CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1068279

Available online at: http://www.iajps.com Research Article

ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF VARIOUS EXTRACTS OF *BERBERIS ARISTATA*

M. Amin Mir[1*], Komal Manhas[2], MMS Jassal[3]

[1]Sai Institute of Paramedical and Allied Sciences, Dehradun

[2] Uttaranchal (PG) College of Science and Technology Dehradun

[3] Deptt. of Chemistry DAV (PG) College, Dehradun

Abstract:

Formation of free radicals in the human body is a daily process and in the present set up of life there occurs inclination in the formation of free radicals in every human body because of life style change. The over production and the accumulation of free radicals leads to the generation of various types of diseases like, diabetes, Cancer, Alzheimer etc. Also in the present age the most dreadful and drastic disease viz diabetes had touched the heights as most of the humans above the age of 40 had affected by the concerned disease. Various types of nourishments are being taken against the free radicals and various types of allopathic medicines are taken against the diabetes, but none finds the complete effect neither against the complete neutralization of free radicals nor against the diabetes. So Scientists finally showed their interest towards the utilisation of phytochemicals or medicinal plants as a whole. So taking these factors into consideration the study in reference have been carried out. Berberis aristata have been collected from the Kishtwar region of Jammu and Kashmir. The plant extracts have been analysed for the antioxidant and antidiabetic properties. All the plant extracts have been found effective against the inhibition of free radicals, and the inhibition of alpha amylase and alpha glycosidase enzymes. Among the carious extracts of the plant, the acetone extracts have been found much effective against the diabetes and also the antioxidant power of the acetone extract of plant have been found to be much more effective than the water and DCM extracts.

Keywords: *Antidiabetic, antioxidant, Berberis aristata, Plant extracts, DPPH, Acarbose*

Corresponding author:

M. Amin Mir,

Sai Institute of Paramedical and Allied Sciences,
Dehradun.
mohdaminmir@gmail.com,
+91-9897635334

QR code

Please cite this article in press as M. Amin Mir et al., ***Antioxidant and Antidiabetic Properties of Various Extracts of Berberis Aristata****, Indo Am. J. P. Sci, 2017; 4[11].*

INTRODUCTION:

Medicinal plants are plants provide medicines-to prevent disease, maintain health and cure ailments. In one form or another, they benefit virtually everyone on earth. No exact definition of medicinal plants is possible. There are related issues, such as for nutrition, toiletry, bodily care, incense and ritual healing. Medicinal plants are the plants used for centuries as remedies for human diseases because they contain components of therapeutic value. About 80% of world population relies on the use of traditional medicine which is predominantly based on plant material. The scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for human health problems including cancer, diabetes and infectious diseases. Therefore the need to develop efficient, safe and inexpensive drugs from plant sources is of great importance.

Berberis aristata (Berberidaceae) is commonly known as daruharidra, garhwal and chitri. It is a shrub found in the northern mountainous region of Pakistan and India, as well as in the Nilgiri Hills of Southern India. B. aristata extract has been used by the natives of Sikkim and Darjeeling as a folklore medicine for the treatment of diabetes. The properties like cholegogue, hepato-stimulant and astringent are useful in treating anorexia, dysentery hepatitis and liver disorders. Antioxidant, antibacterial, anti-inflammatory, analgesic and antipyretic activities have been evaluated. Chemical analysis revealed the presence of alkaloids, amino acids, tannins, terpenes, resins, phenols and reducing sugars as major compounds.

Studies have shown that Berberis aristata (1) root is used in traditional medicine for a number of ailments including antibacterial, anti platelet, anti-inflammatory, analgesic, antipyretic, antioxidant and hepatoprotective activities etc.

Berberis aristata (Berberidaceae) (2) is an important medicinal plant, different region of the world. It has significant medicinal value in the tradition a total phenol and flavonoid content was found to be 0.11%.

Berberis aristata (3) is one of the herbs mentioned in all ancient scriptures of Ayurveda. It is a well-known medicinal plant in hilly areas of Nepal and its occurrence is reported from middle altitude areas. It is used traditionally for treating various disorders including diabetes mellitus.

