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Analysis of phytochemical, antioxidant, and α-amylase inhibition capacity of methanol extract of six plants from Kaski, Gulmi and Rupandehi districts of Nepal

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Abstract

*This study was designed to explore phytochemicals and biological activities of methanolic extract of six traditional medicinal plants (*Clerodendrum trichotomum*,* Mallotus philipinensis*,* *Dioscorea*-bublifera*,* Rubia cordifolia*,* Viscum articulatum*) of Nepalese origin. Plant extracts were prepared in methanol through cold percolation. Analysis of phytochemical constituent, antioxidant capacities and brine shrimp lethality assay of the test plant samples were carried out using standard methods. The 1,1-diphenyl-2-picryl hydrazyl (DPPH) method was used to study antioxidant activity of different plant extracts. Furthermore, starch-iodine method was used to study the inhibition effect of extract on α-amylase. Phytochemical analysis showed the presence of phytochemicals like alkaloids, flavonoids, phenolic content, glycosides, reducing sugars, etc. in six medicinal plants. Brine shrimp lethality assay suggested the presence of pharmacologically active compounds. Total phenolic content and total flavonoid content of* C. trichotomum*'s leaves extract were found to be higher with 212.742 mg GAE/g and 112 mg Q/g respectively with strong antioxidant activity. Similarly, the α–amylase inhibition of* R. cardifolia's root extract and C. trichotomum*'s leaves extract was found to be 252.44±0.55 µg/mL and 293.33± 0.81 µg/mL comparative with IC_{50} value 119.063±0.73 µg/mL of standard acarbose that showed remarkable antidiabetic property among six samples. The results, obtained here suggested that six medicinal plants* i.e. C. trichotomum*,* M. philipinensis, D. deltoidea*,* D.bublifera*,* R. cordifolia*, and* V. articulatum*, Nepal origin showed biological activity by targeting multiple drug targets which justifies their traditional uses. This is the first finding that* C. trichotomum*'s leaves can be a promising source for the development of natural antioxidant and antidiabetic agents.*

Keywords
C. trichotomum, M. philipinensis, D. deltoidea, D. bublifera, R. cordifolia, V. articulatum,.

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1 Introduction

Many diseases have been cured with plants by relating in various formula such as vegetables, fruit, spices, tea, decoction, etc., since ancient time. Today's modern society is slowly returning to ancient medicinal system as the number of people who still prefer using traditional folk medicines is increasing per year. Plants comprise a wide diversity of compounds, especially secondary metabolites with anticancer, antibacterial, antidiabetics, antitumor, antiviral, analgesic and many other activities to a greater or lesser extent. Notable examples of these secondary metabolites include phenols, flavonoids, alkaloids, terpenoids, saponins, glycosides, tannins etc. [1, 2].

Antioxidants compounds have the ability to protect the body from reactive oxygen species. Reactive oxygen species (ROS) become toxic and cause the harmful effects like oxidative stress in absences of proper amount of antioxidants in our body. DPPH scavenging (2, 2-diphenyl-1-picrylhydrazyl) assay is a popular method for screening in-vitro antioxidant of plant extract. DPPH radical in methanol shows strong absorption at 517 nm. On increasing the concentration of extracts decreases the value of maximum absorbance suggest that scavenging of free radical by electron donation [3].

The types of diabetes mellitus according to the American Diabetes Association in 1997 are type 1, type 2, other specific types, and gestational diabetes. It is characterized by high blood sugar (glucose) levels that consequence from defects in insulin secretion, or resistant in its action, or both. Normally when there is an elevated level of glucose in the bloodstream, the pancreas releases insulin which then binds a membrane protein of a cell and results a series of reactions that induce glucose transporters to move into the membrane and facilitate the movement of glucose into the cell. Type 2 Diabetes [T2D] is characterized by an increase in insulin resistance and decrease beta-cell function and chronic hyperglycemia. Inhibition of α amylase activity is one of the alternative pathways for the control of postprandial hyperglycemia in diabetic patients.

