Quest Journals
Journal of Research in Pharmaceutical Science
Volume 7 ~ Issue 3 (2021) pp: 01-07
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Invitro Studies on α-Amylase, α-Glucosidase Inhibition, Antioxidant and free radical scavenging Effects of Ethanolic Extract of *Bryophyllum pinnatum*

Alli SmithYemisi Rufina
Department of Biochemistry, Faculty of Science, Ekiti State University, Nigeria

ABSTRACT: *Diabetes mellitus (DM) is one of the non-communicable life threatening diseases and oxidative stress has been identified to play a major role in the pathogenesis of different clinical conditions such as diabetes. Bryophyllum pinnatum leaves are employed as food and as traditional medicines. This study investigates the antioxidant potentials of the ethanolic extract of the leaves of Bryophyllum pinnatum and the effect on carbohydrate metabolizing enzymes. The antioxidants potentials were evaluated by determining the Total phenolic, flavonoid content and free radical scavenging effects. Its inhibitory effect on α-amylase and α-glucosidase activities were also investigated. The results of the study indicated that Bryophyllum pinnatum leaves contained a considerably high amount of Total Phenol with the highest value (101.2 ± 2.83mg/g GAE) at concentration of 1mg/ml and Total Flavonoids has the highest value(45.8.2 ± 2.82mg/g GAE) at 0.5mg/ml when compared with other tropical plants. The ethanolic extracts was able to scavenge free radicals in a dose dependent manner. Results also showed that ethanolic extract of Bryophyllum pinnatum had a considerable inhibitory effect on α-amylase and α-glucosidase activities at different concentrations when compared with a standard. This study suggest that ethanolic extract of Bryophyllum pinnatum leaves could be considered as potential source of bio-active constituents with excellent antioxidant and anti-diabetic activity.*
Keywords: *α-amylase, α-glucosidase, oxidative stress, antioxidant,anti-diabetic*

Received 01 Mar, 2021; Revised: 12 Mar, 2021; Accepted 14 Mar, 2021
Published with open access at www.questjournals.org

I. INTRODUCTION

Diabetes mellitus, a complex metabolic disorder that results in increased blood glucose level, is considered as one of the major health related problems and is growing rapidly worldwide.[1] It occurs when the pancreas does not produce enough insulin or when the body cannot use the insulin effectively [2]. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030[3].

α-Glucosidase and α-amylase are the key enzymes involved in the digestion of the carbohydrate. α-Amylase hydrolyses the α-linked polysaccharides in to oligosaccharides, and α-glucosidases, membrane bound enzymes which are located in the brush border of the small intestine, catalyze the final step in the digestive process of carbohydrate to release absorbable monosaccharide's including glucose. Hence, inhibitors of these enzymes can slow down the liberation of absorbable monosaccharides from dietary complex carbohydrates, delaying the absorption of glucose into blood steam and thus preventing any sudden rise in meal induced blood glucose level [4-5].

Oxidative stress arises from an imbalanced redox status between the production of Reactive Oxygen Species (ROS) and the biological system able to remove them. ROS, including superoxide (O2−), hydroxyl radical (·OH) and H_2O_2, are constantly generated in aerobic organisms. The endogenous sources usually are oxidative phosphorylation, P_{450} metabolism, peroxisomes and inflammatory cell activation [1,2]. Nonenzymatic antioxidants, like vitamin E, vitamin C, β-carotene, glutathione, and coenzyme Q function to quench ROS [5].

Medicinal plants with antioxidant properties can also help to maintain oxidative balance within the body system. The number of people suffering worldwide multiplies with diabetes. Thus it is a global concern until the successive treatment is discovered. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs[6]. Plants play vital role in the medicinal field, because

of its healing capacity. Plant metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannin, saponin were responsible for potential activities[7].

Bryophyllum pinnatum is a 3 to 5 meters high perennial herb with opposed glabrous leaves. It has a sour taste, hot strength and sugary post digestive effect. The herb contains a wide range of valuable chemicals that could be responsible for its various pharmacological effects[8].

Inhibition of alpha amylase and alpha-glucosidase enzymes can be an important strategy in management of post prandial blood glucose level in type 2 diabetes patient[9]. Thus, the objective of this study is to investigate in-vitro antidiabetic activity and antioxidant potentials of ethanolic extract of *Bryophyllum pinnatum* leaves.

