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IN VITRO ANTIDIABETIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF *Chorispora tenella* (Pall.) DC.

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ABSTRACT

The present study was carried out to assess the various phytochemical profile and antidiabetic activity of *Chorispora tenella* seed extracts. The ethanol and aqueous extract of *Chorispora tenella* seed showed the presence of saponin, flavonoids, terpenoids, alkaloids, anthroquinone, polyphenol, glycoside and coumarins while tannin, steroids were absent. Significant amount of polyphenol and flavonoid were present in *Chorispora tenella* seed extract. This study further confirmed the presence of phytochemicals in *Chorispora tenella* seed extract using histochemical, UV visible, fluorescence. *Chorispora tenella* seed extract from natural sources as potential anti-diabetic agents by monitoring their α-amylase and α-glucosidase inhibition assay. *Chorispora tenella* seed exhibited anti-diabetic activities in dose dependent manner. Overall, it can be concluded from the present study that *Chorispora tenella* seed contains rich source of phytochemicals and anti-diabetic activities.

Keywords: *Chorispora tenella* seed, Qualitative, Quantitative, Histochemical, UV visible, Fluorescence and anti-diabetic.

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INTRODUCTION

Diabetes is a long-term illness in which the body is unable to regulate blood sugar or glucose levels because the pancreas is either not producing enough insulin or the body is not using the insulin that is produced efficiently. One of the most deadly epidemics in India and the rest of the world is diabetes. The International Diabetic Federation (South-East Asia) reports that 72,946,400 cases of diabetes were reported in India in 2017, out of 425 million people worldwide who have the disease, 82 million of whom reside in Southeast Asia. Globally, the number of diabetics is predicted to rise from 425 million in 2017 to 629 million by 2045, with India alone expected to account for the majority of this growth (Prabhakar *et al.*, 2020). Diabetes is a group of metabolic diseases in which elevated blood sugar levels last for an extended length of time because of an insulin production malfunction that impacts the metabolism of different nutrients, including proteins, fats, and carbohydrates (Bai *et al.*, 2019).

Environmental and congenital factors typically change metabolism (Zhu *et al.*, 2021). The pathophysiology of the disease indicates that in addition to other symptoms, patients may have frequent urination, thirst, and hunger. If left

untreated, serious complications like kidney, eye, foot, and other organ failure could worsen. The disease affects 4–5% of adults, and by 2025, that number is expected to increase to 5.4% (Anshika *et al.*, 2022). Due to the high number of deaths it causes, diabetes continues to be a global issue despite advancements in anti-hyperglycemic and antidiabetic medications. Although there are new therapeutic substances such as insulin and oral hypoglycemic agents, their regular use is associated with a number of negative side effects (Bailey, 2010). Diabetes is one of the many diseases that medicinal plants are useful in treating. It has been reported that more than 100 medicinal plants are used to treat diabetes in Morocco. Active substances such as terpenoids and polyphenols are responsible for the antidiabetic and antihyperglycemic effects of these plants (Bnouham *et al.*, 2002). Considering that the goal of this study is to analyze the phytochemicals and assess the *in vitro* anti-diabetic properties of *Chorispora tenella* seeds.

MATERIALS AND METHODS

Collection of plant materials

The *Chorispora tenella* seeds were gathered in January 2025 from Veerakkan in Ariyalur district, Tamil Nadu, India. After being allowed to dry at room temperature, the seeds were ground using a grinder mixture. The powder was kept for later examination.

Preparation for extract

One gram of seed powder should be added to 50 milliliters of ethanol and aqueous solvent. The extract should then shake the mixture thoroughly for 30 minutes by hand and then be left for 24 hours. Whatman filter paper No. 1 was used to filter the extracts, and the filtrate was then used for additional analysis.

Phytochemical screening

The extract was subjected to chemical tests using standard protocols to determine its constituents, as outlined by Sofowara (1993), Trease and Evans (1989), and Harborne (1973 and 1984). Edeoga *et al.* (2005) used a spectrophotometric method to determine the total phenols. Boham and Kocipai-Abyazan's (1994) method is used to determine flavonoids. Histochemical assays (Gersbach *et al.*, 2001; John Peter Paul, 2014). Assessment of plant powder's fluorescence behavior (Rao *et al.*, 2016). UV analysis of the visible spectrum. Functional groups were identified using Ahluwalia and Dhingra's (2004) methodology.

