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Evaluation of phytochemical constituents, *in-vitro* anti-diabetic activity of extracts from *Monstera deliciosa* liebm leaves

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Abstract: *Monster deliciosa is a medicinal plant, which originated from the tropical forests of southern Mexico and Panama. The plant belongs to the family Araceae. There are several chemical compounds in the plant that have distinct pharmacological characteristics. The aim of the present study was to investigate the phytochemical bioactive compound and in vitro anti-diabetic activity from extracts of Monster deliciosa leaves. The experiment results imply that the existence of bioactive chemicals may be the basis for this plant's wide range of therapeutic qualities, including its ability to treat diabetes. Phytochemical screening was carried out using standard protocols. The anti-diabetic activity was evaluated by α-amylase inhibition assay that was performed using 3, 5-dinitrosalicylic acid (DNSA) method. The phytochemical screening study of Monstera deliciosa leaves extracts revealed the presence of important phytochemicals namely Tannins, Steroids, Flavonoids, Alkaloids and Saponins. The results of this study suggest the medicinal importance of this plant due to the presence of various phytochemicals.*

Key Words: *Monstera deliciosa, Phytochemical screening, anti-diabetic activity, α-amylase inhibition assay, 3, 5-dinitrosalicylic acid (DNSA) method.*

1. INTRODUCTION:

The term "medicinal plants" refers to several different kinds of plants employed in herbalism, some of which have therapeutic properties. these medicinal plants are regarded as a rich source of components that can be employed in the creation and synthesis of drugs. The active components that are employed in the manufacture of various medications are regularly extracted from medicinal plants using raw materials. the active components of taxol, vincristine, and morphine, respectively, were found in foxglove, periwinkle, yew, and opium poppies [1].an aberrant metabolism of proteins, lipids, and carbohydrates is a feature of diabetes mellitus, which is defined by high plasma glucose concentrations brought on by either insulin resistance or insufficiency [2].some herbal plants and their chemically active components are crucial in the treatment of diabetes mellitus [3]. amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to α-amylases are starch degrading enzymes that catalyze the hydrolysis of internal α-1,4-o-glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in low molecular weight products, such glucose, maltose and malt triose units. most of the a-amylases are metalloenzymes, which require calcium ions (ca2+) for their activity, structural integrity and stability. they belong to family 13 (gh-13) of the glycoside hydrolase group of enzymes [4].numerous plants are regarded as a major source of effective anti-diabetic medications. Although utilizing insulin and artificial oral hypoglycemic medications are the major technique of controlling diabetes [5]. *monstera deliciosa* also called as fruit salad plant belongs to araceae family native to mexico. Common names include fruit salad plant (in reference to its edible leaves and fruits), ceriman and swiss cheese plant. in the present study, an attempt was made to evaluate the phytochemical screening, anti-diabetic activity and also to determine the alkaloids, tannin, saponins, terpenoids, steroids and flavonoid contents of *monstera deliciosa* leaves extracts of different solvents.

Figure no 1: ***Monstera deliciosa leaves***

Table no 1: scientific classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Order	Alismatales
Family	Araceae
Subfamily	Monsteroideae
Genus	*Monstera*
Species	*M. deliciosa*

2. MATERIALS AND METHODS:

Plant Collection, Identification and Authentication:

Fresh leaves of *Monster deliciosa* were collected locally from Sri Anjanadri nursery and farms and Plant materials was identified and authenticated by research officer (botany) Dr. V. Rama Rao in Central Ayurveda Research Institute.

Preparation of extracts

The collected leaves of *Monster deliciosa* were washed well, shade dried and powdered. The plant parts were then separated and dried which were then powdered using a grinder.

1. Soxhlation technique

Equipped with a 500Ml boiling flask, a weighed sample (20 g) of powdered material was extracted at 60–70 °C for 24 h under reflux with 350 mL methanol in a round bottomed flask heated by using heating mantle and filtered by using Whatman No. 1 filter paper. After filtration and extraction, the content was concentrated on a hot plate. The crude extract wassubsequently oven dried at a temperature of 50 °C to form a powdery residue and the yield of crude was calculated [6].

