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Evaluation of Antioxidant and Antidiabetic Potential of Polyherbal Drug Made with Three Herbs: An *In vitro* Cell Culture Model

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ABSTRACT

In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels, and the possibility that the postprandial “hyperglycemic spikes” may be relevant to the onset of cardiovascular complications has recently received much attention. Medicinal use of herbal medicine in the treatment and prevention of diseases including diabetes has a long history compared to conventional medicine. These plants have no side effects and many existing medicines are derived from the plants. Hence, the current investigation was planned to make a poly herbal drug (PHD) through *Punica granatum* (fruits), *Illicium verum* (flowers) and *Nyctanthes arbor* (leaves) and assess their antioxidant and antidiabetic activities *in vitro* and in HepG2 cell line. The respective plant methanolic extracts and PHD are exposed to phytochemical assessment and to discriminate the bioactive factors by Gas Chromatography–Mass Spectrometry. We evaluated the antioxidant properties 2, 2-diphenyl-1-picrylhydrazyl scavenging, hydrogen peroxide scavenging, thiobarbituric acid reactive substances and total antioxidant activity of individual plant extracts and the PHD. At the same time, *In vitro* and cell culture approaches were used to assess the anti-diabetic activity. The PHD has a higher concentration of secondary metabolites than individual plant extracts, according to our findings. On the other hand, we also notice that PHD demonstrated higher antioxidant capability and considerable *in vitro* glucose-lowering effects along with noteworthy inhibition of α -amylase, glucosidase, lipase, dipeptidyl peptidase-IV, collagenase and protein glycation in HepG2 cell line. In conclusion, this study clearly demonstrated the significant antioxidant and antidiabetic activities of the PHD. Hence, PHD may be used as a potential source in the management of diabetes, hyperglycemia and the related state of oxidative stress.

KEY WORDS: ANTIOXIDANTS, FREE RADICALS, MEDICINAL PLANTS, PHYTOCHEMICALS, POSTPRANDIAL DIABETES.

INTRODUCTION

Diabetes mellitus (DM) is a multifaceted metabolic disorder attributable to a complete deficiency of insulin or comparative be short of insulin as an outcome of defection in insulin production, action or a combination of both. The clinical feature of DM is glucose intolerance, which results in hyperglycemia. Type 1 DM (T1DM) and type 2 DM (T2DM) are the two main types of DM. T1DM, which is linked with full or near-total insulin insufficiency and

autoimmune destruction of pancreatic β -cells, affects around 5-10% of people with DM. T2DM, on the other hand, has evolved into a virulent illness marked by β -cell dysfunction, various degrees of insulin resistance, and increased hepatic glucose production (American Diabetes Association 2015). Nearly 425 million persons have DM; by 2045, this number is expected to rise to 629 million. 79 percent of adults with DM live in low- and middle-income countries, 1 of every 2 (212 million) people with DM is undiagnosed, and 352 million people are at risk of developing T2DM (IDF 2018). In most developing countries, such as India, DM is widespread (Uddand Rao et al. 2019; Cole and Florez 2020).

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According to statistics, India has the world's highest number of DM patients and has been termed the "diabetes capital of the world". At the same time, the limitation of the accessible treatments and also the harmful side effects, the look for new medication continues to be directed against DM (Abate and Chandalia 2007; Parim et al. 2019). The researchers have an interest in looking out new medication to treat DM because of impending hypoglycemic activity and negligible side effects of healing plants (Kavishankar et al. 2011; Cole and Florez 2020). One technique of a therapy to reduce hyperglycemia, the inhibition of carbohydrate hydrolyzing enzymes in the digestive organs, slows down and reduces the digestion and absorption of ingested carbohydrates, especially after a meal. As a result, these inhibitors may be able to minimize the postprandial rise in blood glucose levels (Narkhede, 2011; Gurung et al. 2020). Consequently, natural inhibitors derived from dietary plants have a reduced inhibitory impact on carbohydrate hydrolyzing enzyme activities and can be employed as a possible postprandial hyperglycemia treatment with little adverse effects (Uddandrao et al., 2020).

