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A Study on Quantitative Estimation of Secondary Metabolites And In Vitro Dipeptidyl Peptidase IV (DPP-IV) Inhibitory Activity of Hyptissuveolens seed Extract.

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ABSTRACT

The increasing prevalence of diabetes has driven research towards natural alternatives for managing the disease. DPP-IV (Dipeptidyl Peptidase-IV) inhibitors play a vital part in regulating blood glucose levels by prolonging the activity of incretin hormones. Natural plant extracts rich in bioactive compounds have shown promising potential as DPP-IV inhibitors. This study focuses on the quantitative analysis of phytochemicals and the evaluation of the inhibitory activity of DPP-IV of a hydroalcoholic extract.

Objective: The primary objective of this research has been to quantitatively estimate the Alkaloids, phenols, and flavonoids that make up the phytochemical content of a hydroalcoholic extract and to investigate its DPP-IV inhibitory possibilities using in vitro tests, using sitagliptin as a positive control.

Materials and Methods: The DPP-IV inhibitory activity of the extract was determined by spectrophotometric determination. To assess how effectively the extract compares to sitagliptin, percent inhibition and IC50 values were calculated. Total flavonoid, phenolic, and alkaloid contents were quantitatively measured to investigate any correlation between these compounds and the noted inhibitory activity.

Results: The hydroalcoholic extract showed to be quite potent on DPP-IV with much higher concentration than sitagliptin needed for attaining the same potency. The IC50 value for an extract was 115.38µg/mL, whereas for sitagliptin it was 44.68µg/mL. Further quantitative evaluation resulted in the finding of the following: flavonoids were found in 2.88 mg/100 mg, phenols in 1.90 mg/100 mg, and alkaloids in 1.92 mg/100 mg, thus flavonoids are in majority.

Conclusion: The results indicate that the hydroalcoholic extract possesses notable DPP-IV inhibitory activity, likely driven by its flavonoid content. These results underline the extract's potential as a natural antidiabetic drug and call for more research into its possible medicinal uses.

INTRODUCTION

DM is a chronic metabolic disorder which is characterized by high levels of glucose in the blood and is becoming a serious health issue worldwide. The incidence of diabetes along with its related complications has made alternative and natural remedies to control the disease highly accepted. Plant-derived drugs are less harmful and cause relatively fewer side effects than synthetic drugs (1).

Flavonoids, alkaloids, and terpenoids are secondary metabolites in most plant extracts, which reveal marked pharmacological activities, include anti-inflammatory, antioxidant, antimicrobial, and antidiabetic. They might activate and enhance the generation of insulin, increase the sensitivity of insulin towards blood sugar levels, and thus might be a potentially useful therapeutic agent in diabetes.

Many medicinal plants have shown antidiabetic activity, but most of the mechanisms of action remain not well understood for the majority of them (4). In vitro assays, such as those involving enzyme inhibition tests, are one useful approach to screening potential antidiabetic compounds and lead on to further insight into their mechanisms of action (5).Unlike the conventional pharmacologic therapies of diabetes such as metformin, sulfonylureas and thiazolidinediones that target different areas of glucose metabolism, DPP-IV(Dipeptidyl Peptidase-IV) inhibitors like sitagliptin and saxagliptin also protect the incretin hormones from excessive degradation which plays a crucial role in maintaining normal blood sugar levels with appropriate release of insulin (6,7). Those drugs work by prolonging the availability of two important proteins that play a critical role in the regulation of glucose: GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic peptide) (8). But even though DPP-IV inhibitors do give the desired results, they have adverse effects associated with them such as nasopharyngitis and pancreatitis (9).

Even though synthetic drugs entail various potential pitfalls, interest in the use of natural products for the treatment of diabetes has gained momentum. Phytochemicals have multiple mechanisms of action and hold promise as a promising candidate for novel antidiabetic therapies (10). For instance, natural DPP-IV inhibitors include berberine from Berberis aristata and the flavonoids from various fruits and vegetables with increased potential for improving incretin hormone activity, though their efficacy would not be as good as that of synthetic inhibitors (11). This aims to determine Hyptissuaveolens for its possible potential as an antidiabetic, particularly in the setting of its inhibitory activity on DPP-IV. Assays & quantification of the in vitro phytochemical contents of the plant are carried out to investigate the mechanism involved in action towards contributing to filling the gap in creating plant-based therapies for the management of diabetes.