2. Maceration technique

3g of concentrated residue taken in 250ml beaker and 30ml of solvent was added (Methanol, Chloroform, ethyl acetate, hexane). Cold maceration techniques were used for extraction. The solvent with powder were kept for 24hr at room temperature, with occasional shaking.

3. PRELIMINARYPHYTOCHEMICAL SCREENING:

Phytochemicals are responsible for medicinal activity of plants; these are non-nutritive chemicals that have protected human from various diseases. The major constituents consists of Alkaloids, Flavonoids, Saponins, Phenolic Compounds, Phytosterols, Proteins and amino-acids, gums and mucilage and lignin. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries; the constituents are playing a significant role in the identification of crude drugs [7].

1) Test for alkaloids :
a) Dragendroff's/ Kraut's test Few mL filtrate + 1-2 mL Dragendorff's reagents, Orange Red precipitate or reddish-brown precipitate was taken as positive.
b) Hager's test Few mL filtrate + 1-2 mL Hager's reagents, creamy white precipitate will be taken as presence of alkaloids.
c) Mayer's/ Bertrand's/ Valser's test few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube) A creamy white/yellow precipitate, will be taken as presence of alkaloids.
d) Wagner's test Few mL filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube) a brown/reddish precipitate will be taken as presence of alkaloids [8].

2) Test for flavonoids
a) Ferric chloride test Two ml of the test solution was boiled with distilled water and filtered. Then, few drops of 10% ferric chloride solution were added to the 2 ml of filtrate. A greenish blue or violet coloration indicates the presence of a phenolic hydroxyl group.
b) Shinoda's test Five grams of each extract was dissolved in ethanol, warmed and then filtered. Small pieces of magnesium chips were then added to the filtrate followed by few drops of conc. HCl. The pink, orange, or red to purple coloration indicates the presence of Flavonoids.
c) Sodium hydroxide test Extract of 0.2 gm was dissolved in water and filtered. To this, 2 ml of the 10% aqueous sodium hydroxide was added to produce yellow coloration. A change in colour from yellow to colorless on addition of dilute hydrochloric acid was the indication for the presence of Flavonoids.
d) Lead acetate test Extract of 0.5 gm was dissolved in water and filtered. To the 5 ml of each filtrate, 3 ml of lead acetate solution was added. Appearance of a buff-colored precipitate indicates the presence of Flavonoids.

3) Test for Tannins
a) Gelatin test Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl A white precipitate.
b) Braymer's test 1mL filtrated + 3mL distilled water + 3 drops 10% Ferric chloride solution Blue-green color.
c) NaOH test 0.4mL plant extract + 4mL 10% NaOH + shaken well Formation of emulsion {Hydrolysable tannins}.

4) Test for Terpinoides
2ml chloroform + 5mL plant extract, (evaporated on water bath) + 3mL conc. H2SO4 (boiled on water bath) a grey colored solution.

5) Test for Saponins
Crude powder of 0.5 g was shaken with water in a test tube and it warmed in a water bath. The persistent froth indicates the presence of saponins.

6) Test for carbohydrates
a) Molisch's test Two ml of Molisch's reagent was added to the extract dissolved in distilled water and 1 ml of conc. H2SO4 was dispensed along the walls of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a dull violet colour at the inter phase of the two layers indicates the positive test for carbohydrates.
b) Fehling's test (for free reducing sugars) the crude extracts were treated with 5.0 ml of Fehling's solution (A & B) and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of free reducing sugars.
c) Fehling's test (for Combined Reducing Sugars) Extract of 0.5 g was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. To this, few drops of Fehling's solution were added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.
d) Barfoed's test (for monosaccharide) in distilled water, 0.5 g of the extract was dissolved and filtered. To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharide.

7) Test for steroids
a) Salkowskii test in 2 ml of chloroform, 0.2 g of extract was dissolved and added the conc. H2SO4. The development of reddish brown color at inter phase indicates the presence of steroids.

b) Keller-Killiani test to 0.5 ml of test solution, 2 ml of 3.5% FeCl3, and small amount of glacial acetic acid and 2 ml of conc. H2SO4 were added carefully. Appearance of reddish-brown ring at inter phase is a positive indication for the presence of steroids.

c) Liebermann-Burchard test to 0.2 g of each extract, 2 ml of acetic acid was added and the solution was cooled well in ice followed by the addition of conc. H2SO4 carefully. Color development from violet to blue or bluish-green indicates the presence of a steroidal ring (i.e., Aglycone portion of cardiac glycoside) [9].