Nepal has many plants with medicinal values as it is rich in biodiversity. Some of them are used in traditional medicine that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design and some are still not explored scientifically for medicinal values [3, 4]. The biological activities of the test plants i.e *C. trichotomum*, *M. philipinensis, D. deltoidea*, *D. bublifera*, *R. cordifolia*, *V. articulatum* had been reported by many research articles. Although these plants are reported for their uses such antioxidant, antidiabetic, antimicrobial, reducing high blood pressure, or improving blood circulation, the same plant grown in Gulmi, Rupandehi, Kaski district of Nepal has not been explored yet. The genetic and geographical variation plays significant role for the dissimilarity of chemical constituents of the same plant. The growing stages of the concerned plant at the time of the collection also plays a role for the variation of the chemical constituents of the same plants. Usually synthetic drugs contain a single active compound targeting a specific drug target but plant extracts may contain various active ingredients aiming at multiple drug targets [5].

On the basis of these reports, the present study purposes to calculate the polyphenols and flavonoids in the methanolic extract from different parts of such six medicinal plants i.e. *C. trichotomum*, *M. philipinensis, D. deltoidea*, *D. bublifera*, *R. cordifolia*, *V. articulatum* to determine their antioxidant potential and to evaluate its inhibitory properties on the α-amylase activities.

2 Materials and Methods

2.1 Collection of plant materials and preparation their methanol extract

Various plant parts of six medicinal plants were collected from the farmland of Gulmi, Rupandehi, Kaski district of Nepal. The plants were identified with literatures and matched with the voucher specimens deposited at National Herbarium and Plant Laboratories, Godavari, Kathmandu. The collected plant parts were cleaned, sliced into small pieces, and shade dried for 10-15 days. Then it was ground into a powder and stored. The leaves extract of six plants was prepared by cold percolation method in methanol solvent.

2.2 Chemicals and reagents

The chemicals used in this study were of the commercially available analytical grade. The methanol solvent was purchased from Merck, Germany. Similarly, porcine pancreatic α-amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were purchased from Sigma-Aldrich, USA.

2.3 Qualitative phytochemical analysis

Phytochemicals were identified by various color reactions [6]. In brief, all plant materials were completely extracted by percolation with methanol and subjected to phytochemical screening. The presence of main groups of natural constituents in different extractive solutions was analyzed by using different specific reagents.

2.4 Brine shrimp lethality assay

Brine shrimp lethality assay was carried according to standard protocol [7]. Briefly, sample stock solutions were prepared by dissolving 200 mg of each plant extract in 2 mL acetone. The test was conducted in 15 mL test tubes and sterilized artificial sea water (final volume 10 mL). From each stock solution 500 µL (equivalent to 1000 ppm), 50 µL (equivalent to 100 ppm) and 5 µL (equivalent to 10 ppm) were transferred to total nine test tubes, three test tubes for each dose level. The control was performed by adding the solvent used to dissolve the extract (acetone) in the assay. The solvent was evaporated by standing overnight. After complete evaporation of solvent, 5 mL of sea water was added to each test tube with gentle shaking to ensure that the compounds diffused adequately in the aqueous solution. Ten Salina nauplii were counted macroscopically in the stem of a Pasteur pipette against a lighted background and transferred into each sample tube and the solutions were made to 10 mL with artificial sea water. After 24 hours, the numbers of survivor were counted with the help of disposable pipettes. The entire experiment was performed in a temperature controlled room at 28 °C under the continuous supply of light by table lamp (60 Watt). The surviving nauplii were counted with the aid of a 3x magnifying glass after 24 hours. The mean mortality at the three dose levels for each extract was determined. No death was observed in the control tubes. The LC_{50} (Lethal concentration for 50% mortality) values was determined using the probit method, as the measure of toxicity of the extracts [8].

2.5 Determination of total phenolic content in the plant extracts

Total phenolic content in plant extract was calculated by Folin-Ciocalteu Colorimetric method with some modifications [9]. An aliquot of 1 mL of each leaf extract (0.5 -1.0 mg/mL) were mixed with 5 mL Folin–Ciocalteu phenol reagent (10%) and 4 mL of 7% Na_2CO_3 to get a total volume of 10 mL. The blue colored mixture was shaken well and incubated for 30 minutes at 40 ºC in a water bath. The absorbance of the mixture was measured at 760 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Standard calibration curve for gallic acid in the range of 50–100 g/mL was prepared in the same manner and results were expressed as mg gallic acid equivalent (GAE) per gram of extract.

