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Role of Secondary Metabolites in Alpha-Amylase Inhibition in Diabetes

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ABSTRACT

This study aims to establish the anti-diabetic potential of three medicinal plant yucca, Buxus bonsai , and Syngonium, this study adopted phytochemical screening tests. Widely allowed methods to assess antioxidant strength (DPPH assay), phenolic level (Folin-Ciocalteu method), and antibacterial activity (disc diffusion) were observed to test ethanol fresh leaves extract. The results indicated the differences in the phytochemical compositions of the plants. Yucca was the highest in phenolics (1.7 mg/mL) and the most potent in antioxidant activity (67.8% DPPH scavenging) and Buxus bonsai had the highest anti-diabetic profile with 66.16 percent inhibition of α-amylase. Syngonium failed to exhibit any significant symptom of alpha-amylase inhibition but demonstrated high levels of antibacterial activity. These findings indicate that Yucca possess significant antioxidant properties and Buxus bonsai possesses high anti-diabetic potential. The evidence further requests additional clinical studies and sustains the healing capabilities of these medicinal plants as a treatment of diabetes.

Keywords: Diabetes mellitus, α-amylase inhibition, medicinal plants, Yucca, Buxus bonsai , Syngonium, antioxidant activity, phenolic estimation , DPPH assay

I. INTRODUCTION

Diabetes is metabolic condition that arises as result of elevated raised blood sugar level in the body and this occurs when our body is not able to produce a neat amount of insulin and this can cause a variety of problems in the blood vessels, eye, kidney, heart and nerve(1).Diabetes takes two main types which include the Type 1 Diabetes and Type 2 Diabetes In Type 1 Diabetes, it is an autoimmune disease whereby, a local inflammatory response in and around of the islet precedes the selective destruction of insulin producing beta cells.Type II diabetes Occurs when the body does not react positively to insulin and it is

unable to manufacture sufficient amounts of insulin (3). This is capable of causing severe health conditions such as heart disease, sluggish blood circulation to the limbs, stroke, nerve damage, renal failure etc. The figures however vary quite extensively depending on the location; Bihar has it at a minimum of 4.3 percent and Punjab at a maximum of10 percent. It is also two times higher in cities (11.2%), than in countryside (5.2%). There is a higher rate of diabetes in rich states (13.6%) compared to poor states. In total, about 47 percent of diabetic patients who did not even know they had the condition, which means that 1 out of 2 people have an undiagnosed disorder. One out of every 10 people in India (or 10.3 percent of the total population) has prediabetes or is at risk of diabetes. Diabetes is no longer a rich man kind of disease with the lowest percentages in Mizoram and the worst in Tripura. The leading causes of this epidemic are lack of knowledge, bad diets and city life. India requires better screening and prevention activities, especially in the rural areas, where half of cases remained unidentified .In sum, approximately 8.4 million people in the world had type 1 diabetes. Where of One and a half million (18%) were under 20 years old. 64 percent or 5.4 million were adults between 20 to 59 years. Among the population aged 60 and older, 1.6 million (19 percent) were diagnosed in 2021, with an average age of diagnosis of 29. Approximately 35, 000 individuals died within a year after symptom onset but before a diagnosis (14).Diabetes had a hazard ratio (HR) of 2.00 (95% CI 1.83-2.19) with coronary heart disease and it is 2 times more than those without diabetes. The risk of coronary disease is higher in women and younger people Also, the research examines the death rate of other vascular problems, such as heart failure and hypertensive illness, which has an HR of 1.73 (95% CI 1.51-1.98) in diabetics(15) . The term secondary metabolites is used to describe organic substances produced by the plants but not directly involved in their growth, development, or reproduction (2). Secondary metabolites contrast with primary metabolites, which are substances required to make up and to run plant cells (proteins, lipids, carbohydrates and nucleic acids). In plants, secondary metabolites often play significant roles in plant ecophysiology in defense mechanisms against detrimental organisms as well as interactions with other living organisms (16). In this research, the plants Yucca , Syngonium and Buxus bonsai. YUCCA-The presence of an abundance of secondary metabolites in Yucca plants, such as flavonoids, phenolic compounds, steroidal saponins, is well-known (7). The potential anti-diabetic actions of these metabolites have been tested. As one example, yucca steroidal saponins have been demonstrated to bind to the active site of the enzyme, and inhibit the activity of alpha-amylase and thereby reduce starch digestion (2). SYNGONIUM-The species of syngonium are phenolic rich, especially flavonoid and alkaloids (3). Quercetin is one of the most effective inhibitors of alpha-amylase that belong to the family of flavonoids and kaempferol is also an alpha-amylase inhibitor. Terpene saponins and alkaloids present in Buxus Bonsai have antidiabetic potential where they bind to the active site of the enzyme and inhibit its activity (6) Alpha-amylase activity is inhibited by formation of stable complex by triterpenoids e.g. betulinic acid which lowers its capacity to hydrolyze starch (6).