4. ISOLATION OF ACTIVE CONSTITUENTS FROM THE EXTRACT:

Detection of mobile phase:

The Methanol extract was introduced to activated TLC plates using a capillary tube at 1/2inch besides the lower edge of the TLC plate, and thus the plate was left in a developing chamber bearing a proper solvent system for a specified time. Once the developing solvent reached the top of the top edge of the TLC plate, the plate has been removed from the chamber; the solvent front was marked with lead pencil and dried. Visual detection of compoundbands/spots has been carried out on TLC plate which detected under UV light (254nm) for the presence of specific compounds. The spots of the components in the TLC plate were marked and the *Rf* value of each spot was calculated by the formula:

$$Rf = \frac{\text{distance traveled by compund}}{\text{distance traveled by slovent front}}$$

Column chromatography:

Partial purification of ethyl acetate extract was carried out using silica gel column chromatography. Glass column was packed by wet method. The adsorbents slurry (silica gel; 60-120) was prepared by stirring the adsorbent with the same mobile phase and used as a stationary phase. Then, it was dripped into the glass column (43 cm x 3.5 cm) (sintered glass disk at the bottom) and allowed to remain and settle. The air entrapped was removed by tapping the column with rubber tube. A small amount of sand and cotton was kept at top of the column toprovide the latter a flat base. Excess solvent was run off once the mobile phase level dropped to 1cm just above the upper edge of the sand and cotton layer.3g of methanol extract was mixed with 3g of silica gel as stationary phase loaded onto the column flow rate was set to1ml/min. The column was eluted with methanol, chloroform (0.5:9.5) mobile phase. Separation of bioactive constituents from first mobile phase was carried out by eluting the column at uniform interval (3 drops per minute), the eluents (each of five ml) which were collected in test tube and the progress of separation was monitored by thin layer chromatography (TLC) (silica gel G 60 F254TLC plates of E. Merck, layer thickness0.2mm) using the same solvent system.

***In vitro* anti-diabetic assay**

Assay for α-amylase inhibition

In vitro α-amylase inhibitory studies the α-amylase inhibition assay was performed using the 3, 5-dinitrosalicylic acid (DNSA) method. The leaf extract of *Monstera deliciosa* was dissolved in minimum amount of 10% DMSO and was further dissolved in buffer ((Na2HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 10 to 100 µg/ml. A volume of α-amylase solution (1 units/ml) was mixed with minimum amount of the extract and was incubated for 10 min at 30 °C. Thereafter prepare a 1.0% w/v soluble starch solution was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 96 mM 3, 5-dinitrosalicylic reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3, 5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer.

The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µl of buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (100 µg/ml–2 µg/ml) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

The % α-amylase inhibition was plotted against the extract concentration and the IC50values were obtained from the graph [10].

Inhibition (%) = Abs 540 (control) – Abs 540 (extract) *100 ÷Abs 540(control)

Statistical analysis

All the analyses were carried out in triplicate and the results were expressed in mean ± SD.

5. INFRARED SPECTROSCOPY:

The infrared spectroscopy is suitable for the assessment of chemical compounds based on the plots of light intensity as a function of wavelength within the infrared range of electromagnetic spectrum. It enables the fingerprinting of substances, as well as detection and quantitative analysis of admixtures and additives present in a given substance [10]. the use of midrange infrared for spectral interpretation is a commonplace tool in pharmaceutical laboratories. For most materials, however, a dilute solution or mixture must be made of the substance to be measured. This is due to the high molar absorptivities of compounds in the IR and the fact that most instruments measure in the transmission mode [11].

LC-MS

Liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques became increasingly important for identification and quantification purposes. LC-MS/MS methods are targeting screening analysis for molecules with similar pharmacological activity or chemical structure, including thermolabile and non-volatile compounds, achieving high sensibility and specificity [12].

