Inhibitory Effects of a Specific Phytochemical Combination on Carbohydrate Metabolism, Lipid Digestion, and Free Radicals: An in Vitro Study

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Abstract—**Context: Diabetes, characterized by insulin insufficiency/resistance and oxidative stress, is a global health concern. Traditional plant combinations offer potential as oral diabetes therapy due to their multifaceted pharmacological properties.**

Aims: This study evaluated the antidiabetic, antilipidemic, and antioxidant potential of a phytochemical combination comprising *Salacia* extracts (*Salacia chinensis* and *Salacia oblonga*), Curcumin, and Piperine.

Settings and Design: In vitro experiments assessed the inhibitory activity of the phytochemical combination on key enzymes related to carbohydrate metabolism, lipid digestion, and free radicals.

Methods and Material: A formulation containing *Salacia* extract (80 mg %), Curcumin (15 mg %), and Piperine (05 mg %) per 100 mg total weight was evaluated. Inhibition of carbohydrate metabolism enzymes (α-amylase, α-glucosidase, aldolase, aldose reductase, PTP 1B) and lipid digestion enzymes (cholesterol esterase, lipase) was determined. Antioxidant activity was assessed using DPPH and ABTS assays.

Statistical analysis used: The results were expressed as the average ± standard deviation (SD) based on three replicates. IC50 values were calculated using GraphPad Prism 5.0 software. Principal Component Analysis (PCA) was conducted to assess the relationship between enzyme activity and total phenolic content/antioxidant activity utilizing SPSS® version 20.

Results: The phytochemical combination demonstrated significant inhibitory activity against carbohydrate metabolism enzymes (IC50 values: 37.20 µg/ml for α-amylase, 18.65 µg/ml for α-glucosidase, 59.38 µg/ml for aldolase, 17.52 µg/ml for aldose reductase, 32.54 µg/ml for PTP 1B). Moderate cholesterol esterase inhibition (IC50: 53.50 µg/ml) and antioxidant activity (IC50: 66.12 µg/ml for DPPH, 45.01 µg/ml for ABTS) were observed.

Conclusions: The phytochemical combination exhibits significant inhibitory effects on key enzymes involved in diabetes pathophysiology, suggesting its potential as a primary diabetes therapy. Additional in vivo studies are required to confirm and validate these findings.

Key-words: **Diabetes, Phytochemical combination, Carbohydrate metabolism, Antioxidant activity, Therapeutic potential**

Key Messages: This study showcases the potent inhibitory effects of a phytochemical combination (*Salacia* extracts, *Curcumin*, and Piperine) on enzymes crucial for diabetes pathophysiology, suggesting its potential as a promising therapeutic intervention warranting further validation in clinical settings.

INTRODUCTION

Indeed, numerous plant extracts have been utilized in both Ayurvedic and conventional Chinese medicine for centuries in the treatment of diabetes. The increased prevalence of diabetes mellitus due to various environmental factors has led to the increased recognition and use of these extracts in alternative and complementary medicine [1]. Curcumin, Salacia (*Salacia chinensis* and *Salacia oblonga*) extract, and forskolin are some of the plants known to possess useful phytochemicals with inhibitory activity against type 2 diabetes and hyperglycemia, and oxidative stress [2].

Turmeric (*Curcuma longa*), has been used for the management of diabetes in alternative and complementary medicines [3]. Curcumin and has elicited interest as a potential drug for the cure of diabetes and its complications. These properties have been attributed to the secondary metabolites present in turmeric. Phenolics are the most significant bioactive molecules present in curcuminoids [3, 4]. Besides being relatively safe and inexpensive, the compound has been reported to be efficacious against hyperglycemia and hyperlipidemia in diabetic rat models [4]. Recent literature indicates the applications of curcumin for blood glucose and diabetes-related complications, lipidemia, neuropathy, nephropathy, and other complications [5, 6]. Curcumin reported to have antioxidant and anti-inflammatory properties [7]. The phenolic moieties located in the structure of curcumin have been suggested to define the

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ability of turmeric to scavenge the reactive oxygen species and NO [7, 8]. The antihyperglycemic, antihyperlipidemic, and antioxidant effects of this drug have attracted interest to research the compound for purposes of creating a "super curcumin" with improved bioavailability to encourage its increased use in diabetes management [8].