II. MATERIALS AND METHODS

2.1 Samples Collection and Preparation

Fresh leaves of *Bryophyllum pinnatum* was collected from a local farm at Ora Ekiti, Ekiti State, Nigeria. The plants were authenticated at the Herbarium of section of the Department of Plant Science and Biotechnology, of Ekiti State University, Ado-Ekiti, Nigeria. A voucher specimen was deposited in the Herbarium (Voucher no: UHAE-2020/081). The leaves air dried blended using the electric blender to increase the surface area and to hasten the process of extraction. The leaves in their blended form were stored in a stoppered container with necessary labeling for proper preservation.

2.2 Extraction of *Bryophyllum Pinnatum* with Ethanol

The powered leaves of ***Bryophyllum pinnatum*** (200g) was measured using the standard weighing balance. It was then extracted with 500ml ethanol inside stoppered glass bottles with continuous shaking overnight. The extracted solutions were then allowed to evaporate in open trays for few days inside a well-ventilated room. The resulting air dried extracts was weighed and used for the study.

2.3 Experimental Animals

Three male wistar albino rats weighing between 190 and 250g were purchased from the Animal House, Ekiti State University,Nigeria. They were housed in cages under controlled conditions of a 12hr light/dark cycle at room temperature. The rats were allowed to assess food and water ad *libitum*. The animals used was maintained with the principles and guidelines of the Nigeria Council on Animal Care as outlined in 'Guide for the Care and Use of Laboratory Animals'.

2.4 Preparation of Tissue Homogenates

The rats were decapitated under mild diethyl ether anaesthesia, and the tissues were rapidly dissected and placed on ice and weighed. These tissues were subsequently homogenized in cold saline. The homogenate was centrifuged (KX3400C Kenxin Intl. Co., Hong Kong) for 10 minutes at 3000 ×g.

2.5 Determination of Total phenolic content

The total phenolic content in the crude ethanolic extract of *Bryophyllum pinnatum* was determined according to a well-cited protocol[10]. Briefly, 1.5 mg of an extract was dissolved in 5 ml of methanol from which 40 µL was taken and dissolved in 3.16 ml of distilled water. To this 200 µl of Folin-Ciocalteu reagent was mixed and, after an interval of 8 min, 20% of 600 µl sodium carbonate solution was added. The mixture was incubated at 40 °C for 30 min and absorbance was measured at 765 nm on UV/Visible spectrophotometer. The standard calibration curve was prepared with gallic or tannic acid standard solution (50 to 500 mg/L) following the same procedure. The gallic acid equivalent (GAE/TAE) was determined from the following equation of the standard curve and the results were expressed as µg of GAE/TAE per mg of dried sample.

2.6 Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which absorb maximum at 415nm. 100µl of the sample extracts in methanol (10 mg/ml) will be mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm is then read after 40 minutes. Blank samples will be prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol will be measured under the same conditions. All determinations will be carried out in duplicates[11].

2.7 Determination of ABTS radical scavenging assay

The antioxidant effect of the leaf extracts was studied using ABTS (2,2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid) radical cation discoloration assay[12]. ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the extracts was added to

0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer and the percent inhibition was calculated using the formula.

$$\text{Inhibition (\%)} = \frac{Control-test}{Control} x\ 100$$

2.8 DPPH radical scavenging Assay

The free radical scavenging activities of the samples by DPPH method were determined according to the method reported by [13]. In this method, 2.4 mg of DPPH free radical were dissolved in 100 ml of methanol to prepare its stock solution, which was kept at 20 °C until required. The working solution of DPPH was obtained by diluting its stock solution with methanol till the absorbance was noted to be 0.980 ± 0.02 at 517nm. Then, 3 ml of the working solution was mixed with 100 µL of a sample (1 mg/ml). After incubating the mixture in the dark for 30 min, absorbance was measured at 517nm. The scavenging activity was calculated by using the formula:

% inhibition= [(Absorbance of blank - Absorbance of sample)/Absorbance of blank] × 100 % inhibition = 100-(ABS)*100

2.9 Superoxide Radical Scavenging Assay

To the reaction mixture containing 0.1 ml of NBT (1mg/ml solution in DMSO) and 0.3 ml of the extracts, the compound and standard in dimethyl sulphoxide(DMSO), 1 ml of alkaline DMSO (1ml DMSO containing, 5mMNaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560nm[14].

