IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

Preliminary studies of extraction of Antidiabetic compounds from seeds of *Syzygium Jambolanum* (Black Plum, Jamun)

[1]*Ritwija Bhattacharjee, [2]Pushpa Agrawal

[1,2]Department of Biotechnology, RV College of Engineering, Bangalore-560 059, Karnataka, India

[1]* Corresponding author

Abstract: Diabetes mellitus is a long-term condition marked by elevated blood glucose levels. It is a leading cause of morbidity and mortality worldwide. Diabetes is linked to heart disease, particularly coronary artery disease. Plant-based diets, which include fruits, vegetables, herbs, and spices, are thought to be a good source of phytonutrients that can improve one's health. The presence of non-nutritive secondary metabolites known as phytochemicals, which contain numerous physiologically active moieties, has been related to the capacity of several plant-based meals to reduce the risk of chronic diseases. These phytonutrients' bioactivity has been linked to their antioxidant properties, as they scavenge free radicals, which are responsible for the development of various serious degenerative illnesses such as LDL oxidation, DNA oxidation, inflammation, and ageing. The *Syzygium jambolanum* is one of the valuable antidiabetic plants and a significant medicinal resource in many traditional medical systems, sometimes referred to as black plum and initially indigenous to India. The seeds include phenols, polyphenols, jambosine, alkaloid, and glycoside antimellin or jambolin, inhibiting the diastatic conversion of starch into sugar.

Objective of our study are, preparation and extraction of Jamun seed extracts, and characterization of the extracts using UV-Visible spectroscopy, Phytochemical screening of seed extracts and test on glucose using seed extracts. Here three different extracts, ethanol+water, pure ethanol and pure methanol have been used. All the extracts were placed in UV-Visible spectroscopy to check their absorbance. Glucose solution with different extracts were tested to check the glucose breakdown using UV-Visible spectroscopy.

All three extracts with jamun seed showed promising result in breaking down of the glucose, but pure ethanol extract showed the maximum breaking down of the glucose and it showed highest percentage of phenol and alkaloids are present, so these compounds in various food systems can be used in efficacious dose for humans, it is possible to develop nutraceuticals or functional foods for diabetic patients.

Keywords- Diabetes, *Syzygium jambolanum,* UV-Visible spectroscopy, insulin, jambosine, phenol and alkaloids

INTRODUCTION:

Despite huge advances in the field of medical sciences in the past century, diabetes mellitus, a condition as ancient as mankind, remains poorly controlled. Diabetes is now the biggest endocrine disease in the world and has an estimated impact on approximately 5% of the total. [1] Diabetes is on the rise across the world at an alarming pace owing to changes in lifestyle and has become a worldwide burden needing attention from the most populous nations in which their prevalence is steadily rising. [2] Two major diabetes types: Type I are an autoimmune condition in which T lymphocytes are implicated in the destruction of β-cells of the pancreas and are genetically predisposed. In children as well as young people, type I diabetes is very prevalent. Type II diabetes is very common and it affects 90 percent of the global population. [3] It is because of obesity as well as developing insulin resistance with a dysfunctional pancreas. Various therapy alternatives for diabetes in contemporary medical science are available, which are intended to regulate blood sugar but do not have a permanent cure. Diabetes in diabetic individuals is characterized by impaired sight, tiredness, hunger, polyurea, excess thirst, and loss of weight. [4] Different treatment methods for both type I and II diabetes in medical sciences are available. Diabetes causes many additional conditions, such as nephrotoxicity, cardiomyopathies, neuropathy, wound healing abnormalities as well as cerebrovascular disorders. [5] The worldwide cost of diabetes treatment is extremely expensive and alternative medicinal systems offer diabetic patients more therapeutic options. Ayurveda, an ancient Indian medicine system, is one of the oldest in the world in which diagnosis and management of diabetes are recorded. Experimental investigations conducted according to contemporary medical science provisions have revealed that certain of the polyherbal formulations and medicinal plants produced using Ayurveda plants help avoid both hyperglycaemia and associated consequences. [6] The *Syzygium jambolanum* (Syn Syzygium cumini, Eugenia cumini, Eugenia jambolana) is one of the valuable antidiabetic plants and a significant medicinal resource in many traditional medical systems, sometimes referred to as black plum and initially indigenous to India. It has also been found

