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CHARACTERISATION, *INVITRO* AND *INSILICO* ANTIDIABETIC ACTIVITY IN THE LEAF EXTRACTS OF *MANGIFERA INDICA*

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism. Several phytocompounds are known to modulate glucose metabolism by inhibition of carbohydrate digesting enzyme like α-amylase. Leaves of *Mangifera indica* were subjected to qualitative tests and quantitative total phenolic content assay. It was found that the ethanolic extract had high phenolic content. Ethanolic extract also had maximum DPPH free radical scavenging activity and *invitro* α-Amylase Inhibition activity. GC-MS analysis of the ethanolic extracts of the samples gave several compounds which were docked against the target Human Pancreatic Alpha-Amylase. Ethyl octadecanoate was found to be an active compound through *insilico* docking with the least E value of – 6.35 kcal/mol. This result may pave the way to modulate glucose metabolism using phytocompounds and thereby may lead to the development of more effective chemical entities with antidiabetic property.

KEYWORDS: Glucose homeostasis, α-amylase, Human Pancreatic Alpha-Amylase, Ethyl octadecanoate, docking.

1.0 INTRODUCTION

World Health Organization (WHO) estimates about 70-80% of Indians depend on Indian system of medicine like Unani, Siddha and Ayurvedha **(Gupta and Shaw, 2009).** The use of herbal medicine is usually an integral part of culture around the world, which has been used in medical practice for thousands of years and has made a great contribution for maintaining human health before spread of modern science **(Verma and Singh, 2008)**.

The emerging importance of biologically active medicinal plants and their constituents as possible therapeutic measures has become a subject of active scientific investigation. It is likely that in future safe and effective medicines will be developed from medicinal plants to treat various degenerative diseases. Many pharmaceutical companies show interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects **(Nikhal *et al*., 2010)**.

Diabetes mellitus, a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism results from defects in insulin secretion, insulin action, or both. Diabetes mellitus can be classified under following two categories: Type I is insulin-dependent diabetes mellitus (IDDM), in which the body does not produce any insulin. It most often occurs in children and young adults. Type I diabetes accounts for 5-10% of diabetes. Type II is non insulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough, or improper use of secreted insulin is the most common form of the disease, accounting for 90-95% of diabetes is nearing epidemic proportions, due to an increased number of elderly people and a greater prevalence of obesity and sedentary lifestyles. **(Kirti *et al*., 2008)**. According to WHO, it is estimated that 3% of the World's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% **(Meenakshi *et al*.,2010)**.

The presence of diabetes confers increased risk of many devastating complications such as cardio vascular disease, peripheral vascular disease complications. Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas are available for the treatment of diabetes. The main disadvantages of the currently available drugs are that they have to be given throughout the life characterized with side effects **(Halim, 2003)**. Hence, there is increasing emphasis on the use of plant products rich in phenolic compounds that could be more effective for the management of type II diabetes with few side effects. In addition to their effectiveness and safety, herbal remedies could be a cheaper alternative to the synthetic antidiabetic drugs. Consequently, the World Health Organization recommended that further research on the antidiabetic effects of medicinal plants should be carried out **(WHO, 1980).**

Flavonoids and phenolic compounds are part of the secondary metabolites that constitute the active principles in plant products. These active ingredients are responsible for the therapeutic and or pharmacological activities, such as antidiabetic effects of medicinal plants

(Sumbul *et al*., 2011). Phenolic compounds are known to modulate glucose metabolism by several mechanisms including inhibition of carbohydrate digesting enzyme like α-amylase. The inhibition of carbohydrate metabolizing enzyme such as α-amylase retards the digestion carbohydrates and the subsequent absorption of glucose, leading to a decrease in postprandial blood glucose level **(Hanhineva *et al*., 2010).**

Mangifera indica (Anacardiaceae) is a tree, distributed in rural and semi urban parts of the India. It is one of the most important tropical plants marketed in the world **(Ross, 1999).** *Magnifera indica* is a large evergreen tree in the anacardiaceae family that grows to a height of 10-45 m, dome shaped with dense foliage, typically heavy branched from a stout trunk. Phytochemical research from different parts of *M. indica* has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols **(Singh *et al*., 2004).**

Thus the current study is focused to characterise, evaluate the *invitro* and *insilico* antidiabetic activity in the leaf extracts of *Mangifera indica*.

2.0 MATERIALS AND METHODS

2.1 COLLECTION AND PREPARATION OF PLANT MATERIALS

Healthy fresh leaves of *Mangifera indica* were collected from the nearby areas of Coimbatore district. The leaves were rinsed with distilled water and dried at room temperature under well ventilated shade for 10 days. The dried leaves were powdered and stored in air-tight container for further analysis.

2.2 EXTRACTION OF PLANT MATERIAL

The powdered leaves were extracted in various solvents, viz hexane, ethyl acetate and ethanol **(Gayathri and Jeyanthi, 2013).** One part of the powdered leaves were macerated in three parts of hexane, ethyl acetate and ethanol separately and kept for 24 hours at 37°C. Filtered and collected the solvents. The solvents were evaporated to obtain the hexane, ethyl acetate and ethanol extracts.

2.3 QUALITATIVE ANALYSIS

PHYTOCHEMICAL TESTS

The methods described by **Trease and Evans, 1989** and **Abalaka *et al*., 2011** were used for screening of phytochemicals like tannin, saponin, flavonoids, phenols, cardiac glycosides, terpenoids, steroids, phytosteroids, phlobatannins, alkaloids and carbohydrates.

