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IN VITRO EVALUATION OF ANTI-DIABETIC ACTIVITY OF POLYALTHIA KORINTI EXTRACTS

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ABSTRACT

Diabetes mellitus is a multi-etiology metabolic disorder in which chronic hyperglycemia is caused by deficiencies or changes in either insulin discharge or behaviour resulting in disruptions in the metabolism of carbohydrates, fats and proteins. Medicinal plants are rich in bioactive compounds, making them an important raw material for drug production and a target for the quest for new drugs. To evaluate the anti-diabetic activity of the *P.korintii* leaves and bark, the extracts were tested. The results of the α- amylase inhibition and α-glucosidase inhibition activities showed that all the extracts of *Polyalthia korinti* leaves and bark showed a varying effect on glucose utilization. Out of all the extracts Methanol extract of leaves showed the lowest IC_{50} value. The lowest IC_{50} indicates the strongest ability of the extracts to act as an amylase and α - glucosidase inhibitory activity. The glucose transfer across the yeast cell membrane was enhanced by all sample extracts. When glucose uptake by yeast cells was compared at 5 mM, 10 mM, and 20 mM for the same amount of plant extract, an inverse relationship to the molar concentration of glucose was observed. Out of all the extracts methanol extracts have great potential as antidiabetic. The results can be used to guide future research into how to prepare and optimize plant extracts for structural elucidation and characterization methodologies to identify anti-diabetic bioactive compounds.

Key words: Diabetic mellitus, *Polyalthia korinti* , α- amylase inhibition, α -glucosidase inhibition, glucose uptake.

1. INTRODUCTION

Diabetes mellitus is a multi-etiology metabolic disorder in which chronic hyperglycemia is caused by deficiencies or changes in either insulin discharge or behaviour resulting in disruptions in the metabolism of carbohydrates, fats and proteins (Narmadha and Devaki 2012). The medical community also faces a challenge in treating diabetes without negative side effects, as the most recent diabetes treatments include insulin and a number of oral anti-diabetic agents, all of which have a slew of negative side effects. As a result, it's become important to conduct research and testing on medicinal plants that have therapeutic effectiveness in the treatment of diabetes mellitus.

Several bioactive compounds from plants have been shown to have a hypoglycemic effect involving alkaloids, flavonoids, triterpenoids, and carbohydrates (Perumal et al. 2012; Oboh et al. 2011; Dineshkumar et al. 2010; Sharma et al. 2010; Panda and Kar 2007; Yoshikawa et al. 2007; Contreras et al. 2005). Medicinal plants are rich in bioactive compounds, making them an important raw material for drug production and a target for the quest for new drugs (Priyanga et al. 2014). Many scientists have looked into plants that contain different phytochemicals that have additive and synergistic interactions in antidiabetic properties that have optimistic health-promoting effects (Perumal et al. 2015).

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant material

The leaves and bark material of *Polyalthia korinti* (Dunal.) Thaw. was collected from the Seshachalam Hills. Seshachalam hill ranges of Eastern Ghats lie between 13°38' to 13°55' N latitudes and 79° 07 to 79° 24' E longitudes and spread over two districts viz., Chittoor and Kadapa of Southern Andhra Pradesh.

The Plant was authenticated by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupati, and Andhra Pradesh. Where the voucher specimens were deposited (Herbarium voucher No 566).

The required quantity of plant parts was collected and separated from undesirable materials. Washed with running water followed by distilled water to remove the contaminants. Chopping process was carried out and they were allowed to dry under shade (Mehrotra 1976). The dried material was ground into a coarse powder with the help of a suitable pulverizer. The powder was stored in an airtight container and kept in a cool, dark and dry place

2.2 Extraction Technique

The dried powder of the leaves and bark was extracted sequentially (Wiart et al. 2004) using a soxhlet apparatus (Lin et al. 1999) with various solvents based on their polarity, such as hexane, chloroform, methanol, and water. Using a rotary evaporator, the extracts were concentrated and solvent-free under reduced pressure. To measure the extractive yield, the dried crude concentrated extracts were weighed and stored in an airtight bottle until used for analysis.

2.3 Anti-Diabetic Activity

The anti-diabetic properties of *P.korinti* plant extracts were investigated using three methods: assay of α-amylase inhibition activity, assay of α-glucosidase inhibition activity, and Glucose uptake by Yeast cells method.

2.3.1 Assay of α - amylase inhibition activity

1 gram of starch was dissolved in 100 mL of 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride (NaCl) to make a starch solution (1% w/v). 27.5 mg Porcine pancreatic α-amylase (PPA) was combined in 100 mL of 20 mM phosphate buffer saline (PBS, pH 6.9) with 6.7 mM NaCl to make the enzyme solution. 200 µL porcine pancreatic amylase (2, 4, 8, 10, 15 µg/mL) was added to 100 µL plant extracts and incubated for 20 minutes at 37 ºC. 100µL (1%) starch solution was added to the reaction mixture and incubated for 10 minutes at 37 ºC. The reaction was stopped by adding 200 µL DNSA (1g of 3,5 dinitro salicylic acid, 30g of sodium-potassium tartrate, and 20 mL of 2N sodium hydroxide were added and made up to a final volume of 100 mL with distilled water) and heating it for 5 minutes in a boiling water bath. Absorbance was measured at 540 nm after the reaction mixture was diluted with 2.2 mL of water. Blank tubes were made by replacing the enzyme solution with 200 µL of distilled water for each concentration. The control, which represented 100 percent enzyme activity, was prepared in the same way but without the extract. The amylase inhibitor was tested using acarbose as a positive control. The experiments were carried out three times with the same protocol each time (Ali et al. 2006; Kim et al. 2011).

