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Research Article

In Vitro Study of Antioxidant and Antidiabetic Activity from the Rhizome of *Alpinia Purpurata*

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ABSTRACT

The term diabetes mellitus is defined as a metabolic disorder characterized by multiple etiologies, like chronic hyperglycemia. The effects of diabetes mellitus include dysfunction and long-term damage to various organs. The characteristic symptoms of diabetes mellitus include polyuria, blurred vision, thirst and weight loss. Different preparations of insulin are available, such as beef insulin, pork insulin and human insulin. The most important adverse effects of using insulin are weight gain and hypoglycemia. Insulin binds to sulfonylurea receptors on the β- cell plasma membrane. ATP-sensitive potassium channels lead to depolarized cell membranes. The aqueous extract of the rhizome of *Alpinia purpurata* has been taken for the present study. The aqueous extract was also examined for anti-oxidant activities by using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging method. The different concentrations of extracts exhibited potent radical scavenging activity using DPPH as substrate. The rhizome *Alpinia purpurata* exhibited significant α-amylase (75.609%) and α- glucosidase (77.064%) inhibitory activities at the concentration of 100µg/ml when compared with standard acarbose drug. The GCMS analysis is done to find the active components in the extract. The aqueous extract suggested that the rhizome of *Alpinia purpurata* is a potential source for natural antioxidant and antidiabetic compounds used in the management of diabetesmellitus.

Keywords: Hyperglycemia, hypoglycemia, *Alpinia purpurata*, α-amylase, α- glucosidase, antioxidant and antidiabetic

INTRODUCTION

Herbal medicine is widespread and is used for the treatment of various diseases and disorders. It is adequate enough [1]. The usage of herbal medicines has more advantages and the first advantage is wide availability and simple preparation. Those plants contain proteins, sugars, minerals, and more chemicals, which are interacted with the active chemicals in different ways, may lessen their harsh or toxic side effects and may concentrate to intensify their effect, it is considered for easy digestion or absorption [2]. In the last few years, there is a growth in herbal medicine. The drugs are popular in both developing and developed countries, because of their fewer side effects and natural origin.Medicinal plants, organic matter and minerals are the sources of traditional medicines [3]. Medicinal plants are used for therapeutic purposes, in which any part of the plant is used. Bioactive phytochemicals or bio-nutrients are found in large numbers in medicinal plants. Some medicinal plants are rediscovered for their effectiveness, with no or fewer side effects and contraindications compared to synthetic medicines [4]. Phytochemicals are chemical compounds that occur naturally in plants. They are biologically active. The presence of macronutrients and micronutrients in plant parts such as flowers, fruits, seeds, roots, leavesor stems provides health benefits to humans [5,6]. The most important phytochemical compounds are alkaloids, terpenoids, tannins, saponins, flavonoids, and phenolic compounds [7]. The various compounds like nitro compounds, long-chain hydrocarbons, alcohols, organic acids, steroids, alkaloids, amino acids, and esters are detected by using GCMS. The GCMS is one of the best, most accurate and fast techniques and it requires a small volume of plant extract [8]. The secondary metabolite profiling of plant and non- plant species are found by the GCMS technique in the last few years [9,10,11].

Diabetes mellitus is referred to as a chronic disorder of protein, fat and carbohydrate metabolism [12]. Diabetes mellitus is an endocrine disorder and it is referred to as "sugar" and this occurs due to the absence or deficiency of insulin or impairment of insulin activity (insulin resistance) [13]. The pancreas secretes both glucagon and insulin hormones. In the islets of Langerhans, alpha (α) cells secrete glucagon and beta (β) cells secrete insulin. The blood glucose level is decreased by

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insulin by the process of glycogenesis and glucose is transported into the liver, muscles and adipose tissue. For glucose utilization, insulin is notrequired for neural tissues and erythrocytes whereas alpha (α) cells produce glucagon to control blood glucose and the blood glucose level increases by the glycogenolysis acceleration [14,15]. Type I diabetes mellitus is also known as autoimmune diabetes or ketosis-prone diabetes or juvenile-onset [16]. This type of diabetes mainly affects young adults and children and the onset suddenly happens and threatens life [14]. Type II diabetes mellitus is also called adult-onset diabetes. Frequently, people with type II diabetes are resistant to insulin action [17]. Gestational diabetes mellitus (GDM) is diabetes in which glucose intolerance is diagnosed for the first time or during pregnancy [13]. The cause of diabetes mellitus happens when the cells become starved due to a lack of normal glucose metabolism [13]. The other causes are a renal failure due to nephropathy, retinopathy with potential blindness, neuropathy with foot ulcer, Charcot joint and autonomic dysfunction sexual dysfunction [18]. The plants have antioxidants naturally and herbal medicines are effective due to the presence of antidiabetic compounds like alkaloids, phenolic compounds, tannins and flavonoids that improve the pancreatic tissue's performance by increasing insulin secretion or decreasing glucose absorption in the intestine. Different natural antioxidants like flavonoids, vitamin E, vitamin C and tannins in plants maintain β- cell performance and decrease the level of glucose in the blood [19].

Alpinia purpurata (Vieill) K. Schum. is a medium-sized plant that grows up to 2 meters in height and is a sub-family of Alpinieae [20,21]. The leaves are alternate, oblong with a pointed apex, deep green and sessile. Shoots have attractive inflorescence and have a showy red or pink bract with erect spikes. Leaf stalks and rhizomes release aroma. The inflorescence of some plant species produces plantlets [22]. The consumption of rhizomes is followed in India to enhance appetite, voice and taste [23]. The native of *Alpinia purpurata* is Papua New Guinea of Malesia and New Caledonia, Vanuatu and Solomon Islands of the Southwestern Pacific. In the tropics, it is grown for its ornamental uses [24,25]. The antibacterial, antioxidant, cytotoxic, larvicidal and vasodilator activities are found in the leaves and rhizomes of *Alpinia purpurata* [26].

Therefore, in the present study the in vitro study of antioxidant and antidiabetic activity from the aqueous extract of the rhizome of *Alpinia purpurata* with the GCMS hasundergone.

MATERIALS AND METHODS

Collection of Plant Material

The rhizome of *Alpinia purpurata* has been collected from Kolli Hills, Namakkal, Tamil Nadu, India.

