

ANTIOXIDANT AND ANTIDIABETIC STUDY OF *Senna auriculata*, *Psidium guajava* AND *Syzygium cumini*: A COMPARATIVE ANALYSIS

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ABSTRACT: Diabetes mellitus is a chronic disorder of carbohydrate metabolism characterized by the presence of hyperglycemia. It is frequently accompanied by oxidative stress and by several micro- and macrovascular complications. Medicinal plants have been widely explored for the treatment of diabetes mellitus because they have fewer side effects than synthetic drugs. The present study was aimed at the evaluation of antidiabetic and antioxidant potential of *Senna auriculata*, *Psidium guajava* and *Syzygium cumini* as well as comparing their efficacy among themselves. Plant materials were shade dried and powdered, extracted with suitable solvents and the extracts were evaluated in vitro. The antidiabetic potential was carried out with yeast glucose uptake assay. The antioxidant potential was determined by DPPH radical scavenging and phosphomolybdenum reduction assays. The maximal inhibition observed for the glucose uptake of *Psidium guajava* extract was 83.80% at 30 mM, in the DPPH assay was 90.94% at 120 µg/mL and in the molybdate assay was at 1000 µg/mL. *Senna auriculata* showed the best phosphomolybdenum reduction of 98.78% at a concentration of 120 µg/mL. *Syzygium cumini* reportedly showed the best antioxidant and antidiabetic activity. In general, all plant extracts exhibited strong bioactive properties, with *Psidium guajava* showing the most strong antidiabetic and radical scavenging activities compared to the other tested plants. Collectively, these results support the use of these medicinal plants for the control of diabetes mellitus and other oxidative stress diseases.

KEYWORDS: Hyperglycemia, *Senna auriculata*, *Psidium guajava*, *Syzygium cumini*, DPPH, phosphomolybdenum.

I. INTRODUCTION

Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and /or insulin action both causing by impaired metabolism of glucose, lipids and protein (Scheen et al., 1997). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra R. et al., 2006). The number of people suffering from the disease worldwide has increased at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild S.G. et al., 2004).

Psidium guajava, belonging to the family *Myrtaceae*, grows in tropical and subtropical regions worldwide like India, Pakistan, Bangladesh, Indonesia, South America and Syria. Its various parts, including, leaves, barks, flowers and fruits of guava a rich reservoir of bioactive compounds that have been traditionally used as folkloric herbal medicines, providing many therapeutic applications (J. Jiménez-Escrig et al., 2001). In guava, various compounds with antioxidative properties and phytochemical constituents are present, which includes alkaloids, polysaccharides, essential oils, minerals, vitamins, enzymes, triterpenoids, steroids, glycosides, tannins, flavonoids, and saponins. These include potential anti-cancer and antimicrobial properties. The fruit has showed potential anti-inflammatory, anticancer, antidiarrheal, antidiabetic, hepatoprotective, antioxidant, antimicrobial, anti-allergy, and anti-plasmodial effects. Both guava leaves and fruits have been historically employed to address an array of conditions, including gastroenteritis, hypertension, diabetes, dental caries, and pain relief (Kareem et al., 2024).

Psidium guajava (P. guajava), commonly referred to as guava is a tropical fruit extensively cultivated across various regions of the globe, encompassing countries like Egypt, India, Indonesia, Syria, Pakistan, Bangladesh, and South America (Kumar M. et al., 2021). It belongs to the *Myrtaceae* family and takes the form of an evergreen shrub or a compact tree (Laily N. et al., 2015). Not only utilized as a dietary staple, guava also holds significance in folk medicine, with distinct components of the plant boasting a spectrum of therapeutic attributes. Renowned for its dual role as sustenance and remedy, guava, originating in the tropics, boasts an extensive historical legacy (A. Gutiérrez et al., 2008). Multiple segments of the plant, encompassing leaves and fruits, offer an array of medicinal benefits, spanning from antimicrobial efficacy to potential anti-cancer attributes (Daswani PG et al., 2017). Among the noteworthy medicinal characteristics of guava is its efficacy in treating gastrointestinal infections, notably recognized as a traditional solution for conditions such as diarrhea (Nasser S. et al., 2018). Additionally, the extract derived from guava leaves has demonstrated antinociceptive properties, effectively mitigating pain (Jeffs et al., 1986). Furthermore, guava leaves find application in diabetes management, and the plant is credited with wound-healing capabilities, regulation of blood glucose levels, and enhancement of cardiovascular well-being (Koller et al., 1972).

