Chamaecostus cuspidatus (Nees & Mart.) ethanolic extract: GC-MS analysis and characterization by FTIR and UV

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***Abstract*: This study deals with the phytochemical screening of ethanol leaf extract of Chamaecostus cuspidatus followed by GC-MS analysis. It explores the natural compounds present in the leaf extract. The same extract was utilized to analyze the UV-Vis analysis and FTIR of different wavenumbers of varying compounds. Phytochemical analysis of ethanol leaf extract shows a majority of secondary metabolites like phenols, alkaloids, terpenoids, steroids, tannins and saponins. GC-MS analysis is used to detect the presence of different natural compounds present in the extract. GC-MS analysis detects various compounds, while FTIR detects distinct functional groups. This study shows that chamaecostus cuspidatus methanolic leaf extract could be a good source of bioactive components with antidiabetic and antioxidant properties.**

***Keywords*: Chamaecostus cuspidatus, Phytochemical, FTIR, UV -vis analysis, GC-MS analysis**

I. Introduction

One in ten people, including children, has diabetes and hyperthyroidism in today's world. Many drugs have been technologically advanced to treat various disorders, which can also be cured with botanicals. Nature contains a thriving source of medicinal properties worldwide, and many alternative modern drugs are isolated from natural environmental sources. Many selected medicinal plants are used in daily life and treat the disease in humans. In ancient times, plants were the most valuable natural products that maintain human health; new selected studies for natural therapies are modern. In recent days the use of phytochemicals for pharmaceutical uses has steadily increased in many countries

C. cuspidatus is a South and Central American plant known as flaming costus, Step ladder, Spiral flag, or Insulin plant. It is a novelty to India from America as a herbal diabetic treatment known as the "insulin plant" (Hegde et al., 2014). It is recognized that diabetic persons consume one leaf every day to maintain their blood glucose low, and it is used to control diabetes in India. Tonic, stimulant, carminative, diuretic, digestive, and antibacterial qualities are all used to describe these plants (Rani et al., 2013). The leaves of *C. cuspidatus* can improve renal functions and possess good antioxidant, anti-inflammatory and hypoglycemic properties (Nakkala et al., 2015). *C. cuspidatus* leaves were demonstrated to be high in protein, iron, and antioxidant components such as ascorbic acid, tocopherol, carotene, terpenoids, steroids, and flavonoids phytochemical screening.

Diabetes mellitus (DM) is the most common endocrine disease worldwide. There are numerous diabetes mellitus, one of which is noninsulin-dependent diabetes mellitus (type 2 DM), the most common, afflicting 90–95 percent of the population. This rising trend in type 2 diabetes has become a severe medical concern worldwide, prompting all efforts to find novel therapeutic agents to slow its progression (Elya et al., 2012). Diabetes affects 77 million adults in India, which is anticipated to nearly double 134 million by 2045(Luhar et al., 2020). The purpose of this study was to study the phytochemicals of the standard leaf extracts of *C. cuspidatus*, GC-MS analysis of the leaves extracts, UV vis spectroscopy and FTRI of *C. cuspidatus*.

II. Plant collection and processing

The fresh leaves of *C.cuspidatus* were collected from Tulasi nursery, Hyderabad, in January 2020. The plant has been authenticated by the Herbarium facility of the Department of Botany, Osmania University, Hyderabad. The leaves were collected, shade dried and then pulverized into a dry powder using an electric blender. Until needed, the powdered leaf samples were kept in an airtight container.

III. Extract preparation

The air-dried leaf of *C. cuspidatus* (500 g) was macerated with 2.5 L methanol and extracted by cold maceration for 48 hours at room temperature with agitation. The liquid extract was filtered and the filtrate concentrated under reduced temperature (40EC) using a rotary evaporator to yield the dry extract.

