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ANTIDIABETIC PROPERTIES OF VARIOUS EXTRACTS OF *HIPPOPHAE RHAMNOIDE*

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Abstract:

The various extracts of Hippophae rhamnoide plant have been scrutinized for the anti-diabetic property by the use of alpha-amylase and alpha glycosidase enzymes inhibition method. The various parts of the plant have been extracted by Soxhlet extraction method, in which various solvents have been used in the order of their increasing polarity like Petroleum ether, Ethanol and Water. Acarbose have been used as a reference antidiabetic medicine by the inhibition of alpha-amylase and alpha glycosidase enzyme, the two key enzymes responsible for the degradation of higher carbohydrates into lower glucose molecules. Three different plant parts have been analysed separately viz, root, leaves and fruit. The fruit extracts of the plant have been found to posse's highest inhibition potential followed by leaf extracts and least inhibition of alpha-amylase and alpha-glycosidase have been noticed for all root extracts. As per the solvent polarity, the highest inhibition of alpha-amylase and alpha glycosidase have been noticed for water extracts of the root, leaf and fruit extracts of the concerned plant, followed by ethanol extracts, and less inhibition of alpha-amylase and alpha glycosidase have been observed for petroleum ether extracts.

Keywords: *Hippophae rhamnoide, alpha-amylase, alpha glycosidase, antidiabetic, plant extracts*

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INTRODUCTION:

Medicinal plants have been used for centuries as remedies for various types of diseases, which visit human life from time to time, because they contain components of curative value. Near about 80% of world's population depends on the use of traditional medicines which is predominantly based on plant material. The scientific studies available on a good number of medicinal plants indicate that the phytochemicals can be developed against the human health related problems including cancer, diabetes and infectious diseases. Sea Buckthorn (SBT) is a deciduous and a spiny shrub and belongs to a genus *Hippophae* and family Elaeagnaceae which possess 3 to 15 feet height and some SBT (*Hippophae rhamnoides*) in China have reached 18 m (59 feet), while others have higher than 50 cm (20 inches).

The plant in reference is considered as a rich source of a large number of metabolites like flavonoids, carotenoids, amino acids, tannin acid, antioxidant vitamins and minerals (1, 2, 3). Hippophae grow in five states in India; 3 in the North-West (Lahaul-Spiti districts of Himachal Pradesh, Uttaranchal and river belts of Indus, Nubra, Shyok, Zanskar etc. of ladakh), 2 in the North East (Sikkim and Arunachal Pradesh) Himalaya (4) . The classification of genus Hippophae is still unclear although it has been classified into seven major species; H. tibetana, H. salicifolia, H. rhamnoides, H. neurocarpa, H. litangensis, H. gyantsensis and H. goniocarpa. In India, H. rhamnoides, H. salicifolia and H. tibetana have been described. Of which H. rhamnoides L. ssp. Turkestanica are the major one (5). The plant is used to strengthen the spleen and the stomach, and to promote blood circulation and to remove blood stasis.

EXPERIMENTAL:

The *Hippophae rhamnoides* plant was collected from the Ladakh Region of Jammu and Kashmir. The plant was shade dried and powdered in to mixture.

Extraction

Separately plant root, stem and leaf powder were weighed and then extracted in a Soxhlet Apparatus using thimble in the extraction. Petroleum ether, Ethanol and Water have been used as extraction solvents.

Extraction A: The sample was extracted first with (petroleum ether) in a Soxhlet apparatus for a required period. After the Extraction with Petroleum ether, the extract solution was subjected to filtration, the residue was further extracted with another solvent. The filtrate was collected and evaporated to remove the volatile solvent to its 1/4th volume on water bath at a suitable temperature. The filtrate was then made in solid form (powdered) after being kept in an oven at 40-60^{0}C. The residue was collected, and subjected to further extraction process.

Extraction B: The residue was then extracted with Methanol in a same manner as mentioned above, in extraction A.