For centuries in Asia, the root, stem, and leaf extracts of Salacia species have been utilized for the therapeutic management of diabetes [9]. A wide variety of compounds of the extract has been identified as having anti-diabetic properties. The compounds include kotalagenin 16-acetate, kotalanol, mangiferin, ponkorinol, salacinol, salaprinol, and various de-0-sulfonated compounds as well as proanthocyanidin oligomers [10]. Salacia extracts are known to exhibit inhibitory activity against various enzymes involved in carbohydrate and lipid metabolism, including pancreatic lipase, intestinal α-glucosidase, peroxisomal proliferator-activated receptor-α, glucose transporter-04 mediated glucose uptake, aldose reductase, pancreatic α-amylase, and angiotensin II type 1 receptor [11].

Additionally, Salacia extracts have been reported to have antioxidant activity, hepatoprotective activity, and free radical scavenging capability in rodent models [12]. Studies have shown that Salacia extracts can lead to decreased blood glucose and insulin levels, lower HbA1c levels, and reduced serum lipid levels in human models. Similar outcomes have been noted in both in vitro experiments and rodent models. Furthermore, Salacia extracts are reported to be safe for human consumption [13].

Forskolin is an extract of the plant *Coleus forskohlii* [14, 15]. The mechanism of action of forskolin is through the activation of adenylate cyclase (AC), which raises the levels of intracellular cAMP (an important messenger potentiating insulin secretion). The impacts of cAMP elevation depend on the isoforms of adenylate cyclase as well as the cell types [16]. In pancreatic β-cells, forskolin enhances the release of insulin via glycemic-mediated stimulus of the β-cells insulin [17]. In the Ammon and Müller [17] study, this effect was reported to occur through cAMP elevation, which modulates two pathways in pancreatic cells. Holtz [18] suggested one pathway is mediated by protein kinase A and the other by cAMP-GEF. The activities of forskolin are believed to have the potential ameliorate hyperglycemia and hyperlipidemia in human subjects.

Improper glucose metabolism affects the metabolism of both carbohydrates and lipids during the progeression of type 2 diabetes [19]. Incorrect regulation of glucose metabolism results in elevated levels of postprandial blood glucose. Improper homeostasis for an elongated period gives rise to hyperglycemia which leads to the onset of noninsulin-dependent type 2 diabetes [20]. New methods of prevention and management of the scourge are necessary to alleviate the suffering of diabetic patients. Given the known characteristics of phytochemicals in fighting hyperglycemia and intracellular eradication of free radicals from the human body, knowledge of the effects of a combination of these phytochemicals on elevated blood sugar and antioxidant activities is necessary. The objective of this research was to investigate the inhibitory activity of a phytochemical combination on antioxidant activities and key enzymes involved in carbohydrate metabolism and lipid digestion.

Subjects and Methods

Collection of Phytochemicals

Salacia (*Salacia chinensis* and *Salacia oblonga*) Extract (80%; Batch Number: GC-8004) was received as kind gift from Natural Remedies Pvt. Ltd., Bengaluru, Karnataka 560100, India and Curcumin (95%; Batch Number: PII-5202) was procured as a kind gift from Phytotech Extracts Pvt. Ltd, HRBR Layout, Bangalore-84, India. Finally, Forskolin (95 %; Batch No: RD/COL/394(A)) was procured as a kind gift from Sami Labs Limited, Peenya Industrial Estate, Bangalore-58, India.

Preparation of phytochemical combination (PCC)

Three phytochemicals were mixed at individual concentrations to obtain a final formulation in which, Curcumin (15%), Salacia (*Salacia chinensis* and *Salacia oblonga*) Extract (80%) and Forskolin (05%) per 100 mg of total weight. All the three phytochemicals were blended and dissolved in ethanol and then filtered. The filtered formulation (PCC) was used in the following assays.