Chemicals and Reagents

Alpha (α)-amylase, *p*-nitrophenyl-α-D-Glucopyranose(P-NPG), Alpha (α)- Glucosidase, DPPH (1,1-Diphenyl- 2-picrylhydrazyl), 3,5-dinitrosalicylic acid (DNS), Ascorbic acid & acarbose, soluble starch, phosphate buffer and sodium carbonate.

Preparation of Plant Extract

The rhizome of *Alpinia purpurata* is washed in running water. This is then cut into small pieces. The cut pieces are dried for a week at 35-40^0C. It is then ground into a uniformpowder of 40 mesh size. The extract is prepared by soaking 100g of the dried powder plantmaterial in 1L of aqueous using a Hot-Percolation method. It is continued for 10hr and the extract is filtered through Whatman filter paper No: 42(125mm). All extracts are concentrated to dryness, using a rotary evaporator at reduced pressure. The final dried samples are stored in labeled sterile bottles and kept at 40^0C. The filtrate is used as a sample solution for further isolation (Rathee D *et al.,* 2015).

Phytochemical Screening Test of the Aqueous Extract of *Alpinia Purpurata* (Qualitative Analysis)

Test for Tannins

Extract of 2ml was taken and 0.1% ferric chloride was added and 2 or 3 drops of distilled water is added. The presence of tannins is found by the green color precipitate.

Test for Phlobatannins

Extract of 2ml was taken and dissolved in water and filtered. The filtered is added with2ml of 1% Hydrochloric acid solutions. The presence of phlobatannin is found by the red precipitate.

Test for Saponins (Foam test)

Extract of 2ml was taken and dissolved with 2ml of distilled water in the test tube and the solution is vigorously shaken and observed stable persistent froth and the foam indicates the presence of saponins.

Test for Flavonoids

Extract of 2ml was taken and treated with 2ml of ammonia solution and added a few drops of conc. H_2SO_4. The presence of flavonoids is found by the yellow color.

Test for Steroids

Extract of 2ml was taken and treated with 2ml of chloroform and added a few drops of conc. H_2SO_4. The violet-blue color indicates the presence of steroids.

Test for Terpenoids

Extract of 2ml was taken and dissolved with 2ml of chloroform followed by a few drops of conc. H_2SO_4. The presence of terpenoid is found by the reddish-brown color.

Test for Cardiac Glycosides

Extract of 2ml was taken, dissolved with

1ml of glacial acetic acid and 1ml of ferric chloride and a few drops of conc. H_2SO_4 is added. The presence of violet color is obtained by cardiac glycosides.

Test for Leucoanthocyanins

Extract of 2ml was taken and added with a few drops of isoamyl alcohol. The presence of leucoanthocyanin is found by the red color.

Test for Anthocyanins

Extract of 2ml was taken and added with 2ml of HCl and a few drops of ammonia solution. The pinkish-red color indicates the presence of anthocyanin.

Test for Anthraquinones

Extract of 2ml was taken and a few drops of benzene is treated with 1ml of ammonia solution. The pink or violet or red color indicates the presence of anthraquinone.

Test for Proteins (Xanthoproteins test)

Extract of 2ml was taken and a few drops of conc. H_2SO_4 was added. The presence of proteins is found by the white precipitate.

Test for Coumarins

Extract of 2ml was taken and added with 2ml of 10% sodium hydroxide was added.

The presence of coumarins is found by the yellow color.

Test for Glycosides (Liebermann test)

Extract of 2ml was taken with 1ml of chloroform and a few drops of glacial acetic acidis added. A violet to blue to green color indicates the presence of glycosides.

Test for Phenols

Extract of 2ml was taken with a few drops of ammonia solution. A reddish-orange precipitate is obtained by phenols.

Test for Alkaloids (Hager's test)

Extract of 2ml was taken with a few drops of glacial acetic acid and added with 2ml ofpicric acid and a few drops of ammonium acetate. A yellow color or precipitate indicates the presence of alkaloids.

Test for Xanthoproteins

Extract of 2ml was taken with 2ml of ammonia solution and a few drops of ferric chloride is added. A blue-black color indicates the presence of xanthoproteins.

Test for Emodin

Extract of 2ml was taken with a few drops of ammonium hydroxide and added with 1ml of benzene. A red color precipitate indicates the presence of emodin.

Test for Carbohydrates

Extract of 2ml was taken with a few drops of distilled water and 2 drops of ethanolic alpha naphthol and 2 drops of conc. H_2SO_4 is added. A red-violet ring is obtained by the presence of carbohydrates.

Quantitative analysis of the aqueous extract of *Alpinia purpurata*

Flavonoids

The plant sample extract of 0.5g was taken and added 2ml of 80% aqueous methanol. The entire solution was filtered on filter paper and the filtrate is transferred into the crucible and evaporated. It is then dried and weighed.

Tannins

The plant sample extract of 0.5g was taken and treated with 2ml of distilled water. The entire solution was filtered on filter paper and 1 drop of ferric chloride and 1 drop of 0.008 M potassium ferrocyanide were added to the filtrate. The filtrate was transferred into a crucible and evaporated. It is then dried and weighed.

Alkaloids

The plant sample extract of 0.5g was taken and 2ml of 10% acetic acid (acetic acid andethanol) is added and incubated for 10 minutes and ammonia solution is added drop by drop and filtered through filter paper. The filtrate is transferred into a crucible and evaporated. It is then dried and weighed.

Saponins

The plant sample extract of 0.5g was taken and 2ml of ethanol is added and filtered through filter paper and 1ml of filtrate is transferred in a test tube and 1ml of diethyl ether is added and is vigorously shaken. The ether layer and aqueous layer are recovered, discarded and the transferred aqueous solution in another tube and 2 drops of 1N n-butanol and 1 drop of NaOH were added. The filtrate is transferred into a crucible and evaporated. It is then dried and weighed.

Phenols

The plant sample extract of 0.5g was taken with a few drops of diethyl ether and filteredthrough filter paper. Take 2ml of distilled water, 1 drop of ammonia solution and 1 drop of isoamyl alcohol and is added to the filtrate. The filtrate is transferred into a crucible and evaporated. It is then dried and weighed.

Terpenoids

The plant sample extract of 0.5g was taken and 2ml of ethanol is added. It is filtered with filter paper and 2 drops of petroleum ether were added to the filtrate. The filtrate is transferred into a crucible and evaporated. It is then dried and weighed.

