IN-VITROESTIMATION OF ANTIOXIDANT AND ANTIDIABETIC POTENTIAL OF PLANT EXTRACTS

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Abstract

Medicinal plants have always been the principal sources of medicine worldwide. India sustains a very rich traditional medicinal plant wealth and inherits unique plant and animal communities. Free radicals are implicated in many diseases like diabetes, inflammation, cancer, which leads to gained more attraction of antioxidant therapy. Diabetes is a metabolic disorder which results due to deficiency in insulin and its metabolism. At present, the prevalence of Diabetes has increased worldwide and predicted to increase to greater extent in future generations. Among various therapeutic approaches implemented to prevent diabetes is to regulate the blood glucose levels by various mechanisms. Present study enumerates the *In-Vitro* Evaluation of Antioxidant and Antidiabetic Potential of Plant Extracts. Phytochemical screening showed the presence of alkaloids, glycosides, carbohydrates, steroids and flavonoids in both the extracts. Physical parameters like solubility, MP, ash values, LOD, extractive value etc. has been studied. The antioxidant activity of the extract was done by using DPPH and H_2O_2 method. This is being assessed by assay such as inhibition of α-amylase enzyme suppresses the level of production of glucose. Our results suggested that *D. strictus* extracts showed potential *In-Vitro* antioxidant and anti-diabetic activity which indicates that this extract can be taken further for pharmacological study.

KEYWORDS: *D.strictus,* Physicochemical parameter,Antioxidant effect, Anti-diabetic activity, α-amylase enzyme.

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INTRODUCTION:

Hyperglycemia (high blood sugar) is a symptom of diabetes mellitus (DM), a long-term condition of carbohydrate, lipid, and protein metabolism caused by problems with insulin levels, insulin action, or both. Currently, it is one of the worst global health issues that can cause both microvascular and macrovascular consequences. By 2040, there will probably be 700 million persons with diabetes worldwide. Reactive oxygen species (ROS), which are known to be produced by hyperglycemia, are crucial in the development of diabetic complications. Antioxidants are substances or nutrients found in food that function as free radical scavengers, preventing and repairing damage from reactive oxygen and nitrogen species (ROS) and reactive nitrogen species (RNS). As a result, antioxidants can strengthen the immune

system and reduce the risk of diabetic complications.Although there are synthetic anti-diabetic medications like insulin available, none of them provide long-lasting glycemic control without producing undesirable side effects. As a result, employing herbal treatments to treat DM is becoming more popular. The World Health Organization has placed enough emphasis on using traditional herbs to treat diabetes since they are safe, effective, have few to no adverse effects, and are relatively affordable. The *D. strictus* plant is exhibits various pharmacological activities reported as analgesic, antipyretic, anti-inflammatory, antispasmodic, anthelmintic, antimicrobial & antioxidant activity. Therefore, the extracts of plantpart were subjected to study ***In-vitro*estimation of Antioxidant and Antidiabetic potential of plant extracts.**

MATERIAL AND METHOD

Collection of Plant Material

Plant material was collected from hilly regions of Latur district. Authenticated by the botanists (Department of Botany)& Botanical survey of India. Then it was shade dried, Powdered and extracted with ethanol, methanol and aqueous by maceration.

Preliminary Phytochemical Screening

The preliminary phytochemical analysis was carried out for the active extracts using standard phytochemical methods.

Estimation of Total Phenolic Content

The amount of total phenolic content of crude extracts were determined according to Folin-Ciocalteu procedure [12]. The different concentrations of extracts (100 µl) were introduced into test tubes. 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer).

Estimation of Total Flavonoids

The total flavonoids contained in the extracts were estimated. Briefly, 0.5 mL of 2% AlCl3 ethanolic solution was added to 0.5 mL of the extracts, left for 1 h at 25 C; after which the absorbance was measured at 420 nm. The appearance of yellow colour suggested the presence of flavonoids. Total flavonoid content was expressed as quercetin equivalent (mg/g) using the equation obtained from the calibration curve

ANTIOXIDANT ACTIVITY:

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

The antioxidant activity of the various extracts of *DS* was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH, as described by Turkoglu et al. [24]. In brief, 1 mL of a 0.2 mM DPPH methanol solution was added to 1 mL of various concentrations (0.2–1.0 mg/mL) of the extracts and incubated at 25 C for 30 min. The absorbance of the resulting mixture was measured against blank at 516 nm using UV-visible spectrophotometer). The percentage inhibition rate (I%) on the DPPH radical was calculated using the formula:

$$\text{Percentage inhibition (I\%)} = [(A_{control} - A_{extract})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract.

