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Phytochemical screening, free radical scavenging activity, *In-vitro* Alpha-amylase enzyme and glucose diffusion inhibition activity of ethyl acetate and water extracts of selected medicinal plants of Nepal

Rishiram Baral, Laxman Subedi, Monica Gurung, Sabita Ojha, Basanta Shrestha, Dinesh Chaudhary and Nirmala Jammarkattel

Abstract
Diabetes mellitus is the metabolic disease leading to elevation of blood glucose, which may be due to the lack of enough insulin or responsible tissue become insulin resistance. The main aim of the present study was to evaluate in-vitro glucose diffusion and alpha-amylase inhibition as well as to determine free radical scavenging effects of selected plant sample. The five different plants i.e., *Amomum subulatum* Roxb., *Choerospondias axillaris* (Roxb.) B.L. Burtt, *Nephrolepsis cordifolia* (L). C. Presl, *Musa sp.* And *Myrica esculenta* Buch. -Ham. ex D. Don were collected from different area of Kaski district, Nepal. *Myrica esculenta* stem bark showed potent DPPH free radical scavenging effect in both solvent which showed IC50 value of 5.920µg/ml. *Amomum subulatum, Choerospondias axillaris, Nephrolepsis cordifolia* fruit and leaves and *Musa species* leaves in water have IC50 value of 0.81 mg/ml, 0.69mg/ml, 0.49mg/ml, 0.77mg/ml and 0.61mg/ml showing better alpha amylase inhibition. Glucose diffusion inhibition study revealed that *Amomum subulatum* seed, *Choerospondias axillaries* fruit and *Musa species.* Leaves showed highest GDRI% in water at concentration of 20mg/ml and 40 mg/ml. Thus, *Myrica esculenta* stem bark, *Choerospondias axillaris* fruit and *Musa species* leaves were found potent antioxidant and alpha amylase inhibitors.

Keywords: Diabetes mellitus, alpha-amylase, glucose diffusion, antioxidant activity, phytochemical screening

1. Introduction
Diabetes mellitus is the metabolic disease-causing elevation of blood glucose, which may be due to the lack of enough insulin or responsible tissue become insulin resistance [1]. Global prevalence of diabetes is upsurging each year, mainly in urban areas in developing countries where 90% people are suffering from Type II diabetes and by 2030 the ratio is projected to be increase by 10.1% [2]. Free radical enhances the oxidative stress which further increases the diabetic complication [3]. Therefore, diabetes and its related complication can be treated by using antioxidant agents as well [4]. According to WHO, medicinal plants would be the best source to obtain variety of drugs. In developing countries, about 80% of people utilize traditional medicine and it consists of numerous compounds derived from plants. Various bioactive substances are present in plant such as tannins, alkaloids, carbohydrates, terpenoids, steroid and flavonoid which can be qualitatively and quantitatively identified by phytochemical screening [5]. Free radicals namely Reactive Oxygen Species (ROS), and Reactive Nitrogen Species (RNS) cause tremendous damage to lipids, protein, enzymes, and nucleic acids which result in cell and tissue injury and cell death. These free radical and oxidative stress cause large sum of chronic degenerative diseases including inflammation, cancer, diabetes mellitus, atherosclerosis, liver injury, Alzheimer, and coronary heart pathologies. The ROS and RNS consist reactive entities like superoxide (O_2^-), hydroxyl (OH^-), peroxynitrite ($ONOO^\bullet$), peroxyl ($ROO^\bullet$) and nitric oxide ($NO^\bullet$) along with few non-free radical species like hydrogen peroxide (H_2O_2), nitrous acid (HNO_2) and hydrochlorous acid (HOCl) [6]. Large amount of antioxidant is produced from plant which can arrest the damage and injury made by these ROS and RNS entities [7].
A key enzyme in digestive system, pancreatic alpha amylase hydrolyze starch into smaller oligosaccharides like maltose, maltotriose which further breakdown into glucose and enter the blood stream by absorption [8]. Thus, pancreatic alpha amylase is initially responsible for increasing blood glucose level which should be inhibited for treatment of Type II diabetes.

Inhibitors currently in the clinical use for e.g. Acarbose, imiglitol, and voglibose are known to inhibit a wide range of glucosidase such as alpha-glucosidase and alpha-amylase [9]. But, long term use of allopathic drugs is often associated with serious side effects such as gastrointestinal disorder associated with the use of acarbose, granulocytopenia and hypoglycemia with glibenclamide, lactic acidosis associated with metformin therapy [10]. Glucose diffusion inhibition is a dialysis tubing technique which evaluate the potential of soluble dietary fibers to additionally retard the diffusion and movement of glucose in the intestinal tract [11]. Many medicinal plants are being used in managing diabetes mellitus and the activity is depicted by the presence of active phytoconstituents such as phenolic and flavonoids [12]. Low toxicity and side effect of naturally occurring herbal plants encourage its utilization as medicinally active constituents [13]. Meanwhile, Nepal is rich in biodiversity and almost 90% of Nepalese population from rural part utilizes naturally occurring medicinal plants for different acute and chronic ailment [14]. Utilization of herbal plants and formulation to prevent hyperglycemia is common. Herbal medicines depict anti-diabetic activity resembling insulin like activity, act on insulin secreting beta cell or modify glucose utilization [15]. Despite widespread use of their medicinal plants in Nepal, very few scientific study and research have been done to prove their therapeutic efficacy and safety [16]. This study was carried out by taking locally available medicinal plants from Kaski district of Nepal which consist of Greater cardamom or large cardamom (*Amomum subulatum*Roxb.), Lapsi (*Choerospondias axillaris* Roxb.), Banana (*Musa* species.), Box berry or kafal (*Myrica esculenta* Buch. -Ham.ex D Don), Pani amala (*Nephrolepis cordifolia* (L). C. Presl).