IV. Phytochemical analysis (Method adopted by Srinivas R. et al., 2014)

a) Test for tannins

In 2ml of distilled water, a few drops of ferric chloride solution were added to roughly 2ml of extract. The appearance of green-tinted precipitate confirms the presence of tannins.

b) Test for saponins

In a test tube, 3ml of the extract was mixed with 3ml of distilled water and vigorously shaken for a few moments. The test tube was heated, and creating a stable foam indicates the presence of saponins.

c) Test for flavonoids

1ml of 10 percent lead acetate solution was added to 1ml of extract in a test tube. The appearance of the yellow precipitate can determine the presence of flavonoids.

d) Test for Alkaloids

On a hot water bath, 3ml of the extract was mixed with 3ml of 1% HCL. Then 1ml of the liquid was split between two test tubes. 1 mL of Dragendroff's reagent was added to the first test tube. The presence of orange-red precipitate was regarded as a good sign. 1ml of Mayer's reagent was added to the second test tube. The formation of a buff-coloured precipitate can confirm the presence of alkaloids.

e) Test for terpenoids

2 mL extract was dissolved in 2 mL $CHCl_3$ and evaporated to dryness. After that, 2 mL of concentrated sulphuric acid (H2SO4) was added and heated for about 2 minutes. The emergence of a greyish colour indicates the presence of terpenoids.

f) Test for steroids

Two tests were carried out to perceive the presence of steroids in the extract, and those were the Salkowski's test and the Liebermann test. Salkowski's test**:** 2ml of the organic extract was dissolved in 2ml of chloroform. 2 mL concentrated H_2So_4 was added to this. The presence of steroids is shown by the appearance of red colour in the chloroform portion.

g) Test for Phenols

5ml distilled water was added to 1gm of extract, and the solution was then treated with a 5% ferric chloride solution. The presence of phenols is confirmed by forming a dark green colour.

V. GC-MS Analysis

The *C. cuspidatus* igneous extracts were GC-MS analyzed using a GCMS-QP2010 SHIMADZU, Japan, fused with an Optima 5 ms capillary column (30 0.25 mm) 0.25 m film thickness, as described by Iheagwamet al (2019). Initial column oven temperature (60°C) planned to climb to 160°C at a rate of 10°C/min, then to 250°C with a hold duration of 2 min/increment. An injection volume of 1.0 L in the splitless mode with a split ratio of 1:1 and injector temperature set at 200° C with minor adjustments, The following were the mass spectrophotometer settings: 230°C for the ion source, 250°C for the interface, 4.5 minutes for the solvent delay, and 50–700 amu for the scan range. The multiplier voltage and electron ionization mode were modified to 70 eV and 1859 V. For compound identification, the retention time, fragmentation pattern, and mass spectral data of unknown components in the extracts equated to those in the Wiley and National Institute of Standards and Technology (NIST) libraries (Iheagwam et al.,2019).

VI. UV-Vis Spectroscopy

The Ethanol extract solutions were monitored by measuring the UV–vis spectrum in UV-Vis NIR Cary 300 spectrophotometer of the reaction medium at room temperature operated at a resolution of 1 nm between 200 and 700 nm ranges (Muthukrishnan et al. 119). The UV-Visible spectrophotometric analysis is carried on the ethanolic leaf extract of *Chamaecostus cuspidatus* using the UV-Visible spectrophotometer (UV-1800 Series) with a slit width of 1.0 nm. The examination was done under UV and Visible light in the wavelength ranging between 190.00 to 1100.00 nm.

The extract is first subjected to centrifugation at 3000 rpm for approximately 10 minutes and then filtered through Whatman filter paper No.1. It is followed by diluting the sample with the same solvent (ethanol) in the ratio of 1:10.

VII. FTIR Analysis

The characterization of the plant extract and the resulting silver nanoparticles was analyzed in FTIR. For FTIR, the ethanol extract was centrifuged at 12,000 rpm for 30 min. The pellet was washed three times with 25 ml of de-ionized water to eliminate the free proteins or enzymes (Muthukrishnan et al. 123). The particles were dried, ground with KBr, and analyzed (Perkin Elmer).