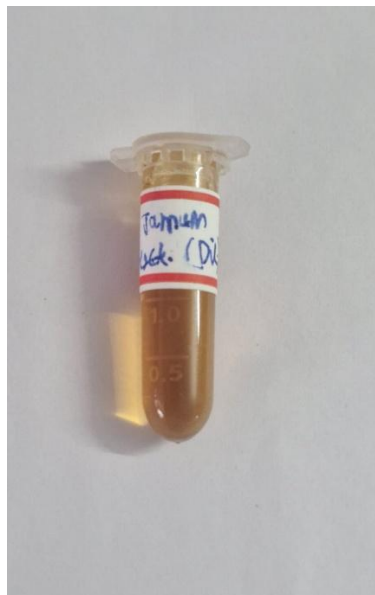
Jamun (*Syzygium cumini*) is an underexploited, important, indigenous fruit crop of our country. It belongs to *Myrtaceae* family and has gained great importance and recognition in recent past not only because of its hardy nature but also for its vast medicinal and nutritional properties (A. K. Sharma et al., 2015). The fruit is a good source of iron, minerals, sugars and proteins. The fruits are used in the preparation of delicious beverages, jam, jellies, squash, vinegar, wine, etc. The vinegar prepared out

of juice extracted from slightly unripe fruits is stomachic, carminative and diuretic, apart from having cooling and digestive properties (P. K. Warriar et al., 1996). The fruit syrup is a good remedy for diarrhoea. Jamun seed powder has antidiabetic properties and is a lotion for the cure of ringworm (A. K. Ayyanar et al., 2011). Maximum number of jamun trees is found scattered throughout the tropical and subtropical regions of the country. Seed contain an alkaloid jambosin and a glycoside, jambolin or antimallin, which reduces or stop diastatic conversion of starch into sugars. The volatile oil from the jamun seeds can be extracted & used as an effective medicine against diabetes, heart & liver trouble (C. G. S. Kumar et al., 2008).

Jamun seeds are refractory and are comparatively high in moisture content and possess a characteristic feature of losing their viability during desiccation. To increase the produce, there should be availability of good planting material along with proper management practices. The crop can be propagated through seeds, sexually and asexually by grafting (J. S. Bal, 2017). Major planting materials are produced from seeds and there are reports that do not present suitable germination (T. K. Bose et al., 2001). Since the seeds exhibit slow and fewer germination, pre-germination treatments may enhance the germination potential of jamun seeds.

In a present investigation, *Senna auriculata* (L.) Roxb leaves were experimented for their antidiabetic, antihyperlipidaemic and antioxidant efficacy. *Senna auriculata* (L.) Roxb (Family: *Fabaceae*) is vastly used in Indian traditional medicines and flowers are used for diabetes (Shanmugam S. et al., 2009); leaves and flowers for treatment of skin diseases (Sandhya B. et al., 2006); leaf juice used to reduce body heat (Latha M. et al., 2003). The flower and leaf extracts showcase antidiabetic activity in clinically induced diabetes rats (Uma Devi P. et al., 2006).

II. SAMPLE MATERIALS METHODS OF PREPARATION:



Syzygium cumini diluted Sample extract



Psidium guajava diluted Sample extract

Preparation of plant extracts for phytochemical screening and antidiabetic studies:

- The *Senna auriculata* flowers were shade dried and powdered by using a mechanical blender (Brinda P. et al., 1981). Hundred grams of powdered *Senna auriculata* leaves was packed in a Soxhlet apparatus and extracted with ethanol (Harborne et al., 1998). The extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract was used for antidiabetic studies (Lala PK, 1993).
- Fresh Jamuns or *Syzygium Cumini* were collected from the local market Chennai. The collected Jamuns were washed and pulped to retrieve the Jamun seeds and washed immediately. Then, the seed coat was manually removed to get the Jamun seed. These Jamun seeds were used for powder preparation. The seeds were washed properly to remove any adhering material. Then the Jamun seed were dried for 48 hours at 45°C by utilising sunlight. The dried materials were grinded in lab grinder into flour and transformed into fine powder by sieving. The powdered sample was then stored in polybag and stored for further use (Sood et al., 2018).