α-amylase Inhibition Assay

This assay was conducted following the protocol reported by Gella et al. [21] with minor amendments. In this study, a pre-incubation mixture consisting of 90 µl (comprising 60 µl of 40 mM phosphate buffer pH 6.9/positive control/test sample at various concentrations and 30 µl of the enzyme at 0.5128 units/ml) was mixed and pre-incubated at 37°C for ten min. Subsequently, 125 µl of substrate (2.3 mM CNP-G3) was added, and the mixture was further incubated at 37°C for eight min. The absorbance of the reaction mixture was measured at 405 nm using a Molecular Devices Versamax Microplate Reader. Inhibition was calculated using the formula:

% inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100.

The IC50 value was determined from the % inhibition versus concentration plot using non-linear regression formulae.

α-glucosidase Inhibition Assay

The α-glucosidase inhibition assay, based on the method by Tomoki Ohta [22] with modifications, involved a pre-incubation mixture of 300 µl (250 µl of 80 mM phosphate buffer pH 7.0/positive control/test sample at varying concentrations and 50 µl of the enzyme), incubated at 37°C for 30 min. Subsequently, 500 µl of substrate (37 mM Sucrose) was added and further incubated at 37°C for 20 min. The reaction was stopped by boiling for two min., followed by cooling. Then, 250 µl of glucose reagent was added to 50 µl of the reaction mixture and mixed. Absorbance was read at 510 nm using a Molecular Devices Versamax Microplate Reader after incubation at 25°C for ten min..

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Inhibition was calculated as % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100.

The IC50 value was determined from the % inhibition versus concentration plot using non-linear regression analysis.

Glucose-6-phosphatase Inhibition Assay

The Glucose-6-phosphatase inhibition assay, adapted from Petrolonis et al. [23] with slight adjustments. In the assay, a pre-incubation mixture of 60 µl (42 µl of 100 mM MES K+ buffer pH 6.5/positive control/test sample at various concentrations, 12 µl of enzyme at 0.28 u/ml, and 18 µl of 6.65 mM glucose-6-phosphate) was incubated at 37°C for 40 min. Following pre-incubation, 240 µl of malachite green was added, and the reaction mixture was incubated at 25°C for 35 min. Absorbance was measured at 560 nm using a Molecular Devices Versamax Microplate Reader.

Inhibition was calculated as % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100.

The IC50 value was determined from the % inhibition versus concentration plot using non-linear regression analysis. Control reactions without the test sample were conducted, and sodium orthovanadate was used as a positive control.

DPP IV (Dipeptidyl Peptidase IV) Inhibition Assay

The DPP IV assay, adapted from Kojima et al. [24] with minor modifications, involved a pre-incubation mixture of 42 µl (34.5 µl of 100 mM Tris-HCl buffer pH 8.2/positive control/test sample at various concentrations and 7.5 µl of dipeptidyl peptidase IV from porcine kidney at 40 m units/ml) pre-incubated at 37°C for 10 min.. Subsequently, 10 µl of 3.8 mM substrate (Gly-pro-p-nitroanilide) was added, mixed, and incubated at 37°C for 30 min.

Absorbance was measured at 405 nm, and inhibition was calculated as % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100. IC50 values were determined using non-linear regression analysis.

Control reactions without the test sample were conducted, and Ile-Pro-Ile (Sigma-Aldrich, USA) was used as a positive control.

Aldolase Inhibition Assay

The aldolase inhibition assay, utilizing Boyer's adaptation of the hydrazine assay, employed the interaction between 3-phosphoglyceraldehyde and hydrazine to produce a hydrazone. The assay was conducted following the method described by Jagannathan et al. [25] with modifications. In summary, a reaction mixture of 400 µl containing 100 µl of 12 mM fructose-1,6-bisphosphate, 200 µl of 3.5 mM hydrazine sulfate, and 100 µl of different concentrations of PCC was incubated for 10 min. Subsequently, 10 µl of aldolase enzyme was added, and the absorbance of the supernatant was read at 240 nm against blanks using a Molecular Devices Versamax Microplate Reader. Inhibition was calculated as % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100. The IC50 value was estimated from the % inhibition versus concentration plot using non-linear regression analysis [25].