6. RESULT AND DISSCUSION:

EXTRACTIVE VALUE

The dry powdered plant materials of *Monstera delicosa* were extracted with methanol, chloroform, ethyl acetate, hexane and water using maceration process. 3g of concentrated residue was taken in 250 ml conical flask with stopper and 50 ml of solvent was added (hexane, ethyl acetate, chloroform, and methanol). Cold maceration technique was used for extraction. The solvent with powder were kept for 24 h at room temperature, with occasional shaking. The mixture was filtered through Whatman No: 1 filter paper. Different extracts were weighed quantitatively and percentage with respect to the weight of the plant material taken was calculated [13]. The methanol extract gives better extractive values. The extractive value in percentage was calculated by using:

Extractive value = $Weight\ of\ the\ dried\ extrct / Weight\ of\ the\ drug \times 100$.

Table no 2: The percentage yield of various extracts of *Monstera deliciosa* leaves

Sl no	Solvent	Extract in (g)	Values
1	Methanol	0.62	20.6%
2	chloroform	0.51	17%
3	Ethyl acetate	0.33	11%
4	Hexane	0.47	15.6%

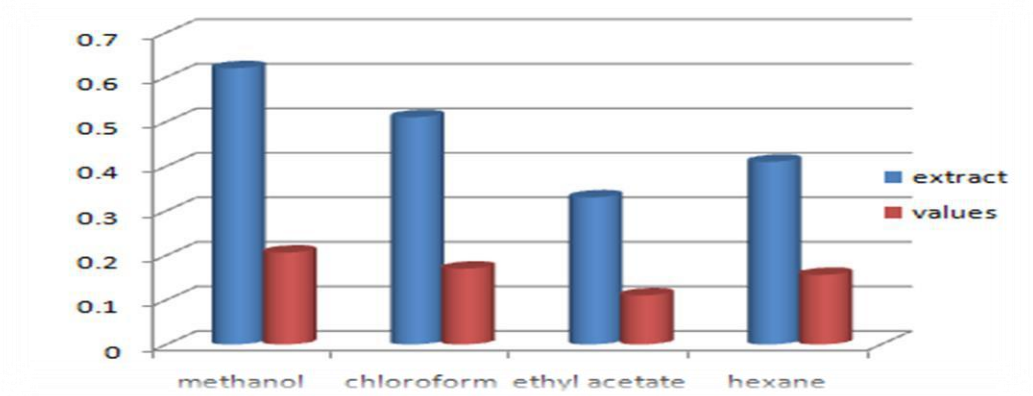

Figure no 2: Extractive values

7. PHYTOCHEMICAL SCREENING:

The results of phytochemical screening of different extracts of *Monstera deliciosa* plant were reported. The phytochemical study revealed the presence of various Phyto compounds in different solvent extracts.

Table no 2.1: Phytochemical screening

Sl no	Phytochemicals	H	C	E	M
1	**alkaloids**	-	+	+	++
2	**Flavanoids**	+	-	-	++
3	**Tannins**	-	+	+	+
4	**Terpenoids**	-	-	-	-
5	**Saponins**	-	-	+	+
6	**Carbohydrates**	-	+	+	+
7	**Steroids**	+	+	++	++

H- Hexane, C- Chloroform, E-Ethyl acetate, M-Methanol.

The phytochemical screening study of *M. deliciosa* leaves extracts showed the presence of various phytochemicals in different solvent extracts (Table-2.1). Tannins, Steroids, flavonoids, alkaloids, terpenoids and Carbohydrates were found to be present in the solvent extracts. Flavonoids were present in all the solvent extracts except the Ethyl acetate and chloroform extract. Alkaloids, tannins and carbohydrates were present all the solvent except in hexane extract. Steroids were present in all solvent extracts. However, Terpenoids were found to be absent in all solvent extracts.

8. ISOLATION OF ACTIVE CONSTITUENTS FROM THE EXTRACT:

Thin layer chromatography (TLC)

About 20 TLC plates were eluted using different solvent in which the TLC plates showing number of bands (chemical compounds) for each fraction, that can be further isolated and purified using Column Chromatography (CC). Out of 20 TLC plates using different solvents (single solvent and in combination), TLC plate with good separation were selected for carrying out the Column Chromatography. The selected mobile phases with good separation compares to other mobile phase are found in TLC plate, methanol: chloroform in the ratio of (0.5:9.5).

