Determination of Phytochemical Constituents and *Oxalis Corniculata* Linn. (Hmo-na-do) By Thin Layer Chromatography Method and Its Nutritional Values

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Abstract

Oxalis corniculata Linn. is a medicinally important plant which is indigenous to tropical and subtropical regions of the world. In this research work, phytochemical investigation of *Oxalis corniculata* Linn. (Hmo-na-do) was done by test tube method. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, -amino acids, carbohydrates, glycosides, phenolic compounds and reducing sugars. But, starch and saponins were not detected in Hmo-na-do sample by test tube method. Thin layer Chromatography studies of the pet-ether, ethyl acetate and ethanol extract of the Hmo-na-do plant samples were carried out using the solvent system PE:EA (2:1). The finding of the phytochemical screening revealed the presence of alkaloids terpenoids, steroids, phenolic compounds and flavonoid compounds with different R_f values and different colour. The nutritional values were found to be 21.77 % of moisture, 17.71 % of protein 21.48 % of fiber, 0.81 % of fat, 11.58 % of ash and 26.65 % of carbohydrate. The energy value was 184.73 kcal per 100 g.

Keywords : *Oxalis corniculata*, Phytochemical, TLC, R_f value, Nutritional values.

Introduction

Traditionally *Oxalis corniculata* Linn. has several medicinal uses. In Ayurvedic medicine, it is used for liver and digestive problems. In Nepal, it is used for stomachaches; the leaves for ritual source (Hemant, *et al*., 2011). In Zairean Pharmacopoeia, it is used as antivenom: (1) paste of whole plant of *Oxalis. corniculata* rubbed on the wound; swallow the juice of the masticated plant (2) make paste with a salted mixture of *Oxalis corniculata* and Aframomum sanguineum, and cover the bite. The plant is well known for its medicinal value as a good appetizer and as a remover of Kapa, vata, anaemia, dyspepsia, cancer, dementia, convulsion and piles (Rajadurai, *et al*., 2009). *Oxalis corniculata* commonly known as yellow wood sorrel is a very common and useful medicinal plant which has been used since ages for the treatment of various ailment and as an emergency food (Imana Pal, *et al.*, 2015). It is a delicate-appearing, low growing, herbaceous plant and abundantly distributed in damp shady places, roadside, plantations, lawns, nearly all regions throughout the warmer parts of India, especially in the Himalayas up to 8,000 ft- cosmopolitan. The present study is aimed to investigate on the *Oxalis corniculata* with respect to its phytochemical properties (by test tube and TLC method), nutritive value, and medicinal use (Achol, *et al*., 1995).

Botanical Aspects of *Oxalis corniculata* Linn.

Botanical name	:	*Oxalis corniculata* Linn
Genus	:	*Oxalis*
Species	:	*O-corniculata*
Family	:	Oxalidaceae
English name	:	Indian sorrel
Myanmar name	:	Hmo – na –do
Part used	:	Whole plant

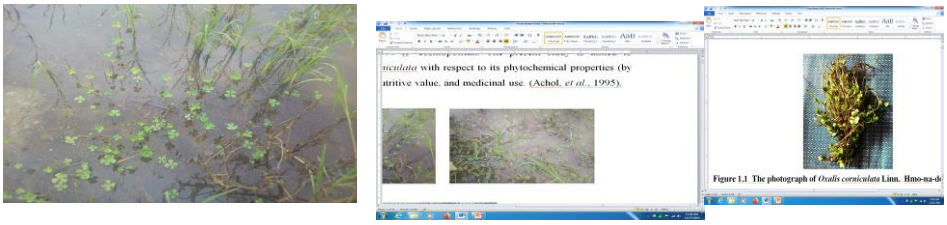


Figure 1. The photograph of *Oxalis corniculata* Linn. (Hmo-na-do) plant

Materials and Methods

Sample collection and Preparation

Fresh Hmo-na-do (*Oxalis corniculata* Linn.) were collected from Htan Tabin Township, Yangon Division in June 2019. These were cleaned from dust, washed with water, cut into small pieces and dried at room temperature for one month. The dry material was made power by using grinding machine and stored in air-tight glass bottle until used.

Phyotochemical Screening of *Oxalis corniculata* Linn. (Hmo-na-do) by Test Tube Method

Phytochemical investigation was carried out on dried powdered leaves, stems and roots of Hmo-na-do with a view to investigate the presence or absence of alkaloids,α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, starch and tannins according to the standard procedure (Harborne, 1984).

