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IN VITRO ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTI-DIABETIC ACTIVITY OF PRUNUS UNDULATA BUCH. -HAM.EX D DON LEAVES

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ABSTRACT
The aim of this research work was to investigate the *in-vitro* antioxidant, Anti-inflammatory and anti-diabetic activity of methanol extracts of *Prunus undulata* Buch. -Ham.ex D Don leaves. Phytochemical screening of methanol extract of *Prunus undulata* Buch. -Ham.ex D Don were analysed by using standard methods. *In-vitro* Antioxidant activity of the methanol extracts of *Prunus undulata* Buch. -Ham.ex D Don leaves was characterized for DPPH (2, 2-diphenyl-1-picrylhydrazy), Reducing power, and hydrogen peroxide radical scavenging activity. *In-vitro* Anti-inflammatory activity such as membrane stabilization and inhibition of protein denaturation analysis was performed in methanol extract. *In-vitro* Antidiabetic activity such as inhibition of alpha amylase enzyme were carried in methanol extract. The phytochemical screening of methanol extract of *Prunus undulata* contains the presence of carbohydrate, Flavonoids, phenol, saponin, steroids and Triterpenoids. The methanol extracts of *Prunus undulata* Buch. -Ham.ex D Don showed potential antioxidant activity in all the assays tested. I*n-vitro* Anti-inflammatory and Antidiabetic activity of *Prunus undulata* Buch. -Ham.ex D Don was also confirmed. The outcome of the present study concluded that the methanol extracts of *Prunus undulata* Buch. -Ham.ex D Don leaves possess significant antioxidant, anti-inflammatory and antidiabetic activity. The potential pharmacological activity of *Prunus undulata* Buch. -Ham.ex D Don leaves might be due to the presence of phytochemicals.

INTRODUCTION

Prunus undulata Buch. -Ham.ex D Don (Rosaceae) is a species of laurel cherry indigenous to south-east Asia, including Nepal, Sikkim, Bhutan, Bangladesh, north-eastern India, Myanmar, South-eastern China, Aceh in Indonesia, Laos, Thailand and Vietnam. A tree reaching 16 m promotes to grow. Although no specific mention has been seen for this species, all members of

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the genus contain amygdalin and prunasin, substances which break down within the water to make prussic acid (cyanide or prussic acid). The leaves are lopped for cattle fodder and located at Mualpheng, Sawleng, dampui, etc. wood reddish-brown [1]. The leaves of *Prunus undulata* Buch. -Ham.ex D Don are boiled in ½ l of water and also the water is filtered out and brought internally for the treatment of heart problems. It's also claimed by some to be of interest within the treatment of cancer. Medicinal plant contain high amount of anti-oxidant plays a vital role in protecting and safeguarding ill health especially within the diseases like cancer. Free radicals are generated by an exogenous and endogenous metabolic within the body causing oxidizing which result in damage or death of cells and tissues [2].

Inflammation is that the response of the body against injury or infection may occurs thanks to pain, swelling, redness, heat, etc. the aim is to safeguard the location of an injury. When inflammation occurred, the body gives response and fights the invading organisms to terminate them and set a stage for healing the injured tissues [3]. This happen due to the delivered of chemical mediators like kinins, prostaglandins and histamines from the injured tissues.

Antidiabetic agents comprise a chemically & pharmacologically heterogeneous group of medicine. The target in treating diabetes is to stop undue rises in blood sugar throughout each successive 24-hour period, without producing clinical hypoglycemia. It's now widely accepted that good control of blood sugar prevents the event of microvascular (retinopathy, nephropathy) and neuropathic long-term complications of the disease in both type 1. This study is to analyse In Vitro Antioxidant, Anti-Inflammatory and Anti-Diabetic Activity of *Prunus undulata* Buch. -Ham.ex D Don Leaves.

MATERIALS AND METHODS

Collection and Authentication of Plants Materials

The fresh leaves of *Prunus undulata* Buch. -Ham.ex D Don was collected on the month of February from Bungzung, Aizawl, Mizoram, India.

The plant was taxonomically identified and confirm by Dr. N. Odyuo, Scientist and Head of Office, Botanical Survey of India, Eastern Region Centre, Shillong. The identification number given was No.: BSI/ERC/Tech/2020/1189.

Preparation of the Plant Material

The fresh leaves gathered were completely cleaned with running water and shade dried for three weeks. Dried leaves were then grounded to fine uniform powder utilizing a mechanical grinder and stored in airtight containers for extraction.