Antioxidant Activity (DPPH Free Radical Scavenging Activity) Determination

The antioxidant activity of the aqueous extract of rhizome of *Alpinia purpurata* is on the basis of the scavenging effect of the stable DPPH free radical activity. The ethanolic solution of DPPH (0.05mM) (300µl) was added to 40 µl of the rhizome of *Alpinia purpurata* with different concentrations (20-100 µg/ml). The freshly prepared DPPH solution was kept in the dark at 4^0C and addition of 96% ethanol (2.7ml) and the mixture is vigorously shaken and kept still for 5

minutes. The absorbance was spectrophotometrically at 540nm. The same amount of the blank sample contains the ethanol and DPPH prepared. Triplicate determinants were performed. The radical scavenging activities of the tested sample's percentage of inhibition were calculated from the equation[1].

$$\text{Percentage inhibition} = [\frac{A-B}{A}] \times 10 \quad \text{eq. (1)}$$

where A=absorbance of control B=absorbance of aqueous extract

α-Amylase Inhibitory Assay

In the alpha-amylase assay, a total of 250µl of aqueous extract of the rhizome of *Alpinia purpurata* (20-100µg/ml) was placed in a tube and 250µl of 0.02M of sodium phosphate buffer and the α-amylase solution containing pH of 6.9 and 0.5mg/ml is respectively added. The solution was pre-incubated for 10 minutes at 25^0C and 250µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at time intervals and further incubated for 25˚C for 10 minutes. The reaction was terminated by adding 500µl of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 minutes and cooled at room temperature, the reaction mixture was diluted with 5ml distilled water and using a spectrophotometer absorbance was measured at 540nm. A control was prepared with the same procedure by replacing the extract with distilled water. The percentage inhibition of α-amylase inhibitory activity was calculated from the equation [1].

α-Glucosidase Inhibitory Assay

The substrate solution of nitrophenyl glucopyranoside (p-NPG) was prepared in a 20mM phosphate buffer with a pH of 6.9. 100µl of α-glucosidase (1.0 U/ml) was preincubated with 50µl of the different concentrations (20-100 µg/ml) of the extracts for 10 minutes. Then 50 μl of 3.0mM (p-NPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37^0C for 20 minutes and stopped by adding 2ml of 0.1M Na_2Co_3. The α-glucosidase activity is measured by yellow-colored para nitrophenol released from p-NPG at 450nm and the results were expressed as a percentage of the blank control. The percentage inhibition of α- glucosidase inhibitory activity was calculated from equation [1].

Gas Chromatography and Mass Spectrometry (GCMS)

GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system was utilized. The system was equipped with an auto-injector (AOC-20i), headspace sampler (AOC-20s), a mass selective detector with an ion source (220 °C) and an interface (260 °C). Rtx-5 MS capillary column having 30 mm X 0.25 mm of length X diameter and 0.25 µm of film thickness was used for MS analyses. The mass range of 40-650 m/z with 1,000 eV of the threshold was purposed. The injector was set in the split injection mode having 250 °C of temperature. The ratio applied for split mode was 10.0. The starting temperature was adjusted to 80 °C (3 min), which afterward increased to 280 °C with a ramp rate of 10 °C/min. Helium (>99.99 %) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow. Components were recognized by their retention time (RT) and elucidation of mass spectra. The spectral fragmentation of unknown components was compared with the known and standard components provided by the databases of WILEY8LIB.

RESULTS AND DISCUSSIONS

Qualitative Analysis

From the phytochemical screening of the aqueous extract of rhizome of *Alpinia purpurata,* the presence of different phytochemical compounds viz, terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, phlobatannins, proteins, coumarins, emodin, anthraquinones, anthocyanins, carbohydrates, leucoanthocyanins, cardiac glycosides, xanthoproteins, phenols were analyzed in the aqueous extract of the rhizome of *Alpinia purpurata*.

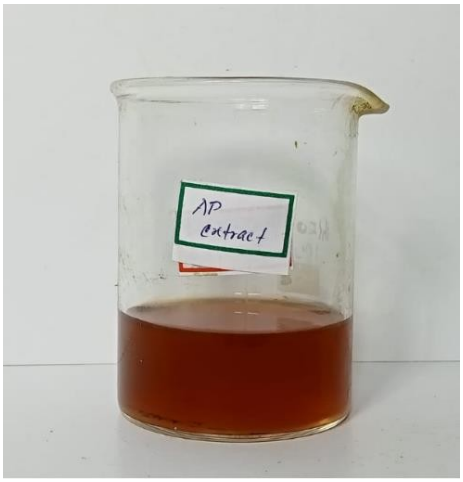


Figure 1: Extract of the rhizome of *Alpinia purpurata*

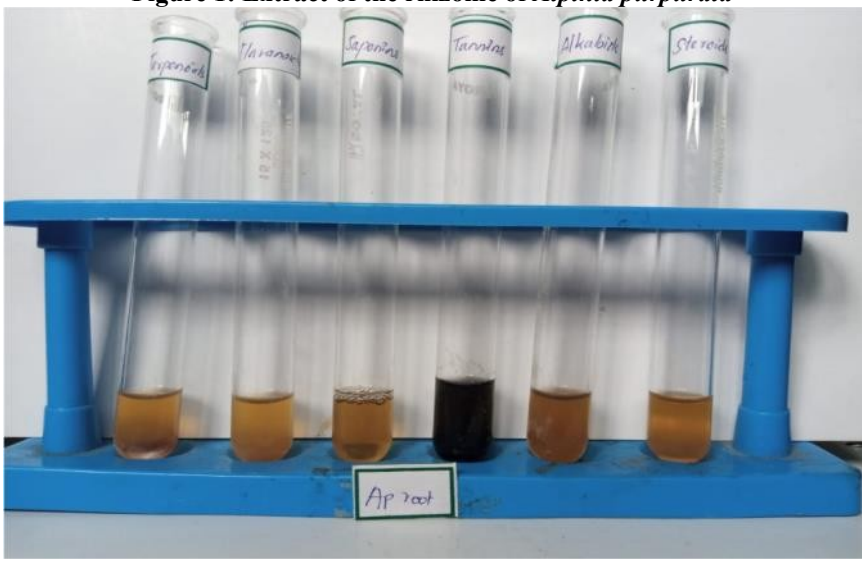


Figure 2(a)