2.3.2 Assay of α -glucosidase inhibition activity

A updated version of a previously published technique (Kim et al. 2011) was used to test the inhibition of α-glucosidase activity. In 100 mL of phosphate buffer, 1 mg of α-glucosidase was dissolved (pH 6.8). After 200µL glucosidase was added to 100 µL plant extracts (2, 4, 8, 10, 15 g/mL), the mixture was incubated at 37°C for 20 minutes. After that, the reaction mixture was added to 100 µl of 3 mM p-nitrophenyl –α-D-glucopyranoside (p-NPG) and incubated for 10 minutes at 37 ºC. The reaction was stopped by adding 2 mL 0.1M Na_2CO_3, and the activity of α-glucosidase was calculated by using a spectrophotometer UV-VIS to measure the amount of p-nitrophenol released from p-NPG. In the α-glucosidase inhibitor test, acarbose was used as a positive control. The IC_{50} value was defined as the extract concentration required inhibiting 50% of α-amylase and α-glucosidase activity under assay conditions.

2.3.3 Glucose uptake by Yeast cells method

Gupta et al. (2003) had previously established a method for preparing yeast cells for glucose uptake. Commercial baker's yeast was washed with distilled water and centrifuged (3,000 g for 5 min) before the supernatant fluids cleared and a 10% (v/v) suspension was obtained. Extracts (10–50µg/mL) were applied to 1 mL of glucose solution (5, 10, and 20mM) and incubated for 10 minutes at 37 °C. The experiment began with the addition of 100 µL of yeast suspension, vortexing it, and then incubating it at 37 °C for 60 minutes. The tubes were centrifuged (3000 g for 5 min) after 60 minutes to determine the glucose content of the supernatant. As a control (reference) drug, metformin was used. The following formula was used to determine the percentage increase in glucose absorption by yeast cells:

% inhibition of glucose uptake = ((Abs sample – Abs control)/Abs control) * 100.

Where Abs control represents the absorbance of the control reaction (which contains all reagents except the test sample) and Abs sample represents the absorbance of the test sample. Triplicates of each experiment were carried out.

3. RESULTS AND DISCUSSION

One of the strategies and approaches used to treat diabetes mellitus is to block carbohydrate digestive enzymes including α-amylase and α-glucosidase from absorbing glucose in the gastrointestinal tract, thus lowering postprandial glucose levels (Mccue et al. 2005). It has been discovered that α-amylase and α-glucosidase inhibitors are successful in lowering postprandial hyperglycemia levels. A number of α -amylase and α-glucosidase inhibitors have been isolated from medicinal plants to serve as an alternative to existing synthetic drugs with better effectiveness and less side effects (Matsui et al. 2006; Matsuda et al. 2002).

3.1 Assay of α - amylase inhibitory activity

The results of the DNSA study and IC_{50} values are summarized in Fig. 1 to 2. All the above extracts of *Polyalthia korinti* leaves and bark showed a varying effect on glucose utilization. At all concentrations (2-15µg/mL), methanol extract of leaves and bark showed maximum inhibition of the enzyme with the highest value of 19.18±0.04% and 18.20±0.04 respectively seen at 15µg/mL concentration of plant extract.

Water extract of leaves and bark showed the next highest value of 17.54±0.05 and 16.82±0.04 respectively seen at 15 µg/mL concentration. Followed by chloroform extract of leaves (16.73±0.05) and bark (16.27±0.05) seen at 15µg/mL concentration. The hexane extract of leaves and bark showed the least inhibition of enzyme with the highest value of 13.46±0.06 and 12.77±0.09 respectively at 15µg/mL concentration. Out of all the extracts Methanol extract of leaves showed the lowest IC_{50} value 40.41±0.080. The lowest IC_{50} indicates the strongest ability of the extracts to act as an amylase inhibitory activity.

Acarbose at a concentration of (2-15 µg/mL) showed inhibitory activity of α-amylase between 5.17±0.04 and 30.57±0.06 with an IC_{50} value of 24.54±0.030µg/mL. A comparison of α –amylase inhibitory activity between the standard drug

and plant extracts has been depicted in Fig. 1. The results proved that *P.korinti* plant extract can also be used as a starch blocker because it prevents or delays the absorption of starch into the body, primarily by preventing the hydrolysis of 1,4-glycosidic linkage of starch and other oligosaccharides into maltose, maltriose, and other simple sugars.