Extraction of plant materials:

The dried plant material was then into powder form. The powder material has been extracted with distinctive solvents depending to its polarity i.e., petroleum ether, chloroform and methanol by soxhlet extraction method for 72 hrs. Solvent elimination under reduced pressure afforded the petroleum ether, chloroform and methanol extract which methanol extract was encourage utilized for in-vitro antioxidant, anti-inflammatory and anti-diabetic activities.

Phytochemical Screening:

The extract of *Prunus undulata* Buch. -Ham.ex D Don leaves were screened for the nearness of phytochemical constituents such as reducing sugar, alkaloids, phenols, tannins, flavonoids and steroids following standard procedures [4].

Evaluation of *In Vitro* Antioxidant Activity:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The free radical scavenging activity of methanol extract and standard Ascorbic Acid were utilizing by using DPPH, which may be a free radical [5]. 3 ml of methanol extract at different concentrations (20,40,60,80,100 µg/ml) were taken in a test tubes and 0.5 ml of 0.1 mM DPPH solution was added to each. It was incubated in the dark for 30 minutes at 37ºC. The absorbance was measured at 517 nm. The IC50 value was calculated. The percentage of DPPH scavenging effect was estimated by using equation

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ is Absorbance of control and A_{sample} is Absorbance of sample.

Hydrogen Peroxide Scavenging Activity

The capacity of methanol extract to scavenge hydrogen peroxide was utilizing by using ascorbic acid as a standard. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 3.4 ml of extract that was dissolved in methanol was

added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide was measured at 230 nm 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide [6].

The percentage of hydrogen peroxide scavenging by the extract and standard (Ascorbic acid) was calculated using the following equation:

$$Scavenged\ H_2O_2\ (\%) = \frac{A_c - A_s}{A_c} \times 100$$

Reducing Power Assay

The reducing power estimation was determined by utilizing the methanol extract. The extract was diluted at different concentrations (20, 40, 60, 80, 100 µg/ml). 1 ml of each dilution was mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50º C for 30 minutes. After cooling, 2.5 ml of 10% TCA was included and centrifuge for 10 minutes at 3000 rpm. 2.5 ml of the supernatant was dilute with 2.5 ml of methanol, to it 0.5 ml of freshly prepared 0.1% ferric chloride solution was added and mixed. The absorbance of the mixture was measured at 700 nm. Ascorbic acid was used as a standard. The higher absorbance indicates increase in the reducing power [7].

***In Vito* Anti-Inflammatory Activity Study of Methanol Extract**

Bovine Serum Albumin (BSA) Denaturation

Test solution (0.5ml) comprises of 0.45ml of BSA (5% w/v aqueous solution) and 0.05ml of test solution (100, 200, 300, 400, 500µg/ml). The control solution (0.5ml) comprises of 0.45ml of BSA (5% w/v aqueous solution) and 0.05ml of Phosphate buffer solution. Standard solution (0.5ml) comprises of 0.45ml of BSA (5% w/v aqueous solution) and 0.05ml of Diclofenac sodium (100, 200, 300, 400, 500µg/ml). All the above solution was balanced to pH 6.3 using 1N Hydrochloric acid. The test samples were incubated at 37ºC for 20 minutes and the temperature was increased to keep the samples at 57ºC for 3 minutes. After cooling, 2.5ml of phosphate buffer saline was added to each solution. The turbidity was measured utilizing UV visible Spectrophotometer (Thermo Fisher Scientific 201) at 660 nm [8].

The percentage inhibition of protein denaturation was calculated by,

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ is Absorbance of control and A_{sample} is Absorbance of sample.

HRBC Membrane Stabilization Method

HRBC method was utilized for the estimation of anti-inflammatory activity in in-vitro. Blood was collected from the healthy volunteer and was mixed with equal volume of sterilized alsevers solution. This blood was centrifuged at 3000 rpm and the packed cell was separated. The packed cell was washed with isosaline solution and a 10% v/v solution was made with isosaline. This HRBC suspension was utilized for the estimation of anti-inflammatory activity. Distinctive conc. of extract (100, 200, 300, 400, 500µg/ml), referenced standard and control were independently mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. All the assay mixtures were incubated at 37ºC for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage haemolysis was evaluated by assuming the haemolysis produced in the control at 100% [9].

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ is Absorbance of control and A_{sample} is Absorbance of sample.