Material and Methods

Plant Material:

Hyptissuaveolens: The seeds of Hyptissuaveolens utilized in this study were gathered from the local area and verified by a botanist, who is an assistant professor in the Department of Botany at SV University, Tirupati. The specimen was assigned a voucher number (0623) for future reference. The collected plant parts were dried in the shade, coarsely powdered, and filtered using a 40-mesh sieve.To stop the fine powder from absorbing moisture, it was kept in an airtight container. One hundred grams of dried powder were soaked in a 60% ethanol hydroalcoholic solution for extraction. The maceration process was conducted over 7 days to extract the phytochemicals from the plant material. To obtain a concentrated hydroalcoholic extract of *Hyptissuaveolens*, the mixture was filtered to remove the extract from the residue, as well as low-pressure evaporation of the solvent have been performed.

Quantitative Estimation:

Calculating the Total Flavonoid content:

The TFC (total flavonoid content) of the hydroalcoholic extract of Hyptissuaveolens has been measured using the aluminium chloride method, following standard procedures [12]. Briefly, 150 µL of 5% sodium nitrite ($NaNO_2$) solution has been includedin a test sample having 1mg/mL of the extract. Following a Five-minute incubation period at ambient temperature, 150µL of a 10% solution of aluminium chloride and 2mL of a 1M sodium hydroxide solution was added. After adding distilled water to reach the desired final volume of 5 mL, the mixture was allowed to rest for 15min. The absorbance have been measured with a spectrophotometer at 510nm. The flavonoid concentration was quantified in milligrams of quercetin equivalents (mg/100 mg) based on a quercetin calibration curve that was produced [13].

Calculating the Total Alkaloid Content

By employing the bromocresol green (BCG) spectrophotometric technique, The entire alkaloid concentration was quantified [14]. After dissolving a 1 mg/mL hydroalcoholic extract in 2N HCl, the mixture was filtered. After being moved to a separatory funnel, the filtrate underwent three chloroform washes. 5mL of BCG mixture together with phosphate buffer were added after 0.1N NaOH was used to bring the pH to neutral. The solution was separated using chloroform after vigorous shaking, and the absorbance have been computed at 470nm. The amount of alkaloid content has been given in milligrams per 100 milligrams (mg/100 mg) [15].

Calculating the Total Phenolic Content

Using the FolinCiocalteu technique, the TPC (total phenolic content) of Hyptissuaveolens was ascertained [16]. Ten milliliters of water that aredistilled along with one milliliter of Reagent Folin Ciocalteu has been combined with a milliliter of extract or a standard gallic acid solution (5–50 µg/mL). After five minutes, 10 milliliters of a 7.5% sodium carbonate (Na2CO3) solution were added, and distilled water have been used to adjust the ultimate volume. A UV-visible spectrophotometer have been utilized to measure the absorbance of both combinations at 760nm after it wasincubated for 30- 45 minutes at room temperature. Milligrams of gallic acid equivalents (mg/100 mg) were used to express the TPC [17].

DPP-IV Enzyme Activity Measurement

The DPP-IV inhibitory action of Hyptissuaveolens has been evaluated utilizinga screening tool for DPP-IV inhibitors [18]. Assay buffershave been used to dissolve test materials at values between 6.25 and 100 µg/mL. The assay buffer was used to dilute the DPP-IV enzyme 1:4. To the control wells, 50µL of the substrate, 10µL of the enzyme, as well as 40µL of assay buffer were added. 50µL of the substrate, 10µL of the test sample, 10µL of enzyme, and 30µL of buffer were put into test wells. For thirty minutes, the plate was incubated at 37 degrees Celsius. 25µL of 25% acetic acid has been added to halt the reaction, as well asutilizing a microplate reader, and the absorbance at 405nm has been computed. Plotting percentage inhibition against extract concentration and using non-linear regression analysis yielded IC_{50} values.