$$\text{Inhibition (\%)} = \frac{Control-test}{Control} x\ 100$$

3.0 Hydroxyl Radicals Scavenging Assay

The assay was performed as described by the method of [15] with minor changes. All solutions were prepared freshly. One millilitre of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer,pH 7.4), 500 µl solution of various concentrations of ethanol extracts (200–1000 lg/ml), 200 µl of 200 µM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 100 µl H_2O_2 (1 mM) and 100 µl ascorbic acid (1 mM). After an incubation period of 1 h at 37^{o}C, the extent of deoxy-ribose degradation was measured by the TBA reaction. The absorbance was read at 532nm against the blank solution. Vitamin C was used as a positive control.

$$\text{Inhibition (\%)} = \frac{Control-test}{Control} x\ 100$$

3.1 Evaluation of α-amylase inhibitory activity

Alpha-amylase activity was determined according to the protocol described by [16]. A volume of 250 µL of *B. pinnatum* leaf ethanolic extract at different concentrations (20 –100 µg mL^{-1}) was incubated with 500 µL of porcine pancreatic amylase (2U mL^{-1}) in 100 mmol L^{-1} phosphate buffer (pH 6.8) at 37°C for 20 min.
250µL of 1 % starch dissolved in 100 mmol L^{-1} phosphate buffer (pH 6.8) was then added to the mixture and incubated at 37 °C for 1 h. One ml of DNS color was then added to the solution and boiled for 10 min. The absorbance of resulting mixture was read at 540 nm and the enzyme inhibitory activity was calculated as percentage of control sample without inhibitors. All assays were applied in duplicate.

$$\alpha\text{−amylase inhibition}(\%)=A_{540}\text{control}-A_{540}\text{sample}/A_{540}\text{control}\times 100$$

3.2 Estimation of α-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was assessed in line with the protocol described by [16]. Briefly, 250µl of *B. pinnatum* leaf aqueous extract and fractions (HF, EAF, BF, AF), at different concentrations (20–100 µg ml^{-1}), was mixed with 500 µl of 1.0 U ml^{-1} α-glucosidase solution in 100 mmol/l phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250 µL of pNPG solution (5 mmol/l) in 100 mmol/l phosphate buffer (pH 6.8) was added and therefore the solution was more mixed at 37°C for 20 min. The absorbance of the free *p*-nitrophenol was read at 405nm and therefore the inhibitory activity was expressed as percentage of a control sample without inhibitors.

$$\alpha\text{−glucosidase inhibition (\%)}=A_{405}\text{control}-A_{405}\text{sample}/A_{405}\text{control}\times 100.$$

III. RESULTS AND DISCUSSION

Enzyme assays have become an extremely valuable means to evaluate the potential health advantages of herbals, dietary supplements, and nutraceuticals for the development and design of functional foods or phyto-pharmaceuticals[17]. Besides, most usual biochemical assays involve major enzymes applicable in metabolic syndrome like diabetes[18]. The inhibition of carbohydrate metabolizing enzymes like α-amylase and α-glucosidase delays the digestion and absorption of starch and later suppresses postprandial symptom.

The α-amylase and α-glucosidase inhibitory activities of ethanolic extract of *Bryophyllum pinnatum* as shows in (Figure1 & 2) indicated that, it could be helpful as an oral antidiabetic drug for the management of high blood sugar in patients with these syndromes.