B. Hydrogen Peroxide (H_2O_2) assay

The capability of scavenging hydrogen peroxide by the extract was determined based on the method. A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the extracted sample (different concentration of 50, 100, 150μg/mL) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. The abilities to scavenge the hydrogen peroxide were calculated based on the following equation: Hydrogen peroxide scavenging activity.

C. FRAP Assay

The reduction property of the extracts were assessed according to the method. Different concentrations of *D. strictus* extracts were added to 1 mL of distilled water and then mixed with 2.5 mL of 0.2 m phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated at 50 C for 20 min before the addition of 2.5 mL of trichloroacetic acid. The resulting mixture was centrifuged at 3000 rpm for 10 min. After this, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% $FeCl_3$. The colour change of the resulting solution was then taken at 700 nm.

IN-VITRO ANTI-DIABETIC ACTIVITY
α-amylase activity (DNSA):

Five concentrations of plant extract were prepared by dissolving in double distilled water. These were 2 µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml. A total of 500µl of plant extract and 500 µl of 0.02M sodium phosphate buffer were incubated for 10 minutes at 25°C.After pre-incubation,500 µl of 1% starch solution in 0.02M sodium phosphate buffer was added to each tube at 5s intervals. This mixture was then incubated for 10 minutes at 25°C.1ml of DNSA color reagent was added to stop the reaction. These test tubes were incubated for 5 minutes and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540 nm.

RESULTS & DISCUSSION

Table 1: Anti-oxidant activity of the ethanol extracts by DDPH assay.

Sr. no	Concentration (µg/ml)	Ascorbic acid (%inhibition)	Ethanolic extract (%inhibition)	Methanolic extract (%inhibition)	Aqueous extract (%inhibition)
1	50	61.95 %	55.20%	57.31 %	57.80 %
2	100	95.12 %	58.38%	59.3 %	81.07 %
3	150	96.22 %	**82.10%**	**79.2 %**	77.31 %

