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PRELIMINARY PHYTOCHEMICAL ANALYSIS, *IN VITRO* ANTIOXIDANT, AND ANTI-DIABETIC ACTIVITY OF *ALOCASIA SANDERIANA* METHANOLIC LEAF EXTRACT

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ABSTRACT

Objective: *Alocasia santeriana W.* The species has been reported to be used in the treatment of anti-diabetic in traditional/folk medicine, which may be explored for its anti-diabetic potential. **Methods:** A preliminary qualitative phytochemical screening of the methanol extract of *Alocasia sanderiana* was performed to detect alkaloids, phenolic compounds, flavonoids, saponins, tannins, glycosides, steroids, carbohydrates and various phytochemical constituents. The UV-Vis profile revealed various absorption bands ranging from 200 to 750 nm, indicating the presence of flavonoids, phenolic compounds, tannins, terpenoids, carotenoids, chlorophyll and alkaloids. 1,1- Diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging, Fe^{3+}–Fe^{2+} transformation assay, ferric reducing antioxidant power (FRAP) assay, superoxide radical ion (O2•−), scavenging, radical, hydroxyls, hydroxylation, etc. Principles, nitric oxide (NO) scavenging, metal chelating and reducing power function. These various antioxidant activities were compared with standard antioxidants such as L-ascorbic acid. **Results** *Alocasia sanderiana* leaf extract exhibited the greatest antioxidant activity via DPPH, superoxide anion scavenging, metal chelator (iron chelator and iron reducing power) and nitric oxide scavenging activity. The antioxidant activity of *Alocasia sanderiana* is due to the presence of flavonoids and polyphenols in the extract. *A.Santeriana* as determined by experimental methods. The α-amylase inhibitory activity and α-glucosidase inhibitory activity methods were used to evaluate the anti-diabetic potential.

KEYWORDS: *Alocasia sanderiana*, α-Amylase inhibiting activity and α-Glucosidase inhibiting activity, Anti Diabetic.

INTRODUCTION

Alocasia sanderiana W. Bull is a plant in the Araceae family. *Alocasia sanderiana W.* Bull is also known as the kris plant because of the resemblance of its leaf edges to the wavy blade of the kalis dagger (also known as kris plant). It is a tropical perennial with upright shiny, V-shaped and deeply lobed leaves. The plant can be upto 6 ft (2 m) tall and large in its native habitat. However, cultivated specimens are smaller. It possesses leaves that are evergreen, pelted, V-shaped, deeply lobed, and a glossy deep-green with large silvery white veins. They are about 12-16 in (30-40 cm) long and 6-8 in (15-20 cm) wide, with red-green undersides. The leaf stem is about 2 ft (60 cm) long. The rhizome of *A. Sanderiana* is vertically placed and is known as root stock. Female flowers are grouped at the lower part of the inflorescence, whereas the male flowers are at the top. According to literature report, *alocasia* is a kris plant native to tropical and subtropical Asia to Eastern Australia. *Alocasia* genus consists of about 79 species of which 28 are cultivated variety. *Alocasia sanderiana* W. Bull plant extract used in nanosilver particles to fight and prevent bacteria in vitro[1] and *Alocasia sanderiana* W.Bull endemic plant available in Tamilnadu[2, 3, 4] India.[5]

Where by the over production of reactive oxygen species related to hyperglycemia likely leads to an imbalance of the quantity of antioxidants inside the body and eventually, to oxidative stress. On the other hand, the blood sugar level is crucially determined by the act of digestive enzymes such as α-amylase and α-glucosidase. While α-amylase is responsible for breaking down long-chain carbohydrates, α-glucosidase directly converts carbohydrate to glucose in the small intestine. The inhibition of α-glucosidase has been acknowledged as a therapeutic target for the control of postprandial hyperglycemia, as well as type 2 diabetes.[6,7] Therefore, simultaneously providing antioxidants and α-

amylase and α- glucosidase inhibitors through nutriments is a potential and feasible method for the management of type 2 diabetes. However, the origin and dose of ingredients should be scrupulously studied before application and production. Additionally, natural products are recommended owing to their long history of medicinal and beneficial effects on human health.[8]

2 MATERIALS AND METHODS

Collection and preparation of plant sample

Alocasia sanderiana was collected from waste lands in and around Poondi village, Thanjavur district, Tamil Nadu, India, where it was found naturally. Fresh leaves of Alocasia sanderiana were collected and stored.Soak fresh leaves in water for 24 hours and then strain. The filtered supernatant was collected in a reagent bottle, tightly capped and stored at 4°C until use.