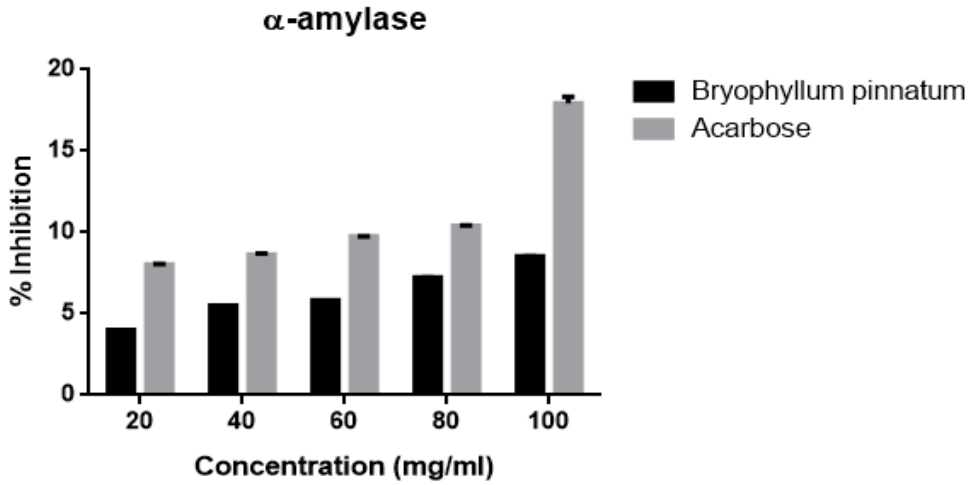

Figure1: Percentage Inhibition Of α- Amylase by ethanolic extract of *Bryophyllum pinnatum*

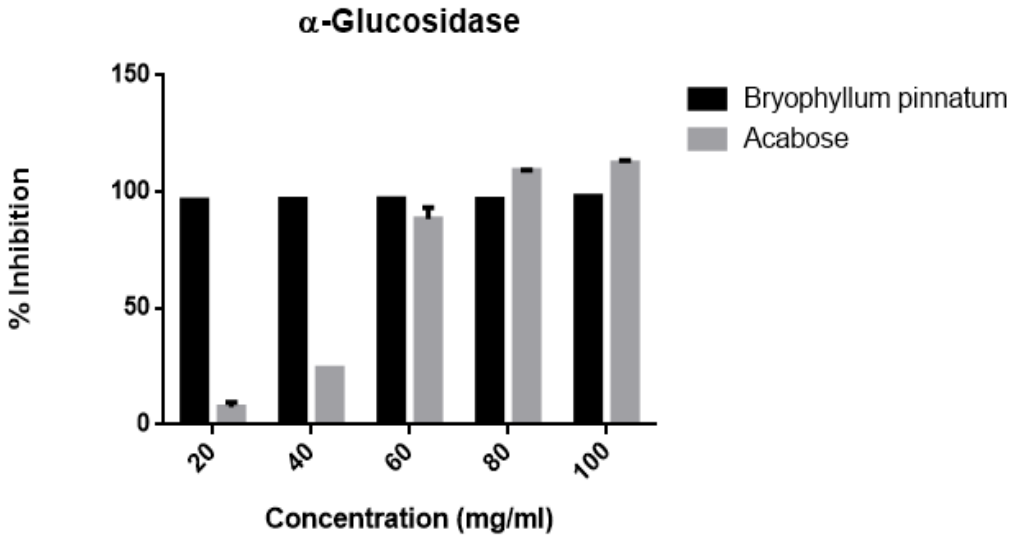

Figure 2: Percentage Inhibition Of α-Glucosidase by ethanolic extract of *Bryophyllum pinnatum*

Inhibitions of these enzymes interrupt macromolecule digestion and overall extend the breakdown time inflicting a discount in the degree of glucose ingestion and thus plummeting postprandial blood sugar[18]. From this study,the percentage inhibition of the α-amylase by the ethanolic extract of *Bryophyllum pinnatum* increases as the concentration of the extract increases which implies that the higher the concentration of the extract, the more potent inhibitor of the enzyme. This is consistent with earlier studies that antidiabetic agents derived from plants are strong inhibitors of α-amylase and α-glucosidase[19] and [20]. *Bryophyllum pinnatum* shows a very high percentage inhibition for α-glucosidase when compared with other plants and synthetic standard drugs[Acabose]. This implies that ethanolic extract of *Bryophyllum pinnatum* leaves could offer better pharmacological effect than the common synthetic drugs.