2.4 QUANTITATIVE ANALYSIS

ESTIMATION OF TOTAL PHENOLIC CONTENT

Total phenolic content of the extracts were assessed according to the Folin–Ciocalteau method **(Slinkard & Singleton, 1977)** with some modifications. Briefly, 0.1 ml of the extracts with varying concentrations (200, 600 and 1000µg/ml), 1.9 ml distilled water and 1.0 ml of Folin–Ciocalteau's reagent were seeded in a tube, and then 1.0 ml of 100 g/l Sodium carbonate was added. The reaction mixture was incubated at 25°C for 2 hours and the absorbance of the mixture was read at 765 nm. The readings were taken in triplicates. The total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Amount TPC = Sample OD/Standard OD * Respective Amount of extract

Where, Sample OD refers to optical density of sample and Standard OD refers to optical density of standard.

2.5 *INVITRO* ANTIOXIDANT ACTIVITY

DPPH FREE RADICAL SCAVENGING ASSAY

The ability of the extracts to annihilate the DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) was investigated by the method described by **Blois, 1958.** Stock solution of extracts were prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 600 and 1000 µg) of extracts were added at an equal volume to 1.0 ml of methanolic solution of DPPH (0.1mM). The reaction mixture was incubated for 30 minutes at room temperature and the absorbance was recorded at 517 nm. Ascorbic acid was used as standard. The readings were taken in triplicates. The annihilation activity of free radicals was calculated in % inhibition according to the following formula,

% of Inhibition = (Abs of control – Abs of sample)/Abs of control * 100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

2.6 *INVITRO* ANTIDIABETIC ACTIVITY

α- AMYLASE INHIBITION ASSAY

Invitro α -amylase inhibition of the extracts were determined using the procedure reported by **Bernfeld *et al.*, 1955** with slight modifications as proposed by **Abirami *et al.*, 2014**. In α -amylase inhibition method, the enzyme solution was prepared by dissolving α -amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5 mg/ml. 1.0 ml of the extract in various concentrations (250, 500, 750, 1000 µg/ml) and 1.0 ml of enzyme solution were mixed together and incubated at 25°C for 10min. After incubation, 1.0 ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10 minutes. The reaction was then stopped by adding 2.0 ml of dinitro salicylic acid (DNS, colour reagent) heating the reaction mixture in a boiling water bath (5min). After cooling, the absorbance was measured colorimetrically at 565 nm. Metformin was used as a standard for the assay. The readings were taken in triplicates. The inhibition percentage was calculated using the given formula,

$$\% \text{ of Inhibition} = (\text{Abs of control} - \text{Abs of sample})/\text{Abs of control} * 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

2.7 GAS CHROMATOGRAPHY—MASS SPECTROMETRY

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. The ethanol leaf extracts of *Mangifera indica* and *Costus igneus* which had high *in vitro* antioxidant activity were subjected to GC-MS analysis using the software XCALIBUR (ver-2.2). The GC-MS analysis of unknown compounds deals by using a TSQ QUANTUM XLS Gas Chromatography. It ionizes compounds and measures their mass number equipped with the column DB-5MS (30m X 0.25mm X 0.25um) and mass detector which was operated at in EI mode. The experiment was programmed with total run time 34 min, helium was used as the carrier gas at the flow rate of 1 ml / min. The injector was operated at 280˚C and the oven temperature was programmed as follows: 70˚C @ 8˚C /min to 150˚C (1 min) @ 8˚C / min to 280˚C (10 min). Injection volume was 1µl with scan mass range 30m/z – 600m/z having positive polarity (+ve). The identification of components was based on comparison of their mass spectra with NIST-011S library.

2.8 *INSILICO* ANALYSIS

In the first step protein structure file of Human Pancreatic Alpha-Amylase (PDB ID-4X9Y) was retrieved with resolution of about 1.07A°. The target protein was visualized with RasMol. In next step chemical structures were retrieved from PubChem and Lipid maps database. The chemical structures of n-hexadecanoic acid, 1-Octadecene, Pentacosane, Dotriacontane and Ethyloctadecanoate were used for docking studies by Argus Lab. The docking analysis of ligand with Human Pancreatic Alpha-Amylase was carried by Argus lab docking software using default parameters. The values were obtained in terms of energy (e-value) Kcal/mol. Lesser the E-value greater the acceptability of compound as a drug **(Srivastava *et al*., 2008).** Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme (Human Pancreatic Alpha-Amylase) fit together and dock to each other well. The molecules binding to a receptor, inhibit its function, and thus act as drug.

3.0 RESULTS AND DISCUSSIONS

3.1 COLLECTION AND PREPARATION OF PLANT MATERIALS

Healthy fresh leaves of *Mangifera indica* are collected from the nearby areas of Coimbatore district. The leaves are rinsed with distilled water and dried at room temperature under well ventilated shade. The dried leaves are powdered and stored in air-tight container for further analysis.

3.2 EXTRACTION OF PLANT MATERIAL

The extract is prepared by adding 150 ml of hexane, ethyl acetate and ethanol to 50 g of powdered leaves. After 24 hours, the solvent is allowed to evaporate at room temperature to obtain the hexane, ethyl acetate and ethanol extracts.

3.3 QUALITATIVE ANALYSIS

Phytochemicals are the potent bioactive components that provide the therapeutic effect in medicinal plants **(Doss *et al*., 2009).**

The results of phytochemical screening of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the table 1.