Amomum subulatum is a well-known flavoring species member of *Gingeberaceae* family which is being used traditionally to treat stomach pain, flatulence, belching, indigestion, vomiting, constipation. The root juice is being used even as blood glucose reductant, expectorant, stimulant, appetizer and diuretics [17, 18]. *Choerospondias axillaris* belonging to family *Anacardiaceae* is a deciduous tree whose fruit is rich in Vitamin C and known for antibacterial, antimicrobial and antioxidant activities. Traditionally, increased consumption of the fruitis believed to reduce the incidence of chronic diseases such as cancer, cardiovascular diseases and other ageing related pathologies [19, 20]. *Musa species* of family *Musaceae*which is being consumed as a nutritious food is also being used in traditional folk medicine for cure of various ailments. The flower part of *Musa species* are being used to treat heart pain, asthma, endocrine problem like diabetes, painful menses and menopausal bleeding, dysentery etc. and water juices from flowers, fruits and leaves is being used in burns [21, 22]. *Myrica esculenta* of *Myricaceae* family is also being used traditionally for the treatment of wider ranges of disorders. The stem bark is being used by several ethnic community of Kaski district, Nepal for the treatment of cough, asthma, fever, chronic bronchitis, diarrhea, rheumatism, and inflammation. Root of this plant is being used in bronchitis, asthma, cholera and flowers is being used to treat earache, diarrhea, paralysis, etc [23, 24]. *Nephrolepis cordifolia* of Nephrolepidaceae family is also being used in traditional folk medicine. Its root, stalk, leaves and whole grass can be used as medicine. Root tuber juice is given in fever, indigestion, cough, cold, headache and to lower blood glucose level while rhizome is reported for the treatment of cough, rheumatism, chest congestion, nose blockage and loss of appetites [25].

This study was conceived to figure out the scientific background of these selected medicinal plants by taking their traditional and local use as a reference basis for selection of these plants. The main aim of this study is to perform phytochemical screening, free radical scavenging activity, In-vitro alpha-amylase enzyme inhibition and glucose diffusion inhibition of these selected medicinal plants.

2. Materials and Methods

2.1 Chemicals and Reagents

1,1 Diphenyl-2 picryl hydrazyl radical (DPPH) is purchased from Tokyo Chemical Industry, Japan. α-amylase (1,4-α-D-Glucan-glucano hydrolase), Dialysis membrane, L-ascorbic acid were purchased from Himedia Laboratories, India. Starch, Sodium hydroxide pellets, Dimethyl sulfoxide, Benedict's reagent, Mercuric chloride, and Hydrochloric acid were purchased from Thermo Fisher Scientific, India.3,5-Dinitrosalicyclic acid was purchased from Central Drug House, New Delhi. Dextrose anhydrous purified, Potassium sodium tartrate tetra hydrate, Di-sodium hydrogen phosphate, Benzene, Copper sulphate pentahydrate, Sodium anhydrous, 1-napthol were purchased from Merck specialties, India. Reference drug Voglibose IP was obtained from Asian pharmaceuticals Pvt. Ltd, Bhairahawa, Nepal. All chemicals and reagents used were of analytical reagent grade.

2.2 Collection and Identification of plants sample

Selected medicinal plants (listed in table 1) were collected from different area of Kaski district, Nepal. The herbaria were prepared and identified with the help of taxonomist from National Herbarium and Plant Laboratories, Godawari, Kathmandu, Nepal. The voucher specimen of each collected medicinal plants was deposited in the crude drug museum of School of Health and Allied Sciences, Pokhara University. The collected sample was chopped into small pieces and was shaded dried. It was incubated in hot air oven at 40˚ C for complete removal of moisture, which is detected by weight variation test at different time intervals. After complete drying of sample, it was powdered with the help of grinder.

Table 1: List of Plants

Scientific Name	Family	Local Name	Parts Used	Crude Drug Voucher No.	Sample No.
Amomum subulatum Roxb.	Zingiberaceae	Alaichi	Seed	PUCD-2019-12	S1
			Outer cover	PUCD-2019-13	S2
Choerospondias axillaries (Roxb.) B.L. Burrt	Anacardiaceae	Lapsi	Fruits	PUCD-2019-14	S3
Musa sp.	Musaceae	Kera	Leave	PUCD-2019-15	S4
			Leaves vein	PUCD-2019-15	S5
Myrica esculenta Buch. -Ham.ex D Don	Myricaceae	Kafal	Stem bark	PUCD-2019-16	S6
			Small branches	PUCD-2019-17	S7
Nephrolepis cordifolia (L). C. Presl	Nephrolepidaceae	Pani amala	Fruits	PUCD-2019-18	S8
			Leaves	PUCD-2019-19	S9

2.3 Sample Extraction
Extraction of crude drug was done by single maceration for 24 hours. The crude drug was macerated with ethyl acetate and water separately in the ratio 1:5 (w/v). Then the extract obtained were filtered and concentrated in rotary vacuum evaporator to obtain dried extracts for further study.

2.4 Phytochemical Screening
Phytochemical analysis of all extracts was carried out as per the procedure described by Trease and Evans, 1989, Yadav *et al*., 2014 and Stanley I *et al*. for qualitative determination of active components [26, 27].

