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PHYTO CHEMICAL INVESTIGATION, OPTICAL CHARACTERIZATION AND ANTI-DIABETIC STUDIES OF SENNA AURICULATA (AVARAM POO)

Physics

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ABSTRACT

Cassia auriculata Linn., commonly called Tanner's Cassia in English and in Tamil as "Avarai". C. auriculata is an evergreen shrub that has attractive yellow flowers grows in many parts of India and in other parts of Asia. The major chemical groups in the ethanol fractions of Cassia auriculata flower extract and their structures were elucidated, on the basis of GC-MS data. The GC-MS spectrum reveals the presence of nine bioactive compounds. The FT-IR and UV spectrum of the extract exhibits the functional groups and the characteristic absorption of the ethanol extract Cassia auriculata flower. The anti-diabetic capability of the Senna auriculata flower extract was measured using DNS assay and found to exhibits significant activity against diabetic disorder.

KEYWORDS

Cassia auriculata, GC-MS, Phytochemicals, Hyperglycemia and anti-diabetic activity

INTRODUCTION

According to the World Health Organization (WHO), in the world there are over 347 million people suffering from diabetes mellitus (DM). Projections indicate that by 2030 diabetes mellitus will become the seventh leading cause of death worldwide, and type 2 DM (T2DM) will be the responsible for 90% of cases [1]. Diabetes mellitus is basically a metabolic disorder associated with excess accumulation of glucose in blood. The two main forms of diabetes are type 1 or insulin dependent diabetes mellitus (IDDM) and type 2 diabetes or non-insulin-dependent diabetes mellitus (NIDDM) [2]. Both types are characterized by progressive β-cell failure. Medicinal plants are nontoxic and easily affordable so it plays a vital role not only in pharmacological research and drug development, but also used directly as therapeutic agents and a starting materials for the synthesis of drugs [3]. Traditional herbal medicines are generally considered to be safer than synthetic drugs. Over 50% of all modern clinical drugs are natural products that play a vital role in drug development in pharmaceutical industries. Cassia auriculata Linn., commonly called Tanner's Cassia in English and in Tamil as "Avarai". C. Auriculata [4] (family: Cesalpinaceae) is an evergreen shrub that has attractive yellow flowers grows in many parts of India and in other parts of Asia. This plant reported for its uses in the Ayurvedic and Siddha system of medicine for treating various diseases. Its medicinal properties have been extensively studied. The flower, leaves, stem, root, and unripe fruit are profoundly used in Ayurvedic medicine as a remedy for diabetes. The flower extract articulate its anti-diabetic effects by regulating antioxidant effects by regulating antioxidants levels in plasma, liver and pancreas

Cassia auriculata L. (Family: Caesalpiniaceae) is a shrub with large bright yellow flowers which is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant has been reported to possess antipyretic [5], hepatoprotective [6], antidiabetic, antiperoxidative and antihyperglyceamic [7] and microbicidal activity [8]. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation [9]. They are one of the constituent of polyherbal formulation 'Diasulin' in the concentration range of 40 mg/dl which is proven to have anti-diabetic activity [10]. It is also one of the major components of a beverage called "kalpa herbal tea" which has been widely consumed by people suffering from diabetes mellitus, and mixture called "avaraipanchagachoornam" which is prepared from dried and powdered plant parts and commonly used for opthalmia, conjunctivitis, diabetes, and urinary infections. Some published preliminary work describing the phytochemical content of the plant, a systematic activity-directed isolation study on C. auriculata has not yet been carried out. .Among the phenolic constituents, the pale yellow and poorly soluble substances such as flavonol, group of flavonoids are widely present in 80% of higher plants.