On the other hand, oxidative stress plays a crucial function in DM pathophysiology; thus, the interest of using natural antioxidants as beneficial tools exists (Uddandrao et al. 2018). There is a rising interest in using therapeutic plants and their phytoconstituents as natural sources because of their well-known capability to scavenge free radicals. Plants are effective sources of natural antioxidant molecules with a variety of pharmacological activities and few or no side effects that protect human health from a variety of diseases (Omari et al. 2019). The idea of poly herbal drug (PHD) is well known in the therapeutic system. Plant formulations and concentrated concentrations of plants are employed as a contrast to individual ones in traditional Indian drug procedures. The Ayurvedic herbals are set up in a variety of measuring structures in India's traditional helpful framework called Ayurveda, with the most of them including PHD (Parasuraman et al. 2014; Hill-Briggs et al. 2020).

Since PHD is a result of nature, they are modestly less expensive, environment-friendly, and promptly introduced than allopathic medications (Parasuraman et al. 2014; Hill-Briggs et al. 2020). Because of the rapid rise in DM, safe and efficient treatments are required, and one potential strategy is to minimize postprandial hyperglycemia by delaying glucose absorption by inhibiting carbohydrate-hydrolyzing enzymes and scavenging free radicals (Uddandrao et al. 2020). There was no scientific data available about the PHD made with *Punica granatum* (fruits), *Illicium verum* (flowers) and *Nyctanthes arbor* (Leaves) and their antioxidant and anti-diabetic properties so far. Therefore, in this study we made an effort to prepare PHD with three herbs as mentioned above and evaluated their antioxidant and antidiabetic activities through *in vitro* and cell culture models.

MATERIAL AND METHODS

For the sample collection, The *P. granatum* fruits were taken from a local garden in Erode, Tamil Nadu, the *I.*

verum flowers was taken from the neighborhood garden, Tiruchengode, Namakkal, Tamil Nadu and *N. arbor* (Leaves) was procured from local garden, Salem, Tamil Nadu, India. The plants *P. granatum* (Voucher No: BSI/SRC/5/23/2020/Tech/510), *I. verum* (Voucher No: BSI/SRC/5/23/2020/Tech./509) and *N. arbor* (Voucher No: BSI/SRC/5/23/2020/TECH/511) were authenticated by the Southern Regional Centre, Botanical Survey of India, TNAU Campus, Coimbatore, Tamilnadu, India. For sample preparation, the *P. granatum* (fruits), *I. verum* (flowers), and *N. arbor* (leaves) were rinsed with cleaned water and on a fresh sheet, air-dried for seven days at room temperature. The dry plant materials were made of powder and extraction was carried out with the help of soxhlet apparatus in the presence of methanol. Then the samples were evaporated to waterlessness at decreased pressure at 40°C by rotary evaporator (Superfit, Precision Scientific, India). A PHD was made through extracts of *P. granatum*, *I. verum* and *N. arbor*. A variety of concentrations and combinations were tried and a last valuable formulation with equal volume of each one plant extracts (1:1:1 ratio) was fixed for experiments.

For better formulation stability during shelf life, several additives such as Tween-80, Sodium CMC, sweetening agent (Sodium saccharin), and flavouring agent (Lemon oil) were incorporated. Preliminary phytochemical screening was done by Kokate's techniques to test the plant individual extracts and PHD for the presence of a range of phytoconstituents (Kokate 1986). For quantitative estimation of phytochemicals, we used the individual concentrates and PHD to quantify secondary metabolites based on the elemental phytochemical examination results; for example, total phenolic content, total flavonoids, and total tannins were measured in accordance with the guidelines (Folin and Ciocalteu 1927; Singleton and Rossi 1965; Ordóñez et al. 2006).

