Research Article

In vitro Antidiabetic Activity of Root and Bioactive Fractions in Extract of *Decalepis hamiltonii* Wight & Arn

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ABSTRACT

Plants are naturally produced important sources of medicines. Today, a large number of drugs in use is derived from plants. A *Decalepis hamiltonii* (Asclepiadaceae) to treat the various diseases of to human such as diabetic and cancer. To present investigate on antioxidant activity, invitro antidiabetic activity and *In silico* approaches for antidiabetic activity of the plant *Decalepis hamiltonii*. The methodology includes, DPPH, ABTS and In vitro diabetic assay α-glucose and α-amylase. *In silico* approaches for antidiabetic activity of major compounds for GC-MS. Docking analysis was carried using PBRY protein and ERRα receptor using for protein target gene interaction. The results showed that methanolic root extract of *Decalepis hamiltonii* has higher percentage of inhibition for DPPH (93%), ABTS (90%) which is comparable with respective standards and dose increase in percentage antidiabetic enzymes activity α-glucose (82% to 92%) and α-amylase enzyme (73% to 90).

Keywords: DPPH, ABTS, *Decalepis hamiltonii,* Ascorbic acid**,** α-amylase, α-glucosidase.

INTRODUCTION

Plants that possess medicinal properties and wield beneficial pharmacological effects on the human body are generally designed as medicinal plants. Medicinal plants have been used as a seedbed of drugs by mankind for thousands of years. Ancient man depended on green plants for his day-to-day needs of medicaments. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. However, plants were used as a basis of some of the most important drugs, even in the modern system of medicine. Traditional system of medicine continues to be widely practiced on many accounts.

Population growth, insufficient supply of drugs, high cost of treatments, side effects of synthetic drugs and development of resistance to currently used drugs for infectious disease have led to increased accent on the use of plant materials as a source of medicine for a wide variety of human illness[1] (Schulz).

Diabetes mellitus is a various disorder characterized by derangement of carbohydrate, protein and lipid metabolism. It arises from complex interactions between multiple genetic and environmental or lifestyle factors.

Antidiabetic effect of medicinal plants can be studied *in vitro* using various test systems like testing inhibitory activity of α--amylase, α--glucosidase, inhibition of intestinal glucose uptake using isolated diaphragm, secretion of insulin from beta-cells of pancreas using various cell-lines and *in vivo* using animal models. *In vitro* tests can play a very significant role in the evaluation of the antidiabetic activity of drugs as initial showing tools where the screening of beneficial candidates may be required. However, previous reports have also indicated that excessive inhibition of pancreatic alpha amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in the colon and therefore mild α - amylase inhibition activity is useful (Bharathi). They might provide useful information on the mechanism of action of therapeutic agents. Biological material used in these includes perfused whole organs, isolated tissues, cell culture systems, or tissue slice preparations, while *in vivo* biological systems use live animals.

Data from experiments carried out under *in vitro* systems can establish mechanisms and define toxicities while *in vivo* systems are necessary to study how such mechanisms behave under clinical or pathophysiological conditions (Thorat).

In silico technique help identifying drug target for bioinformatics tools. They can also be used to explore the target structures for possible active sites, generate candidate molecules, dock these molecules with the target, rank them according to their binding affinities and further optimize the molecules to develop binding characteristics (Bharathi). *Decalepis hamiltonii* Wight& Arn. Also known as swallow root, is a woody climber belonging to the family Asclepiadaceae.

In vitro, *in vivo* and *in silico* approaches can help in screening and identification of lead pharmacological principles from medicinal plants.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Gallic acid (GA), Ascorbic acid, BHT, Quercetin, ABTS were purchased from Himedia (Mumbai, India).

All other chemical reagents used were of analytical grade.

Collection of Material

The Root of *Decalepis hamiltonii* has been collected from Kolli hills, Namakkal district of TamilNadu, India. The taxonomic identification of the plant was done with the help of the Flora of Presidency of Madras, by Gamble J.S., 1921.