2.5 Antioxidant Activity Study
2.5.1 DPPH free radical scavenging activity
The DPPH free radical scavenging activity was carried out according to the method of Irene p *et al*., 2000 [28]. In brief, 2 ml of solution of different concentration (1 µg/ml, 10 µg/ml, 100 µg/ml) of plant sample was mixed with 2 ml of DPPH solution (60 µM). The mixture was kept in dark for 30 minutes to perform the complete reaction. Finally, the absorbance of the sample was measured at 517 nm by using UV-visible spectrophotometer. Free radical scavenging activity of each sample was calculated by using following formula:

$$\%\ DPPH\ Scavenging\ activity = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\%$$

Where,
$Abs_{control}$ = Absorbance of control
Abs_{sample} = Absorbance of sample

2.6 Alpha- amylase Inhibition Assay
Alpha-amylase inhibition activity study was done by starch iodine method according to method given by Gebretsadkan H T *et al*., 2019 [29]. Briefly, 390 µl of phosphate buffer containing different concentration of plant extracts (0.25 mg/ml, 0.5 mg/ml, 1 mg/ml, and 2 mg/ml) was mixed with 10 µl of alpha-amylase solution of 0.025 mg/ml concentration. Then, the solution was incubated at 37°C for 10 minutes. After incubation 100 µl of 1% starch solution was added and was kept at incubation for 1 hour. Then, 0.1 ml of 1 % Iodine solution was added. Finally, 5 ml of RO water was added, and absorbance of the solution was measured at 565 nm. Voglibose standard solution was prepared in the same way replacing plant extracts with the voglibose IP. Blank solution (no alpha-amylase) was prepared by taking 400 µl of phosphate buffer, 100 µl of starch solution, 0.1 ml of Iodine and 5ml of RO water. Control solution (no starch) was prepared by taking 390 µl of phosphate buffer, 10 µl of alpha amylase, 100 µl of RO water, 0.1 ml of Iodine and finally diluted with 5 ml of RO water. Inhibition of enzyme activity was measured by using following formula.

$$Inhibition\ of\ enzyme\ activity(\%) = \frac{(A - C)}{(B - C)} * 100\%$$

Where,
A= Absorbance of sample
B= Absorbance of blank (no alpha amylase)
C= Absorbance of control (no starch)

2.7 Glucose Diffusion Inhibition Assay
2.7.1 Measurement of Glucose Diffusion Inhibition Activity
Glucose diffusion inhibition activity was done according to the method given by Archit *et al*., 2013 with some modification [30]. 5 cm strip of dialysis membrane (12000 MW Cut off) was taken, and it was kept in boiled water for 25-30 min to remove the sulphate contained on the membrane. Then, the membrane was filled with 1 ml of 5% dextrose containing 0.15 M NaCl and 1ml of plant extract. Membrane was tied at the end using nylon thread. 1 ml of RO water will be taken in place of plant extract in negative control. Then, membrane was placed in 250 ml conical flask containing 40 ml of 0.15 M NaCl and 10 ml of RO water. This equalized the internal and external strength. Conical flask was then placed in shaking water bath at temperature 37°C and 100 rpm. 500 µl of sample was taken from each conical flask at 30-minute time interval and glucose concentration in them was tested every hour up to 180 min. This procedure was repeated for three times.

2.7.2 Determination of Reducing Sugar (Glucose) from the Withdrawn Sample by 3,5 Dinitro salicylic Acid (DNSA) Method
1 ml of DNSA reagent was combined with 500 µl of sample and 2 ml of RO water was poured over it. Then, the test tube was placed on 95°C for 5 min, so that the reaction occurs between DNSA and glucose present in sample to give reddish color solution. The test tube was then cooled thoroughly, and 3.5 ml of RO water was added to each tube. Absorbance of the solution was measured at 540 nm. To determine the glucose/starch present in plant sample, direct plant sample solution and DNSA reagent was reacted, and absorbance was measured outside the membrane.
Plant sample, 5% dextrose solution and DNSA reagent was also reacted outside the membrane and absorbance was measured to determine the maximum absorbance shown by the sample. After determination of glucose concentration glucose diffusion inhibitory study was done by using the following formula
Glucose Diffusion Retardation Index (GDRI %)

$$GDRI\% = 100 - \frac{glucose\ content\ with\ addition\ of\ sample}{glucose\ content\ of\ control} * 100\%$$

To determine concentration of glucose different concentration of dextrose (0.1%, 0.5%, 1%, 5%, 10%) were taken as standard. Calibration curve was plotted by taking concentration as standard. Then, concentration of glucose in solution was determined as dextrose equivalent from graph.

3. Results
3.1 Phytochemical screening
The qualitative determination of different secondary metabolites (phytoconstituents) present in ethyl acetate and water extracts was carried out using different chemical test method. Result obtained for qualitative screening of phytochemicals in different plant extracts are present in Table 2 and Table 3. Terpenoid, Tannin, Flavonoid, Saponin and Glycoside are found in large extent in all plant extracts of both solvents.