PTP 1B (Protein Tyrosine Phosphatase-1B) Inhibition Assay

The PTP-1B assay followed the protocol provided in the product brochure of PTP-1B from Sigma, USA. A pre-incubation mixture of 90 µl containing 38 µl of NPP buffer pH 7.2/vehicle buffer/positive control/test solution at various concentrations and 7 µl of enzyme was prepared. Additionally, 45 µl of BSA (100 µg/ml) was added to the mixture, which was then pre-incubated at 37°C for 10 min.. After pre-incubation, 50 µl of 50 mM substrate was added and thoroughly mixed. The reaction mixture was incubated at 37°C for 40 min., and absorbance was measured at 405 nm using a spectrophotometer. Inhibition was calculated using the formula: % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100. The IC50 value was determined from the % inhibition versus concentration plot using non-linear regression analysis.

Aldose Reductase Inhibition Assay

Aldose Reductase activity was assessed using the method described by Kinoshita et al. [26] with minor adjustments. The incubation mixture (250 µl) comprised 67 mM Potassium Phosphate buffer (pH 6.2), 0.4 M Lithium Sulphate, 150 µM NADPH, 220 µM DL-Glyceraldehyde, and 1384 µg/ml of enzyme preparation for the control. Different concentrations of PCC were added for the test, while the blank contained all components except DL-Glyceraldehyde. The reaction was initiated by adding NADPH, and absorbance was read at 340 nm after 10 min.. The activity was calculated considering 1 unit activity as the change in absorbance of 0.001 and expressed as units/min/mg of protein. The difference was indicated as % inhibition.

DPPH Radical Inhibition Assay

The DPPH assay, following the method by Vani et al. [27], involved a total reaction volume of 250 µl. This included 200 µl of methanol, positive control, or various concentrations of the test solution, and 50 µl of DPPH solution at a concentration of 0.659 mM. The mixture was thoroughly mixed and incubated at 25°C for 20 min.. After incubation, absorbance was measured at 510 nm using a Microwell plate reader. Inhibition was calculated using the formula: % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100 [27]. The IC50 value was determined from the % inhibition versus concentration plot using non-linear regression analysis.

ABTS Radical Inhibition Assay

ABTS radical scavenging activity was evaluated following Auddy et al.'s [28] procedure. A total reaction volume of 250 µl was prepared with various concentrations of 10 mM PBS pH 7.4, positive control, or test solution, and 230 µl of ABTS radical solution. Absorbance was measured at 734 nm using a Microplate reader immediately after mixing. Inhibition was calculated using the formula: % inhibition = (Absorbance of

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Control – Absorbance of Test) / Absorbance of Control × 100 [28]. The IC50 value was determined from the % inhibition versus concentration plot using non-linear regression analysis.

Cholesterol Esterase Inhibition Assay

The assay, based on Pietsch et al.'s [29] method with modifications, involved incubating phosphate buffer pH 7.0, test sample, or positive control with sodium taurocholate, enzyme, and substrate. Inhibition was calculated as % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100, and IC50 was determined from the % inhibition versus concentration plot using non-linear regression analysis [29].

Lipase Inhibition Assay

The lipase inhibition assay followed Lott et al.'s [30] protocol. A total reaction volume of 265 µl, including buffer from the Randox-Lipase assay kit, positive control, or PCC as test sample at various concentrations, lipase, and substrate, was incubated at 37°C for 15 min. Absorbance change was measured at 340 nm using a Fluostar Optima Microplate Reader, with ursolic acid as a positive control. Inhibition was calculated using % inhibition = (Absorbance of Control – Absorbance of Test) / Absorbance of Control × 100, and IC50 was estimated from the % inhibition versus concentration plot using non-linear regression analysis [30].

Determination of Total Phenolic Content

The total phenolic content (TPC) was assessed using the Folin-Ciocalteu method as described by Chun et al. [31]. In summary, 0.5 ml of the plant extract mixture was combined with methanol fractions (1.0 mg/ml) in a 10-volumetric flask. Folin-Ciocalteu reagent was added, and the mixture was left at room temperature for 5-8 min.. Then, 0.2 ml of a 7.0% sodium carbonate solution was added, and the volume was adjusted to 10 ml with distilled water. After 2 hours, the absorbance was measured at 725 nm to determine the TPC concentration. Gallic acid was used to create the standard calibration curve. Results were expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g) [31].