Fig: 1 Assay of α - amylase inhibitory activity of *P.korinti* leaves extracts

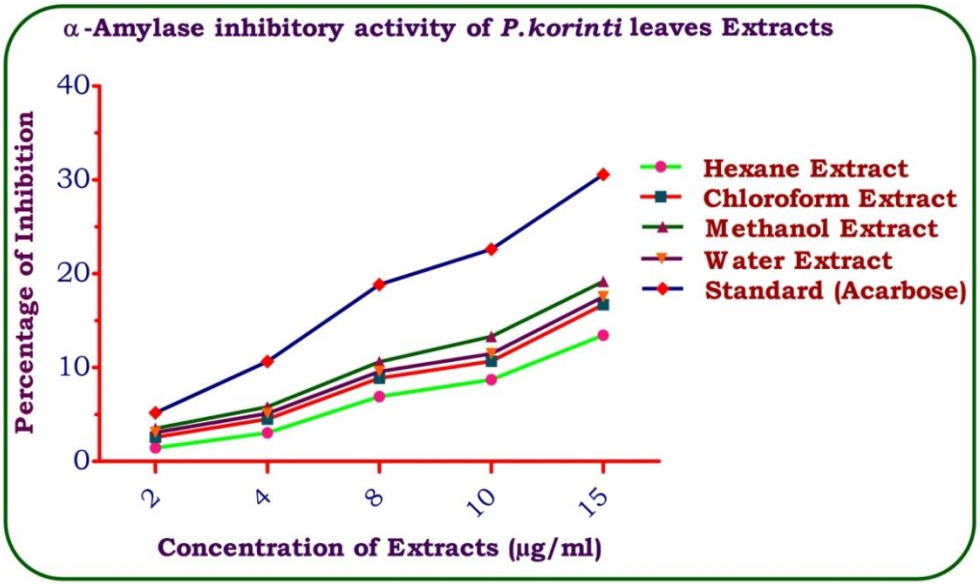
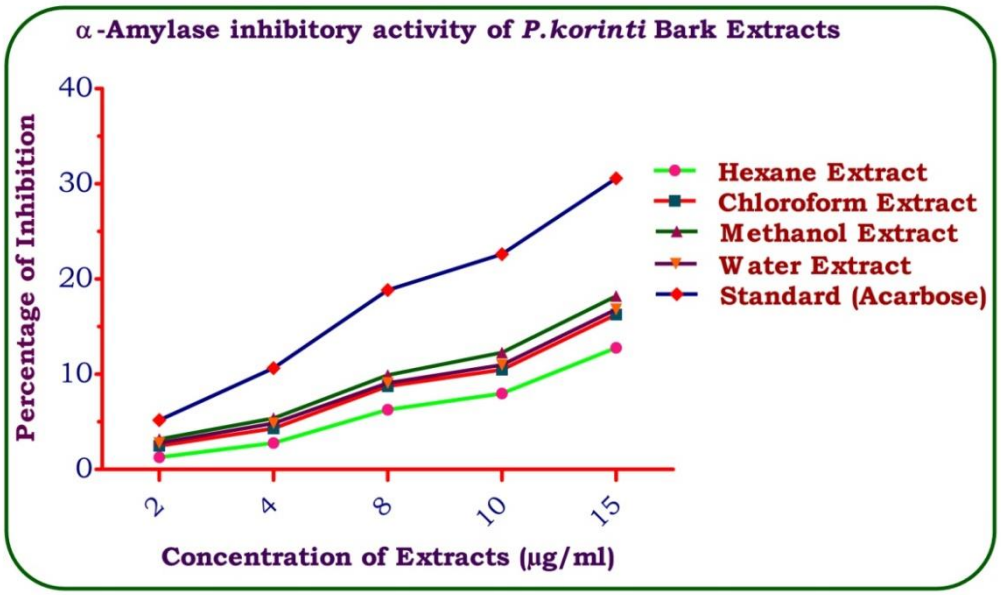


Fig: 2 Assay of α - amylase inhibitory activity of *P.korinti* bark extracts



In our study, the methanol extract of leaves showed maximum α- amylase inhibitory activity (40.41±0.080 µg/mL), which may be attributed to the presence of polyphenols and flavonoids, because polyphenols can reduce oxidative stress as well as inhibit carbohydrate hydrolyzing enzymes due to their ability to bind with proteins (Keerthana et al. 2013).

Our findings support a previous study that found a connection between total polyphenol and flavonoid content and the ability to inhibit pancreatic α-amylase (Mai et al. 2007; Ramkumar et al. 2010).

3.2 Assay of α -glucosidase inhibitory activity:

Fig. 3 and 4 demonstrate the effects of antidiabetic activity using α-glucosidase inhibitory assays of *P.korinti* leaves and bark extracts, as well as the IC_{50} values for each extract. The extracts revealed a significant inhibitory action of an α-glucosidase enzyme. The percentage of inhibition showed a dose-dependent increase in the percentage of inhibition at 2-15 µg/ mL extract concentrations.

