ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF LEAVES OF *ALPINIA PURPURATA* BY ALPHA AMYLASE AND ALPHA GLUCOSIDASE ASSAY METHOD

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Abstract

Diabetes mellitus (DM) is a kind of disorders distinguished by high levels of blood glucose arising from lacks in insulin secretion, insulin function, or both. The symptoms of diabetes mellitus regard long-term damage, dysfunction and failure of various organs. Therefore alternative treatments are of high interest means by using medicinal plants or phytotherapy. The present study performedto qualitative and quantitative analysis of bioactive compound from the leaves of *Alpinia purpurata*and antimicrobial activity of *Alpinia purpurata*leaves by disc diffusion assay method.The antidiabetic and antioxidant activity of leaves of *Alpinia purpurata* were done by alpha amylase and alpha glucosidase assay method. The methanolic extract was also explored for its antioxidant activities by using free radical 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging method. The study revealed that the different concentration of plant extracts exhibit potent radical scavenging activity by using DPPH as substrate. The leaves of *Alpinia purpurata*showed significant α-amylase (74%) and α-glucosidase (78%) inhibitory activities at the concentration 100 µg/ml severally and well analyzed with standard acarbose drug. Therefore, it is recommended that the methanolic extract of leaves of *Alpinia purpurata* is a potential source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetes mellitus.

Key words: *Alpinia purpurata* leaves, Alpha amylase inhibitory activity, Alpha glucosidase inhibitory activity, Antioxidant activities, Antimicrobial activity.

INTRODUCTION

In recent years, there has been revived interest within the treatment against totally different diseases victimisation seasoner medication, as they're usually non-toxic and World Health Organization has counseled itseffectiveness rather than the precarious modern drugs. Plant derivatives with hypoglycemic properties have been used in ayurvedic medicine and traditional healing systems around the world (Yeh et al., 2003) from ancient time.Despite, the introduction of symptom agents from natural and artificial sources, diabetes and its secondary complications continue to be a major medical problem to people (Ravi et al., 2005).Medicinal plants accustomed treat symptom and hyperglycemic conditions ar of tidy interest to ethnobotanical community because the plants contain valuable healthful properties in its totally different components.In traditional medicine, diabetes is treated with diet, physical exercise and medicinal plants.Even although, more than 1200 plants were used in the control of diabetes mellitus, approximately 30% of the antidiabetic plants were pharmacologically and chemically investigated (Alarcon et al., 2002).

The use of herbal medicine is now wide spread for the treatment of various diseases and disorders, it is redundant (Schulz et al., 2001).The use of pharmaceuticals has led to unforeseen side effects such as genetic alterations, bio magnifications and even death.

Unforeseen side effects often appear after a drug has been on the market for years and is taken by many.Drug testingdoesn'tnoticethese effects, as the number of patients in trials is not generally high enough.Also, trials are controlled by the company that wants the medicine approved, they are slanted to find efficacy and safety (Nicholas et al., 2008).

On the other hand, the use of herbal medicines has several advantages. One advantage is its wide availability and simple in preparation.Plantswillcontain sugars, minerals, proteins and other chemicals that interact with the active chemical in a variety of ways viz.they may concentrate or intensify its effect, they may make it easier to digest or absorb, or they may lessen its harsh or toxic side effects (Jellin et al., 2002).

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Alpinia purpurata* were collected in the month of December from the mullipatti, pudukkottai, Tamil Nadu, India. The plant was identified and leaves of *Alpiniapurpurata* authenticated and confirmed from Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu. The voucher specimen number SGP001 (17.12.2018).

Preparation of plant Extracts : The Plantmethanol extractions were prepared by using standand protocal. The extracts were filteredby using whatman filter paper No. 42 (125mm) to remove all unextractable matter, including cellular materials. Thetotal amount of extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labelled sterile bottles and kept at -20^{o}C. The obtainedfiltrate was used as sample solution for the further isolation (Rich*et al*., 2015).

QualitativePhytochemical screening:

Various Phytochemical screenings such as Tannins, Phlobatannins, Saponins,Flavanoids, Steroids Terpenoids, Cardiac Glycosides, Leucoanthocyanin, Anthocyanins, Anthroquinone,Proteins, Coumarins, Glycosides, Phenols, Alkaloids Xanthoproteins, Emodin, Carbohydrates wereperformed separately in Leaves of *Alpinia purpurata* using standard procedures(Grover*et al.*, 2014).The presence of above phytochemicals confirmed by visual observations of colour change orthe precipitates formationafter the addition of specific reagents(Mahmuda *et al.*, 2018)(Table 2).

Antioxidant Activity (DPPH Free Radical Scavenging Activity) Determination:

The leave of *Alpinia purpurata* antioxidant activity was studied on the basis of the scavenging effect on the stable DPPH free radical activity (Braca et al., 2002). The radical scavenging activities of the tested samples expressed as percentage of inhibition were calculated according to the following equation (Yen and Duh, 1994).

Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100

Where B and A are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

Alpha-Amylase Inhibitory Assay : This assay was carried out using a modified procedure of McCue and Shetty, 2004. The percentage of inhibition was calculated by α-amylase inhibitory activity.