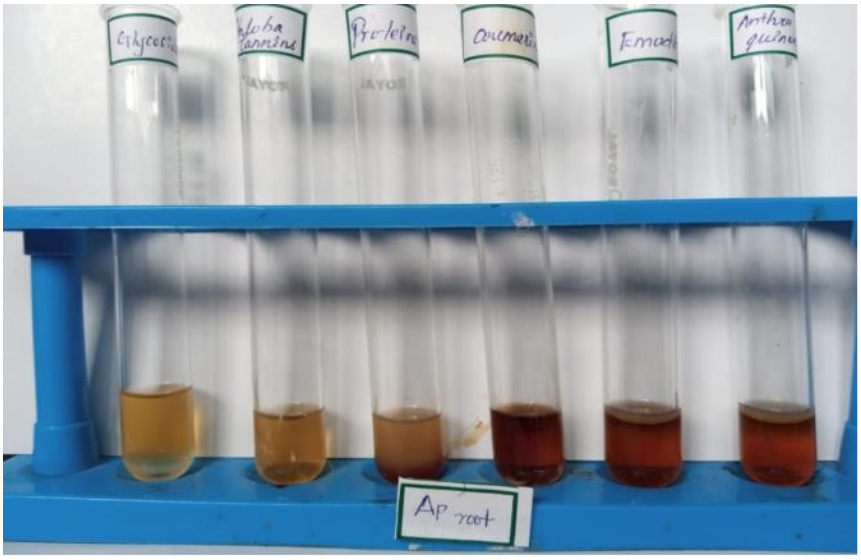


Figure 2(b)

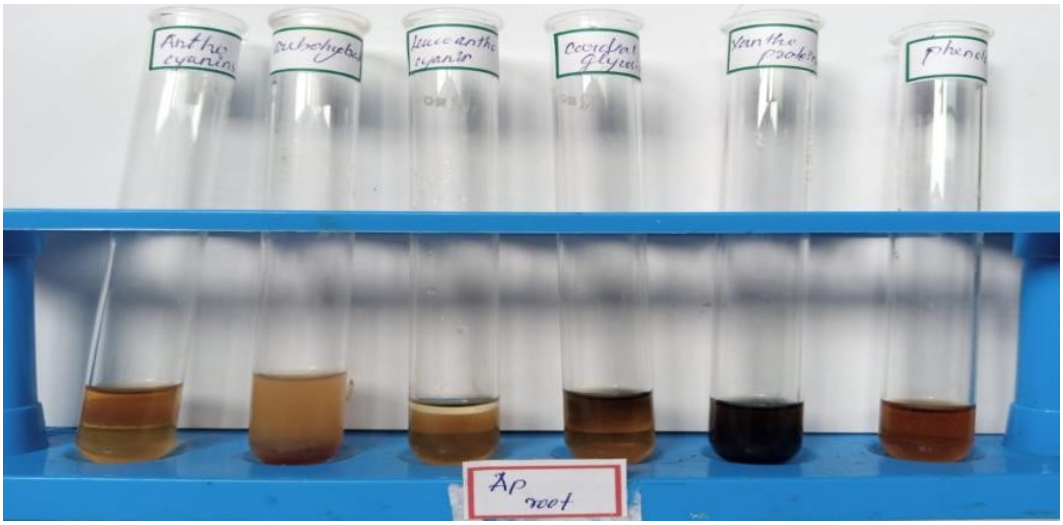


Figure 2(c)

Figure 2: Qualitative analysis of the aqueous extract of rhizome of *Alpinia purpurata*

Table 1: Qualitative analysis of the aqueous extract of rhizome of *Alpiniapurpurata*

S.No.	Phytochemical Constituents	*Alpinia Purpurata*
1.	Terpenoids	+++
2.	Flavonoids	+++
3.	Saponins	+++
4.	Tannins	+++
5.	Alkaloids	+++
6.	Steroids	+++
7.	Glycosides	++
8.	Phlobatannins	++
9.	Proteins	+++
10.	Coumarins	+++
11.	Emodin	+++
12.	Anthraquinones	+++
13.	Anthocyanins	+++
14.	Carbohydrates	++
15.	Leucoanthocyanins	+++
16.	Cardiac glycosides	+++
17.	Xanthoproteins	+++
18.	Phenols	+++

(+ = Slightly present, ++ = moderately present, +++ = Strongly present)

The aqueous extract of the rhizome of *Alpinia purpurata* indicated the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, phlobatannins, proteins, coumarins, emodin, anthraquinones, anthocyanins, carbohydrates, leucoanthocyanins, cardiac glycosides, xanthoproteins, phenols.

Similarly, the ethanolic extract of the leaves of *Alpinia purpurata* has terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, phlobatannins, coumarins, emodin, anthraquinones, anthocyanins, leucoanthocyanins, cardiac glycosides, xanthoproteins, phenols[27].

Quantitative Analysis

Quantitative analysis of important phytochemicals in the medicinal plant *Alpinia purpurata* contains these phytochemicals in varying amounts in the rhizome. The phytochemicals with the highest quality are tannins followed by saponins, phenols, alkaloids, flavonoids and terpenoids. The highest concentration of tannin (0.013 mg/g), saponin (0.054 mg/g), phenol (0.007 mg/g), alkaloids (0.025 mg/g), flavonoids (0.014 mg/g), terpenoids (0.010 mg/g) respectively.

The previous study reported that the ethanolic extract of the leaves of *Alpinia purpurata* has 0.007 mg/g of flavonoids, 0.024 mg/g of tannins, 0.002 mg/g of alkaloids, 0.001 mg/g of saponins, 0.008 mg/g of terpenoids and 0.002 mg/g of phenol [27].