GC-MS (Gas Chromatography-Mass Spectrophotometry) analysis, the plant individual extracts and PHD were subjected to identify bioactive compounds by GC-MS as described in the previous studies (Uddandrao et al. 2020). Analysis of the mass spectrum the compounds were identified using GC-MS and the National Institute of Standards and Technology (NIST) database. For evaluation of antioxidant activity, plant individual extracts and PHD was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Hydrogen peroxide (H₂O₂) scavenging assay, total antioxidant activity as determined by the phosphomolybdate assay proposed in the previous studies and Thiobarbituric acid reactive substances (TBARS) assay (Ruch et al. 1989; Shimada et al. 1992; Kikuzaki and Nakatani 1993; Prieto et al. 1999). For assessment of *in vitro* antidiabetic effects, the inhibitory activities of α -amylase, α -glucosidase (Becerra-Jimenez et al. 2008) and glucose diffusion inhibition were measured as per customary protocols.

All experiments were performed in triplicate, with results expressed as a percentage of inhibition (Gallagher et al. 2003; Thalapaneni et al. 2008). For determination of antidiabetic activity by cell culture model, the HepG2 cell

culture was incubated at 37°C in a humidified environment with 5% CO₂, and growth media, consisting of RPMI 1640 media supplemented with 10% foetal calf serum, was replaced every 2-3 days. Venter et al. (2008) described a technique for determining glucose consumption in HepG2 cells (Venter et al. 2008).

The difference between the cell-free and cell-containing wells was used to compute the amount of glucose utilized. The percentage of glucose intake was calculated in contrast to the untreated controls. For Assay of metabolic enzymes inhibition potential of PHD in HepG2 cell line, the inhibition of α-amylase, α-glucosidase, lipase, dipeptidyl peptidase-4 (DPP-IV), and collagenase were measured as per respective protocols (Sancheti and Seo 2010; Lewis and Liu 2012; Odeyemi 2015; Idowu et al. 2018). The percentage of inhibitory activity was calculated by using the following formulae: % Inhibition = $(1 - A/B) \times 100$, A = Absorbance of the untreated (Control), B = Absorbance of the test well.

For protein glycation assay, the protein glycation assay was performed according to the methodology described by Idowu et al. (2018). The studies were done three times, and the percentage of inhibition was estimated using the formula below: % Inhibition = $(1 - A/B) \times 100$
A = Fluorescence of test well, B = Fluorescence of negative control. For Statistical analysis, the investigational data were presented as the average of three replicates' standard deviations (SD). Wherever relevant, the results be treated to a unidirectional analysis of variance (ANOVA) and the significant difference (p < 0.05, 0.01) between means was decided by the least significant difference (LSD) using Statistical Package for Social Sciences (SPSS) version 15.0 for Windows.

RESULTS AND DISCUSSION

DM is a metabolic disorder characterized by hyperglycemia and glucose intolerance, as well as abnormalities in insulin production and insulin action to increase glucose uptake. The high prevalence of morbidity and death, as well as higher health-care costs for management and treatment, make this condition a global burden. Herbal medications are made from a variety of plant sources, either in its natural state or after processing with various ingredients. Despite the fact that a variety of oral hypoglycemic medications, as well as insulin, are used to treat DM, there is a revived curiosity among patients in using natural medicines with anti-diabetic properties (Brahmanaidu et al. 2017; Vadivel et al. 2018; Unuofin and Lebelo 2020). A large number of conservative or native medications are also driving the cutting-edge prescription arrangement. In comparison to a single herb, the PHD offers a greater and broader therapeutic potential, particularly for DM (Petchi et al. 2014; Williams et al. 2020).

