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Antidiabetic Activity; *Gossypium Herbaceum*; HPLC; Phyto Chemicals

Review Article

In Vitro Antioxidant Potentials, Antidiabetic Activities and Compounds Identification of Aqueous Extracts from *Gossypium Herbaceum* (L.) Leaf

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin production or insulin resistance. As a result, there is a growing interest in exploring natural alternatives for the treatment and prevention of diabetes. *Gossypium herbaceum* has been traditionally used in various traditional medicine systems, including Ayurveda, for its therapeutic benefits. Therefore, the aim of this study was to evaluate the *in vitro* antioxidant potentials, antidiabetic and neuroprotective properties of ethanolic extract of Gossypium herbaceum leaves as well as its HPLC-identified phytoconstituents. The results oh HPLC analysis showed the presence of thirteen bioactive compounds such as Gossypol, choline, catechin, epicatechin, gallocatechin, epigallocatechin, sitosterol, ergosterol, gossycaerulin, gossypupurin, flavan-3-ol, cyanidin, delphinidin with their heights and retention times. Also, according to the antioxidant potentials of ethanolic extract of *Gossypium herbaceum*, it revealed higher Total Phenolic Contents (TPC) than the Total Flavonoid Contents (TFC) as well as a significant ($p<0.05$) inhibitory effect against DPPH and ABTs in concentration-dependent manner. In addition, ethanolic extract of *Gossypium herbaceum* demonstrated a significant ($p<0.05$) inhibitory effect against α-amylase and α-glucosidase in concentration-dependent manner. Similarly, the extract had a significant ($p<0.05$) inhibition against Acetylcholinesterase (AChE) and Butylrylcholinesterase (BChE) in concentration-dependent manner. Therefore, finding of this study suggest that ethanolic extract of *Gossypium herbaceum* could probably have demonstrated antidiabetic and neuroprotective effects owing to the presence of bioactive phytonutrients as revealed by the HPLC technique. Furthermore, this property could be explored in the management of these afore-mentioned human diseases and beneficial pharmaceutical products.

Introduction

Gossypium herbaceum, commonly known as Levant cotton or tree cotton, is a plant species belonging to the Malvaceae family. It is native to the Indian subcontinent and has been cultivated for centuries for its fibers, which are used in the production of textiles. Besides its economic importance, *Gossypium herbaceum* has also been recognized for its medicinal properties, particularly in the management of diabetes [1]. Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin production or insulin resistance. The prevalence of diabetes has been rapidly increasing worldwide, posing a significant burden on public health. As a result, there is a growing interest in exploring natural alternatives for the treatment and prevention of diabetes. *Gossypium herbaceum* has been traditionally used in various traditional medicine systems, including Ayurveda, for its therapeutic benefits. Recent scientific studies have focused on investigating the anti-diabetic activities of *Gossypium herbaceum* and its potential role in the management of diabetes [2].

The plant contains various bioactive compounds, such as flavonoids, phenolic acids, terpenoids, and alkaloids, which are known for their antioxidant and anti-inflammatory properties. These compounds have been found to possess significant anti-diabetic effects by regulating blood glucose levels, enhancing insulin sensitivity, and protecting pancreatic beta cells. Several studies have demonstrated the hypoglycemic activity of *Gossypium herbaceum* extracts in both *in vitro* and in vivo models. These extracts have shown the ability to inhibit carbohydrate-digesting enzymes, such as α-amylase and α-glucosidase, thereby reducing the absorption of glucose from the digestive system. Additionally, *Gossypium herbaceum* extracts have been reported to stimulate glucose uptake in skeletal muscle cells and adipocytes, promoting glucose utilization and improving insulin sensitivity. Moreover, *Gossypium herbaceum* extracts have exhibited antioxidant properties, which can help mitigate oxidative stress, a contributing factor in the development and progression of diabetes complications. By reducing oxidative damage, *Gossypium herbaceum* may protect pancreatic beta cells and improve their insulin-producing function. *Gossypium herbaceum*, a plant with a rich history in textile production, possesses promising anti-diabetic activities (Abubakar et.at, 2017). The bioactive compounds present in the plant exhibit hypoglycemic effects, enhance insulin sensitivity, and protect against oxidative stress. Further research and clinical trials are necessary to elucidate the mechanisms of action and evaluate the therapeutic potential of *Gossypium herbaceum* in the management of diabetes. Harnessing the medicinal properties of this plant may offer new avenues for the development of natural and effective treatments for diabetes [3].