Table 1: Phytochemical screening of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

S.No	Phytochemical Tests	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Tannin	+	+	+
2	Saponin	-	-	+
3	Flavonoid	+	+	+
4	Phenol	+	+	+
5	Cardiac glycoside	-	-	-
6	Terpenoid	+	+	+
7	Steroid and Phytosteroid	Steroid +	Steroid +	Steroid +
8	Phlobatannin	-	-	-
9	Alkaloid	+	+	+
10	Carbohydrate	+	+	+

+ = Present - = Absent.

Preliminary phytochemical screening of the extracts reveal the presence of various bioactive components like alkaloid, flavonoid, steroid, phenol, terpenoid, carbohydrate and tannin in hexane, ethyl acetate and ethanol extracts. Phlobatannin and Cardiac glycoside are absent in all the extracts. Saponin is present only in ethanol extract. The above results are similar to the study of **(Somkuwar and Kamble, 2013)** in which the presence of the alkaloids, carbohydrates, tannins, flavonoids were reported in the extracts of *Mangifera indica*. Terpenoid is attributed for analgesic and anti-inflammatory activities and flavonoids had been reported to possess many useful properties, including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant properties **(Harborne and Williams, 2000).** Flavonoid, phenol and alkaloid have hypoglycemic activities. Tannins isolated from plant species *Solanum trilobatum* Linn exhibited antibacterial activities **(Doss *et al*., 2009).**

3.4 QUANTITATIVE ANALYSIS

TOTAL PHENOLIC CONTENT

Phenolic compounds are among the most important plant components as they possess a variety of biological activities including antioxidant activity, therefore it is quite important to evaluate the total phenolic content in tested extracts **(Elzaawely and Tawata, 2010).**

- **Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica***

Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the table 2.

Table 2: Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Total Phenolic Content		
Concentration (µg/ml)	**Name of the extract**	**Amount of Phenol***
200	Hexane	23.10 ± 1.24
600		54.07 ± 0.79
1000		69.72 ± 1.50
200	Ethyl acetate	33.93 ± 0.97
600		55.05 ± 1.76
1000		74.06 ± 0.91
200	Ethanol	46.74 ± 0.34
600		58.85 ± 1.35
1000		84.14 ± 1.76

*Amount of Phenol is expressed as mg of catechol per gram of extract

** (Values are expressed as mean ± SD).

Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the figure 1.

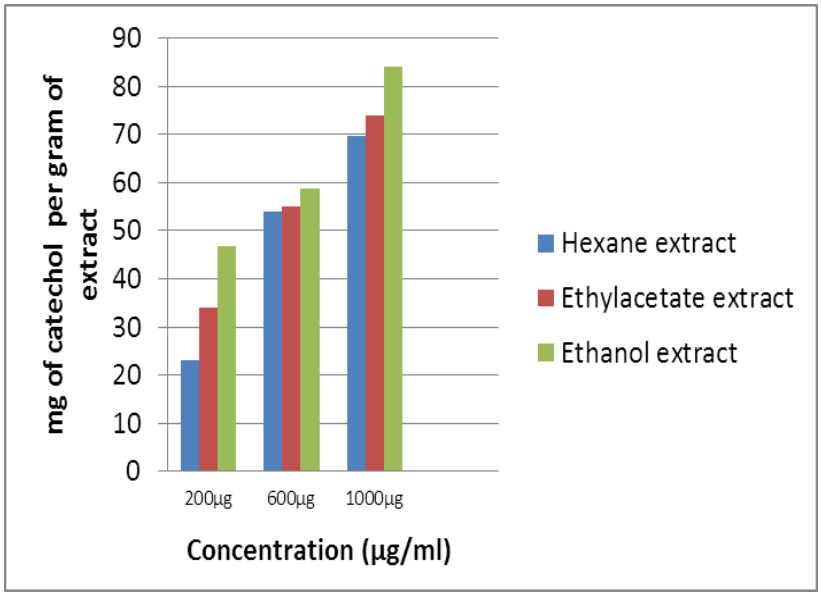

Figure 1: Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

The amount of total phenolics in *Mangifera indica* - hexane, ethyl acetate and ethanol extracts are presented in the Figure 1. The ethanol extract has high amount of total phenolics (84.14 mg of catechol per gram of extract) at 1000µg/ml concentration than that of hexane and ethyl acetate extracts. This is in accordance with the study of (**Elzaawely and Tawata, 2010)** in which the ethanol extract of *Mangifera indica* had high phenolic content.

3.5 *INVITRO* ANTIOXIDANT ACTIVITY

DPPH FREE RADICAL SCAVENGING ASSAY

Scavenging of the stable radical DPPH is considered a valid and easy assay to evaluate scavenging activity of anti oxidants **(Nanjo *et al.*, 1996).** It is quite important to evaluate the DPPH scavenging activity of plant extracts.

- **DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica***

DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the table 3.

Table 3: DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Concentration (µg/ml)	Name of the extract	Control	DPPH Scavenging Activity	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Ascorbic acid*
200	Hexane	1.2339	21.36 ± 0.76	57.04 ± 1.22
600		1.2339	31.91 ± 0.45	74.87 ± 1.42
1000		1.2339	36.59 ± 0.67	89.46 ± 1.31
200	Ethyl acetate	1.2339	27.93 ± 0.56	57.04 ± 1.29
600		1.2339	34.16 ± 0.97	74.87 ± 1.56
1000		1.2339	43.19 ± 1.12	89.46 ± 1.62
200	Ethanol	1.2339	43.19 ± 1.07	57.04 ± 1.86
600		1.2339	61.01 ± 0.99	74.87 ± 1.76
1000		1.2339	65.88 ± 0.77	89.46 ± 1.91

*Ascorbic acid = Standard.