Table 2: Phytochemical Analysis of Ethyl acetate Extracts of Plants Sample

Phytochemical Constituents	Specific Tests	S1	S2	S3	S4	S5	S6	S7	S8	S9
Alkaloid	Mayer	-	-	-	-	-	+	+	-	-
	Wagner	+	+	-	+	+	+	+	+	+
Carbohydrate	Molish	-	-	-	+	-	+	+	-	+
	Benedict	-	-	-	-	-	-	-	-	-
Glycoside	Modified Borntrager	-	+	+	-	+	+	+	+	+
Saponin	Foam	+	+	+	+	+	+	+	+	+
Phenol	Ferric Chloride	-	-	+	+	-	+	+	-	-
Flavonoid	Alkaline Reagent	+	+	+	+	-	+	+	-	+
Tannin	Gelatin	+	+	+	+	+	+	+	+	-
Terpenoid	Salkowaski	+	+	+	-	+	+	+	+	+

Phytochemicalscreening of the ethyl acetate extracts of selected medicinal plants revealed that glycosides, saponins, flavonoids, tannins and terpenoids are highly present on these extracts. It also revealed that ethyl acetate extracts of *Myrica esculenta* stem bark and small branches are rich in all phytoconstituents. Positive wager test revealed that ethyl acetate extract is also rich in alkaloid content while carbohydrate is found to be rarely distributed among the extracts.

Table 3: Phytochemical Analysis of Water Extracts of Plant Samples

Phytochemical Constituents	Specific Tests	S1	S2	S3	S4	S5	S6	S7	S8	S9
Alkaloid	Mayer	+	+	-	+	+	+	+	+	-
	Wagner	+	+	-	+	+	+	+	-	-
Carbohydrate	Molish	+	+	+	-	+	+	+	-	+
	Benedict	-	-	-	-	-	-	-	+	+
Glycoside	Modified Borntrager	+	+	+	+	+	+	+	-	+
Saponin	Foam	+	+	+	+	+	+	+	+	+
Phenol	Ferric Chloride	-	-	-	-	-	+	+	+	-
Flavonoid	Alkaline Reagent	+	+	+	+	+	+	+	-	-
Tannin	Gelatin	+	+	+	+	+	+	+	+	+
Terpenoid	Salkowaski	-	-	-	+	+	+	+	+	+

Phytochemical analysis of water extracts revealed the presence of alkaloids, flavonoids, tannins, saponins and glycoside on almost all plant extracts. Water extracts of *Nephrolepis cordifolia* was found to be devoid of most of the major phytoconstituents like alkaloids, flavonoids, and glycosides. Phenols was found to be poorly distributed in water extracts of plant samples. Result revealed that carbohydrate is present on almost all water extracts and among *Amomum subulatum* and *Choerspondias axillaries* are devoid of terpenoids.

3.2 DPPH Free Radical Scavenging Activity

The hydrogen atom or electron donating ability of extract against DPPH free radical was measured from the bleaching of violet color of methanol solution of DPPH. The DPPH radical absorbs at 517 nm and antioxidant activity was determined by monitoring the decrease in absorbance. The scavenging effects of ethyl acetate and water extracts of different plant sampleat different concentration are shown:

Table 4: DPPH Free Radical Scavenging Activity and $IC_{50}$of Ethyl acetate Extracts of Selected Plants

Plants	Concentration			IC_{50}
	1 µg/ml	10 µg/ml	100µg/ml	
S1	3.95±1.01	32.014±1.01	54.49±2.79	82.03
S2	2.51±1.01	3.77±1.78	10.07±0	>100
S3	5.39±1.01	17.08±5.34	54.13±1.27	89.19
S4	5.39±2.03	13.66±1.52	20.86±2.03	>100
S5	2.51±2.54	3.05±1.18	10.61±0.25	>100
S6	30.03±1.27	58.09±4.32	94.42±1.78	7.40
S7	17.08±0.76	21.76±3.3	90.46±0.25	46.99
S8	5.4±0.5	11.69±2.28	31.83±0.25	>100
S9	13.3±1.01	21.22±1.01	35.61±1.01	>100
Ascorbic acid	23.96±4.26	96.34±0.22	97.62±0.22	4.23

Data are expressed as mean ± standard deviation (n=3).

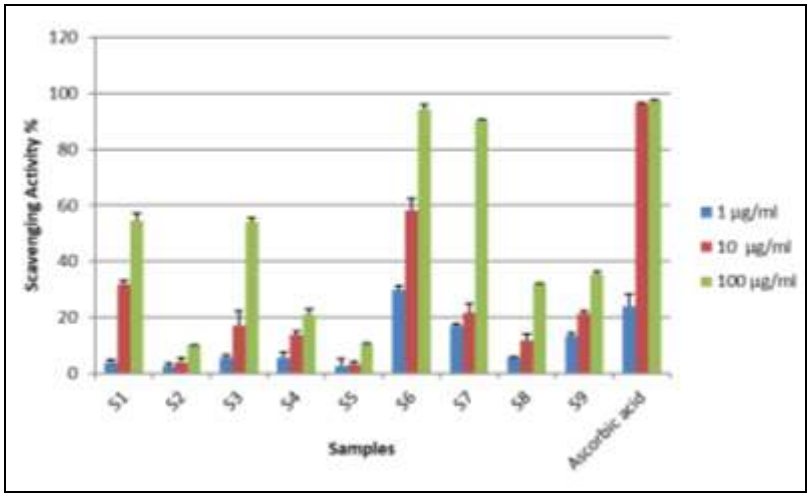

Fig 1: DPPH Free Radical Scavenging Activity of Ethyl acetate Extracts

Ethyl acetate extract of *Myricaesculenta* stem bark was proved to be very potent for DPPH free radical scavenging activity which showed IC_{50} value of 7.41 µg/ml which is comparable to ascorbic acid having IC_{50} value of 4.23 µg/ml. *Myricaesculenta* small branches showed 90.46±0.25% DPPH activity at 100 µg/ml with IC_{50}value 46.99 µg/ml.