Materials and Methods

Chemicals and reagents used

All chemicals and reagents will be of analytical grades and prepared in all-glass apparatus using sterilized distilled water.

Preparation of Samples

Sample collection and preparation: The *Gossypium herbaceum* leave samples were obtained from a popular market place in A do-Ekiti, Ekiti State, Nigeria. The voucher sample was taken to the herbarium of Plant Science Department in Ekiti State University, Ado-Ekiti, Ekiti State, where it was authenticated. Thereafter, the sample was treated and pulverized using a laboratory blender and the fine powders obtained stored at moderate temperature until further use.

Extraction of free phenolic-rich extract

The aqueous extracts of *Gossypium herbaceum* was carried out according to the method reported by Chu et al [4] Sample (120 g) of the ground powdered samples was soaked for 48 h and filtered using Whatman no. 2. The filtrate was then evaporated using a rotary evaporator under vacuum at 45 °C until about 90% of the filtrate had been evaporated. The extracts were kept in the refrigerator for further analyses.

Phytochemical assays

Determination of total phenolic content: The total phenolic content of free /bound phenolic-rich extracts of *Gb* was determined by the method of Singleton et al. [5]. 0.2 ml of the extract was mix with 2.5 ml of 10 % folin-ciocalteau's reagent and 2 ml of 7.5 % Sodium carbonate. The reaction mixture was subsequently incubated at 45 °C for 40 min, and the absorbance was measured at 700 nm with gallic acid as standard. The result was expressed as mg gallic equivalent/ gram dry sample (mg GAE/g dry sample).

Determination of total flavonoid content

The total flavonoid content of free /bound phenolic-rich extracts of *Gossypium herbaceum* was determined using a colorimeter assay developed by Bao [6]. Extract (0.2 ml) of *Gb* was added to 0.3 ml of 5 % $NaNO_3$ at zero time. After 5 min, 0.6 ml of 10 % $AlCl_3$ was added and after 6 min, 2 ml of NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg quercetin equivalent/ gram dry sample (mg QE/g dry sample).

In vitro antioxidant activity assays

Determination of ferric reducing assay power: The reducing property of free /bound phenolic-rich extracts of *Gossypium herbaceum* was determined by method of Pulido et al. [7]. 0.25 ml of the extract was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50 °C for 20 min, thereafter 0.25 ml of 10% TCA was also added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of 1% ferric chloride and the absorbance was measured at 700 nm. The result was expressed as mg Ascorbic acid equivalent/ gram dry sample (mg AAE/g dry sample).

ABT free radical scavenging assay

ABT free radical scavenging ability of PEHc was carried out using the methods of Zhao et al. [8]. With some modifications. The sample (0.2 ml) in various concentrations was mixed with 2.0 ml diluted ABTS radical cation solution (7 mM ABTS dissolved in 0.01 M PBS, pH 7.4). The reaction mixture was left at room temperature for 20 min, absorbance was measured immediately at 734 nm in the spectrophotometer. ABTS free radical scavenging ability was subsequently calculated and expressed as % inhibition against the control with BHT as standard.

DPPH free radical scavenging ability

The free radical scavenging ability of the free /bound phenolic-rich extracts against DPPH (1,1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Gyamfi et al. [9]. Briefly, appropriate dilution of the extracts (1 mL) was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm in the spectrophotometer. The DPPH free radical inhibitory ability was subsequently calculated for each extract and expressed in % inhibition.

Quantification of phytochemicals using HPLC coupled with diode array detector

Chromatographic analysis was performed using uBandapak C18 reversed-phase column (250 mm × 4.6 mm) packed with 5-µm diameter particles; the mobile phase (carrier) was acetonitrile: water (70:30 v/v) stabilized with ethanol. This mobile phase was filtered through a 0.45-µm membrane filter (Millipore), then deaerated ultrasonically prior to use. The mobile phase flow rate was 2 ml/min at injection volume of 5 µl. Appropriate detection wavelengths were used for detection of different compounds in the PEHc. The chromatographic peaks of the analytes were thereafter confirmed by comparing their retention time (Rt) and UV spectra with those of the reference standards. All chromatographic operations were carried out at ambient temperature following the analytical protocol by [10].