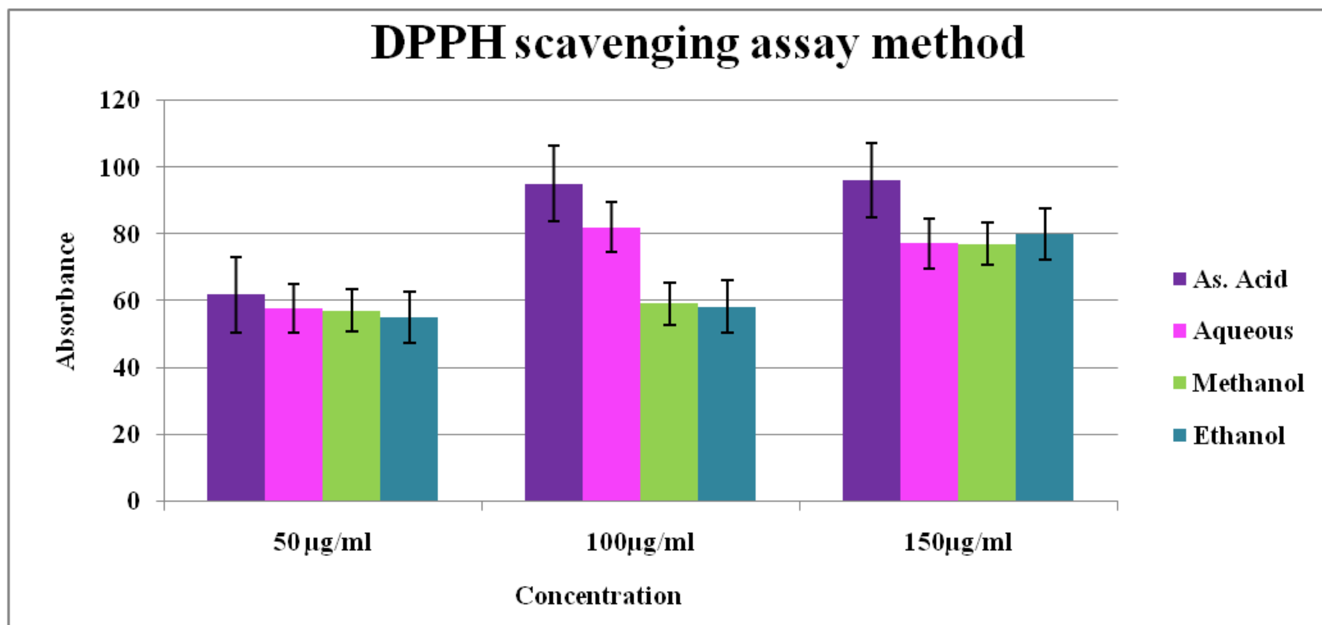

Table 2: Anti-oxidant activity of the ethanol extracts by H_2O_2 assay.

Sr. no	Concentration (µg/ml)	Ascorbic acid (%inhibition)	Ethanolic extract (%inhibition)	Methanolic extract (%inhibition)	Aqueous extract (%inhibition)
1	50	62.35 %	56.10%	56.32 %	55.78 %
2	100	96.21 %	59.83%	60.13 %	82.74 %
3	150	97.24 %	**84.11%**	**80.21 %**	78.31 %

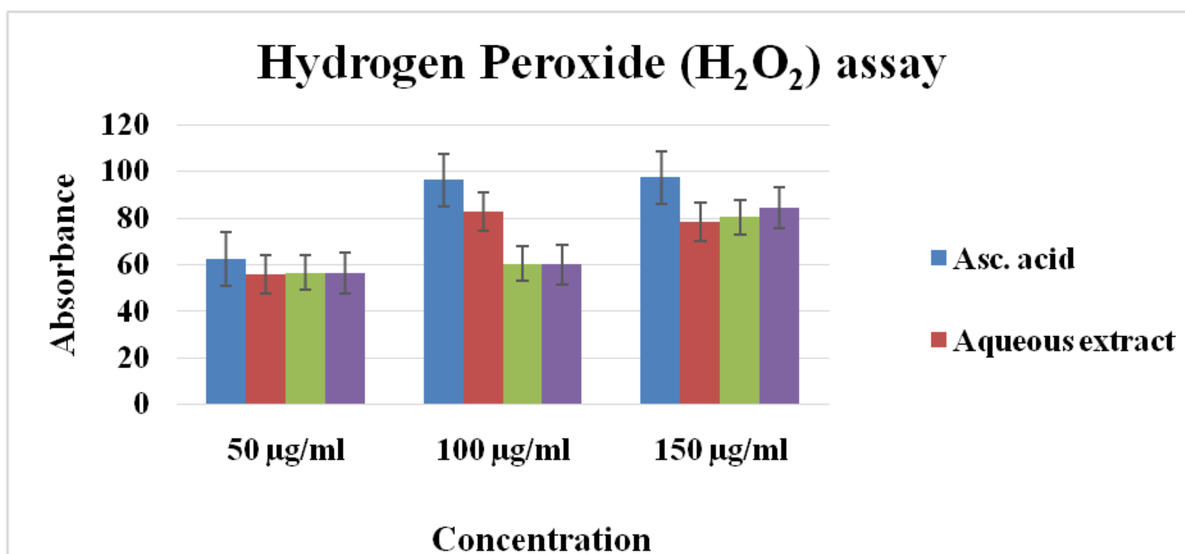

Table 3: Anti-oxidant activity of the ethanol extracts by FRAP assay

Sr. no	Concentration (µg/ml)	Ascorbic acid (%inhibition)	Ethanolic extract (%inhibition)	Methanolic extract (%inhibition)	Aqueous extract (%inhibition)
1	50	61.85 %	58.98 %	57.69 %	56.87 %
2	100	95.22 %	61.84 %	62.57 %	83.21 %
3	150	98.12 %	**86.33 %**	**81.23 %**	79.47 %