Phytochemical Screening

Preliminary qualitative phytochemical screening of aqueous extract of *Alocasia sanderiana* was performed to detect alkaloids, phenolic compounds, flavonoids, saponins, tannins, glycosides, steroids, carbohydrates, carbohydrates andvarious phytochemical constituents.[10]

Test for alkaloids

About 50 mg of solvent-free aqueous extract was stirred with 5 ml of dilute hydrochloric acid and filtered. To the filtrate, 2 ml of Hager's reagent (aqueous solution of picric acid) was added. A yellow precipitate appears, indicating the presence of alkaloids.[10]

Test for phenolic compound

About 50 mg of the solvent-free aqueous extract was stirred with 5 mL of dilute hydrochloric acid and filtered. To the filtrate, 2 mL of Hager's reagent (aqueous solution of picric acid) was added. A yellow precipitate appears, indicatingthe presence of alkaloids.[9]

Test for tannins

The aqueous extract (500 mg) was added to 10 ml of freshly prepared 10% potassium hydroxide (KOH) in a beaker and shaken well to dissolve. The formation of a dirty precipitate indicates the presence of tannins in the sample.[10]

Test for flavonoids

Aqueous solution of plant extract was treated with 10% ammonium hydroxidesolution. The appearance of a thick white precipitate indicates the presence of flavonoids.[11]

Test for terpenoids

About 50 mg in 1mL chloroform. This was mixed well and added to acetic anhydride with concentrated sulfuric acid from the sides of the tubes. The appearance of red and blue-green color indicates the presence of steroids and triterpenoids.

Test for saponins

The aqueous extract (50 mg) was diluted with distilled water to 10 ml. The suspension was shaken in a graduated cylinder for 15 min; An increase in the foam layer indicates the presence of saponins.[12]

Test for glycosides

About 50mg extract was hydrolyzed with concentrated hydrochloric acid on a water bath for 2 hours and filtered. To 2 ml of the filtrate was added 3 ml of chloroform and shaken well. The chloroform layer was separated and 10% ammonia solution was added to it. The formation of pink color indicates the presence of glycosides.

Test for steroids determination

Two ml of chloroform and 1 ml of concentrated sulfuric acid were mixed with 10 drops of aqueous extract of isopropyl alcohol, slowly until a double phase was formed. A dish-brown color in the middle layer indicates the presence of a steroidalring.

Antioxidant activity of *Alocasi sanderiana*

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging activity was assayed by the method of Shimada *et al*. (1992).[13]

Determination of total antioxidant capacity

The antioxidant activity of the *Alocasia sanderiana* leaves extract was evaluated as per the method of Prieto *et al.* (1999).[14]

Superoxideanion scavenging activity assay

The superoxide radicals scavenging activity was determined as per the methodof Liu *et al.* (1997).[15]

Fe^{2+}chelating activity assay

The metal chelating activity of the *Alocasia sanderiana* leaves extract was assayed by the method of Dinis *et al*., (1994).[16]

Nitric oxide scavenging activity assay

Nitricoxideradical scavenging activity was determined according to the method reported by Garrat (1964).[17]

Phytochemical analysis of leaves extract of *Alocasia sanderiana*

Qualitativeanalysis

Phytochemical characters of *Alocasia sanderiana* leaves were studied and summarized in fig 4.1..*Alocasia sanderiana* leaves showed the presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthroquinone, polyphenol and glycosides, while ethanol and aqueous extracts did shown Of the two extracts, the ethanol extract showed the richest content of phytochemicals and was used for further experimental studies.

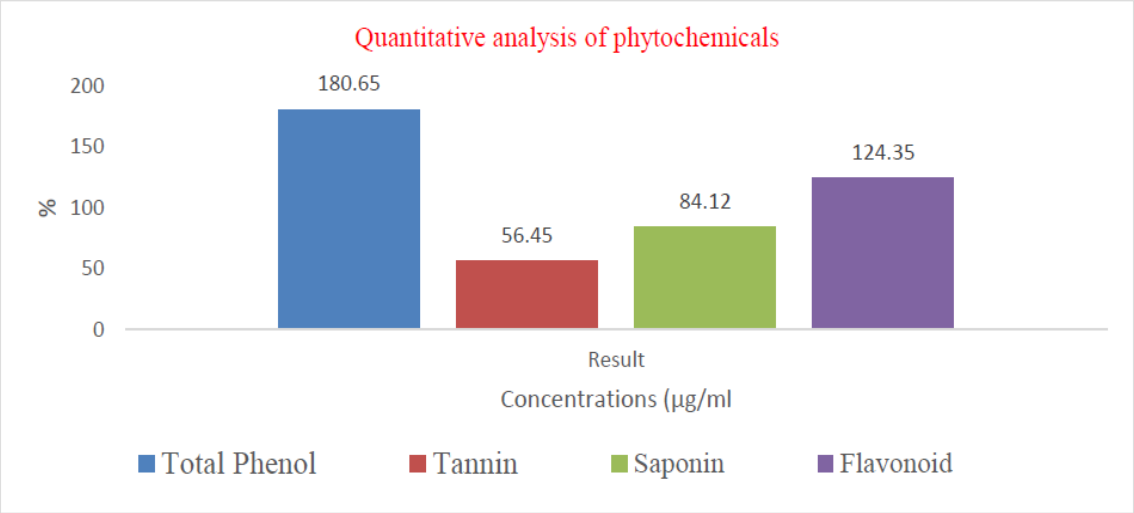
Table 4.1: Preliminary Qualitative phytochemical screening.

