In vitro antidiabetic evaluation guided by GC-MS analysis of ethyl acetate leaves extract of *Acacia auriculiformis* Benth.

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Graphical Abstract

Abstract:

Acacia auriculiformis Benth. is an evergreen shrub traditionally used as a remedy in the cure of itching, aches, allergy, rheumatism, and sore eyes. But recently, this shrub has been explored for antidiabetic activity. Here in this work, we have attempted to evaluate various leaves extracts of *Acacia auriculiformis* Benth. for α-glucosidase assay and α-amylase assay and thereafter the bioactive ethyl acetate leaves extract was subjected to GC-MS analysis. The *in vitro* antidiabetic evaluation showed promising results. The % inhibition of ethyl acetate leaves extract in the α-glucosidase assay and the α-amylase assay was found be 94.259% and 95.259% respectively, at a concentration of 10 μg/ml. The LC-MS results revealed the presence of phenolic compounds (phenol, 2,4-bis(1,1-dimethylethyl); 2,4-Ditert-butylphenol) and various esters (hexadecanoic acid, methyl ester; 1,2-benzenedicarboxylic acid, butyl octyl ester; hexadecanoic acid, 2,3 bis[(trimethylsilyl)oxy]propyl ester). Those compounds could be responsible for the desired antidiabetic activity.

Keywords: *Acacia auriculiformis* Benth.; Antidiabetic activity; α-Glucosidase Assay; α-Amylase Assay; GC-MS.

Introduction

- Diabetes mellitus (DM) is a complicated chronic systematic disorder usually assisted by basic metabolic disorders like hyperglycaemia, hypertriglyceridemia and hyperinsulinemia.
- It has been estimated that by the end of 2035, the number of DM patients will be increased from 422 million to 592 million.
- DM is mainly classified into two types: type1 diabetes mellitus and type 2 diabetes mellitus.
- Type 1 diabetes mellitus is identified by deficiency in insulin because of the destruction of β-cells of pancreas and is a rare disease.
- Whereas the type 2 diabetes mellitus is characterized by deficiency in insulin secretion and insulin resistance. 95% of the individuals are affected by type 2 diabetes.
- From ancient time, natural products including herbal formulations, crude extracts and isolated phytoconstituents are being used in the treatment of various human ailments including diabetes mellitus.

- *Acacia auriculiformis* A. Cunn.ex Benth. belonging to family Mimosaceae or Fabaceae, is an ornamental, evergreen shrub widely distributed in India as well as in several parts of world.
- It was traditionally used as a medical ailment for aches, sore eyes, itching, allergy and rheumatism .
- The phytochemical investigations disclose the presence of tannins, flavonoids, and triterpenoidal saponin glycoside in the plant extracts .
- The phytochemical extracts and phytoconstituents of *Acacia auriculiformis* has been evaluated for various pharmacological activities like cestocidal, anti-mutagenic, antidiabetic activity, hepatoprotective, antioxidant, spermicidal, wound healing, CNS depressant activity, chemopreventive, antimicrobial, antimalarial, and anti-filarial.
- Here, in this work an attempt has been made to highlight the *in vitro* antidiabetic activity of leaves extracts of *Acacia auriculiformis* Benth.
- The gas chromatography-mass spectroscopy (GC-MS) analysis of the bioactive extract was performed to identify the phytoconstituents responsible for respective biological activity.
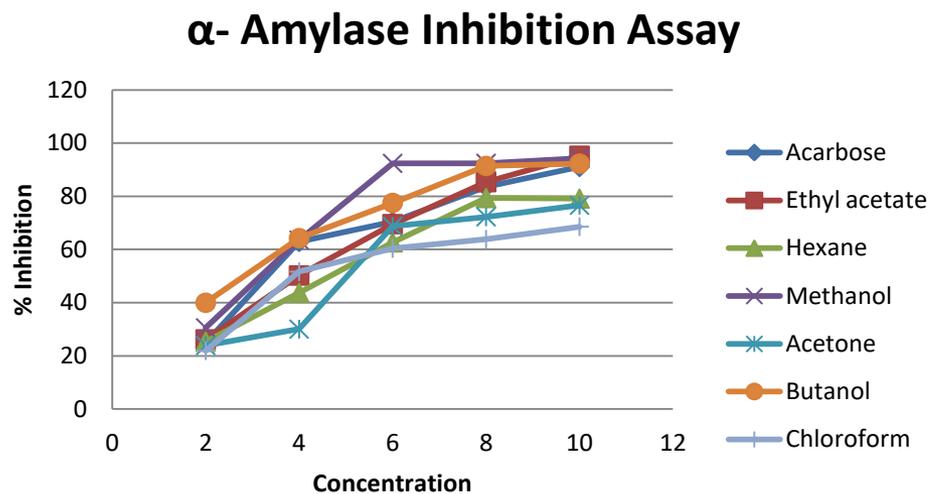
Results and discussion