VIII. Results

i. Phytochemical analysis

The qualitative chemical tests for the extracts were performed using standard procedures. The Phytochemical composition of Chamaecostus cuspidatus in leaf is stated in Table 1. The investigation showed that Ethanolic leaf extract contains tannins, flavonoids, Steroids and Alkaloids, phenols, whereas Silver nanoparticles leaf extract contains tannins, saponins, alkaloids and steroids. However, saponins and terpenoids are absent in ethanol extract, silver nanoparticles extract, flavonoids, terpenoids, and phenols.

Table 1 Result pf Phytochemical analysis

Test	Result (In Ethanol)
Test for tannins	Present
Test for saponins	Absent
Test for flavonoids	Present
Test for alkaloids	Present
Test for terpenoids	Absent
Test for steroids	Present
Test for phenol	Present

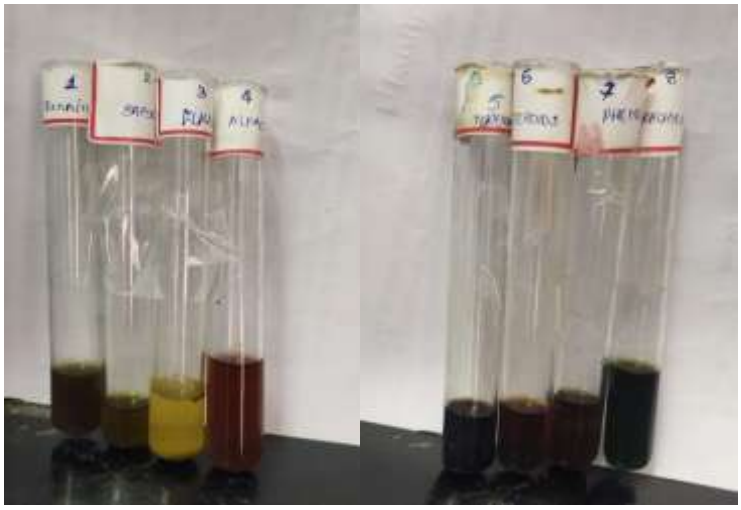

Fig.1 Ethanol extract result

ii. GCMS Analysis

Identification of possible chemical ingredients may necessitate qualitative examination of chemical substances. A gas chromatogram paired with mass spectroscopy is a must-have tool for these types of analyses. It was characterized by a mass spectrum and offered qualitative chemical ingredients. Twelve significant medicinal components were discovered through GC-MS analysis from *C. cuspidatus* leaf extract (Fig.4). The name of chemical components, molecular weight, molecular formula and their potential activities are shown in Table II**.**

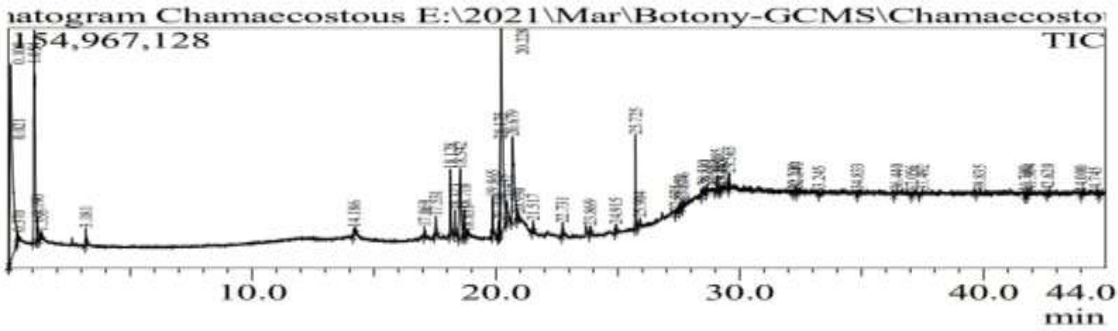

Figure 2 Gas Chromatography-Mass Spectrum of leaves extract of *Chamaecostus cuspidatus*

iii. FTIR Analysis

Based on the peaks values in the IR radiation region, the FTIR spectrum was applied to categorize the functional groups of the active components present in the extract. The functional groups of components were separated based on the peak ratio when the extract passed through FTIR. The characterization of the plant extract and the resulting ethanol extract was analyzed in FTIR Fig.5. The absorbance bands analysis in bioreduction are observed in the 400–4000 cm−1 are 1629.9, 2856.7, 3389.04 cm−1. Significant peaks were observed at 2231.71 cm−1 that could be assigned to nitrile groups' C N stretching vibrations (Sigmaaldrich, 2021). 3531.78 cm−1 – OH Stretch, 3389cm−1 – primary amine NH Stretch, 3026 cm−1 – Alkenes, and 1745.64 cm−1 might be contributed by the ester groups of the polysaccharides in the aqueous extract of the plant.