Table 2: Quantitative analysis of the aqueous extract of rhizome of *Alpinia purpurata*

S.No.	Phytochemical Constituents	*Alpinia purpurata* (mg/g)
1.	Flavonoids	0.014
2.	Tannins	0.013
3.	Alkaloids	0.025
4.	Saponins	0.054
5.	Terpenoids	0.010
6.	Phenols	0.007

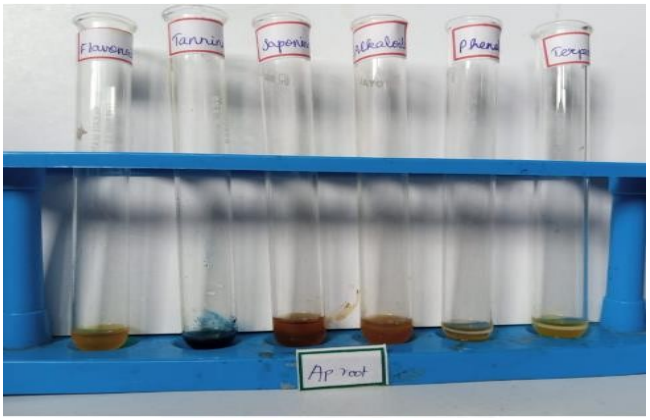


Figure 3(a)

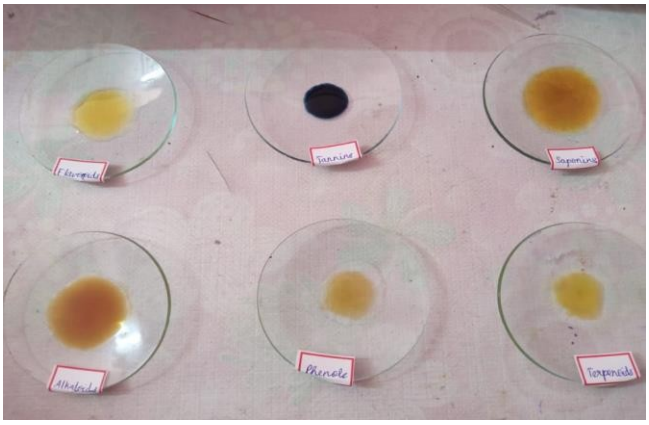


Figure 3(b) – Before drying

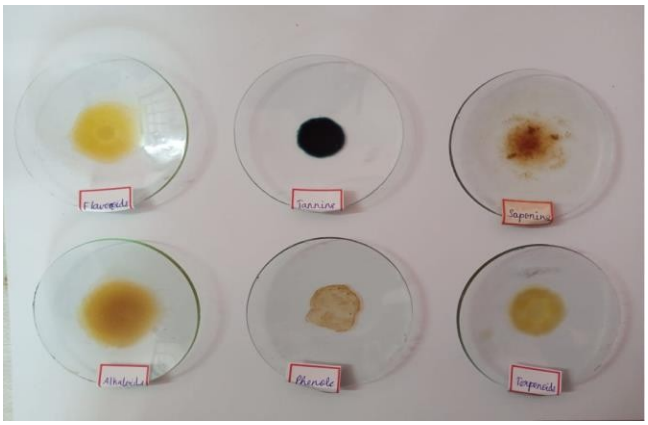


Figure 3(c) – After drying

Figure 3: Quantitative analysis of the aqueous extract of rhizome of *Alpinia purpurata*

Antioxidant Activity of the Rhizome of *Alpinia Purpurata* – DPPH Method

The result showed that the extract from the rhizome of *Alpinia purpurata* has a better

percentage of antioxidant activity at high concentrations when compared with ascorbic acid. The extract showed 84.496% activity at the higher concentration of 100µg/ml while ascorbic acid showed 89.147% at the same concentration.

Similarly, the previous study done with the ethanolic extract of the leaves of *Alpinia purpurata* by the DPPH method showed 70% of antioxidant activity while ascorbic acid showed 94.69% at the concentration of 100 µg/ml [27].

Figure 4: Antioxidant activity of the standard ascorbic acid by DPPH activity

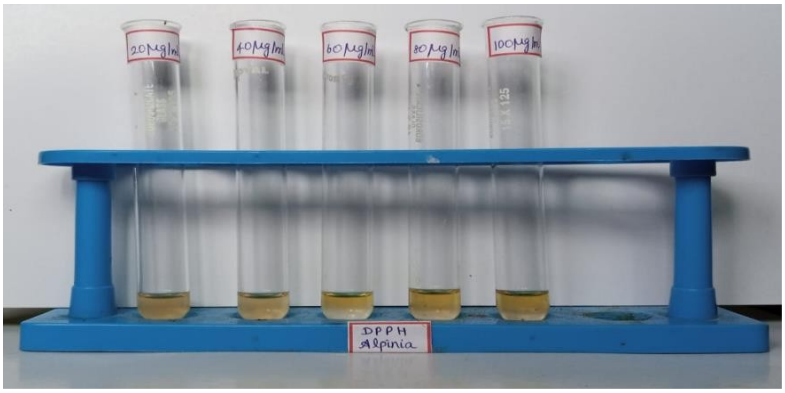


Figure 5: Antioxidant activity of the rhizome of *Alpinia purpurata* by DPPH activity

Table 3: Antioxidant activity of the rhizome of *Alpinia purpurata* by DPPH activity

S. No.	Concentrations C=1.29	Scavenging Effect (%) Rhizome of *Alpinia purpurata*	Ascorbic acid
1.	20(µg/ml)	65.891	69.767
2.	40(µg/ml)	70.542	79.069
3.	60(µg/ml)	74.418	82.170
4.	80(µg/ml)	82.945	85.271
5.	100(µg/ml)	84.496	89.147

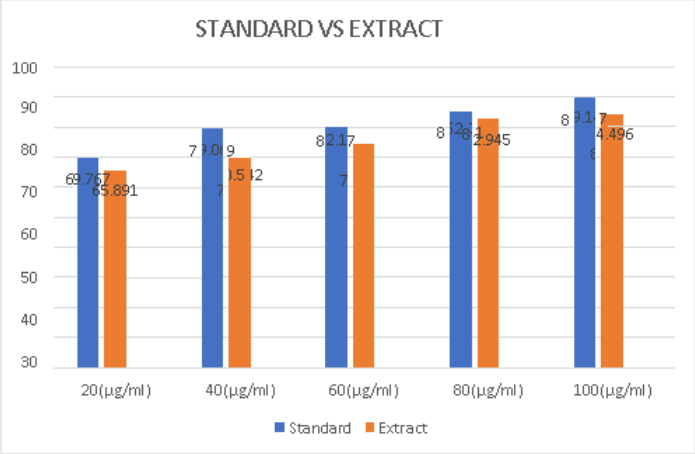


Figure 6: The comparison between the standard ascorbic acid and the extract to findthe antioxidant activity of the rhizome of *Alpinia purpurat*

In vitro α-amylase inhibitory assay

The study of in vitro α-amylase inhibitory activities of the aqueous extract of the rhizome of *Alpinia purpurata* was examined. The result showed an increase in the percentage of inhibitory activity against the α-amylase enzyme. In a dose-dependent manner, the *Alpinia purpurata*'s rhizome of the various concentrations (20-100µg/ml) exhibits potent α-amylase inhibitory activity. The rhizome of *Alpinia purpurata* showed inhibitory activity of 75.609% at a concentration of 100µg/ml. The standard drug for α-amylase inhibition is Acarbose. Acarbose showed α-amylase inhibitory activity from 87.804% at the same concentration of 100 µg/ml. A comparison between the standard drug and the extract for α-amylase inhibitory activity was done.