***In Vito* Anti-Diabetic Activity Study of Methanolic Extract**

Inhibition of Alpha Amylase Enzyme

Add up to 500µl of test samples and standard drug metformin (100-500µg/ml) were included to 500µl of containing alpha amylase (0.5mg/ml) solution and were incubating at 25ºC for 10 minutes. After these, 500µl of a 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was included to each test tube and were incubate at 25ºC for 10 minutes. The reaction was stopped with ml of 3, 5-dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes, brought to room temperature. The reaction mixture was then diluted after including 10ml distilled water and absorbance was measured at 540nm [10].

The percentage inhibition of alpha amylase was calculated by,

$$\%\ inhibition = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

Statistical Analysis

All values were appeared as Mean ± SEM. Statistical analysis was performed using a one-way analysis of variance (ANOVA) taken after by Dunnett's t-test (compare all. vs control). A P-value of less than 0.05 was considered statistically significant $*P<0.05$ and $**P<0.01$ when compared with control. All analysis was made with the statistical software Graphpad In.

RESULTS AND DISCUSSION

Extractive Value

721.2 gm of powdered materials of the leaves of *Prunus undulata* Buch. -Ham.ex D Don was extracted successively in a soxhlet apparatus with the solvent's petroleum ether, chloroform and methanol separately (table 1). The extraction was carried out exhaustively and the solvents were recovered by Rotary evaporator. The concentrated extracts were kept in refrigerator at 4ºC for further use.

Table 1: Extraction yield for different solvents.

S. No	Individual solvents	Wt. of drug (gm)	% yield (w/w)
1	Petroleum ether	721.2	0.91 %
2	Chloroform	721.2	1.28 %
3	Methanol	721.2	9.95 %

Phytochemical Screening

The results for Preliminary phytochemical screening of different solvents extracts of leaf part of the investigated plant showed presence of different phytochemical groups (Table 2).

Table 2: Results for Preliminary phytochemical screening for different solvents.

Extract constituents	Petroleum ether	Chloroform	Methanol
Alkaloids	-	-	-
Carbohydrates	+	+	-
Reducing sugar	-	-	-
Flavonoids	-	-	+
Tannins	-	-	+
Saponins	+	+	+
Steroids and triterpenoids	+	+	+
Protein	-	-	-
Glycoside	+	+	+

(-) indicates absent, (+) indicates present

Evaluation of *In Vitro* Antioxidant Activity:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

The radical scavenging activity of the extract was estimated by DPPH method. DPPH radical has been usually used to analyze the scavenging activities of natural compounds. When reaction take place, the color changed from purple to yellow since it was scavenged due to the donation of hydrogen, forming a non-radical DPPH. The percent inhibitions on DPPH radical of the extract were compared with the standard Ascorbic Acid. The study was performed in triplicate, methanol extract showed the activities with increasing concentrations of the extract. IC_{50} values were calculated for standard as well as for extracts. The mean IC_{50} of *Prunus undulata* Buch. -Ham.ex D Don *was found to be* 98.32±0.85 µg/ml as compared to standard (Ascorbic acid) which indicated 4.37±0.3 µg /ml as shown in **table 3** and **fig 1.** The result indicates that *Prunus undulata* Buch. -Ham.ex D Don exhibit antiradical activity, however, the standard Ascorbic acid showed significantly higher DPPH activity than the sample *Prunus undulata* Buch. -Ham.ex D Don.

Table 3: % Inhibition of DPPH radical scavenged rates of methanol extract of *Prunus undulata* Buch. -Ham.ex D Don and standard Ascorbic acid.

Concentration (µg/ml)	% Inhibition	
	Extract Mean ± SEM	Ascorbic acid Mean ± SEM
20	15.96±1.73**	54.51±0.34**
40	19.79±0.6**	60.41±0.60**
60	37.15±0.35**	61.45±0.60**
80	39.23±0.34**	62.74±0.40**
100	51.38±1.25**	74.30±0.30**

All value is Mean ± SEM, n=3. One-way Analysis of Variance (ANOVA) taken after by Dunnett's test was performed as the significance. The minimum value of $*p<0.05$ considered significant, $**p<0.01$, $***p<0.001$ as compared with control group.