Daruharidra (Berberis aristata) (4) has been used in Ayurveda and traditional Chinese medicine for more than 3000 years. It is a red-listed endemic medicinal plant species of conservation concern and has become very important in recent years due to its rarity and huge demand in the medicinal plant sector. Plants belonging to Berberis (5) are reported in several folklore medicinal pharmacopeias and are used in traditional medicines in Asia and European countries. The plants have been used in the preparation of various traditional and synthetic medicines since pre-historic times for wound healing, fever, eye disease, jaundice, vomiting during pregnancy, rheumatism, kidney and gall balder stones, and several other illnesses.

EXPERIMENTAL:

The *Berberis aristata* was collected from the Kishtwar region of Jammu and Kashmir. The plant parts were segregated shade dried and powdered in mixture. The powder was used for experiment.

Extraction

50 gms of the plant root, and leaf powder were weighed separately and accurately and then extracted in a Soxhlet Apparatus using thimble in order to get the best extract. Various solvents were used depending upon their polarity index with increasing polarity (DCM, Acetone and Water).

Extraction A:

The sample was extracted with a particular solvent (DCM) in a Soxhlet apparatus for a required period, till no extract was coming out of the sample, as being examined by taking a small amount of the extracted solvent from the main chamber of the Soxhlet apparatus over the watch glass for the appearance of precipitate. After the Extraction with DCM, the extract solution was subjected to filtration to remove the residue from extract. The filtrate was then collected and evaporated to remove the volatile solvent to its $1/4^{th}$ volume on water bath at a suitable temperature. The whole filtrate was then made in solid form (powdered) after being kept in an oven. The residue was collected, and subjected to further extraction process.

Extraction B:

The residue from the first extraction was then extracted with acetone in a same manner as mentioned above, in extraction A.

Extraction C:

The residues from extract B were then subjected to water extraction by decoction technique. In this technique the extract was dissolved in 500 ml of water. The whole solution was heated over water bath to remove all the water from the extract. Finally additional 500 ml of water was added to the extract, the extracted solution was finally evaporated to remove nearly 250ml of water. The solution was then

subjected to filtration. The filtrate was then evaporated to remove nearly 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30-50^{0}C.
The percentage yield of all the extracts were determined as w/w.

Antioxidant Property
DPPH Method
DPPH Scavenging activity was measured by the spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol. Decrease in the absorbance in presence of sample extract at different concentration (10-125 µg/ml) was noted after 15 min. IC_{50} was calculated from % inhibition.

Protocol for DPPH Free Radical Scavenging Activity
Preparation of stock solution of the sample:-
.100 mg of extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.
2.Dilution of test solution: 10, 20, 30, 40, 50,60,70,80,90,100 µg/ml solution of test were prepared from stock solution.
3.Preparation of DPPH solution: 15 mg for DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminium foil to protect from light.
4.Estimation of DPPH scavenging activity: 75 µl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm.
Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction = Control absorbance – Test absorbance/ Control absorbance x 100
Calculation of IC_{50} value using graphical method.

***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 color was measured at 540nm.**Krishnaveni, S., B. Theymoli, and Sadasivam, S. (7)**.

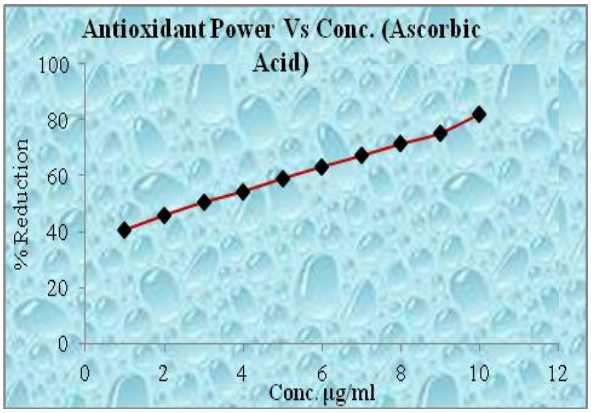
Calculation of 50% Inhibitory Concentration (IC_{50})
The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (% I) was calculated by

% I = (Ac-As)/Ac X 100 [Shai, L. J., P. Masoko., M.Eloff, J.N. – 2010 …… (8)
Where ***Ac*** is the absorbance of the control and ***As*** is the absorbance of the sample.