The α-amylase inhibitory activity of the methanolic extract of the root of *Alpinia purpurata* showed 66.10% at the concentration of 100 µg/ml and the standard acarbose drug showed 85.87% at the same concentration [28].

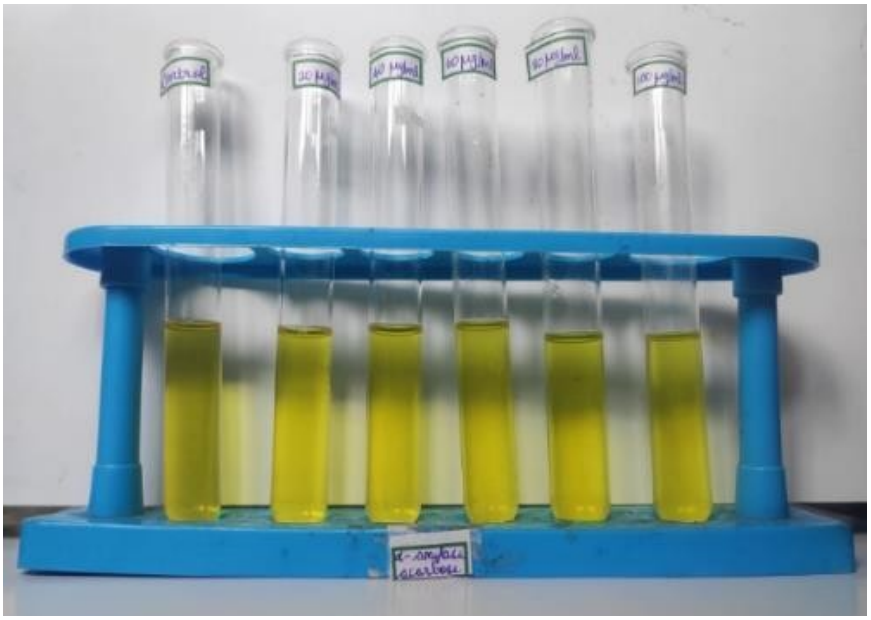


Figure 7: α-amylase inhibitory activity of the standard acarbose

Figure 8: α-amylase inhibitory activity of the rhizome of *Alpinia purpurata*

Table 4: A comparison between the rhizome of *Alpinia purpurata* and the standardacarbose using the α-amylase method to find the antidiabetic activity

S. No.	Concentration	α-amylase % Rhizome of *Alpinia Purpurata*	Acarbose
1.	20(µg/ml)	57.723	72.357
2.	40(µg/ml)	64.227	74.796
3.	60(µg/ml)	69.105	83.739
4.	80(µg/ml)	73.983	85.365
5.	100(µg/ml)	75.609	87.804

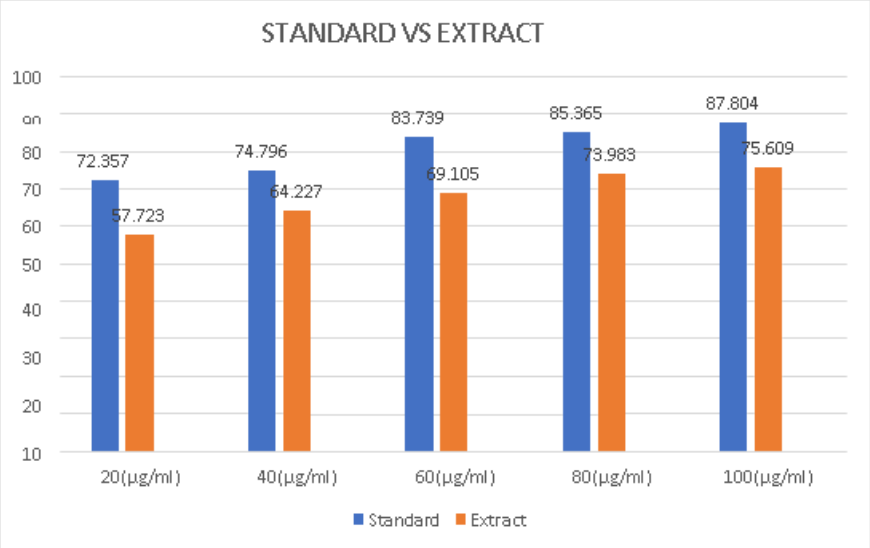


Figure 9: The comparison between the standard acarbose and the extract from the rhizome of *Alpinia purpurata* to find the in vitro antidiabetic activity using α-amylase method

In vitro α-glucosidase inhibitory assay

The result of antidiabetic activity using α-glucosidase inhibitory assay of the aqueous extract of rhizome of *Alpinia purpurata*, revealed the significant inhibitory action against α- glucosidase enzyme. The inhibition at 20-100µg/ml concentrations of extracts showed a dose- dependent increase in percentage inhibition. The percentage inhibition was 77.064% for the highest concentration of 100µg/ml. Acarbose at the same concentration of 100µg/ml showed the α-glucosidase inhibitory activity of 85.321%. It indicates that the rhizome of *Alpinia purpurata* has a very potent α-amylase and α-glucosidase inhibitory activity in comparison with acarbose.

The α-glucosidase inhibitory activity of the methanolic extract of the root of *Alpinia purpurata* showed 78.70% at the concentration of 100 µg/ml and the standard acarbose drug showed 95.68% at the same concentration [28].