- Raw guavas or *Psidium Guajava* were washed, halved and cut into slices. It was subjected to drying in a convection oven. The convection oven was operated by directed sunlight. For sample extraction, samples were ground using a lab grinder. The powder was then extracted with 50 ml of methanol, and transferred into a 100 ml conical flask. The flask was then placed on an orbital shaker for 1 hour (Siow et al., 2013). Results suggest that oven drying is a viable option for the production of a functional ingredient that would improve the phenolic content of cereal foods while adding desirable guava flavour (Shaikh et al., 2018).

Preparation of Diluted Test Sample:

The test sample was diluted with ethanol at the ratio of 1:10 and stored it the cooler for further use (J. Sambrook et al., 2001).

Preparation of Yeast Suspension:

1% yeast sample (1g in 100 ml) was taken and incubated overnight. The incubated yeast sample was centrifuged and pelleted to collect the supernatant. The yeast supernatant was diluted at the ratio of 1:9 using distilled water to produce 10% Yeast suspension (Keith Wilson et al., 2010).

Antidiabetic Assay:

The assay has been performed according to the established method of Cirillo with some modifications. Commercial baker's yeast was dissolved in distilled water to make a 1% yeast suspension. The suspension was incubated at room temperature (25°C) overnight. The next day, a yeast cell suspension was centrifuged at 4200 rpm for 5 minutes. By repeating the procedure with the addition of distilled water to the pellet, a clear supernatant was obtained. A 10%v/v yeast cell suspension was made by combining exactly 10 parts clear supernatant fluid and 90 parts distilled water (Cirillo et al., 1962). Different test samples and standard drugs with concentrations ranging from 50µg to 1600µg were prepared in distilled water. 1ml of 5mM glucose was combined with 1 ml of test samples or standard drug. At 37°C, the mixture was incubated for 10 minutes. The reaction was started by adding 100µl of yeast suspension to the sample and glucose mixture, then vortexing and incubating for 60 minutes at 37°C. After incubation, the tubes were centrifuged for 5 minutes at 3800 rpm and the absorbance has been recorded by using a UV spectrophotometer at 520 nm (S. S. Nielsen, 2017). The percent increase in glucose uptake was calculated by the formula:

$$\% \text{ increase in glucose uptake} = \frac{((\text{Abs. of control} - \text{Abs. of sample}))}{(\text{Abs. of control})} \times 100$$

where control solution contains all the reagents except the test sample. Metformin was used as standard drug.

Antioxidant Assay:

[1] DPPH Radical Scavenging Assay

The antioxidant activity of the sample extract was measured by DPPH (1, 1- diphenyl 2-

picrylhydrazyl) free radical scavenging activity. 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (20-120µg/mL) of plant extracts. The mixture was then allowed to stand for 30 min incubation in dark (T. Yamaguchi et al., 1998). Distilled water was used as the reference standard. 1 mL methanol and 1 mL DPPH solution was used as the control (Blois et al., 1958). The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH radical inhibition} = \frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 100$$

[2] Phosphomolybdenum Reduction Assay

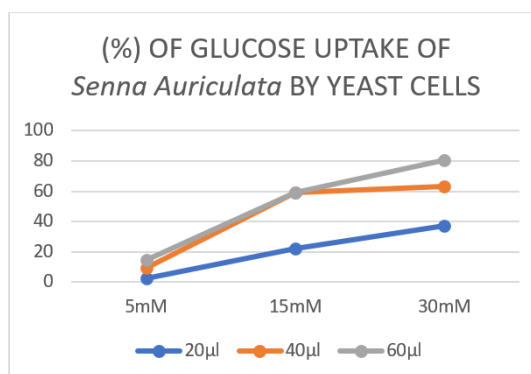
The antioxidant capacity of the sample extract was assessed by phosphomolybdenum reduction assay method. Extract with concentrations ranging from 20 - 120µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM) (Jayaprakasha et al., 2001). The reaction mixture was incubated in water bath at 90°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Distilled water was used as standard reference (Prieto et al., 1999). The percentage of inhibition was calculated using the following formula:

$$\% \text{ of Phosphomolybdenum Reduction} = \frac{(\text{Sample} - \text{Control})}{\text{Sample}} \times 100$$