Recently, phenolic compounds have attracted great interest for their potential use in the development of new nutraceuticals or pharmaceuticals due to their remarkable antioxidant, anti-inflammatory, anticancer or antibacterial activities [21]. Hence, several of the possible protective effects of polyphenols could rely upon their concentration[22]. Flavonoids are major classes of phenolics and many studies have documented their biological and pharmacological activities [21]. The concentration of total phenols and total flavonoids of the ethanolic extract of *Bryophyllum pinnatum* Leaves in mg/gGAE is shown on (Table 1)**.**

Table 1: Total Phenolic and Flavonoids content Of Ethanolic Extract of *Bryophyllum pinnatum* leaves

Concentration(mg/ml)	Total Phenol (mg/g GAE)	Total Flavonoid(mg/g GAE)
0.5	67.2 ± 2.82	45.8 ± 2.72
1.0	101.2 ± 2.83	44.8 ± 5.65
1.5	49.2 ± 2.82	40.8 ± 1.41

Value represent mean ± standard deviation, number of samples n =2

The results indicated that the leaves at the concentration of 1mg/ml has the highest concentration of Total phenol while at 0.5mg/ml has the highest concentration Flavonoids.This gives the plant the ability to protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells and are strong antioxidants, capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases [21]. It was also reported that compounds containing phenolic could play an important role in eliminating radicals.

The ethanolic extract (Figure 3-6) of *Bryophyllum pinnatum* leaves shows that, it could eagerly inhibit ABTS, DPPH, Superoxide anion and Hydroxyl radicals in a concentration dependent manner specifying the presence of secondary metabolites like flavonoids, phenolic which validate their action [12].

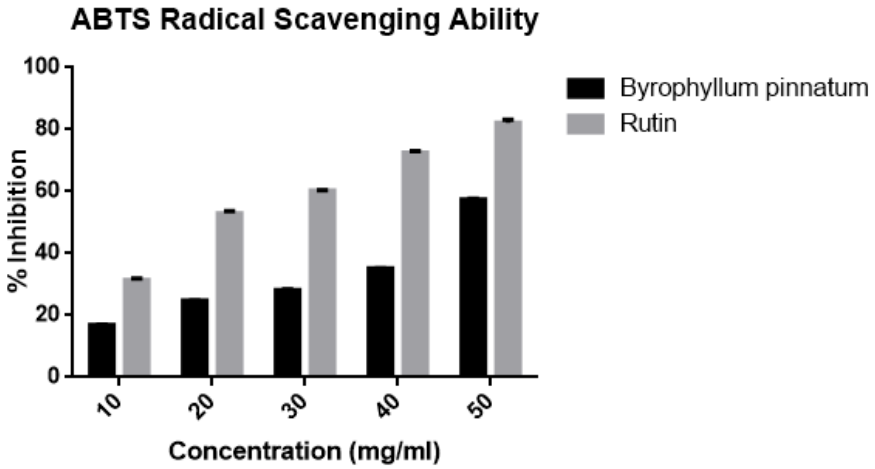

Figure 3: ABTS radical Scavenging ability of different concentrations of ethanolic extracts of *Bryophyllum pinnatum* leaves

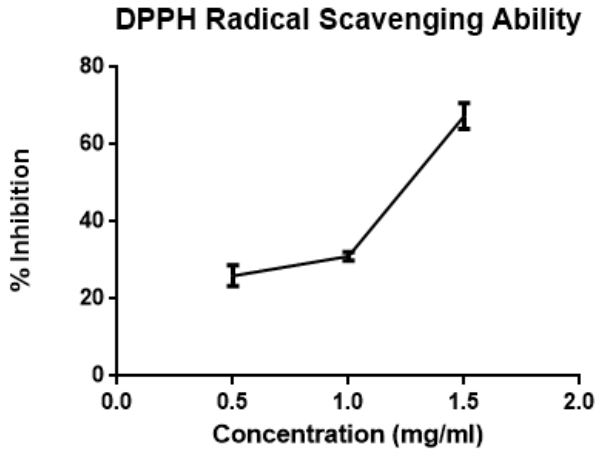

Figure 4: DPPH radical Scavenging ability of different concentrations of ethanolic extracts of *Bryophyllum pinnatum* leaves