Preparation of Crude Extracts from *Oxalis corniculata* Linn. (Hmo-na-do) Plant

The dried powder sample (20 g) was percolated with pet-ether (60-80˚C) (60 mL) for one week and filtered. The filtrates were evaporated at room temperature and pet-ether soluble extract was obtained. The defatted mare (powdered samples left after extraction with pet-ether) was then extracted with ethyl acetate (60 mL) for one week. The filtrates were evaporated by means of a water bath. Consequently ethyl acetate soluble extract was obtained. The defatted marc (powdered samples left after extraction with ethyl acetate) was then extracted with ethanol (60 mL) for one week. The filtrates were evaporated by means of a water bath. Consequently, ethanol soluble extract was obtained. In this way, pet-ether soluble extracts and ethyl acetate soluble extract of plant samples were prepared.

Qualitative Determination of Phytoconstituents in Petroleum ether, Ethylacetate and Ethanol Crude Extracts by TLC Method

Preliminary detection and identification of the phytoconstituents were performed by thin layer chromatographic technique. In this present work, the solvent systems for PE and EtOAc extracts used were the ratio of PE: EtOAc (2:1 v/v) for all extracts respectively.

The TLC plates were checked by spraying with various reagents such as Dragendorff's reagent, Liebermann-Burchard reagent, 10% $FeCl_3$ solution and NH_3 vapour to classify the compounds and their functional groups present in the crude extracts according to the standard procedures (Marini-Bettolo, *et al.*, 1981)

Determination of R_f (relative flow) Values

The R_f (relative flow) values of developing spots on chromatograms were calculated by the following formula.

Distance travelled by the solute from the position of origin

Distance travelled by the solvent from the position of origin

Investigation of Nutritional Composition of *Oxalis corniculata* Linn.

The nutritional composition of *Oxalis corniculata* sample such as moisture, nitrogen and protein, fiber, fat, ash and soluble carbohydrate contents were investigated by AOAC method.

RESULTS AND DISSICUSSIONS

Phytochemical Screening of *Oxalis corniculata* Linn.

Phytochemical investigation was carried out to know the type of phytoorganic constituents present in selected *Oxalis Corniculata* Linn (Hmo-na-do). These test were based on the result of colour, which indicates that the presence of classes of organic constituents containing in the plant sample. It was found that there were alkaloids, -amino acids, carbohydrates, phenols compounds, flavonoids, glycosides, tannins, and reducing sugar. But starch and saponins were not detected in sample. The results of test tube method were shown in Table 1 and Figure 2.

Table 1. Phytochemical Investigation Result of *Oxalis Corniculata* Linn (Hmo-na-do)

No	Tests	Extract	Test reagent	Observations	Results
1	Alkaloids	1%HCl	Mayer's reagent Wagner's reagent	White ppt Reddish brown	(+) (+)
2	Flavonoids	EtOH	Alcoholic HCl and Mg	Pink Colour	(+)
3	Tannins	EtOH	Few gelatin, 10% FeCl3	Greenish-yellow	(+)
4	α-amino acids	H_2O	Ninhydrin	Purple spot	(+)
5	Carbohydrates	H_2O	10% α-napthol, conc:H_2SO_4	Red ring	(+)
6	Glycosides	H_2O	10% lead acetate	White ppt	(+)
7	Phenolic compounds	H_2O	10% Ferric chloride & $K_3Fe(CN)_6$	Deep blue colour	(+)
8	Reducing sugars	$5NH_2SO4$	Benedict's reagent	Reddish brown ppt	(+)
9	Starch	H_2O	Iodine solution	No blackish blue ppt	(-)
10	Saponins	H_2O	Distilled water	No forthing	(-)

(+)=present (-)= absent (ppt)= precipitate

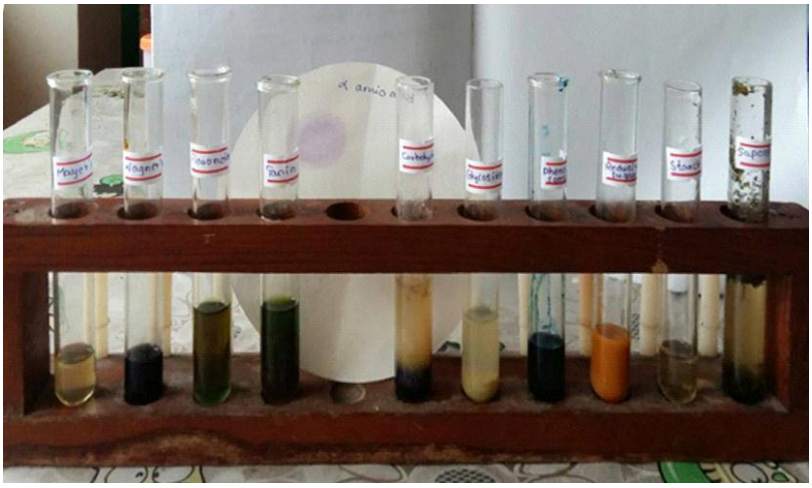


Figure 2. Observation of Phytochemical Test Using Test Tube Method

Preparation of Crude Extracts from *Oxalis corniculata* Linn. (Hmo-na-do) Plant

After carrying out the preliminary phytochemical tests, some crude extracts were prepared according to the general procedure mentioned in Figure 3.