% Inhibition = [(Abs control – Abs methanol extract) / Abs control] x 100Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

Alpha-Glucosidase Inhibitory Assay : The α-glucosidase activity of methanolic extract was determined according to the method described by Kim *et al.*, 2005 using α-glucosidase from *Saccharomyces cerevisiae.* The percentage of inhibition was calculated by α-glucosidase inhibitory activity.

%Inhibition = [(Abs control – Abs methanol) / Abs control] x 100Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

Antimicrobial Activity of leaves of *Alpinia purpurata*

Specific Aim: The purpose of this study was to examined the antibacterial and antifungal activity of the crude methanolic extracts toward selected pathogens using disc diffusion method. Antibacterial activity of methanolic extract of leaves of *Alpinia purpurata* (Disc diffusion method).

Collection of test organisms: To examine the antibacterial activity of plant extract, three strains *Escherichia coli* (MTCC 25922), *Enterococcus aerogenes* (MTCC 29212). *Pseudomonas aeruginosa* (MTCC 27853),] were prepared as test organisms. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on the nutrient agar (Difco, USA) slant at for 4ºC.

Screening of Antibacterial Activities: Antibacterial activity of crude methanolic extract was determined using the disc diffusion method. The zone of inhibition was measured in millimeters and the experiment was repeated twice (Karumaran *et al*., 2016).

Screening of Antifungal Activities: The clinical fungal test organisms used for study are *Candida albicans* (MTCC 282), *Candida tropicalis* (MTCC No.184) *Aspergillus niger*, (MTCC 227), were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India. Antifungal activity of methanolic extract of leaves of *Alpinia purpurata*was determined using disc diffusion method. The zone of inhibition was recorded in millimetres (Vivek et al., 2013).

RESULT AND DISCUSSION:

Phytochemical screening of methanolic extract of leaves of *Alpinia purpurata*

Qualitative analysis

Presence of different phytochemical compounds viz, flavonoids, tannins phlobatannins, saponin, flavonoids, steroids, terpennoids, cardiac glycosides, leucoanthocyanin, anthocyanins, anthoquines, proteins, coumains, glycosides, phenols, alkaloids, xanthoproteins, emodine and carbohydrate were analysed in methanol extract of leaves of *Alpinia purpurata*.(figure:1)

The methanolic extract of leaves of *Alpinia purpurata* indicated the presence of phlobatannin, saponin, flavonoids, tannin, steroids, texpenoids, cardiac glycosides, leucoanthocyanin, protein, coumarin, glycosides, phenol, alkaloids, and carbohydrate and absence of anthoquione, xanthoprotein and emodine.(Table:1)

The previous study suggested that the presence of alkaloids, tannins, steroids, flavonoids, saponins, phlobanninns and phenolics (Juna beegum *et. al.*, 2014), steroids, sugars and alkaloids (Babita agrawal *et. al.*, 2011), in the ethanolic extract of whole plant of *Alpinia purpurata*.

The *Alpinia purpurata* leaves contain rich amount of alkaloids and steroids including ursolic acid and hypoxanthine. These alkaloids and hypoxanthine which is responsible for the antioxidant and antidiabetic activity (Amarnath satheesh. and Pari. 2004).

Juna beegum and her co-workers reported that phlobatanninn was found only in methanolic extract. They observed all the phtochemical compounds in methanolic extract and flavonoids having antioxidant property and it protect tissue against oxygen free radicals. The main role of flavonoids is to prevent the atherosclerosis, cancer, chronic inflammation(Juna beegum. *et al*., 2014).

The punarnava was used for the treatment of hepatic disorders (Babita et. al., 2011). Punarnava that it contain alkaloids, carbohydrates, glycosides, triterpenoids, steroids, phenols and tannins (Meera sumanth and Mustafa 2007). *Alpinia purpurata* leaves contains alkaloids, flavonoids, amino acids, lignans-sitosterols, tetracosanoic, esacosanoic, stearic and ursolic acids. It was reported by Kuldeep rajpoot.

Table:1- qualitative analysis of ethanol extract of leaves of *Alpinia purpurata*

S.No	**Phytochemical Constituents**	***Alpinia Pirpurata***
1	Tannin	+++
2	Phlobatannin	+++
3	Saphonin	+
4	Flavonoids	+++
5	Steroids	+++
6	Terpenoids	+++
7	Cardiac glycosides	+++
8	Leuco anthocyanin	+
9	Anthocyanine	+++
10	Anthoquione	+++
11	Protein	-
12	Coumarin	++
13	Glycosidase	-
14	Phenol	+++
15	Alkaloids	+++
16	Xanthoprotein	+++

17	Emodine	+++
18	Carbohydrate	-

(+ =slightly present, ++ = moderately present , +++ = strongly present)

Quantitative determination of phenolic compounds ofcrude extract

Determination of total phenolic content (TPC): Quantitative anlaysis of important phytochemicals in the medicinal plant of *Alpinia purpurata* contain these phytochemicals in varying amounts in the leaves. The phytochemical with the highest quantity was alkaloids followed by saponin, flavonoids, phenol, tannin and terpenoids respectively, as shown in (table 2). The highest concentration of Alkaloids (0.002 mg/g), Saponin(0.001mg/g), Flavonoids(0.007mg/g), Phenol (0.002mg/g), Tannin(0.024mg/g) and Terpenoids(0.008mg/g) respectively.(figure :2)

Federico ferrered et. al., 2005 were use HPLC-ESI/MS method to derive the phenolic compounds from the leaf and roof of *Alpinia purpurata* .The root and leaves of *Alpinia purpurata* contain high level of flavonoids and S.angustifolia. Flavonoids used to prevent the oxidative cell damage, have strong anticancer activity (Aliyu *etal*.,2008).