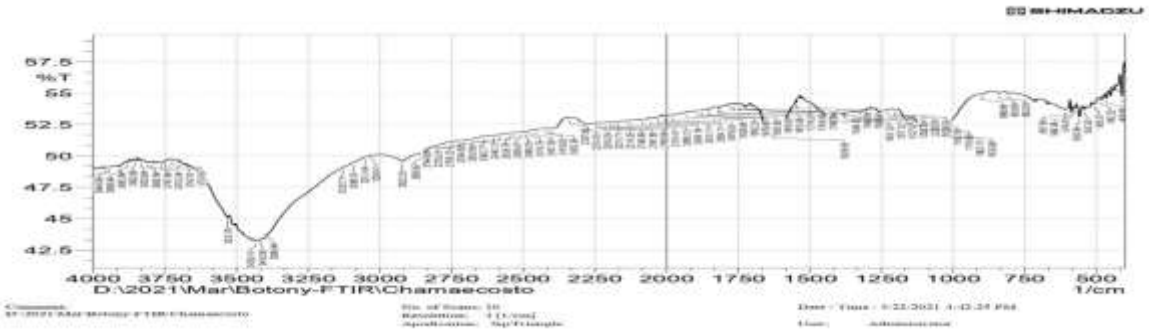

Figure 3 FTIR analysis Graph

Table II Phytocomponents identified by GC-MS analysis

S.No	Name	Retention Time	Area%	Molecular Formula	M.Wt	Compound
	2-Nonynoic acid	0.020	1.33	$C_9 H_{14} O_2$	154	Fatty acid
	Carbamodithioic acid	0.370	0.40	$C_8 H_9 NS_2$	183	Organosulfur compounds
	Tetraborane	1.055	12.51	B4H10	54	Hydride compounds
	Cyclohexanone	3.181	0.61	$C_6 H_{100}$	98	Organic compound (Ketone)
	9-Hexadecanoic acid	18.130	17.32	$C_{17} H_{34} O_2$	270	Fatty acid
	11,14-Eicosanoic acid	20.175	2.38	$C_{21} H_{38} O_2$	322	Fatty acid
	octadecanoic acid	20.109	0.42	$C_{19}H_{38}O_2$	296	Fatty acid
	1,2-Benzenedicarboxylic acid	0.100	24.90	$C_{20} H_{30} O_4$	334	Phthalic acid
	9-Hexadecenoic acid	20.228	17.32	$C_{16} H_{30} O_2$	254	Fatty acid
	Tetra decanoic acid	20.452	1.50%	$C_{14} H_{28} O_2$	228	Fatty Acid
	Diosgenin acetate	27.552	0.41	$C_{29} H_{44} O_4$	456	Saponin
	d-Xylitol	42.620	0.33	$C_{15} H_{22} O_{10}$	362	Carbohydrate
	Cyclotrisiloxane	37.056	0.30	$C_6H_{18}O_3Si_3$	222	Silicon
	1-Benzopyrylium	41.904	0.37	$C_{15}H_{11}O$	207	Phenol
	Benzoic acid	27.375	0.26	$C_{14} H_{24} O_3 Si_2$	296	Carboxylic acid

Table III Compounds analyzed through FTIR analysis

Wavenumber (cm^{-1})	Possible compounds
3531.78-3389.04	Alcohols, Phenols (O-H)
3389.04	Aliphatic primary amines (N-H)
3126.71	Amines
3126.71-2509.47	Carboxylic acid
3026.41	Alkenes (C = C-H)
2856.7	Alkanes (C-H)
2131.41	Alkynes
2214.35	Nitrile
1745.64	Ester (RCOOR)
1629.9	Aromatics

iv. UV analysis

Due to the clarity of the peaks and suitable baseline, the qualitative UV-VIS spectrum profile of ethanol extract of *Chamaecostus cuspidatus* was preferred at a wavelength range of 190 to 1100 nm. The profile showed the peaks at 222.20, 221.70 and 266.20 nm with the absorption of 4.000, 3.954, and 2.143, respectively.