** (Values are expressed as mean ± SD).

DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the figure 2.

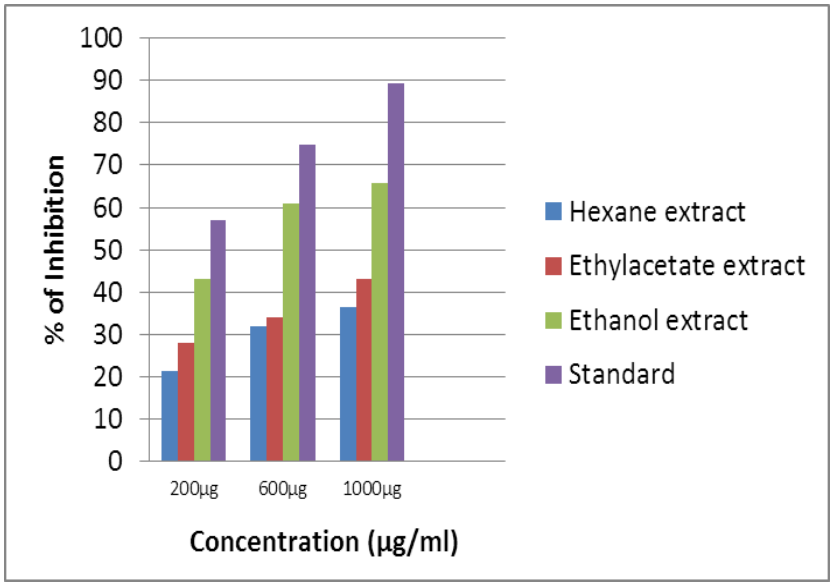

Figure 2: DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Figure 2 shows the *in vitro* antioxidant potential of hexane, ethyl acetate and ethanol extract of *Mangifera indica* at different concentrations. The result shows the maximum DPPH scavenging activity of ethanol extract of *Mangifera indica* appeared to be potent as standard ascorbic acid with inhibition of 66% at 1000µg/ml concentrations of plant extracts. These activities are lesser than ascorbic acid. These results are in accordance to the work of **(Kaur *et al.*, 2015)** who stated that the ethanol extracts of *Mangifera indica* had high DPPH scavenging activity. The more rapidly the absorbance decreases the more potent is the antioxidant activity of the extract.

3.6 *INVITRO* ANTIDIABETIC ACTIVITY

α – AMYLASE INHIBITION ASSAY

The intestinal digestive enzymes alpha-amylase plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in management of blood glucose **(Shreedhara *et al.*, 2009).** Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha linked polysaccharide such as starch to yield high levels of glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide **(Abirami *et al.*, 2014).** Therefore it is quite important to evaluate the α – Amylase inhibitory activity of the extracts.

- **Inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* on α – Amylase activity**

The inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* on α – Amylase activity are shown in the table 4.

Table 4: Inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* on α – Amylase activity

Concentration (µg/ml)	Name of the extract	Control	α – Amylase inhibition assay	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Metformin*
250	Hexane	0.94	28.72 ± 0.98	60.63 ± 0.54
500		0.94	35.10 ± 0.78	68.08 ± 0.65
750		0.94	45.74 ± 0.87	77.65 ± 0.68
1000		0.94	56.38 ± 0.65	81.91 ± 0.71
250	Ethyl acetate	0.94	30.85 ± 0.43	60.63 ± 0.75
500		0.94	37.23 ± 0.54	68.08 ± 0.77
750		0.94	51.06 ± 0.64	77.65 ± 0.82
1000		0.94	63.82 ± 0.67	81.91 ± 0.88
250	Ethanol	0.94	35.10 ± 0.56	60.63 ± 1.04
500		0.94	43.61 ± 0.45	68.08 ± 1.21
750		0.94	58.51 ± 0.35	77.65 ± 1.34
1000		0.94	67.02 ± 0.21	81.91 ± 1.43

*Metformin = Standard.

** (Values are expressed as mean ± SD).

The inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* on α – Amylase activity are presented in the figure 3.

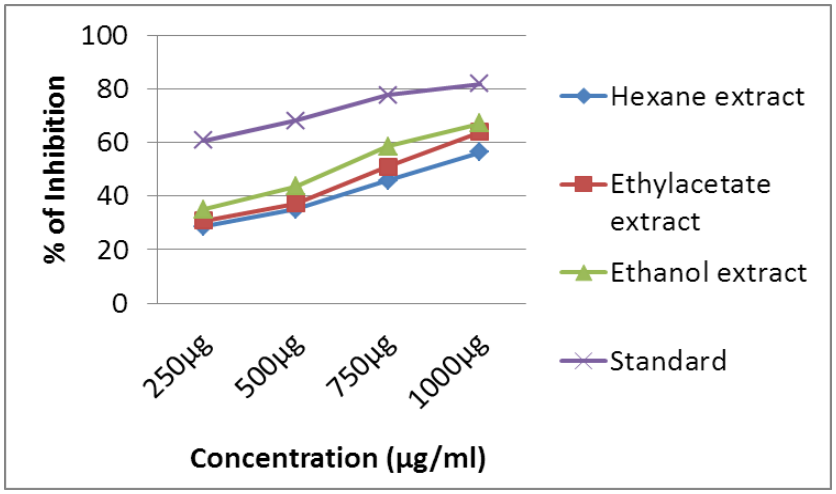

Figure 3: Inhibitory effect of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* on α – Amylase activity

Alpha amylase inhibitory activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the Figure 3. Ethanol extracts of *Mangifera indica* has high percentage of inhibition than hexane and ethyl acetate. The percentage inhibition of ethanol extract of *Mangifera indica* is found to be 67% at 1000 µg/ml concentrations of plant extract. The activity is less than metformin. It is to note that the ethanol extracts of *Mangifera indica* and *Azadirachta indica* showed maximum alpha amylase inhibitory activity **(Kumar *et al.*, 2008).** This showed a dose dependent increase in the percentage inhibition.