III. OBSERVATIONS AND RESULTS

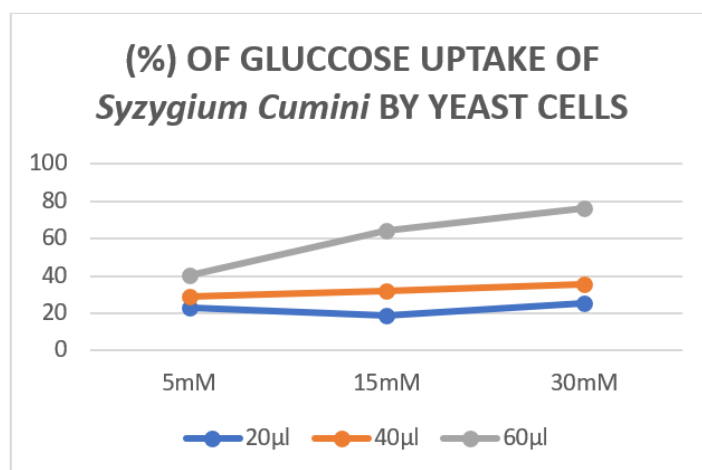
Antidiabetic Assay Observations:

1. *Senna Auriculata*



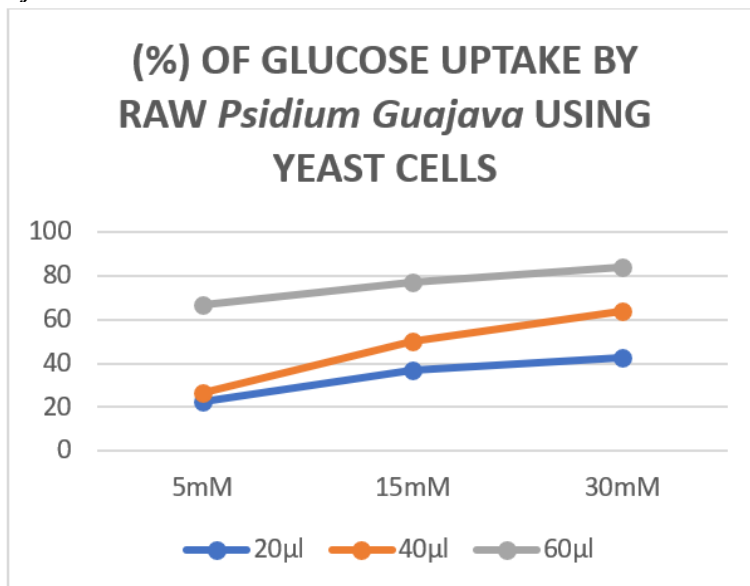
S. No.	CONCENTRATION	5mM	15mM	30mM
1.	20µl	2.38	21.87	36.95
2.	40µl	9.52	59.37	63.04
3.	60µl	14.3	59.37	80.43

2. *Syzygium Cumini*



S. No.	CONCENTRATION	5mM	15mM	30mM
1.	20µl	23.2	18.6	25.2
2.	40µl	28.8	31.8	35.6
3.	60µl	40.2	64.34	76.3

3. *Psidium Guajava*

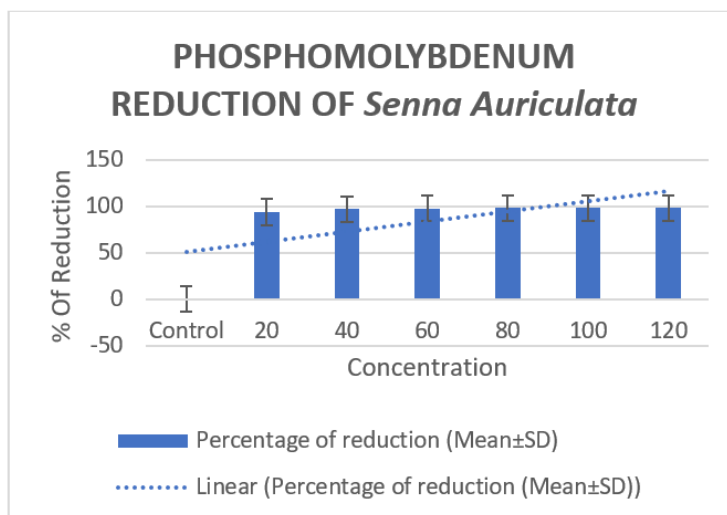


S. No.	CONCENTRATION	5mM	15mM	30mM
1.	20µl	22.3	37.04	42.7
2.	40µl	26.4	50	63.8
3.	60µl	66.7	76.85	83.8

Antioxidant Assay Observations:

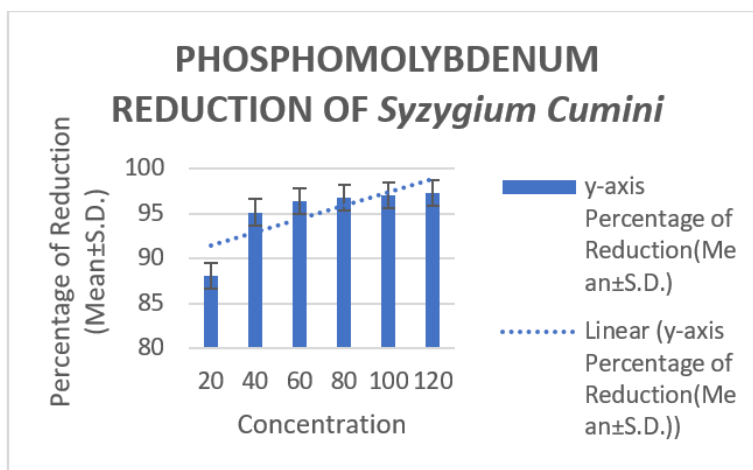
- **Phosphomolybdenum Reduction Assay:**

1. *Senna Auriculata*



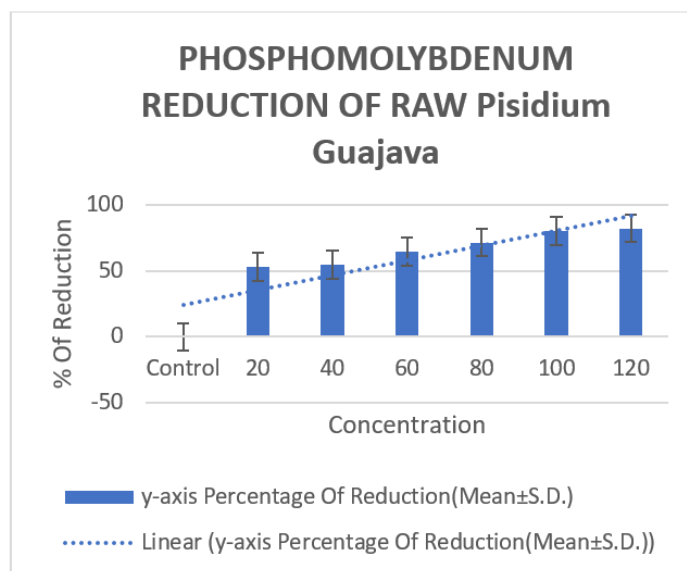
CONCENTRATION	A1	A2	A3	%R1	%R2	%R3	MEAN	S.D.
Control	0.017	0.006	0.011					
20	0.2	0.211	0.206	91.5	97.15	94.66	94.43	2.831
40	0.407	0.435	0.427	95.8	98.62	97.64	97.35	1.431
60	0.655	0.791	0.729	97.4	99.24	98.49	98.37	0.925
80	0.849	0.832	0.837	98	99.27	98.68	98.65	0.635
100	0.885	0.907	0.894	98.07	99.33	98.76	98.72	0.630
120	0.888	1.008	0.973	98.08	99.4	98.86	98.78	0.663

2. *Syzygium Cumini*:



CONCENTRATION	A1	A2	A3	%R1	%R2	%R3	MEAN	S.D.
Control	0.021	0.026	0.024					
20	0.2	0.198	0.199	89.5	86.9	87.9	88.1	1.311
40	0.491	0.491	0.492	95.7	94.7	95.12	95.17	0.502
60	0.667	0.668	0.669	96.85	96.1	96.41	96.45	0.377
80	0.727	0.722	0.725	97.2	96.4	96.7	96.77	0.404
100	0.808	0.825	0.813	97.4	96.8	97	97.07	0.305
120	0.884	0.921	0.899	97.62	97.2	97.3	97.37	0.219