Extraction C: The residue from extract B was subjected to Water extraction by decoction technique in which the extract was dissolved in 500 ml of water. The whole mixture was heated over water to 1/5th of its original volume. The further 500 ml of water was added to the extract, the extracted solution was further evaporated to remove nearly 300 ml of water. The obtained solution was subjected to filtration and then the filtrate was evaporated to 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30- 50^{0}C.

In-vitro inhibition of extracts by alpha amylase, alpha glycosidase enzymes

Inhibition of alpha amylase Enzyme

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by **Laila A. Shekib, Samir M. El-Iraqui, Taisser M Abo-Bakr (6).** In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), different concentrations of (Acarbose std drug /Plant extracts), 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer. The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator (635mg Iodine and 1gm potassium iodide in 250 ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. % inhibition was calculated and all the tests were performed in triplicate.

Inhibition of alpha-glucosidase Enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm.

Krishnaveni, S., B. Theymoli, and Sadasivam, S. (7)

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (% I) was calculated by

% I = (Ac-As)/Ac X 100 [Shai, L. J., P. Masoko, M. Eloff, J.N. (8)

Where ***Ac*** is the absorbance of the control and ***As*** is the absorbance of the sample.

Observations and Results

Hippophae rhamnoides plant has been analyzed for the antidiabetic potential determination by the inhibition of alpha-amylase and alpha-glycosidase enzymes. All the plant extracts (fruit, root and leaf) have been analyzed and are respectively shown in the figures (1-20). The concentration range was made between (0.5-2.5 µg/ml).

Antidiabetic Property of *Hippophae rhamnoides* plant extracts by inhibition of Alpha-amylase)

The alpha-amylase inhibition by various plant extracts of the concerned have been analyzed, and it was found that **(water extract of fruit)** posses the highest inhibition potential followed by **(ethanolic extract of root).** The percentage inhibition was found to increases with the increase in the concentration of the plant extracts. The **IC_{50}** value was determined from the straight line graph. The **IC_{50}** value of reference compound **(ACAROSE)** was found lesser than the other plant extracts. The **IC_{50}** value of various plant extracts follows the order **(fruit water, root ethanol, root petroleum ether, root water, fruit ethanol, leaf ethanol, fruit petroleum ether, leaf water, leaf petroleum ether)** was found to be **(32.95, 33.14, 33.30, 33.34, 33.45, 33.85, 34.95, 35.15, 35.65)** respectively and are presented in **(Tables 2-10).**

Table 1: Report of % inhibition of (alpha-amylase) by ACAROSE Standard for in vitro antidiabetic studies).

Conc. of (µg/ml)	% Inhibition	IC_{50}
0.5	35.12	**32.55**
1.0	56.22	
1.5	74.23	
2.0	87.22	
2.5	93.97	

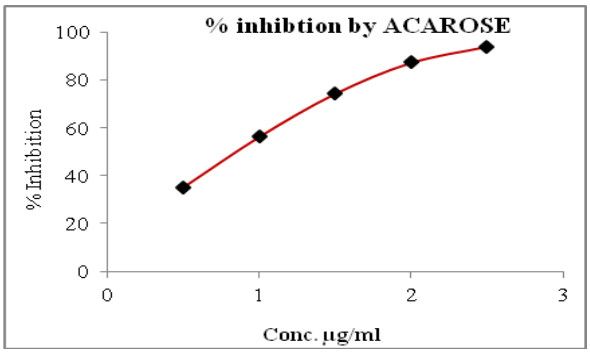


Fig 1: Represents the (% Inhibition of alpha amylase enzyme) by ACAROSE

Table 2: Report of % inhibition of (alpha-amylase) by petroleum ether extract of leaf

Conc. µg/ml)	% Inhibition	IC_{50}
0.5	42.5	35.65
1	47.3	
1.5	48.26	
2	64.62	
2.5	77.11	