3.7 GAS CHROMATOGRAPHY—MASS SPECTROMETRY

The study of the organic compounds from plants and their activity has increased by the combination of a best separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds **(Grover *et al.*, 2013).** The ethanol extract of *Mangifera indica* which has high *in vitro* antioxidant activity were subjected to GC-MS analysis.

In present investigation total seventy nine bioactive chemical constituents are identified in ethanol extract of *Mangifera indica* with important properties. The GC-MS chromatogram of ethanol extract of *Mangifera indica* is shown in the figure 4.

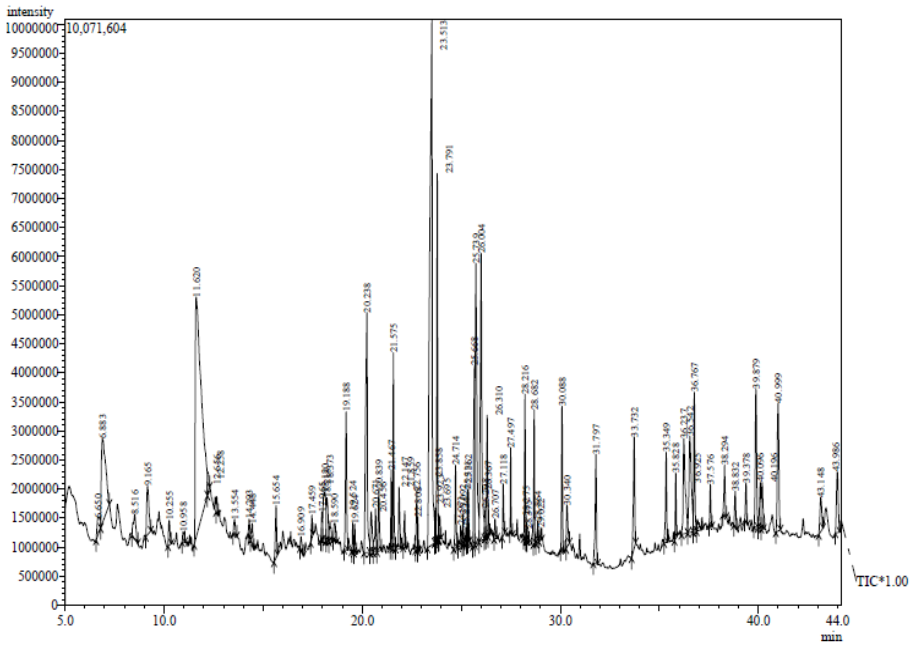

Figure 4: GC-MS chromatogram of Ethanol extract of *Mangifera indica*

The constituents in Ethanol extract of *Mangifera indica* are presented in the table 5.

Table 5: Constituents in Ethanol extract of *Mangifera indica*

S.No	Compound name	Molecular formula	Retention time	Area %
1.	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	6.650	0.52
2.	1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	6.883	3.78
3.	2-Hydroxybenzoic Acid Methyl Ester	$C_8H_8O_3$	8.516	0.66
4.	2,3-Dihydroxypropyl Acetate	$C_5H_{10}O_4$	9.165	1.12
5.	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	10.255	0.37
6.	n-Decanoic acid	$C_{10}H_{20}O_2$	10.958	0.17
7.	1,2,3-Benzenetriol	$C_6H_6O_3$	11.620	14.81
8.	10-(1-Methylallyl) tricyclo[6.3.1.0 (2,7)]dodeca-2(7),3,5-trien-10-ol	$C_{16}H_{20}O$	12.258	0.28
9.	6,10-Dimethyl-5,9-undecadien-2-one	$C_{13}H_{22}O$	12.656	0.16
10.	2,3,4,5-Tetramethyl-2-cyclopentenone	$C_9H_{14}O$	13.554	0.25
11.	1-(2,3,6-Trimethylphenyl)-3-Buten-2-One	$C_{13}H_{16}O$	14.293	0.23
12.	6,6-Dimethylhexahydro-1h-Cyclopenta[3,4]Cyclobuta[1,2-A]Benzene-3a,4,7a(3bh,7bh)-Triol	$C_{13}H_{22}O_3$	14.448	0.31
13.	Dodecanoic acid	$C_{12}H_{24}O_2$	15.654	0.73
14.	Cedran-8-Ol	$C_{15}H_{26}O$	16.909	0.20
15.	2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl ester	$C_{16}H_{26}O_2$	17.459	0.23
16.	4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-Cyclohexen-1-one,	$C_{13}H_{20}O_2$	17.965	0.29
17.	4-hydroxy-3-methoxy -Benzeneacetic acid	$C_9H_{10}O_4$	18.120	1.31
18.	4,4,5,8-Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	18.218	0.79
19.	2-Hexyl-1-octanol	$C_{14}H_{30}O$	18.373	0.37
20.	2,6,10,10-Tetramethyl-1-oxaspiro[4.5] decan-6-ol	$C_{13}H_{24}O_2$	18.590	0.32
21.	4-(3-Hydroxybutyl)-3,5,5-Trimethyl-2-Cyclohexen-1-One	$C_{13}H_{22}O_2$	19.188	1.80
22.	3,7,11,15-Tetramethylhexadecan-1-Ol	$C_{20}H_{42}O$	19.524	0.25
23.	3,7,11,15-Tetramethylhexadecan-1-Ol	$C_{20}H_{42}O$	19.624	0.29
24.	Tetradecanoic acid	$C_{14}H_{28}O_2$	20.238	4.47
25.	5,6,7,7A-Tetrahydro-6-Hydroxy-4,4,7a-Trimethyl-, (6s-Cis)- 2(4h)-Benzofuranone	$C_{11}H_{16}O_3$	20.456	0.74
26.	1-Octadecene	$C_{18}H_{36}$	20.671	0.61
27.	4-Hydroxy-3,5,6-Trimethyl-4-[(1e)-3-Oxo-1-Butenyl]-2-Cyclohexen-1-One	$C_{13}H_{18}O_3$	20.839	0.85
28.	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{20}H_{38}$	21.467	0.58
29.	6,10,14-Trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	21.575	1.69
30.	Pentadecanoic acid	$C_{15}H_{30}O_2$	21.859	0.80
31.	2,6,10-Trimethyl,14-Ethylene-14-Pentadecene	$C_{20}H_{38}$	22.147	0.41
32.	3,5a,9,9 Tetramethyldecahydrobenzo	$C_{15}H_{26}O_2$	22.756	0.64

