plants as medicine are becoming more popular due to fewer side effects, low cost and greater effectiveness than the synthetic drugs. This signifies the importance of active phytochemicals for curing different problems of the human body.

The medicinal plant contains bioactive compounds such as alkaloids, flavonoids, tannins, glycosides, etc. These constituents have their own biological functions. Alkaloids are heterocyclic nitrogen-containing compounds which possess antimicrobial, analgesic, anthelmintic, and antidiarrhoeal activity [8]. Flavonoids are plant pigments that produce the color of the flowers and possess significant biological functions as an antimicrobial, antioxidant, antidiarrhoeal, anti-inflammatory [9] and anticancer [10]. Tannins are polymeric phenols having antimicrobial, and anthelmintic functions [8].

In this investigation, we carried physicochemical and phytochemical screening of five species of medicinal plants collected from the forest of Letang Municipality situated in province 1 of Nepal. *Cheilocostus speciosus* (J.Koenig) C.D. Specht, is a perennial, rhizomatous plant commonly called crepe ginger from family Costaceae. Traditionally the plant is used in the treatment of asthma, fungal diseases, rheumatism, diabetes and hepatoprotective disorders [11]. It is also reported that plant has various biological activities such as antibacterial, antifungal, anthelmintic, antioxidant, anti-inflammatory, analgesic, antipyretic, antistress and larvicidal [12]

*Curcuma caesia* Roxb., belongs to the family Zingiberaceae and is a nonconventional medicinal plant with common name Black turmeric [13]. The ethnobotanical study concludes its use in the treatment of asthma, bronchitis, epileptic, gonorrhoeal discharges, impotency, leprosy, menstrual disorders, rheumatic arthritis pain, wounds, piles, toothache, tumors, and vomiting [14]. Several studies have also reported its various biological functions such as antiallergic, antibacterial, anthelmintic, anti-inflammatory and antioxidant [15].

*Drymaria cordata* (L.) Willd. ex Schult., commonly known as tropical chickweed belongs to the family Caryophyllaceae. The plant has various pharmacological, traditional and nutritive values. To date, there are fewer reports available on its phytochemical profile [16]. Roots are applied externally to heal pain and are alexipharmic. Pharmacologically, the plant has been reported to possess antiurolithiatic [17] and anti-inflammatory activities [18].

*Leea macrophylla* Roxb. ex Hornem., locally known as "Ekle Galeni" of family Leeaceae is herbaceous shrub with big leaves. The plant has traditional uses in tonsillitis, tetanus, nephrolithiasis, rheumatism, arthritis, snake bites, sore, pain, and blood effusion [19, 20]. Although the plant has great medicinal properties, there is no proper data available on the phytochemical profile [21].

*Plumbago zeylanica* L., commonly known as Ceylon leadwort or Wild leadwort is a multipurpose medicinal herb of family Plumbaginaceae. In several traditional systems, the plant is used in the treatment for skin diseases, rheumatic swellings, rheumatic pains, tuberculosis, leprosy, scabies diarrhea, gonorrhoea, dyspepsia and ulcers [22]. It is reported that plant shows sufficient antidiarrheal properties [23], antiallergic, appetizer, anti-saturative, antianorexic, anti-haemorrhoidal, pain-reliever [24], insecticidal, antidiabetic [25], and hepatoprotective properties.

## 2. Methods

### Plant collection

The reported plants were collected from the Mahavarat hilly area of Letang Municipality of Morang district in province 1, Nepal, during mid-November of 2017. It has the highest altitude of

494 m and rich in plant diversity though it falls under moist tropical forest (locally called Charkoshe jungle). All of the plants were collected from their wild habitat. People also cultivate a few of them in their field, garden and private forest for quick access during their needs. The plants were identified with the help of taxonomic literature and herbarium specimens deposited in Botany Department of Mahendra Morang Adarsh Multiple Campus, Biratnagar. The voucher specimens were deposited in the same Department.

### Extraction

The rhizomes of *Cheilocostus speciosus*, and *Curcuma caesia*, aerial parts of *Drymaria cordata*, tuberous roots of *Leea macrophylla* and root of the *Plumbago zeylanica* were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered. From each sample, 5 g powder was taken and soaked them for 24 hours in 50 ml of water, ethanol, and chloroform separately. The extracts were filtered using Whatman filter paper 1. The filtrates were stored at 4 °C for further use.