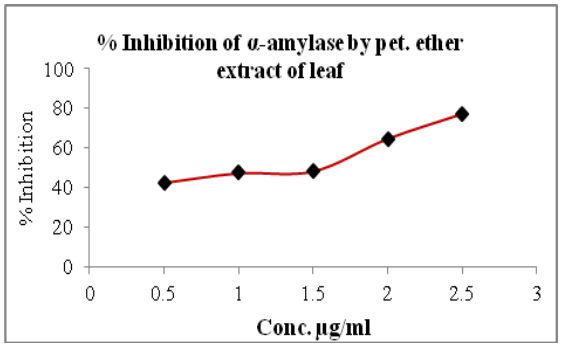


Fig 2: Represents the (% Inhibition of alpha amylase enzyme) by petroleum ether extract of leaf

Table 3: Report of % inhibition of (alpha-amylase) by Ethanolic extract of leaf

Conc. (µg/ml)	% Inhibition	IC_{50}
0.5	18.26	33.85
1	46.15	
1.5	57.69	
2	71.57	
2.5	87.3	

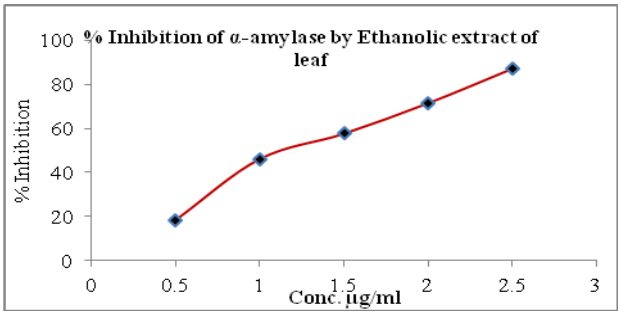


Fig 3: Represents the (% Inhibition of alpha amylase enzyme) by Ethanolic extract of leaf

Table 4: Report of % inhibition of (alpha-amylase) by water extract of leaf

Conc. (µg/ml)	% Inhibition	IC_{50}
0.5	10.57	**35.15**
1	27.89	
1.5	32.6	
2	43.26	
2.5	61.54	

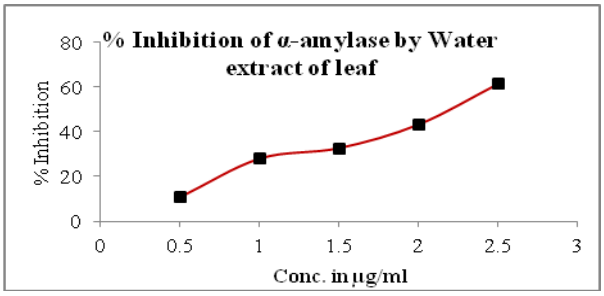


Fig 4: Represents the (% Inhibition of alpha amylase enzyme) water extract of leaf

Table 5: Report of % inhibition of (alpha-amylase) by petroleum ether extract of fruit

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	33.65	34.95
1	48.08	
1.5	50.10	
2	69.23	
2.5	96.15	

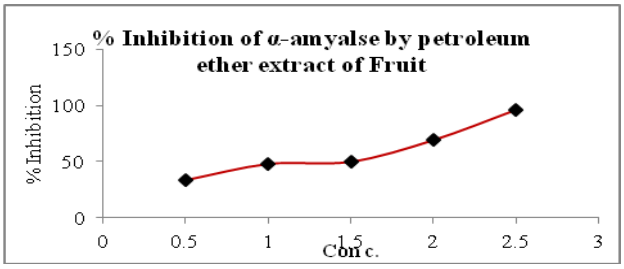


Fig 5: Represents the (% Inhibition of alpha amylase enzyme) petroleum ether extract of fruit

Table 6: Report of % inhibition of (alpha-amylase) by Ethanolic extract of fruit

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	**33.65**	**33.45**
1	**47.11**	
1.5	**78.85**	
2	**87.5**	
2.5	**88.46**	

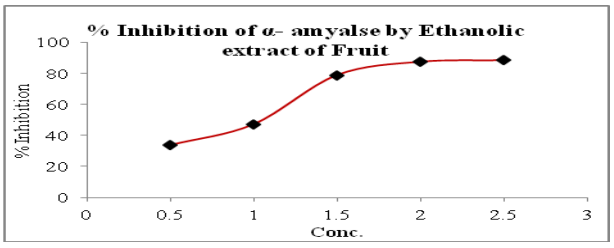


Fig 6: Represents the (% Inhibition of alpha amylase enzyme) ethanolic extract of fruit