S.No	Phytochemical analysis	Ethanolextract	Aqueousextract
1	Tannin	+	+
2	Saponin	++	+
3	Flavonoids	++	+
4	Steroids	+	+
5	Terpenoids	++	+
6	Triterpenodis	+	+
7	Alkaloids	+	+
8	Anthroquinone	+	+
9	Polyphenol	++	+
10	Glycoside	+	+

(+)Presence,(++)High concentration,(-)Absence.

Quantitativeanalysis

Quantitative analysis of *Alocasia sanderiana* showed that total phenol(180.65 ± 21.25mg/g), tannins (56.45 ± 8.45mg/g), saponin (84.12 ± 13.26mg/g) and flavonoids (124mg/g) were 6.1 mg/kg. Table 4.1 and Figure 4.1). The above phytochemicals were tested according to standard methods.

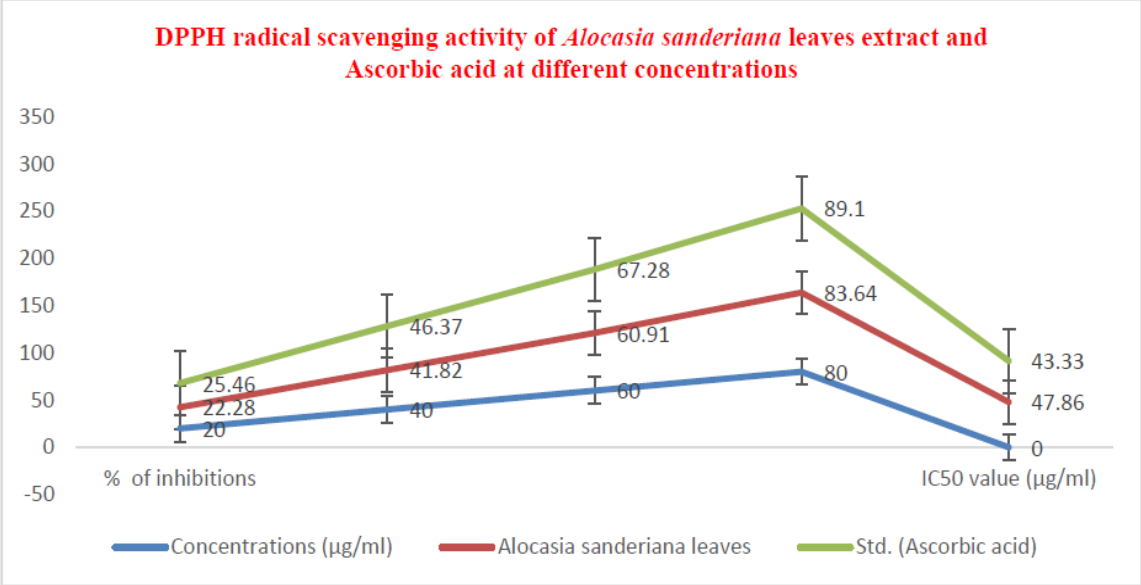


Values are expressed as Mean ± SD for triplicates

***In vitro* antioxidant activity of *Alocasia sanderiana* leaves**

4.1 DPPH radical scavenging activity

The DPPH assay is one of the most widely used methods to screen the antioxidant activity of *Alocasia santeriana* leaf extracts. DPPH radical scavenging activity of *Alocasia sanderiana* leaves and ascorbic acid-like constant is presented in Fig.4.2..Half inhibitory concentration (IC50)of leaves extract and ascorbic acid were 47.86µg/ml and 43.33µg/ml respectively. *Alocasia sanderiana* extract exhibited significant dose-dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to concentration. DPPH assay activity is close to that of ascorbic acid.



Values are expressed as Mean± SD for triplicates

Fig. 4.2: DPPH scavenging activity of leaves extract and Ascorbic acid atdifferent concentrations.

4.2 Total antioxidant activity

Alocasia sanderiana leaf extract and its total antioxidant capacity are given inFigure 4.3. The study revealed that the antioxidant activity of the extract increased with increasing concentration of *Alocasia sanderiana* leaf extract.

The half inhibitory concentration (IC50)of *Alocasia sanderiana* leaves extract and ascorbic acid was 47.70µg/ml-1 and 42.72µg/ml, respectively.