**Table 1:** Preliminary phytochemical tests for plant extracts.

S.N.	Phytochemicals	Test	Observation	References
1	Alkaloid	2 ml of extract + 2 ml of HCl+ boil and cool + 2 drops of Wagner's reagent	Brownish red coloration indicated the presence of alkaloid	[8]
2	Tanin	2 ml extract + 2 ml water + 2-3 drops of Ferric chloride (5%)	Green precipitate indicated the presence of tannin.	[26]
3	Flavonoid	3 ml extract + evaporated to dryness. Residue + 1-2 ml CH <sub>3</sub> CH <sub>2</sub> OH (50%) + heat + Mg ribbon + 4-5 drops HCl	Red or orange color indicated the presence of flavonoid.	[27]
4	Steroid	5 ml extract + 2 ml CHCl <sub>3</sub> + 3 ml conc. H <sub>2</sub> SO <sub>4</sub>	The reddish brown precipitate formed at the interface indicated the presence of steroid.	[28]
5	Glycoside	2 ml extract + 2 ml CHCl <sub>3</sub> + 1-2 ml CH <sub>3</sub> COOH	Formation of violet or blue to green coloration indicated the presence of glycoside.	[29]
6	Saponin	2 ml extract + evaporated to dryness. Residue + 1 ml water + shaken vigorously.	The persistent foam (1 cm in the test tube) confirmed the presence of saponin.	[30]
7	Emodol	2 ml extract + 1-2 ml NH <sub>3</sub> (25%) + Shaken.	A cherries red color indicated the presence of emodol	[31]
8	Quinone	1 ml extract + 1 ml conc. H <sub>2</sub> SO <sub>4</sub>	Formation of red color indicated the presence of quinone.	[32]
9	Coumarin	2 ml extract + 3 ml NaOH (10%).	Formation of yellow coloration indicated a positive result for coumarin.	[33]
10	Anthocyanin	2 ml extract + 2 ml HCl (2N) + 1 ml NH <sub>3</sub>	Formation of pinkish red to bluish violet coloration indicated the presence of the anthocyanin.	[29]
11	Protein	2 ml extract + 1 ml NaOH (40%) + 1-2 drops 1% CuSO <sub>4</sub> solution.	Formation of violet color indicated the presence of protein.	[34]
12	Reducing sugar	1 ml extract+ 2 ml water + 1 ml Fehling solution (1 and 2) + heated in a water bath.	A brick red precipitate indicated the presence of reducing sugar.	[32]

## Physicochemical analysis

### Moisture content

In a dry and clean Petri dish, 5g of powdered material was weighted accurately. The sample was transferred in an oven at 110 °C. It was dried until two consecutive weighings do not differ by more than 5 mg. Loss of weight in terms of percentage was calculated.

### Total ash

About 2 g of dried plant material was weighed accurately in a previously ignited clean crucible. It was ignited by gradual heating until it became white. It was cooled for a while and weighed. The content of total ash in terms of percentage was calculated.

### Acid-insoluble ash

To the crucible containing total ash, 25 ml of 70 % hydrochloric acid was added and boiled gently for 5 minutes by covering with a watch glass. The cover glass was rinsed with 5 ml of hot water and the liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate became neutral. The filter paper containing insoluble matter in the crucible was ignited until a constant weight appeared. Then it was cooled and weighed. The content of acid insoluble ash in terms of percentage was calculated.