Observations and Result
Antioxidant Power Determination

Table 1: showing the Antioxidant Power of Ascorbic Acid

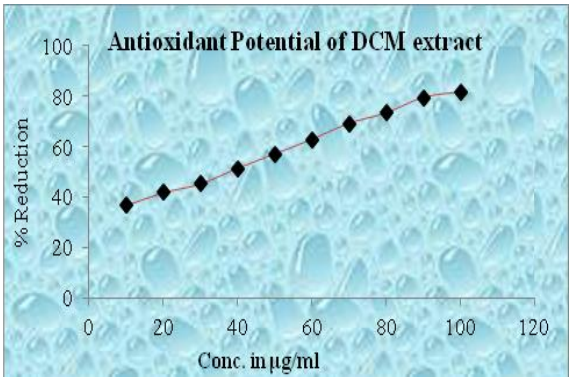
S. No	Conc. (µg/ml)	Abs. of Ascorbic acid	% Red	IC_{50} Value
1	10	0.292	40.63	26
2	20	0.269	45.90	
3	30	0.244	50.60	
4	40	0.226	54.45	
5	50	0.202	59.10	
6	60	0.181	63.33	
7	70	0.162	67.21	
8	80	0.141	71.45	
9	90	0.122	75.30	
10	100	0.088	82.16	



Graph 1: showing the Antioxidant Power of Ascorbic Acid

Table 2: showing the Antioxidant Activity of DCM Extract of *Berberis aristata*

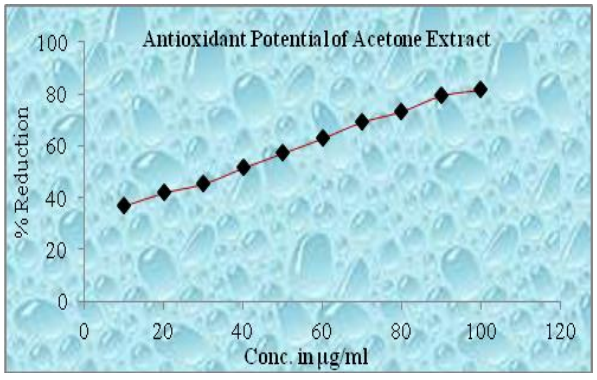
S. No.	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	10	0.280	42.85	50
2	20	0.274	44.48	
3	30	0.252	46.93	
4	40	0.216	47.55	
5	50	0.201	49.59	
6	60	0.174	53.06	
7	70	0.153	60.26	
8	80	0.141	64.8	
9	90	0.119	69.38	
10	100	0.093	72.44	



Graph 2: showing the Antioxidant Power of DCM extract of *Berberis aristata*

Table 3: Showing the Antioxidant Activity of Acetone Extract of Berberis aristata

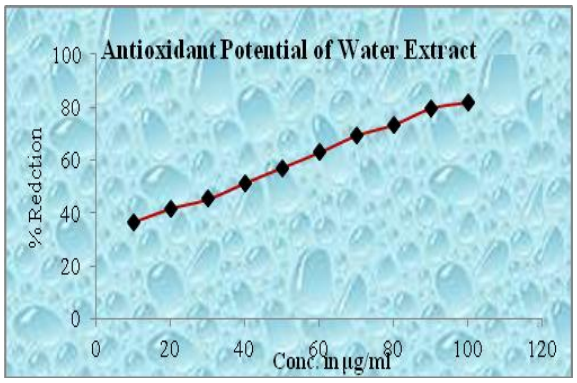
S. No.	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1.	10	0.288	43.82	30
2.	20	0.261	46.93	
3.	30	0.241	49.38	
4.	40	0.230	52.65	
5.	50	0.211	57.14	
6.	60	0.181	61.22	
7.	70	0.162	66.32	
8.	80	0.141	71.42	
9.	90	0.117	73.26	
10	100	0.072	77.55	