The following formula was used to get the % "inhibition:

$$\%\text{Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test Sample}}{\text{Absorbance of Control}} \times 100$$

Results

Quantitative Phytochemical Analysis of Hyptis suaveolens

Total Flavonoid Content (TFC):

The total flavonoid content of the hydro-alcoholic extract of Hyptis suaveolens was determined to be 2.88 mg/100 mg, based on the quercetin standard curve equation ($y = 0.0188x + 0.0022$, $R^2 = 0.9996$)

Total Alkaloid Content:

The alkaloid content of Hyptis suaveolens was found to be 1.92 mg/100 mg, with the atropine standard curve equation being $y = 0.0036x + 0.0238$ ($R^2 = 0.9919$).

Total Phenolic Content (TPC):

The total phenolic content of Hyptis suaveolens was calculated as 1.90 mg/100 mg, using the gallic acid standard curve ($y = 0.0189x + 0.0478$, $R^2 = 0.9981$.

Invitro DPP-IV Enzyme Inhibitory Activity

The hydro-alcoholic extract of Hyptis suaveolens demonstrated significant DPP-IV inhibitory activity, with an IC_{50} value of 115.38 µg/mL. In comparison, the IC_{50} value for sitagliptin was 44.68 µg/mL.

Table1. Total Flavonoid, Alkaloids and Phenolic contents in hydroalcholic extract of Hyptissuaveolens (HAHS) seed.

S.no.	Plant used	Plant used	Phytochemical constituents	Concentration
1	HAHS	SEED	Flavonoids	2.88 of quercetin/100gm
			Alkaloids	1.92 of atropine/100gm
			Phenols	1.90 GAE/100gm