	[2,3]cyclohepta[1,2-b]oxiren-3-ol			
33.	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	22.808	0.21
34.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	23.513	13.44
35.	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	23.695	0.33
36.	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$	23.791	3.17
37.	1-Iodooctatetracontane	$C_{48}H_{97}I$	23.858	0.16
38.	14B-Pregnane	$C_{21}H_{36}$	23.923	0.21
39.	Heptadecanoic Acid	$C_{17}H_{34}O_2$	24.714	0.86
40.	n-Pentadecanol	$C_{15}H_{32}O$	24.973	0.23
41.	Heptadecanoic Acid, Ethyl Ester	$C_{19}H_{38}O_2$	25.092	0.22
42.	5-heptyldihydro-2(3H)-Furanone	$C_{11}H_{20}O_2$	25.234	0.16
43.	Palmitaldehyde Diallyl Acetal	$C_{22}H_{42}O_2$	25.311	0.37
44.	3,7,11,15-Tetramethylhexadec-2-En-1-Ol	$C_{20}H_{40}O$	25.362	0.40
45.	9,12-Octadecadienoic Acid	$C_{18}H_{32}O_2$	25.668	2.18
46	22-Tricosenoic acid	$C_{23}H_{44}O_2$	25.739	4.71
47.	Octadecanoic acid	$C_{18}H_{36}O_2$	26.004	4.70
48.	2-Phenyl-1,3-dioxolan-4-yl)methyl (9E,12E)-9,12-octadecadienoic acid	$C_{28}H_{42}O_4$	26.208	0.35
49.	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	26.310	1.26
50.	Hexadecane	$C_{16}H_{34}$	26.367	0.20
51.	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	26.707	0.22
52.	14B-Pregnane	$C_{21}H_{36}$	27.118	0.47
53.	n-Triacontane	$C_{30}H_{62}$	27.497	0.58
54.	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	28.216	1.21
55.	Icosanoic Acid	$C_{20}H_{40}O_2$	28.275	0.17
56.	2,2-dimethyl-3-(4-methyl-4-pentenyl)-Cyclopropanecarboxylic acid	$C_{12}H_{20}O_2$	28.375	0.19
57.	n-Triacontane	$C_{30}H_{62}$	28.682	1.05
58.	3,6-Dimethyl-5-hepten-1-ol acetate	$C_{11}H_{20}O_2$	28.684	0.21
59.	22-Stigmasten-3-one	$C_{29}H_{48}O$	29.022	0.16
60.	Pentacosane	$C_{25}H_{52}$	30.088	1.39
61.	2-hydroxy-1-(hydroxymethyl)ethyl ester Hexadecanoic acid	$C_{19}H_{38}O_4$	30.340	0.61
62.	Dotriacontane	$C_{32}H_{66}$	31.797	1.44
63.	Dotriacontane	$C_{32}H_{66}$	33.732	1.57
64.	Dotriacontane	$C_{32}H_{66}$	35.349	1.07
65.	Squalene	$C_{30}H_{50}$	35.828	0.58
66.	Delta-tocopherol	$C_{27}H_{46}O_2$	36.237	2.19
67.	Delta-tocopherol	$C_{27}H_{46}O_2$	36.542	2.49
68.	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	36.767	1.54
69.	Methyl commate B	$C_{31}H_{50}O_3$	36.925	0.25
70.	Delta-tocopherol	$C_{27}H_{46}O_2$	37.576	0.44
71.	Dotriacontane	$C_{32}H_{66}$	38.294	0.61
72.	Stigmasta-4,7,22-trien-3.alpha.-ol	$C_{29}H_{46}O$	38.832	0.44
73.	gamma.-Tocopherol	$C_{28}H_{48}O_2$	39.378	0.45
74.	Cholesta-4,6-Dien-3-Ol, Benzoate	$C_{34}H_{48}O_2$	39.879	2.06
75.	Tetracosane	$C_{24}H_{50}$	40.096	0.69
76.	1-Heptacosanol	$C_{27}H_{56}O$	40.196	0.71

77.	Alpha.-Tocopherol-.beta.-D-mannoside	$C_{35}H_{60}O_7$	40.999	2.15
78.	Ergost-5-En-3-Ol	$C_{28}H_{48}O$	43.148	0.68
79.	Stigmasterol	$C_{29}H_{48}O$	43.986	1.19

The bioactive constituents identified possess various therapeutic activities such as antidiabetic, anti-inflammatory, antibacterial, anticancer, antifungal, anti aging activities.