At all concentrations (2-15µg/mL), methanol extract of leaves (58.35±0.06) and bark (55.96±0.05) showed maximum inhibition of the enzyme seen at 15µg/mL concentration, with a lowest IC_{50} value 12.25±0.009 and 12.76±0.017 respectively. The lowest IC_{50} indicates the strongest ability of the extracts to act as α -glucosidase inhibitory activity. Water extract of leaves (53.08±0.04) and bark (51.21±0.05) showed the next highest value of seen at 15 µg/mL concentration. Followed by chloroform extract of leaves (48.61±0.05) and bark (46.07±0.05) seen at 15µg/mL concentration. The hexane extract of leaves and bark showed the least inhibition of enzyme with the highest value of 44.26±0.07 and 42.11±0.07 respectively at 15µg/mL

concentration. Thus, inhibition of -glucosidase activity by *P.korinti* leaves methanol extract would delay carbohydrate degradation, resulting in a decrease in glucose absorption and, as a consequence, a reduction in postprandial blood glucose level elevation (Manikandan et al. 2013). A comparison of α-glucosidase inhibitory activity between regular medication and plant extracts is shown in Fig. 3. Acarbose was also used as a reference drug for the α-glucosidase inhibitor in this research. The concentration of acarbose (2-15 µg/mL) showed an inhibitory activity of α-glucosidase of 16.27±0.09 to 78.59±0 percent, with an IC_{50} value of 7.99±0.002 µg/mL.

Fig: 3 Assay of α - glucosidase inhibitory activity of *P.korinti* leaves extracts

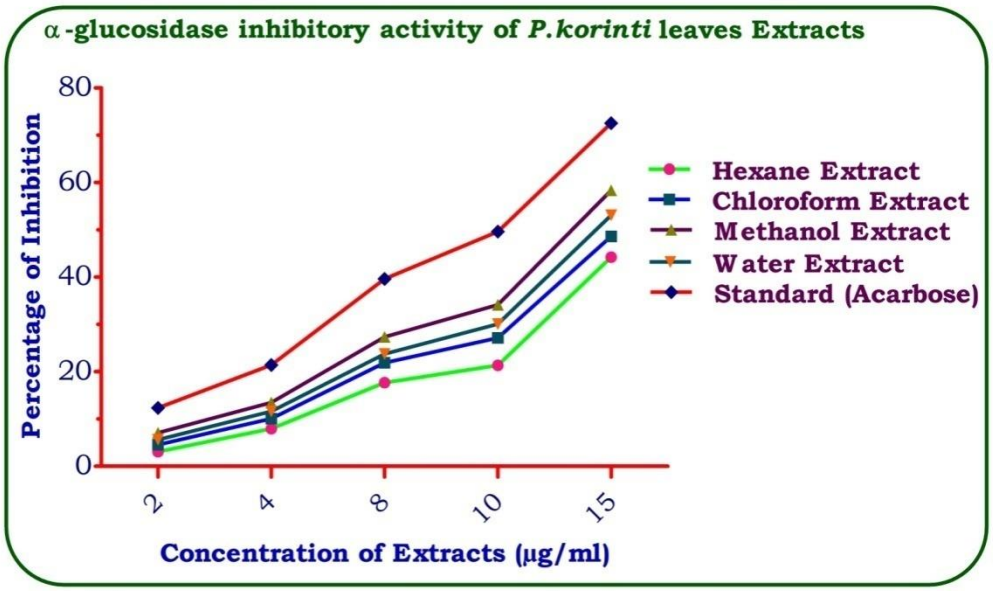
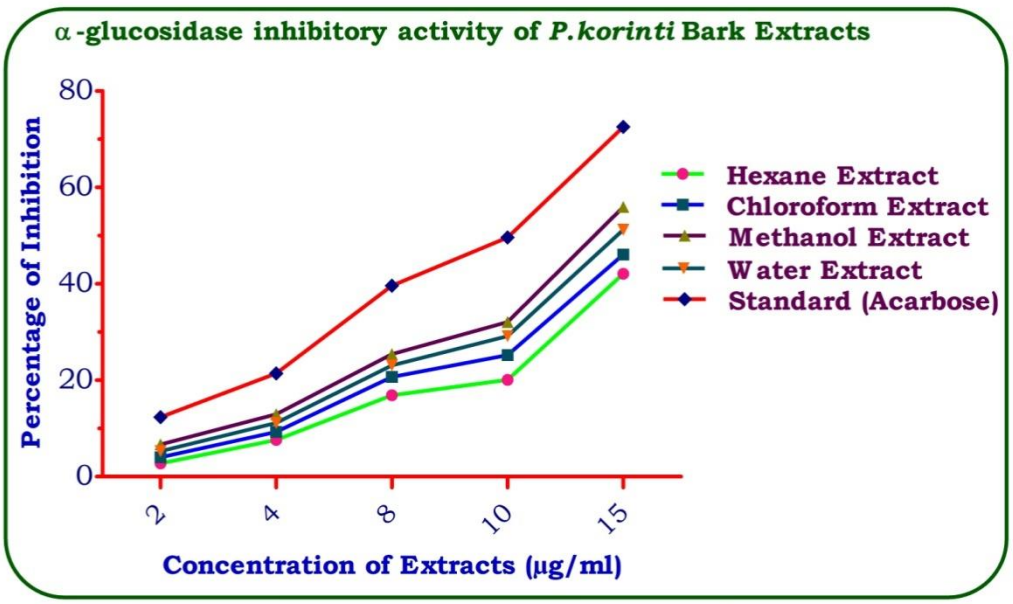


Fig: 4 Assay of α - glucosidase inhibitory activity of *P.korinti* bark extracts



The methanol extract of *P.korinti* leaves is a very potent inhibitor of α-amylase and α-glucosidase when compared to acarbose. This may be explained by the fact that the presence of bioactive compounds in the extract, such as phenols, flavonoids, saponins, steroids, alkaloids, and terpenoids, which may be effective α-amylase and α-glucosidase inhibitors.