2.6 Determination of total flavonoid content in the plant extracts

Total flavonoid content was determined using the aluminum colorimetric method using quercetin as the standard [10]. An aliquot of 1 mL leaf extract of each concentration range from 0.5-1 mg/ mL in methanol was poured to 15 mL test tube containing 4 mL of double distilled water. At the 0, 5 and 6 minutes' time interval, 0.3 mL 5% $NaNO_2$, 0.3 ml of 10% $AlCl_3$ and 2 mL of 1 M NaOH was added to the test tube respectively. Immediately volume was maintained 10 mL by adding 2.5 mL double distill water. Absorbance of the obtained pink color mixture was determined at 510 nm versus blank containing all reagents except quercetin. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Standard calibration curve for quercetin in the range of 50–100 g/mL was prepared in the same manner. The concentration of flavonoid was expressed as mg quercetin equivalent (QE) per gram of extract.

2.7 Antioxidant assay

The antioxidant activity of extract of the test plants and standard (ascorbic acid) was carried by DPPH assay method, following standard protocol [11]. Different concentration of plant extracts (10- 100 µg/mL) and ascorbic acid (10-100 µg/mL) were prepared in methanol on the clean and clear test tubes. The sample volume was taken 2 mL. To this sample volume, 2 mL of this 0.2 mM DPPH solution was added. The tube was shaken vigorously for the uniform mixing. These tubes were allowed to stand in dark for half an hour. The control was prepared as above without the plant extract or ascorbic acid. Methanol was used to collect the baseline on the spectrophotometer (Thermo Fisher Scientific, Genosystem-10-50. The absorbance was taken on the spectrophotometer at 517 nm.

Now the radical scavenging activity was calculated by using the following formula.

$$\%\text{Radical scavenging activity} = \frac{\text{Control}_{\text{abs}} - \text{Sample}_{\text{abs}}}{\text{Control}_{\text{abs}}} \times 100\% \quad (1)$$

Standard graph was plotted by taking the concentration on the X- axis and percentage free radical scavenging activity on the Y-axis. Based on this graph, IC_{50} value of each sample was calculated and these values of the different species were compared. The species having the lowest IC_{50} is considered to have the best antioxidant property.

2.8 Alpha-amylase inhibition assay

α-amylase inhibition assay was performed following the standard protocol [11]. This protocol is based

on that α-amylase converts starch into sugars and plant extracts inhibits the action of α-amylase as acarbose. Substrate was prepared by dissolving 200 mg starch in 25 mL of NaOH (0.4 M) by heating at 100 ºC for 5 minutes and adjusting pH was adjusted to 7.0 after cooling. 400 µL of substrate solution was pre-incubated at 37 ºC for 15 min. Termination of the reaction was carried out by adding 800 µL of HCl (0.1 M). Then, 1000 µL of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. The assay was carried out in triplicates using spectrophotometer. Percentage of inhibition was calculated using the formula

$$\%Inhibition = \left(1 - \frac{Abs2 - Abs1}{Abs4 - Abs3}\right) \times 100 \quad (2)$$

where Abs1 is the absorbance of the incubated mixture containing sample, starch, and α-amylase, Abs2 is the absorbance of the incubated mixture of sample and starch, Abs3 is the absorbance of the incubated mixture of the of starch and α-amylase, Abs4 is the absorbance of the incubated solution containing starch.

3 Results and Discussion

3.1 Plant collections

The plants samples on this research work were selected and collected from the farmland of Gulmi, Rupandehi, Kaski district of Nepal (Table 1).

Table1: List of plants, parts used and therapeutic use.

S.N.	Scientific name	Common name	Used part	Therapeutic use
1.	*Clerodendrum-trichotomum*	Lapche	leaves	Antioxidant Antidiabetic Antibacterial
2.	*Mallotus-philipinensis*	Sindoor	leaves	Antioxidant Antifilarial
3.	*Diosocrea- deltoidea*	Vyakur	rhizomes	Anti-inflammatory Antiviral Antidiarrhoic
4.	*Dioscorea-bublifera*	Gitto	rhizomes	Antioxidant Anti-inflammatory Antidiabetic
5.	*Rubia- cordifolia*	Majitho	roots	Antidysentric Antioxidant
6.	*Viscum- articulatum*	Harchur	bark	Anticancer Antioxidant

Table 2: Phytoconstituents of test plants extract in methanol.