Graph 3: showing the Antioxidant Power of Acetone extract of *Berberis aristata*

Table 4: Showing the Antioxidant Activity of Water Extract of *Berberis aristata*

S. No.	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1.	10	0.310	40.81	42
2.	20	0.291	42.85	
3.	30	0.271	44.48	
4.	40	0.231	48.97	
5.	50	0.215	56.93	
6.	60	0.171	64.89	
7.	70	0.150	70.81	
8.	80	0.131	75.3	
9.	90	0.112	78.57	
10	100	0.071	83.26	



Graph 4 showing the Antioxidant Power of water extract of *Berberis aristata*

Antidiabetic Property

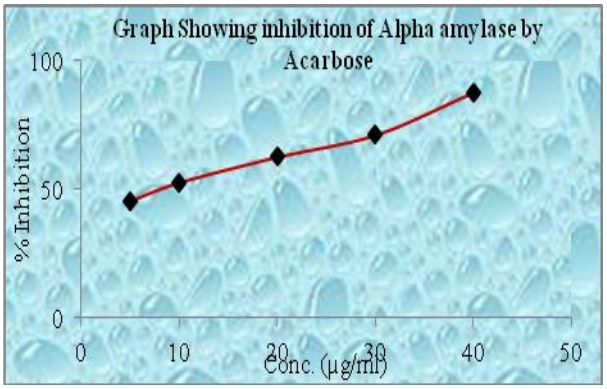
Berberis aristata plant has been analyzed for the anti-diabetic potential determination by the inhibition of alpha-amylase and alpha-glycosidase enzymes. All the plant extracts have been analyzed and are respectively shown in the figures. The concentration range was made between (0.5-2.5 µg/ml).

Anti-diabetic Property of *Berberis aristata* extracts by inhibition of Alpha-amylase)

The alpha-amylase inhibition of various *Berberis aristata* extracts (Acetone, DCM and Water) extracts have been analyzed, and it was found that **(water extract)** possess the highest inhibition potential followed by **(DCM extract)**. The percentage inhibition by plant extracts was found to be concentration dependent, percentage inhibition 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 all the plant extracts was found lesser than the reference compound **(ACAROSE).** The **IC_{50}**value of various plant extracts follows the order **(water extract, DCM extract and Acetone Extract)** was found to be **(12.6, 17.5 and 22.4)** respectively and are presented in **(Tables).**

Table 5: Report of % inhibition of (alpha-amylase) by ACAROSE Standard

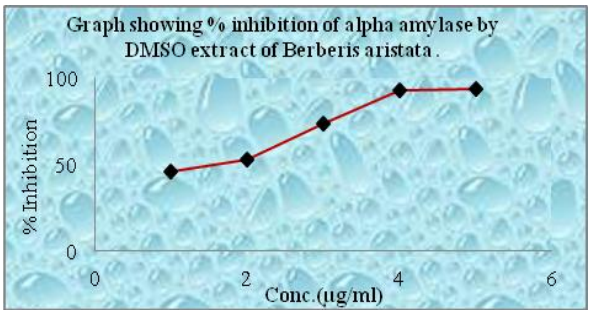
S. No.	Conc. (µg/ml)	Absorb of Acarbose	% Red	IC_{50}
1.	**5**	**0.081**	**44.66**	**9.3**
2.	**10**	**0.074**	**57.81**	
3.	**20**	**0.051**	**64.71**	
4.	**30**	**0.039**	**79.14**	
5.	**40**	**0.032**	**81.61**	



Graph 5: Report of % inhibition of (alpha-amylase) by ACAROSE Standard

Table 6: Showing the Anti-diabetic property of DCM extract of *Berberis aristata*