II. MATERIALS AND METHOD –

3 plants are sourced out of the local nursery at Manimajra (Chote lal Nursery). To obtain fresh leaves of the selected plant, they were cleaned initially with the help of distilled water so that to remove dust or any other impurity. This was followed by softening of the leaves by letting them soak in distilled water for fifteen minutes. After immersion, the water was filtered out and then the leaves were pounded using a mortar and a paste. Subsequently crushed leaves were extracted with ethanol until the active ingredients were obtained. The above mixture was pass through filter papers to filter and the clear liquid that was obtained thereof collected as the plant extract (17) .

PLANT	FAMILY NAME	LOCAL NAME	PART USED
Yucca	Asparagus	Dakota	Leaves
Buxus Bonsai	Buxaceae	Boxwood	Leaves
Syngonium	Araceae	Arrowhead	Leaves

PHYTOCHEMICAL ANALYSIS-

Phytochemical testing aims at determining secondary metabolites such as flavonoids, phenolic compounds, tannins, alkaloids and other bioactive ingredients.

Detecting Alkaloids A. Dragendorff /Krauts test A. Few ml filterate + 1-2 ml of Dragendroffs reagents when the alkaloids are present a Reddish Brown Precipitate will form .

B. Mayer / Bertrand / Valser is as follows: A few ml of filterate with the addition of 1-2 drops of Mayer Reagent (by the sides of the test-tube) when Alkaloids are present, a creamy white, or yellow precipitate is formed.

Reducing Sugar detection name - Benedicts test- 0.5ml filterate +0.5 ml benedicts reagent +boil for 2 min green/yellow/red color appeared.

Proteins and amino acids identification- xanthoproteic test- plant extract + a few drops of concentrated nitric acid in case proteins and amino acids are present a yellow coloured solution will be appeared.

detection of flavanoids - conc. H 2 SO 4 test -plant extract+ conc. H 2 SO 4 In case flavonoids are to be detected then an orange colour will appear.

Phenolic compounds detection A. Iodine test -1 ml extract + few ml of dilute iodine solution if phenolic compounds will be present there will be formation of a transient red coloured solution.

B. Ferric chloride test- Aqueous solution + A drop or two of 5 % ferric chloride solution in case of phenolic compounds, dark green / bluish black colour will be observed.

Tannin-detection -0.4 to 0.4 ml of derivative of the plant extract combined with 4 ml of 10 percent NaOH (NaOH - 10 percent) mixture and shaken very well in case tannins are to be found there will occur the formation of emulsion (Hydrolyzable tannins).

Anthocyanins detection Hcl Test- 2 ml plant extract and 2 ml 2 N HCL (+ few ml ammonia)

In case of anthocyanins pink red solution that becomes blue violet on addition of ammonia appear.