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

to have chemo preventive, antineoplastic as well as radioprotective effects in the management of diabetes mellitus, inflammatory diseases, diarrhoea and ulcers in preclinical trials.[7] The plant is high in anthocyanin, ellagic acid, glucoside, myrecetin and kaempferol-containing substances. The seeds include phenols, polyphenols, jambosine, alkaloid, and glycoside antimellin or jambolin, inhibiting the diastatic conversion of starch into sugar. [8] Jamun was utilized in several alternatives and complementary medical systems and even in Europe, it was a frontline antidiabetic treatment before insulin was discovered. The brew made by boiling Jamun seeds in boiling water was utilized in many ancient Indian medical systems. A whole Jamun consists of an edible portion, kernel and seed coat [9].

Figure 1 - Jamun plant

Figure 2 – Jamun seed

The edible portion of Jamun entire fruit is about 75 percent. In the edible Jamun portion, protein of 0.7 percent, moisture of 83.7%, the fibre of 0.9%, fat of 0.3%, ash of 0.4% and carbohydrate of 14% were discovered. Jamun seed consists of 0.66% lipid, 6.63% protein, 1.32% insoluble dietary & fibre 75.4% carbohydrates. Jamun seed powder is a strong source of fat and cholesterol, vitamin B and C complexes and also dietary fibre, potassium, iron which are low in cholesterol and fat. It also includes some important minerals, like potassium, phosphorus sodium, calcium, and magnesium [1].

The effects on the pancreas of Jamun fruits are generally recognised as extremely beneficial for medical reasons, particularly for diabetes. [10] The fruit, juice and seed contain a biochemical product termed 'Jamboline,' supposed to

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

monitor the pathological conversion. From starch to sugar in case of enhanced glucose production. Jamun seeds are recognized to treat diarrhoea, diabetes, dysentery and blood pressure for their therapeutic characteristics [1].In the present review, Jamun seed and some of its components are proven anti-diabetic properties, previously many studies are available on Jamun seed but in this review, different extracts were prepared, checked for the presence of phytocompounds and test the activity on glucose solution.

MATERIALS AND METHODOLOGY:

Collection of fruits and processing:

Fresh Jamuns fruits were obtained from Bangalore's local market. The fruits were rinsed with water and pulped to obtain the Jamun seed and immediately rinsed. The seed coat was then peeled manually to obtain the Jamun seed. The seeds were washed thoroughly using distilled water and utilized to make a powder. Then the Jamun seed have been kept in dyer overnight at 60° Celsius. The dry materials were turned into flour by using a lab grinder and converted to a very fine powder via sieving and again keeping it overnight in a dryer in a sterilized condition. The powdered material was placed in a sterilized jar and retained for future use. Seeds were crushed, dried and ground to fine powder *of 60* mesh sieve size.

Figure 3 - collection of seed

Figure 4 – crushing and drying of seeds

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

Figure 5 – seed powder

Extraction of Phytochemicals:

The prepared seed powder was used for extraction using various solvents, i.e., ethanol (50ml), methanol (50 ml) and ethanol (25ml) + distilled water (25ml). Approximately 50g sample was put in volumetric flasks after adding solvent. The volumetric flasks were then put in an orbital shaker at 280 rpm and overnight at 50°C temperature. All extracts were subsequently filtered. The filtrate was then evaporated using a 40°C rotary evaporator with lower solvent removal pressure. The extracts then were kept for future use in corked bottles.

UV-Visible spectroscopy:

All the three jamun seed extracts (ethanol, methanol, ethanol+water) were made in triplicates. Samples were carefully placed in UV-Visible spectroscopy. UV Spectroscopy is a technique that uses ultraviolet light to determine a substance's absorbency. In simple terms, the approach maps and measures the interaction between light and matter. When matter absorbs light, it experiences either excitation or de-excitation, resulting in a spectrum. A UV-Visible spectroscopy measures the intensity of light passing through a sample solution in a cuvette, and compares it to the intensity of the light before it passes through the sample. Water was used as blank. Carefully analysis of absorbance graph was done to find the concentration of each metabolite present in the sample Due to unexpected increase in the values, the samples were diluted to get the perfect values.