Table 5: DPPH Free Radical Scavenging Activity and $IC_{50}$of Water Extracts of Selected Plants

Plants	Concentration			IC_{50}
	1 µg/ml	**10 µg/ml**	**100µg/ml**	
S1	1.9±2.24	16.19±0	17.14±1.34	>100
S2	2.22±1.79	22.22±2.24	24.44±0.44	>100
S3	8.57±4.48	10.79±3.59	45.71±0	>100
S4	4.13±4.93	5.71±0.44	74.76±4.26	67.72
S5	1.59±0.44	3.8±0.44	26.66±1.79	>100
S6	4.13±5.38	87.94±0.44	92.06±0	5.920
S7	1.43±0.67	50.48±2.69	83.97±0.67	9.91
S8	6.67±0.45	48.25±0.89	79.05±3.14	15.11
S9	8.25±0.89	19.52±2.02	84.44±0.44	52.25
Ascorbic acid	23.96±4.26	96.34±0.22	97.62±0.22	4.23

Data are expressed as mean ± standard deviation (n=3)

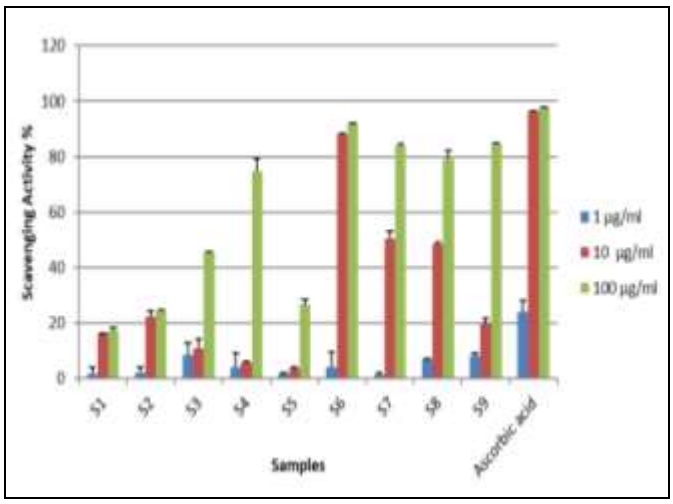

Fig 2: DPPH Free Radical Scavenging Activity of Water Extracts

Myricaesculenta showed very potent DPPH free radical scavenging activity in which stem bark showed IC_{50}value of 5.92 µg/ml and small branches showed IC_{50}value of 9.91 µg/ml. *Nephrolepis cordifolia* fruit was also found to be highly potent with IC_{50}value of 15.11µg/ml. Therefore, *Myricaesculenta* stem bark showed very potent DPPH free radial scavenging activity with extract in all solvents with value as comparable as ascorbic acid.

3.3 Alpha amylase Inhibition Activity

Drug that inhibits carbohydrates hydrolyzing enzyme have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion. The alpha-amylase absorbs at 540 nm and its inhibitory activity was determined by monitoring the decrease in absorbance. The inhibitory effects of plant extracts at different concentration in different are solvents as shown in Table 6 and Table 7:

Table 6: Alpha-amylase Inhibitory Activity and IC_{50} Value of Ethyl Acetate Extracts of Selected Plant Samples

Plants	Concentration				IC_{50}
	0.25 mg/ml	**0.5 mg/ml**	**1 mg/ml**	**2 mg/ml**	
S1	16.41±0.61	20.99±0.81	35.56±2.66	48.71±0.67	>2
S2	3.23±0.61	7.61±0.81	12.86±2.66	21.11±0.67	>2
S3	16.53±0.7	26.8±1.19	40.62±0.54	51.3±0.53	1.87
S4	15.73±0.08	26.48±0.89	35.68±0.65	52.89±0.3	1.83
S5	2.47±0.61	8.92±0.67	18.24±0.89	19.32±0.72	>2
S6	14.26±0.65	20.71±1.04	33.57±1.6	51.57±0.38	1.91
S7	4.82±0.84	7.53±0.35	10.43±0.3	20.71±3	>2
S8	2.588±0.73	8.36±1.07	9.96±0.81	23.66±0.67	>2
S9	13.1±0.92	18.52±0.78	32.3±0.96	50.5±0.77	1.97
Volgibose	48.71±1.08	69.16±4.14	82.2±4.15	94.31±2.45	0.26

Data are expressed as mean ± standard deviation (n=3)

Choerspondias axillaris and *Musa species.* Leaves show potent alpha-amylase inhibition with IC_{50} value of 1.87 mg/ml and 1.83mg/ml respectively in ethyl acetate extracts.

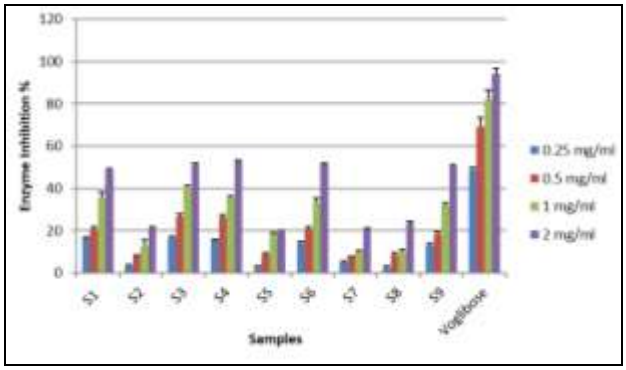

Fig 3: Alpha-amylase Inhibitory Activity of Ethyl Acetate Extracts

Table 7: Alpha-amylase Inhibitory Activity and IC_{50} Value of Water Extracts of Selected Plant Samples