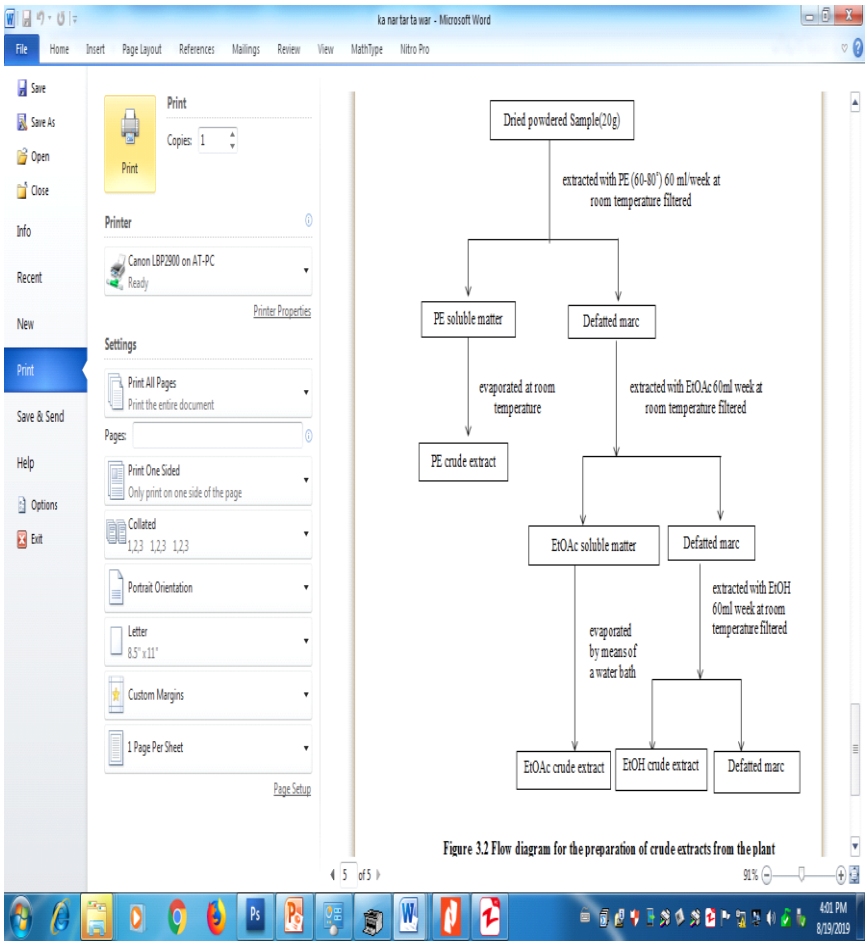


Figure 3. Flow diagram for the preparation of crude extracts from the plant sample

Qualitative Determination of Phytoconstituents in Petroleum ether, Ethylacetate and Ethanol Crude Extracts by TLC Method

TLC Method was used for qualitative determination of possible number of component from the extract of PE, EtOAc and EtOH. A solvents system was optimized in order to get maximum separated on plate and the presence of various phytochemicals was confirmed by the use of different spraying reagents. It was found that the ratio of PE:EtOAc (2:1 v/v) is suitable for all extracts. The presence of flavonoids, steroids, terpenoids, alkaloids and phenolic compounds were confirmed by the TLC results.

Presence of alkaloids was confirmed by Drangedroff's reagent whereas steroid and steroids and terpenoids were detected visually by spraying with Lieberman-Burchard reagent followed by heating. Phenolic compounds showed coloured reaction by 10% ferric chloride solution whereas flavonoids were confirmed by detecting with NH_3 vapour. The observed colours and behaviours on TLC chromatograms were summarized in Table 2, 3, 4 and Figure 4, 5, 6.

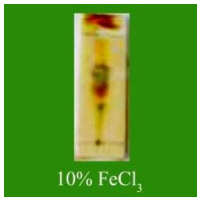
Table 2. Qualitative Determination of phytoconstituents in Petroleum Ether Crude Extract of *Oxalis Corniculata* Linn (Hmo- na- do) by TLC Method

Solvent System	No of Spots	R_f value	Observation				Remark
			Drangedroff's	Libermann-Burchard, Δ	10% $FeCl_3$	NH_3 Vapour	
PE:EA (V/V) 2:1	6	0.8,0.72, 0.6,0.5, 0.2,0.13	orange	-	-	-	Alkaloid
	4	033,0.58, 0.68,0.93	-	Pink and green	-	-	Terpenoid and steroid
	5	0.95,0.83, 0.63,0.43, 0.33	-	-	Black	-	Phenolic compound
	6	0.93,0.8 0.6,0.5 0.33,0.3	-	-	-	Yellow	Flavonoid compound