In vitro Anti-diabetic Activity

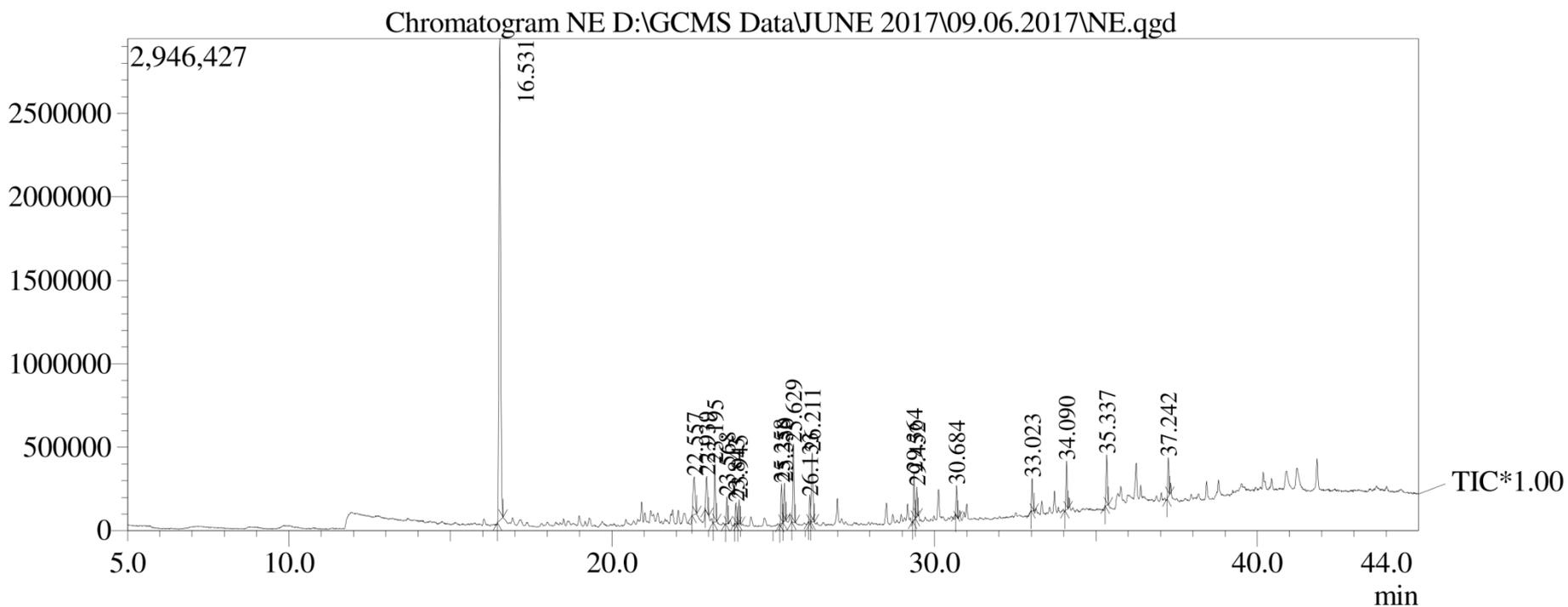### α- Glucosidase Inhibition Assay

α- Glucosidase Inhibition Assay

α- Amylase Inhibition Assay
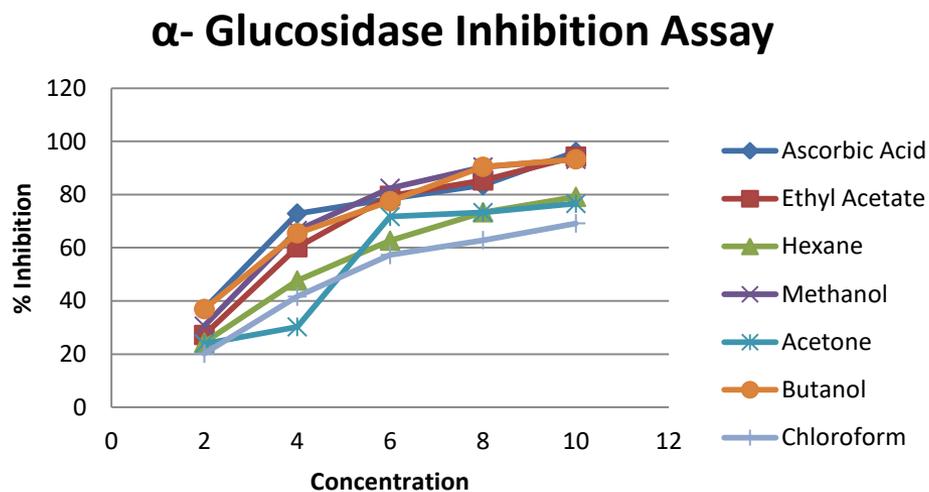
α- Amylase Inhibition Assay