Alpinia purpurata contain high concentration of flavonoids followed by alkaloids, glycosides and sterols have been reported to be present in the alcoholic root extracts. The antistress activity of *Alpinia purpurata* that contain antioxidant activity (kuldeep rajpoot, Mishra, 2011). As can be seen from the above results that the methanolic extract showed that the highest concentrations levels for alkaloids, saponin, phenols, tannin and terpenoids.

Table.2: Quantitative analysis of ethanolic extract of leaves of *Alpinia purpurata*

S.No	**Phytochemical Constituents**	***Alpinia purpurata*(Mg/g)**
1.	Flavonoids	0.007
2.	Tannin	0.024
3.	Alkaloids	0.002
4.	Saponin	0.001
5.	Terpenoids	0.008
6.	Phenol	0.002

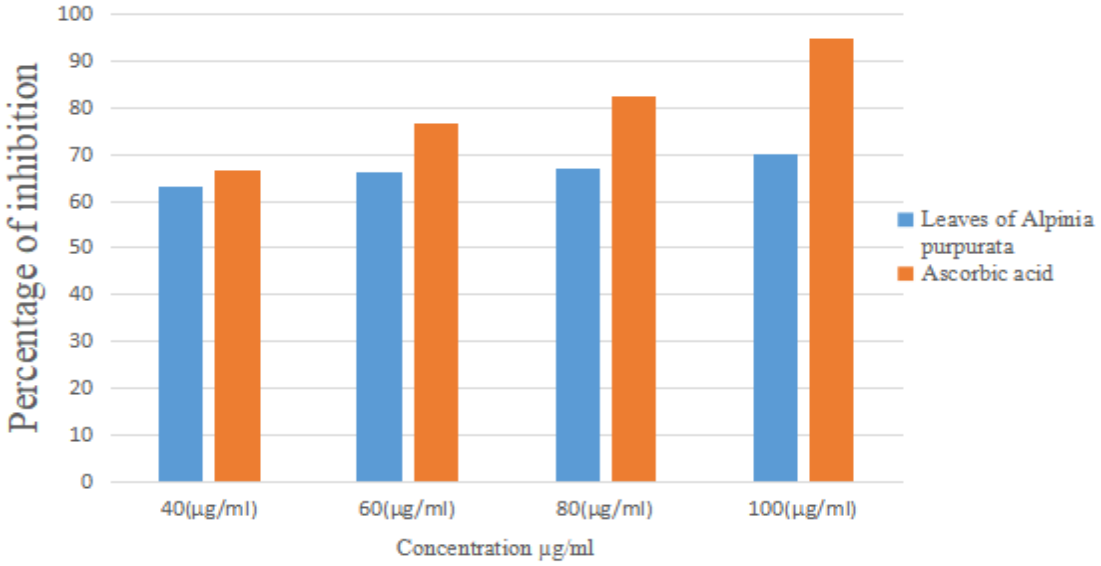
Antioxidant activity of leaves of *Alpinia purpurata* by DPPH method

The result showed that the leaves of *Alpinia purpurata* had better percentage antioxidant activities at high concentrations when compared with ascorbic acid (Table 3). The compound showed 70 % activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration (fig. 3). The previous study suggested that the lupeol has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes levels (Manikandan R.*et al.*, 2013)

Table-3: Antioxidant activity of leaves of *Alpinia purpurata* by DPPH activity

S.No	Concentrations	Scavenging Effect (%)	
		Leaves of *Alpinia purpurata*	**Ascorbic acid**
1	20 (µg/ml)	58±0.72	41.60±1.33
2	40 (µg/ml)	63±0.63	66.85±1.37
3	60 (µg/ml)	66±0.59	76.74±1.42
4	80 (µg/ml)	67±0.56	82.34±1.47
5	100 (µg/ml)	70±0.52	94.69±1.50