Preparation Solvent Extraction

50gm of *Decalepis hamiltonii* root was packed in Soxhlet apparatus for extraction and 500 ml of Petroleum ether, chloroform Ethylacetae and methanol was used as solvent. Soxhlet was kept running for 72 hours, until the solvent colour appears in the collection tube. The residue was then placed in an oven at 40°C for about 48hours to remove the moisture. The resulting dried mass was then powdered and used for further studies.

DPPH Scavenging Assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method (Shimada). Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. L-Ascorbic acid was used as the standard. Where $A_{C=}$ control is the absorbance of the control and $A_{S\ =}$ sample is the absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates (n = 3), and the average values were calculated.

ABTS Scavenging Assay

The antioxidant effect of the leaf extracts was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method (Re). ABTS radical cations ($ABTS^{+}$) were produced by reacting ABTS solution (7mM) with 2.45mM potassium persulphate.

The mixture was incubated at room temperature in the dark for 12 to 16 hrs to yield a dark-colored solution containing $ABTS^{\bullet+}$ radicals and diluted for an initial absorbance of about 0.700 (±0.02) at 734 nm. Aliquots (10µl) of the different concentrations of the extract were added to 1ml of ABTS solution. The absorbance was read at 734nm after 6 minutes in a spectrophotometer. L-Ascorbic acid was used as the standard. Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicate and the percent of inhibition was calculated using the formula.

In *vitro* Alpha-Amylase Assay

The extracts were tested for alpha-amylase inhibitory potential of the method of Bernfeld with slight modifications as described below. The reducing sugars produced by the action of α-amylase react with dinitrosalicylic acid and reduce it to a coloured product, which is measured at 540 nm. The amylase inhibitor inhibits the action of amylase that hydrolyses starch to maltose. In brief, 100 µL of various extracts (Petroleum ether, chloroform Ethylacetae and methanol) were allowed to react with 200 µL of porcine pancreatic α-amylase enzyme (dissolved 1 mg α-amylase / mL of 0.1 M sodium acetate buffer, pH 4.7) and µL of 0.1 M sodium acetate buffer, pH 4.7. After 20 min of incubation 0.5 mL of 1% starch was added. The same was performed for the control where 200 µL of enzyme was replaced by the buffer. Incubated for 15 minutes and added 1 mL of DNS (dissolved by stirring 1 g dinitrosalicylic acid, 200 mg crystalline phenol in 100 mL of 1% NaOH solution, 50 mg sodium sulphite added at the time of use) to both the control and test. The tubes were kept in a boiling water bath for 10 minutes. At hot conditions added 0.5 mL of 40 % potassium sodium tartarate and made up the volume to 5 mL with distilled water. The absorbance was recorded at 540 nm using a spectrophotometer and the percentage of α-amylase inhibition was calculated using the formula,

$$Inhibition\ (\%) = 100\left(\frac{Absorbance\ Control - Absorbance\ Test}{Absorbance\ Control}\right)$$

***In vitro* Alpha-Glucosidase Assay**

The extracts to be tested for their inhibitory property of α-glucosidase activity were prepared as mentioned various concentration. The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from p-nitrophenyl α-D glucopyranoside as given by (Sun *et al*1995). The assay mixtures for these experiments contained 0.3 mL of 10 mM paranitrophenyl alpha-D-glucopyranoside, 1.0 mL of 0.1 M potassium phosphate buffer, pH - 6.8, 0.2 mL of enzyme solution and 0.2 mL of various extracts (Petroleum ether, chloroform Ethylacetae and methanol) all in a final volume of 1.7 mL. Following an incubation time of 30 min at 37°C, the reaction was terminated by the addition of 2.0 mL of 100 mM sodium carbonate. The liberated p-nitrophenol was determined at 400 nm using spectrophotometer. The percentage inhibition rates were calculated using the formula.

