Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X

RESEARCH ARTICLE

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Received on: 20/06/2014
Accepted on: 30/07/2014
Published on: 15/08/2014

Govindappa M
Department of Biotechnology, Shridevi Institute of Engineering & Technology, Sira Road, Tumkur-572 106, Karnataka, INDIA
Email:endophytessiet@gmail.com

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Conflict of Interest: None Declared !

DOI: 10.15272/ajbps.v4i34.520

In Vitro Antidiabetic Activity of Three Fractions of Methanol Extracts of *Loranthus Micranthus,* Identification of Phytoconstituents by GC-MS and Possible Mechanism Identified by GEMDOCK Method

Channabasava, Govindappa M*, Chandrappa CP and Sadananda TS

Natural Products Laboratory, Department of Biotechnology, Shridevi Institute of Engineering & Technology, Sira Road, Tumkur-572 106, Karnataka, INDIA

Abstract

Three (17, 20, 21) fractions of methanol extracts of *Loranthus micranthus* yielded different phytochemicals was confirmed by GC-MS analysis. The fraction 17 yielded 13 different phytochemicals followed by 20 (11 compounds) and 21 (12 compounds). More antidiabetic compounds were observed in fraction 17. In vitro antidiabetic activity was performed using these three column fractions for inhibition of α-amylase, α-glucosidase, sucrase and glucose. Strong antidiabetic activity was observed in 17 fraction and they inhibited all the above enzymes at rate followed by 21 and 20. The common phytochemical in all the extract is octadecenoic acid. It was used for GEMDOCK to study the interaction with enzymes. octadecenoic acid can be seperated and used as antidiabetic agent.

Keywords: *Loranthus micranthus,* antidiabetic, GC-MS, enzymatic inhibitions, GEMDOCKing

Cite this article as:
Channabasava, Govindappa M*, Chandrappa CP and Sadananda TS. *In Vitro* Antidiabetic Activity of Three Fractions of Methanol Extracts of *Loranthus Micranthus,* Identification of Phytoconstituents by GC-MS and Possible Mechanism Identified by GEMDOCK Method. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (34); 2014; 34-41.

INTRODUCTION

Diabetes mellitus is a multiple metabolic disorder characterized by high blood glucose level and its improper management leading to multiple chronic complications. Increased level of blood glucose may be due to inefficiency of pancreatic cells in insulin secretion or cellular resistance towards insulin uptake. International Diabetes Federation`s (IDF) recent estimates indicate that 8.3% of adults – 382 million people worldwide and 65.1 million people in India have diabetes, and the number of people with the disease is set to rise beyond 592 million in less than 25 years [1].

Herbal drugs are being the choice over synthetic drugs for having multiple therapeutic properties and fewer side effects. Even there are more than 1000 plant species being used for the treatment of Type 2 diabetes mellitus (advanced stage) worldwide [2], the diabetic cases are increasing at an alarming rate. This shows the necessities and the importance of more alternate and effective antidiabetic drugs and their systematic studies.

Loranthus micranthus (Loranthaceae) is a hemiparasitic shrub commonly known as African mistletoe. It has been reported to have antidiabetic, antimicrobial and studies reveal that composition and biological activities of mistletoe are dependent on harvesting period, locality and host tree species [3], immunomodulatory and antimotility activities [4]. It decreases the blood glucose level and controls the loss of body weight in diabetes mellitus [5].

The review of literature reveals no reports on mistletoe, which are present in India. Our present work was aimed to carry out in vitro antidiabetic activities by enzymatic inhibition studies of Loranthus micranthus. Solvent, methanol was selected for extraction. α-amylase, α-glucosidase, sucrase and glucose diffusion models were used for antidiabetic studies. Further, it has been confirmed by GC-MS studies in the identification of responsible antidiabetic phytoconstituents present in Loranthus micranthus extract.

The protein-ligand docking is the prediction of ligand conformation and orientation relative to the active site of a target protein. A computer aided docking process, identifying the lead compounds by minimizing the energy of intermolecular interactions, is an important approach for structure based drug design [6] and it is a very useful tool to give strong evidence for in vitro experiments. Ligand-molecule interaction study was conducted by using GEMDOCK method [7] to know systemic evaluation in identifying scoring functions. This method will know how our molecules interact with ligands and how they inhibit their function.

MATERIALS AND METHODS:

Chemicals and reagents

p-nitrophenyl-α-d-glucopyranoside, *p*-nitrophenyl-β-d-glucopyranoside, β-glucosidase from almonds and 3-5-dinitrosalisylic acid were purchased from Sisco Research Laboratory, India. A glucose oxidase/peroxidase assay kit was purchased from Agappe Diagnostics, India. α-amylase (23 u/mg solid) was purchased from Sigma-Aldrich, India. All the chemicals and reagents used in the study were of extra pure analytical grade.