Results and Discussion

Results

In vitro antidiabetic studies

The global prevalence of diabetes mellitus and neuro genetic diseases has necessitated the need for alternative approaches to diabetes mellitus management. Current treatment modalities for diabetes primarily focus on glycaemic and lipid control or lifestyle changes, these therapeutic approaches are insufficient to control oxidative stress and subsequent diabetes mellitus complications. Medicinal plants are rich sources of phytochemicals notably flavonoid and phenolic compounds which are believed to have several pharmacological benefits. In recent years, scientific exploration of these medicinal plants has shown the potential for the development of diversified diabetes mellitus treatment and management options (Negi et al., 2021). Hence, the purpose of this study was to assess the antidiabetic and antioxidative properties of these bioactive compounds in *Gossypium herbaceum* and predict their drug likeliness.

The *in vitro* antioxidant potential of the ethanolic extracts of *Gossypiums herbaceum* leaf was evaluated in Figure 1. The results demonstrated that the ethanolic extract had a higher total phenolic content. However, ethanolic extracts of *Gossypium herbaceum* leaf revealed relatively low TFC values. *Gossypium herbaceum* is a rich source of polyphenols, including hydroxybenzoic acid, caffeoylquinic acid derivatives, and cinnamic acids (Aruajo et al., 2021). However, Amado et al. (2019) found that leaf ethanolic extracts from several varieties of *Gossypium herbaceum*, including the Fortuna variety, on the other hand, contained lower levels of phenolic chemicals. Work done by Tesfaye et al. (2022) found that the flavonoid content of *Gossypium herbaceum* leaf extracts was substantially high. The ferric reducing antioxidant power, total antioxidant capacity and DPPH radical scavenging power of ethanolic extracts of *Gossypium herbaceum* leaf was evaluated (Figure 2 & 3). The results showed that *Gossypium herbaceum* leaf posses high antioxidative power with ethanoloic extract in each assay. Studies by Palot et al. (2020) show that *Gossypium herbaceum* leaf extracts exhibit potent ABTS radical scavenging activity in a concentration-dependent manner. The DPPH radical scavenging method is frequently used to diagnose free radical elimination. Non-radical DPPH-H is dissolved in alcohol, resulting in the production of DPPH, a radical source, which is subsequently effectively scavenged by antioxidant agents of one hydrogen donor. *Gossypium herbaceum* leaf extracts serve as DPPH radical scavengers and lower the colour intensity of the test solutions (Öztaşkın et al., 2016). The phenolic compounds found in *Gossypium herbaceum* extracts have DPPH scavenging and ferric ion and phosphomolybdate lowering capacities, according to the correlation between DPPH, and TAC.

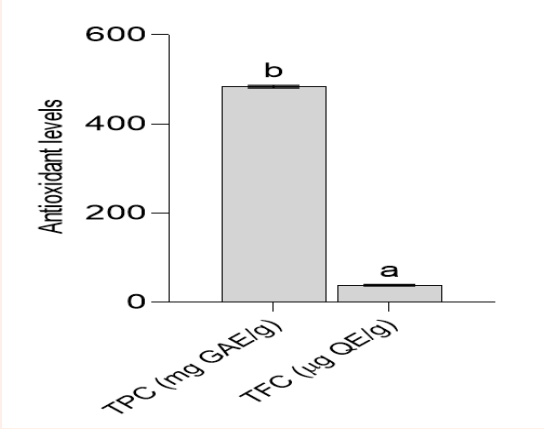

Figure 1: Total phenolic content of *Gossypium herbaceum* and total flavonoid content.

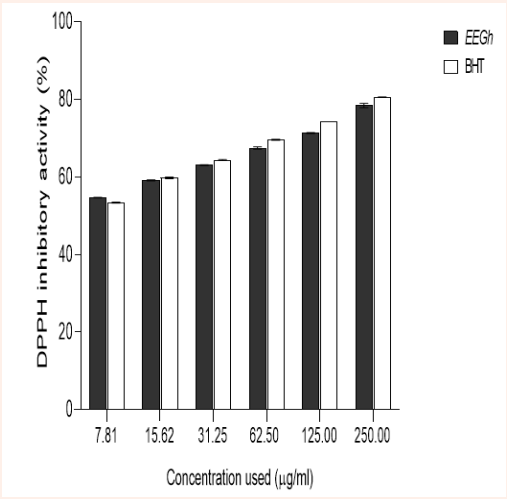

Figure 2: Inhibitory effect of EEGh against DPPHactivity. Results are expressed as mean ± SD of duplicate trials (n=2).