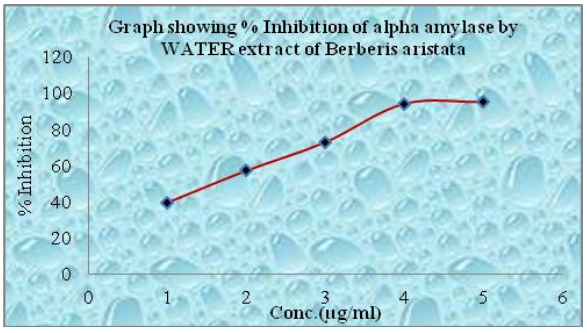
S. No.	Conc. (µg/ml)	Absorb	% Red	IC_{50}
1	10	0.165	47.90	17.5
2	20	0.153	57.11	
3	30	0.120	69.77	
4	40	0.091	89.11	
5	50	0.040	92.44	



Graph 6: Showing the Anti-diabetic property of DCM extract of Berberis aristata.

Table 7: Showing the Anti-diabetic property of water extract of Berberis aristata

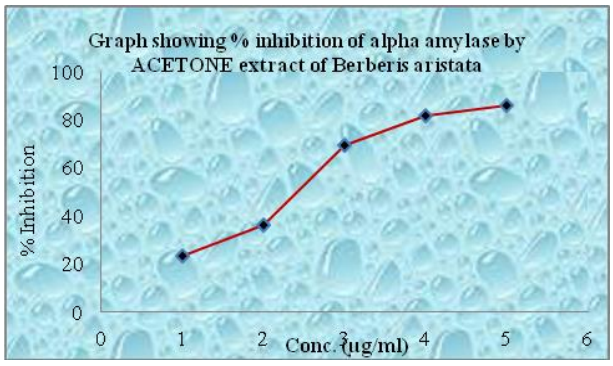
S. No.	Conc. µg/ml	Absorb	% Red	IC_{50} Value
1	10	0.175	31.34	12.6
2	20	0.121	56.04	
3	30	0.073	78.78	
4	40	0.043	81.09	
5	50	0.023	96.08	



Graph 7: showing the Anti-diabetic property of Water extract of Berberis aristata.

Table 8: Showing the Anti-diabetic property of Acetone extract of Berberis aristata

S. No.	Conc. µg/ml	Absorb	% Red	IC_{50} Value
1	10	0.233	28.61	22.4
2	20	0.181	35.14	
3	30	0.081	71.10	
4	40	0.043	83.46	
5	50	0.031	86.85	



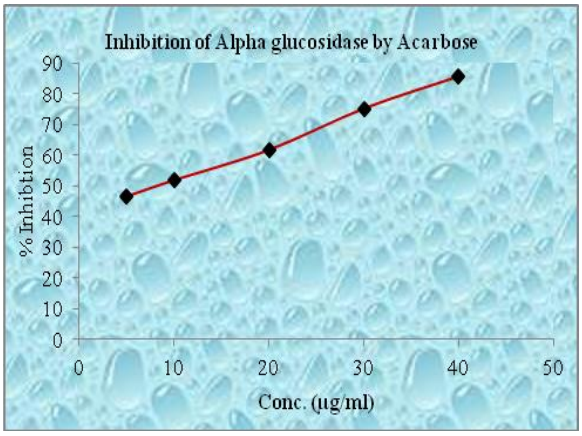
Graph 8: Showing the Anti-diabetic property of Acetone extract of Berberis aristata.

Alpha glucosidase inhibition

The ***Berberis aristata*** plant extracts have been analyzed 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. The lower **IC_{50}** value indicates higher inhibition percentage. Among the all plant extracts the higher inhibition of alpha glucosidase was shown by (water extract of fruit) and the **IC_{50}**value as determined was found to be **(10.4).** The various plant extracts follow the order as per their inhibition potential as **(Acetone and DCM**). 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 less than the reference compound **(ACAROSE)** as per their percentage inhibition is taken into consideration. The **IC_{50}**value of various plant extracts is as **(12.4, 22.35, and 42.20)** respectively for **(Water, Acetone and DCM).**

