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Evaluation of In vitro Antioxidant and Antidiabetic activity of a Polyherbal formulation

Research Article

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Abstract

Recent years have seen significant improvements in the identification, management, and study of metabolic illnesses, particularly diabetes. In addition to the present synthetic medicines, current research is focused on developing an alternate source of medication from natural resources for managing diabetes. Objective: To investigate the antioxidant and antidiabetic efficacy of a poly-herbal formulation (PHF) by *in vitro* models. Methods: The Polyherbal formulation was screened for the presence of phytochemical constituents. The total phenolic and flavonoids content of the formulation was also analysed. The *in vitro* antioxidant and antidiabetic activity of the formulation (PHF) were calculated. Results: The Polyherbal formulation showed presence of phenols, flavonoids, steroids, and saponins. The IC_{50} value of PHF4 was found to be 42.70µg/ml in DPPH radical scavenging assay. It has shown high levels of phenols (172.6±0.68 mg/g), flavonoids (137.3±0.91 mg/g) and had IC_{50} values of 422.50 µg/ml and 438.71 µg/ml for amylase and glucosidase, respectively. Conclusion: The results indicate that Polyherbal Formulation (PHF4) had significant antioxidant activity as well as a strong inhibitory effect on α -amylase and α -glucosidase. However, for further validation of the above findings, *in vivo* antidiabetic study of polyherbal formulation will be carried out using animal models.

Keywords: Diabetes, Herbal plant, Antioxidant, Antidiabetic, Polyherbal, Treatment.

Introduction

Diabetes Mellitus (DM) is a metabolic disease characterized by the pancreas inability to produce enough insulin. The major two types of diabetes are type 1 and 2. Type 1 diabetes mellitus is referred to as insulin-dependent, juvenile, or childhood-onset diabetes, and is autoimmune reaction in which pancreas is unable to produce adequate insulin, while type 2 diabetes mellitus is non-insulin-dependent or adult-onset diabetes because of the less efficiency of insulin hormone (1). According to the World Health Organization (WHO), the population with diabetes in 1980 was 108 million which is increased to 422 million in 2014. By 2045, there will be 700 million adults with diabetes worldwide (2).

Polyherbal formulations contain various essential components of different plants species. polyherbal products are considered to be better with the extended therapeutic potentials as compared to single herbal medicines. It has been reported that polyherbal formulations may produce a synergetic effect because of the presence of various constituents and have lesser side effect as compared to the current synthetic medicines (3). The polyherbal formulation (PHF) consists of *Lycopersicon pimpinellifollium* L (Solanaceae), *Moringa oleifera* (Moringaceae), *Abelmoschus esculentus L* (Malvaceae), *Artocarpus heterophyllus Lam* (Moraceae) and *Brassica oleracea var. italica* (Brassicaceae). From the literature survey it is well known that the ingredients present in the formulation showed antioxidant and antidiabetic activity (4, 5,6,7,8). But till now, the prepared Polyherbal formulation is not investigated for its antioxidant and anti-diabetic potential. Hence, the present work was conducted to study antioxidant and antidiabetic potential of polyherbal formulation (PHF) by *in vitro* models.

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Materials and Methods

Collection, identification and authentication of plant materials

In this study, the fruits of *Lycopersicon* pimpinellifollium L (Wild tomato), *Moringa oleifera* (Drumstick), *Abelmoschus esculentus L* (Lady finger), *Brassica oleracea var. italic* (Broccoli) and seeds of *Artocarpus heterophyllus Lam* (Jackfruit) were collected in june, 2022, from Rangiya, Assam, India. The plants were identified and authenticated by Dr. Minaram Nath, Professor, Department of Botany, Royal School of life Sciences, The Assam Royal Global University, Guwahati, Assam, India.

Fig 1: Broccoli **Fig 2: Lady finger** **Fig 3: Wild tomato** **Fig 4: Drumstick** **Fig 5: Jackfruit (Seed)**

Preparation of Polyherbal Formulation

The powder of the dried fruits of *Lycopersicon pimpinellifollium* L (Wild tomato), *Moringa Oleifera* (Drumstick), *Abelmoschus esculentus L (Lady finger), Brassica oleracea var. italic(Broccoli)* and *seeds* of *Artocarpus heterophyllus Lam (Jackfruit)* was stirred using a magnetic stirrer with 500 ml mixture of ethanol:water (6:4) (hydro-alcoholic extract) for 15 hours; then the mixture was centrifuged at 2850 x *g* and the supernatant decanted (9). The process was repeated again with the precipitated pellet. The supernatants were collected, concentrated in a rotary evaporator at room temperature and freeze dried in a lyophilizer. Furthermore, five formulations (PHF1-PHF5) were prepared by mixing different ratios of plant materials as shown in Table 1.