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM



In vitro alpha amylase inhibitory assay

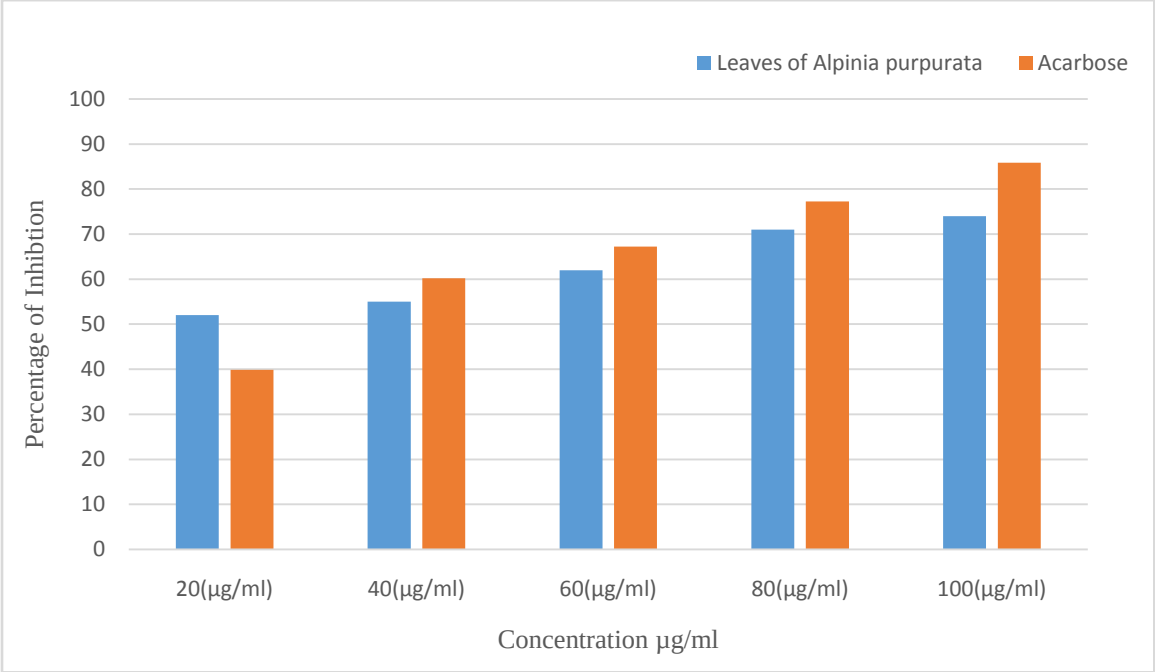
In this study the in vitro alpha amylase inhibitory activities of the methanolic extract of leaves of *Alpinia purpurata* was investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against alpha amylase enzyme. The leaves of *Alpinia purpurata* (20-100 µg/ml) of the various concentrations exhibited potent α-amylase inhibitory activity in a dose dependent manner. The leaves of *Alpinia purpurata* showed inhibitory activity from 52±0.62 to 74±0.33% at concentration 100 µg/ml (Table 4). Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 39.85±0.24 to 85.87±0.37% at the same concentrations 100 µg/ml. A comparison of α–amylase inhibitory activity between the standard drug has been depicted in fig. 4. Our results are in accordance with the previous study wherein, there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal α-glucosidase and pancreatic α-amylase (Sincy Joseph et al., 2016). The isolated compounds were tested for their antidiabetic potential in vitro by inhibition of α-amylase enzyme. Total saponins,

Lupeol and stigmasterol showed higher alpha amylase inhibitory activity which confirms its antidiabetic potential was reported (Space JC et al., 2003).

Table-4:*In vitro* antidiabetic activity of the leaves of *Alpinia purpurata* using alpha amylase method and comparison with standard drug acarbose.

S.No	Concentrations	Alpha amylase (%)	
		Leaves of *Alpinia purpurata*	**Acarbose**
1	20 (µg/ml)	52±0.62	39.85±0.24
2	40 (µg/ml)	55±0.53	60.21±1.37
3	60 (µg/ml)	62±0.49	67.20±1.42
4	80 (µg/ml)	71±0.37	77.25±1.47
5	100 (µg/ml)	74±0.33	85.87±0.37

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM



In Vitroα-glucosidase inhibitory assay

The results of antidiabetic activity using α- glucosidase inhibitory assay of the methanolic extract of leaves of *Alpinia purpurata* are shown in Table 5. The extracts revealed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition at 20-100 µg/ ml concentrations of extracts showed a dose dependent increase in percentage inhibition.

The percentage inhibition varied from 44±0.67 -78±0.26 for highest concentration to the lowest concentration. Thus the inhibition of the activity of α-glucosidase by extracts would delay the degradation of carbohydrate, which would in turn cause a decrease in

the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation. A comparison of α–glucosidase inhibitory activity between the standard drug has been depicted in fig. 5.