Table IV UV readings

Wavelength	Absorption sp
1097.90	0.052
673.90	0.186
305.90	1.620
295.10	1.640
267.70	2.133
266.20	2.143
264.40	2.137
258.00	2.082
256.30	2.072
222.20	4.000
963.70	0.046
641.40	0.145
305.40	1.613
294.70	1.639
267.30	2.128

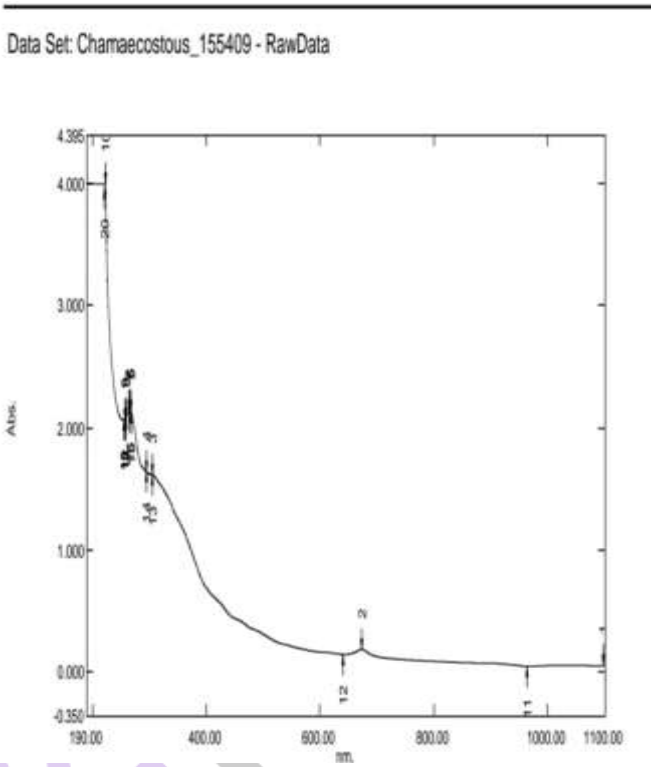

Fig 4 UV Graph

IX. Discussion

Diabetes mellitus is a metabolic disorder, a life-threatening disease increasing day by day. Natural chemical constituents reported by GCMS analysis demonstrated potential glucose reduction capacity. Many researchers identified various therapeutic compounds through GC-MS (Sahaya Sathish, Janakiraman, Johnson, 2012; Gopinath et al., 2013). Such compounds possess antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic and anticancer activities. According to prior research, n-hexadecanoic acid is an essential chemical ingredient with antioxidant, hypocholesterolemic, nematicide, insecticide, and lubricating properties. In the research, Jesus et al. (2016) Diosgenin, a steroid saponin present in various plant species, has been identified as a promising bioactive biomolecule. It includes a few medical qualities, like hypolipidemic, hypoglycaemic, antioxidant, anti-inflammatory, and antiproliferative effects. Tetradecanoic acid is a straight-chain, fourteen-carbon, long-chain saturated fatty acid primarily found in milk fat. The earlier study has shown the presence of phytochemical components in methanol extract, and the researchers are progressing to work on synthesizing secondary metabolites. This study shows the existence of secondary metabolites such as saturated and unsaturated fatty acids, saponins, and phenols. This research also focuses on the characterization of *Chamaecostus cuspidatus* methanolic extract using FTIR and UV analysis, which depicts the distinct functional groups present in the extract and the presence of likely compounds based on the wavenumber.

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