Table 1: Composition of polyherbal formulation

SL. No	Code	Formulation	Ratio
1	PHF-1	LF:JF:DS:BC: WT	300 mg:200 mg:100 mg: 100mg:100 mg
2	PHF-2	LF:JF:DS:BC: WT	200 mg:200mg :100mg:100mg:100mg
3	PHF-3	LF:JF:DS:BC: WT	100 mg:200 mg:100 mg:100 mg:200 mg
4	PHF-4	LF:JF:DS:BC: WT	100mg:100 mg:100 mg:300 mg:100 mg
5	PHF-5	LF:JF:DS:BC: WT	200 mg:300 mg:100 mg:100 mg:100 mg

*LF-Lady finger, JK-Jackfruit, DS-Drumstick, BC-Broccoli, WT- Wild tomato

Preliminary Phytochemical Analysis

All the five extracts were screened for the presence of different phytochemical constituents such as alkaloids, phenols, flavonoids, glycosides, tannins, steroids, saponin etc (10).

In vitro anti – diabetic activity of polyherbal formulation

α-amylase inhibition assay

The assay was carried out by utilizing the DNSA reagent (11). A volume of 500 µl of enzyme solution was mixed with 1ml of various concentrations of extract and was incubated at 37° C for 10 min. Thereafter, 500 µl of the starch solution was added to each test tube and they were incubated for 10 minutes at 37° C. The reaction was terminated by the addition of 1ml of DNSA solution and was incubated for 5 min in a boiling water bath. It was then cooled, diluted with 10ml of water, and measured at 540 nm in UV spectrophotometer. Acarbose was used as standard.

The inhibition percentage was calculated by the following equation:

$$\text{Inhibition (\%)} = [(Abs1 - Abs2)/Abs1] \times 100$$

where, Abs1=sample and Abs2 = control

Alpha-Glucosidase Inhibitory Activity

The plant extracts (50 µL) at various concentrations levels (50 to 1000 µg/mL) were incubated with 10 µL of α-glucosidase (maltase), yeast (Sisco Research Laboratories Pvt. Ltd.), and enzyme solution (1 U/mL) for 20 min at 37ºC and 125 µL of 0.1M phosphate buffer (pH 6.8) (12). After 20 min of incubation, the reaction was initiated by adding 20 µL of 1M pNPG (substrate) and then incubated for 30 min. The reaction was terminated by adding 0.1N of Na2CO3 (50 µL) and the final absorbance was calculated at 405 nm using a microplate reader. Besides, Acarbose drug was used as a positive control at different dilutions levels (50 to1000 µg/mL) . The results of this analysis were expressed as percentage inhibition, calculated with the formula below:

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100$$

where, As represents the absorbance of the test substance while Ac is the absorbance of control.

DPPH Free Radical Scavenging Assay

A 0.2 mM DPPH solution was freshly prepared by dissolving DPPH in ethanol (13). The freshly prepared solution was then kept aside (in dark) until further use. About 1 ml of different concentrations of extract (50, 100, 200, 400, 800, 1000 µg) was added to 1 ml DPPH solution. The control was prepared by replacing the extract with ethanol solution. The resulting mixture was then incubated in the dark for about 30 minutes under ambient temperature. After 30 minutes, the resulting absorbance was measured using a UV-Visible spectrophotometer (Perkin-Elmer, USA) at 517 nm. Quercetin was used as a standard. All the determinations were done in triplicate. The percentage radical scavenging activity of the extracts was calculated using the following formula,

$$\text{\% DPPH radical scavenging activity} = \{(A0 - A1)/A0\} \times 100$$

where, ABS- Absorbance

Determination of total Phenoilc content

Phenolic content was determined using Folin-Ciocalteu (FC) reagent method (14). The reaction mixture was prepared by mixing 0.5 mL of sample solution, 2.5 mL of 10% Folin-Ciocalteu reagent dissolved in water and 2.5 mL of 7.5% of Na2CO3. Blank was concurrently prepared using 0.5 mL distilled water instead of PHF solution. The samples were thereafter incubated in an incubator at 45°C for 45 min. The absorbance was read on a spectrophotometer (Shimadzu UV-1900) at 765 nm. The phenolic content was calculated as Gallic acid equivalent from the calibration curve of Gallic acid standard solutions (range: 20–100 µg/mL). The phenolic content was expressed in terms of mg gallic acid equivalent (mg of GAE/g of the PHF).