In vitro anti-diabetic activity

This *in vitro* α-amylase and α-glucosidase inhibition assay was performed using Apostolidis *et al.* (2007) methodology.

Statistical analysis

Three distinct experiments were tested in triplicate. Using MS-Windows software, a linear regression method was used to graphically determine the result. Graphical results and mean ± standard deviation were used.

RESULTS AND DISCUSSION

Natural products, like plant extracts, offer countless possibilities for the development of new drugs due to their unparalleled chemical diversity, which can be found in both pure compounds and standardized extracts (Sasidharan *et al.*, 2011). It continues to be a very valuable source for the creation of valuable chemical entities that can be used to treat some complex diseases, according to recent evidence from pharmaceutical companies (Chin *et al.*, 2006). The current investigation demonstrated the presence of medicinally active constituents in the seed extract of *Chorispora tenella. Chorispora tenella* seed phytochemical characteristics were examined and compiled in Table 1 and Figure 1. Saponin, flavonoids, terpenoids, alkaloids, anthroquinone, polyphenol, glycoside, and coumarins were detected despite the absence of tannin and steroids in the ethanol and aqueous extract of *Chorispora tenella* seed. The seed extract from *Chorispora tenella* contained a considerable amount of flavonoids (60.00±21.58) and polyphenols (235.00±38.12). According to Ranjitham *et al.* (2013), these medicinal plants may contain phenolic compounds, alkaloids, titerpenoids, steroids, and other substances that prevent the growth of different microorganisms. In addition to these, phytochemicals found in plant extracts have a broad range of applications in biological activity (Swarnalatha *et al.*, 2019).

Table 1: Qualitative analysis of *Chorispora tenella* seed extract

S. No	Phytochemicals	Aqueous extract	Ethanolic extract
1	Tannin	-	-
2	Saponin	+	+
3	Flavonoids	+	+
4	Steroids	-	-
5	Terpenoids	+	+
6	Alkaloids	+	+
7	Antroquinone	+	+
8	Polyphenol	+	+
9	Glycoside	+	+
10	Coumarins	+	+

(+) Presence and (–) Absence

Table 2: Quantitative analysis of *Chorispora tenella* seed

Secondary metabolites	Result (mg/gm)
Flavonoid	60.00 ± 21.58
Total Phenol	235.00 ±38.12

Values were expressed as Mean ± SD.

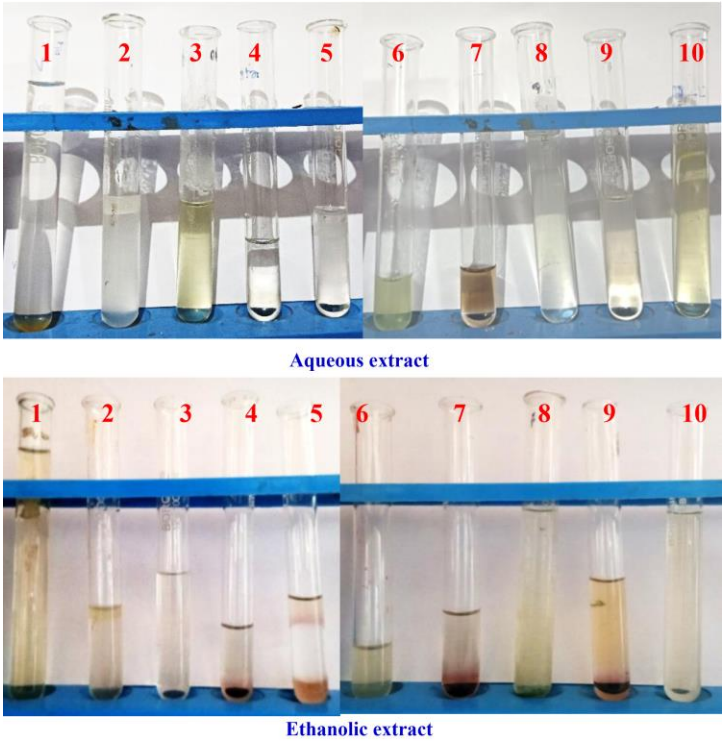

Figure 1: Qualitative analysis of *Chorispora tenella* seed extract
(1. Tannin, 2. Saponnin, 3. Flavonoids, 4. Steroids, 5.Terpenoids, 6. Alkaloids, 7. Anthroquinone, 8. Polyphenol, 9.Glycoside and 10. Coumarins)