Molecular Docking

Compound Identification for GC-MS Analysis (Gas Chromatography-Mass spectroscopy)

Compounds Screened

The compounds namely 2-hydroxy-4-methoxybenzaldehydewere identified by GC-MS analysis were screened against the antidiabetic protein. The compound details were retrieved from the Pubchem database and the chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Chemsketch Software (www.acdlabs.com).

Protein Data Bank

The Protein Data Bank (PDB) archive is the single worldwide repository of information about the 3D

structures of large biological molecules, including proteins and nucleic acids. The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank, WWPDB.

Preparation of Protein Structure

The structural information of the macromolecules determined by x-ray crystallographic and NMR methods are available in the PDB. The 3D structureantidiabetic protein Receptor (PDB I.D: 4IFI) was downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) the water molecules were removed from protein file **4IFI** before docking. Energy minimization by applying for CHARMM (Chemistry at Harvard Macromolecular Mechanics) force fields, It's a program for macromolecular dynamics; it can be used for energy minimization, normal modes and crystal optimizations and also incorporates free energy methods for chemical and conformational free energy calculations.

Pubchem

The Pubchem bioassay data (http://pubchem.ncbi.nlm.nih.gov) is a public repository for biological Activities of small molecules and small interfering RNAs (siRNAs) hosted by the US National Institutes of Health (NIH). It archives experimental descriptions of assays and biological test results and makes the information freely accessible to the public. A Pubchem Bioassay data entry includes an assay description, a summary and detailed test results. Each assay record is linked to the molecular target, whenever possible, and is cross-referenced to other National Center for Biotechnology Information (NCBI) database records. 'Related Bioassays' are identified by examining the assay target relationship and activity profile of commonly tested compounds. A key goal of PubChem Bioassay is to make the biological activity information easily accessible through the NCBI information retrieval system- Entrez, and various web-based PubChem services. An integrated suite of data analysis tools is available to optimize the utility of the chemical structure and biological activity information within PubChem, enabling researchers to aggregate, compare and analyze biological test results contributed by multiple organizations. Describe the PubChem BioAssay database, including data model, bioassay deposition and utilities that PubChem provides for searching, downloading and analyzing the biological activity information.

Preparation of Ligand Structures

The identified Chemical compound namely 2-hydroxy-4-methoxybenzaldehydewas derived from *Decalepis hamiltonii* Wight & arn. and this compound structure were retrieved from Pubchem online server both of these compounds were under investigation of Chemsketch (Chemically intelligent drawing interface free ware developed by Advance Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands.

The ligand molecules were generated and the three dimensional optimizations were done and then saved MOL file (a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule).

Drug Likeliness Prediction

Ligand property was predicted by using "Lipinski drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp). Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipinski filter helps in early preclinical assessment and thereby avoiding costly late stage preclinical and clinical failures.

Docking Analysis

Thedocking analysis is performed by Arguslab 4.0.1 for the antidiabetic protein interacts with GC-MS of major compound 2-hydroxy-4-methoxybenzaldehyde.

The compound or ligand selected for based on Lipinski's rule of five. Fitting points are added to hydrogen bonding groups on the protein.

The interaction between the binding pockets of target antidiabetic protein and investigation compound to find out the accurate binding model for the active site of protein.

The mechanism of ligand placement is based on binding site position.

The protein ligand docking energy values performance of this compound was based on the Scoring functions which is implemented in docking program to make various assumptions and simplifications to fit best complexes, which includes terms of hydrogen bonds employed by Arguslab 4.0.1 to rank the docked bases and to assess the binding site and the number of rotatable bonds present.

Ligand Binding Sites Prediction

After docking the docked structure was saved as ".pdb" file and further explored to predict the binding sites using "ligand explorer" software.

The predicted binding sites, based on the binding energy, and amino acids make up the binding cavity. Here ligand binding site represents the site where the ligands most efficiently bind with the protein, among all the active site.