3.8 MOLECULAR DOCKING

Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme Human Pancreatic Alpha-Amylase fit together and dock to each other well. Human pancreatic α-amylase (HPA) inhibitors offer an effective strategy to lower postprandial hyperglycemia via control of starch breakdown. The compounds binding to a receptor, inhibit its function and thus act as drug (Srivastava et al., 2008).

The following are the bioactive compounds from the ethanol extract of *Mangifera indica* with good docking activity

1. n-hexadecanoic acid
2. 1-Octadecene
3. Pentacosane
4. Dotriacontane
5. Ethyl octadecanoate

The docking results are presented in the table 6.

Table 6: Docking Results

S.No	Compound Name	E value(kcal/mol)	Figure number
1.	Ethyl octadecanoate	-6.35	05
2.	Dotriacontane	-6.11	06
3.	n-hexadecanoic acid	-5.86	07
4.	Pentacosane	-5.65	08
5.	1-Octadecene	- 4.69	09

The following are the figures which represents the ligands in complex with Human Pancreatic Alpha-Amylase:

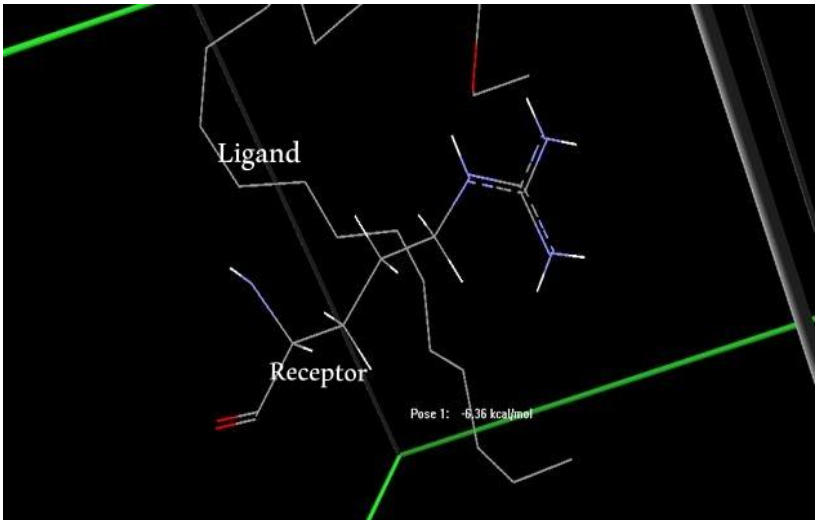

Figure 5: Ethyl octadecanoate in complex with Human Pancreatic Alpha-Amylase

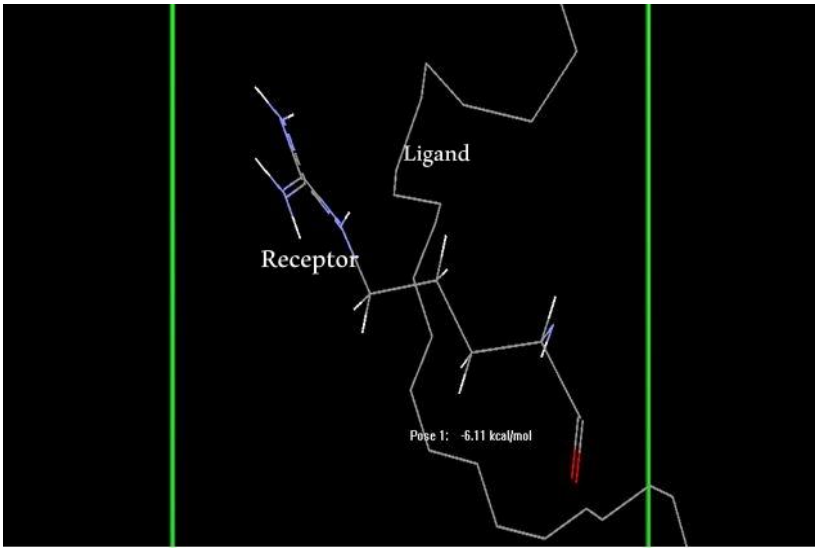

Figure 6: Dotriacontane in complex with Human Pancreatic Alpha-Amylase

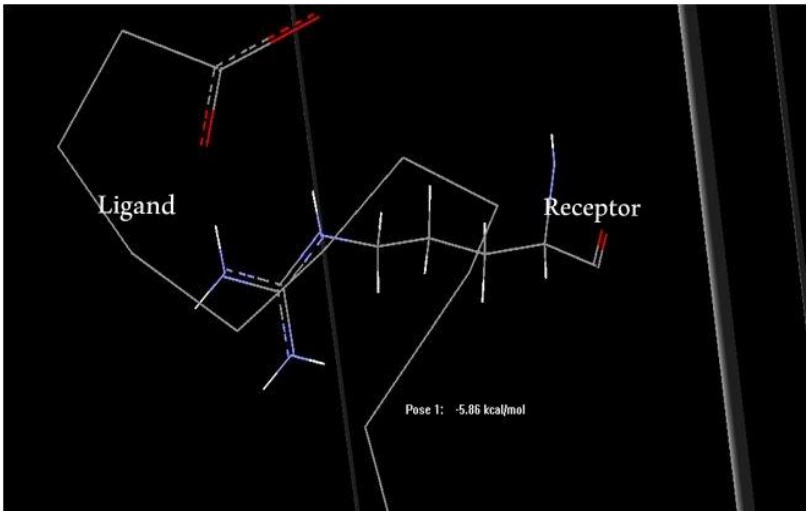

Figure 7: n-hexadecanoic acid in complex with Human Pancreatic Alpha-Amylase

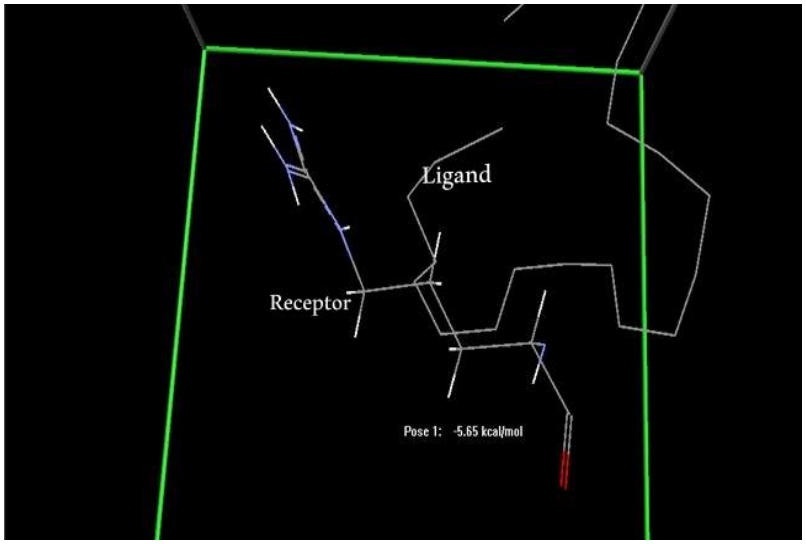

Figure 8: Pentacosane in complex with Human Pancreatic Alpha-Amylase

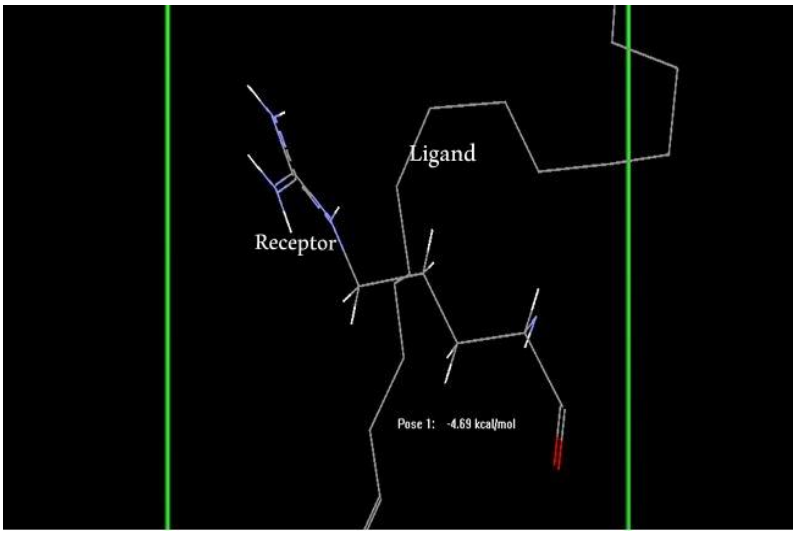

Figure 9: 1-Octadecene in complex with Human Pancreatic Alpha-Amylase