The plants contain preliminary phytochemical constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, saponins, proteins, carbohydrates and anthraquinone derivatives and, these are responsible for the pharmacological activity. The flower extract of Cassia auriculata has significant effect against diabetic rats and reduces the blood glucose and lipid level drastically in the diabetic rats [11]. The disease is estimated to affect 4-5% of the population and patients are generally diabetes type 1 or type 2. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components, volatile essential oil, fatty acids and lipids [12, 13]. There is a lack of scientific data on the quantification and identification of chemical composition in C.auriculata. The present investigation was designed to evaluate the phytochemical contents (GC-MS), Optical characterization (FT-IR and UV) and anti-diabetic (on 3T3 cell lines) properties of ethanolic extract of Cassia auriculata flowers.

CLASSIFICATION

FIG.1 CASSIA AURICULATA

Kingdom : Plantae
Order : Fabales
Family : Fabaceae
Sub family : Caesalpinioideae
Tribe : Cassiea
Genous : Cassia
Species : C. auriculata

RESULTS AND DISCUSSION

GC-MS- PHYTOCHEMICAL INVESTIGATION

The GC/MS spectra of the extract were obtained using the JEOL GCMATE II GC: Maximum resolution: 6000 and maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI) and Chemical ionization (CI).GC-MS is combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyse complex organic and biochemical mixtures. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according to their mass-to-charge ratio (m/z). The name and structure of the components of the test materials were ascertained. Table 1 and Fig.3.1 to 3.10, shows the phytochemical constituents that have been identified from various fractions of Senna auriculata flower extract by GC-MS spectral analysis.

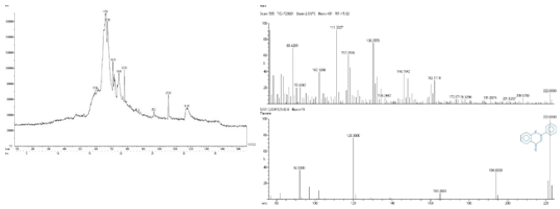

Fig 3.1:GC-MS with ethanol extract of cassia auriculata(L)
Fig3. 2: Mass spectrum of flavones avaram flower

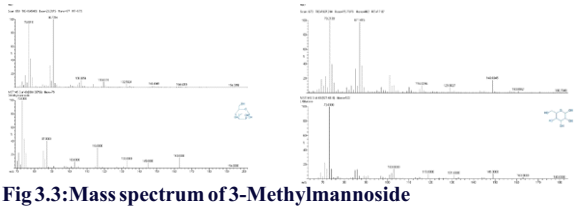

Fig 3.3:Mass spectrum of 3-Methylmannoside
Fig3.4: Mass spectrum of L-Glucose

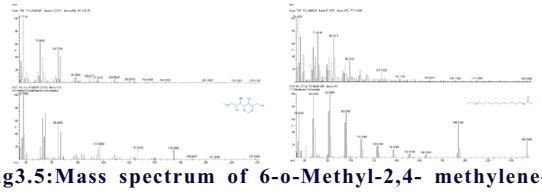

Fig3.5:Mass spectrum of 6-o-Methyl-2,4- methylene-a-sedoheptitol
Fig3.6: Mass spectrum of 11-Tetradecen-ol acetate

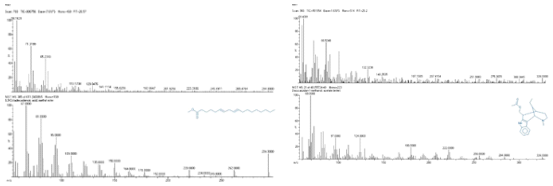

Fig 3.7: Mass spectrum of6,9-octadecadienoic acid, Methyl ester
Fig3.8: Mass spectrum of Dasycarpidan-1-methanol, acetate(ester)

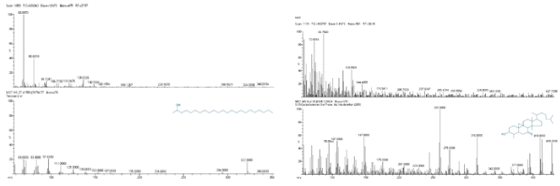

Fig3.9: Mass spectrum of Tricosan-2-ol
Fig 3.10: Mass spectrum of 9, 19-cyclocholestan-3- o 1-7-one 4a,14a dimethyl-(20R)