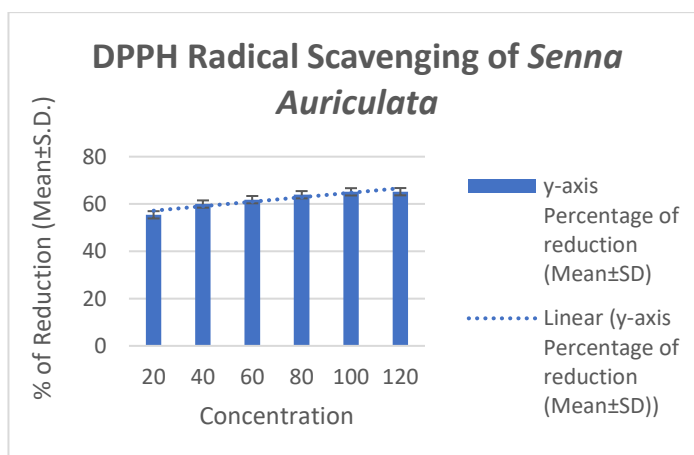
3. *Psidium Guajava*:



CONCENTRATION	A1	A2	A3	%R1	%R2	%R3	MEAN	S.D.
Control	0.241	0.247	0.244					
20	0.514	0.537	0.523	53.11	53.01	54.13	53.41	0.620
40	0.551	0.532	0.545	56.26	53.57	55.23	55.02	1.357
60	0.677	0.702	0.689	64.4	64.81	64.6	64.60	0.205
80	0.86	0.857	0.858	72.0	71.2	71.56	71.59	0.401
100	1.227	1.236	1.232	80.35	80.01	80.2	80.19	0.170
120	1.38	1.381	1.381	82.53	82.11	82.33	82.32	0.210

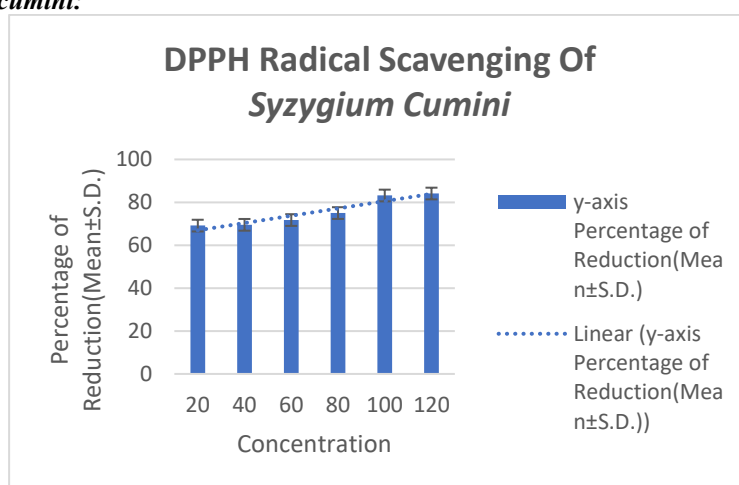
• DPPH Radical Scavenging Assay:

1. *Senna Auriculata*:



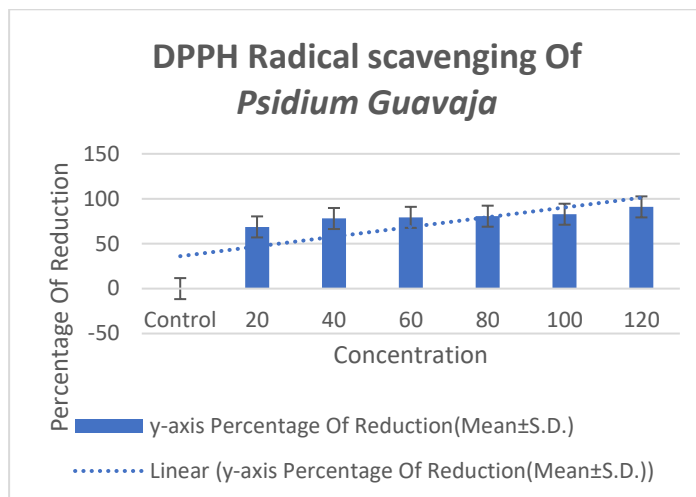
CONCENTRATION	A1	A2	A3	%I1	%I2	%I3	MEAN	S.D.
Control	0.235	0.257	0.269					
20	0.115	0.11	0.113	51.06	57.19	58	55.42	3.795
40	0.104	0.1	0.102	55.74	62.08	62.08	59.97	3.660
60	0.092	0.101	0.097	60.85	60.7	63.94	61.83	1.828
80	0.089	0.094	0.091	62.13	63.42	66.17	63.91	2.063
100	0.089	0.089	0.089	62.13	65.36	67.91	65.14	2.897
120	0.088	0.088	0.088	62.55	65.75	67.28	65.19	2.414