Table 9: Report of % inhibition of (alpha-amylase) by ACAROSE Standard

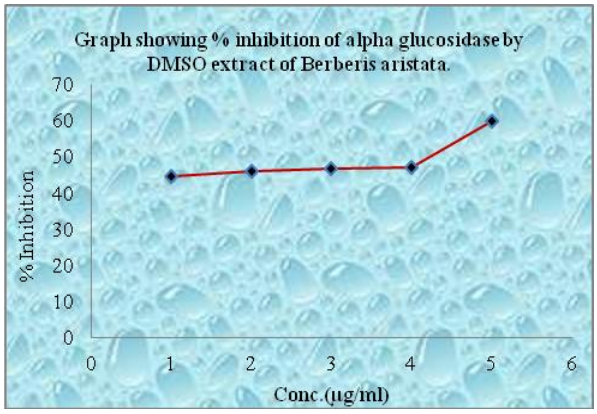
S. No.	Conc. µg/ml	Absorbance	% Reduction	IC_{50} Value
1	5	0.230	47.61	8.1
2	10	0.189	52.92	
3	20	0.165	59.73	
4	30	0.142	74.16	
5	40	0.134	84.57	



Graph 9: Report of % inhibition of (alpha-amylase) by ACAROSE Standard

Table 10: Showing the Anti-diabetic property of DCM extract of Berberis aristata

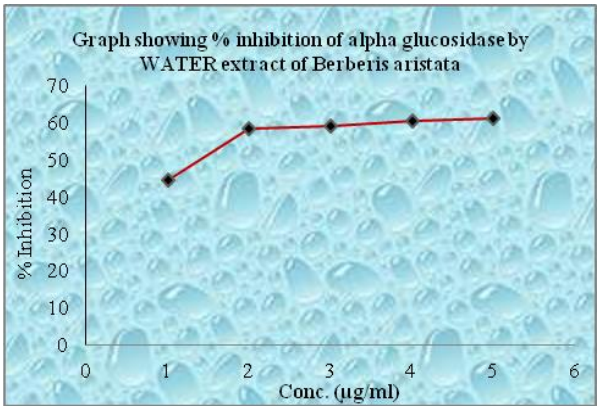
S. No.	Conc. µg/ml	Absorbance	% Reduction	IC_{50} Value
1	10	0.179	43.592	42.2
2	20	0.165	46.91	
3	30	0.153	47.51	
4	40	0.148	48.82	
5	50	0.112	58.61	



Graph 10: Showing the Anti-diabetic property of DCM extract of Berberis aristata.

Table 11: Showing the Anti-diabetic property of WATER extract of Berberis aristata

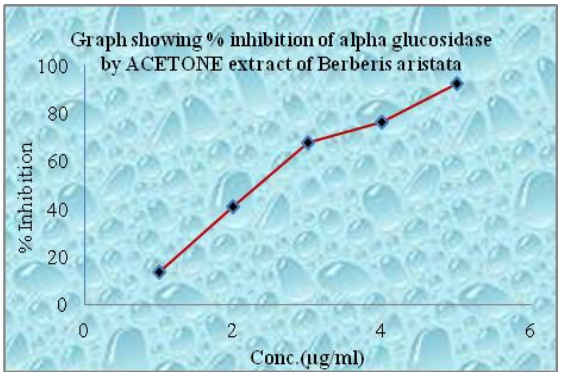
S. No.	Conc. (µg/ml	Absorbance	% Reduction	IC_{50} Value
1	10	0.173	45.26	12.4
2	20	0.122	54.36	
3	30	0.121	57.01	
4	40	0.126	63.32	
5	50	0.113	64.98	



Graph 11: showing the Anti-diabetic property of Water extract of Berberis aristata.

Table 12: Showing the Anti-diabetic property of ACETONE extract of Berberis aristata

S. No.	Conc. µg/ml	Absorbance	% Reduction	IC_{50} Value
1	10	0.262	14.77	22.35
2	20	0.181	38.98	
3	30	0.093	69.86	
4	40	0.071	74.06	
5	50	0.026	90.45	



Graph 12: showing the Anti-diabetic property of Acetone extract of *Berberis aristata*.