Determination of total flavonoids content

The total flavonoids were determined using AlCl3 method (15). A volume of 0.5 mL of 2% AlCl3 aqueous solution was added to 0.5 mL of sample. The contents were mixed well and incubated for 1 hour at room temperature for yellow colour appearance; the absorbance was read on a spectrophotometer (Shimadzu UV-1900) at 420 nm. Concurrently, the same procedure was used for the standard solutions of quercetin (concentrations: 20-100 µg/mL). Total flavonoids content was calculated using the calibration curve and expressed as mg quercetin equivalent (mg of QUE/g of the PHF).

Results

The phytochemical investigations of polyherbal extracts were tabulated in Table 2. The alpha-amylase inhibition assay was performed for the formulation extracts viz. PHF1, PHF2, PHF3, PHF4 and PHF5 and the results were tabulated in Table 3. The percentage inhibition of alpha-amylase by PHF4 was ranging from 11.12±0.21 % to 79.10±0.36 % in a dose dependent manner at 1000µg/ml. PHF4 showed strong alpha amylase inhibitory activity than other formulation (PHP1, PHP2, PHP3 and PHP5). IC_{50} values of the Polyherbal preparations were tabulated in Table 4 and represented in Figure 6. The IC_{50} of PHF 1, PHF2, PHF3, PHF4 and PHF5, were found to be 561.17 µg/ml, 608.12 µg/ml, 505.43 µg/ml, 422.50 µg/ml and 786.73µg/ml, respectively. The lower IC_{50} value indicates the significant antidiabetic potential of PHF4 (422.50 µg/ml) and it is closer to Standard Acarbose (IC_{50} 392.79 µg/ml), thus taken for further analysis. PHF4 (IC_{50} 438.71 µg/mL) showed potent inhibition of *α*-glucosidase whereas standard acarbose had IC_{50} value of 394.64 µg/mL as shown in Table 5 and represented in Figure 7. DPPH radical scavenging activity of PHF4 and Quercetin at various concentrations (50, 100, 200, 400, 800, 1000 µg/ml) were shown in Table 6 and represented in Fig 8. The total phenolic and flavonoids contents were 172.6 mg Gallic acid equivalent/g and 135.3 mg quercetin equivalent /g of the PHF4, respectively as shown in Table 7 and represented in Figure 9 and 10.

Table 2: Preliminary phytochemical investigation of various extracts of formulation

Phyto-chemical Test	Drum stick (Fruit)	Lady finger (Fruit)	Broccoli (Fruit)	Wild Tomato (Fruit)	Jack fruit (seed)
Alkaloid	----	+++	--	--	++
Glycosides	+	+	-	-	+
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Saponin	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Steroids	+	+	+	-	+
Tannins	+	+	+	+	+

(+)=Presence of constituents, (-) = Absence of constituents

Table 3: Percentage inhibition of alpha-amylase for different extracts of polyherbal formulation

SL. No.	Concen-tration (µg/ml)	PHF1	PHF2	PHF3	PHF4	PHF5	Acar-bose
1	50	9.25± 0.20	3.57± 0.59	8.2±0. 76	11.12 ±0.21	4.72± 0.31	12.24 ±0.25
2	100	27.23 ±0.34	18.43 ±0.65	28.30 ±1.34	31.12 ±0.53	16.23 ±1.30	32.41 ±0.36
3	250	45.67 ±0.25	32.41 ±0.36	46.21 ±0.29	47.24 ±0.75	32.10 ±1.57	48.04 ±0.28
4	500	56.14 ±0.54	54.10 ±0.24	62.14 ±1.69	68.18 ±0.27	42.17 ±1.79	69.14 ±0.38
5	1000	65.51 ±0.36	69.10 ±0.30	71.12 ±0.62	79.10 ±0.36	57.10 ±0.62	84.12 ±0.49

The values are denoted in mean ± SEM. (n=3), PHF: Polyherbal formulation.