Plants	Concentration				IC_{50}
	0.25 mg/ml	**0.5 mg/ml**	**1 mg/ml**	**2 mg/ml**	
S1	8.12±0.23	48.1±1.31	51.1±0.49	54.52±2.31	0.81
S2	3.01±0.78	12.14±1.55	18.99±0.59	36.63±2.33	>2
S3	38.47±0.83	40.78±1.11	64.83±0.99	67.78±0.36	0.69
S4	30.94±0.43	48.78±0.41	53.88±0.66	54.91±0.53	0.61
S5	8.48±0.9	20.82±0.67	35.08±0.6	51.61±2.82	1.90
S6	2.5±0.31	3.42±0.49	7.56±0.99	9.04±0.3	>2
S7	3.14±0.41	15.49±0.48	35.64±0.3	59.41±0.36	1.60
S8	21.43±0.73	51.25±0.94	68.14±0.61	71.17±0.56	0.49
S9	0.52±0.48	24.61±0.35	71.21±0.49	73.91±0.67	0.77
Volgibose	48.71±1.08	69.16±4.14	82.2±4.15	94.31±2.45	0.26

Data are expressed as mean ± standard deviation (n=3)

Water extracts of *Choerspondias axillaris* fruit and *Musa species* leaves showed positive result for alpha-amylase inhibition activity with IC_{50} value of 0.691 mg/ml and 0.62 mg/ml, respectively. *Nephrolepis cordifolia* fruit and leaves also showed potent alpha-amylase inhibition in water extracts with IC_{50} value of 0.49 mg/ml and 0.77 mg/ml, respectively.

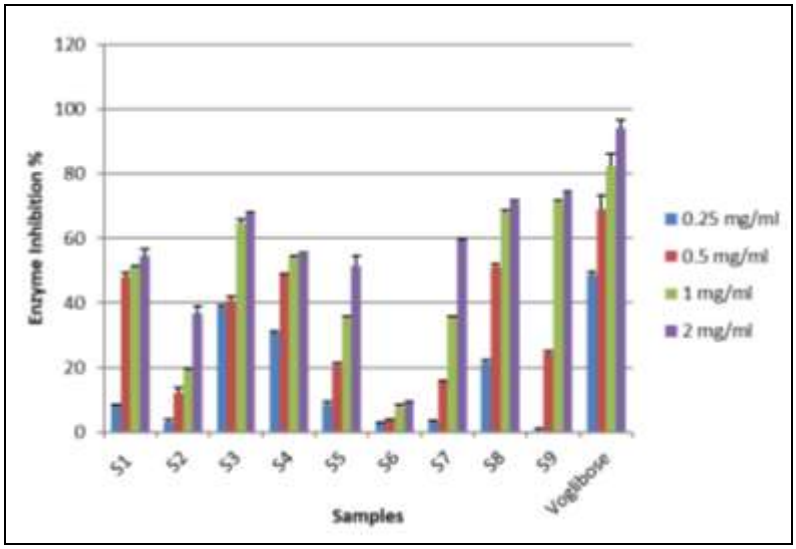

Fig 4: Alpha-amylase Inhibitory Activity of Water Extracts

3.4 Glucose Diffusion Inhibition Assay

Five different concentration of standard dextrose were taken and poured on the outer solution of the conical flask. Standard calibration curve was plotted as shown in Fig 5. This curve was taken as reference standard to determine the glucose concentration out of dialysis membrane.

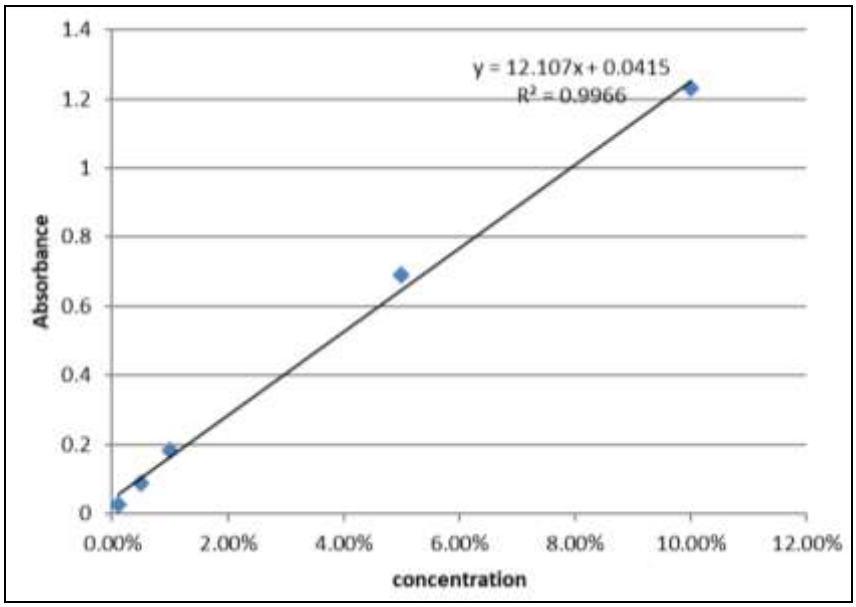

Fig 5: Calibration Curve of Dextrose Standard Directly Kept Outside of Membrane

Table 8: Concentration of Glucose Diffused out of Dialysis Membrane at Different Time Interval in Presence of Different Concentration of Water Extracts