Statistical Analysis

All experiments were performed in triplicate, presenting data as mean ± standard deviation (SD), calculating percentage inhibition of individual enzyme activity against a range of concentrations, determining the IC50 value using GraphPad Prism 5.0 software, and conducting Principal Component Analysis (PCA) on the mean activity values to correlate with TPC/antioxidant activity across different assays using SPSS® version 20.

RESULTS

Antidiabetic activity of phytochemical combination

Antidiabetic potential of the PCC was investigated by conducting inhibition assay for certain enzymes viz., α-amylase, α-glucosidase, glucose-6-phosphatase, aldolase, aldose reductase, DPP IV and PTP 1B and the results were shown in the Table 1. The study findings indicated that, the PCC has shown significant inhibitory function against the enzymes such as α-amylase (IC_{50}=37.20 µg/ml), α-glucosidase (IC_{50}=18.65 µg/ml), aldolase (IC_{50}=59.38 µg/ml), aldose reductase (IC_{50}=17.52 µg/ml) and PTP 1B (IC_{50}=32.54 µg/ml), the enzymes that have the significance in increasing blood glucose levels and suppressing normal carbohydrate metabolism in diabetics. While, the PCC showed either lesser or no inhibition against glucose-6-phosphatase (3.51±1.27 % at 500 µg/ml) and DPP IV (3.96±1.03 % at 100 µg /ml). The inhibitory potential of PCC has been compared with the standard inhibitors for respective enzymes (Table 1).

TABLE 1. PERCENT (%) INHIBITORY EFFECTS OF PCC ON A-AMYLASE, A-GLUCOSIDASE, GLUCOSE-6-PHOSPHATASE, ALDOLASE, PTP-1B, DPP IV AND ALDOSE REDUCTASE ACTIVITY

No	Assay	Sample/Standard	Concentration tested (µg/ml)	% Inhibition	IC_{50} µg/ml
1	α -amylase	PCC	10	17.96±5.03	37.20
			25	32.37±0.71	
			50	65.23±0.59	
			100	74.83±0.82	
		Acarbose			0.26
2	α -glucosidase	PCC	1	06.62±1.84	18.65
			5	27.52±1.00	
			10	37.92±1.01	
			50	66.95±0.70	
			100	79.91±0.18	
		Acarbose			0.78
3	Glucose-6-phosphatase	PCC	5	0	
			25	0	--
			50	0	
			100	0	
			500	3.51±1.27	
		Sodium orthovanadate			8.91
4	Aldolase	PCC	10	6.92±0.43	59.38
			25	19.26±0.74	
			50	29.14±0.71	
			100	79.40±1.35	

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		Copper Sulphate Pentahydrate			1.63
5	Aldose reductase	PCC	5	11.48±1.00	17.52
			10	37.94±0.66	
			25	65.57±0.11	
			50	80.56±0.99	
			100	86.46±0.83	
		Quercetin			3.09
6	PTP-1B	PCC	5	11.67±0.50	32.54
			10	17.03±2.93	
			25	35.92±1.50	
			50	56.91±5.93	
			100	88.07±1.27	
		Sodium orthovanadate			12.91
7	DPP-IV	PCC	5	0	-
			10	0	
			25	0	
			50	1.03±0.56	
			100	3.96±1.03	
		Ile-Pro-Ile			6.59

Antioxidant activity of phytochemical combination

Antioxidant potential of PCC was studied by performing scavenging activity by using DPPH and ABTS radicals and the results were presented in the table 2. The PCC showed significant scavenging activity DPPH (IC_{50}=66.12 µg/ml) and ABTS (IC_{50}=45.01 µg/ml) radicals and the results were compared with standard compound gallic acid (Table 2).