Table 4: IC_{50} value of polyherbal formulations and standard in *in-vitro* α-amylase assay

Sl.No.	PHF	IC_{50} (µg/ml)
1	PHF1	561.17
2	PHF2	608.12
3	PHF3	505.43
4	PHF4	422.50
5	PHF5	786.73
6	Acarbose	392.79

Figure 6: Graphical representation of IC_{50} values of polyherbal formulations and Standard (Acarbose)

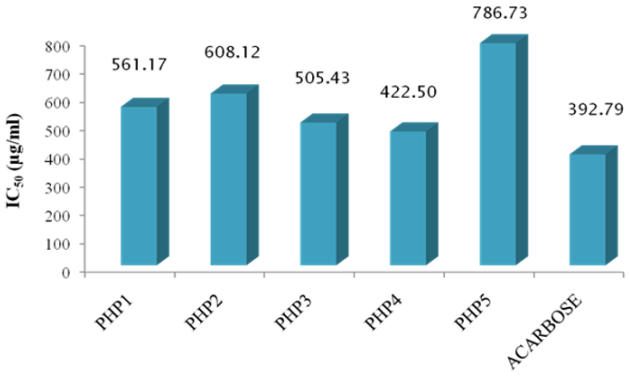

Table 5: *In- vitro* α-glucocodase assay of PHF4 and standard (Acarbose)

Sl.No.	Concentration (µg /ml)	PHF4	Acarbose
1	50	9.34±2.98	10.21±3.1
2	100	23.28±3.2	25.10±1.4
3	250	44.20±4.9	45.34±2.6
4	500	62.23±7.2	69.16±3.5
5	1000	89.10±6.2	95.14±7.1
	IC_{50}	**438.71**	**394.64**

The values are denoted in mean ± SEM. (n=3)

Figure 7: Graphical representation of the α-glucosidase inhibition assay

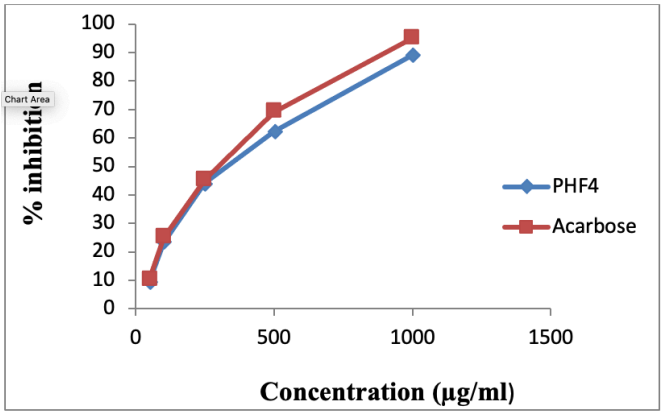

Table 6: DPPH scavenging activity and IC_{50} values of PHF4 and Quercetin

SL No.	Concentration(µg /ml)	PHF4	Quercetin
1	50	37.25±0.25	38.20±0.15
2	100	48.59±0.19	52.10±0.23
3	200	68.12±0.20	71.21±0.67
4	400	79.97±0.54	85.24±0.29
5	800	87.52±0.39	92.10±0.42
6	1000	89.34±0.56	97.41±0.67
	IC_{50}	42.70	12.94

Each value represents a mean ± SD (n = 3)

Figure 8:Graphical representation of DPPH radical scavenging activity of PHF4 and Quercetin at various concentration