Phytochemical	**CT**	**MP**	**DD**	**DB**	**RC**	**VA**
Reducing Sugars	-	-	-	+	-	+
Polyphenols	+	+	-	+	+	+
Alkaloids	+	+	+	+	+	+
Glycosides	-	-	+	+	+	+
Quinones	+	-	+	-	+	+
Saponins	+	-	-	+	+	-
Coumarins	-	-	-	+	-	+
Flavonoids	+	+	+	+	+	+

where, (+) present and (-) absent, CT = *C. trichotomum*, MP = *M. philipinensis, DD = D. deltoidea*, DB = *D. bublifera*, RC = *R. cordifolia*, and VA = *V. articulatum*

3.2 Phytochemical screening

The results obtained from the phytochemical screening indicating the presence and absence of different types of phytoconstituents are tabulated in table 2.

The different extracts of plants depict the presence of various secondary metabolites like alkaloids, glycosides, coumarins, saponins, flavonoids, terpenoides etc. These compound are supposed to be biologically active and act as anticancer, antioxidant, antidiabetic etc. [12]. Due to presence of such secondary metabolites *V. articulatum* be the good source of such biologically active compounds and

D. deltoidea and *C. trichotomum* can be inferred to be potent antioxidant, antidiabetic and antimicrobial [13–15]. Flavonoids, alkaloids and phenolic compounds are a major source of compounds that act as primary antioxidants or free radical scavenger. Subsequently, plants containing these phytochemicals may be used for the preparation of drug in a systematic way which may be lead to the cure of many ailments in the future [16]. So, many of selected medicinal plants can be inferred as the good source of biologically active compounds.

3.3 Brine shrimp bioassay

Results obtained from the Brine Shrimp Lethality assay are presented in table 3.

Table 3: LC_{50} Value of Plant samples in Brine shrimp bioassay.

Name of Plant Extracts	LC_{50} Value µg/mL
C. trichotomum	184.79
M. philipinensis	100
D. deltoidea	193.06
D. bublifera	135.93
R. cordifolia	184.79
V. articulatum	46.39

From the above calculations, sample VA (bark of *V. articulatum*) had shown the lower LC_{50} value which indicates that it contains highly active compound. The result showed that LC_{50} of different plant extracts ranged from 46.39 to 193.06 µg/mL.

V. articulatum can be inferred to have very strong cytotoxic effect. Likewise, *M. philipinensis*, *D. bublifera*, *C. trichotomum*, *R. cardifolia*, and *D. deltoidea* were biologically active with LC_{50} values 100, 135.93, 184.79, 184.791, and 193.06 µg/mL respectively. So, these plants can be suggested to use as therapeutic agents as the toxicity activity on them is nominal. Plant extract resulting in LC_{50} less than 1 mg/mL were considered toxic to the larvae. This brine shrimp lethality test was also a guide for active antitumor agent [17,18].

3.4 Total Phenolic and Flavonoid Contents

The TPC and TFC were expressed as the GAE/g, and QE/g of extract using a calibration curve of gallic acid and quercetin, respectively which is shown in fig. 1 & 3.

The total phenolic content of all selected medicinal plant extracts showed varied data ranging from 54.742±0.025 to 212.742±3.34 mg GAE/g in *C. trichotomum* and *D. deltoidea* respectively which is shown in figure 2. The total phenol content of rest of plant extract lied between these two extremes.

The results showed that the total phenolic content in *C. trichotomum* (CT) was highest among six selected medicinal plants. Leaf extract of *C. trichotomum* (CT), bark of *V. articulatum* (VA) also showed relatively high phenolic content.

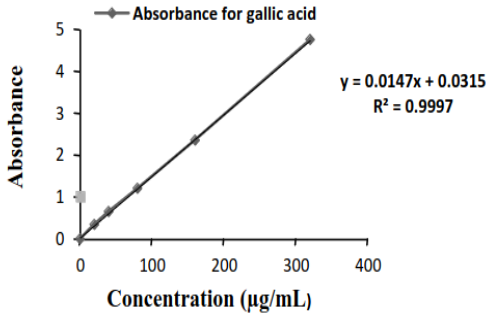

Figure 1: Calibration Curve of Gallic acid.

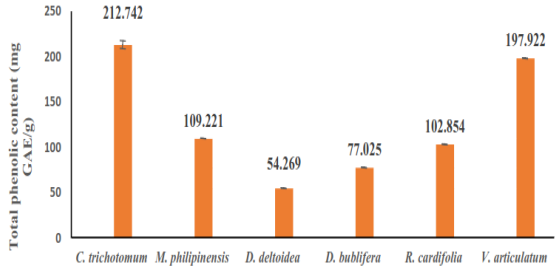

Figure 2: Comparison of TPC in different plant extracts.