Histochemical studies

A strong tool for locating trace amounts of substances found in biological tissues is histochemistry, the area of histology that deals with identifying the chemical components of cells and tissues. Major phytocompounds distribution and time course of deposition have been studied, as well as their structure and development, using histochemical techniques (Krishnan *et al.*, 2001). Histochemistry is just as widely used as other

markers in the solution of important biosystematic issues. Histochemical characters are now frequently used in taxonomic conclusions, according to botanical literature. Histochemical analyses of *Chorispora tenella* seed powder are shown in Table 3 and Figure 2. The phytochemical content of the *Chorispora tenella* seed extract was further validated by this investigation.

Table 3: Histochemical studies of *Chorispora tenella* seed powder

S. No.	Secondary metabolites	Result
1	Saponnin	+
2	Flavonoids	+
3	Terpenoids	++
4	Polyphenol	+
5	Glycoside	++

Note: (+) Presence; (++) present with high intensity of the colour

Figure 2: Histochemical studies of *Chorispora tenella* seed powder

Fluorescence analysis

The components can be precisely and accurately determined over a satisfactory concentration range using fluorescence analysis, which is sufficiently sensitive and eliminates the need for time-consuming dilution steps before other pharmaceutical sample analyses. Each compound has a unique fluorescent color. When treated with different chemical reagents, different plant materials produce different colors (Reddy and Chaturvedi, 2010). It serves as a pharmacognostic criterion for distinguishing and standardizing a specific medication from its counterfeit counterparts. When exposed to UV light, various crude drugs displayed varying wavelengths of fluorescence (Kavitha, 2014). This is a result of the drug's numerous chemical constituents. Following treatment with a variety of chemical and organic reagents, the fluorescence analysis of powdered *Chorispora tenella* seed material was examined under visible light, short UV light (254 nm), and long UV light (365 nm). Table 4 shows the behavior of the fluorescence. The findings show that the plant contained the fluorescence compounds (Table 4 and Figure 3).

Table 4: Fluorescence analysis of *Chorispora tenella* seed

S. No	Test	Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Ash	Ash	Black
2	Plant powder treated with distilled water	Brown	Brown	Black
3	Plant powder treated with Hexane	Ash	Ash	Black
4	Plant powder treated with Chloroform	Ash	Ash	Black
5	Plant powder treated with Methanol	Ash	Ash	Black
6	Plant powder treated with Acetone	Ash	Ash	Black
7	Plant powder treated with 1N Sodium Hydroxide	Brown	Black	Black
8	Plant powder treated with 1N HCL	Ash	Black	Black
9	Plant powder treated with sulphuric acid with equal volume of water	Black	Brown	Black
10	Plant powder treated with HNO_3 diluted with an equal volume of water	Yellow	Light green	Black