In this study, we attempted to make PHD from *P. granatum* (fruits), *I. verum* (flowers), and *N. arbor* (leaves) and evaluated their antioxidant and anti-diabetic properties. Preliminary phytochemical screening: The methanolic extract of PHD contained secondary metabolites such as

alkaloids, carbohydrates, phenols, flavonoids, amino acids, proteins, terpenoids, Saponins and Tannins. Quantitative estimation of phytochemicals: The quantification of secondary metabolites such as phenolics, flavonoids, and tannins in *P. granatum*, *I. verum*, *N. arbor*, and the PHD is shown in Figure 1. PHD has the highest concentration of phenolics (Fig. 1A), flavonoids (Fig. 1B), and tannins (Fig. 1C) when compared to individual plant extracts. The levels of phenolics were found to be larger than flavonoids and tannins, according to these calculations. As shown in figure 1A-C, the individual extracts of *P. granatum*, *I. verum*, and *N. arbor* extracts showed a noticeable quantity of these secondary metabolites. Since DM may pose a hazard to our populace, scientists all around the world are scouring phytochemicals for natural active agents (Pimple et al. 2011; Chan et al. 2021).

Figure 1: Quantitative estimation of secondary metabolites such as (A) phenolics, (B) flavonoids, and (C) tannins present in the *P. granatum*, *I. verum*, *N. arbor* and the PHD. Values are mean ± SD, n=3. Values are significant at *P<0.05, **P<0.01.

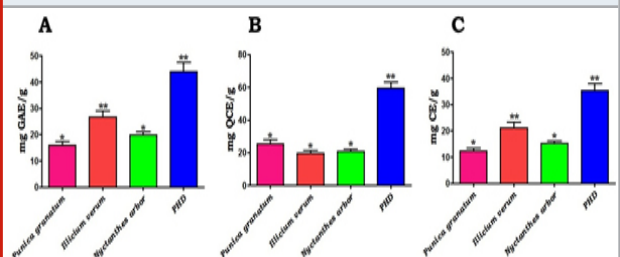
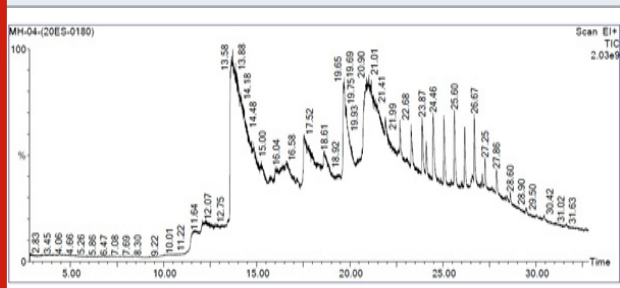


Figure 2: GC-MS chromatogram of the methanolic extract of PHD.



The antioxidant efficacies of phenolic compounds have been shown to be robust, and they play an important role in the management of chronic diseases like DM. Flavonoids are polyhydroxy polyphenolic chemicals with a wide range of applications in herbal medicine. Flavonoids, which are divided into flavanols, flavones, and flavanones, have a variety of therapeutic benefits, including anti-diabetic qualities (Roman et al. 1995; Adefegha et al. 2013). GC-MS analysis: GC-MS analysis of the PHD (Table 1, Fig 2) revealed that several compounds as exposed tables 1. Antioxidant activity: For antioxidant activity, when compared to the plant extracts individually, PHD had the DPPH radical scavenging activity is greatest at doses ranging between 100µg/mL to 1000µg/mL (Fig. 3A). Similarly, PHD demonstrated H₂O₂ decomposition activity in a dose-

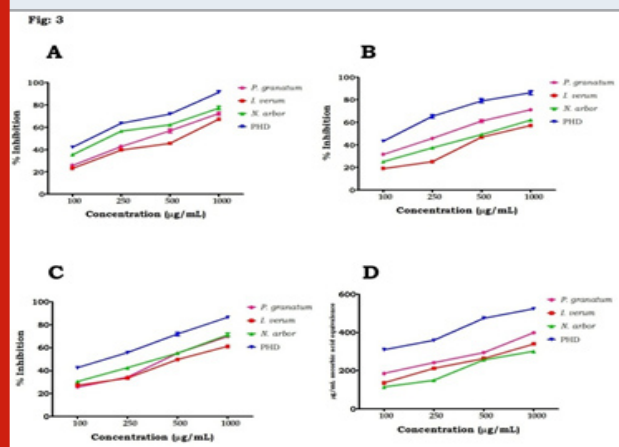
dependent manner, with the maximum activity reported at 1000 µg/mL. At the same time, Individual plant extracts were shown to have moderate H₂O₂ scavenging capabilities when compared to PHD (Fig. 3B). Figure 3C shows the % inhibition of *P. granatum*, *I. verum*, *N. arbour*, and PHD at various doses using the TBARS test. When compared