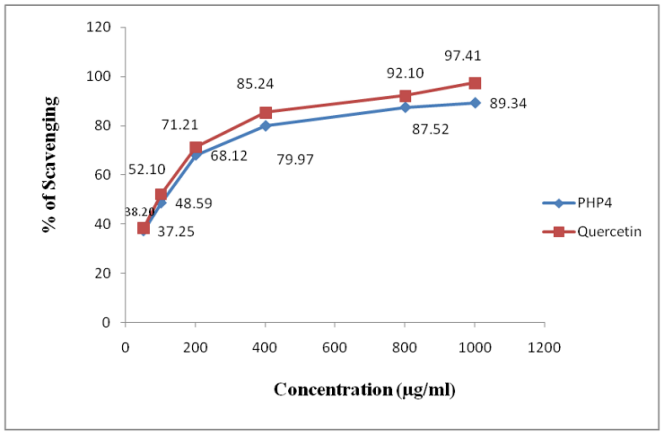

Figure 9: Standard calibration curve for Gallic Acid

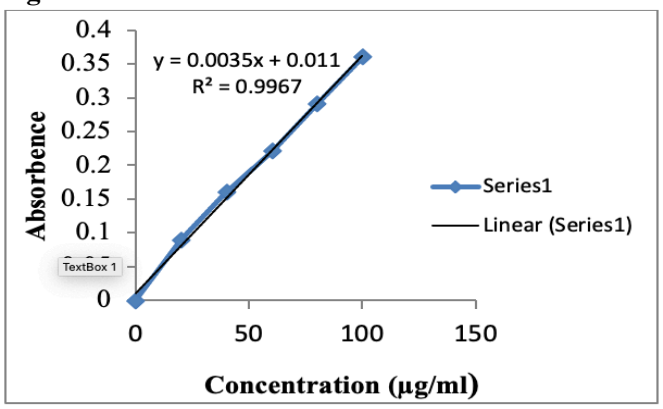

Figure 10: Standard calibration curve for Quercetin

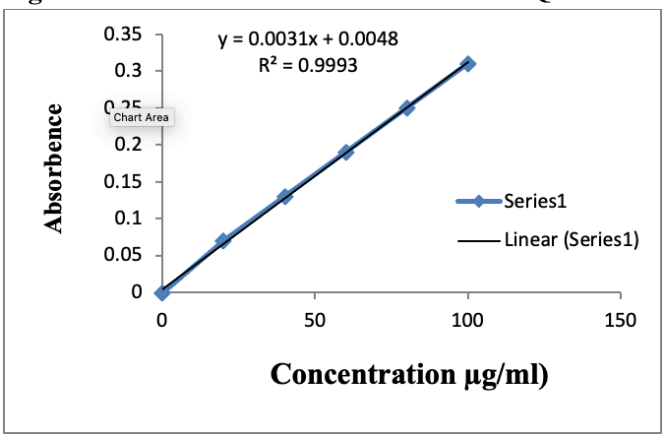

Table 7: Quantitative estimation of the PHF4

Sample	Total Phenolic Content	Total Flavonoids Content
PHF4	172.6±0.68	137.3±0.91

Discussion

Qualitative chemical test has shown the presence of alkaloids, flavonoids, phenols, saponins, glycosides, tannins and carbohydrates. It is an important step in the detection of the essential bioactive constituents present in herbal plants which may helpful for drug discovery and development. The *in-vitro* antidiabetic activity was performed with alpha amylase and alpha glucosidase assay. α-amylase inhibition activity of PHF4 at the concentration of 50, 100, 250,500 and 1000 µg/ml were found to be11.12±0.21, 31.12±0.53, 47.24±0.75, 68.18±0.27 and 79.10±0.36 respectively while as α-glucosidase inhibition activity of PHF4 at the concentration of 50, 100, 250, 500 and 1000 µg/ml were found to be 9.21±2.98, 25.13±3.2, 45.20±4.9, 65.21±7.2 and 87.10±6.2 respectively. The α-amylase and α-glucosidase inhibition activity of PHF4 was compared with the standard control drug acarbose. The present study shows that PHF4 acts by α-amylase and α-glucosidase inhibitory mechanism. It has been reported that due to hydrogen donating ability, the antioxidants may shows effect on DPPH (16). The present study suggests that PHF4 (IC_{50} 42.70µg/ml) showed radical scavenging activity by their electron transfer or hydrogen donating ability. The total phenolic and flavonoids content were found to be 172.6 mg Gallic acid equivalent/g and 135.3 mg quercetin equivalent /g of PHF4, respectively. Phenols and flavonoids may play an essential role in protecting cellular oxidative damage in many chronic diseases such as diabetes, obesity, heart related problem and ageing (17)(18) and also shows antioxidant properties. Herbal drugs containing polyphenol compounds such as flavonoids are supposed to be responsible for the radical scavenging effect. Epidemiological studies have shown that the consumption of flavonoids is effective in lowering the risk of coronary heart diseases (19). Radical scavenging activities are regarded as an important method to prevent the harmful role of free radicals in different diseases, including diabetes, cancer etc.

Conclusion

The present *in vitro* study of a poly-herbal combination of plants (PHF4) showed strong antioxidant effects. The polyherbal extract significantly reduced the alpha amylase as well as alpha glucosidase levels. The above results showed that the selected polyherbal extracts have polyphenols and flavonoids, which might be responsible for their antioxidant as well as antidiabetic effect. For further validation of above findings, *in vivo* antidiabetic study of the polyherbal formulation (PHF4) will be carried out using animal models.

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