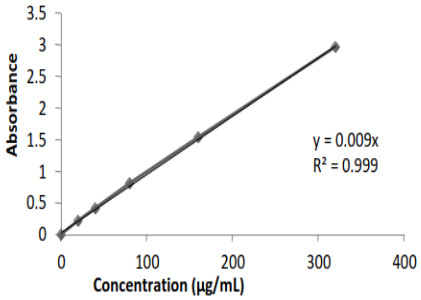

Figure 3: Calibration Curve of Gallic acid.

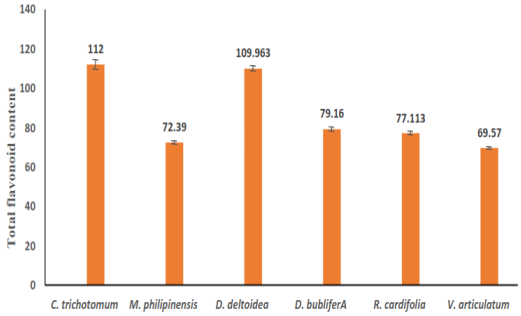

Figure 4: Comparison of TPC in different plant extracts.

From the calculation of total flavonoid content in different plant extract, it was found that sample (CT) *C. trichotomum* has highest TFC. It was also seen that TFC in (DD) *D. deltoidea* was high enough. The total flavonoid content of different medicinal plants was found and the result revealed that the TFC varied from 72.39±112 mgQE/g in *M. philipinensis* to 109.964±1.25 mgQE/g is *D. deltoidea.* All the remaining plant extract showed the TFC in two extremes as shown in fig 4. The literature revealed that presence of phenols and flavonoids in plants extract had been reported to be associated with antioxidative action in biological system, acting as scavengers of singlet oxygen and free radicals.

Thus, greater TPC and TFC had positive correlation with greater antioxidant activities. In this study, the high value TPC (212±2.742 mgQE/g) and TFC (112±2.42 mgQE/g) could be attributed to antioxidant property of *C. trichotomum* [19].

3.5 DPPH free radical scavenging activity

The free radical scavenging activity of methanol extractives of *C. trichotomum*, *M. philipinensis, D. deltoidea*, *D. bublifera*, *R. cordifolia*, and *V. articulatum* were evaluated by DPPH assay. Table 4 show the value of % DPPH free radical scavenging activity at different concentrations of ascorbic acid, *C. trichotomum*, *M. philipinensis, D. deltoidea*, *D. bublifera*, *R. cordifolia*, and *V. articulatum.* The graph of concentration against the corresponding percentage radical scavenging activity of different samples was plotted fig 5 and IC_{50} value was determined. Ascorbic acid was used as the standard in this experiment. Among six different plant extract, methanol extract of leaves of *C. trichotomum* had the IC_{50} value 40.62±0.16 g/mL and *rhizome of D. deltoidea* had the IC_{50} value 41.18±0.81 very close to that of ascorbic acid i.e. 26.64±0.14 g/mL respectively. Remaining plants *M. philipinensis's* leaves (60.45 ± 0.99 g/mL), *V. articulatum's* bark (63.12±0.85g/mL), *D. bublifera's* rhizome (69.58±0.15 µg/mL), *R. cordifolia's root* (89.71±0.13 µg/mL) extract had moderate antioxidant activity table 5.

Literature revealed that the majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarins, lignans, catechins and isocatechins. The obtained IC_{50} of methanolic extract of test samples are supported by previously reported value of the same plants from different region with slight variation in value [20, 21]. Compounds like trichotomoside (phenylpropanoid glycoside), isoacteoside, jionoside D had been isolated and proved to be responsible for the antioxidant activity of *C. trichotomum* [22–24]. Strong antioxidant activity for acetone and ethanol extract of *V. articulatum* bark had been reported by Kakpure, 2020 [25]. While weak antioxidant activity of methanol extract of *V. articulatum* bark extract had been reported for plant collected from Kathmandu valley by Kumal et al 2020 [26].