to separate plant extracts, our results clearly demonstrated that the PHD might possibly prevent lipid peroxidation in a dose-dependent manner. Phosphomolybdate test was used to investigate the total antioxidant capabilities of *P. granatum*, *I. verum*, *N. arbour*, and PHD at various doses, and the findings revealed dose-dependent antioxidant activity (Fig. 3D) (Zhang et al. 2020).

Table 1. Compounds identified in the methanolic extract of PHD with GC-MS

RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
11.662	1,2-Cyclohexanedione	C ₆ H ₈ O ₂	112	1.197
12.252	Cyclohexanone, 2-Methyl-	C ₇ H ₁₂ O	112	0.977
12.307	Cyclohexanone, 2-Methyl-	C ₇ H ₁₂ O	112	1.051
12.882	5-Trans-Methyl-1r,3-Cis-Cyclohexanediol	C ₇ H ₁₄ O ₂	130	0.844
13.713	Benzene, 1-Methoxy-4-(1-Propenyl)-	C ₁₀ H ₁₂ O	148	27.781
16.619	Benzene, (1-Methoxyethyl)-	C ₉ H ₁₂ O	136	12.014
17.534	Phenol, 4-(2-Propenyl)-, Acetate	C ₁₁ H ₁₂ O ₂	176	8.983
18.615	2-Cyclopenten-1-One, 2-(2-Butenyl)-4-Hydroxy-3-Methyl-, (Z)-	C ₁₀ H ₁₄ O ₂	166	4.328
19.690	N-Hexadecanoic Acid	C ₁₆ H ₃₂ O	256	8.856
21.011	Dodecanoic Acid, 9-Decen-1-Yl Ester	C ₂₂ H ₄₂ O ₂	338	17.146
21.901	3-(5-Benzyloxy-3-Methylpent-3-Enyl)-2,2-Dimethyloxirane	C ₁₇ H ₂₄ O ₂	260	17.146
22.681	Oxalic Acid, Isohexyl Nonyl Ester	C ₁₇ H ₃₂ O ₄	300	3.813
23.291	Octane, 3,4,5,6-Tetramethyl-	C ₁₂ H ₂₆	170	2.387
23.867	Octadecane, 3-Ethyl-5-(2-Ethylbutyl)-	C ₂₆ H ₅₄	366	1.183
24.097	Cyclopropyl 2-(5'-Methyl-2'-Furyl) Cyclopropyl Ketone	C ₁₂ H ₁₄ O ₂	190	0.974
24.462	Hexatriacontane	C ₃₆ H ₇₄	506	1.595
25.042	Heptacosane	C ₂₇ H ₅₆	380	0.883
25.602	Ethanol, 2-(Octadecyloxy)-	C ₂₀ H ₄₂ O ₂	314	0.836

Figure 3: (A) DPPH radical scavenging activity, (B) H₂O₂ scavenging activities, (C) TBARS and (D) total antioxidant capacities of *P. granatum*, *I. verum*, *N. arbor* and PHD at different concentrations, Values are mean ± SD, n=3.



When compared to individual plant extracts, the PHD demonstrated much higher activity. Free radicals, such as reactive oxygen species (ROS), have been linked to DM, obesity, inflammation, cardiovascular disease, atherosclerosis, ageing, and cancer (Meriga et al. 2017; Brahmanaidu et al. 2017). It's critical to strike stability between the pace of free radical creation and exclusion. Excessive cell radical formation is typically harmful; in any event, oxidative cellular stress occurs when there is a significant increase in excessively radical formation or a reduction in radical clearance from the cell (Valko et al. 2007; Li et al. 2019).