DISCUSSION:

The antioxidant potential of the concerned plant was analysed by DPPH method in which ascorbic acid had been used as a reference compound. As per the antioxidant power of the various extracts of the shrub are taken into consideration, the extracts responded very well, as all the extracts showed good antioxidant power. The fruits of the plant are mostly consumed by the humans, but the leaves are the best source of nutrients for cattle. The wood of the shrub is golden colour, and had been used as medicinal plant from times immoral. All the extracts of the concerned plant posses the neutralization potential of free radicals. ***Berberis aristata*** plants extracts could help to overcome the diseases which are of free radical origin. So the plant could be used as source of medicine. Among the plant extracts, it had been found that the acetone extract could be much more beneficial as compared to DCM and water extracts of the concerned plant as per their antioxidant potential.

Also one more analysis the antidiabetic potential determination of the various plant extracts had been carried out, by the inhibition of alpha amylase and alpha glycosidase enzymes, the two key enzymes responsible for the disease in reference. All the extracts of the plant viz, DCM, Acetone and water showed the inhibition of both the enzymes, but every plant extract inhibits the concerned enzymes in varying degree. The best inhibition of both the enzymes was shown by the water extract of the concerned plant followed by the acetone extracts, and least inhibition was noticed for the DCM extracts of the concerned plant. The observed results get agreed with the results as noticed by Amin et.al (9), in which polar solvent extracts marginally showed higher antidiabetic effect as compared to the less polar solvent extracts.

CONCLUSION:

In conclusion it could be concluded that Ayurvedic system of medicine is touching the heights in the medical field nowadays, as most of the medicines being consumed by various patients against the concerned diseases are much more effective and of least side effects, where as the allopathic medicines are associated with side effects in one way or another way. Also more research needs to be done on the medicinal plants as every type of disease finally gets cured by the phytochemicals isolated from the plants.

REFERENCES:

1.Anil Pareek, Manish Suthar. “Antidiabetic activity of extract of Berberis aristata root instreptozotocin induced diabetic Rats”. Pharmacology. 2010; 2: 179-185.

2.Dinesh K. Patel, Kanika Patel, S. P. Dhanabal. “Standardization of Berberis aristata extract through conventional andmodern HPTLC techniques’’. Asian Pacific Journal of Tropical Disease. 2012; 136-140.

3.Bharati, Amatya, Rokeya, Bhoumik, Gharti. “Study on hypoglycemic effect of Berberis aristata on type 2 diabetic model rats”. Journal of Institute of Medicine. 2013; 3(2):58-64.

4.S. Tamilselvi, S. P. Balasubramani, Padma Venkata Subramania. “A review on the phamacognosy and pharmacology of the herbals traded as Daruharidra”. Int J Pharm Bio Sci. 2014;5(1): 556 – 570.

5.Ibrahim Khan, Syed Najee-bullah, Muhammad Ali and Zabta Khan Shinwari. “Phyto pharmacological and ethno-medicinal uses of the Genus Berberis (Berberidaceae). Tropical Journal of Pharmaceutical Research. 2016;15 (9): 2047-2057.

6.Laila A. Shekib Samir, M. El-Iraqui, Taisser M. Abo-Bakr. Studies on amylase inhibitors in some Egyptian legume seeds. Plant Foods Human Nutr. 1988;38:325–332.

7.Krishnaveni S. B. Theymoli, Sadasivam S. Phenol Sulphuric Acid method. *Food Chem.* 1984;15: 229.
8.Shai L. J. P. Masoko, M. P. Mokgotho, S. R. Magano, M. A. Mogale, N. Boaduo, Eloff J. N. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African J. Bot.* 2010; 76: 465 - 470.
9.Amin MM, Sawhney SS, Jassal MS (2013) Comparative Antioxidant Power Determination of *Taraxacum officinale* by FRAP and DTPH Method. Pharmaceut Anal Acta 4: 221. doi:10.4172/2153-2435.1000221.