Collection and processing of plant

The fresh leaves of *Loranthus micranthus* growing on the host plant *Azadirachta indica* collected in the month of April, 2009 during the flowering period at DC Bunglow, Tumkur, Karnataka, India and identified using authenticated herbarium from the Department of Studies in Botany, University of Mysore, Mysore. The plant material was washed with distilled water three times, shade-air dried (26 ± 2^0C) and pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and stored in airtight container for further work.

Preparation of crude extracts

25 g/100ml of powdered *Loranthus micranthus* was kept for solvent extraction in a rotary shaker at 37^0C, 72 rpm for 48 h. The methanol was used with increasing order of their polarity. The solvent extract was centrifuged at 6000 rpm for 10 min and then filtered with Whatman No. 1 filter paper and evaporated at a constant temperature of 62^0C in hot air oven until a very concentrated extract was obtained.

Column chromatography

Column chromatography is a type of adsorption chromatography technique. Here stationary phase is a silica gel packed in a vertical column. Cotton wool was plugged at the bottom of knob to hold stationary phase to allow only the solvent and sample. Silica was activated at 110^0 C for 20 min to remove moisture content. Silica slurry was prepared by starting eluent and packed sufficiently in the column. The column was eluted with an initial eluent to remove further impurities. The extract sample to be separated was placed on the top of packed stationary phase without disturbing the silica bed. The gradient elution was carried out using solvents as an increase in their polarity. The solvents used are hexane, hexane and toluene (2:1, 1:1, 1:2), toluene, toluene and ethyl acetate (2:1, 1:1, 1:2), ethyl acetate, ethyl acetate and methanol (2:1, 1:1, 1:2) and methanol in different ratios. 25 eluted fractions were periodically collected at regular volumes of 5 ml each. Further TLC was carried out for each fraction to confirm the separation of single compounds.

GC – MS analysis

GC-MS analysis of methanolic fractions of *Loranthus micranthus* was carried out with Agilent 7890-A having an MS detector 5975-C, ionization for MS is electron impact ionization. Mass analyzer was Quadrupole. The peaks were analyzed using data analysis software NIST-2008. An experiment was carried in column HP- 5 ms, dimensions- 30m L x 0.25mm ID x 0.25um film thickness. The initial temperature ramp was maintained at 40ºC, hold time -2 min. At the end the temperature ramp was 310ºC and hold time was 10 min. The rate of temperature ramp was 10ºC/min. The experiment was programmed with total run time 34 min, helium was used as a carrier gas at a constant flow rate of 1.0 ml/min, split less flow 1ml/min. Injection volume was 1µl with scan mass range 30m/z – 600m/z having positive polarity (+ve).

Identification of phytoconstituents

Interpretation of GC-MS mass-spectra were carried out using the database of National Institute Standard and Technology- 2008 (NIST-2008) having more than 62,000 patterns. The spectrums of the unknown components were compared with the spectrum of known components of NIST library and the parameters viz., molecular weight, structure of the components, total ionic chromatograms and ionization chromatograms were ascertained in naming the particular compound.

***In vitro* antidiabetic activity**

Assay of α-amylase inhibitory activity

The effect of three fractions of methanol extract of *Loranthus micranthus* on α-amylase activity was studied using an enzyme–starch system [8]. Three fractions (1–5%) were mixed by stirring with 25 mL of 4% potato starch in a beaker; 100 mg of α-amylase was added to the starch solution, stirred vigorously and incubated at 37°C for 60 min. After the incubation period, 0.1M NaOH was added to terminate enzyme activity. The mixture was centrifuged (3000 g; 15 min) and the maltose content in the supernatant was determined.

Assay of α-glucosidase inhibitory activity

α-glucosidase inhibitory activity was assayed according to the method of Honda and Hara [9]. Enzyme solution (10 µL) and three fractions of methanol extract (10–50 µL) were incubated together for 10 min at 37°C and the volume was made up to 210 µL with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 µL of 2 mM *p*-nitrophenyl-α-d-glucopyranoside solution and further incubated at 37°C for 30 min. The reaction was terminated by treating the mixture in a boiling water bath for 5 min. After the addition of 1.0 mL of 0.1 M disodium hydrogen phosphate solution, absorption of the liberated *p*-nitrophenol was read at 400 nm.

Assay of sucrase inhibitory activity

The effect of three fractions of methanol extract of *Loranthus micranthus* on sucrase activity was assayed according to the method of Honda and Hara [9]. The enzyme solution (10 µL) and three fractions of methanol extract (10–50 µL) were incubated together for 10 min at 37°C and the volume was made up to 200 µL with maleate buffer (pH 6.0). The enzyme reaction was started by adding 100 µL sucrose solution (60 mM). After 30 min, the reaction was terminated by adding 200 µL of 3, 5-dinitrosalicylic acid reagent and treating the mixture in a boiling water bath for 5 min. The absorbance of the solution was read at 540 nm. The percent inhibitory activities were calculated using the following formula:

$\%Inhibition = (Abs_{Control} - Abs_{Sample}) \times 100 / Abs_{Control}$

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.

An untreated enzyme solution was used as the control. All experiments were carried out in triplicate.