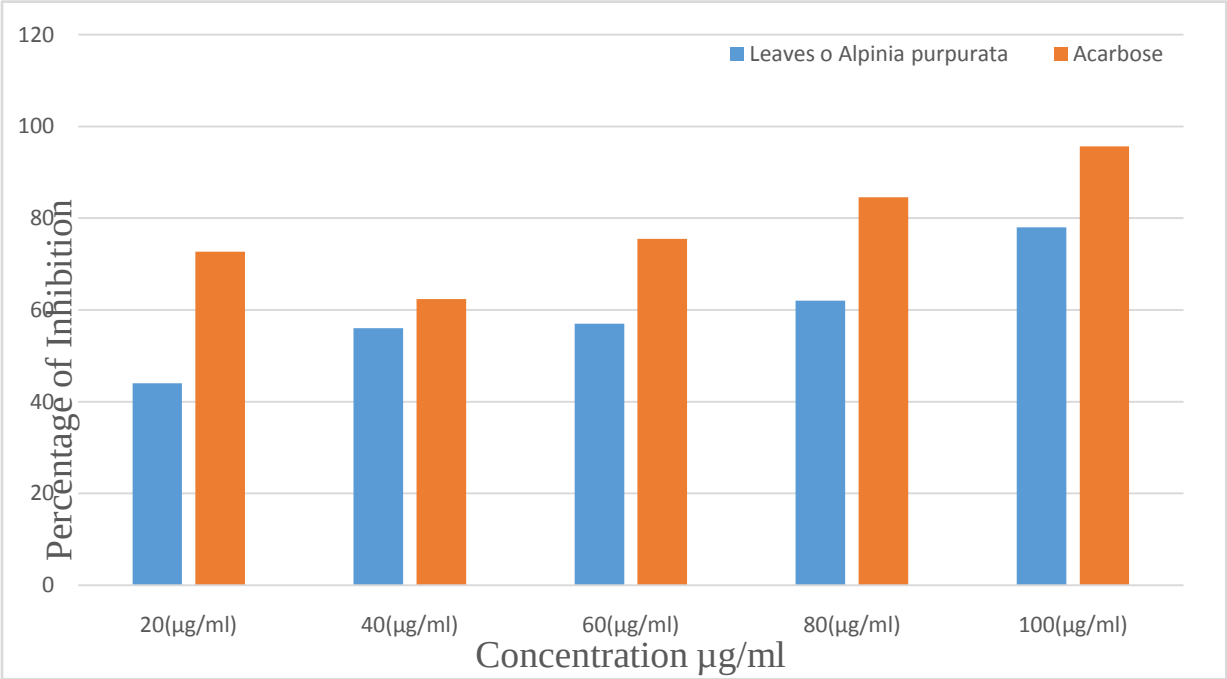
In this study acarbose was also used as a standard drug for α-glycosidase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-glucosidase inhibitory activity from 72.70 ±1.40 to 95.68±1.38 % with an IC50 value 45.03±1.03 µg/ml. This indicates that the leaves of *Alpinia purpurata* is very potent α-amylase and α-glucosidase inhibitor in comparison with acarbose (Mai, TT et al., 2003).

The hypoglycemic activity of crude extracts and isolated compounds (lupeol acetate, cis-p-coumaric acid, lupeol, β-sitosterol, trans-p-coumaric acid, linoleic acid, (+)-catechin, afzelin and quercitrin) was assessed by the ability to inhibit α-amylase and α-glucosidase enzymes (María et al., 2016.)

Table-5: *In vitro* antidiabetic activity of the leaves of *Alpinia purpurata* using alpha glycosidase method and comparison with standard drug acarbose.

S.No	**Concentrations**	**Alpha glycosidase (%)**	
		leaves of *Alpinia purpurata*	**Acarbose**
1	20 (µg/ml)	44±0.67	72.70 ±1.40
2	40 (µg/ml)	56±0.53	62.34±1.37
3	60 (µg/ml)	57±0.52	75.48±1.42
4	80 (µg/ml)	61±0.47	84.54±1.47
5	100 (µg/ml)	78±0.26	95.68±1.38

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM



Antibacterial activity of methanolic extract of leaves of *Alpinia purpurata* by disc **diffusion assay method**

The results of the antibacterial activity of crude extracts were tested against pathogens by disk diffusion method are shown in (Table 6). The crude extracts showed growth inhibitory activity against and *Escherichia coli* (10 mm) at concentration 250 mg/ml. At concentration 200 mg/ml, the crude extracts exhibited the antibacterial activity all the five bacteria, but was more susceptible against *Escherichia coli*. However, the crude extract showed better inhibitory actions against pathogens at a concentration 150, 200 and 250 mg/ml than at lower concentration. As the concentration of extracts increased from 50-250 mg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study. (Fig: 6)

Table 6: Antibacterial activity of methanolic extract of leaves of *Alpinia purpurata*

Plant extracts	**Concentrations (µg/ml)**	**Organisms/Zone of inhibition (mm)**		
		methanolic extract of leaves of *Alpinia purpurata*		
		Escherichia coli	***Enterococcus aerogenes***	***Pseudomonasaeruginosa***
Extracts	50	6	0	6
	100	7	0	6
	150	8	0	6
	200	9	7	7

	250	10	8	8
Methanol	10 µl/disc	0	0	0

Antifungal activity of methanolic extract of leaves of *Alpinia purpurata*

Results of the antifungal susceptibility test of the different plant extracts and against the test organisms. From the result, the methanolic extracts were the most effective and the highest activity was demonstrated against Aspergillus niger (11 mm zone of inhibition) at 250 mg\ml, followed by the highest activity against Candida albicans (10 mm zone of inhibition) at 250 mg\ml) and At concentration 200 mg/ml, the crude extracts exhibited the antifungal activity all the five bacteria, but was more susceptible against Aspergillus niger (10 mm), Candida albicans (9 mm). However, the crude extract showed better inhibitory actions against pathogens at a concentration 150, 200 and 250 mg/ml than at lower concentration. As the concentration of extracts increased from 50-250 mg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study. (Fig: 7)