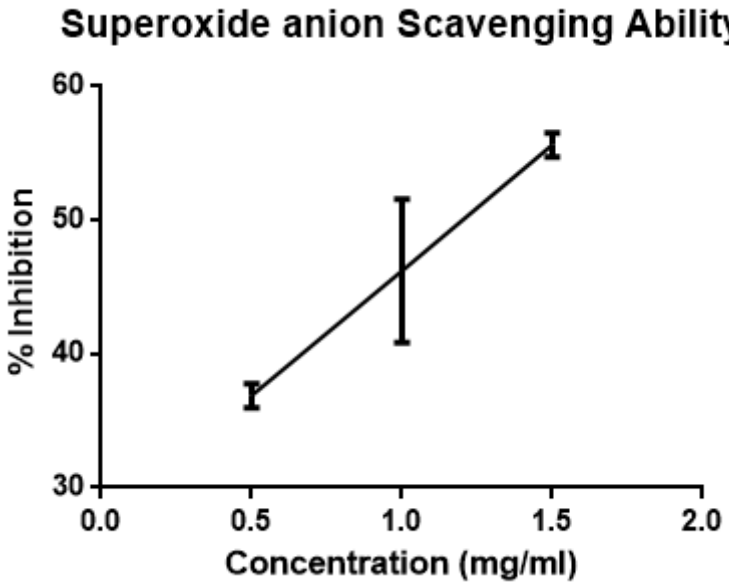

Figure 5: Superoxide anion radical Scavenging ability of different concentrations of ethanolic extracts of *Bryophyllum pinnatum* leaves

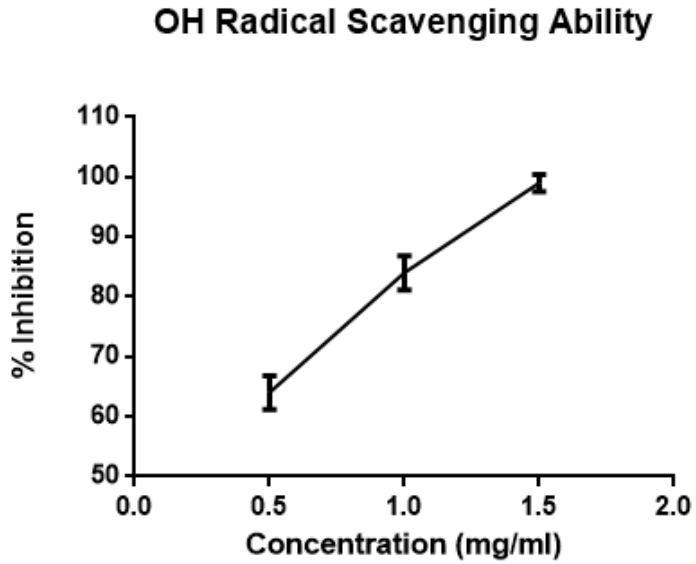

Figure 6: Hydroxyl radical Scavenging ability of different concentrations of ethanolic extracts of *Bryophyllum pinnatum* leaves

The findings of this study indicated that the ethanolic extract contained a considerably amount of ABTS and DPPH radical scavenging activity that shows B. pinnatum leaves were rich in biologically active components; thus, could be employed to formulate new plant-based pharmaceutical and nutraceutical drugs to improve human health. As shown in (Figure 5 and 6), the increased hydroxyl radical and superoxide anion scavenging activity with increase in concentration of the extract added (0.5–1.5 mg. mL-1) to the system, indicate that it contained appreciable O_2 scavenging activity in a dose-dependent manner.

Bryophyllum pinnatum leaves showed a dose dependent inhibitory activity towards superoxide and hydroxyl radical generation and the phenolic moiety of the structure was presumably responsible for its ability to scavenge free radicals[12]. Based upon these results, we could infer that the inhibitory mechanism of B. pinnatum is due to scavenging of O_2 by the action of Superoxide dismutase, which destroys the O_2 by converting it to H_2O_2 that can in turn be destroyed by catalase or glutathione peroxidase reactions.

IV. CONCLUSION

This research work shows the ability of *Bryophyllum pinnatum* to inhibit alpha amylase and alpha glucosidase when compared with other plants and synthetic drugs. This suggest that, it could offer a better pharmacological effect than the common synthetic drugs in the treatment of hyperglycemia. Its antioxidant activity indicates that it could be used in the management of diseases associated with oxidative stress.

REFERENCES

[1]. Pallavi, B., et al., In vitro evaluation of antidiabetic and antioxidant activity of Seabuckthorn (*Hippophae rhamnoides* L.). Journal of Medicinal Plants Research. 2015. 9(35): p. 929-932.

[2]. Arumugam, G., et al., A review: antidiabetic medicinal plants used for diabetes mellitus. Journal of Acute Disease.,2013. 13(2): p. 196-200.

[3]. Wild,S.G., et al., Global prevalence of Diabetes estimates for the year 2000 and projections for 2030. Diabetes care, 2004. 27:p. 1047-1053.