2. *Syzygium cumini*:



CONCENTRATION	A1	A2	A3	%I1	%I2	%I3	MEAN	S.D.
Control	0.26	0.252	0.256					
20	0.08	0.079	0.079	69.72	68.65	69.1	69.16	0.537
40	0.077	0.079	0.078	70.4	68.65	69.5	69.52	0.875
60	0.069	0.076	0.072	73.5	69.8	71.9	71.74	1.856
80	0.064	0.065	0.064	75.4	74.6	75	75	0.4
100	0.042	0.044	0.043	83.8	82.53	83.2	83.18	0.635
120	0.042	0.039	0.041	83.8	84.5	84	84.1	0.360

3. *Psidium Guajava*:



CONCENTRATION	A1	A2	A3	%I1	%I2	%I3	MEAN	S.D.
Control	0.234	0.23	0.232					
20	0.073	0.072	0.073	68.8	68.7	68.53	68.68	0.136
40	0.05	0.052	0.051	78.63	77.4	78.01	78.01	0.615
60	0.048	0.048	0.048	79.48	79.13	79.31	79.31	0.175
80	0.045	0.044	0.046	80.76	80.86	80.17	80.6	0.373
100	0.039	0.041	0.04	83.4	82.17	82.75	82.77	0.615
120	0.021	0.02	0.022	91.02	91.3	90.51	90.94	0.400

IV. CONCLUSION

Diabetes mellitus, one of the major public health problems worldwide, is a metabolic disorder characterized by high blood glucose levels with disturbances of carbohydrate, fat and protein metabolism as a result of defects in insulin secretion and/or insulin action. About 422 million people worldwide have diabetes, particularly in low- and middle-income countries, making diabetes mellitus one of the leading causes of death. Chronic hyperglycemia during diabetes causes the glycation of proteins, which in turn leads to complications (damage) affecting the eyes, kidneys, nerves, and arteries. Given the important role of oxidative stress in the pathogenesis of many clinical conditions and aging, antioxidant therapy could positively affect the natural history of several diseases. Hyperglycemia activates a particular metabolic route that involves diacylglycerol, protein kinase C, and NADPH-oxidase, culminating in reactive oxygen species (ROS). It is suggested that ROS is induced by hyperglycemia in diabetic patients through mitochondrial respiratory chain enzymes, xanthine oxidases, lipooxygenases, cyclooxygenases, nitric

oxide synthases, and peroxidases. Even though there are many conventional drugs such as metformin (MET), Thiazolidinediones (TZDs), sulfonylureas (SUF), dipeptidyl peptidase 4 (DPP-4) inhibitors, glucagon like peptide 1 (GLP-1) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, side effects still exist. As numerous plant extracts with antidiabetic effects have been widely reported, they have the potential to be a great therapeutic agent for type 2 diabetes with less side effects. In this study, three plant extracts such as *Senna auriculata*, *Syzygium cumini* and *Psidium guajava* were used to test glucose uptake assay by yeast cells. The *Psidium guajava* extract showed maximum of 83.80% inhibition of glucose uptake by yeast cells at 30 mM concentration and showed maximum DPPH radical scavenging activity of 90.94% at 120 µg/mL concentration. The *Senna auriculata* extract showed maximum phosphomolybdenum reduction of 98.78% at 120 µg/mL concentration. Based on the results *Psidium guajava* extract showed better glucose management as well as radical scavenging activities.

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