The compounds were docked against the target Human Pancreatic Alpha-Amylase (PDB ID-4X9Y) using Argus Lab with default parameters. From the table 6, it is clear that Ethyl octadecanoate, a compound present in the ethanol extract of *Mangifera indica* has good docking activity with the least E value of – 6.35 kcal/mol. Lesser the E-value greater the acceptability of compound as a drug and so it is considered as an effective ligand in inhibiting Human Pancreatic Alpha-Amylase.

4.0 CONCLUSION

The current study helps us to conclude that the hexane, ethyl acetate and ethanol extracts of *Mangifera indica* leaves are found to be rich in carbohydrates, tannins flavonoids, steroids and alkaloids. Ethanol extract of *Mangifera indica* possess a high phenolic content than ethyl

acetate and hexane extracts. *In vitro* scavenging effect is observed good in the ethanol extract of *Mangifera indica*. It is also observed that ethanol extract of Mangifera indica possess *in vitro* antidiabetic activity by inhibiting α-amylase.This property is beneficial in discovery of novel phytomedicine for the treatment of diabetes. *In silico* analysis also shows that a compound 'Ethyl octadecanoate' present in ethanol extract of *Mangifera indica* has a good docking activity with the least E value (– 6.35 kcal/mol) when molecularly docked against Human Pancreatic Alpha-Amylase using ArgusLab and may lead to the development of more effective chemical entities with antidiabetic property.

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