Table 7 Antifungal activity of methanolic extract of leaves of *Alpinia purpurata*

Plant extracts	**Concentration (µg/ml)**	**Organisms/Zone of inhibition (mm)**		
		Methanolic extract of leaves of *Alpinia purpurata*		
		Candida albicans	***Candida tropicalis***	***Aspergillus niger***
Extracts	50 100 150 200 250	0 0 8 9 10	0 0 6 7 8	0 8 9 10 11
Methanol	10 µl/disc	0	0	0

Figure:1- qualitative analysis of methanolic extract of leaves of *Alpinia purpurata*

Figure2: quantitative analysis of methanolic extract of leavesof *Alpinia purpurata*

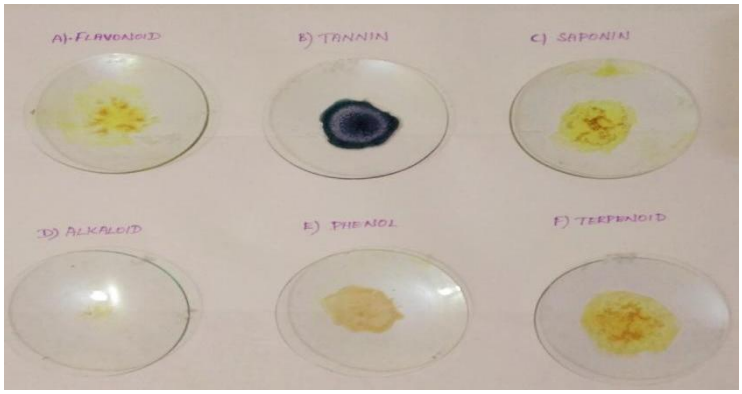


Fig.3. Antioxidant activity of leaves of *Alpinia purpurata* by DPPH activity

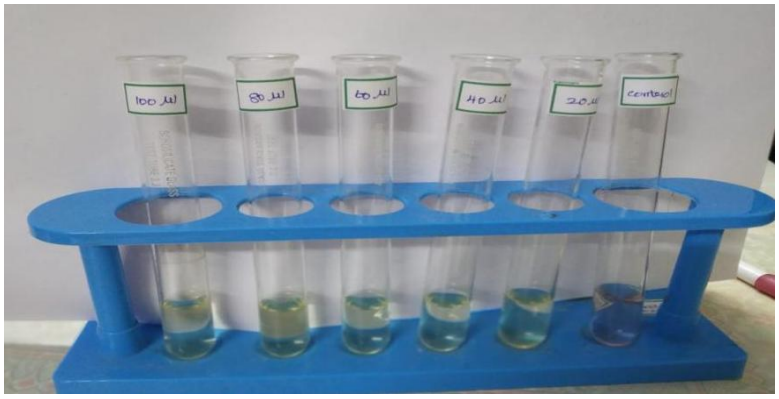


Fig. 4. α-Amylase inhibitory activity of Acarbose vs leaves of *Alpinia purpurata*

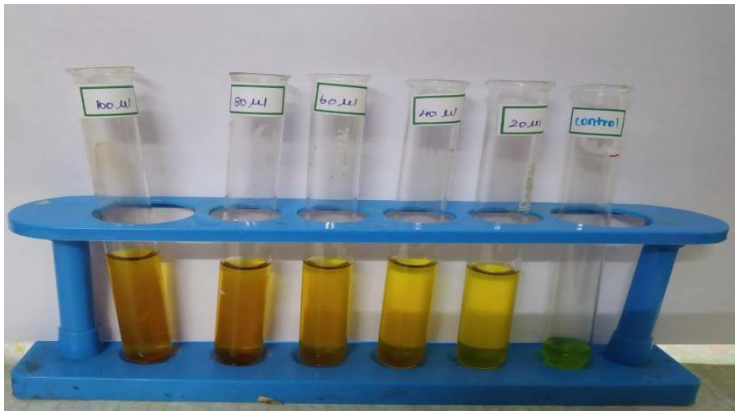


Fig. 5. **α**- glycosidase inhibitory activity of Acarbose vs leaves of *Alpinia purpurata*