Table 4: % inhibition of ascorbic acid, CT, MP, DD, DB, RC, VA at different concentrations

Concentration (μ g/mL)	% Inhibition						
	Ascorbic acid	CT	MP	DD	DB	RC	VA
20	64.06±0.56	52.5±3.23	35.97±0.85	64.37±0.51	45.23±2.82	1.54±4.04	23.73±2.34
40	75.45±0.05	61.76±2.74	45.27±2.65	65.52±0.08	50.59±2.8	2.97±3.63	55.49±7.74
60	86.79±0.06	69.86±1.52	55.52±0.52	64.85±1.33	51.27±2.86	20.91±2.56	57.99±0.65
80	92.5±0.06	75.40±2.17	60.44±0.99	64.22±3.31	52.44±2.85	54.89±2.01	58.49±19.25
100	94.06±1	78.09±0.64	65.23±0.66	65.55±0.20	52.77±3.73	59.02±3.56	59.62±23.22

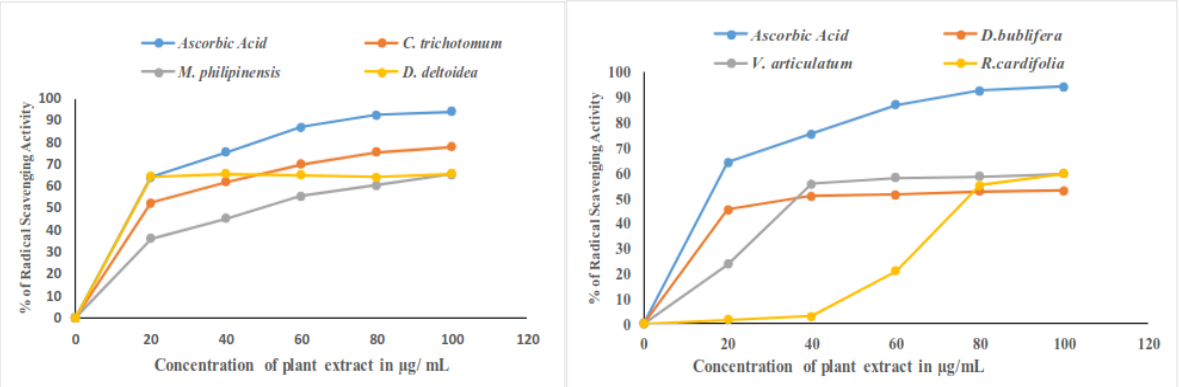

Figure 5: A plot of % free radical scavenging of methanol extracts against concentration of plant extract, and ascorbic acid.

IC_{50} values of the plant extracts along with the standard Ascorbic acid is tabulated below in table 5.

Table 5: Comparison of IC_{50} values of different plant extracts with ascorbic acid

Sample	**IC_{50} (µg/mL)**
Ascorbic acid	26.64±0.14
C. trichotomum	40.62±0.16
M. philipinensis	60.45±0.99
D. deltoidea	41.18±0.81
D. bublifera	69.58±0.16
R. Cardifolia	89.71±0.13
V. articulatum	63.12±0.91

IC_{50} values are expressed as mean±SD (n = 3)

3.6 Alpha-amylase inhibition test

Alpha-amylase inhibitory activity of plant extracts was determined from quantitative starch-iodine method. Alpha-amylase inhibition % values at different concentrations of standard acarbose and six plant extracts are shown in the below table 6. The graph of concentration against the corresponding percentage radical scavenging activity of different samples was plotted fig 6 and IC_{50} value was determined. The percentage inhibition of different plant extracts versus concentration and antidiabetic activity in term of IC_{50} values of different extract are given in table 7.

Table 6: % Inhibition at different concentrations of Plant extract.

Concentration (μg/mL)	% Inhibition						
	Acarbose	CT	MP	DD	DB	RC	VA
40	53.07±1.02	30±0.29	22.89±4.89	13.81±3.06	45.01±3.99	60.96±3.05	37.37±5.33
80	60.73±0.95	41±0.82	30.46±0.39	13.61±1.23	59.01±3.5	63.09±3.09	53.56±0.25
160	67.12±0.95	49±1.13	40.22±0.20	18.77±3.42	57.31±2.4	57.21±2.01	57.12±2.8
320	81.31±0.59	65±5.47	45±2.56	32.08±2.42	62.46±3.2	75.20±1.89	54.92±0.21
640	88.20±0.58	70±0.29	47.24±4.21	38.92±1.23	64.16±4.5	70.52±1.55	56.1±0.69

where CT = *C. trichotomum*, MP = *M. philipinensis*, DD = *D. deltoidea*, DB = *D. bublifera*,RB =*R. Cardifolia*, VA = *V. articulatum*. Mean % Inhibition are expressed as means±SD (n = 3).