[4]. Mata, R., et al., Mexican Antidiabetic Herbs: Valuable sources of inhibitors of α-glucosidases. Journal of Natural Products,2013. 76(3): p. 468-483.

[5]. Van de Laar, F.A., et al., Alpha-Glucosidase inhibitors for patients with type 2 diabetes results from a cochrane systematic review and meta-analysis. Diabetes Care, 2005. 28(1): p.154-163.

[6]. Syamsudin, S., Standardization of extract of *Leucaena leucocephala* (lmk) De Wit seeds by -glucosidase inhibitor. International Journal of Phytomedicine, 2010. 2: p. 430-435.

[7]. Nittya, K.D., Phytochemical analysis and in vitro antioxidant studies of *Plumeria obtusa* L. leaves. Indian Journal of Pharmaceutical Science, 2016. 78(1): p.169-171.

[8]. Jaiswal, S and Sawhney, S., Correlation of epiphyllous bud differentiation with foliar senescence in crassulacean succulent Kalanchoepinnata as revealed by thidiazuron and ethrelapplication. Journal of Plant Physiology, 2006. 163(**7**): p. 717-722.

[9]. Karthic, K.S., et al., Identification of - amylase inhibitors from *Syzygium cumini* Linn seeds. Indian Journal of Experimental Biology, 2008. 6: p. 677- 680.

[10]. Singleton, V.L and Rossi, J.A., Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American Journal of Enol Vitic, 1965. 16: p.144–158.

[11]. Mervat, M.M., et al., Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. Australian Journal of Basic and Applied Science, 2009. 3(4): p.3609-3616.

[12]. Gülçin, I., et al., Antioxidant activity of bisbenzylisoquinoline alkaloids from Stephania rotunda: Cepharanthine and fangchinoline. Journal of Enzyme Inhibition and Medicinal Chemistry, 2010b. 25:p. 44-53.

[13]. Brand-Williams, W., et al.,Use of a free radical method to evaluate antioxidant activity. LebensmWiss & Technol/Food Science and Technology, 1995. 28:p. 25–30.

[14]. Van den Ende W., et al., 'Disease prevention by natural antioxidants and prebiotics acting as ROS scavengers in the gastrointestinal tract', 2011. 22(12): p. 689-697.

[15]. Elizabeth, K and Rao, M. N. A., Oxygen radical scavenging activity of curcumin. International Journal of Pharmaceutics, 1990.58: p. 237–240.

[16]. Bräunlich, M., et al., Extracts, anthocyanins and procyanidins from *Aronia melanocarpa* as radical scavengers and enzyme inhibitors. Nutrients, 2013. 5(3): p.663-678.

[17]. Bahadori, M.B., et al., Chemical composition and antimicrobial, cytotoxicity, antioxidant and enzyme inhibitory activities of Salvia spinosa L. Journal of Functional Foods, 2015. 18:p. 727–736

[18]. Zengin,G., et al., Anthraquinone profile, antioxidant and enzyme inhibitory effect of root extracts of eight *Asphodeline* taxa from Turkey: can *Asphodeline* roots be considered as a new source of natural compounds?, Journal of Enzyme Inhibition and Medicinal Chemistry,2016. 31(5): p. 754-759,

[19]. Ojo, O A., et al., Ethyl acetate fraction of bark of *Bridelia ferruginea* Benth. Inhibits carbohydrate hydrolyzing enzymes associated with type 2 diabetes (α-glucosidase and *α*-amylase). Advances in Bioresearch, 2016,.7(**3**): p.126–33

[20]. Kazeem, M.I., et al., *In vitro* study on the hypoglycemic potential of Nicotianatabacum leaf extracts. Bangladesh Journal of Pharmacology, 2014. 9(2):p.140–145

[21]. Ajiboye, B.O., et al .,. Inhibitory effect of key enzymes relevant to acute type-2-diabetes and antioxidative activity of ethanolic extract of *Artocarpus heterophyllus* stem bark. Journal of acute disease, 2016. 5(**5**): p.423–429

[22]. Sanchan, A.K., et al., Invitro studies on the inhibition of α-glucosidase and *α*-amylase by hydro-alcoholic extract of *Pluchea lanceolata*. Phamacognosy .Research, 2019. 11: p. 310-314