Table 7: Report of % inhibition of (alpha-Amylase) by water extract of fruit

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	**50**	32.95
1	**62.5**	
1.5	**87.5**	
2	**90.3**	
2.5	**92.3**	

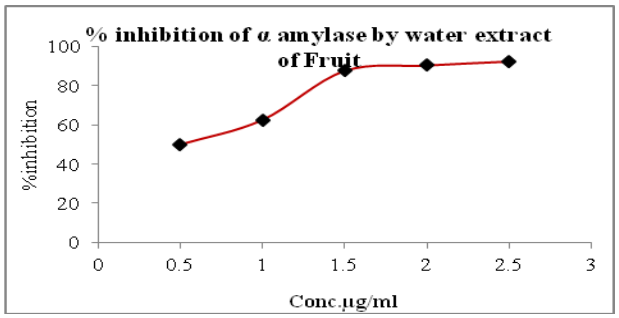


Fig 7: Represents the (% Inhibition of alpha amylase enzyme) by water extract of fruit

Table 8: Report of % inhibition of (alpha-amylase) by petroleum ether extract of root

Conc. µg/ml	% Inhibition	IC_{50}
0.5	48.6	33.3
1	50.56	
1.5	51.54	
2	52.5	
2.5	53.5	

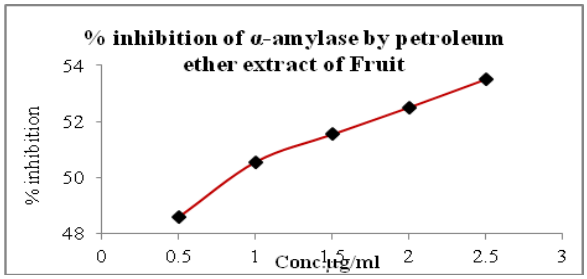


Fig 8: % Inhibition of alpha amylase enzyme) by petroleum ether extract of root

Table 9: Report of % inhibition of (alpha-amylase) by water extract of root

Conc. µg/ml	% Inhibition	IC_{50}
0.5	45.19	33.34
1	51.92	
1.5	59.61	
2	63.46	
2.5	65.38	

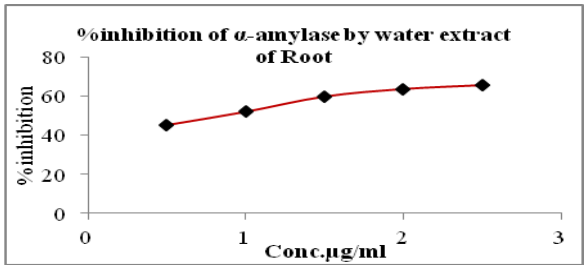


Fig 9: Represents the (% Inhibition of alpha amylase enzyme) by water extract of root

Table 10: Report of % inhibition of (alpha-amylase) by ethanolic extract of root

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	44.42	**33.14**
1	59.81	
1.5	66.54	
2	67.54	
2.5	68.46	

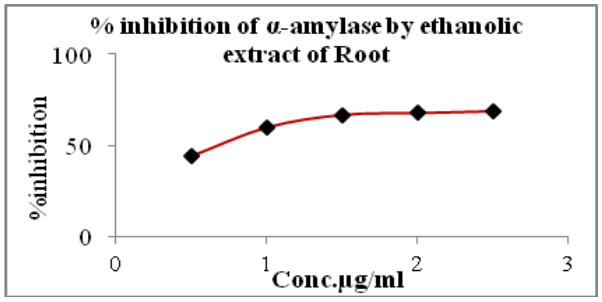


Fig 10: Represents the (% Inhibition of alpha amylase enzyme) by ethanolic extract of root

Antidiabetic Property of Various Extracts of *Hippophae rhamnoides* by inhibition of (Alpha-glycosidase)