Discovery Studio Visualizer

The docking results were visualized using Accelyrs Discovery Studio 4.1 Visualizer. The discovery studio

visualizer is also a free viewer that is designed to offer an interactive environment for viewing and editing molecular structures, sequences, X-ray reflection data, script and other data.

DS Visualizer is handier for analyzing the docking results.

Discovery Studio is designed for use in the Life Sciences, with focus on the study of biologically relevant structures. These range from small molecules, such as drugs and inhibitors, to larger molecules such as proteins and nucleic acid biopolymers. Form typically determines function of molecular systems.

A large body of experimentally determined and theoretically modeled structural information allows you to understand and predict molecular properties.

In addition to atomistic representations, you can also create abstractions such as molecular surfaces are created for protein to Ligand complex.

Statistical Analysis

All experiments were carried out in triplicates, and data were expressed as mean 7 standard deviation (SD). Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc. Chicago, USA).

Differences among the samples were evaluated by using analysis of variance (ANOVA) and Duncan's multiple comparison method. Significant difference was assumed at $p < 0.05$.

RESULTS AND DISCUSSION

DPPH (1,1- diphenyl - 2 - picrylhydrazyl hydrate radical scavenging activity)

Free radical scavenging potential of methanolic root extract along with the standard Ascorbic acid at different concentration was tested by the DPPH method are shown in Fig.1.

The percentage inhibition of the methanolic root extract and ascorbic acid (62.5-500µg/ml) are about 16, 29, 55, 93% and 21, 30, 68 and 95% respectively, and it was obvious from the results that the values of the standard antioxidant were equal with our methanolic root extract.

$ABT^{S\bullet+}$ Scavenging Effects (2,2'-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid)

The ABTS radical scavenging method is one of the most extensively used antioxidant assays of plant samples. The methanolic root extract efficiently scavenged ABTS radicals, generated by the reaction between ABTS and ammonium persulfate.

The activity was found to be increased in a dose dependent manner from 14 to 90% at a concentration of 62.5-500µg/ml which was comparable with the standard BHT (Fig.2). A similar type of work has also been carried out using the whole plants *A.benthamii* (Ganie) and significant DPPH activity has also been documented against *A.densiflora* root extract (Orhan). Methanol solvents generally used for antioxidant ability assays, are strongly hydrogen bond -accepting, therefore the hydrogen- abstracting reaction occurs very slowly (Macdonald-wicks).

The presence of acids or bases in methanol may greatly influence the ionization equilibrium of phenols and cause either a reduction or an increase of the measured rate constants (Huang).

Therefore, the ABTS radical scavenging activity of methanolic root extract of *Decalepis hamiltonii* indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction.

Further the antioxidant activity of the extract by this assay implies that action may be by either inhibiting or scavenging properties of antioxidant towards this radical have been reported in earlier studies (Rice-Evans, 1997).

Antidiabetic Activity

Inhibition of α-glucosidase and α-amylase

Inhibition of α-glucosidase and α-amylase activities by Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts prepared from the root of *D. hamiltonii* is presented in Figure 1 and 2.

Among all the extracts tested, the methanolic root extract of *Decalepis hamiltonii* exhibited highest inhibition of 94 % and 85% of α-glucosidase and α-amylase activities respectively. This was followed by Ethyl acetate, Petroleum ether and Chloroform extracts respectively.

Depending upon the efficiency of the solvents in extracting the active principles, various solvent extracts exhibit difference in their inhibition of α-glucosidase and α-amylase.

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The methodology includes, DPPH, ABTS and *In vitro* diabetic assay α-glucose and α-amylase. *Insilco* approaches for antidiabetic activity of major compounds for GC-MS.

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The results showed that methanolic root extract of *Decalepis hamiltonii* has higher percentage of inhibition for DPPH (93%), ABTS (90%) which is comparable with respective standards and dose increase in percentage antidiabetic enzymes activity α-glucose (82% to 92%) and α-amylase enzyme (73% to 90).