According to research and scientific data, oxidative stress is linked to the onset and development of DM (Rösen et al. 2001). Using DPPH radical and H₂O₂ scavenging action, TBARS test, and total antioxidant activity, we evaluated the antioxidant properties of PHD and individual plant extracts in this study. DPPH is a stable, nitrogen-focused free radical that is transformed to diphenylpicryl hydrazine

after absorbing hydrogen from the polyphenolic extract's antioxidants (Vladimir et al. 2011). The drop in DPPH seen by the PHD was caused by either a hydrogen atom exchange or an electron exchange. Additionally, phenolic compounds and flavonoids are abundant hydrogen benefactors, making them excellent antioxidants (Michalak 2006; Yaribeygi et al. 2020).

H₂O₂ is a mild oxidising agent that may inactivate a few enzymes directly, often by oxidising crucial thiol groups. H₂O₂ may quickly permeate cell membranes, and once inside, it interacts with Fe²⁺ and perhaps Cu²⁺ ions to produce hydroxyl radicals, which are harmful to cells. Because of their hydrogen-giving and scavenging capacities, secondary metabolic products may act as free radical scavengers (Beyhan et al. 2010). PHD had a substantially greater overall antioxidant capacity than individual plant extracts, according to our research. The total phenolic content and antioxidative activity have been linked in several studies. Natural antioxidants' efficiency is determined in part by the chemical composition and chemical structures of the extract's active components (Hossain et al. 2015; Jiao et al. 2019).

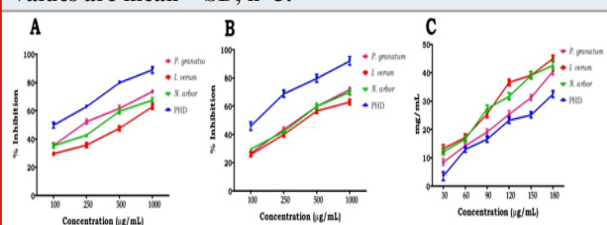
We observed high quantities of total phenolic content, flavonoids, tannins, and bioactive components in PHD using GC-MS throughout this investigation. PHD's high antioxidant action might be attributed to these components. *In vitro* antidiabetic activity of PHD: the current research found a dose-dependent increase in % inhibitory efficacy against the α -amylase (Fig. 4A) and α -glucosidase enzymes (Fig. 4B). The PHD demonstrated considerable inhibitory action ($p < 0.05$) against these enzymes, with the maximum dosage of 1000g/mL having the maximum inhibitory action. The individual extracts of *P. granatum*, *I. verum* and *N. arbour* did not demonstrate substantial inhibitory activity against α -glucosidase and α -amylase as compared to PHD.

The current study used a variety of biochemical and cell-based tests to determine the probable mechanism(s) of *P. granatum*, *I. verum*, *N. arbour*, and PHD extracts antidiabetic effects. Glucose absorption inhibition activity: When compared to metformin, the results of this study on glucose uptake in HepG2 cells indicated that PHD increased glucose uptake in HepG2 cells. This suggests that the PHD works similarly to metformin in that it increases glucose absorption in the liver. Metformin can have a hypoglycemic effect by activating the AMP activated protein kinase in the liver, which can lead to a variety of pharmacologic effects, such as glucose inhibition, lipid synthesis inhibition, and enhanced hepatic insulin sensitivity (Viollet et al. 2012). Phytochemicals such as phenols, terpenoids, flavonoids, and flavanols have been shown to limit glucose release from the liver while also increasing glucose absorption in the hepatic cells, therefore altering the intracellular signalling pathway (Hanhineva et al. 2010; Peng et al. 2019).