The *Hippophae rhamnoides* plant extracts **(Fruit, Root, leaf)** have been analysed for the inhibition of (alpha glucosidase). The inhibition percentage was determined by spectrophotometric method, and **IC_{50}** value of all the plant extracts was determined. Among the all plant extracts the higher inhibition of alpha glucosidase was noticed for (water extract of fruit), for which the **IC_{50}** value was found to be **(36.21).** The various plant extracts follow the order as per their inhibition potential as **(water fruit, root ethanol, root water, leaf ethanol, fruit ethanol, leaf petroleum ether, fruit petroleum ether, root petroleum ether, leaf water**). The percentage inhibition was found to be concentration dependent as the value of percentage inhibition increases correspondingly with the increase in the concentration of plant. The **IC_{50}** values of all the plant extracts have been found to be more than the reference compound **(ACAROSE) (Table 11)** as per their percentage inhibition is taken into consideration. The **IC_{50}** value of various plant extracts is as **(36.21, 36.30, 36.50, 36.70, 36.82, 37.00, 37.20, 37.30, 37.60)** respectively for **(water fruit, root ethanol, root water, leaf ethanol, fruit ethanol, leaf petroleum ether, fruit petroleum ether, root petroleum ether, leaf water**).

Table 11: Report of % inhibition of (alpha glucosidase) by (Acarbose)

Conc. (µg/ml)	% Inhibition	IC_{50}
20	**31.7**	**35.5**
40	**53.9**	
60	**68.2**	
80	**76.5**	
100	**89.4**	

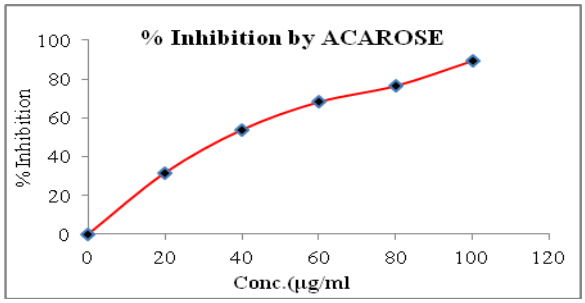


Fig 11: Represents the (% Inhibition of alpha glycosidase enzyme) by (ACAROSE)

Table 12: Report of % inhibition of (alpha glucosidase) by petroleum ether extract of fruit

Conc. (µg/ml	% Inhibition	IC50
0.5	45.11	37.2
1	46.25	
1.5	47.5	
2	55.12	
2.5	58.75	

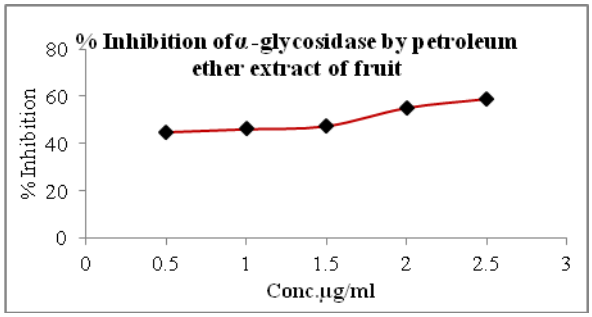


Fig12: Represents the (% Inhibition of alpha glycosidase enzyme) by petroleum extract of fruit

Table 13: Report of % inhibition of (alpha glucosidase) by Ethanolic extract of fruit

Conc. (µg/ml	% Inhibition	IC50
0.5	17.5	36.82
1	22.5	
1.5	61.25	
2	62.5	
2.5	68.75	

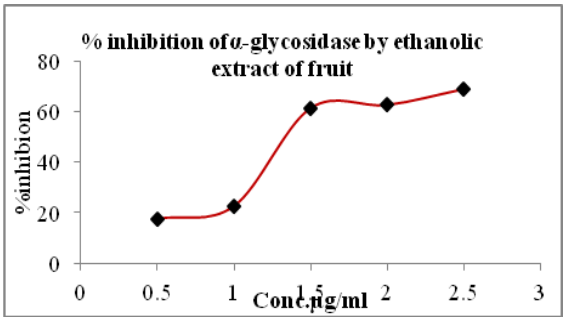


Fig 13: Represents the (% Inhibition of alpha glycosidase enzyme) by Ethanolic extract of fruit