### Phytochemical screening

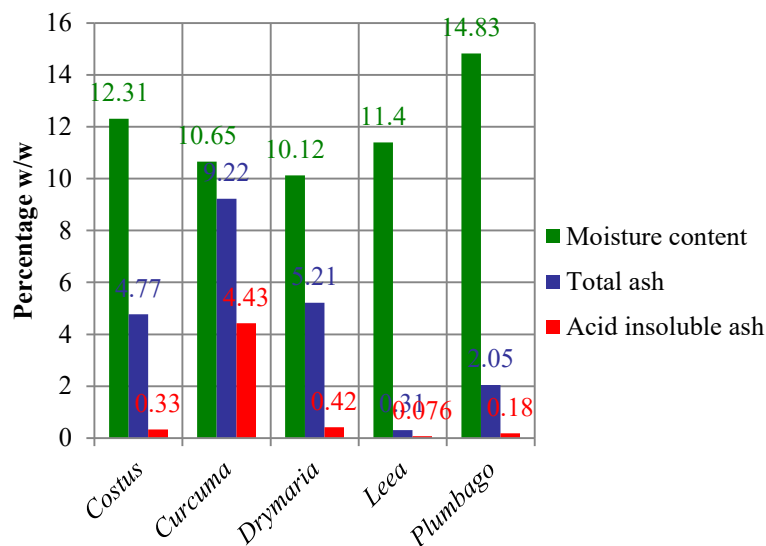
The bioactive compounds were analyzed by the qualitative tests of extracts. It was screened for alkaloids, steroidal compounds, flavonoids, saponins, phenolic compounds, tannins, steroids, coumarins, and glycosides by using standard procedures. The methodology and the screening tests are presented in Table 1.

## 3. Results

### Physicochemical analysis

The results of the physicochemical analysis are presented in Figure 1. Among 5 plants, the highest moisture content (14.83%) was found in *Plumbago zeylanica*, higher total ash (9.22%) and acid insoluble ash (4.43%) was observed in *Curcuma caesia*. Although these plants showed variation in data, the moisture content and ash content are associated with the purity and authenticity of plants

[35]. These parameters of physicochemical analysis are essential for checking the quality of herbs, standardization of medicinal plants, crude drug formulation [36].



**Fig. 1:** Result of physicochemical analysis of five medicinal plants.

### Phytochemical screening

The phytochemical screening of five plants in three extracts revealed the presence of alkaloid in all except in the chloroform extract Table 2. Tannin was found in all the plants except in *Curcuma caesia*. In the *Cheilocostus speciosus* and *Plumbago zeylanica*, tannin was absent in aqueous and chloroform extract but in *Drymaria cordata* and *Leea macrophylla*, tannin was absent only in chloroform extract. Flavonoid was present in all five plants except in *Curcuma caesia*, whereas all the three extracts of *Plumbago zeylanica* revealed the presence of flavonoid. In *Cheilocostus speciosus* it was absent in ethanolic extract and in *Leea macrophylla* it was absent in aqueous extract.

The steroid was found in all the plants and in all the extracts of *Cheilocostus speciosus* and *Curcuma caesia*, but it was absent in aqueous extract of *Drymaria cordata*. In *Leea macrophylla* it was only present in chloroform extract and in *Plumbago zeylanica* it was only present in ethanolic extract. Glycoside showed positive result in all the five plants except *Curcuma caesia* and in all the extracts of *Plumbago zeylanica*, but absent in chloroform extract of *Cheilocostus speciosus* and

*Drymaria cordata*. In *Leea macrophylla* it was only present in the aqueous extract. Saponin was present only in four plants except in *Curcuma caesia*. In the *Cheilocostus speciosus*, *Leea macrophylla* and

*Plumbago zeylanica* it was only absent in chloroform extract but in *Drymaria cordata* it was only present in ethanolic extract.

**Table 2:** Results of phytochemical screening of five medicinal plants.

Name of plant	Extracts	Alk.	Tan.	Flav.	Ster.	Gly.	Sap.	Emo.	Qui.	Coum.	Anth.	Pro.	RS.
<i>Cheilocostus speciosus</i>	Aqueous	+	-	+	+	+	+	+	-	-	+	+	+
	Ethaonic	+	+	-	+	+	+	-	+	-	-	+	+
	Chloroform	-	-	+	+	-	-	+	-	-	+	-	-
<i>Curcuma caesia</i>	Aqueous	+	-	-	+	-	-	-	-	-	-	+	+
	Ethaonic	+	-	-	+	-	-	-	+	+	-	-	+
	Chloroform	-	-	-	+	-	-	-	-	-	-	-	+
<i>Drymaria cordata</i>	Aqueous	+	+	-	-	+	-	-	-	-	+	+	+
	Ethaonic	+	+	-	+	+	+	-	-	+	-	+	-
	Chloroform	-	-	+	+	-	-	-	-	-	-	+	+
<i>Leea macrophylla</i>	Aqueous	+	+	-	-	+	+	-	-	+	-	+	+
	Ethaonic	+	+	+	-	-	+	-	-	+	+	+	-
	Chloroform	-	-	+	+	-	-	-	-	-	-	-	-
<i>Plumbago zeylanica</i>	Aqueous	+	-	+	-	+	+	+	+	+	-	+	+
	Ethaonic	+	+	+	+	+	+	+	+	+	-	-	+
	Chloroform	-	-	+	-	+	-	-	-	+	-	-	-