3.3 Determination of Glucose Uptake by Yeast Cells:

The effect of *P.korinti* extracts on glucose transport across cell membranes was investigated in vitro using yeast cells suspended in glucose solutions of various concentrations (5, 10, and 20 mM) in the presence of extracts at various concentrations (Fig. 5 to 10). The effect of the *P.korinti* extracts on glucose transport across cell membranes was studied in-vitro system comprising of yeast cells suspended in glucose solution of varying concentration (5, 10 and 20 mM) in the presence of the extracts at different concentrations (Fig. 5 to 10). The amount of glucose left in the medium after a certain period of time is used to determine how much glucose the yeast cells have absorbed. The entire sample extracts significantly enhanced glucose transfer across the yeast cell membrane. At various samples and glucose concentrations, methanol extracts from leaves and bark improved glucose absorption, followed by extracts from water, chloroform, and hexane. Glucose absorption increased in a dose-dependent manner and was proportional to sample concentration. However, the percentage increase in yeast cells' glucose absorption was inversely proportional to the glucose concentration and decreased with an increase in the molar concentration of the glucose solution.

Moreover, *P.korinti* leaves extract shows more effective than that of bark extracts in the uptake of glucose from the medium at all concentrations of the sample. The glucose uptake capacity at 1mg/mL of methanol extracts of leaves was >58% (58.14±0.15) that has reached 83% (83.60±0.16) when 5 mg/mL was used at 5mM glucose concentration. This means that increasing the extract concentration allows yeast cells to absorb more glucose from the surrounding environment. Fig. 5 to 10 showed a linear increase in glucose absorption by yeast cells as the concentration of the test sample increased. When glucose uptake by yeast cells was compared at 5 mM, 10 mM, and 20 mM for the same amount of plant extract, an inverse relationship to the molar concentration of glucose was observed. From the results, it is concluded that the lower the concentration of glucose in the solution, the higher the uptake by yeast cells.

Fig: 5 Effect of *P.korinti* Leaves extracts on Yeast glucose uptake assay with 5mM Glucose concentration.

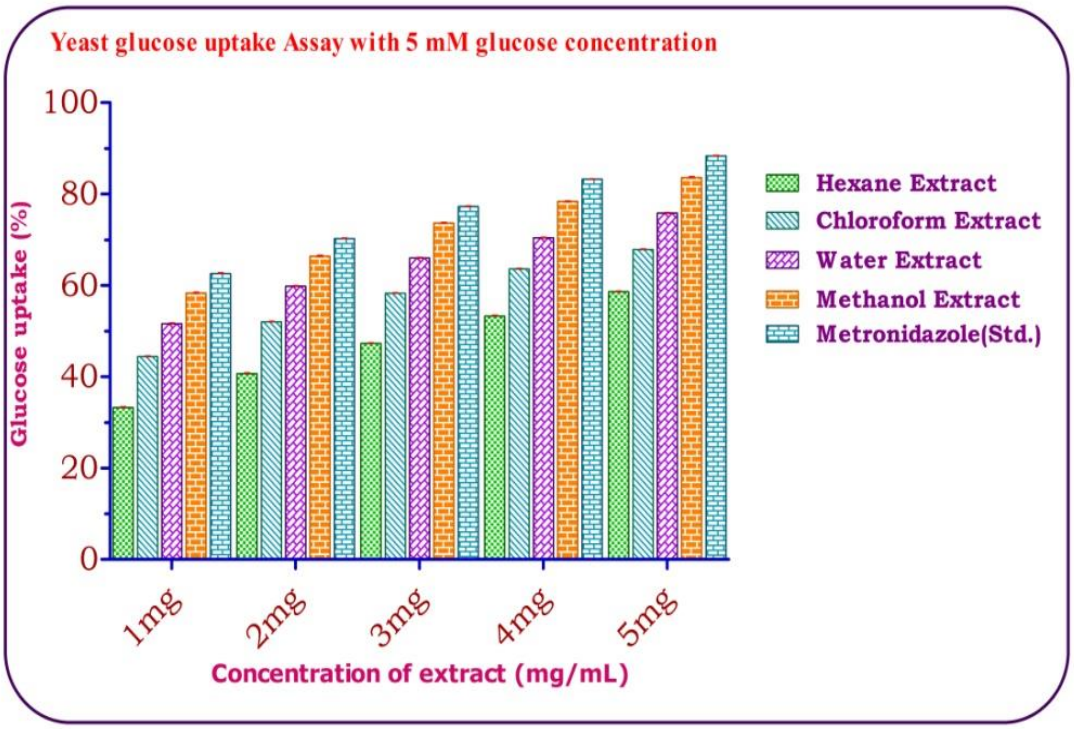


Fig: 6 Effect of *P.korinti* Bark extracts on Yeast glucose uptake assay with 5mM Glucose concentration.