Table 14: Report of % inhibition of (alpha glucosidase) by water extract of fruit

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	42.5	36.21
1	55	
1.5	67.5	
2	68.5	
2.5	71.25	

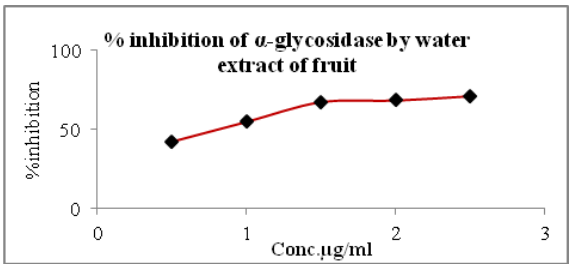


Fig 14: Represents the (% Inhibition of alpha glycosidase enzyme) by water extract of fruit

Table 15: Report of % inhibition of (alpha glucosidase) by ethanolic extract of root

Conc. (µg/ml	% Inhibition	IC50
0.5	37.5	36.3
1	56.25	
1.5	57.5	
2	58.75	
2.5	61.25	

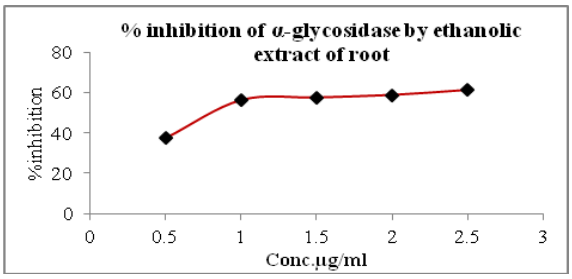


Fig 15: Represents the (% Inhibition of alpha glycosidase enzyme) by Ethanolic extract of root

Table 16: Report of % inhibition of (alpha glucosidase) by water extract of root

Conc. In µg/ml	% Inhibition	IC50
0.5	32.5	36.5
1	50	
1.5	57.5	
2	65	
2.5	66.25	

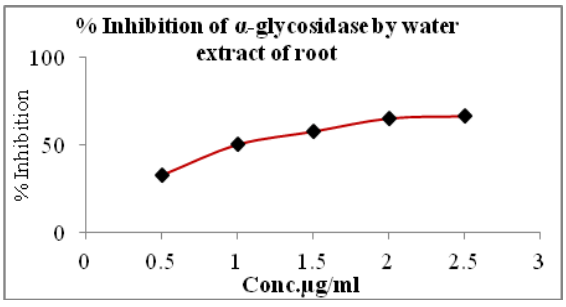


Fig 16: Represents the (% Inhibition of alpha glycosidase enzyme) by water extract of root

Table 17: Report of % inhibition of (alpha glucosidase) by petroleum ether extract of root

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	40	37.3
1	42.5	
1.5	43.75	
2	46.25	
2.5	47.55	

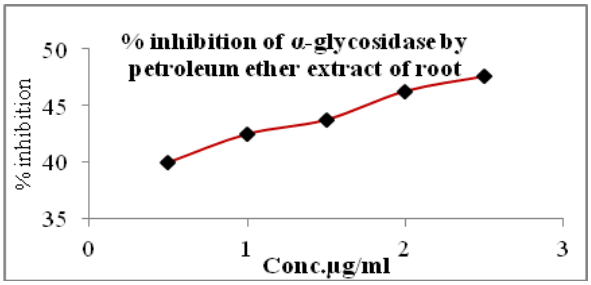
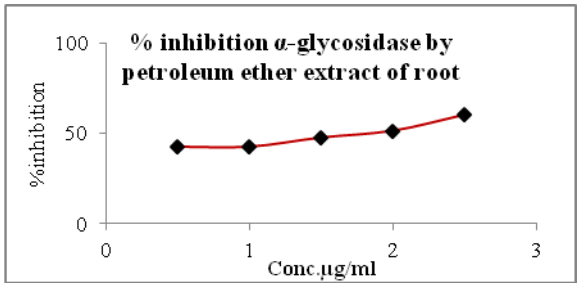