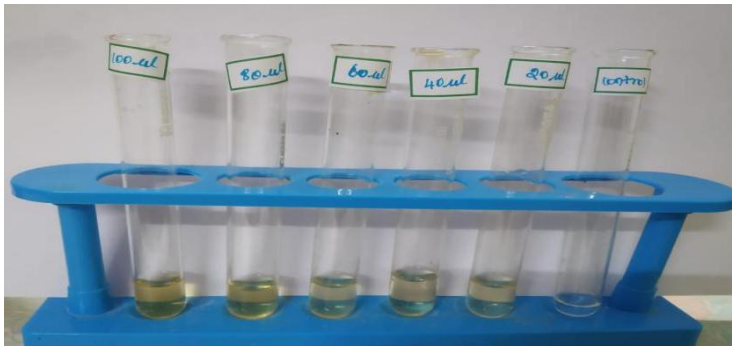
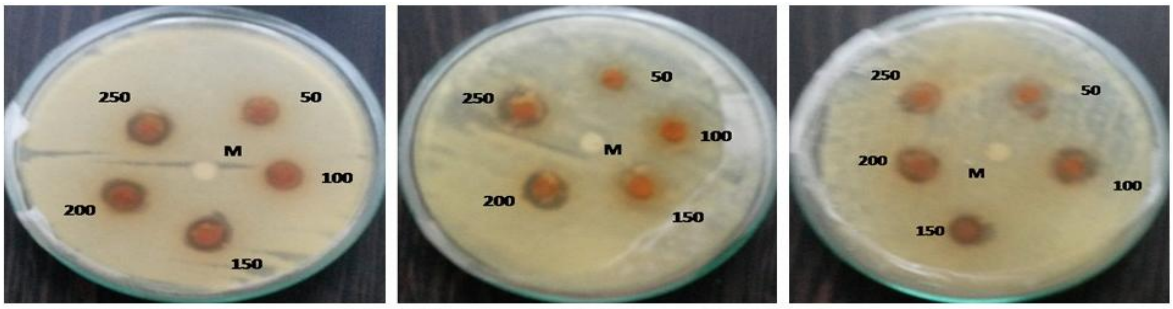
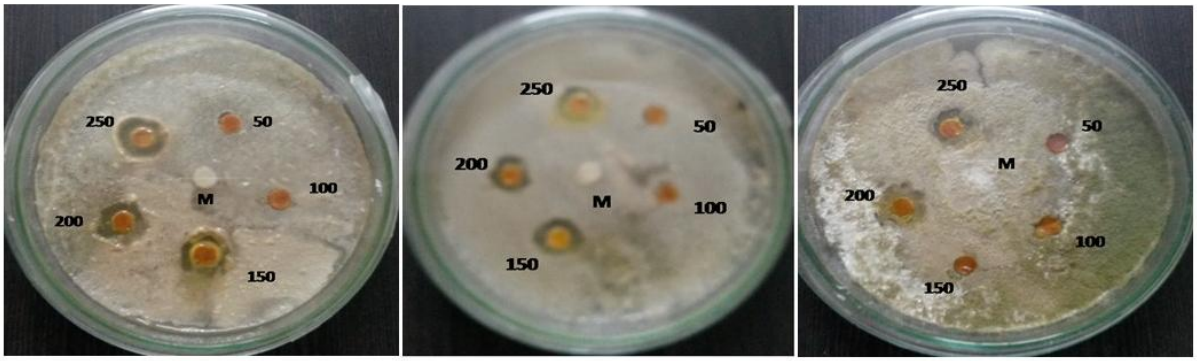


Fig: 6 Antibacterial activity of methanolic extract of leaves of *Alpinia purpurata*



Activity of Alpinia purpurata against Escherchia coli | Activity of Alpinia purpurata against *Enterococcus aerogenes* | Activity of Alpinia purpurata against *Pseudomonas aeruginosa*

Fig: 7Antifungal activity of methanolic extract of leaves of *Alpinia purpurata*



Activity of Alpinia purpurata against *Candida albicans* | Activity of Alpinia purpurata against *Candida tropicalis* | Activity of Alpinia purpurata against *Aspergillus niger*

SUMMARY AND CONCLUSIONS

The methanolic extract of leaves of *Alpinia purpurata* indicated the presence of phlobatannin, saponin, flavonoids, tannin, steroids, texpenoids, cardiac glycosides, leucoanthocyanin, protein, coumarin, glycosides, phenol, alkaloids, and carbohydrate and absence of anthoquione, xanthoprotein and emodine. The phytochemical with the highest quantity was alkaloids followed by saponin, flavonoids, phenol, tannin and terpenoids respectively. The highest concentration of alkaloids (0.002 mg/g), saponin(0.001mg/g), flavonoids(0.007mg/g),phenol (0.002mg/g), tannin(0.024mg/g) and terpenoids(0.008mg/g) respectively.

The result showed that the leaves of *Alpinia purpurata* had better percentage antioxidant activities at high concentrations when compared with ascorbic acid. The compound showed 85 % activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration. The various concentrations of leaves of *Alpinia purpurata* (20-100 µg/ml) exhibited potent α-amylase inhibitory activity in a dose dependent manner. The leaves of *Alpinia purpurata* showed inhibitory activity from 28±0.25 to 70±0.37% at concentration 100 µg/ml. The extracts showed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition at 20-100 µg/ ml concentrations of extracts showed a dose dependent increase in percentage inhibition.

The *Alpinia purpurata*leaves showed significant enzyme inhibitory activity. So the *Alpinia purpurata*leaves compound which are responsible for inhibiting activity. The *Alpinia purpurata*leaves have to be done for the usage of antidiabetic agent. The antioxidant and antidiabetic activities of the methanolic extract of leaves of *Alpinia purpurata* has been investigated and analysed successfully. As a result, we found that the extracts have free radical scavenging activity and inhibitory activity against

α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus.

However further research on detailed isolation of another active phytoconstituents possessing the therapeutic activity and clinical study for the evaluation of safety and efficacy of the drug needs to be assessed.