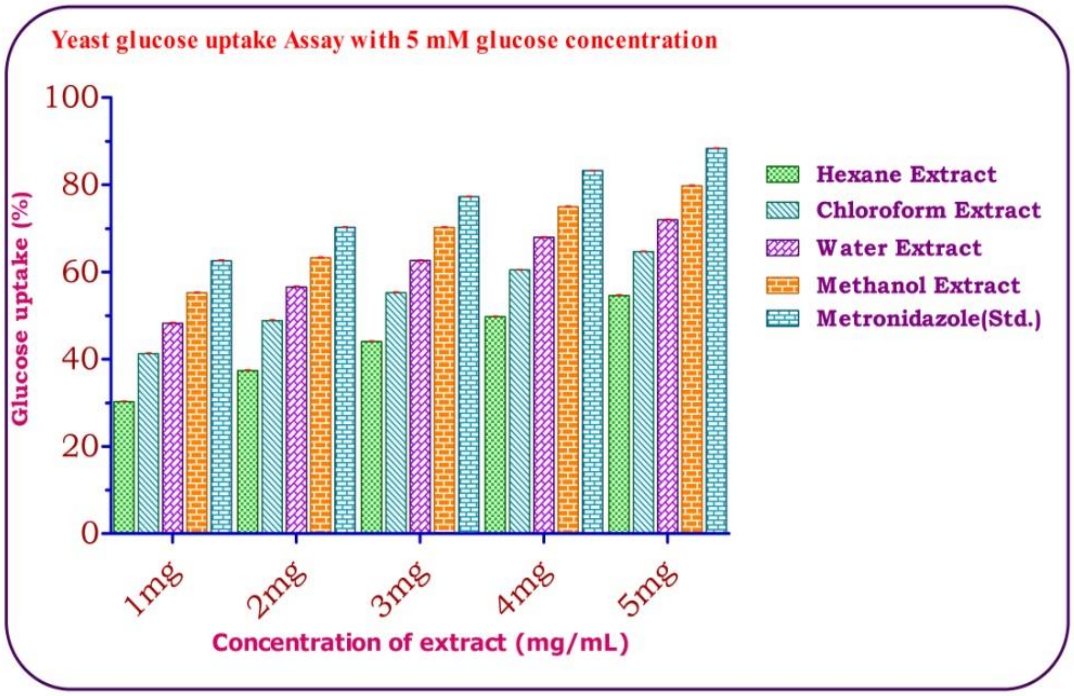


Fig: 7 Effect of *P.korinti* Leaves extracts on Yeast glucose uptake assay with 10mM Glucose concentration.

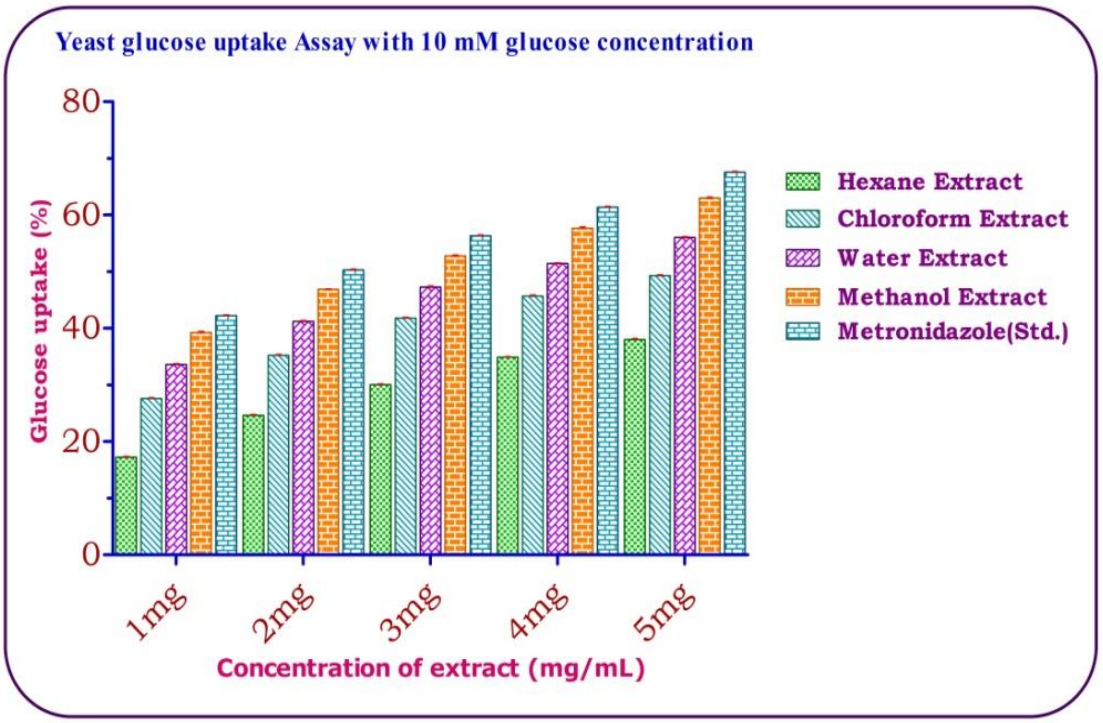


Fig: 8 Effect of *P.korinti* Bark extracts on Yeast glucose uptake assay with 10mM Glucose concentration.