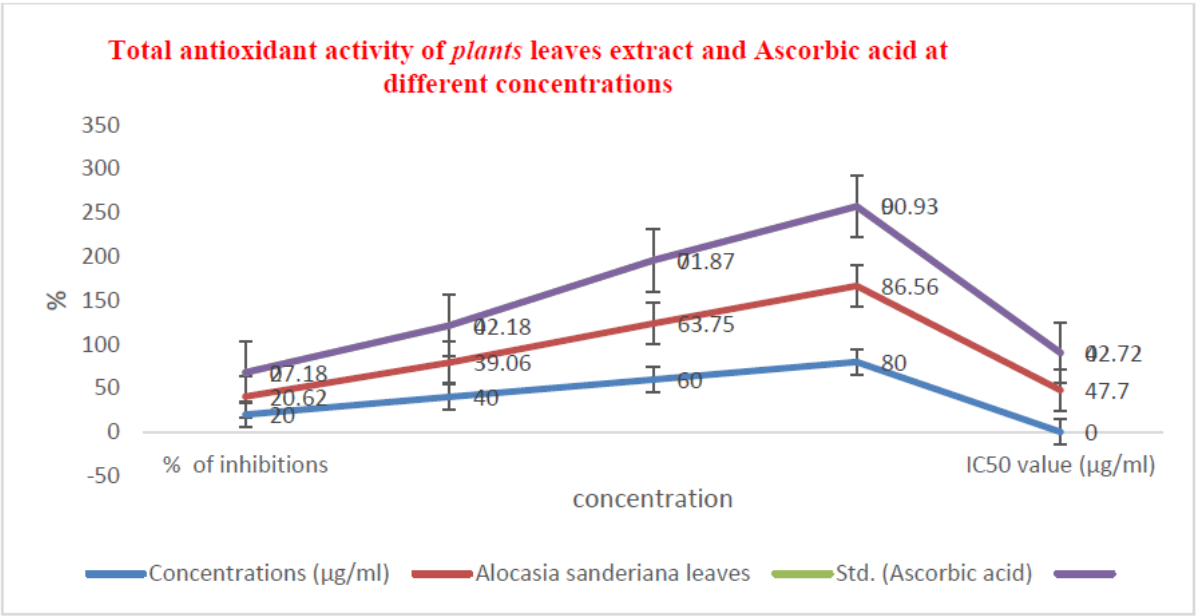


Fig. 4.3: Total antioxidant activity of *Alocasia sanderiana* leaves extract and Ascorbic acid at different concentrations.

4.3 Superoxide Scavenging Activity

Superoxide anion radical scavenging activity of *Alocasia sanderiana* leaf extract by PMS-NADH system is shown in Figure 4.4. The superoxide scavenging activity of *Alocasia sanderiana* increased significantly with increasing concentrations. Half inhibitory concentration (IC50)of *Alocasia sanderiana* was 44.95µg/ml and ascorbic acid was 36.32µg/ml respectively.

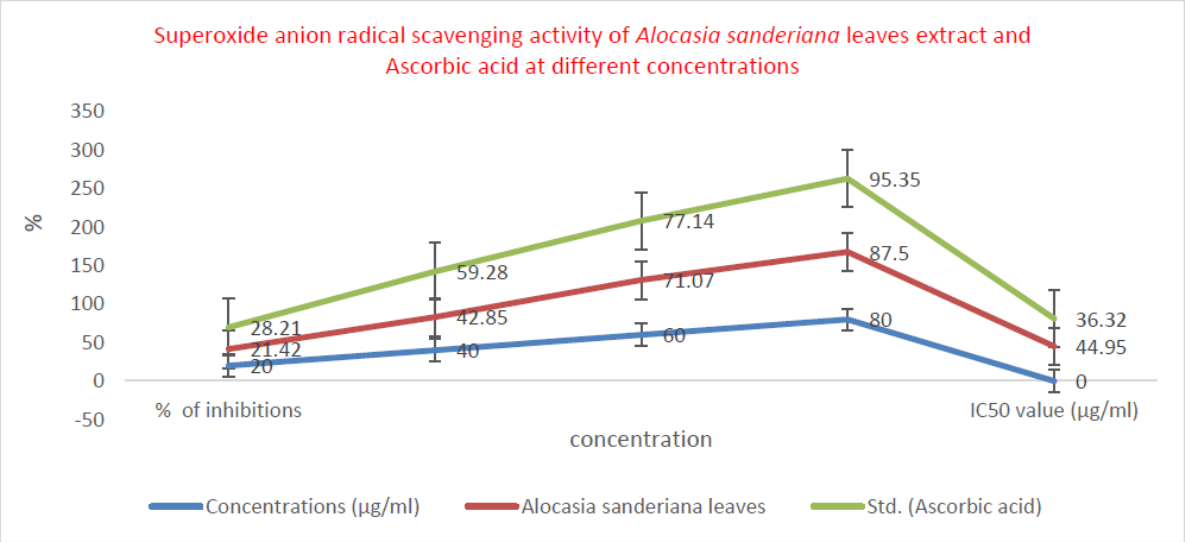


Fig. 4.4: Superoxide radical scavenging activity of *Alocasia sanderiana* leaves extract and Ascorbic acid at different concentrations.

4.4 The ferrous ion chelating activity

The formation of ferrocine-Fe2+ complex was inhibited in the presence of *Alocasia sanderiana* leaf extract which had chelating activity with IC50 of 45.76µg/ml and ascorbic acid 41.72µg/ml respectively (Fig. 4.5).