GC-MS Analysis of the Bioactive Ethyl acetate Leaves Extract

Chromatogram NE D:\GCMS Data\JUNE 2017\09.06.2017\NE.qgd

Phytoconstituents identified from GC-MS analyis of ethyl acetate leaves extract

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	16.531	10876767	47.84	2884942	39.71	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	191.15
2	22.557	938655	4.13	227134	3.13	(-)-LOLIOLIDE	111.10
3	22.939	678740	2.99	212993	2.93	$$ (-)-VINCADIFFORMINE	124.10
4	23.195	980954	4.31	346151	4.77	3-Heptadecanol	59.05
5	23.568	414531	1.82	162119	2.23	Isopropyl myristate	60.05
6	23.843	296618	1.30	126532	1.74	Neophytadiene	68.10
7	23.945	355022	1.56	145360	2.00	Hexahydrofarnesyl acetone	58.05
8	25.258	616897	2.71	236119	3.25	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	57.10
9	25.350	534395	2.35	230613	3.17	DOCOSANE	57.10
10	25.629	1258063	5.53	467406	6.43	HEXADECANOIC ACID, METHYL ESTER	74.10
11	26.133	454174	2.00	146696	2.02	3-PENTANOL, 3-ETHYL-	87.10
12	26.211	1176949	5.18	413237	5.69	1,2-BENZENEDICARBOXYLIC ACID, BUTYL OCTYL ESTER	149.10
13	29.364	719392	3.16	277301	3.82	Eicosane	57.10
14	29.452	419235	1.84	181176	2.49	OCTADECANOIC ACID, METHYL ESTER	74.05
15	30.684	424759	1.87	182612	2.51	1-Heneicosanol	57.10
16	33.023	498566	2.19	194685	2.68	OCTADECANE	57.10
17	34.090	724270	3.19	292452	4.03	Eicosyl trifluoroacetate	57.10
18	35.337	810797	3.57	303956	4.18	HEXADECANOIC ACID, 2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER	130.10
19	37.242	555720	2.44	232919	3.21	1-Heptacosanol	57.10

- The leaves plant extracts showed inhibitory action against α –glucosidase and α–amylase enzymes.
- The ethyl acetate leaves extract showed highest % inhibition(94.259% and 95.259%) on α –glucosidase and α –amylase respectively at a concentration of 10 µg/ml.
- The carbohydrate metabolism involves α-amylase and α-glucosidase enzymes and therefore these enzymes are considered to be the effective target for in vitro antidiabetic evaluation.
- The α-amylase and α-glucosidase inhibition results in prolonged carbohydrate digestion and glucose absorption.
- Several drugs like Acarbose, voglibose, metformin and miglitol that inhibit the α-amylase and α-glucosidase enzymes are available in the market.
- Acarbose is the first dual enzyme inhibitor i.e. it inhibits both the enzymes.
- But the notable side effects of the above enzyme inhibitors are flatulence, abdominal distention, and diarrhea, there is still a need to search the effective and safe inhibitor from natural sources.

- The ethyl acetate leaves extract of *Acacia auriculiformis* Benth. inhibited effectively both the target enzymes which might be due to the presence of specific phenolics.
- Further GC-MS analysis of bioactive ethyl acetate extract suggested the presence of phenolic compounds like Phenol, 2,4-bis(1,1-dimethylethyl) and esters like Hexadecanoic acid, methyl ester, 1,2-benzenedicarboxylic acid, butyl octyl ester, Octadecanoic acid, methyl ester, Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester and various other compounds like (-)-Loliolide, SS (-)-Vincadifformine, 3-Heptadecanol, Isopropyl myristate, Hexahydrofarnesyl acetone, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Docosane, Eicosane, 1-Heptacosanol etc.
- From the above-analyzed compounds by GC-MS analysis the phenolic compounds may be responsible for imparting the desired antidiabetic activity.

Conclusions

- From the present findings, it is concluded that the ethyl acetate leaves extract of *Acacia auriculiformis* Benth. possess significant antidiabetic activity due to the presence of phenolic compounds.
- The GC-MS analysis also revealed the various compounds including phenolics.
- This confirmed the correlation of desired pharmacological activity (antidiabetic activity) with the phenolic compounds.
- Further, this bioactive extract is being explored for *in vivo* antidiabetic studies and also the isolation of bioactive compounds from bioactive ethyl acetate leaves extract is in process.

Acknowledgments