H_2O_2 was considered poorly active because of its weak oxidizing and reducing capability. Biologically, it reacts as a toxicant to the cell by changing itself into hydroxyl radical in bearing of metal ions and superoxide anion and also produces singlet oxygen through reaction with superoxide anion or with hypochlorous acid (HOCl) or chloramines in living systems. Hydrogen peroxide can degrade certain haeme proteins, such as

haemoglobin, to release Fe ions and therefore the hydrogen radical scavenging activity was calculated [2]. Scavenging activity of the extract and ascorbic acid as reference compound against hydrogen peroxide in terms of effective concentration were shown in figure 2. IC_{50} values were calculated for standard as well as for extracts. The mean IC_{50} values for H_2O_2 radicals by methanol extract of *Prunus undulata* Buch. -Ham.ex D Don and ascorbic acid was found to be 58.41 µg/ml and 36.54 µg/ml respectively. The results for H_2O_2 radical scavenging activity were given below (Table 4 and fig. 2).

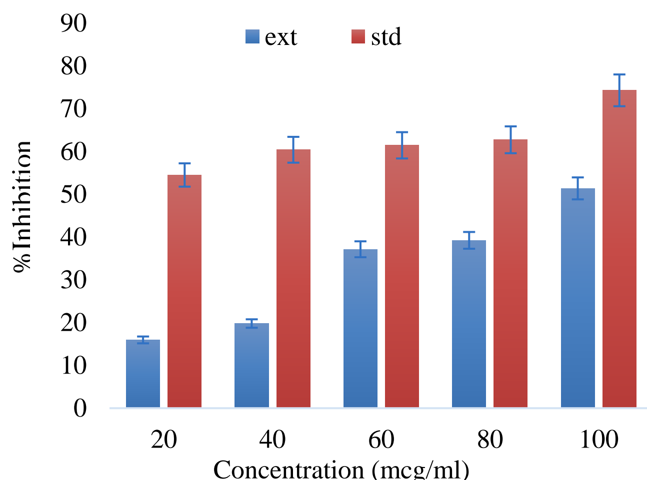

Fig no 1: DPPH radical scavenging activity of the methanol extract of *Prunus undulata* Buch. -Ham.ex D Don extract

Determination of hydrogen peroxide radical scavenging activity

Table 4: Hydrogen peroxide scavenged rates of methanol extract of *Prunus undulata* Buch. -Ham.ex D Don and standard Ascorbic acid.

Concentration (µg/ml)	% Inhibition	
	Extract Mean ± SEM	Ascorbic acid Mean ± SEM
20	12.956±0.042**	36.652±0.0318**
40	39.093±0.031**	55.991±0.0319**
60	49.513±0.111**	65.916±0.047**
80	70.481±3.247**	74.852±0.042**
100	80.548±0.016**	81.267±0.073**

All value is Mean ± SEM, n=3. One-way Analysis of Variance (ANOVA) taken after by Dunnett's test was performed as the significance. The minimum value of *p<0.05 considered significant, **p<0.01, ***p<0.001 as compared with control group.

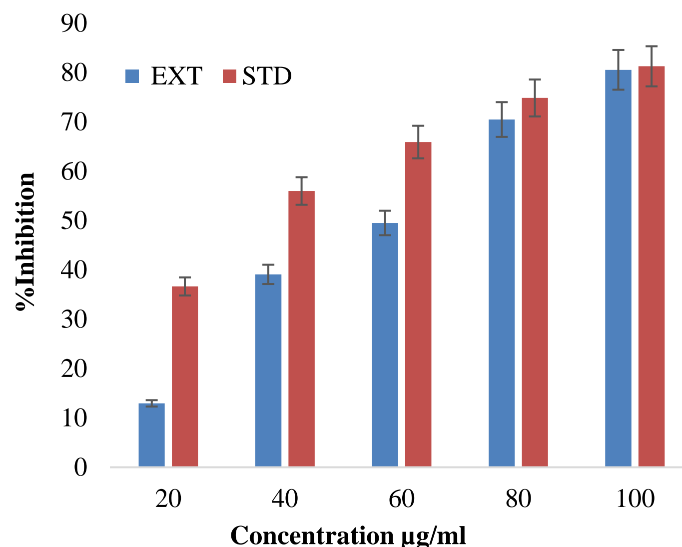

Fig 2: Hydrogen peroxide radical scavenging activity of *Prunus undulata* Buch. -Ham.ex D Don extract.