(a) Qualitative determination of alkaloids by TLC method

(b) Qualitative determination of steroid, terpenoid by TLC method

(c) Qualitative determination of phenolic compound by TLC method



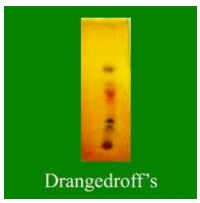
(d) Qualitative determination of flavonoid compound by TLC method

Figure 4. TLC chromatograms of PE crude extract from *Oxalis corniculata* Linn. (Hmo-na-do)

Table 3. Qualitative Determination of phytoconstituents Ethyl Acetate Crude Extract of *Oxalis Corniculata* Linn (Hmo- na- do) by TLC Method

Solvent System	No of Spots	R_f value	Observation				Remark
			Drangedroff's	Libermann-Burchard, Δ	10% $FeCl_3$	NH_3 Vapour	
PE:EA (V/V) 2:1	6	0.6,0.49, 0.43,0.37, 0.22,0.16	orange	-	-	-	Alkaloid
	4	0.75,0.63, 0.55,0.35	-	Pink and green	-	-	Terpenoid and steroid
	5	0.67,0.5, 0.38,0.23, 0.08	-	-	Black	-	Phenolic compound
	7	0.73,0.63, 0.45,0.37, 0.31,0.14, 0.08	-	-	-	Yellow	Flavonoid compound

(a) Qualitative determination of alkaloids by TLC method



(b) Qualitative determination of steroid, terpenoid by TLC method

(c) Qualitative determination of Phenolic compound by TLC method

(d) Qualitative determination of flavonoid compound by TLC method

Figure 5. TLC chromatograms of EtOAc crude extract from *Oxalis corniculata* Linn. (Hmo- na-do)

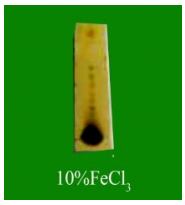
Table 4. Qualitative Determination of phytoconstituents Ethanol Crude Extract of *Oxalis Corniculata* Linn (Hmo- na- do) by TLC Method

Solvent System	No of Spots	R_f value	Observation				Remark
			Drangedroff's	Libermann-Burchard, Δ	10% $FeCl_3$	NH_3 Vapour	
PE:EA (V/V) 2:1	5	0.75,0.6, 0.55,0.27, 0.13	orange	-	-	-	Alkaloid
	3	0.65,0.3, 0.1	-	Pink and green	-	-	Terpenoid and steroid
	7	0.72,0.65, 0.55,0.45, 0.38,0.28, 0.23	-	-	Black	-	Phenolic compound
	7	0.8,0.67, 0.58,0.48, 0.43,0.3, 0.1	-	-	-	Yellow	Flavonoid compound

- Qualitative determination of alkaloids by TLC method

(b) Qualitative determination of steroid, terpenoid by TLC method

(c) Qualitative determination of Phenolic compound by TLC method



(d) Qualitative determination of Flavonoid compound by TLC method

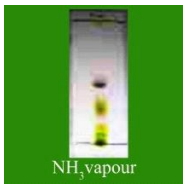


Figure 6. TLC chromatograms of EtOH crude extract from *Oxalis corniculata* Linn. (Hmo- na-do)

Investigation of Nutritional Composition of *Oxalis corniculata* Linn.

The determination of nutritional composition of the dried *Oxalis Corniculata* powder was carried out to know the nutritional value of *Oxalis Corniculata* sample. The results are shown in Table 5 and Figure 7.

No	Nutrients	Content (%)
1	Moisture	21.77
2	Protein	17.71
3	Crude fiber	21.48
4	Crude fat	0.81
5	Ash	11.58
6	Carbohydrate	26.65

Conclusion

In this research work, the *Oxalis corniculata* was collected for chemical analysis. According to the phytochemical investigation of the *Oxalis corniculata* plant sample, it has revealed the presence of alkaloids, flavonoids, tannins, - amino acid, carbondydrates, glycosides, phenolic compounds and reducing sugars. But, starch and saponins were not detected in *Oxalis corniculata* sample by test tube method. Thin layer chromatography screening reveals the presence of alkaloids, terpenoids, steroids, phenolic and flavonoid compounds with different R_f values and different colour. From the result of nutritional values, it was observed that the *Oxalis corniculata* contains many nutritional values which have benefits to humans. Among them, the soluble carbohydrate content was found to be the highest values (26.65%) in the *Oxalis corrniculata* plant sample. Energy value of plant sample was 184.73 kcal per 100 g. It can be concluded that the *Oxalis corrniculata* plant sample contains the valuable phytochemicals for human's health and nutritional values for the maintenance of the body.

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