ANTIBACTERIAL ACTIVITY-

Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi are the two Gram-negative bacteria and one Gram-positive bacterium that have been studied with an aim of determining the antibacterial potential of Yucca, Buxus bonsai and Syngonium. They prepared twenty milliliters of molten or liquefied agar medium in every clean Petri dish and followed by letting the medium to turn into solid agar media under aseptic environment. After solidification the bacterial cultures were dispersed well on the surface of the media. Under the disc diffusion methodology, 50 microliter of plant extracts was poured on sterilized discs and let it sit for 15 mins and after then positioned on the inoculated media. The plates were then incubated at 37 o C after for 24 hours. The antibacterial activity was identified using the diameter of the developed zones of inhibition surrounding each disc (mm) after incubation. These zones were measured in a millimeter scale and the findings recorded accordingly.

ANTIMICROBIAL SUSCEPTIBILITY TEST (AST)-

The antibacterial test included the use of the disc diffusion technique (18). In the method, Petri plates which were sterilized were used. The nutrient agar medium was prepared in aseptic conditions, poured in the plates and allowed to settle down. Salmonella typhi, Staphylococcus aureus, and Pseudomonas aeruginosa had an equal distribution all over the surface of the agar following its solidification.The sterile discs were dipped in 50 microliter of respective plant extracts and air dried for ten-fifteen minutes. Dried discs were then put on the surface of the inoculation plates, carefully. The common antibiotic was doxycycline. The plates were incubated at 37 oC during a period of twenty-four hours.After incubation

period, the zones of inhibition that surround the discs were measured in millimeters (mm) using a ruler.

TOTAL PHENOLIC ESTIMATION-

The overall content of phenolic compounds of the extracted plant was calculated using the Folin-Ciocalteu method. To do this, the extract of the plants 0.1 mL was dispersed in 0.9 mL of distilled water, after which 0.2 mL of the Folin-Ciocalteu reagent was added to the mixture. A 7% solution of sodium carbonate (Na 2 CO 3) was then added after the reaction was in progress after five minutes to the reaction mixture amounting to one milliliter. This mixture was subsequently allowed to incubate at 30 o C following a period of 90 minutes.The absorbance was determined by UV-Vis spectrophotometer at 750 nm after incubation. The calibration standard used was Gallic acid with a range of 0.2- 1.0 mg/mL(10).

III.DPPH ASSAY-

Sample Preparation: To individual test tubes add 0.5 mL of extract of the three plants (yucca, Buxus bonsai ,and Syngonium). Take 1.5 ml of DPPH solution and add it to each of the test tubes. Preparation of Control: In the case of the control mix 0.5 mL of distilled water with 1.5 mL of DPPH solution. Take ethanol as a blank . Incubation: All the them (sample and control) are to be incubated in the dark at the room temperature of 30 minutes. Absorbance: Perform measurements using a spectrophotometer after incubation with an absorbance measurement at 519 nm. The percentage inhibition of the free radical (DPPH) scavenging activity is calculated; [(OD value of control-OD value of test sample)/OD value of control] x 100.

IN VITRO ALPHA AMYLASE INHIBITION-

Phosphate Buffer (0.1 M, pH 6.5 -7.0) requires the following chemicals:Solution K 2 HPO 4 0. 1 M Solution and KH 2 PO 4 at 0. 1 M. They will be prepared by making individual 0.1 M solutions of K 2 HPO 4 and K H 2 PO 4. To obtain desired PH add one solution to the other while maintaining a consistent pH using a pH meter. When the pH has reached required point (pH level between 6.5 and 7.0), cease mixing.2. STARCH SOLUTION (1000ppm): Weigh and put 0.01 grams of soluble starch in a 5 milliliters of distilled water. Bring to a slow heat, till the starch is thoroughly dissolved. After dissolving, pour in the phosphate buffer which was prepared and bring down the volume to 10 mL. 3. DNSA Reagent or Dinitrosalicylic Acid reagent:Components required (50 mL):0.5g of NaOH 20 g of NaK tartrate, or sodium potassium tartrate 0.5g of DNSA, or 3,5-dinitrosalicylic acid, 0.125g of phenol.Just before using add 0.3 g of sodium sulfate (Na 2 SO 4).(NOTE-first of all dissolve the NAOH, then NAK tartrate in H2O after this add 3,5 dinitrosalicyclic acis and then phenol . Make up to the volume of 50 mL by adding distilled water as enough. Add a 0.3 g sodium sulfate just before using the reagent, and come to good mix. Enzyme activity is defined as μM of substrate consumed or product formed per unit time under specific conditions. The unit of enzyme activity is IU (International units) and Katal. IU is defined as μM of product produced or substrate consumed per min under defined set of conditions. Katal is mM of product formed per second under defined condition. To determine the enzyme activity of any enzyme we require the analysis methods for the estimation of substrate or product. For example to determine the amylase activity, there is requirement of analysis methods of substrate starch (Iodine methods) or product glucose (3, 5 dinitrosalicylic acid method). Amylases converts starch (substrate) into glucose (product) which is determined by the DNSA method spectrophotometrically. The Aminonitrosalicylic acid is formed by reaction of DNSA with glucose (reducing sugar) in the presence of sodium potassium tartrate as catalyst, which has maximum absorbance at 540nm. Amylase activity is defined as μM of glucose formed per ml of enzyme solution in 1 min under defined set of conditions (pH, temperature etc)