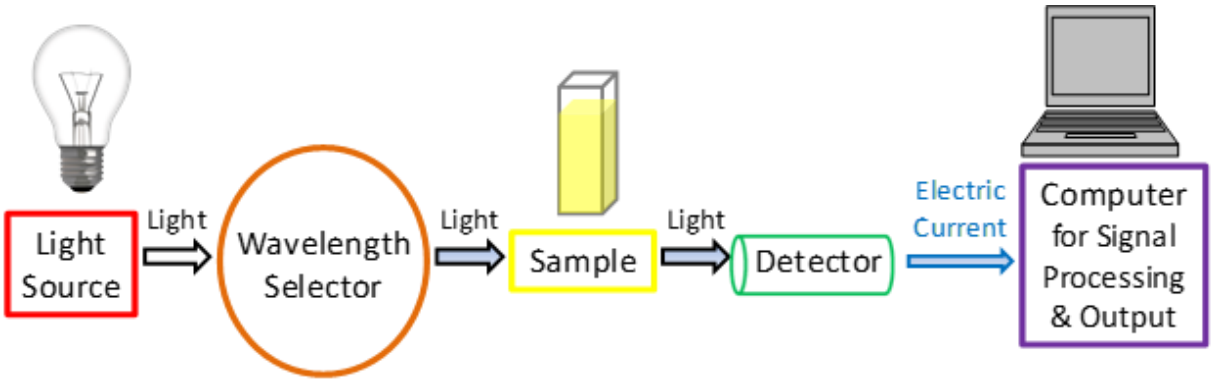


Figure 6 - UV-Visible spectroscopy .

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

Figure 7 - UV-Visible spectroscopy

Phytochemical screening:

All *Syzygium jambolanum* seed extracts samples were tested for the presence of phytochemical components Flavonoids Test: Alkaline Reagent Test - 2ml 2% NaOH with crude plant extract has been mixed. The concentrated yellow colour generated indicates that flavonoids are present. Tannins Test: In 0.5ml of crude extract, 10ml of bromine water was added. Bromine water discolouration indicates the presence of tannins. Steroids Test: Chloroform 2ml and sulphuric acid 0.5 ml of the crude extract have been added. The presence of steroids was confirmed by the formation of red colour in the lower chloroform layer. Saponins Test: 5ml distilled water has been put in a test tube with 0.5ml of raw extract and vigorously mixed. The appearance of the foam indicated saponins. Glycosides Test: Keller-kiliani Test-glacial acetic acid of 4ml and one drop of 2% FeCl3 has been added with crude extract of 1ml and then concentration Sulphuric acid of 1ml was added slowly. A brown ring will be formed which reveals the presence of glycosides.Terpenoid Test: Added 2ml chloroform to 0.5ml of crude extract, then boiled 3ml sulphuric concentration. The grey colour formation indicates the presence of terpenoids. Alkaloids Test: hydrochloric acid was added with crude extracts and filtered. Filtrate has been treated with a solution of potassium mercuric iodide (Mayer's reagent). The formation of a precipitate of white yellow or cream indicates the presence of alkaloids. Test for Phenols: 1-2 FeCl3 drops with 1ml of crude extract were added. The formation of green, blue, red, purple colours shows the presence of alkaloids.

Figure 8- phytochemical screening

Test on glucose degradation using seed Extract:

Known quantity glucose solution was prepared adding 30mg of glucose powder in 100ml of distilled water, which was used as stock solution. Standard graph was plotted by recording absorption at 270 nm with known concentration of samples. The Standard graph was used to determine concentrations of various materials present in three extracts in triplicate. In 12 conical flasks are marked, 1-3 conical flask for standard , 4-6 for ethanol+ water seed extract, 7-9 conical flask for pure ethanol seed extract and 10-12 conical flask for pure methanol extract. In each conical flask 60ml of the

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

glucose sample was added i.e. 30mg/100ml concentration of glucose. In conical flask 4-6 , 30ml of ethanol+water extract was added In conical flask 7-9, 30 ml of pure ethanol extract was added In conical flask 10-12, 30ml of methanol extract was added . The experimental conical flask was incubated for 30 minutes. Absorbance was detected for each one using UV-Visible spectroscopy. Atomic excitation is caused by the absorption of UV-Vis radiation, which refers to the transition of molecules from a low-energy ground state to an excited state. Based on their absorption characteristics, a UV-Visible spectroscopy can quantify the analytes in a sample.