Effect of PHD on glucose utilization in HepG2: when compared to the control, PHD had the greatest potential for preventing the flow of glucose molecules over the dialysis membrane (Fig. 4C). In comparison to PHD, individual

plant extracts were less efficient at stopping glucose molecules from diffusing. Figure 5A shows the findings for glucose uptake in HepG2 cells in the presence of the plant extract at concentrations of 25, 50 and 100 μ g/mL. When compared to the untreated control, the PHD generated a significant ($p < 0.05$) greater increase in glucose absorption in HepG2 cells at all concentrations in a concentration-dependent manner, tests were conducted. Phytochemicals have beneficial effects through a variety of pathways, including glucose and lipid metabolism modulation, insulin secretion, cell stimulation, the NF- κ B signalling pathway, inhibition of gluconeogenic enzymes, and ROS protection. Insulin deficiency has an impact on glucose, protein, and fat metabolism, as well as water and electrolyte balance (Frier et al. 2006; Saeedi et al. 2020).

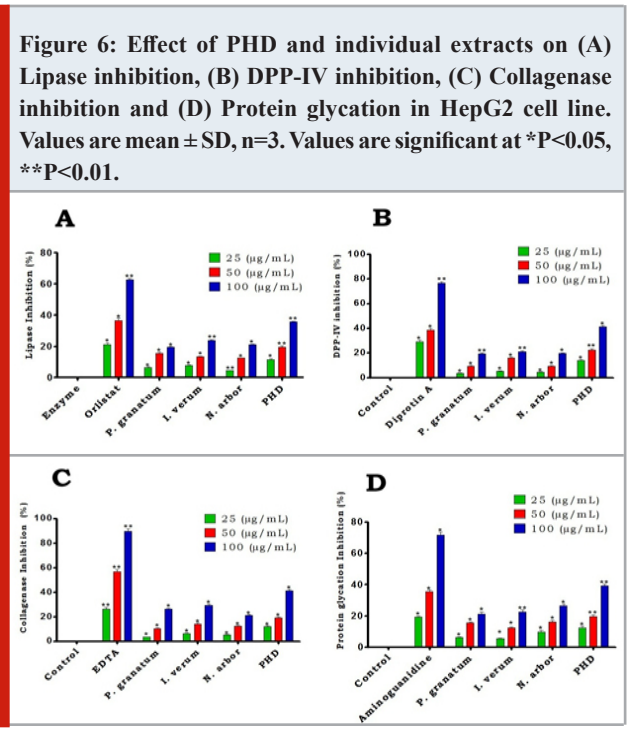
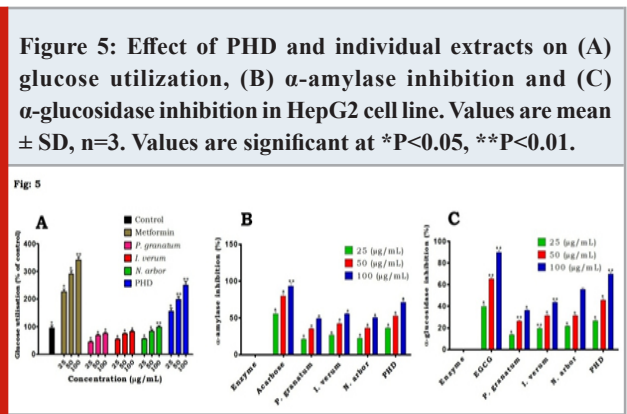
Figure 4: *In vitro* anti-diabetic activities of individual plant extracts and PHD, (A) α -amylase inhibitory activity, (B) α -glucosidase inhibitory activity and (C) glucose diffusion, Values are mean \pm SD, n=3.



A high postprandial blood glucose response is connected to micro- and macrovascular issues in people with DM, and is more strongly linked to cardiovascular disease risk than fasting blood glucose. α -glucosidase enzymes in the intestinal lumen and the brush border membrane convert starch and oligosaccharides to monosaccharides before absorption, which is essential for carbohydrate digestion. Inhibiting the activity of these digestive enzymes was thought to cause a delay in the decomposition of starch and oligosaccharides, leading to a decrease in glucose absorption and, as a result, a decrease in postprandial blood glucose levels rising (Puls et al. 1977; Lachin et al. 2021).