Procedure:Mix 0.5 ml each of starch and enzyme solution. Incubate at 40˚C for 20 min (Reaction time

T1). In this time enzyme will convert starch (substrate) in to glucose (product).To determine the glucose formed in the reaction follow the 3, 5 dinitrosalicylic acid (3, 5, DNSA) method. Add 3 ml of DNSA solution and keep in boiling water for 15min.Remove the test tubes from the water bath and add 15ml of distilled water in each test tube. Take optical density at 540 nm. Determine the glucose concentration (ppm or µg/ml or mg/L) from the standard curve of glucose (as per given below) and determine the enzyme activity in terms of IU (International Units) as mentioned in general calculation.Preparation of glucose standard curve by DNSA method Procedure: Prepare different concentration of glucose (50-5000ppm) using stock solution of 5000 ppm and make final volume 1ml with distilled water. Add 3ml of DNSA solution and keep in boiling water for 15min . Add 15ml of distilled water in each test tube and take optical density at 540nm. Plot the standard curve, optical density (Y axis) versus glucose (X axis, ppm or µg/ml or mg/L) concentration.Calculation for the enzyme activity

Enzyme activity (IU) X (µg glucose formed in reaction calculated from standard curve)

= Reaction time (T1) × X × MW

T1=Reaction time (T1).

X= Volume of enzyme used in reaction.

Mw= Molecular weight of Product (Note: In this case product is glucose; so molecular weight 180 AMU).

The percentage inhibition (I%) = [ODcontrol - ODsample/ ODcontrol]x100.

IV. RESULT AND DISCUSSION-

The phytochemical screening is done on three plants yucca, buxus bonsai and syngonium. Medicinal properties of the plants are determined by phytochemical plant species [8] The aqueous extract of yucca product give positive test of proteins and aa,flavonoids, lowering sugars, tannins and negative of alkaloids, phenolic compounds, antocyanins, in case of buxus bonsai positive test is given to alkaloids, reducing sugars, proteins and aa, flavanoids and negative to anthocyanins, tannins, phenolic compounds as shown in table I,II,III.

Table I Phytochemical analysis for yucca

Sr. No.	Phytochemicals	Results
1.	Alkaloids	Negative
2.	Reducing Sugar	Positive
3.	Proteins and AA	Positive
4.	Flavanoids	Positive
5.	Phenolic compounds	Negative
6.	Tannins	Positive
7.	Anthocyanins	Negative

Table II Phytochemical Analysis for Buxus Bonsai

Sr. No.	Phytochemicals	Results
1.	Alkaloids	Negative
2.	Reducing Sugars	Positive
3.	Proteins and AA	Positive
4.	Flavanoids	Negative
5.	Phenolic compounds	Positive
6.	Anthocyanins	Negative

Table III Phytochemical Analysis for Syngonium

Sr. No.	Phytochemicals	Result
1.	Alkaloids	Positive
2.	Reducing Sugars	Positive
3.	Proteins and AA	Positive
4.	Flavanoids	Positive
5.	Phenolic compounds	Positive
6.	Tannins	Negative
7.	Anthocyanins	Negative