TABLE 2. INHIBITORY EFFECTS OF PCC AGAINST DPPH AND ABTS RADICALS

No	Assay	Sample/ Standard	Concentration tested (µg/ml)	% inhibition	IC_{50} µg/ml
1	DPPH	PCC	10	8.69±1.63	66.12
			25	13.30±2.20	
			50	42.38±0.82	
			100	65.60±1.08	
		Gallic acid			1.15
2	ABTS	PCC	10	16.31±0.54	45.01
			25	24.72±2.88	
			50	46.80±1.07	
			100	81.24±0.87	
		Gallic acid			1.47

Antilipidimic activity of phytochemical combination

Antilipidimic (anti-lipogenic) potential of PCC was investigated by conducting inhibition assays for cholesterol esterase and lipase and the results were given in the Table 3. The PCC showed significant cholesterol esterase (IC_{50}=53.50 µg/ml) inhibition activity and the activity was compared against the areca nut extract (IC_{50}=172.99 µg/ml). The PCC showed less or no activity against lipase (6.59±1.38% at 500 µg/ml) and the activity was compared against standard ursolic acid (IC_{50}=57.09 µg/ml).

TABLE 3. INHIBITORY EFFECTS OF PCC ON CHOLESTEROL ESTERASE AND LIPASE ENZYME

No	Assay	Sample/ Standard	Concentration tested (µg/ml)	% inhibition	IC_{50} µg/ml
1	Cholesterol Esterase	PCC	1	1.72±1.66	53.50
			5	22.73±2.73	
			25	32.41±5.30	
			50	42.01±1.32	
			100	66.69±6.41	
		Areca Nut Extract			172.99
2	Lipase	PCC	10	0	--
			25	0	
			50	0	
			100	3.21±0.76	
			500	6.59±1.38	
		Ursolic Acid			57.09

Total phenolic contents

Calibration curve for Gallic acid (4-20 µg/ml) was assessed by performing single measurement at several analyte concentrations with a coefficient of determination (r^2) value of 0.9989 (Figure 1), which gives good linearity. The total phenol content of PCC was found to be 279.35±8.34 mg gallic acid equivalent/g of dry extract.

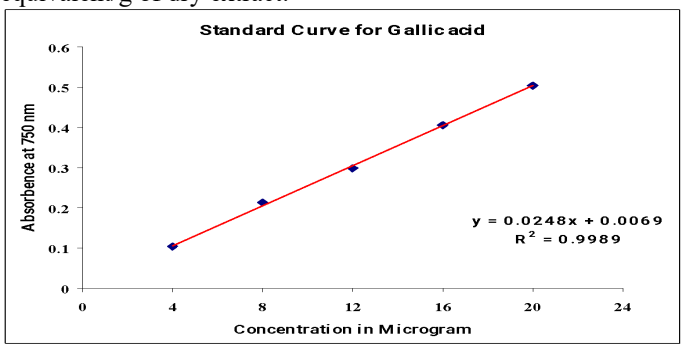

Figure 1. Calibration curve for Gallic acid

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Descriptive Findings

The inhibitory effect of PCC on the different enzymes was expressed as percentages, mean (SEM), SD for all the data was also calculated (Table 4).

TABLE 4. DESCRIPTIVE FINDINGS

Parameters	N	Min	max	Mean(SEM)	SD
Antidiabetic effect					
α-amylase	3	17.96	65.23	38.52(13,4)	24.22767
α-glucosidase	3	6.62	37.92	24.02 (9.2)	15.94083
G6PH	3	0	0	0	0
Aldose	3	6.92	29.14	18.44(6.4)	11.13267
Aldose reductase	3	11.98	65.57	38.4967(14.4)	26.79934
PTP-IB	3	11.67	35.92	21.54(7.4)	12.73855
DPP-IV	3	0	0	0	0
Effect on lipid profile Cholesterol	3	1.72	32.41	18.9533(9.1)	15.68969
Lipase	3	0	0	0	0
Antioxidant effect DPPH	3	8.69	42.38	21.456(10)	18.26616
ABTS	3	16.31	46.8	29.2767(9.1)	15.74746

Principle component analysis

Principle component analysis was performed to correlate the TPC/antioxidant activity with the different assays in this study (Table 5). After statistical analysis of all the data the PCA correlated positively with the total phenolic content and showed that the PCC had high content of phenolic components. PCA also showed a good relationship with the variables of the ABTS and DPPH assays indicating a relatively higher antioxidant activity of the phenolic compounds.