The equation of Beer's law is a straight line with the general form of y = mx +b. where the slope, m, is equal to εl. The absorbance found for your unknown, along with the slope of best fit line, to determine c, the concentration of the unknown solution. The absorption values were then plotted in standard graph to find the concentration of glucose present and based on this the percentage of glucose degraded was calculated.

RESULT AND DISCUSSION:

Collection of plant material and extraction:

The prepared seed preparation was used for extraction was done using ethanol + water , ethanol and methanol. The seed powder was stored in the sealed jar with label on it for future use. And also, the extracts were kept for future use in corked bottles.

Figure 9 - collection and drying of seeds

Figure 10 – grinding of seed into powder and drying

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

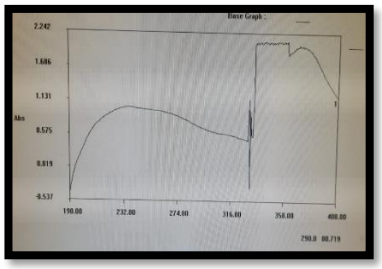
Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

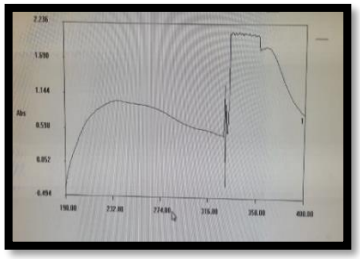
Figure 11 - extraction with three different extracts

UV-Visible spectroscopy:

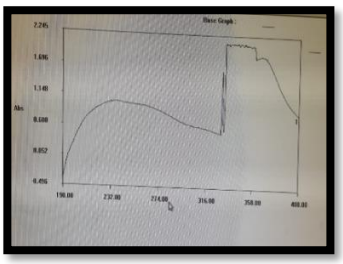
UV-Visible spectroscopy was used to determine the presence of phytochemicals in the seed extracts. The different absorption bands obtained in the UV-Vis region corresponded to the presence of alkaloids, flavonoids, phenolic acids, tannins in the seed extracts. From the graphs percentage of the phytochemicals present in the different seed extracts was calculated, which is shown in table 3. Ethanol extract showed the maximum phenol content which is an antidiabetic compound, followed by methanol extract and ethanol+water extract. Phenols are present in all the extracts so it can be said that it is soluble both in water and alcohol. Tannins were only present in ethanol+water extracts so it can be said that it is only water soluble. Ethanol+water showed highest terpenoid content which is also an antidiabetic compound. Alkaloids which is also known for its antidiabetic effect was maximum in ethanol extract.



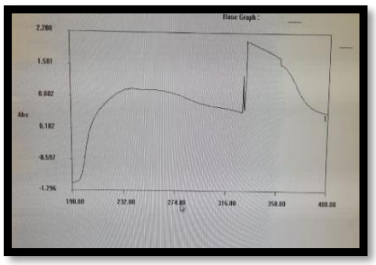
Graph-1 Ethanol+water (t1)



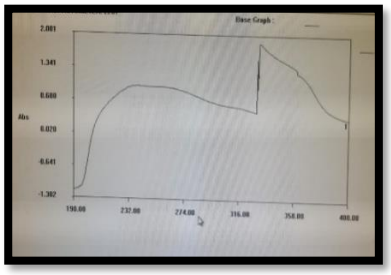
Graph-2 Ethanol+water (t2)



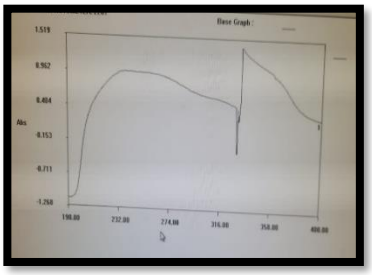
Graph-3 Ethanol+water (t3)