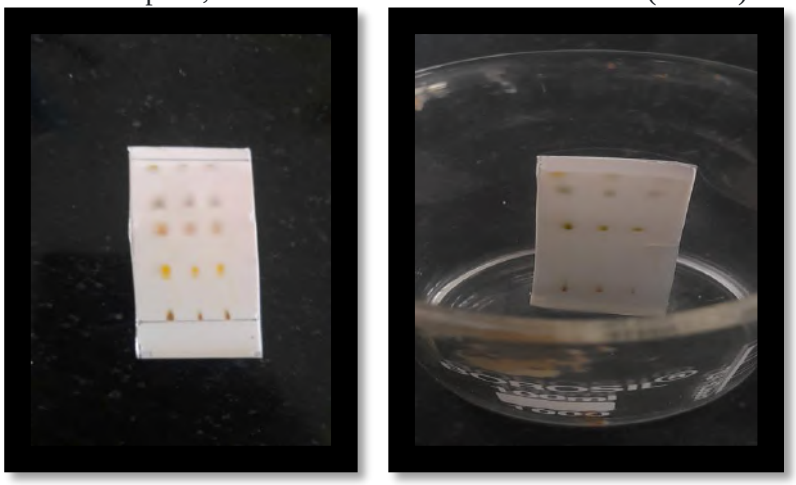

Figure no 2.3: Methanol: Chloroform in the ratio of (0.5:9.5).

ISOLATION OF PHYTOCONSTITUENTS FROM METHANOL EXTRACTS OF *MONSTERA DELICIOSA* LEAVES.

Column chromatography

The same solvent system which found by TLC chromatography, Methanol: Chloroform (were used for column chromatography. For first run of the column, 4 fractions were collected. Incidence of number of spots was deliberated as benchmarks for selection of fraction for isolation of pure compound. All the fractions were checked with TLC plate and the selection has been done based on the selecting the fraction with same Rf value.

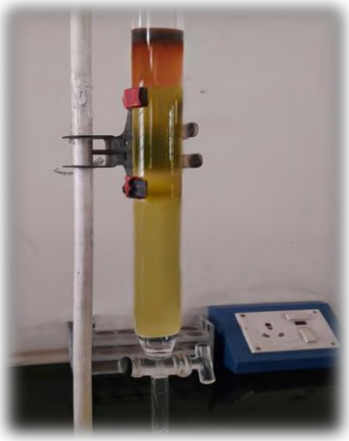

Figure no 2.4: Separation of phytoconstituents using column chromatography technique.

IN VITRO ALPHA-AMYLASE INHIBITORY STUDIES

The plant extracts were prepared sequentially with methanol, chloroform, ethyl acetate and hexane. Each extract was evaporated using rotary evaporator. Different concentrations (10, 20, 40, 60, 80, and 100 µg/mL) of each extract were made by using 3, 5-dinitrosalicylic acid (DNSA) method and subjected to α-amylase inhibitory assay using Acarbose as a substrate. The absorbance was read at 540 nm using spectrophotometer. Using this method, the percentage of α-amylase inhibitory activity and IC50 values of each extract was calculated.

Table 2. 2: Alpha-amylase inhibitory effects of acarbose standard α-amyalse inhibitor.

Drug	Concentration (µg/mL)	% of Inhibition	IC50 value (µg/mL)
Acarbose	10	18.75	84.53 ± 18.4
	20	23.41	
	40	28.02	
	60	38.71	
	80	42.22	
	100	58.45	