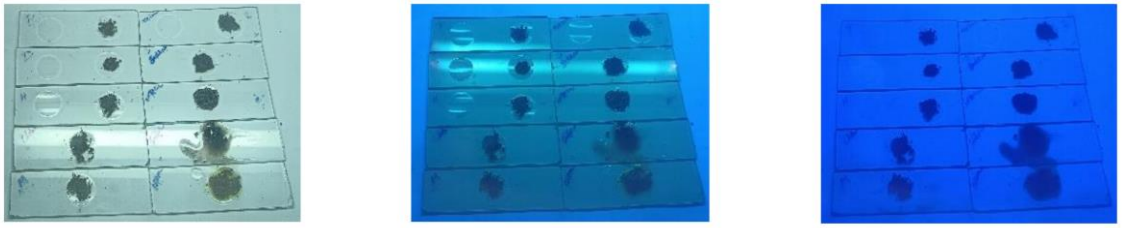

Figure 3: Fluorescence analysis of *Chorispora tenella* seed

UV-visible spectrum of *Chorispora tenella* seed extract

The use of spectroscopy in phytochemical analysis has grown in effectiveness and dependability. Photon spectroscopy in the UV-visible range is associated with ultraviolet-visible spectrophotometry (UV-Vis). This method makes use of light that falls within the electromagnetic spectrum's visible ranges. Absorption is influenced by the color of the chemicals involved, and in these ranges, molecules undergo electron transitions. The *Bauhinia variegate* stem was examined under visible UV-Visible spectrum. The sample was scanned in the wavelength ranging from 340-800nm. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded. The UV spectrum profile showed the peaks at 370, 400 and 640 nm and identified phytochemicals are Flavonoids, alkaloids, phenolic, terpenoids and chlorophyll respectively (Table 5). Figure 4 shows the absorption spectrum of *Bauhinia variegate* stem extract and these are almost transparent in the wavelength region of 340-800 nm (Mabasa *et al.*, 2021). Previous research indicates that these absorption bands are typical of phytochemicals such as phenols, alkaloids, flavonoids, saponins, and terpenoids (Kalaichelvi and Dhivya, 2017).

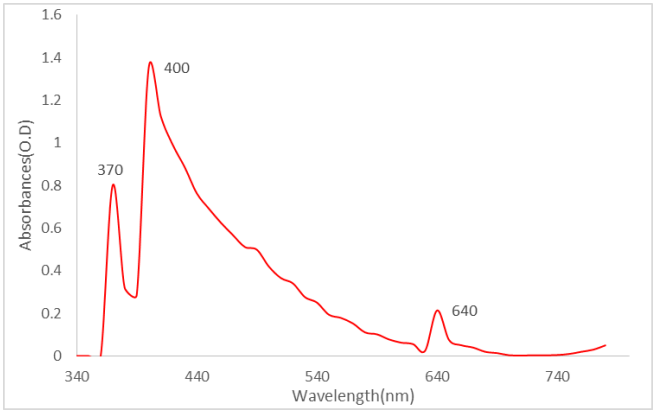

Figure 4: UV visible spectrum analysis *Chorispora tenella* seed extract

Table 5: UV visible spectrum analysis *Chorispora tenella* seed extract

S. No.	Absorption maxima (Wavelength ranges) nm	Phytochemical compounds (metabolites)
1.	370	Flavonoids, alkaloids and phenolic
2.	400	Terpenoids
3.	640	Chlorophyll

Qualitative functional group analysis of *Chorispora tenella* seed

The group of atoms or bonds that give a substance its distinct chemical reactions is known as a functional group. The functional

groups of a molecule are identified through qualitative functional groups analysis. The purpose of this study was to qualitatively screen for functional groups in the plant seed extract. The alcohol, phenol, aliphatic amines, aldehyde, ketone, carboxylic acid are presence in *Chorispora tenella* seed indicating the phytochemicals (Table 6 Figure 5).

Table 6: Functional group analysis of *Chorispora tenella* seed

S. No	Functional group	Results
1	Alcohol	++
2	Phenol	++
3	Aliphatic amines	++
4	Aldehyde	++
5	Ketone	++
6	Carboxylic acid	++

(+) Presence; (++) present with high intensity of the colour

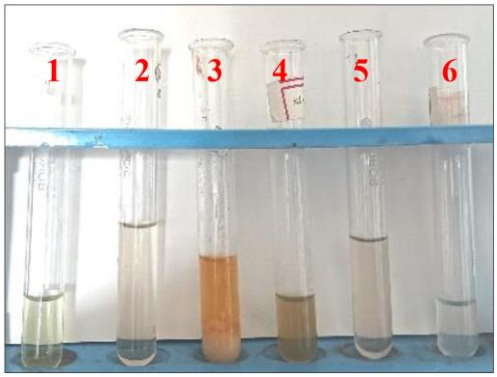

Figure 5: Functional group analysis of *Chorispora tenella* seed

***In vitro* anti-diabetic activity**

The inhibitory activity and α-amylase/α-glucosidase levels of the standard medications have been compared. Our findings are consistent with a prior study from Sincy Joseph *et al.* (2016) that found a positive correlation between the total polyphenol and flavonoid content and the capacity to inhibit pancreatic α-amylase. By inhibiting the enzymes a-amylase and a-glucosidase in vitro, the current study aimed to assess the possible antidiabetic effects of *Chorispora tenella* seed extract. *Chorispora tenella* seed extract was found to have the best inhibitory effect based on the *in vitro* a-amylase and a-glucosidase results shown in Fig. 6, 7 and Table 7, 8. The seeds of *Chorispora tenella* showed dose-dependent anti-diabetic effects. As reported by ɘtefănescu *et al.* (2022), ethanolic extract was found to exert the best antidiabetic activity in another species (*Quercus robur*) from the same family.