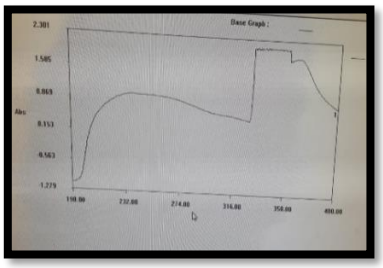
Graph-4 100% Ethanol (t1)



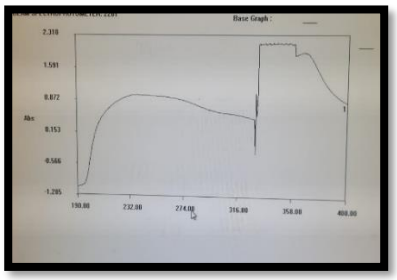
Graph-5 100% Ethanol (t2)



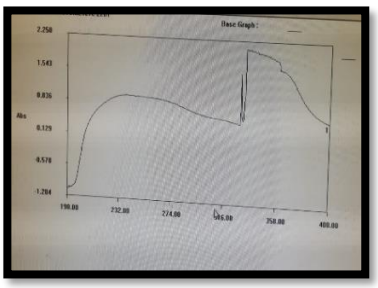
Graph-6 100% Ethanol (t3)



Graph-7 100% Methanol (t1)



Graph-8 100% Methanol (t2)



Graph-9 100% methanol (t3)

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

Table 2- absorbance of the seed extracts

Absorbance of test sample Extracts	Test1	Test2	Test3	Average after standard deviation
Ethanol + Water	1.201	1.178	1.13	1.169
Ethanol	1.200	1.152	1.23	1.194
Methane	1.101	1.109	1.117	1.109

From the above graph and table, the percentage of the phytochemicals present in the extracts was calculated.

Table 3- percentage of phytocompounds present in the seed extracts

Extracts Phytochemicals	Ethanol + water	Ethanol	Methanol
Phenol	50.51%	60.41%	51.79%
Terpenoids	15.16%	-	5.86%
Alkaloids	1.49%	33.34%	-
Tannins	30.83%	-	-
Glycoside	-	4.29%	33.33%

Phytochemical screening :

Preliminary phytochemical screening was done with Jamun seed extracts to test the presence of steroids, flavonoids, tannins, Glycosides, saponins, terpenoids and alkaloids. Ethanol + water extracts display the presence of tannins, terpenoids, alkaloids and phenols. Ethanol extracts shows the presence of phenol and alkaloids. Methanol extracts display the presence of phenol, terpenoids and glycoside. Previous studies have shown these compounds have a promising antidiabetic effect . According to the literature review, phenols decrease hyperglycaemia and improve acute insulin secretion and insulin sensitivity. The results given in Table 1 demonstrated that the maximum extraction of phytochemicals when ethanol is used as an extracting solvent. The maximum extraction of phytochemicals in ethanol is because of the polarity of the solvent that dissolves the polar molecules from plant samples.

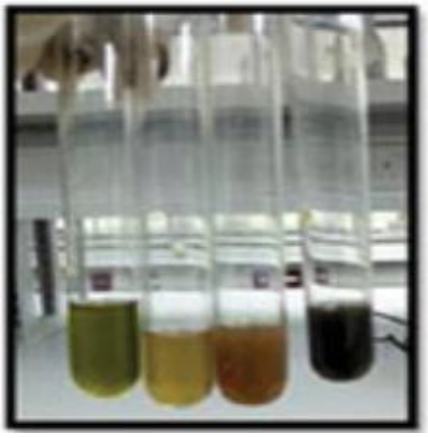


Figure 12 - phytochemical screening

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222

Table 4 – presence of phytochemicals in different seed extracts

Compounds	Ethanol+ water	Ethanol	Methanol
Flavonoids	-	-	-
Tannins	+	-	-
Steroids	-	-	-
Alkaloids	+	+	-
Phenols	+	+	+
Saponins	-	-	-
Glycosides	-	-	+
Terpenoids	+	-	+