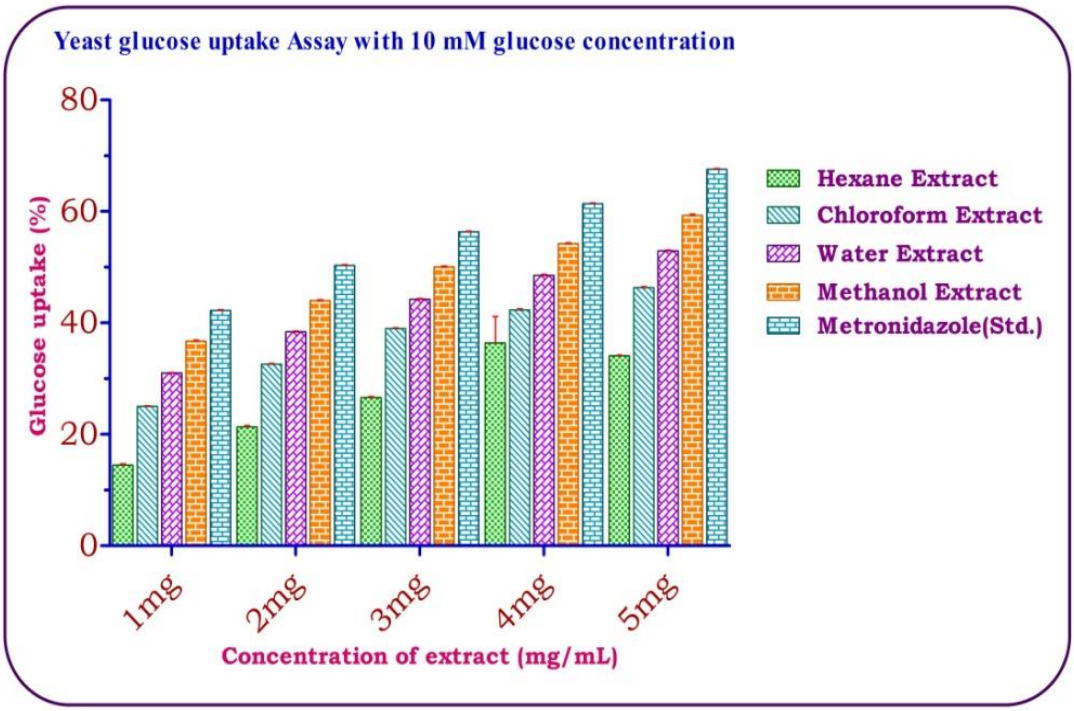


Fig: 9 Effect of *P.korinti* Leaves extracts on Yeast glucose uptake assay with 20mM Glucose concentration.