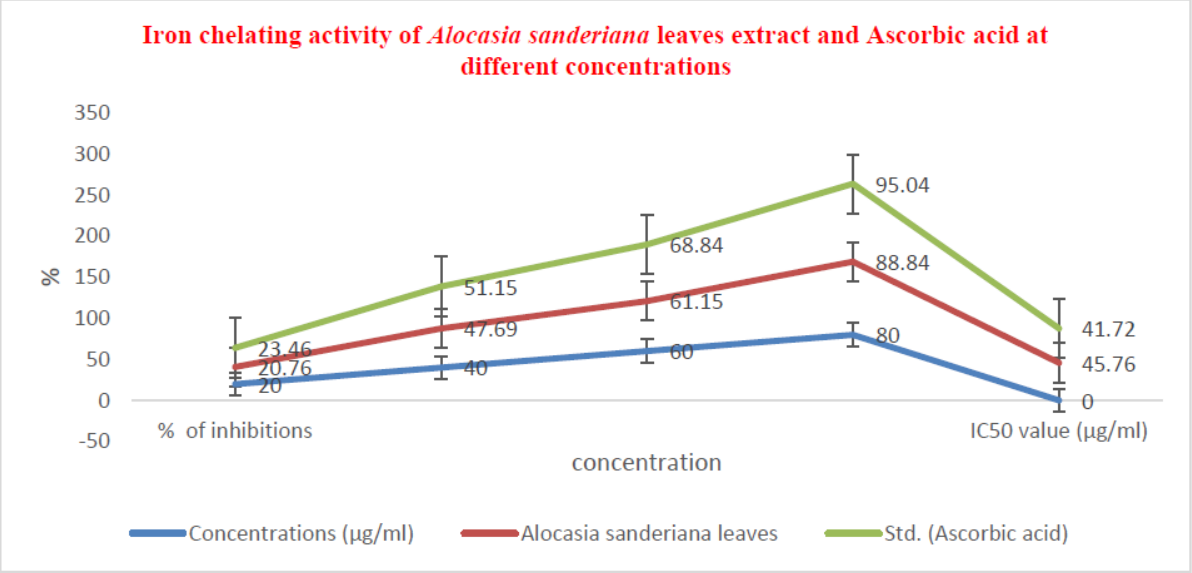


Fig. 4.5: Iron chelating activity of *Alocasia sanderiana* leaves extract and Ascorbic acid at different concentrations.

4.5 Hydroxyl radical scavenging activity

Figure 4.26. Depicting the hydroxyl radical scavenging activity of *Alocasia santeriana* leaves. The hydroxyl radical scavenging activity of *Alocasia sanderiana*leaves increased with increasing. All measures showed significant activity near control. The half inhibitory concentration (IC50)of *Alocasia sanderiana* leaves extract and ascorbic acid was 47.91µg/ml and 38.80µg/ml respectively.

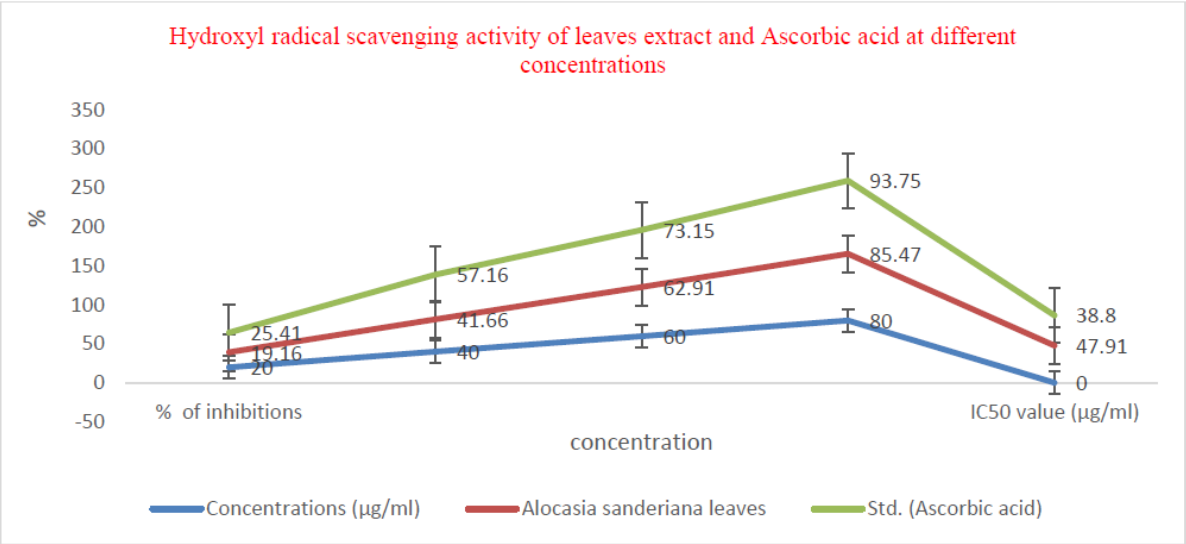


Fig. 4.6: Hydroxyl radical scavenging activity of *Alocasia sanderiana* leavesextract and Ascorbic acid at different concentrations.