Tests on glucose by extracts to study glucose degradation:

Absorbance was found out using UV-Visible spectroscopy. Atomic excitation is caused by the absorption of UV radiation, which refers to the transition of molecules from a low-energy ground state to an excited state. Based on their absorption characteristics, a UV-Visible spectroscopy can quantify the analytes in a sample. Standard graph was plotted with known concentration of samples. Using the standard graph, the concentration of glucose after extract treatment was 16.1mg/100ml for ethanol+water extract, 13.7mg/100ml for ethanol extract and 17.2mg/100ml for methanol extract. From the concentration percentage for glucose breakdown was calculated, 46.33% of glucose was broken down in ethanol+water seed extract, 54.33% of glucose was broken down in ethanol seed extract and 42.66% of glucose was broken down in methanol seed extract. It showed that all the phytochemicals present in the extracts are responsible in breaking down of the glucose.

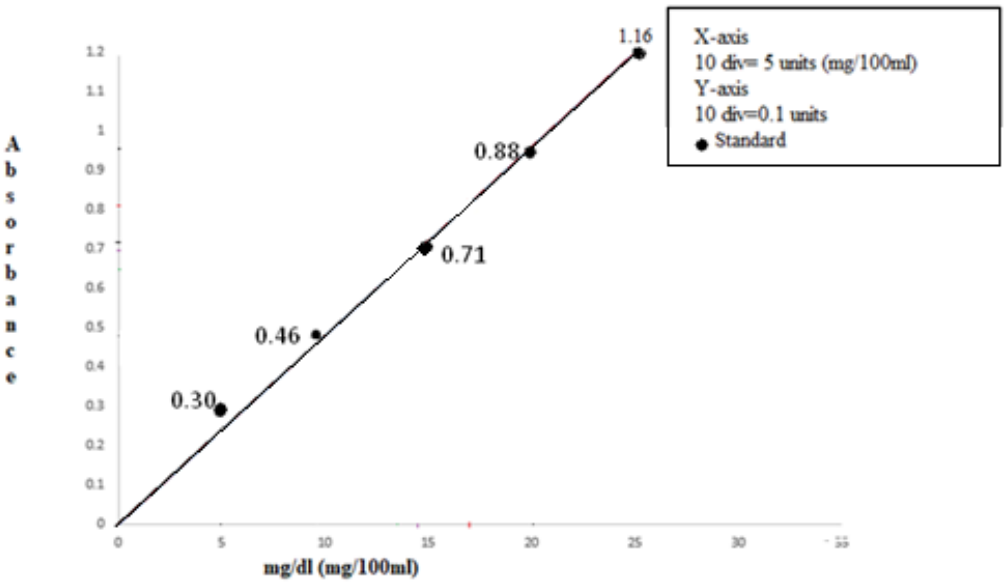
Figure 13 – glucose solution after treatment with extracts

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222



Graph 10 – Glucose standard concentration graph

Table 5- Absorption of known concentrations of glucose solution at 270 nm.

Concentration of glucose mg/ml	Glucose solution ml	Water ml	Absorbance
5	2	8	0.30
10	4	6	0.46
15	6	4	0.71
20	8	2	0.94
25	10	0	1.16

Table 6-absorbance of glucose solution after extracts treatment at 270 nm.