Plants	Time (minute)					
	30 min	60 min	90 min	120 min	150 min	180 min
Standard	3.18±0.01	4.11±0.00	4.50±0.04	4.79±0.02	4.85±0.02	4.86±0.06
S1 20 mg	3.10±0.03	3.73±0.05*	4.33±0.22	4.52±0.02*	3.87±0.01*	4.44±0.05*
S1 40 mg	1.56±0.26*	2.50±0.12*	3.58±0.36	3.89±0.37	4.03±0.27	4.22±0.18*
S3 20 mg	2.80±0.16	3.71±0.06*	3.95±0.09*	4.45±0.05*	4.51±0.02*	4.48±0.04*
S3 40 mg	2.81±0.29	3.58±0.01*	3.94±0.08*	4.00±0.35	4.27±0.25	4.29±0.06*
S4 20 mg	3.03±0.05	3.48±0.07*	4.02±0.07*	3.86±0.09*	3.91±0.04*	3.70±0.08*
S4 40 mg	2.52±0.04*	3.54±0.01*	3.89±0.01*	4.27±0.08*	3.97±0.09*	3.51±0.09*
S5 20 mg	3.12±0.03	3.97±0.05	4.29±0.06	4.72±0.04	4.76±0.02	4.40±0.04*
S5 40 mg	3.11±0.02	4.27±0.61	4.27±0.07	4.29±0.28	4.32±0.02*	4.23±0.21
S6 20 mg	3.10±0.02	3.98±0.01*	4.21±0.06*	4.25±0.04*	4.42±0.04*	4.22±0.02*
S6 40 mg	2.86±0.03*	3.83±0.11	4.19±0.51	4.19±0.18*	4.43±0.08*	4.41±0.25
S7 20 mg	3.10±0.02	3.99±0.07	4.48±0.03	4.75±0.04	4.80±0.05	4.85±0.00
S7 40 mg	2.35±0.07*	3.44±0.25	3.78±0.18*	3.96±0.31	3.84±0.02*	3.94±0.03*
S8 20 mg	2.99±0.04*	3.79±0.08*	4.27±0.02*	3.49±0.02*	4.72±0.03*	4.5±0.06*
S8 40 mg	2.81±0.29	3.58±0.01	3.94±0.08*	4.00±0.35	4.27±0.25	4.26±0.02*
S9 20 mg	3.01±0.05*	3.88±0.06*	4.31±0.22	3.85±0.02*	4.52±0.19	4.80±0.09
S9 40 mg	2.67±0.02*	3.49±0.15*	3.76±0.19*	4.39±0.31	4.22±0.21	4.06±0.01*

Data are expressed as mean± standard deviation (n=2), *p<0.05, when compared to control (base line value).

Amomum subulatum seed shows 20% GDRI at 20mg/ml and the GDRI spontaneously upsurge and reached 50% as concentration is doubled to 40mg/ml. *Musa species* leaves showed almost 25% glucose diffusion inhibition at both concentrations. *Nephrolepis. cordifolia* fruit and leaves showed better glucose diffusion inhibition at lower concentration as compared to the increase in concentration. *Musa species* leaves vein revealed negative GDRI result at 40 mg/ml concentration which may be due to the carbohydrate content within the plant sample.

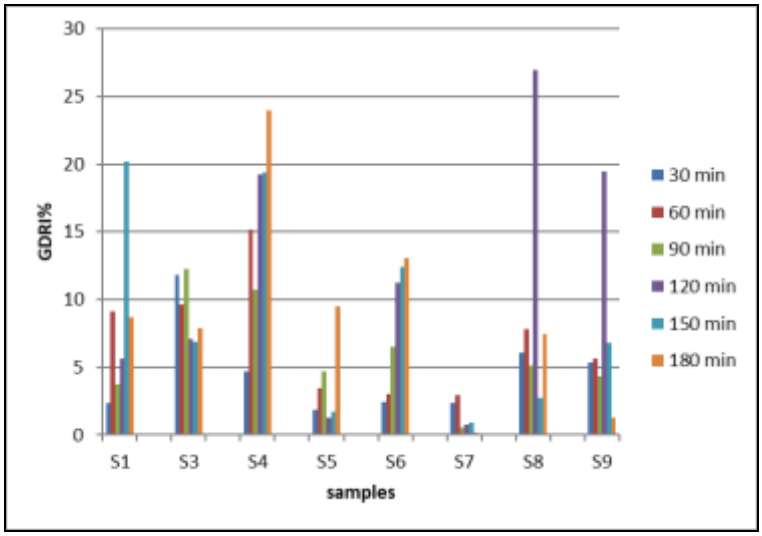

Fig 6: GDRI% of Water Extracts at 20 mg/ml

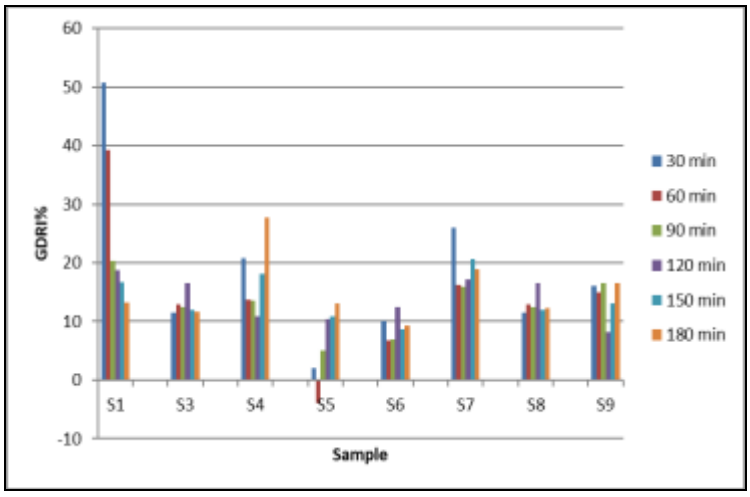

Fig 7: GDRI% of Water Extracts at 40 mg/ml

4. Discussion

The basis behind the selection of these five different medicinal plants in this study was the traditional and ethnomedicinal consumption of those natural plant samples by the indigenous people of different part of Nepal for lowering blood glucose level. From the result of phytochemical screening, it was revealed that glycosides, saponins, flavonoids, tannins and terpenoids are present in higher amount in both ethyl acetate and water extracts which reveals that these extracts show desirable pharmacological activities.