Alk.- Alkaloid, Tan.- Tanin, Flav.- Flavonoid, Gly.- Glycoside, Sap.- Saponin, Emo.- Emodol, Qui.- Quinone, Coum.- Coumariin, Anth.- Anthocyanin, Pro.- Protein and RS.- Reducing sugar, '+' for Present and '-' for absent.

Emodole was found in *Cheilocostus speciosus* and *Plumbago zeylanica*. In *Cheilocostus speciosus* it was absent in ethanolic extract and in *Plumbago zeylanica* it was absent in chloroform extract. Quinone was present only in three plants *Cheilocostus speciosus*, *Curcuma caesia* and *Plumbago zeylanica*. In *Cheilocostus speciosus* and *Curcuma caesia* it was present only in ethanolic extract and in *Plumbago zeylanica* it was absent in chloroform extract. Coumarin was present in all the plants except *Cheilocostus speciosus* and all the three extracts of *Plumbago zeylanica*. But in the majority of other plants it was absent in chloroform extract. Protein and reducing sugar were present in all the plants.

#### 4. Discussion

The present study justifies the presence of active metabolites in plants and the medicinal behavior is due to the presence of bioactive chemicals in them. The earlier reports have also suggested the anticancer activities of water-soluble phenolic compounds like tannins, flavonoids, coumarin[37]. The bioactivity of identified phytochemicals has also established by the previous studies [27,38]. In every research of phytochemistry, it is fundamental to choose a suitable solvent in order to dissolve desired bioactive compounds of plants inducing medicinal properties in human. In this study, the number of phytochemicals tested is in decreasing order of polarity as aqueous > ethanol > chloroform extract. The aqueous extract being the most polar organic extract amongst others has dissolved all the phytochemicals. The ethanol extract has also dissolved all the phytochemicals, however the frequency was high in aqueous extract. The chloroform extract was unable to dissolve alkaloid, tannin, saponin, emodol, quinone, and anthocyanin.

The present study has reported the presence of phytochemical ingredients such as alkaloid,



flavonoid, tannins, glycoside, reducing sugar in *Costus speciosus*, similar to the earlier studies [12]. The physicochemical properties and phytochemicals analyzed in *Curcuma caesia* showed correlations with previous research works [39,40]. In the *Drymaria cordata* having less former research on phytochemistry revealed the identified phytoconstituents to be same as found in earlier researches [41,42]. In the existing works, it was also found that alkaloid, flavonoid, tannin, steroid, saponins, glycoside, reducing sugar and proteins are present in *Leea macrophylla* [21]. The results obtained from physicochemical and phytochemical analysis of *Plumbago zeylanica* correlates with earlier works [22,43,44]. Among all the plants emodol, quinone, and anthocyanine have not been analyzed previously. The *Cheiloostus speciosus* and *Curcuma caesia* both have fleshy rhizomes and collected from the same habitats showed much variation in physicochemical properties and phytochemistry.

The few variations in physicochemical and phytochemical properties might be due to the time of harvesting, age of the plant, environmental factor, grinding process, etc. The plants are active natural chemicals, easily available, free of costs and devoid from side effects due to which they have been considered as really helpful medicinal drug in the studied area from a long time.

## 5. Conclusion

The bioprospecting of medicinal plants through standardization with physiochemical parameters, analysis of active metabolites and reducing over exploitation of medicinal plants should get major concern in Letang and other parts of Nepal. The present study provides the evidence that plants contain bioactive metabolites like alkaloid, tannin, flavonoid, glycoside, saponin, steroid etc and justifies their therapeutic use. Further ample work has to be performed in isolation, quantification and characterization of bioactive metabolites of flora for application in pharmaceutical industry. Dissemination of current findings to the society through the concerned authorities will be fruitful to understand the importance of medicinal plants, with their cultivation and conservation urgencies leading to their processing and selling, which certainly decrease the unemployment and poverty in the region.

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