Reference

Alarcon, A.F.J. Roman, R.R. Flores, S.J.L. and Aguirre, G.F. 2002. Investigation on the hypoglycaemic effects of extracts of four mexican medicinal plants in normal and alloxan-diabetic mice, Phytother. Res., Vol.16, pp.383–386.

Aliyu AB, Musa, AM, Abdullahi MS, Oyewale AO (2008). Phytochemical and antibacterial properties of Ludwigia suffruticosa (Willd.) Oliv.ex.

Braca A, Sortino C, Politi M. Antioxidant activity offlavonoids from Licanialicaniaeflora. J Ethnopharmacol.2002; 79:379-381.

Deepti.et al., "HPTLC densitometric quantification ofstigmasterol and Lupeol from Ficus religosa": Arabian journal of Chemistry, 2015; 8: 366 -371.

Grover J.K., Yadav S., Vats V. 2002. Medicinal plants of India with antidiabetic potential. J. Ethnopharmacol; 8: 81–100.

Jellin, J.M. Gregory, P.J. Batz, F. and Hichens, K. Pharmacist's letter/Prescriber's letter natural medicines comprehensive database (4th ed.,). Stockton, CA: Therapeutic Research Faculty, pp.1131-1384, 2002.

Juna Beegum, G. R., Suhara Beevy, S. and Sugunan V.S., (2014). Nutritive and anti-nutritive properties

Kar, A, Choudhary, B.K. and Bandyopadhyay, N.G. 2003. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats, J. Ethnopharmacol., Vol.84, pp.105–108.

Karumaran S, Nethaji S, Rajakumar R. Antimicrobial and antioxidant activity of leaf extracts of *Aegle marmelos*, 2016; 7(3): 205-208.

Kim SK, Jung SM, Ahn KH, Jeon HJ, Lee DH, Jung KM, Jung SY, Kim DK (2005). Identification of three competitive inhibitors formembrane -associated, Mg^{2+}dependent and neutral 60kDa sphingomyelinase activity. Arch. Pharm. Res., 28(8): 923-929.

Kuldeep Rajpoot, RN Mishra.2011 International Journal of Pharmaceutical and Biomedical Research 2 (4), 1451-1460.

Mahmuda Ferdous, Mohammad Anwarul Basher, Ibrahim Khan, Firoz Ahmed, Md. Shariful Islam Sobuz and AFM Shahid Ud Daula, Evaluation of phytochemicals, antioxidant andantibacterial potentials of Alpinia calcarata, Journal of Medicinal Plants Studies 2018; 6(2): 152-158

Manikandan R., Vijaya A., Muthumani GD. (2013): Phytochemical and in vitro antidiabetic activity of methanolic extract of Psidium guajava leaves. International Journal of Current Microbiology and Applied Sciences, 2(2): 15-19.

McCue pp,Shetty K.Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro, Asia pacific Journal of clinical nutrition .2004;13(1)101-106.

Nicholas, P.H. Pietro, R.G. and Robert, H.C. 2008. Prevention and Treatment of Type 2 Diabetes: Current Role of Lifestyle, Natural Product, and Pharmacological Interventions, Pharmacol. Ther., Vol.118, No.2, pp.181–191.

Ravi, K. Rajasekaran, S. and Subramanian, S. 2005. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin induced diabetes in rats, Food. Chem. Toxicol., Vol. 43, pp.1433 – 1439.

Rich Goel, Daksh Batia, Sadaf Jamal Gilani, Deepti Katiyar. Medicinal Plants as Anti-diabetic: A Review, International Bulletin of drug Research; 2015, 1(2):101.

Schulz, V. Hänsel, R. and Tyler, V.E. Rational Phytotherapy. A Physician's Guide to Herbal Medicine, 4th Ed., Berlin, Springer-Verlag, 2001.

Shaw, D. 1998. Risks or remedies? Safety aspects of herbal remedies. J. Roy. Soc. Med., Vol.91, pp.294–296.

Sincy Joseph., Lekha Kumar and V Narmatha Bai. (2016): Evaluation of anti-diabetic activity of Strobilanthes cuspidata in alloxan induced diabetic rats and the effect of bioactive compounds on inhibition of α-amylase enzyme. Journal of Pharmacognosy and Phytochemistry, 5(3): 169-175.

Space JC; Imada CT, 2004. Report to the Republic of Kiribati on invasive plant species on the islands of Tarawa, Abemama, Butaritari and Maiana. Cont. no.2003-006 to the Pac. Biol. Surv. USDA Forest Service and Bishop Museum, Honolulu.

Vivek MN, Sachidananda Swamy HC, Manasa M, Pallavi S, Yashoda Kambar, Asha MM, Chaithra M, Prashith Kekuda TR, Mallikarjun N, Onkarappa R. Antimicrobial and Antioxidant activity of leaf and flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* Journal of Applied Pharmaceutical Science, 2013; 3(8): 064-071.

Yeh, G.Y. Eisenberg, D.M. Kaptchuk, T.J. and Phillips, R.S. 2003. Systematic review of herbs and dietary supplements for glycemic control in diabetes, Diabetes Care, Vol.26, pp.1277–1294.

Yen, G.C. and Duh, P.D. (1994) Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species. Journal of Agricultural and Food Chemistry, 42, 629-632.