ANTIBACTERIAL ACTIVITY-

In varied concentration, aqueous extracts of Yucca, Buxus bonsai and Syngonium plant exhibited antibacterial effect on different strains of bacteria including pseudomonas aeruginosa , salmonella and staphylococcus aureus. Syngonium has great inhibitory activity with 25mm against staphylococcus aureus and 24mm against salmonella and 23mm after

side pseudomonas aeruginosa then yucca and buxus bonsai appears (12mm and 11 mm) against staphylococcus aureus.(9mm and 10mm) against salmonella and yucca shows 9mm against pseudomonas aeruginosa whereas Buxus bonsai shows no zone of inhibition against pseudomonas aeruginosa.

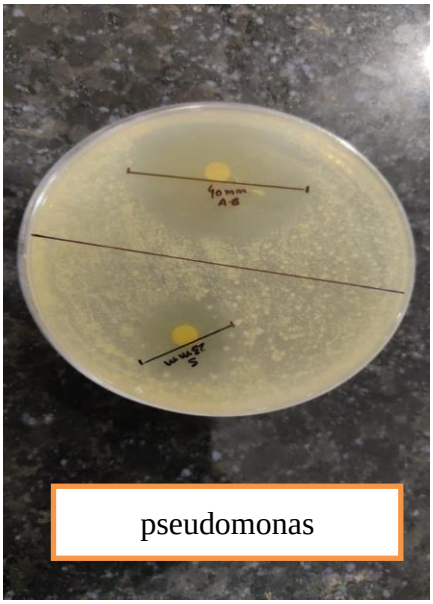


Fig 1 Antibacterial activity of syngonium extract against salmonella , staphylococcus areus and pseudomonas aeruginosa.

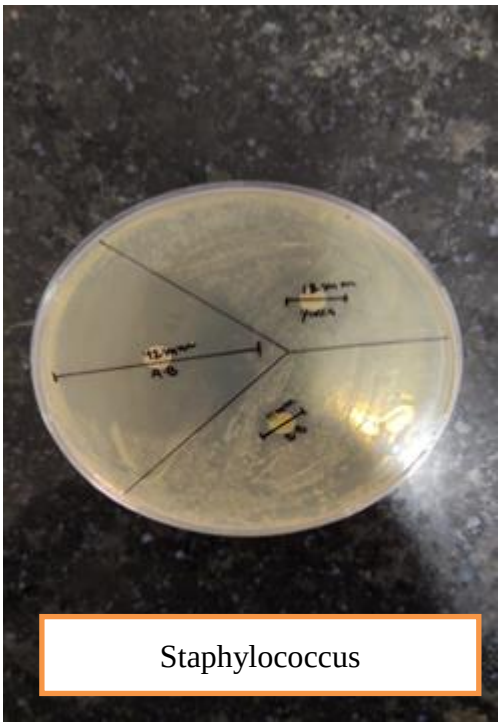


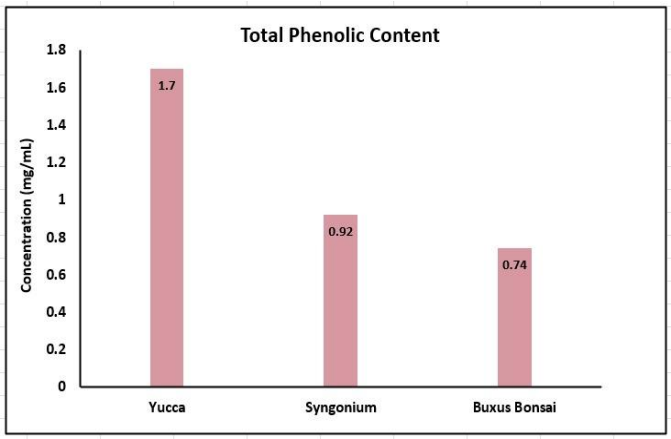
Fig 2 Antibacterial activity of yucca and buxus bansoi against , staphylococcus areus ,salmonella and pseudomonas aeruginosa .