Figure 10: α-glucosidase inhibitory activity of the standard acarbose

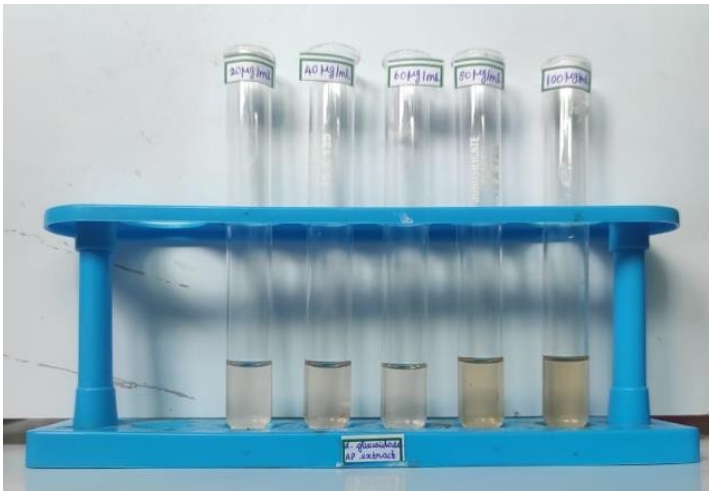


Figure 11: α-glucosidase inhibitory activity of the rhizome of *Alpinia purpurata*

Table 5: A comparison between the rhizome of *Alpinia purpurata* and the standardacarbose using the α-glucosidase method to find the antidiabetic activity

S. No.	Concentrations	α-glucosidase (%) Rhizome of *Alpinia Purpurata*	Acarbose
1.	20(µg/ml)	47.706	72.477
2.	40(µg/ml)	55.045	74.311
3.	60(µg/ml)	58.715	81.651
4.	80(µg/ml)	70.642	82.568
5.	100(µg/ml)	77.064	85.321

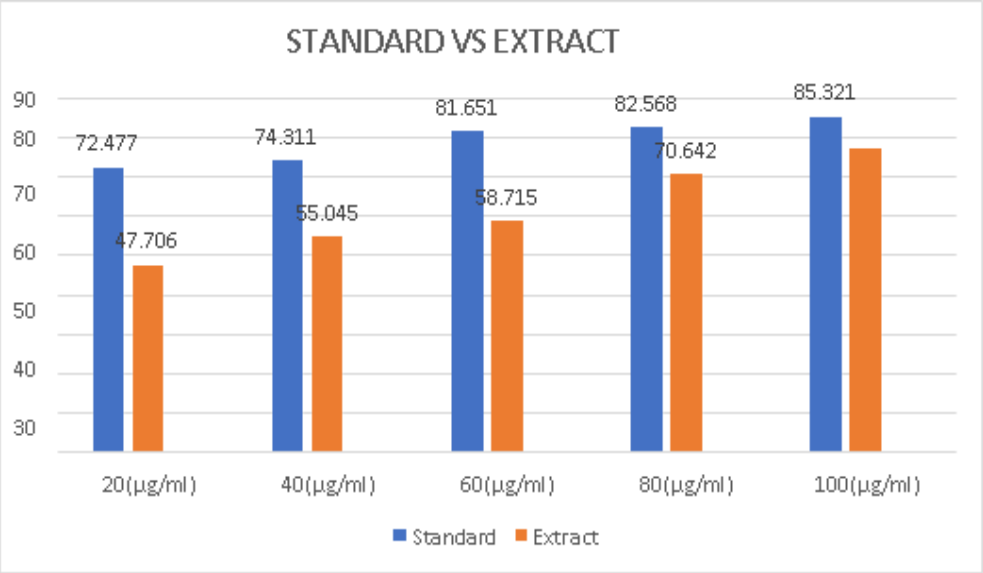


Figure 12: The comparison between the standard acarbose and the extract from the rhizome of *Alpinia purpurata* to find the in vitro antidiabetic activity using α-glucosidasemethod

Gas Chromatography and Mass Spectrometry (GCMS)

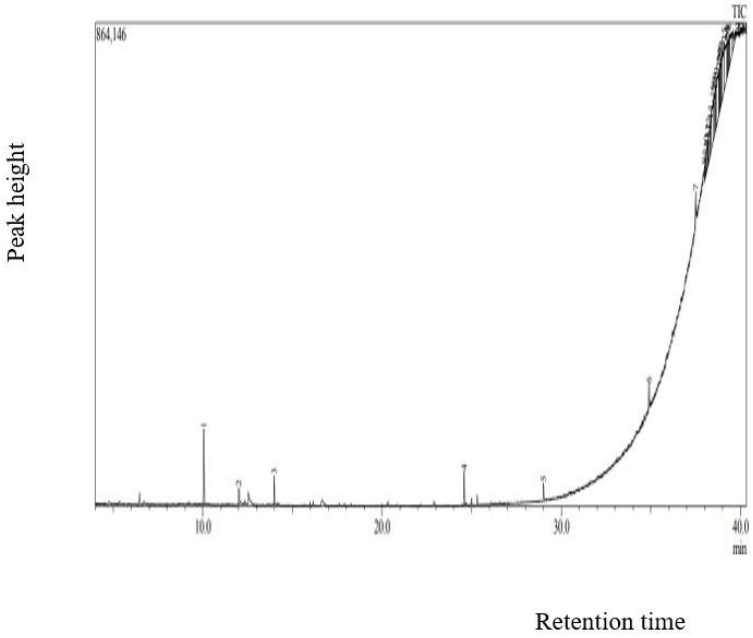
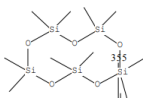
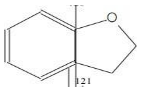
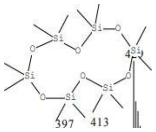
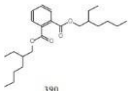
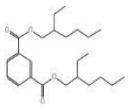
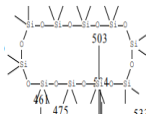
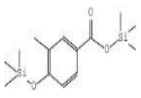
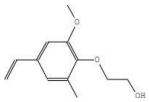
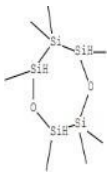
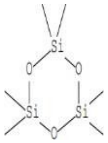
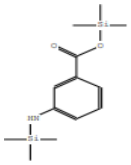
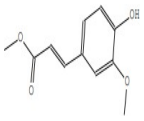