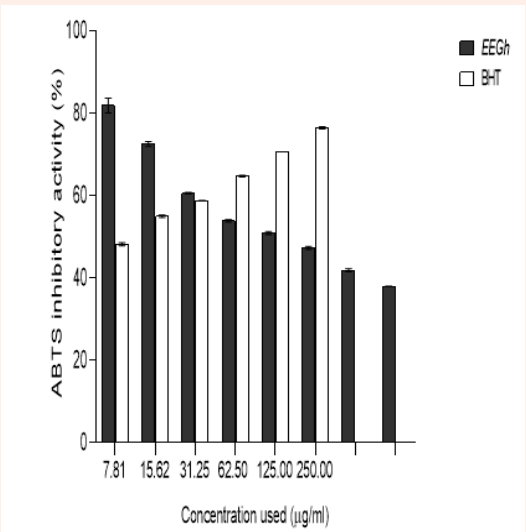

Figure 3: Inhibitory effect of EEGh against ABTS activity. Results are expressed as mean ± SD of duplicate trials (n=2).

The evaluation of the antioxidant activity of ethanolic extracts of *Gossypium herbaceum* leaf by DPH assay, Total antioxidant content showed that the ethanolic extracts had the best antioxidant activities. These results could be explained by the fact that the yield efficiency of bioactive compounds is higher for water than for ethanol because of their polarity; Water being more polar than ethanol will extract more compounds and this could also be confirmed by the results obtained from the HPLC analysis. In this study, we evaluated the *in vitro* inhibitory activities against α-amylase and α-glucosidase of ethanolic extracts of *Gossypium herbaceum* (Figure 4 & 5). The results revealed that ethanolic extracts of *Gossypium herbaceum* leaf inhibited α-amylase and α-glucosidase in a concentration-dependent pattern. However, the ethanolic extracts exhibited better inhibitory activity against α-amylase and α-glucosidase. This ability of phytochemicals presents in *Gossypium herbaceum* to inhibit α-amylase and α-glucosidase is supported by various other findings. Ajani et al. (2014) found that *Gossypium herbaceum* leaf inhibited α-amylase and α-glucosidase in a dose-dependent pattern with the peel exhibiting the highest (P< 0.05) α-amylase and α-glucosidase inhibitory potential.

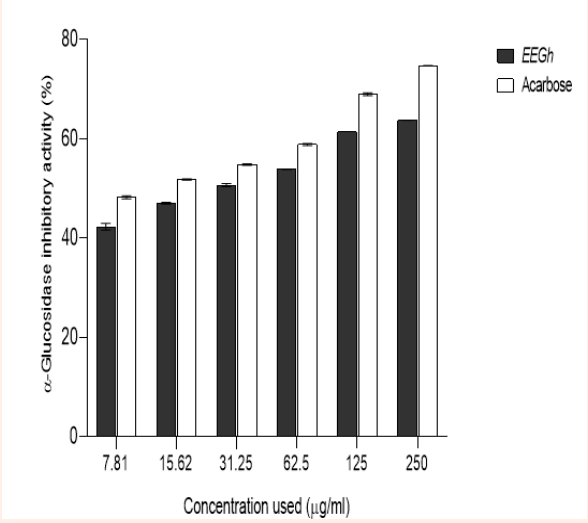

Figure 4: Inhibitory effect of EEGh against α-Glucosidase activity. Results are expressed as mean ± SD of duplicate trials (n=2).

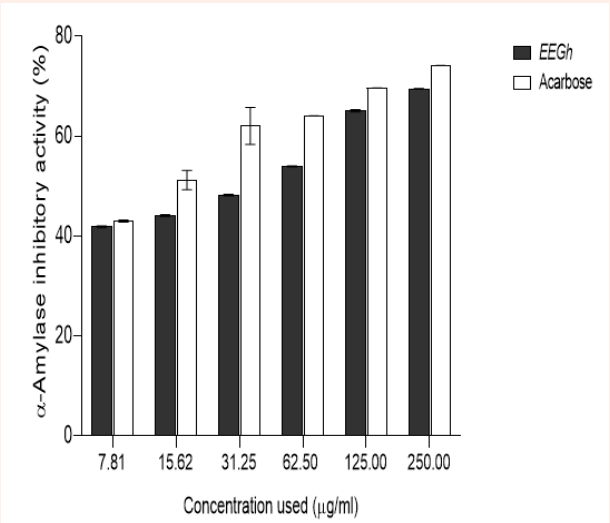

Figure 5: Inhibitory effect of EEGh against α-amylase activity. Results are expressed as mean ± SD of duplicate trials (n=2).