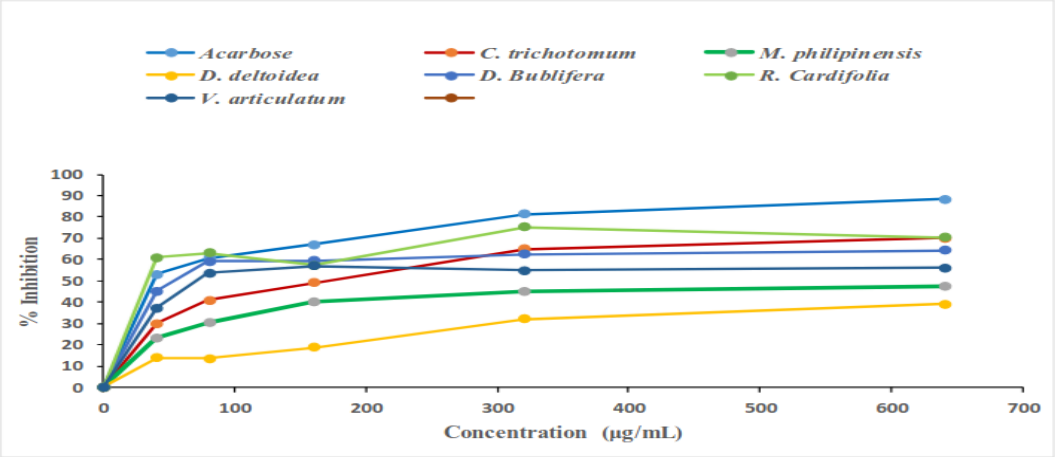

Figure 6: Inhibition of α-amylase activities by plant extracts.

IC_{50} values of the six plant extracts along with the standard acarbose is tabulated below in table 7.
Table 7: Comparison of IC_{50} values of different plant extracts with standard acarbose

Sample	**IC_{50}(µg/mL)**
Acarbose	119.063 ± 0.73
Clerodendrum trichotomum	293.33 ± 0.81
Mallotus philipinensis	554.18 ± 0.75
DIoscorea deltoidea	785.15 ± 0.92
Dioscorea bublifera	352.71± 0.59
Rubia cordifolia	252.44 ± 0.55
Viscum articulatum	344.58 ± 0.61

IC_{50} values are expressed as mean±SD (n = 3)

Here, IC_{50} value of standard acarbose was found to be 119.063 ± 0.73 µg/mL. *C. trichotomum, M. philipinensis, D. deltoidea, D. bublifera, R. Cardifolia, V. articulatum* showed α-amylase inhibitory activity with IC_{50} value 293.33±0.81 µg/mL, 554.18±0.75 µg/mL, 785.15±0.92 µg/mL, 352.71±0.59 µg/mL, 252.44±0.55 µg/mL, 252.44± 0.55µg/mL respectively in table 7. There was a dose dependent increase in percentage inhibitory activity against α-amylase by all the six plant extracts. The result obtained here are in good correlation with previously reported results [27,28]. Strong antioxidant and antimicrobial activity of leaves of *C. trichotomum* collected from Dhankuta had been reported by Subba et al, 2016 [29]. Among six medicinal plants, leaves of *C. trichotomum* is found to be good property as antidiabatic, antioxidant, phenolic and flavonoid content with low cytotoxic effect.

4 Conclusion

The present study revealed that the methanolic extract of the *C. trichotomum's* leaves, *M. philipinesis's* leaves, *D. deltoidea's* root, *D. bublifera's* root, *R. cardifolia's* stem, *V. artculatum's* bark has notable antioxidant potential and inhibitory activity on the α-amylase enzyme. These effects would be due to its important phenolic composition, whose quantitative study has revealed the varied presence of polyphenols and flavonoids. These results could justify the use of these plants in traditional medicine for the treatment various health problem including type 2 diabetes and complications. Our study is the first examination of anti-α-amylase capacities of *C. trichotomum* leaves extract. Study of chemical constituents of these plants using sophisticated technologies like NMR, HPLC, etc. can provide a way for extensive research that can be used for commercial drug production with lesser or no side effects.

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Central Department of Chemistry, Tribhuvan University, Kirtipur.

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