Reducing Power Assay

The ability of extract to reduce Fe^{3+} to Fe^{2+} (reducing effect) was determined according to the method descried by [7]. The reduce capacity of a compound may perform as a significant indicator of its potential antioxidant activity [11]. Compounds with reducing power recommended that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so, that they can take action as primary and secondary antioxidants. The absorbance kept increasing with increased in concentration which showed that the extract has a good reducing potential and electron ability for stabilizing free radical. Reducing power of the extract *Prunus undulata* Buch. -Ham.ex D Don was significantly higher compared to the standard ascorbic acid (Table 5 and fig. 3). However, the antioxidant presents in the sample caused the reduction of Fe^{3+} to Fe^{2+} and thus proving its reducing power.

Table 5: **Rate of reducing power between concentration and absorbance.**

Concentration (µg/ml)	Absorbance	
	Extract Mean ± SEM	Ascorbic acid Mean ± SEM
20	0.256 ± 0.0003	0.026 ± 0.003
40	0.340 ± 0.0003	0.187 ±0.0003
60	0.441 ± 0.0003	0.255 ± 0.0025
80	0.650 ± 0.0003	0.431 ± 0.00057
100	0.756 ± 0.0011	0.957 ± 0.0057

All value is represented as Mean ± SEM, n=3

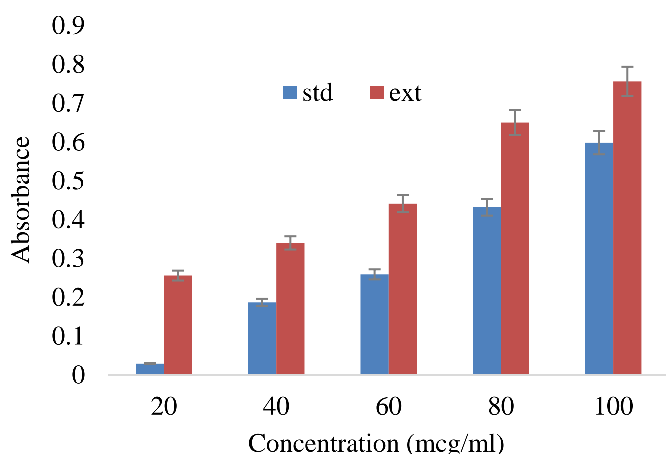

Fig. no 3: Reducing power activity of *Prunus undulata* Buch. -Ham.ex D Don extract

In Vito Anti-Inflammatory Activity Study of Methanol Extract

5.7.1. Bovine Serum Albumin (BSA) Denaturation

Protein denaturation implies loses of biological properties of protein molecules. Denaturation of proteins is primarily capable for the cause of inflammation is conditions like diabetes, rheumatoid arthritis, cancer etc. Hence, by prevention of protein denaturation also help in preventing inflammatory conditions. The denaturation inhibition capability of the methanol extract of *Prunus undulata* Buch. -Ham.ex D Don was found that the concentration increases the absorbance decreases so we can say that it is a dose dependent manner (Table 6 and fig. 4). IC_{50} values were calculated for standard as well as for extracts. The IC_{50} values of both diclofenac sodium standard and the methanol extract of *Prunus undulata* Buch. -Ham.ex D Don was found to be 289.65 µg/ml and 139.60 µg/ml individually.

Table 6: Percent inhibition of bovine serum albumin denaturation of Diclofenac sodium and *Prunus undulata* Buch. -Ham.ex D Don extract.

Concentration (µg/ml)	% Inhibition	
	Extract Mean ± SEM	Diclofenac Sodium Mean ± SEM
100	44.736 ± 0.387**	35.964 ± 1.343**
200	57.017 ± 0.380**	41.228 ± 1.025**
300	66.666 ± 1.025**	49.122 ± 0.775**
400	72.807 ± 0.671**	57.894 ± 0.771**
500	83.333 ± 1.021**	70.175 ± 0.387**

All value is Mean ± SEM, n=3. One-way Analysis of Variance (ANOVA) taken after by Dunnett's test was performed as the significance. The minimum value of $*p<0.05$ considered significant, $**p<0.01$, $***p<0.001$ as compared with control group.