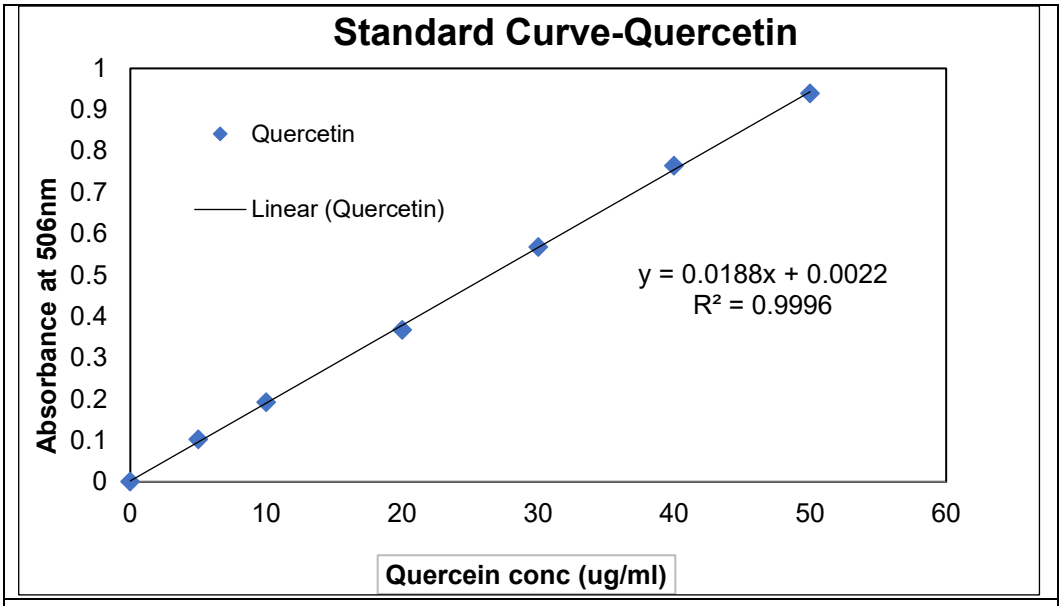

Fig 1. Calibration Curve of Quercetin for Flavonoid Determination

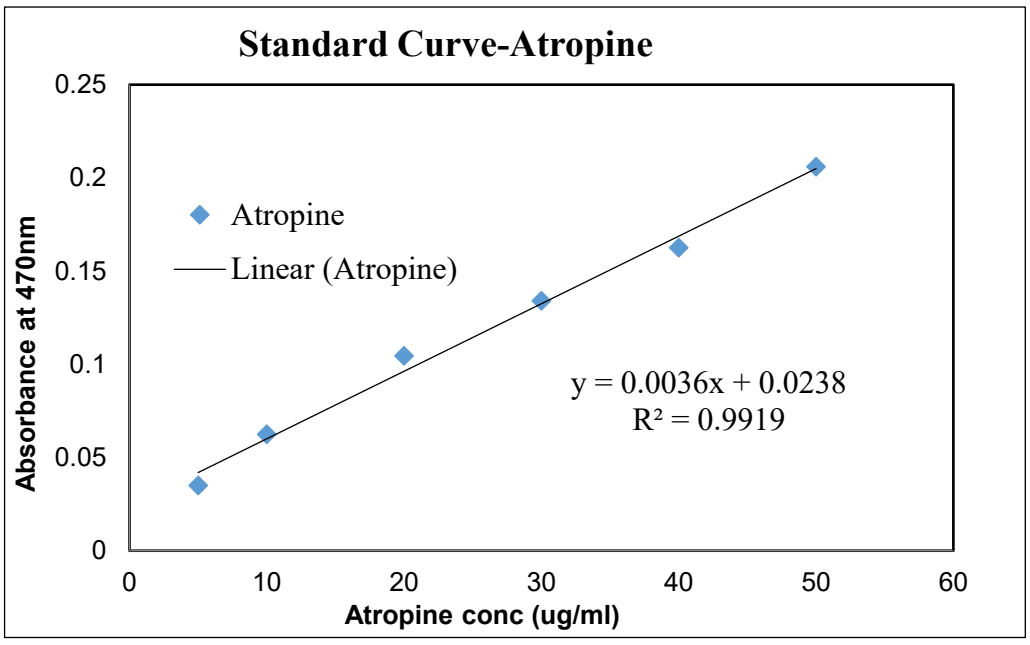

Fig 2. Atropine's Calibration Curve for Alkaloid Detection

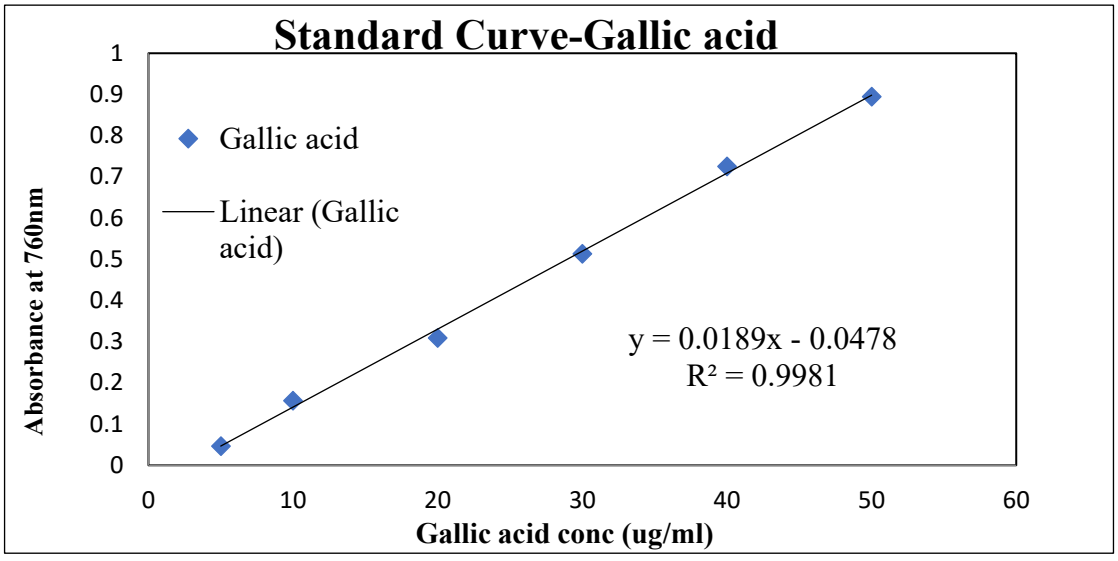

Fig 3. Gallic acid Calibration Curve for Phenol Determination

Table 2: The Comparative results of in vitro DPP-IV enzyme inhibitory activity of different Test compounds.