PHENOLIC CONTENT ESTIMATION

To determine the contents of phenolic, Folin-Ciocalteu method (9) was determined as result in figure 1. The highest level of phenolic content was in yucca extract at 1.7 mg/mL. Their concentrations of Syngonium and Buxus Bonsai extract amount to 0.92 and 0.74 mg/mL, respectively. These values are expressed as gallic acid equivalents, and the uniform way to report phenolics (10). The Yucca phenolic was nearly two times the content of the other two plants. The various phenolic compounds found in each of the three extracts appeared to differ between species implying that each species had different secondary metabolite synthesis(11).

TABLE 1 Phenolic content result

Plant	Phenolic Content (mg/mL)
Yucca	1.7
Syngonium	0.92
Buxus Bonsai	0.74



DPPH ASSAY-

In evaluating the antioxidant activity of plant extracts, a assay of DPPH was performed based on the use of vitamin C as a reference of antioxidant activity of the extracts at a concentration of 0.5 mg/mL and 1 mg/mL. The highest activity was observed in Yucca because it ranged to 0.5 mg/mL with 67.8 percent, followed by Syngonium (25.9 percent) and Buxus bonsai (58.2 percent). At 1 mg2/mL, activities decreased to 39.1 percent, 23.3 percent and 3.1 percent, respectively. Vitamin C was 82.9 percent and 47.2 percent active at its concentration of 0.5 and 1 mg/mL, respectively. Of the examined extracts, Yucca had the highest potential of an antioxidant action when measured using the DPPH radical scavenging test (11).

Table 1. Antioxidant activity (%) of plant extracts and vitamin C at different concentrations

sample	0.5mg/ml	1mg/ml
Yucca	67.8	39.1
Buxus bonsai	58.2	23.3
Syngonium	25.9	3.1
Vitamin c (control)	82.9	47.2

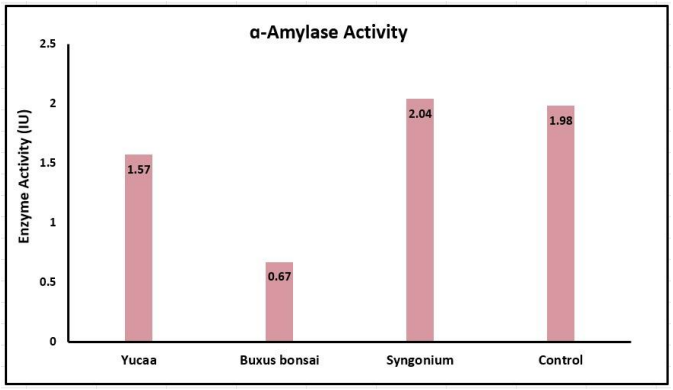
ALPHA AMYLASE ACTIVITY AND INHIBITION ANALYSIS-

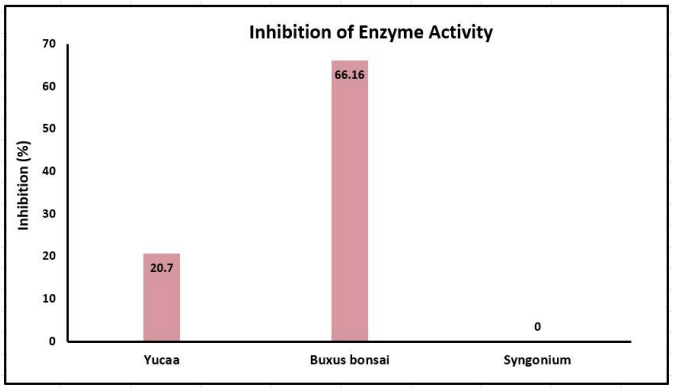
Three plant extracts were tested in their ability to inhibit α-amylase and their α-amylase activity. The result depicted in Table 1 reveals that the Buxus bonsai showed the best enzyme inhibition result of 66.16 percent, which means it is the most stunning blood sugar regulator.