4.6 Nitric oxide scavenging activity

Figure 4.7. *Alocasia santeriana* leaves depict nitric oxide scavenging activity. Nitric oxide scavenging activity of *Alocasia sanderiana* increased with increasing concentrations. All measures showed significant activity near control. The half inhibitory concentration (IC50)of *Alocasia sanderiana* leaves extract and ascorbic acid were 47.31µg/ml and 40.93µg/ml, respectively.

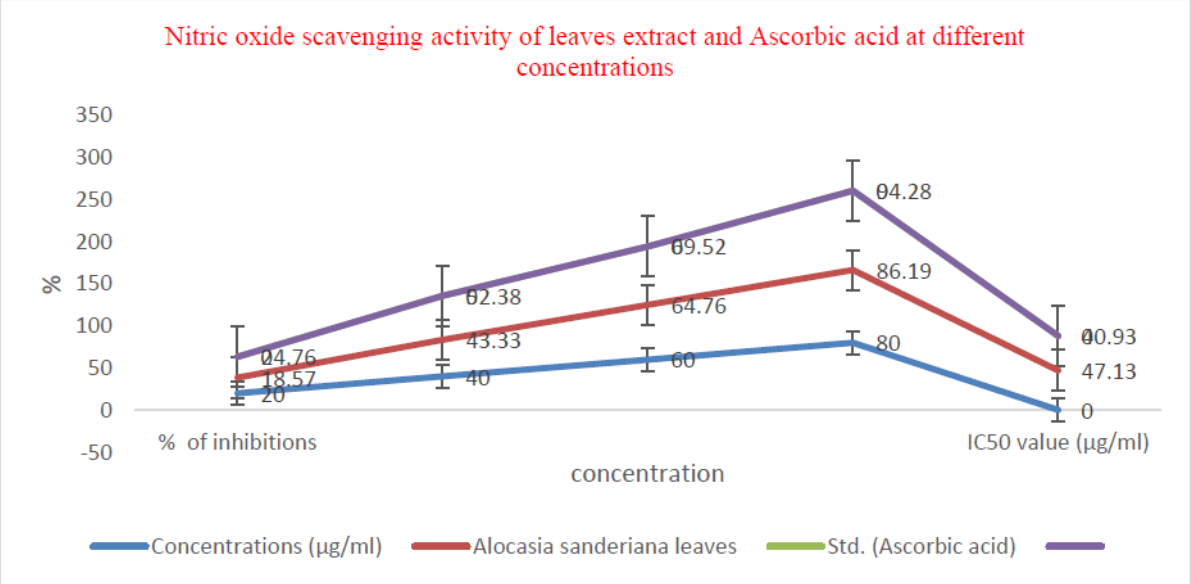


Fig. 4.7: Nitric oxide scavenging activity of *Alocasia sanderiana* leaves extract and Ascorbic acid at different concentrations

4.7 Reducing power activity

Figure 4.8 depicts the reducing effect of *Alocasia santeriana* leaves. Similar to the antioxidant activity, the reducing power of *Alocasia santeriana* leaves increased with increasing dose .*Alocasia sanderiana* leaves have been shown to contain hydrophilic polyphenolic compounds.

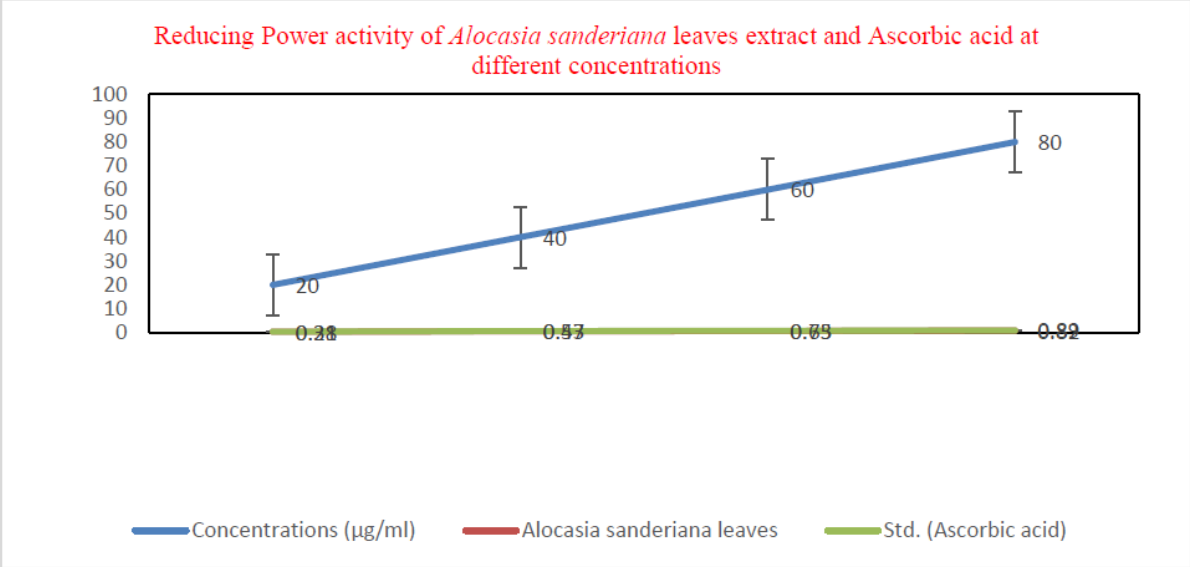


Fig. 4.8: Reducing Power activity of *Alocasias anderiana*leaves extract and Ascorbic acid at different concentrations.