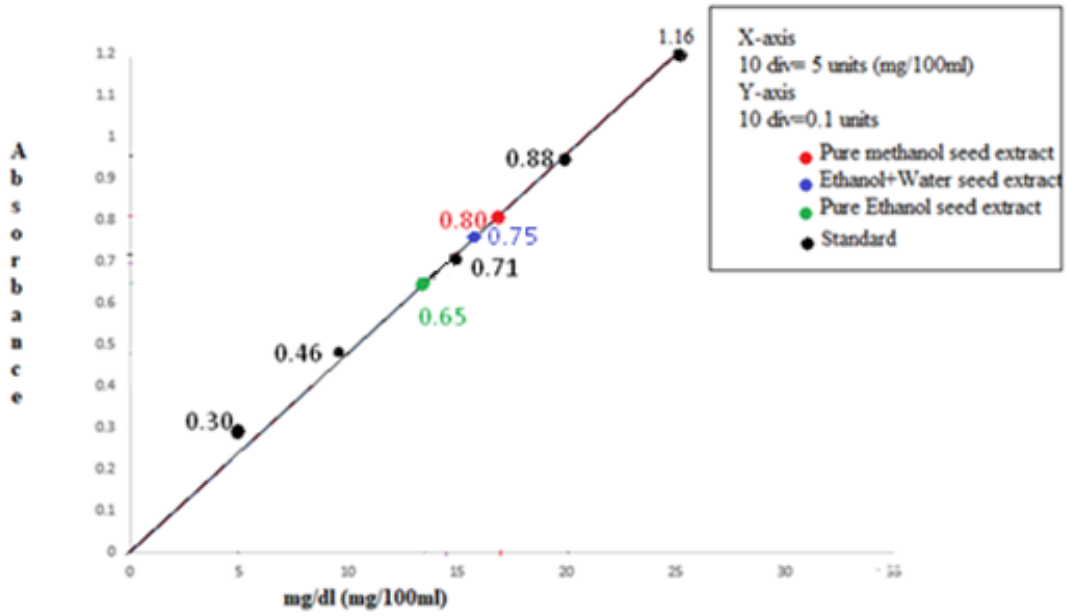
Extracts	Absorbance			Mean Absorbance	Concentration
	T1	T2	T3		
Ethanol+water	0.69	0.70	0.71	0.75	16.1 mg/100ml
Ethanol	0.63	0.66	0.66	0.65	13.7 mg/100ml
Methanol	0.81	0.80	0.79	0.80	17.2 mg/100ml

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9222



Graph 11 – Glucose standard concentration graph with extract samples.

CONCLUSIONS:

The ethanol + water Syzygium jambolanum seed extracts showed the presence of phenols, tannins, terpenoids and alkaloids. Ethanol *Syzygium jambolanum* seed extracts showed the presence of phenol and alkaloids. Methanol *Syzygium jambolanum* seed extracts showed the presence of phenol, glycoside, terpenoids. In 100% ethanol extract highest percentage of phenol and alkaloids are present. In ethanol+water *Syzygium jambolanum* seed extracts highest percentage of tannins is present. All the three extracts result shows that the jamun seed extract is useful for breaking down of glucose. The compounds showed in literature , like phenols, tannins, terpenoids, alkaloids which are present in these extract helps in breaking down glucose.Tannins are water soluble and are only present in ethanol+water . All extracts can be used breaking down of glucose, but pure ethanol extract showed highest capacity to breakdown glucose These phytochemical compounds in various food systems can be used in efficacious dose for humans, it is possible to develop nutraceuticals or functional foods for diabetic patients after a systematic research and development for drug delivery systems

REFERENCES

1. Marufa MA, Das PC, Iqbal A. Utilization of Jamun seed powder in composite cake formulation. Journal of the Bangladesh Agricultural University. 2019 Dec 31;17(4):599-605.
2. IDF Diabetes Atlas, 2017. Global Picture. In: Karuranga S, Fernandes JR, Huang Y, Malanda B, ediotrs. 8th ed. International Diabetes Federation. 2017:40–63.
3. Sicree R, Shaw J, Zimmet P. The Global Burden. Diabetes and Impaired Glucose Tolerance. Prevalence and Projections. In: Gan D. Editor. *Diabetes Atlas.* 3rd ed. Brussels: International Diabetes Federation; 2006. P. 16–103.
4. Mccrimmon RJ, Sherwin RS. Hypoglycemia in type 1 diabetes. *Diabetes*. 2010;59(10):2333–2339.
5. You WP, Henneberg M. Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth. *BMJ Open Diabetes Res Care*. 2016;4(1):e000161.
6. Herman WH, Zimmet P. Type 2 diabetes: an epidemic requiring global attention and urgent action. *Diabetes Care*. 2012;35(5):943–944.
7. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet*. 1997;349(9061):1269–1276.
8. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2009;32(Suppl 1):s62–s67.
9. Singh R, Teel C, Sabus C, et al. Fatigue in type 2 diabetes: impact on quality of life and predictors. *Plos One*. 2016;11(11):e0165652.
10. Khan SA, Saqib MN, Alim MA. Evaluation of quality characteristics of composite cake prepared from mixed jackfruit seed flour and wheat flour. Journal of the Bangladesh Agricultural University. 2016;14(2):219-27.
11. IDF Diabetes Atlas, 2017. Global Picture. In: Karuranga S, Fernandes JR, Huang Y, Malanda B, ediotrs. 8th ed. International Diabetes Federation. 2017:40–63.