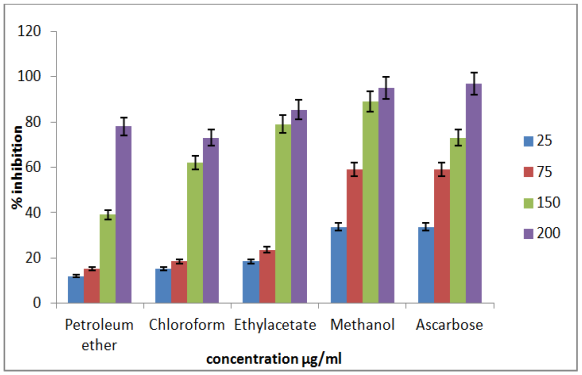
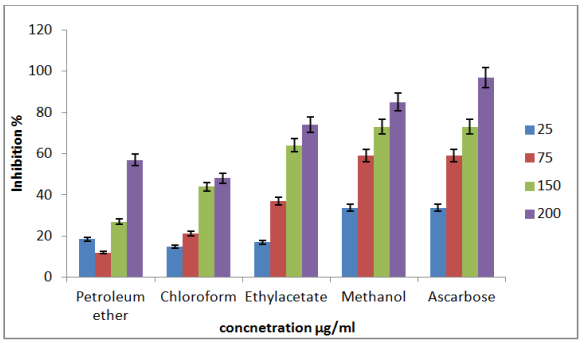


Figure 1: α - glucose assays inhibition of root extracts of *Decalepis hamiltonii*



Results are expressed as mean ± Standard deviation

Figure 2: α - amylase assays inhibition of root extracts of *Decalepis hamiltonii*

Spectroscopic Analysis

GC-MS Analysis

Interpretation on GC-MS was conducted using database of National Institutes of Standard and Technology (NIST) having more than 62,000 patterns.

The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library.

The GC-MS characterization of methanol root extract of *Decalepis hamiltonii* was identified and presented in (Fig.3 and Table.1). The three major compounds identified viz., 2-Hydroxy-4-methoxybenzaldehyde ($OHC_6H_3C(OH_3)CHO$), Hexadecanoic acid ($C_{16}H_{32}O_2$), Cis-vaccenic acid($C_{18}H_{34}O_2$).

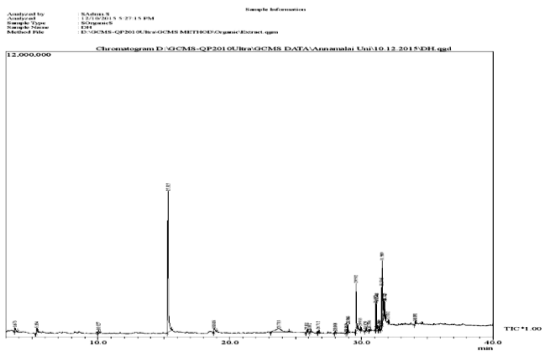


Figure 3: GC-MS Analysis of methanolic root extract of *Decalepis hamiltonii*

In silico* Analysis of Antidiabetic Activity of 2-hydroxy-4-methoxybenzaldehyde from root extract of *Decalepis hamiltonii

The molecular interaction between the ligand and the protein (receptor) was investigated using the Ligand fit protocol. The 2-Hydroxy-4-methoxybenzaldehyde was docked into active site of **ERRα** receptor. The green dotted line denoted the hydrogen bonds. The interactions of amino acid residues involved in molecular interactions with Benzaldehyde, 2-hydroxy-4-Methoxy (Figure 4).