Test compounds	% inhibition at Conc. (ug/ml)					IC 50 (ug/ml)
	6.25	12.5	25	50	100	
Sitagliptin	12.62±0.02	24.02 ±0.01	43.88±0.01	68.54±0.00	89.58±0.01	44.68
HAHS Seed	1.06 ±0.00	2.51 ±0.00	9.44 ±0.01	22.06±0.01	42.86±0.01	115.38

n=3; the values are given as mean± SD

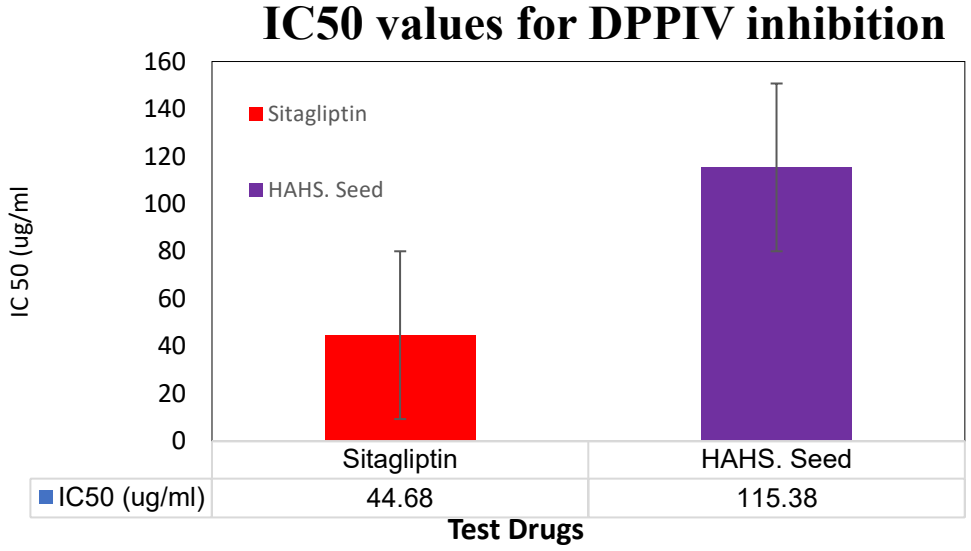

Fig 4: Comparative bar graph depicted the IC50 values in relation to Alpha DPPIV inhibition capacity of Hyptis and Sitagliptin compounds used for the current study.

DISCUSSION

The pharmacological potential of *Hyptis suaveolens* has garnered attention due to its diverse bioactive compounds. The purpose of this work was to examine the phytochemical profile as well as the DPP-IV inhibitory action of hydroalcoholic extracts obtained from *Hyptis suaveolens seeds*.

Phytochemical Content

The quantitative analysis revealed significant levels of flavonoids, alkaloids, and phenolic compounds in *Hyptissuaveolens*. It was discovered that there were 2.88 mg of flavonoids per 100 mg, indicating that this plant may contribute to the antioxidant properties commonly associated with flavonoids, such as quercetin [19]. Flavonoids are known to exhibit many health advantages, which includes anti-inflammatory and anti-oxidants that are essential for the treatment of chronic illnesses like diabetes [20].

1.92 mg/100 mg of total alkaloid concentration was found. Alkaloids are well recognized for their pharmacological activities, including antidiabetic effects [21]. The presence of alkaloids in *Hyptissuaveolens* suggests its potential as an availability of biologically active compounds that may be helpful in the management of diabetes and related metabolic disorders.

The total phenolic content was measured at 1.90 mg/100 mg, corroborating previous studies that highlight the significance of phenolic compounds in imparting various health benefits [22]. Antioxidant qualities of phenols are well recognized, and they aid in reducing oxidative stress, a condition which contributes to the development of diabetes and its complications. [23].

DPP-IV Inhibitory Activity

The DPP-IV inhibitory activity of this extract was found to be IC_{50} = 115.38 µg/mL. This concentration, although less potent than sitagliptin (IC_{50} = 44.68 µg/mL), shows that Hyptissuaveolens extracts have high inhibitory activity of this enzyme. DPP-IV inhibitors, a class of antidiabetic drugs, elevate glucose-dependent insulin production as well as decreases glucagon levels and thus plays an important role in the management of T2D (type 2 diabetes)[24]. It, therefore, appears that the observed activity of the phytochemical constituents of Hyptissuaveolens can be used further for the management of diabetes.

This DPP-IV inhibitory activity aligns with findings with other studies where other plants have been reported to possess similar effects, thus suggesting a larger therapeutic role of phytochemicals in the control of diabetes. For example, the flavonoid and phenolic contents of Hyptissuaveolens may be responsible for its DPP-IV inhibitory activity, as observed in other plants where these compounds were shown to regulate the activity of DPP-IV [25].

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