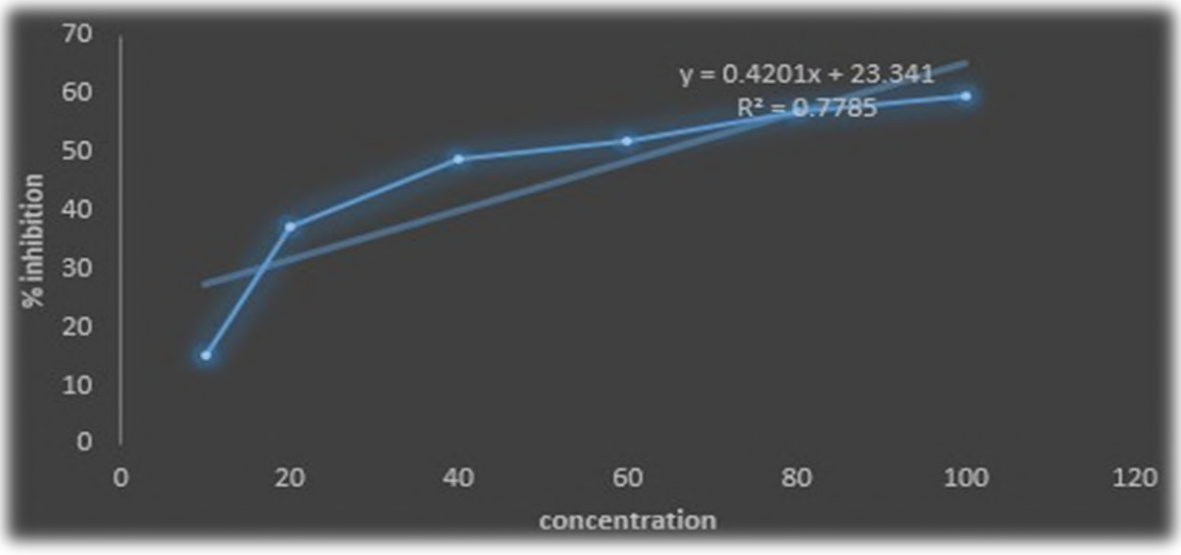

Figure no 2.5; Alpha-amylase inhibitory effects of acarbose standard α-amyalse inhibitor.

Table 2.3: Alpha-amylase inhibitory effects of leaves extracts of *Monstera deliciosa*

Drug	Concentration (µg/mL)	% of Inhibition	IC50 value (µg/mL)
Leaves extract	10	15.09	63.47 ± 11.06
	20	37.49	
	40	48.79	
	60	52.14	
	80	56.88	
	100	59.89	

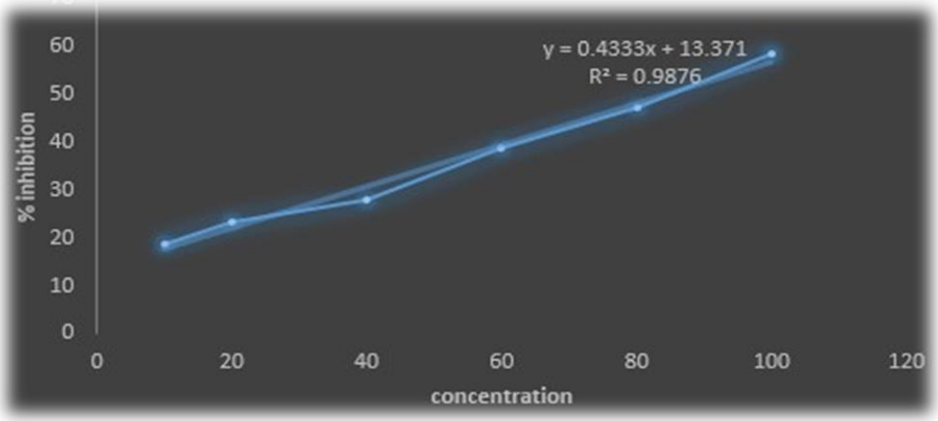

Figure 2.6: Alpha-amylase inhibitory effects of leaves extracts of *Monstera deliciosa.*

Different plant extracts showed appreciable α-amylase inhibitory effects when compared with acarbose. It may be due to the presence of more chemical constituents such as lignans (phyllanthin and hypophyllanthin), terpenes, flavonoids (quercetin, quercetrin, rutin), and alkaloids like glycoalkaloids (α-solanine and α-chaconine) in the different leaves extract. The plant-based α-amylase inhibitor offers a prospective therapeutic approach for the management of diabetes. In this study, leaves part of the plants of *Monstera deliciosa* showed considerable α-amylase inhibitory effects when compared with acarbose. Acarbose (at a concentrations 100 µg/mL) showed 92.27% inhibitory effects on the α-amylase activity with an IC50 value 62.86 ± 18.4 µg/mL [Table 4]. The Methanol extracts of *Monstera deliciosa* leaves (at a concentration 100 µg/mL) exhibited 61.11% of α-amylase inhibitory activity with an IC50 values 37.66 ± 10.02 µg/mL [Table 5]. However, the methanol extracts of *Monstera deliciosa* leaves showed appreciable α-amylase inhibitory effects when compared with acarbose.