This result is also in support of the work done by Lawal (2022) which showed that ethanolic extract of *Gossypium herbaceum* leaf possesses significant (P < 0.05) inhibitory activity on α-glucosidase close to that of a standard drug (acarbose). Research done by Alhassan et al, (2017) revealed that α-amylase and α-glucosidase activity reduces

significantly in the presence of the ethanolic extract of *Gossypium herbaceum* leaf. The inhibition of carbohydrate digesting enzymes (α-amylase and α-glucosidase) activity, as well as delaying the digestion, and absorption of carbohydrates from the small intestinal tract is considered one of the major treatment options for Type 2 diabetes mellitus. By inhibiting these key enzymes, minimal amounts of glucose would be absorbed into the bloodstream, hence the plasma glucose will not spike after a meal.

Similarly, the importance of plant extracts has been reported in the treatment of diabetes mellitus and neurodegenerative diseases (Aruajo et al., 2021). So far, most of the active drugs currently approved for the treatment of diabetes mellitus and neurogenetic diseases are inhibitors of AChE and BChE that directly contributes to regulation and memory processes (Yan, et al., 2004). In diabetes mellitus, there is cholinergic impairment characterised by elevated activity of AChE, making it an appropriate target for diabetes mellitus and neurodegenerative diseases medication development [11]. Moreover, the inhibition of AChE plays a key role not only enhancing cholinergic transmission in the brain, as well as reducing the formation and aggregation of amyloid beta peptide in diabetes mellitus (Yan, et al., 2004). As indicated in this report, the PEHc caused a remarkable inhibition of AChE and BChE (Figure 6 & 7). It could therefore, be suggested that PEHc might possess a neuroprotective potential that are useful in the management of diabetes mellitus and neurogenetic diseases options (Negi et al., 2021), having demonstrated inhibitory effect against these enzymes [12-30]. High-Performance Liquid Chromatography (HPLC) is an analytical technique used for the separation of compounds soluble in a particular solvent. Result of the HPLC analysis on the ethanolic extracts of *Gossypium herbaceum* presented in Figure 8 & Table 1 respectively showed some characteristic peaks believed to represent the presence of about 13 bioactive compounds. The 13 bioactive compounds, their heights and retention time as well as their pharmacological properties presented in Figure 8 suggests that the extract has antioxidant, anti-inflammatory, anticancer, hepatoprotective, neuroprotective, anticonvulsant, antihyperglycemic and cytoprotective properties among others [31-50].

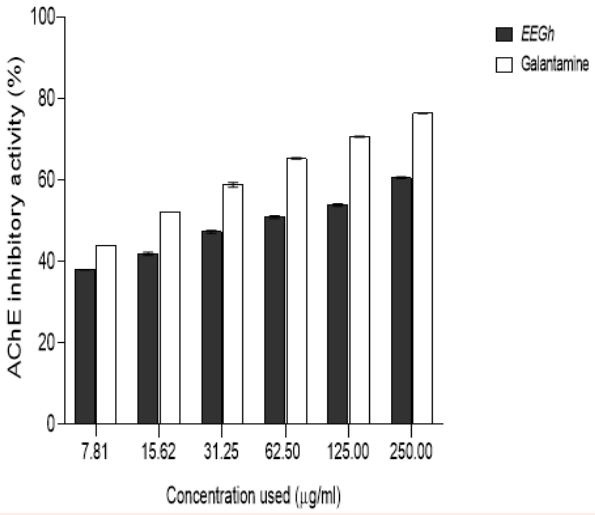

Figure 6: Inhibitory effect of EEGh against AChE activity. Results are expressed as mean ± SD of duplicate trials (n=2).