Table 7: *In vitro* anti-diabetic activity (Alpha amylase) of *Chorispora tenella* seed extract

Concentration (µg/ml)	% of inhibitions	
	Chorispora tenella	**Std. (Acarbose)**
100	16.90±0.08	21.28±0.10
200	33.50±0.12	42.32±0.15
300	61.00±0.34	62.78±0.36
400	66.40±0.37	79.45±0.40
500	88.00±0.51	92.27±0.58
IC_{50} (µg/ml)	281.95	246.30

Values are expressed as mean ± SD for triplicates

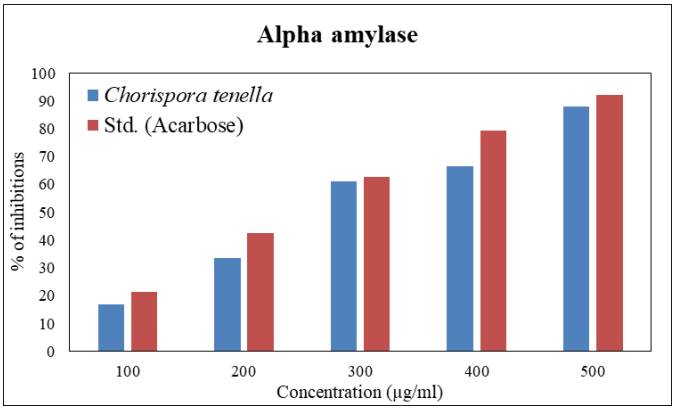

Figure 6: *In vitro* ant-diabetic activity (Alpha amylase) of *Chorispora tenella* seed extract

Table 8: *In vitro* anti-diabetic activity (Alpha-glucosidase) of *Chorispora tenella* seed extract

Concentration (µg/ml)	% of inhibitions	
	***Chorispora tenella* seed**	**Std. (Acarbose)**
100	10.70±0.05	19.57±0.12
200	23.90±0.16	40.12±0.27
300	61.80±0.22	63.78±0.26
400	80.80±0.38	81.75±0.42
500	94.70±0.53	95.24±0.57
IC_{50} (µg/ml)	280.52	247.66

Values are expressed as mean ± SD for triplicates

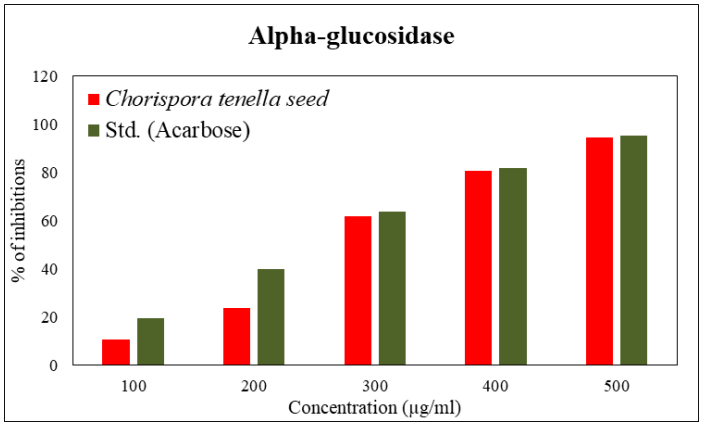

Figure 7: *In vitro* ant-diabetic activity (Alpha-glucosidase) of *Chorispora tenella* seed extract

The α-amylase enzyme was inhibited *in vitro* to test the isolated compounds' antidiabetic potential. The antidiabetic potential of phenol, flavonoids, saponins, and terpenoids was confirmed by their higher alpha amylase inhibitory activity (Mai *et al.*, 2007).

CONCLUSION

The findings of this study suggest that the seeds of *Chorispora tenella* contain several phytocompounds that could be ideal for a variety of therapeutic uses. Overall, it can be said that the extract from *Chorispora tenella* seeds has a wealth of phytochemicals and has been shown to have antidiabetic properties.

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