IDENTIFICATION AND DETECTION OF SUBSTANCES WITH FT-IR

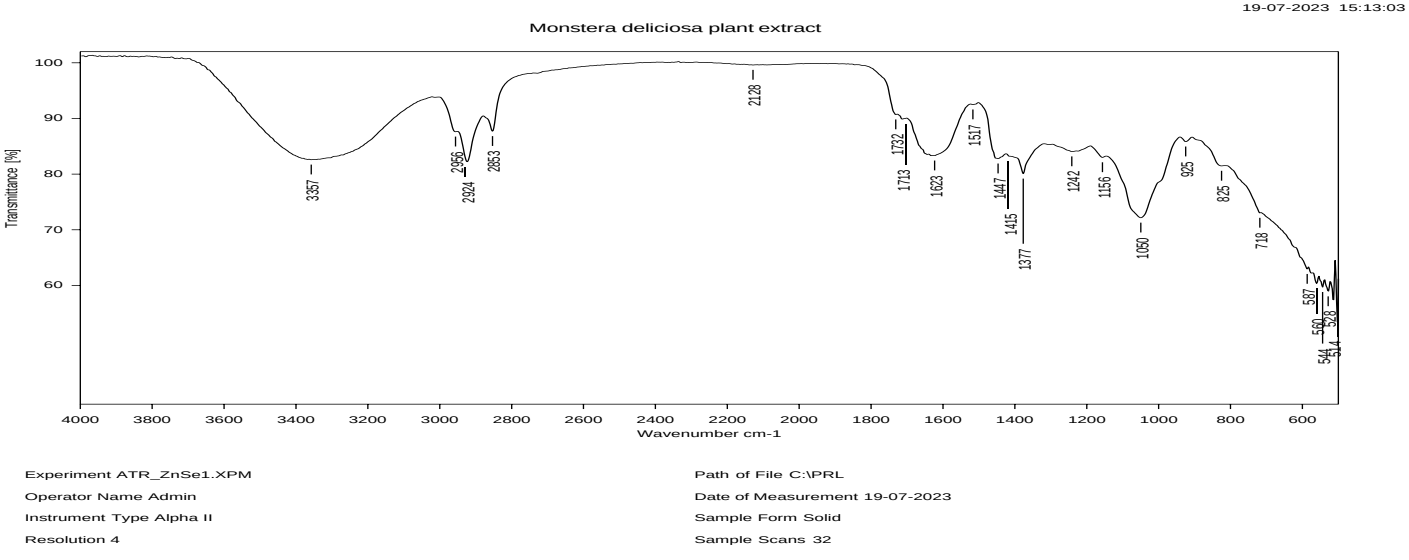

Figure no 2.7: IR spectra of Methanol extract of *Monstera deliciosa*

Table no 6: IR Interpretation Values

IR Values	Functional group
3550-3200	OH stretching
2100-1650	Aromatic group
1750-1735	C=O stretching
1650-1600	C=C stretching
1415-1380	S=O stretching
1275-1200	C-O stretching
850-556	C-Cl stretching