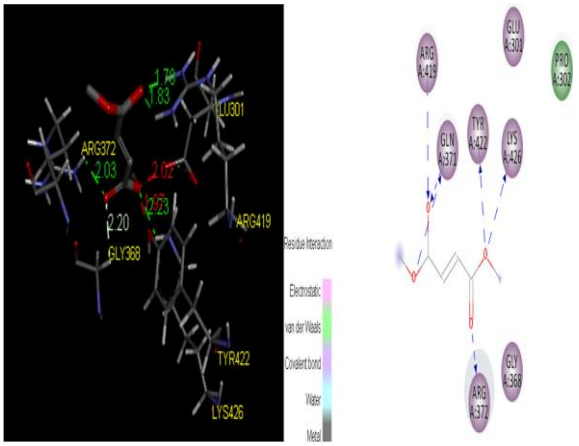


Figure 4: 3D and 2D structures for amino acid regions for Benzaldehyde, 2-hydroxy-4- Methoxy interact with Targeted protein

Screening methods were routinely and extensively used to reduce cost and time of drug discovery. It has been clearly demonstrated that the approach utilized in this study was successful in finding novel anti-diabetic inhibitors from plant source. The plant compounds that targeted the PBRY gamma protein were screened and ranked based on their dock score. The lipinkis prediction helped in the identification of more suitable ligand towards target protein. The dock score and other scores (CYS325, MET506, PHE495) were observed the more the vander-walls interactions show that the ligand structure is having number of alkyl groups due to which Vander-walls interactions can be formed. If the hydrophobic interactions are more it shows that the ligand is having groups that can participate in the hydrophobic interactions. If the charge interactions are presents it helps finding more appropriate binding and so shows greater affinity to the receptor, contributing more potency. Lipinkis rule of five is used as a first step filter to perform virtual screening of compounds, in an effort to quickly eliminate lead candidates that have poor physicochemical properties for oral bio availability. However the compound 2HMB having good dock scores which had similar action like standard drugs (Figure 4). Future perspectives understanding the interactions between proteins and ligand are crucial for the pharmaceutical and functional food industries.

Alpha-amylase and alpha-glucosidase inhibitors have potential value in the control of the kinetics of carbohydrate digestion and absorption which could be

exploited in the prevention and control of diabetes, obesity and hyperlipidaemia (Alagesan). Inhibitors of these enzymes have been recently developed from natural sources.

In the present study methanolic root extract of *Decalepis hamiltonii* was found to have maximum alpha-glucosidase 94%and alpha-amylase 85% at a concentration of 200µg/ml.

Similar results were observed by Gomathi who had reported a dose-dependent increase in alpha-amylase inhibition by the ethanolic extracts of *Evolvulus alsinoides* and Manikandan also reported the inhibition of alpha-amylase and alpha-glucosidase by the methanolic extracts of *Psidium guajava* leaves.

The polar solvent extracts namely Methanol and Ethyl acetate extracts of *Decalepis hamiltonii* was more potent inhibitors of α-glucosidase and α-amylase activities, whereas the non-polar solvent extracts namely Petroleum ether and Chloroform extracts showed moderate inhibition of these enzyme activities. This may be attributed due to the difference in the class of phytochemicals getting extracted in various solvent systems exhibiting alpha-glucosidase and alpha-amylase inhibition.

Many herbal extracts have been reported for their anti-diabetic activities and are being used in ayurveda for the treatment of diabetes.

The results of *in vitro* inhibition of alpha-amylase and alpha-glucosidase indicate that the root of *Decalepis hamiltonii* can act as good sources of natural inhibitors for these enzymes.

CONCLUSION

Hence, the proposed drug is presented to the scientific community for further investigational confirmation.

The results of the present study clearly demonstrated the *in silico* molecular docking studies of PBRY enzyme exhibited binding interactions and warrants further studies needed for the development of potent PBRY inhibitor for the treatment of Type-II diabetes.

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Table 1: GC-MS Analysis of Phyto Components Identified in the Root of *Decalepis hamiltonii*

S. No	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
1	15.315	Benzaldehyde 2-Hydroxy-4-methoxy	$OHC_6H_3C(OH_3)CHO$	152.15	40.94
2	29.592	Hexadecanoic acid	$C_{16}H_{32}O_2$	116.15	9.92
3	31.589	CIS-Vaccenic acid	$C_{18}H_{34}O_2$	282.46	13.72

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