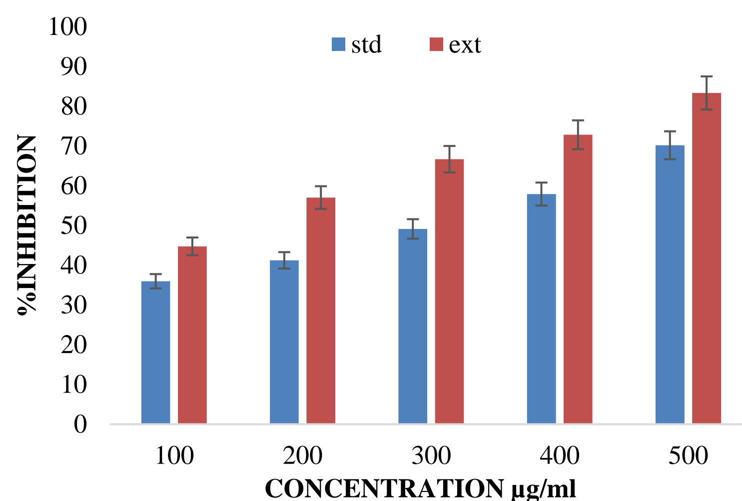

Fig 4: Percent inhibition of BSA denaturation of Diclofenac sodium and *Prunus undulata* Buch. -Ham.ex D Don.

5.7.2 HRBC membrane stabilization method

Methanol extract of *Prunus undulata* Buch. -Ham.ex D Don was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is comparable to the lysosomal membrane and its stabilization shows that the extract may moreover well stabilize lysosomal membranes membrane is shown in table 7 and figure 5. It possesses significant activity comparable with that of the standard diclofenac sodium [12]. IC_{50} values were calculated for standard as well as for extracts. The IC_{50} values of Diclofenac sodium and the extract of *Prunus undulata* Buch. -Ham.ex D Don were found to be 70.194 µg/ml and 300.17 µg/ml individually.

Table 7: Effect of *Prunus undulata* Buch. -Ham.ex D Don and standard diclofenac sodium on HRBC membrane stabilization.

Concentration (µg/ml)	% Inhibition	
	Extract Mean ± SEM	Diclofenac Sodium Mean ± SEM
100	34.523 ± 0.271**	24.404 ± 0.275**
200	44.642 ± 0.472**	30.357 ± 0.477**
300	51.19 ± 0.275**	37.5 ± 0.47**
400	55.357 ± 0.47**	52.38 ± 0.27**
500	64.285 ± 0.477**	57.738 ± 0.275**

All value is Mean ± SEM, n=3. One-way Analysis of Variance (ANOVA) taken after by Dunnett's test was performed as the

significance. The minimum value of *$p<0.05$ considered significant, **$p<0.01$, ***$p<0.001$ as compared with control group.

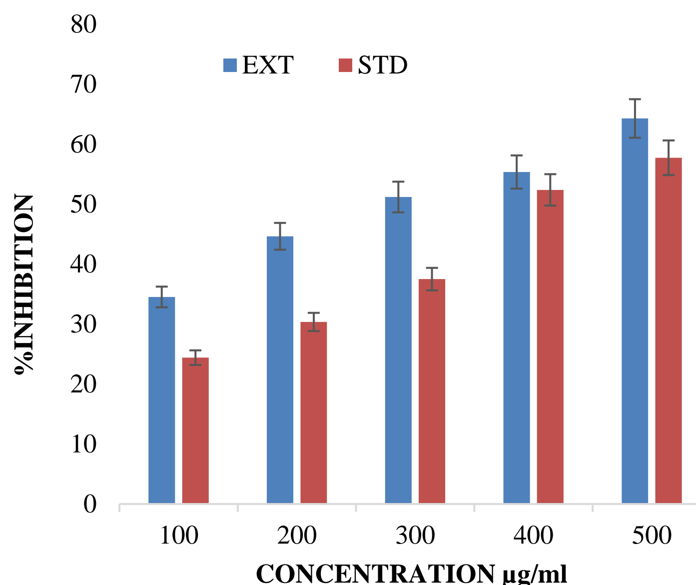

Fig 5: Effect of methanol extract of *Prunus undulata* Buch. -Ham.ex D Don on HRBC membrane stabilization.

5.8. In Vito Anti-Diabetic Activity Study of Methanolic Extract

5.8.1. Inhibition of alpha amylase enzyme:

α-Amylase is one of the main enzymes in human body that is mindful for the breakdown of starch to more simple sugars. α-Amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glycosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels. Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes [13]. *Prunus undulata* Buch. -Ham.ex D Don showed potential inhibitory effects on these enzymes. Thus, a Metformin act as a standard. The methanol extract of *Prunus undulata* Buch. -Ham.ex D Don showed a significant activity (Table 8 and fig. 6). IC_{50} values were calculated for standard as well as for extracts. The IC_{50} values of Metformin standard and the extract of *Prunus undulata* Buch. -Ham.ex D Don were found to be 308.18 µg/ml and 152.40 µg/ml individually.