Anti-diabetic activity

Anti-diabetic activity of the leaf, a Methanolic extracts of sanderiana were determined by experimental methods. The α-amylase inhibitory activity and α- glucosidase inhibitory activity methods were used to evaluate the Anti-diabetic potential. Inhibits α-amylase activity in vitro.

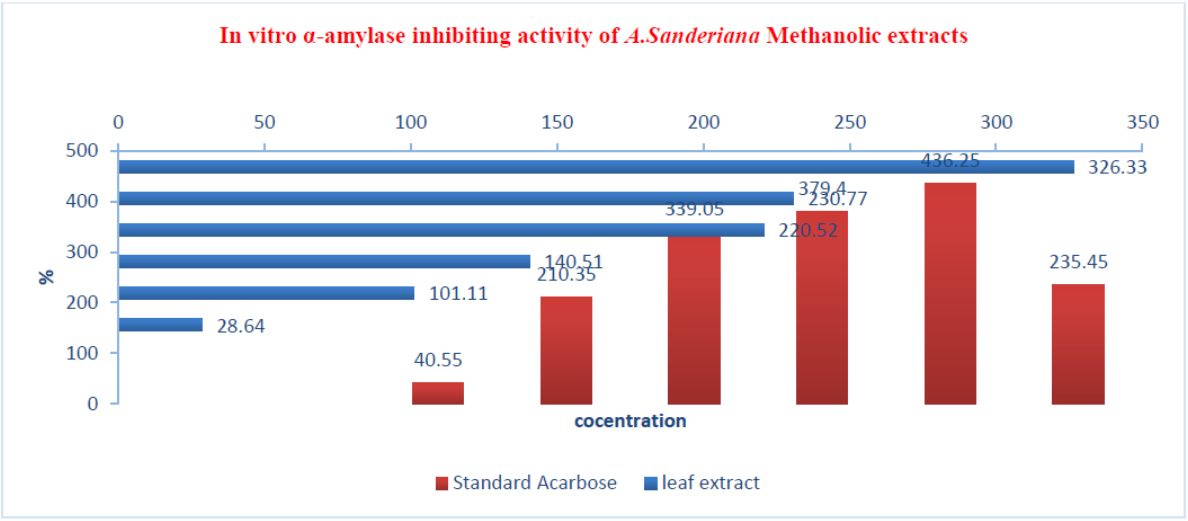


Fig 4.9: *In vitro* α-amylase inhibiting activity of *A.Sanderiana* Methanolicextracts.

Table 4.2: *In vitro* α-amylase inhibiting activity of leaf extracts.

Extract concentration (µg/mL)	leaf extract	Standard Agarose
100	28.64	40.55
200	101.11	210.35
300	140.51	339.05
400	220.52	379.4
500	230.77	436.25
IC_{50} (µg/mL)	326.33	235.45

*Values are means of three independent analysis ± Standard Deviation (n=3)

***In vitro* α-amylase inhibiting activity of leaf extracts**

Methanolic leaf extract exhibited alpha amylase inhibitory activity at different concentrations as shown in Table 9. It showed maximum inhibition % of 23.77±1.75at 500 µg/mL. The IC50 value for the leaf extract was found to be 326.33±28.42 µg/mL.Agarose was used as

standard. The percent inhibition values of the sample leaf extract were comparable to that of agarose. In vitro α-glucosidase inhibition assay of leaf extract.

The alpha glucosidase inhibitory activity of methanolic leaf extract of A.Sanderiana was investigated in this study and the results are shown in Table 4.2 and Figure 17. The extract of leaf, stem and rhizomes were compared with percentage of alpha glucosidase inhibition (IC50 values). value of standard agarose (235.45±0.10%) as shown in Figure 4. 19.