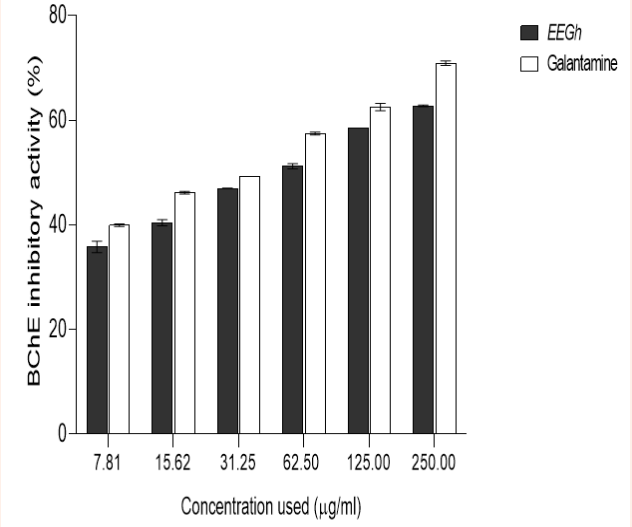

Figure 7: Inhibitory effect of EEGh against BChE activity. Results are expressed as mean ± SD of duplicate trials (n=2).

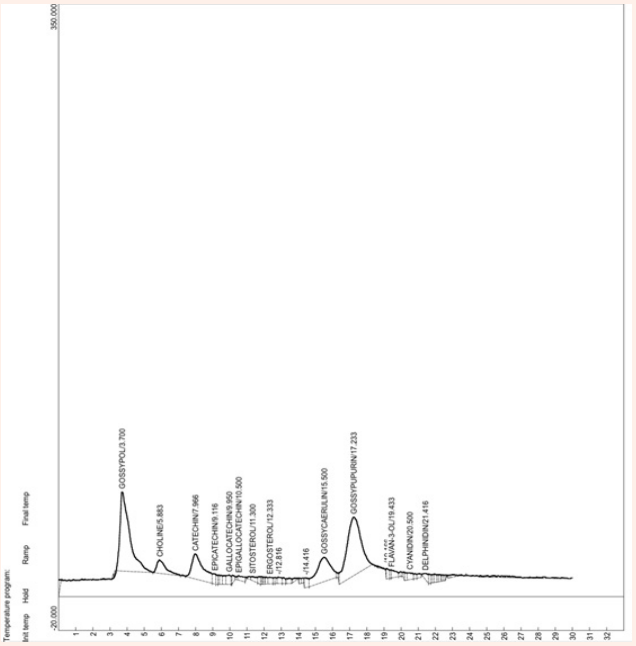

Figure 8: Chromatograms of HPLC bioactive compounds in *Gossypium herbaceum*.

Table 1: HPLC-identified bioactive compounds in *Gossypium herbaceum.*

Components name	Retention time (minutes)	Area	Height	External Units (ppm)
Gossypol	3.700	1844.297	46.677	218.0371
Chloine	5.883	295.2030	7.879	29.5203
Catechin	7.966	715.2640	14.717	0.0000
Epicatechin	9.116	75.3795	6.043	0.0000
Gallocatechin	9.950	93.1570	5.486	0.0000
Epigallocatechin	10.500	66.8940	2.516	0.0000
Stiosterol	11.300	61.5420	2.310	0.0000
s	12.333	66.6550	3.852	0.0000
Gossycaerulin	15.5	823.7040	14.456	0.0000
Gossypupurin	17.233	1982.123	34.523	0.0000
Favan-3-Ol	19.433	88.9235	4.726	0.0000
Cyanidin	20.500	85.4030	4.031	0.0000
Delphinidin	21.416	74.6095	3.614	0.0000
		6273.1045		247.5574

Conclusion

This study evaluated the neuroprotective and antidiabetic properties of the extract of *Gossypium herbaceum* leaf. The presence of various bioactive compounds was confirmed using HPLC technique with the extract indicating antioxidant potential (TPC and TFC), antidiabetic activities (α-amylase & α-glucosidase) as well as acetylcholinesterase and butylrylcholinesterase inhibition in concentration-dependent manner [51-62]. Therefore, finding of this study suggest that ethanolic extract of *Gossypium herbaceum* could probably have demonstrated antidiabetic and neuroprotective effects owing to the presence of bioactive phytonutrients as revealed by the HPLC technique. Furthermore, this property could be explored in the management of these afore-mentioned human diseases and beneficial pharmaceutical products.

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