GC-MS SPECTRA OF SENNA AURICULATA FLOWER EXTRACT

Table 1: Chemical compounds identified from the ethanolic extract of Cassia auriculata flower

S. No	Rt	Name Of The Compound	Molecular Formula	Molecular Weight	Peak Area
1	15.92	Flavone	$C_{15}H_{10}O_2$	222.239	2.6
2	17.5	3-Methylmannoside	$C_7H_{14}O_6$	194.182	23.2
3	17.87	L-Glucose	$C_6H_{12}O_6$	180.155	15.7
4	18.75	6-o-Methyl-2,4-methylene-a-sedoheptitol	$C_9H_{18}O_7$	238.235	12.6
5	19.68	11-Tetradecen-ol acetate	$C_{16}H_{30}O_2$	326.433	5.1
6	20.57	6,9 octadecadienoic acid, Methyl ester	$C_{19}H_{34}O_2$	294.472	7.6
7	25.2	Dasycarpidan-1-methanol, acetate(ester)	$C_{20}H_{26}N_2O_2$	326.44	1.6
8	27.57	Tricosan-2-ol	$C_{23}H_{48}O$	340.627	10
9	30.45	9,19-cyclocholestan-3- o l-7-one 4a,14a dimethyl-(20R)	$C_{29}H_{48}O_2$	428.690	1.4

FT-IR SPECTRAL ANALYSIS

The Fourier transform infrared spectrum of the Cassia auriculata flower extract was recorded in the range of 400-4000cm-1, Model FT/IR-4100typeA and is shown in Fig. 2.1. In this research work the recorded spectrum was used to study the different modes (stretching /bending) of vibrations involving chemical bonds present in the compound. The frequencies assigned for various peaks in the spectrum are listed in the Table 2.

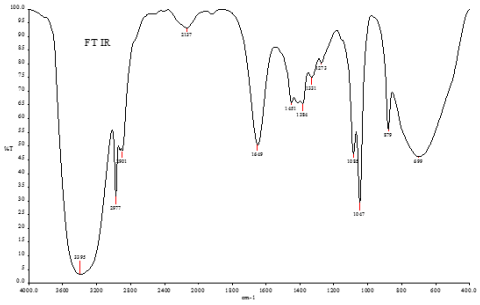

Fig. 2.1 FTIR SPECTRUM

Table 2 Frequency Assignment

Literature values(cm^{-1})	Band region (cm^{-1})	Intensity	Mode Assignment
3446.87	3400	Very strong/ broad	O- H Stretching
2926.14	2900	Moderate intensity	Aromatic C- H stretching
1613.62	1600	Sharp/ medium	C= O Stretching
1511.29	1400	Band/Medium	CH bending
1072.47	1100	Sharp/ strong	C- O stretching
518.87	Near 800, 600	Sharp medium/ broad strong	C- H deformation, out of plane bending deformation

UV-VIS SPECTRAL INVESTIGATION

UV-Vis. absorption spectrum of Senna auriculata flower extract was recorded in the range 250- 900 nm (Fig.3) using JASCO UV, V-650 model. A sharp absorption peak of Cassia auriculta flower extract was found at 284nm. From the absorption peak the energy of absorption was calculated and it is equal to 4.31eV. The absorption energy of Cassia auriculata flower extract was calculated using the absorption wavelength obtained from the spectrum using the relation E = hu eV, where 'h" is the planck's constant; υ=c/λ, here 'c' is the velocity of light in vacuum and 'λ' is the wavelength corresponding to the absorption peak in the spectrum.