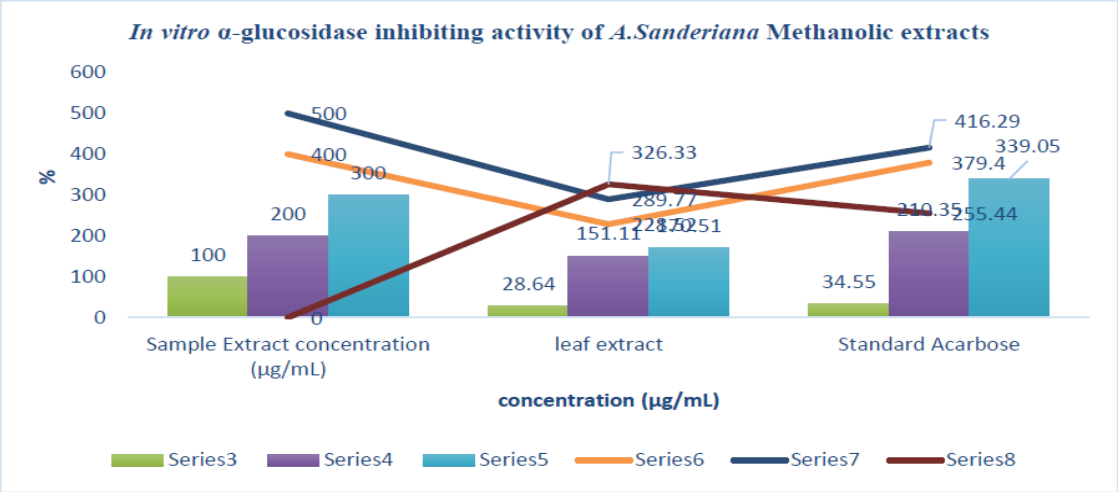


Fig 4.10: ***In vitro*** **α-glucosidase inhibiting activity of** ***A.Sanderiana.***

Table 4.3: ***In vitro*** **α-glucosidase inhibiting activity of** ***A.Sanderiana*** **Methanolic extracts.**

Sample Extract concentration(µg/mL)	leaf extract	Standard Acarbose
100	28.64	34.55
200	151.11	210.35
300	170.51	339.05
400	228.52	379.4
500	289.77	416.29
IC_{50} (µg/mL)	326.33	255.44

*Values are means of three independent analysis ± Standard Deviation (n=3)

Methanolic leaf extract exhibited alpha glucosidase inhibitory activity at different concentrations as shown in Table 10. It showed maximum inhibition % of 34.55±1.05 at 500µg/mL. IC50 value was found to be **326.33**±5.42% for leaf extract. Acarbose was used as standard. Sample leaf extract percentage inhibition values arecomparable with that of acarbose.

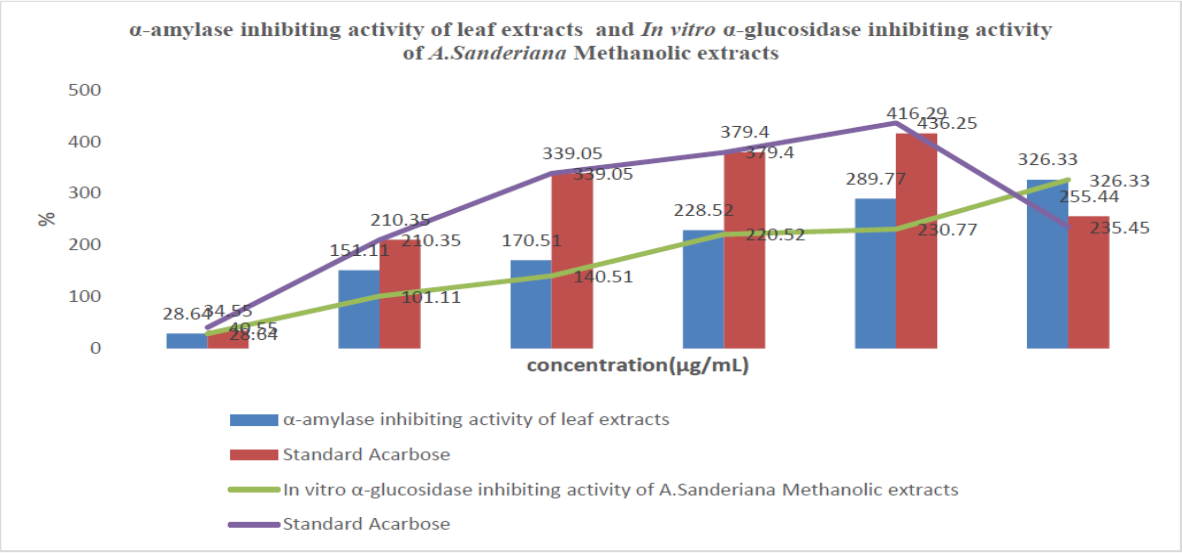


Fig.4.11: ***In vitro*** **α-glucosidase & α-amylase inhibiting activity of** ***A.Sanderiana*** **Methanolic extracts.**

CONCLUSION

The qualitative phytochemical characters of *Alocasia sanderiana* leaf extract include tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthroquinone, polyphenol and glycosides. Total phenol (180.65 ± 21.25mg/gm), tannins (56.45 ± 8.45mg/gm), saponin (84.12 ± 13.26mg/gm) and flavonoids (124.35± 1m/gm) were provided. *Alocasia sanderiana* leaf extract exhibited the greatest antioxidant activity through DPPH, superoxide anion scavenging, metal chelator (iron chelator and iron reducing power) and nitric oxide scavenging activity. The α- amylase inhibitory activity and α-glucosidase inhibitory activity methods were used to evaluate the anti-diabetic potential.

Overall, it can be concluded from the present study that *Alocasia santeriana* leaves are rich sources of phytochemicals and natural antioxidants with anti-diabetic activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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