TABLE 5. PEARSON CORRELATION BETWEEN PCC AND TPC OF THE EXTRACT AND ACTIVITY OBSERVED

Variables	PCC	TPC
α-Amylase inhibition	0.989	0.908
α-glucosidase inhibition	0.848	1
Aldolase inhibition	0.908	0.989
Aldose reductase activity inhibition	0.938	0.975
PTT-1B inhibition	0.995	0.867
DPPH scavenging activity	1	0.821
ABTS radicals	0.993	0.894
Cholesterol esterase activity	0.838	1

DISCUSSION

Turmeric and salacia species have been used since the ancient types for hypoglycemic reasons. Besides, the usefulness of forskolin in the management of diabetes and other health complications has attracted considerable interest. The active ingredient of interest in turmeric is curcumin – a polyphenol with immense benefits to human health – that is rapidly metabolized in the liver and excreted in feces. The production of insulin by pancreatic cells entails various reactions which are the focus for polyphenols actions which was shown to be significantly high in turmeric.

Phenolic groups function by forming non-covalent associations with the enzymes [31]. There exist both galloyl and hydroxyl groups in the polyphenols molecular structure. The polar groups of the enzymes and phenolic groups of the Curcumin phenols form hydrogen bonds. Conversely, numerous hydrophobic amino acids exist in proteins (enzyme) [32]. The galloyl groups present in the polyphenols exhibit hydrophobicity hence the binding of enzymes via hydrophobic bond. The galloyl moiety has a crucial role of interaction with the human α-glycosidase and α-amylase and they normally affect efficiency [33]. The mutual effect of formation of hydrogen bond and hydrophobic association between the carbohydrate digestive enzymes and polyphenols enhance the control of Hyperglycemia, commonly observed in patients diagnosed with type II diabetes.

The polyphenols effect of Curcumin as an anti-oxidant occurs in various diverse mechanism [33, 34]. The polyphenols can scavenge free radicals like reactive nitrogen and oxygen species (RNS and ROS) [35]. The polyphenols reduce the NADPH oxidases, ROS secreted by mitochondria, and the endothelial NO synthase alongside up-regulation of several antioxidant enzymes [35, 36]. The curcumin can modify the glutathione (GSH) activity, Superoxide dismutase (SOD), and catalase enzymes which are functional during the neutralization of the free radicals. The polyphenols can also inhibit ROS- producing enzymes like xanthine, lipoxygenase/cyclooxygenase, and oxidase/ hydroxygenase [36]. Moreover, since curcumin is lipophilic, it is therefore an effective peroxyl radical scavenger hence considered as an effective antioxidant like vitamin E.

A previous study indicated that curcumin with half maximal inhibitory concentration (IC_{50}) of about 51.32 μM has a competitive inhibitory activity against α-amylase. This value is significantly higher than those of synthetic drugs such as acarbose (290.6 μg/ mL). The same inhibitory profile has been reported for Saliacia extract and forskolin against α-amylase and α-glucosidase.

In the present study, the application of PPC demonstrated antihyperglycemic, antihyperlipidemic, and antioxidant activity. The half maximal inhibitory concentration (IC_{50}) of PCC was 37.20 μg/ml against α-amylase, 18.65 μg/ml against α-glucosidase, 59.38 μg/ml against aldolase, 17.52 μg/ml aldose reductase, and 32.54 μg/ml PTP 1B. As compared to the individual compounds alone, the combination of the phytochemicals shows higher inhibitory potency. Besides, the antioxidant activity of the phytochemical combination was evaluated at DPPH (IC_{50}=66.12 μg/ml) and ABTS (IC_{50}=45.01 μg/ml) which are significantly higher than the IC_{50} of individual compounds. The result implies that the phytochemical combination is a potent inhibitor of alpha- amylase and alpha glucosidase, which is of significant pharmacological importance.