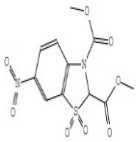
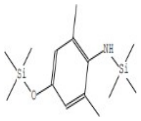
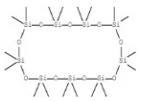
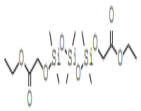
Figure 13: The peak obtained from the analysis of the Gas chromatography and massspectrometry (GCMS)

Table 6: The compounds found with their Rt value, area, area%, height, height%, chemical formula, molecular weight and molecular structure

S.No	Compound Name	Rt Value	Area	Area %	Height	Height %	Molecular Formula	Molecular Weight	Molecular Structure
1.	Cyclopentasiloxane, decamethyl-	10.058	270530	3.99	135740	7.21	C10H30O5Si5	370	
2.	Benzofuran,	12.011	84592	1.25	29078	1.54	C8H8O	120	

	2,3-dihydro-								
3.	Cyclohexasiloxane, dodecamethyl-	13.99	92950	1.37	51658	2.74	C12H36O6Si6	444	
4.	Neophytadiene	24.581	127288	1.88	61398	3.26	C20H38	278	
5.	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R- [R*, R*-(E)]]- (T-Phytol)	29.001	77831	1.15	31961	1.7	C20H40O	296	
6.	1,2-benzenedicarboxylic acid	34.893	89395	1.32	41188	2.19	C24H38O4	390	
7.	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	37.504	95231	1.4	42994	2.28	C24H38O4	390	
8.	Silikonfett	37.99	53793	0.79	30078	1.6	-	9999	-
9.	Silikonfett	38.033	101827	1.5	39306	2.09	-	9999	-
10.	Silikonfett	38.095	107779	1.59	42401	2.25	-	9999	-
11.	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane #	38.125	158702	2.34	42836	2.28	C20H60O10Si10	740	
12.	Silicone Grease, Silikonfett	38.195	95275	1.41	47764	2.54	-	9999	-
13.	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy] benzoate	38.225	298047	4.4	55479	2.95	C14H24O3Si2	296	
	Ethanol, 2-[4-vinyl-2-								

14.	methoxy-6-methyl] phenoxy-	38.32	320618	4.73	61676	3.28	$C_{12}H_{16}O_3$	208	
15.	Silikonfett	38.461	354011	5.22	78637	4.18	-	9999	-
16.	2,2,3,5,6,6,7-Heptamethyl [1,4,2,3,5,6,7] dioxapentasilepane	38.51	360129	5.31	79353	4.22	$C_7H_{24}O_2Si_5$	280	
17.	Hydrochlorothiazide N-butyl Boronate	38.57	106966	1.58	76425	4.06	$C_{11}H_{15}BCl_3O_4S_2$	363	-
18.	Cyclotrisiloxane Hexamethyl-	38.6	242670	3.58	79173	4.21	$C_6H_{18}O_3Si_3$	222	
19.	Silikonfett	38.66	690674	10.19	78984	4.2	-	9999	-
20.	Silikonfett	38.8	188680	2.78	81239	4.32	-	9999	-
21.	Silikonfett	38.84	236773	3.49	86180	4.58	-	9999	-
22.	3-Aminobenzoic acid, 2TMS derivative	38.89	120351	1.78	83097	4.41	$C_{13}H_{23}NO_2Si_2$	281	
23.	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl Ester	38.92	237051	3.5	84299	4.48	$C_{11}H_{12}O_4$	208	
24.	Silikonfett	38.965	573032	8.45	82819	4.4	-	9999	-
25.	Silikonfett	39.1	636123	9.38	85619	4.55	-	9999	-

26.	Dimethyl 6-Nitro-1,3-Benzothiazole-2,3(2H)-Dicarboxylate 1,1-Dioxide #	39.25	161472	2.38	65372	3.47	$C_{11}H_{10}N_2O_8S$	330	
27.	14,14-D (2)-Matrine	39.285	185752	2.74	59671	3.17	$C_{15}H_{22}D_2N_2O$	250	-
28.	Silanamine, N-[2,6-dimethyl-4-[(Trimethylsilyl)oxy] Phenyl]- 1,1,1-Trimethyl-	39.345	155101	2.29	55518	2.95	$C_{14}H_{27}NOSi_2$	281	
29.	Cyclononasiloxane, Octadecamethyl-	39.4	390485	5.76	54293	2.88	$C_{18}H_{54}O_9Si_9$	666	
30.	Ethyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilatetradecan-1- oate	39.564	165360	2.44	37982	2.02	$C_{14}H_{32}O_8Si_3$	412	

From the previous GCMS analysis, the different phytochemicals like Phenanthrene, 3- methyl-(CAS), trans-13-Octadecenoic acid, Benzene, Squalene, Synaptogenin B, 2- Hexadecen-1-ol and other compounds are found from the n-hexane leaf extract of *Alpinia purpurata* (Vieill) K. Schum [29].

CONCLUSION

Diabetes mellitus is a metabolic disorder. The effects of diabetes mellitus include dysfunction, long term damage to various organs. The characteristic symptoms of diabetes mellitus are polyuria, blurred vision, thirst and weight loss.

The aqueous extract of the rhizome of *Alpinia purpurata* has terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, phlobatannins, proteins, coumarins, emodin, anthraquinones, anthocyanins, carbohydrates, leucoanthocyanins, cardiac glycosides, xanthoproteins, phenols. The phytochemical with the highest quantity is tannin followed by flavonoids, terpenoids, alkaloids, saponins and phenols. The highest concentration of flavonoids (0.014mg/g), tannin (0.013mg/g), saponin (0.054mg/g), alkaloids (0.025mg/g), phenols (0.007mg/g), terpenoids (0.010mg/g) are found. The antioxidant activities at a higher concentration of 100µg/ml when compared with ascorbic acid are obtained as 84.496%. At the same concentration of 100µg/ml, 75.609% of α-amylase and 77.064% of α-glucosidase are obtained when compared to the standard acarbose.

The rhizome of *Alpinia purpurata* shows the significant enzyme inhibitory activity and so the compound is responsible for the inhibitory activity and can be used as an antidiabetic agent. From gas chromatography and mass spectrometry analysis of the extract of rhizome of *Alpinia purpurata* showed the presence of various compounds.

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