DOI: 10.17148/IARJSET.2022.9222

12. M.S. Baliga, S. Fernandes, K.R. Thilakchand, P. D'souza, S. Rao ,Scientific validation of the antidiabetic effects of *Syzygium jambolanum* DC (black plum), a traditional medicinal plant of India J Altern Complement Med, 19 (3) (2013), pp. 191-197

13. Sicree R, Shaw J, Zimmet P. The Global Burden. Diabetes and Impaired Glucose Tolerance. Prevalence and Projections. In: Gan D. Editor. *Diabetes Atlas.* 3rd ed. Brussels: International Diabetes Federation; 2006. P. 16–103.

14. Warrier PK, Nambiar VPK, Ramankutty C. Indian medicinal plants. Hyderabad, India: Orient Longman Ltd., 1996: 225–228

15. A. Singh, M.A. Quraishi,The extract of Jamun (*Syzygium jambolanum*) seed as green corrosion inhibitor for acid media Res Chem Intermedia, 41 (5) (2013), pp. 2901-2914

16. Achrekar S, Kaklij GS, Pote MS, Kelkar SM. Hypoglycemic activity of Eugenia jambolana and Ficus bengalensis: Mechanism of action. In Vivo 1991;5:143–147.

17. N. Ayya, V. Nalwade, T.N. Khan,Effect of jamun (*Syzygium jambolanum* L.) Seed powder supplementation on blood glucose level of type-II diabetic subject Food Sci Res J, 6 (2) (2015), pp. 353-356

18. J. Grover, V. Vats, S. Rathi Anti-hyperglycemic effect of Eugenia jambolana and Tinospora cordifolia in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism J Ethnopharmacol, 73 (3) (2000), pp. 461-470

19. Forbes wf. light absorption studies: part xix. the ultraviolet absorption spectra of iodobenzenes. Canadian Journal of Chemistry. 1961 Nov 1;39(11):2295-305.

20. Li L, Long W, Wan X, Ding Q, Zhang F, Wan D. Studies on quantitative determination of total alkaloids and berberine in five origins of crude medicine “Sankezhen”. Journal of chromatographic science. 2015 Feb 1;53(2):307-11.

21. Rosas JA, Rodriguez JA, Fonseca AD, Aguilar AE, Macedo CM, Vázquez VS, Vázquez CS, Rosas ER, Rodríguez LA. Encapsulation of Glycosides from Extract Plantago Major in zno Matrices by Sol-Gel. Journal of Materials Science and Engineering A. 2015;5(3-4):154-8

22. Barbasz A, Oćwieja M, Barbasz J. Cytotoxic activity of highly purified silver nanoparticles sol against cells of human immune system. Applied biochemistry and biotechnology. 2015 Jun;176(3):817-34.

23. Walker Jr RD, Hawkins JE. The ultraviolet absorption spectra of some terpene hydrocarbons. Journal of the American Chemical Society. 1952 Aug;74(16):4209-10.

24. Sisa M, Bonnet SL, Ferreira D, Van der Westhuizen JH. Photochemistry of flavonoids. Molecules. 2010Aug;15(8):5196-245.

25. Helmstadter, “Syzygium cumini (L.) Skeels (Myrtaceae) Against Diabetes: 125 Years of Research,” Pharmazie, Vol. 63, No. 2, 2008, pp. 91-101.

26. K. Ravi, B. Ramachandran and S. Subramanian, “Protective Effect of Eugenia jambolana Seed Kernel on Tissue Antioxidants in Streptozotocin-Induced Diabetic Rats,” Biological & Pharmaceutical Bulletin, Vol. 27, No. 8, 2009, pp. 1212-1217