Syngonium did increase enzyme activity compared to the control and it did not show any inhibition (0%) at all. Since the Buxus bonsai can reduce the 2 of 3 enzyme activity quite significantly, the results show that it does have the potential to fight diabetes to a significant level.

Table 1: α-Amylase Activity and Inhibition by Plant Extracts

Plant extract	Enzyme activity (IU)	Inhibition %
Yucca	1.57	20.7
Buxus Bonsai	0.67	66.66
Syngonium	2.04	0
Control	1.98	-





V. DISCUSSION

The given research study hypothesizes the anti-diabetic effects of three traditional medicinal plants based on extensive phytochemical and biological activity . Their differences in phytochemicals found when comparing the three plants indicate that they have variable biosynthesis patterns in secondary metabolites which is directly proportional to therapeutic efficacy.

Buxus bonsai was identified as the best anti-diabetic prospect with the ability to inhibit 66.16 percent of alpha amylase, which is much stronger than Yucca (20.7 percent) and Syngonium (0 percent). Such significant depressant effect can be explained by the fact that Buxus bonsai contains alkaloids and other phenolic compounds that were found to interact effectively with the active sites of enzymes before. The inhibition of the α-amylase is very important in the treatment of diabetes because they slow down carbohydrate digestion process and lower the glucose levels after meals.

The highest expression of phenolic (1.7 mg/mL) and antioxidant (67.8% DPPH scavenger) was observed in yucca and it can therefore be exploited in diabetes management due to its ability to mitigate oxidative stress related to diabetes challenges. The linear relationship that is observed between the phenolic contents and the antioxidant activity confirms earlier reports that phenolic compounds are the major contributors to radical scavenging capacity . Though Yucca exhibited inhibitory effect on alpha -amylase (with moderate inhibition) it has also been shown to have antioxidant features that can be used as a complementary treatment in diabetic patients.

Syngonium also had no amylase inhibitory activity, but had excellent antibacterial activities because it exhibited zones of 25mm, 24mm, and 23mm against the bacteria tested. This antibacterial effect is especially applicable to diabetic patients since they are at risks of developing and being infected with bacteria because of weakened immune systems.

The results of the study correspond to other studies on anti-diabetic treatments involving plant-based therapies that observed various mechanisms of action which sum to the overall treatment effectivity 77% The enzyme inhibition, antioxidant and antimicrobial properties imply that such plants may be useful in the integrative care of diabetes.

Nevertheless, the drawbacks are that characterization of extraction conditions should be standardized, that dose response experiments should be done as a well as safety analyses. To confirm these in vitro results, in vivo studies and clinical trials should also be pursued in the future to determine therapeutic doses of this finding to use in clinical practice.

VI. CONCLUSION

In this study, the anti-diabetic potential of Yucca, Buxus bonsai , and Syngonium was evaluated based on the measurements of the α-amylase inhibition activities, antioxidants stability, antimicrobial activity, and phytochemical determination.

Phytochemical screening showed that the three plants exhibited different secondary metabolite profiles. Buxus bonsai exhibited the highest anti-diabetic potential that indicated great therapeutic potential in the management of diabetes that inhibited 66.16 of alpha-amylase. Yucca possessed the highest phenolic content (1.7 mg/mL) and ideal antioxidant performance DPPH scavenging (67.8%) that made it practical in regulating the oxidative stress in diabetics. It also showed relatively small 20.7 % alpha-amylase inhibition. Though Syngonium did not show any alpha amylase inhibitory activity, it exhibited significant antibacterial findings with all the bacteria used.

Yucca also contains additional antioxidant advantage as per the results whereas Buxus bonsai has the maximum active potential to prohibit diabetes due to their significant enzyme inhibiting power. The findings mean that Buxus bonsai should undergo further investigations in the form of in vivo studies and clinical trials and the fact that plant-based medication could be used to treat diabetes. Nevertheless, standardization of the extraction procedures and lengthy safety tests are yet to be completed before any clinical applications can be proposed.

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