IDENTIFICATION AND DETECTION OF SUBSTANCES WITH LC-MS

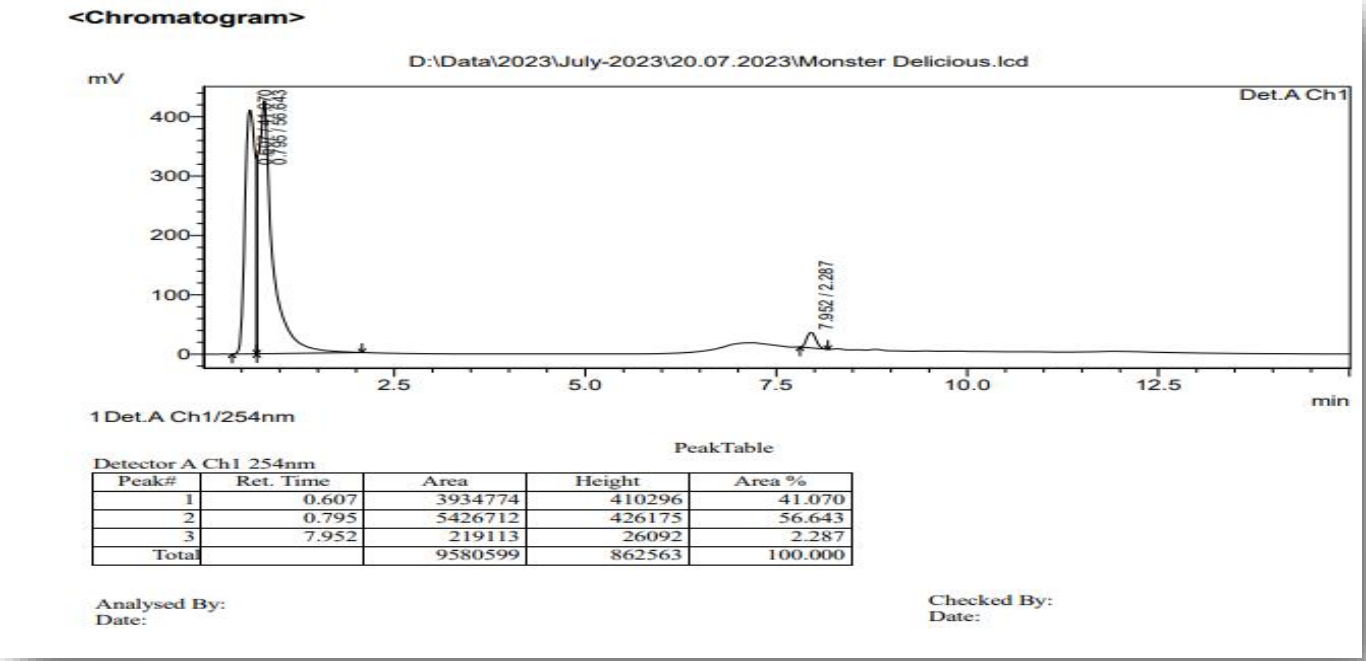

Figure 2.8: LC-MS spectra of Methanol leaves extract of *Monstera deliciosa*

Monstera deliciosa contains glycoalkaloids a prototypical glycoalkaloid is solanine (composed of the sugar solanose and the alkaloid solanidine). The alkaloidal portion of the glycoalkaloid is also generically referred to as an aglycone. The high molecular weight also indicates steroids such as stigmasetrol, etc.It also reveals that the plant possesses endophytic bacteria that release secondary metabolites such as peptide antibiotic. *Monstera species* peptide antibiotic reported was coronamycins whose fragments were found to be 435,546,631,960. It's UV detection range between 260-280nm.

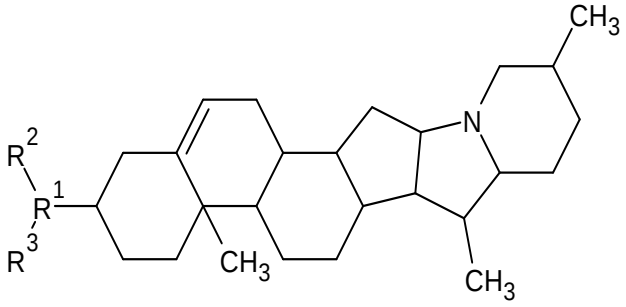

Figure 2.9: glycoalkaloids α-solanine and α-chaconine

The structure of glycoalkaloids **α-solanine and α-chaconine** (R 1 =β-D-galactose, R 2 =β-D-glucose and R 3 =αL-rhamnose for α-solanine, and R 1 =β-D-glucose and R 2 =r 3 =α-L-rhamnose for α-chaconine).

9. CONCLUSION:

The results of study indicate that methanol extracts of *Monstera deliciosa* leaves showed appreciable α-amylase inhibitory effects. From the results, it can be concluded that use of these plant extracts will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes by decreasing the post-prandial hyperglycemia. Future studies will provide an insight for the molecular mechanisms by which these plant and their active compounds regulate glucose homeostasis. This study supports the ayurvedic concept; *Monstera deliciosa* leaves could be useful in management of diabetes.

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