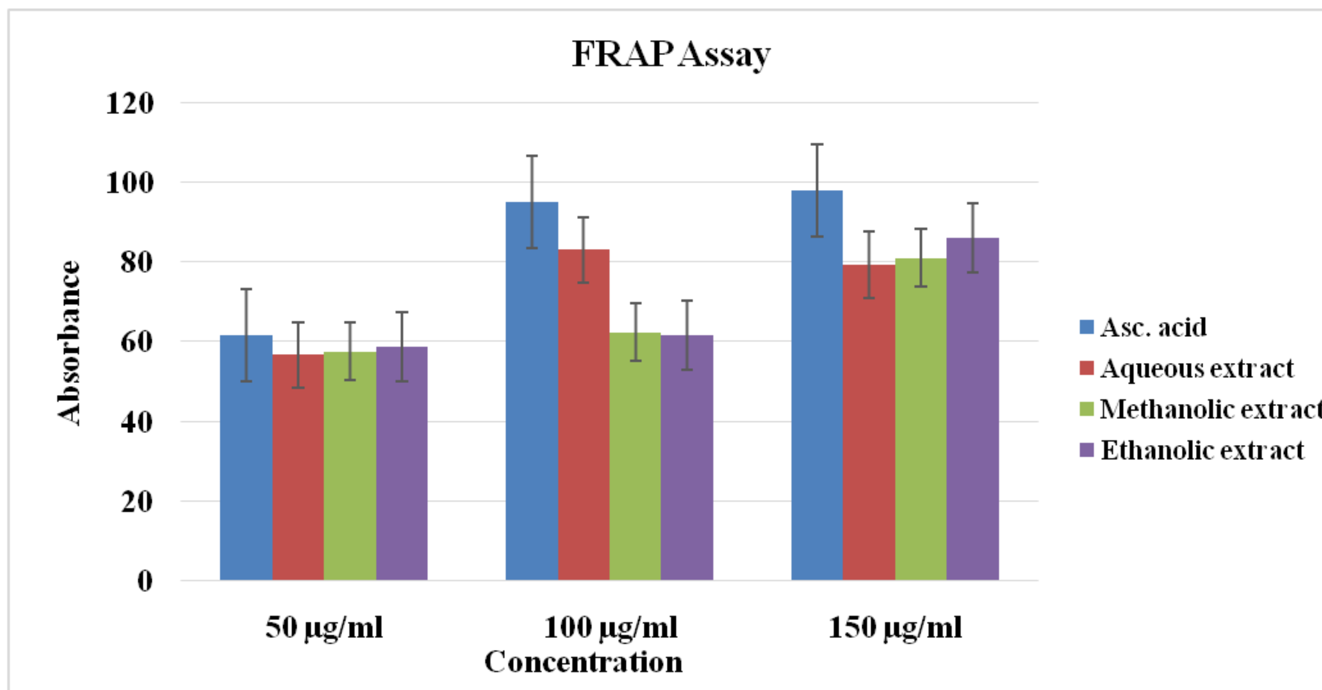

Table 4. *In-vitro* antidiabetic activity of the plant extract using α-amylase method and comparison with standard drug Acarbose

Sr. no.	Concentration µg/ml	% of Inhibition of Acarbose	IC 50 µg dry extract	% of Inhibition *D. strictus* Extract	IC 50 µg dry extract
1	2	6.99±0.03	0.31	2.38±0.02	0.76
	4	11.58±0.05		4.10±0.04	
	6	26.45±0.02		9.96±0.01	
	8	41.64±0.06		12.80±0.07	
	10	56.17±0.05		18.04±0.01	

The ethanol extracts (2-10µg/ml) of the plant exhibited potent α-amylase inhibitory activity in a dose dependent manner. The extracts showed inhibitory activity with IC_{50} value of 0.76 µg dry extract respectively (Table 4). Acarbose is a standard drug and itsconcentration of (2-10 µg/ml) showed α-amylase inhibitory activity with IC_{50} value 0.31 µg dry extract. The ethanol extracts of the plant showed maximum α-amylase inhibitory activity (IC_{50} = 0.76 µg dry extract) which could be attributed to the presence of polyphenols and flavonoids because polyphenols are not only capable of reducing oxidative stress but also of

inhibiting carbohydrate hydrolyzing enzymes because of their ability to bind with proteins.

SUMMARY AND CONCLUSION

The α-amylase inhibitory activity showed by *D. strictus* extract has a significant role in management of diabetes. These overall activities due to bioactive phytochemicals were present in the extract. The plant might be a source of natural antidiabetic agent, which has contribution of most significant therapeutic agents responsible for prevent and management of diabetes. Thus, it was concluded that *D. strictus* extract showed *In-vitro* antidiabetic activity.

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