Phenolic compound and flavonoids have been reported to be associated with anti-oxidative action on living organisms, as it acts as scavengers of singlet oxygen and free radicals [31]. Tanins are also effective antioxidant and show strong anti-cancer activity [32]. Positive result of phenols, flavonoids, and tannins in the ethyl acetate extracts of *Choerspondias axillaries* and *Myrica esculenta* stem bark and small branches can be correlated with potent DPPH scavenging activity having lower IC_{50} value. Water extracts of *Myrica esculenta* stem bark and small branches showed very lower IC_{50} value which is comparable to that of standard ascorbic acid. It can be correlated with high presence of phenols, flavonoids and tannins in *Myrica esculenta* plant extracts [33]. According to Rawat *et al*., 2011, phenolic compound showing antioxidant properties in *Myrica esculenta* may be presence of gallic acid, which is efficiently absorbed in human body, chlorogenic acid, present in *Myrica esculenta* fruit, catechin and P-coumaric acid which are effective in preventing oxidative injuries in human epithelial cells under in vitro [34]. The potent DPPH scavenging activity of *Choerspondias axillaries* on all solvent may be due to the presence of Vitamin C [35]. From our study we can say that *Myrica esculenta* stem bark and small branches, *Nephrolepis cordifolia* fruit and leaves, *Choerspondia. axillaries* as well as *Musa species* are effective as free radical scavengers.

The inhibition of digestive enzymes such as alpha-amylase and alpha-glucosidase has been considered to be effective strategy to control blood glucose [36]. Uddin *et al*., 2014, mentioned that the presence of saponins, steroids and terpenoids are responsible for the therapeutic activity showing alpha-amylase inhibition. Natural polyphenols have been reported to inhibit the activity of carbohydrates hydrolyzing enzyme like alpha-amylase, alpha-glucosidase. Oleanane, ursane, lupane group of terpenoids represents promising source for biologically active natural compounds which have alpha-amylase inhibition activity. Lupeol, a terpenoids compound has been isolated which is potent alpha-amylase inhibitors [35]. According to Picot *et al*., 2013, plant extracts even decreases the Km (the affinity of the enzyme for the substrate) and Vm (velocity of reaction). This tends to suggest the uncompetitive mode of inhibition [36]. *Amomum subulatum* seed, *Choerspondiasaxillaries* fruit, *Musa species* and *Nephrolepis cordifolia* fruit and leaves showed potent alpha-amylase inhibition with IC_{50} value of low concentration which is comparable to the IC_{50} value of Voglibose. As these extracts showed positive phenols, flavonoids, terpenoids and saponins test, the potent alpha-amylase inhibition may be due to the presence of these phytoconstituents [36]. In particular flavonoids, epicatechin, epigallocatechin gallate is considered as promising hypoglycemic agents [37]. These phytochemicals inhibit hydrolysis step of starch by alpha-amylase therefore lower blood glucose level [38]. From various studies it is revealed that, compounds showing free radical scavenging activity even show alpha amylase inhibition due to the presence of phenols, flavonoids, and tannins. Gallic acid and hydroxycinnamic acids are the phenolic acid found commonly in plants which showed both antioxidant and alpha-amylase inhibition activity [39]. *Myrica* stem bark, *Choerspondias axillaris* fruit and *Musa species.* leaves showed better alpha-amylase inhibition reducing absorption of starch into the body which could be due to potent antioxidant properties.

Meanwhile, the glucose entrapment ability in vitro of selected medicinal plants was investigated. The retardation in glucose diffusion *in vivo* might be attributed to the physical obstacles, insoluble fiber particle which entrap glucose molecule within fiber network preventing postprandial glucose rise [40]. GDRI is a useful in vitro index to predict the effect of a fiber on the delay in glucose absorption in the intestinal tract. A higher GDRI indicates higher retardation index of glucose by the sample [41]. *Myrica esculenta*, *Amomum subulatum* seed, *Choerspondias axillaries* fruit and *Musa species* leaves showed higher GDRI showing potent action as glucose diffusion inhibitors. This result may be due to the presence of complex polysaccharides and fibers containing materials on plant extracts which might be attributed to physical obstacles. Insoluble fiber particles may have entrapped glucose molecule with the fiber network preventing surge of glucose diffusion to outer solution of membrane [42]. In the body there are various transporters which work in synchronization with other molecules to transport glucose. In the body this is typically achieved by sodium ions [43]. Similarly, as reported from other studies, the retardation of glucose diffusion *in vivo* is also due to the inhibition of alpha amylase, thus limiting the release of glucose from starch [44].

5. Conclusion

The plants may essentially contain bioactive compounds inhibiting enzyme activity and free radicals. Eventually, these studies have supported scientifically to the evidence of using the selected medicinal plants locally and traditionally to lower the blood glucose level. Finally, this study paved the marvelous path for further scientific validation, research, and investigation to understand the therapeutic potential of these medicinal plants for improving glycemic control in diabetic subjects and confirm their anti-diabetic mode of action.

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7. Conflict of Interest

The author declares no conflict of interest.

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