Effect of PHD on α -amylase in HepG2: when compared to various plant extracts, the results showed that PHD had the most significant impact on α -amylase at all doses tested (Fig. 5B). Both PHD and individual extracts had a considerable impact on α -amylase at the maximum concentration (100 μ g/ml) tested. α -glucosidase inhibition potential of PHD in HepG2: figure 5C depicts that PHD demonstrated that a highest significant effect on α -glucosidase at all the tested concentrations and highest at 100 μ g/ml than the individual plant extracts tested. However, as positive controls, EGCG was somewhat more efficacious than the extract and untreated control in the respective experiments. Lipase inhibition assay in HepG2: PHD exhibited significant inhibition against pancreatic lipase in a concentration dependent manner (Fig. 6A). On the other hand, individual plant extracts demonstrated less lipase inhibition activity when compared to PHD and orlistat. This shows that PHD (100 μ g/ml) may have anti-diabetic properties through processes involving lipase inhibition (Lachin et al. 2021).

Dipeptidyl peptidase-4 inhibition assay in HepG2: the PHD exhibited highest significant inhibition against Dipeptidyl peptidase-4 at all the tested concentrations as similar like standard diprotin A (Fig. 6B). When compared to the untreated control and individual plant extracts, there was a high significant inhibition of the PHD. Collagenase inhibition activity of PHD in HepG2: In this study, the extracts of *P. granatum*, *I. verum*, *N. arbor* exhibited moderate inhibition activity of collagenase when compared to PHD (Fig. 6C). On the other hand, PHD demonstrated that the highest activity at 100 µg/ml concentration similar like EDTA.



Protein glycation assay in HepG2: the findings demonstrated that the PHD extract inhibited protein glycation in a dose-dependent manner at all concentrations tested (Fig. 6D). At the same time, individual plant extracts demonstrated only moderate activity when compared to PHD and aminoguanidine. The inhibition of lipase and collagenase enzymes by secondary plant chemicals has the potential to manage postprandial hyperglycemia and hyperlipidemia, while inhibiting enzyme catalysis will

reduce the number of monosaccharides and fatty acids accessible for absorption (Villa-Rodriguez et al. 2018). Pancreatic lipase is accountable for the hydrolysis of dietary lipids and its inhibition outcome in reduced fat absorption, contributing to DM (Conforti et al. 2012). Anti-diabetic medicines are used to treat or manage DM, and their mechanisms of action are known. Lipase and DPP-IV enzyme inhibition are among them (Saeedi et al. 2020).

When compared to the individual positive controls, our results showed that the extract inhibited lipase and DPP-IV significantly. This suggests that the anti-diabetic mechanism of PHD is as a result of through the inhibition of these enzymes. On the other hand, protein glycation has been established in studies to be one of the outcomes of abnormally high blood glucose in DM individuals (Ulrich and Cerami 2001; McKay et al. 2019). Protein glycation is a reversible process in which a reducing sugar and a free amino group of a protein form adducts that yield glycation products over time. These responses play a crucial role in the aetiology of DM complications. When compared to relevant standards, at the concentrations investigated, the PHD showed significant suppression of collagenase and protein glycation. However, this is the first study to look at both the PHD's protein glycation and anticollagenase properties (Kiho et al. 2000; Eckel et al. 2021).

CONCLUSION

The findings of the present study demonstrated that the PHD has contained abundant phytoconstituents and showed a significant free radical scavenging activity and glucose lowering effects *in vitro*. On the other hand, PHD exhibited noteworthy inhibition of α-amylase, α-glucosidase, lipase, DPP-IV, collagenase and protein glycation in HepG2 cell line. Hence the results obtained from this recommended that PHD may be considered for the treatment of postprandial glycemia in people with type 2 diabetes mellitus. However, further preclinical studies are crucial in advanced animal models to validate its antioxidant and anti-diabetic activities.

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Conflict of Interests: Authors declare no conflicts of interests to disclose.

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