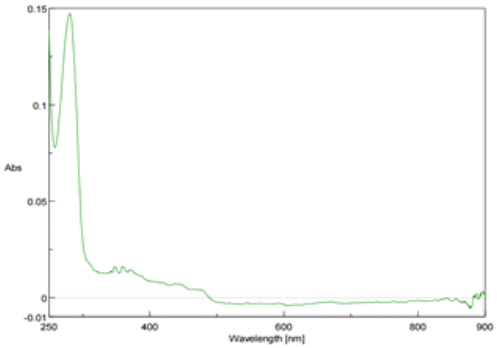

Fig.3.1 UV-VIS SPECTRUM

ANTI-DIABETIC STUDIES ON 3T3 CELL LINE

3T3 cell line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37°C. The anti-diabetic capability of the sennaauriculata flower extract was measured using DNS assay. The optical density and the concentration from the standard graph over a period of 0-25 hrs were estimated and the values are tabulated in Table 3.

TABLE 3. DNS ASSAY

S.No.	Samples	0th hr		5th hr		10th hr		15th hr		20th hr		25th hr	
		OD	Conc. (µg)	OD	Conc.(µg)	OD	Conc. (µg)	OD	Conc. (µg)	OD	Conc. (µg)	OD	Conc. (µg)
1	Control	1.76	3.4	1.70	3.25	1.65	3.2	1.60	3.05	1.45	2.8	0.32	0.70
2	Sample	1.46	2.8	1.44	2.7 5	1.38	2.65	1.32	2.5	1.26	2.4	0.007	0.04

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in the metabolism of carbohydrate, fat and protein and an associated deficiency of insulin secretion. Some of the plants which are being used for the treatment of diabetes have received scientific scrutiny. One such traditional plant is Senna auriculata, whose flower, leaves, stem, root, and unripe fruit are profoundly used in Ayurvedic medicine as a remedy for diabetes, conjunctivitis, joint and muscle pain (rheumatism), opthalmia, jaundice, liver disease, and urinary tract disorders. Considering the exponential increase of diabetes among the population, we have initiated the present study and it is clear from the recorded values of anti-diabetic activity of ethanolic extract of Cassia auricualta flower that it exhibits significant activity against diabetic disorder and hence this flower can be an excellent anti-diabetic agent, but need to be supported by in-vitro analysis.

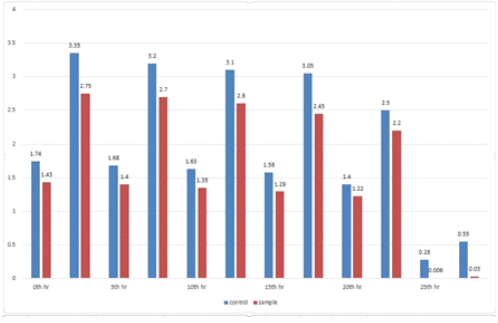

Fig.2.3 BAR GRAPH REPRESENTING THE ESTIMATED ACTIVITY OF THE SAMPLE IN COMPARISON WITH THE CONTROL

It is clear from the bar graph representing the estimated activity of the Cassia auriculata flower in comparison with the control (Fig 2.3) that it exhibits significant activity (≈ 80 % as that of the control) against diabetic disorder and hence this flower can be an excellent anti-diabetic agent.

CONCLUSION

The flowers of Cassia auriculata Linn were collected from Udumalpet, Tamil Nadu and the ethanolic extract of Cassia auriculata flower was prepared and subjected to GC-MS study which reveals the presence of eight bioactive compounds. The potential functional groups have been identified from the FTIR spectrum and the recorded peaks were assigned with different modes according to the literature values. UV-Vis. absorption spectrum of Senna auriculata flower extract was recorded in the range 250-900 nm and a sharp absorption peak was found at 284nm. Anti-diabetic studies on 3T3 Cell Line was also performed for the Cassia auriculata flower extract which reveals its excellent anti-diabetic property. From these research findings primarily we may communicate that the flower of Senna auriculata can be a promising anti-diabetic medicine but need in-vitro investigation.

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