Table 8: Percentage inhibition of alpha amylase enzyme of standard drug Metformin and *Prunus undulata* Buch. -Ham.ex D Don extract.

Concentration (µg/ml)	% Inhibition	
	Extract Mean ± SEM	Metformin Mean ± SEM
100	41.666 ± 0.646**	11.111 ± 0.371**
200	55.555 ± 0.648**	47.222 ± 0.646**
300	70.37 ± 0.749**	53.703 ± 0.374**
400	76.851 ± 0.374**	60.185 ± 0.377**
500	84.259 ± 0.990**	72.222 ± 0.648**

All value is Mean ± SEM, n=3. One-way Analysis of Variance (ANOVA) taken after by Dunnett's test was performed as the significance. The minimum value of *$p<0.05$ considered significant, **$p<0.01$, ***$p<0.001$ as compared with control group.

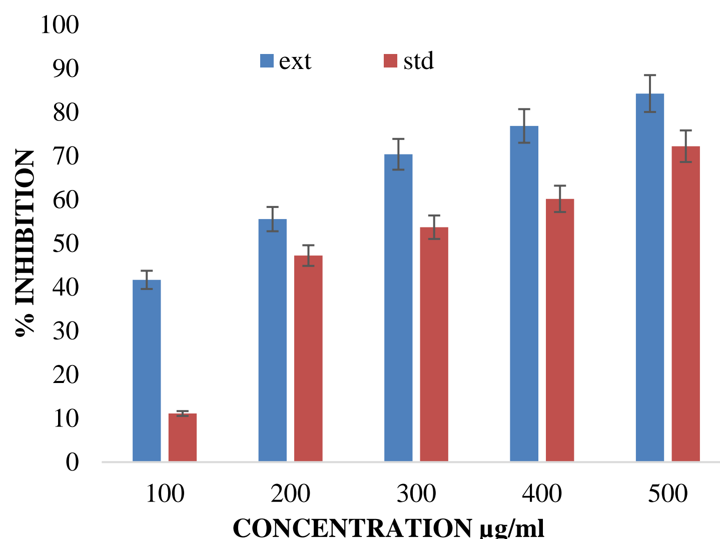

Fig 6: Percentage inhibition of alpha amylase enzyme of standard drug Metformin and *Prunus undulata* Buch. -Ham.ex D Don extracts.

CONCLUSION

Prunus undulata Buch. -Ham.ex D.Don (Rosaceae) is a species of laurel cherry indegenous to south-east Asia, including Nepal, Bhutan, Sikkim, Bangladesh, north-eastern India, Myanmar, South-eastern China, and Aceh in Indonesia, Laos, Thailand and Vietnam. A tree reaching 16 m preomotes to grow. The leaves of *Prunus undulata* Buch. -Ham.ex D.Don are boiled in ½ l of water and the water is filtered out and taken internally for the treatment of heart problems. It is also claimed by some to be of interest in the treatment of cancer.

Extraction of the crude plant leaf was obtained by successive extraction process using petroleum ether, chloroform and methanol and the extractive yield were found to be 0.91%, 1.28% and 9.95% respectively. In this study, the methanolic leaf extract of *Prunus undulata* Buch. -Ham.ex D.Don was subjected to phytochemical screening and *in vitro* studies.

Results for the antioxidant activity studies conclude that the methanol extract of the leaf of *Prunus undulata* Buch. -Ham.ex D.Don possess an antioxidant activity. It also revealed that the plant possesses a significant antioxidant activity as there is presence of flavonoids and phenols. This recommend that the plant can be viewed as a potential source of natural antioxidants which can provide precious functional ingredients useful for prevention of diseases related to oxidative stress.

The results for anti-inflammatory activity studies indicated that the methanol extract of the leaf of *Prunus undulata* Buch. -Ham.ex D.Don possess an anti-inflammatory. It also revealed that the plant possesses certain percentage of inhibition of inflammation as there is presence of flavonoids when compared to the standard drug diclofenac sodium.

From the results, it can be concluding that the use of this plant extract of *Prunus undulata* Buch. -Ham.ex D.Don exhibit remarkable α-amylase inhibitory activity in the crude methanol extract.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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