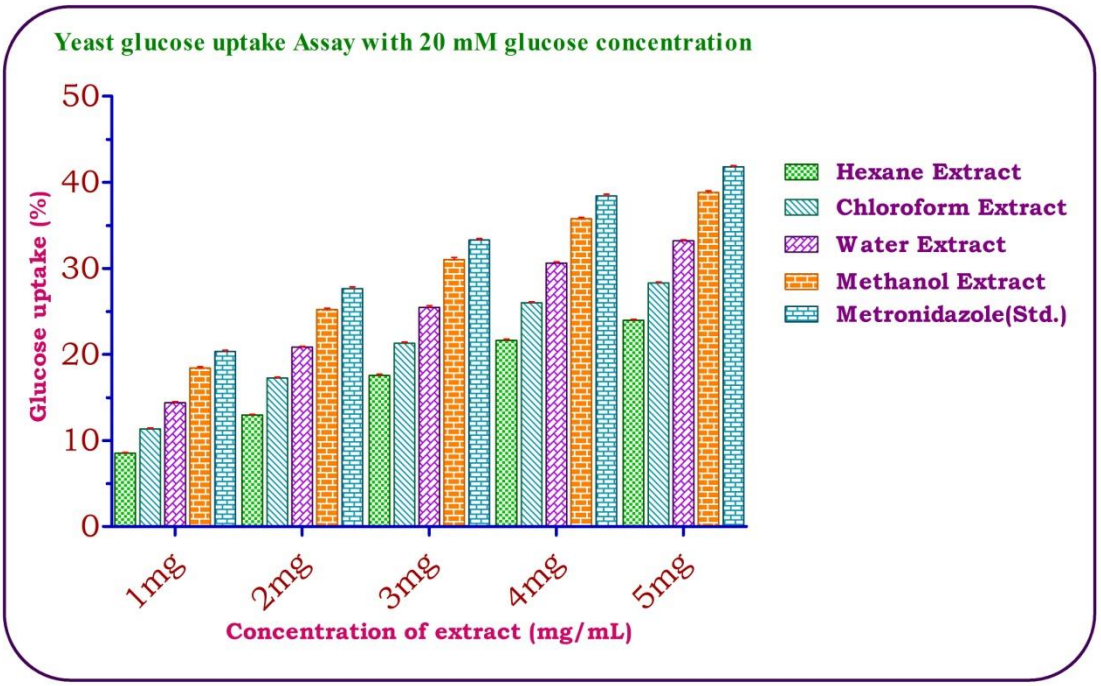
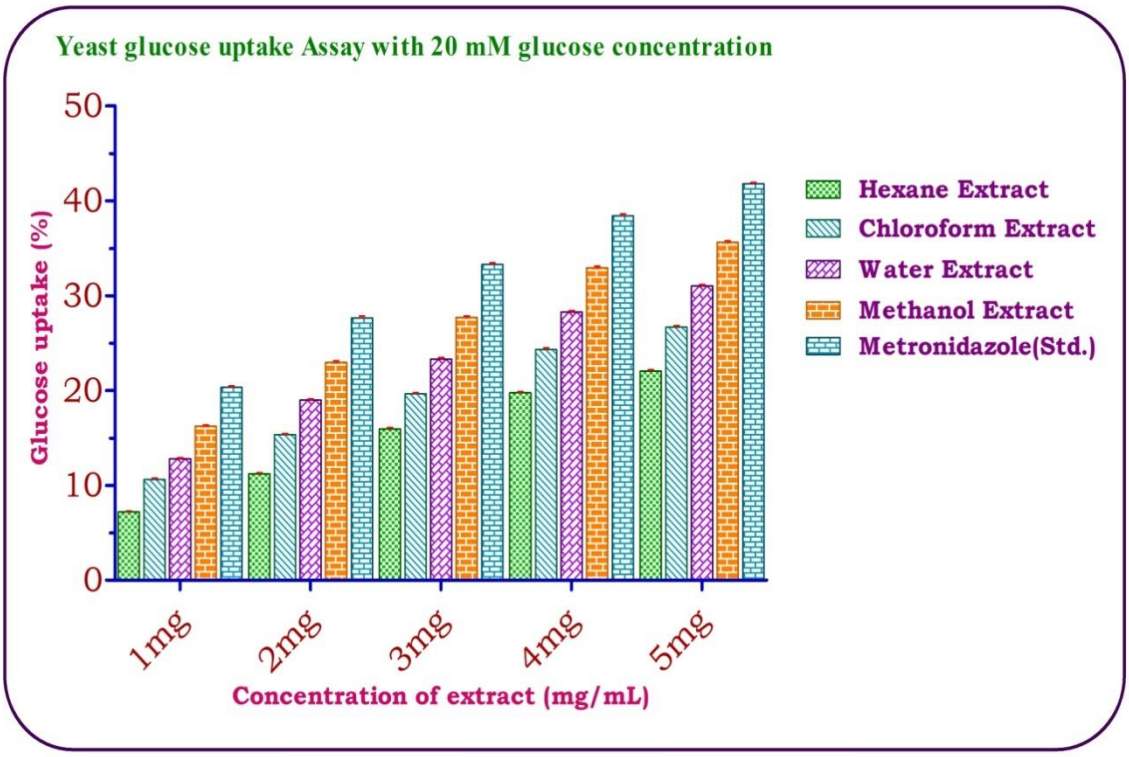


Fig: 10 Effect of *P.korinti* Bark extracts on Yeast glucose uptake assay with 20mM Glucose concentration.



In the current study, bioactive compounds present in *P.korinti* extracts were found to aid glucose absorption. Glucose uptake by yeast cells can be influenced by a number of factors, including the glucose concentration within the cells and the subsequent glucose metabolism. If most of the inner sugar is readily converted into other metabolites, the concentration of internal glucose will be low, and high glucose absorption into the cell will be desired. Glucose uptake in the presence of *P.korinti* extract is likely due to both facilitated diffusion and enhanced glucose metabolism, similar to yeast cells. It would be critical to investigate the natural extract's function in vivo, which could help in the body's enhanced glucose absorption by muscle cells and adipose tissues. The extract had the ability to bind glucose and move it across the cell membrane for metabolism.

In the current investigation, the glucose adsorption capacity of the sample was also found to be proportional to the concentration of plant extract. The presence of soluble and insoluble dietary fibers in *P.korinti* extracts may aid in the understanding of their adsorption properties. Glucose adsorption by extract in the intestinal lumen can help lower blood glucose levels after a meal (Das and Devi, 2015). According to Ou et al. (2001), dietary fiber can help decrease postprandial hyperglycemia through three different mechanisms. Firstly, they will make the small intestinal fluids more viscous, making glucose transport from the lumen to the circulation more difficult. Secondly, glucose can attach to these fibers, resulting in a decrease in their concentration in the small intestinal lumen. Finally, α-amylase (a starch-digesting enzyme) inhibitors found in dietary fibre reduce starch digestion and lower postprandial hyperglycemia.

4. Conclusion

The presence of phytochemicals in *P.korinti* leaves and bark extracts may explain the inhibitions of α – amylase and α – glucosidase, as well as increased glucose absorption, which was discovered in this research. When glucose uptake by yeast cells was compared at 5 mM, 10 mM, and 20 mM for the same amount of plant extract, an inverse relationship to the molar concentration of glucose was observed. Out of all the extracts methanol extracts have great potential as antidiabetic. The results can be used to guide future research into how to prepare and optimize plant extracts for structural elucidation and characterization methodologies to identify anti-diabetic bioactive compounds.

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