Fig 17: Represents (% Inhibition of alpha glycosidase enzyme) by petroleum ether extract of root

Table 18: Report of % inhibition of (alpha glucosidase) by petroleum ether extract of leaf

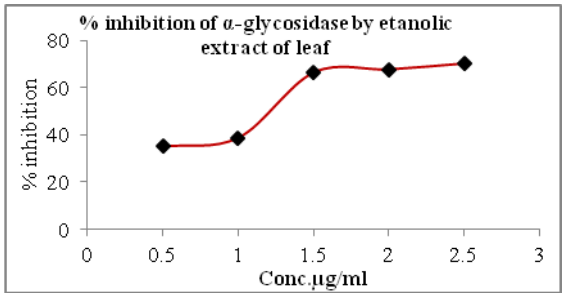
Conc. (µg/ml	% Inhibition	IC_{50}
0.5	42.5	37.00
1	42.5	
1.5	47.5	
2	51.25	
2.5	60	



Graph 18: Report of % inhibition of (alpha glucosidase) by petroleum extract of leaf

Table 19: Report of % inhibition of (alpha glucosidase) by Ethanolic extract of leaf

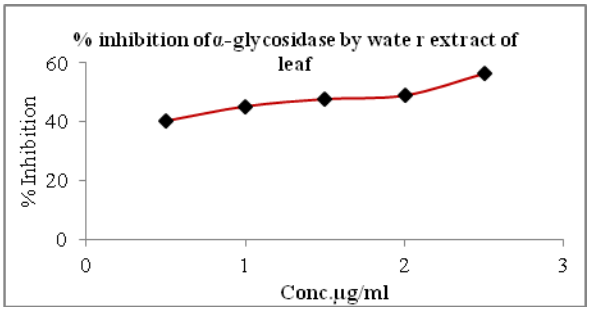
Conc.(µg/ml	% Inhibition	IC_{50}
0.5	35	36.7
1	38.75	
1.5	66.25	
2	67.5	
2.5	70	



Graph 19: Report of % inhibition of (alpha glucosidase) by Ethanolic extract of leaf

Table 20: Report of % inhibition of (alpha glucosidase) by water extract of leaf

Conc. (µg/ml	% Inhibition	IC_{50}
0.5	40	37.6
1	45	
1.5	47.5	
2	48.75	
2.5	56.25	



Graph 20: Report of % inhibition of (alpha glucosidase) by water extract of leaf

DISCUSSION:

The diseases which effect Liver at an alarming sequence appear to increase in our society. Chemical medicines are found to be cost effective in overcoming such types of diseases but are also associated with side effects which are the biggest problem in the present life. So scientists approach towards Herbal drugs so has gained importance in recent years because of their efficacy without side effects **Subramonium A, Pushphagandan P (9).** A large number of plants and polyherbal formulations are claimed to have hepatoprotective activities. But in actual practice, only few plants stand pharmacologically evaluated for their efficacy. ***Hippophae rhamnoides*** plant extracts have been evaluated for its antidiabetic properties by in-vitro inhabitation of alpha amylase and alpha glucosidase

enzymes. The results so far obtained are encouraging by showing inhibition of these enzymes to a large extent. The ethanol and water extracts of all the plant parts show the highest inhibition as compared to petroleum ether extracts. The results obtained in this study are in line with the already obtained results for the antidiabetic potential of the plant. The polar solvent extracts being showing the highest potential against the alpha amylase and alpha glucosidase enzyme. The plant-based antidiabetic drugs or agents contains diversity of major active constituents such as phenols, Coumarins, lignans, terpenoids, carotenoids, glycosides, flavonoids, organic acids, alkaloids and xanthenes.

CONCLUSION:

In conclusion it can be mentioned that plants bear a good potential to overcome every problem in a human body. The concerned plants possess a high degree of value as source of antidiabetic drug.

Every type of activity concerned with a plant is due to the phytochemicals which remain in built within these plants .The antidiabetic activity of the plant is due its potential in habit amylase productions, which in turn leads to non decomposition of higher carbohydrates into lower there by make blood glucose leval within required limit of the cell.

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