The use of conventional medicines such as Acarbose in the management has reported drawbacks. The major disadvantage is related to the excessive inhibition of alpha-glucosidase. The use of herbal drugs in the management of postprandial blood sugar could address these drawbacks. A mild inhibitory activity on alpha-amylase and a stronger activity on alpha-glucosidase are preferred to avoid the side-effects of over-regulation. The reported adverse effects of conventional diabetes drugs include diarrhea, flatulence, and abdominal distention associated with

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excessive amylase inhibition [37]. Flatulence and distention are mainly due to abnormal bacterial fermentation in the colon.

Consumption of carbohydrate and fatty foods leads to elevated levels of glucose and lipids in the blood. Consequently, the management of these profiles involves regulation of the pancreatic α-amylase and α-glucosidase to control postprandial glucose elevation. Ros [38] demonstrated the use of lipid digestion and uptake delay as a new a viable method of prevention of hyperlipidemia. The hydrolysis of dietary fats can be delayed by inhibition of lipase and esterase enzymes in the pancreas [39]. Other mechanisms reported to lower the level of cholesterol in blood include forming insoluble bile acid complexes and increasing their excretion. Besides, reducing the formation of micelle can significantly decrease the level of blood cholesterol [40]. Also, consumption of carbohydrate and lipid-rich diet increase postprandial oxidative stress [41]. The results of this study are significant especially concerning the regulation of hyperglycemia and hyperlipidemia by PCC through its inhibitory activities on cholesterol esterase, pancreatic α-amylase, intestinal α-glucosidase, and pancreatic lipase.

Furthermore, the study explored the potential antioxidant activity of PCC with satisfactory results. Previous studies revealed curcumin has antiglycemic and lipid peroxidation activity in rat models [42, 43]. Another study found that Salacia (*Salacia chinensis* and *Salacia oblonga*) extract ameliorated postprandial hyperglycemia in diabetic rats [44]. Also, reported the antioxidant effect of forskolin which is similar to that of vitamin E and probucol whereby it protects against the intracellular effects of hydrogen peroxide. In diabetic mice models, forskolin has been demonstrated to modify oxidative stress [45]. The combination of these three herbal extracts in the present study appears to have potent antihyperglycemic, antihyperlipidemic, and anti-oxidant activity.

Several studies support these findings, indicating that the combination of phenolic compounds exhibits inhibitory effects on intestinal α-glucosidase, pancreatic lipase, cholesterol esterase, and pancreatic α-amylase [45, 46]. Additionally, these studies corroborate previous findings suggesting a positive correlation between phytochemicals present in plant extracts and the inhibition of pancreatic lipase, pancreatic cholesterol esterase, and antioxidant activity [47]. This body of evidence underscores the importance of phytochemical compounds in exerting inhibitory effects on pancreatic lipase, pancreatic cholesterol esterase, and antioxidant activity.

These findings suggest that the optimal combination ratios of plant extract compounds could hold significant therapeutic value for the management of diabetes mellitus. This is particularly relevant considering that conventional drugs such as Acarbose have been associated with adverse effects in diabetic patients. Herbal combinations have the potential to mitigate the limitations of these antihyperglycemic drugs.

Conclusion

From the results of the study, it can be concluded that the polyherbal combination (PCC) exhibits multifaceted properties relevant to diabetes management. Firstly, PCC interferes with the assimilation process of carbohydrate hydrolytic products, particularly polysaccharides. Secondly, PCC demonstrates significant inhibition of aldose reductase enzyme activity, which is implicated in various diabetic complications such as retinopathy, cataract, nephropathy, and neuropathy. Additionally, PCC exhibits notable antioxidant activity, as confirmed by DPPH and ABTS assays. Furthermore, the inhibition of cholesterol esterase enzyme by PCC suggests a potential mechanism for controlling the bioavailability of dietary cholesterol, which may have implications for managing cholesterol levels in individuals with diabetes. Overall, PCC is a promising antidiabetic combination worthy of further investigation in vivo. Its potential therapeutic benefits in managing type II diabetes mellitus make it a candidate for further exploration in combinational therapy approaches.

Abbreviations

PTP 1B: Protein Tyrosine Phosphatase-1B, DPP IV: Dipeptidyl Peptidase IV, ABTS: 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, PCC: Phytochemical Combination, EC: Enzyme Commission, G6PH: Glucose-6-phosphatase.

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