Assay of *Loranthus micranthus* on glucose diffusion

A method described by Gallagher *et al*. [10] was used to evaluate the effects of three fractions of methanol extract of *Loranthus micranthus* on glucose movement *in vitro*. The *in vitro* model consisted of a dialysis tube (6cm X 29.31 mm) (Himedia LA393-5MT-2010) in which 6 ml of plant extract and 2 ml of 0.15 M NaCl containing 1.65 mM D-glucose was added. The dialysis tube was sealed at each end and placed in a centrifuge tube containing 45 ml 0.15 M NaCl. The tubes were placed on an orbital shaker water bath and incubated at 37^0 C for 3 h. The movement of glucose into the external solution was provided. The concentration of glucose within the dialysis tube was measured and control tests were conducted in the absence of plant extracts. Glucose concentrations were analyzed by enzymatic method using the glucose oxidase kit. All tests were carried out in triplicate and the results were presented as means $\pm$ SD.

GEMDOCK Parameters

We developed a molecular docking approach termed GEMDOCK (Generic Evolutionary Method for molecular DOCKing). The GEMDOCK software is available on the web at http://gemdock.life.netu.edu.tw. Setting of GEMDOCK parameters, such as an initial step size, family competition length (L-2), population size (N=300) and combination probability (pc=0.3) in this work. The GEMDOCK optimization stops when either the convergence is a certain threshold value or the interactions exceeds a maximal present value, which was set to 70. Therefore, GEMDOCK generated 1200 solutions in one generation and terminated after it

exhausted 84, 000 solutions in the worse case. These parameters were decided after the experiments were conducted to recognize complexes of test docking systems with various values. Based on GC-MS analyses, predominantly found that octadecenoic acid esters in the extracts were subjected to GEMDOCK analysis.

Three fractions phytochemicals were analyzed by GC-MS. The literature survey indicates that some of the compounds which are present in three fractions already exhibiting antidiabetic activity by possessing different mechanism and they isolated from other plants. We selected some of the compounds predominantly present in all the fractions were selected for this study and this will help us to know how our compounds acts on enzyme inhibition and is the best tool to give strong evidence of *in vitro* studies.

RESULTS AND DISCUSSION

Column chromatography

The methanol extract shown good distinguishable separation of phytochemicals in preparative TLC, it was chosen for column chromatography for partial purification of phytochemicals. 25 column fractions were collected and carried out TLC. TLC studies shown significant separation of phytochemicals in fractions 17, 20 and 21 were chosen for further studies (Figure 1 and 2).

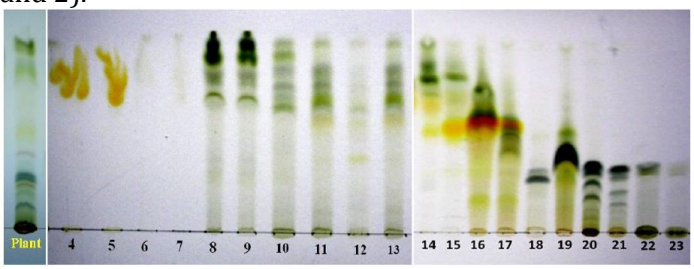

Figure 1: TLC of column fractions (F2-F23) from *Loranthus micranthus* methanolic extract
Mobile phase: Toluene: Ethyl acetate: Methanol (4:0.5:0.5)
Visualization: Iodine vapors

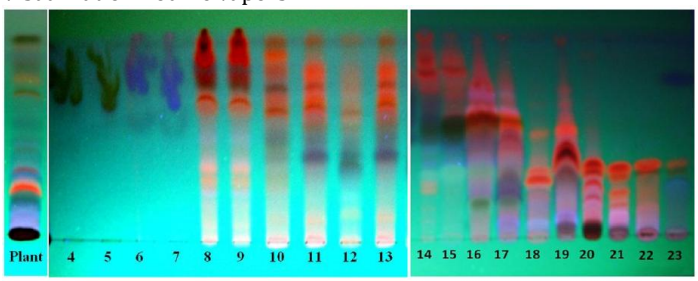

Figure 2: TLC of column fractions (F3-F19) from *Loranthus micranthus* methanolic extract
Mobile phase: Toluene: Ethyl acetate: Methanol (4:0.5:0.5)
Visualization: UV light

GC-MS studies

Out of 25 column fractions of methanolic extract, fractions 17, 20 and 21 were chosen for GC-MS studies based on a clear separation of phytochemicals in TLC (Figure 3-5 and Table 1-3). In fraction 17, 13 prominent compunds were identified and out of which 5 compounds (1,2,3-propanetriol, diacetate, Hexadecanoic acid, octadecenoic acid and eicosanoic acid) have already proved as antidiabetic activity by possessing insulin secretion, insulin stimulation, α-glucosidase inhibitors [11-13]. Fraction 20 yielded 11 different compunds in which three compunds (ar-tumerone, 3,7,11,15-tetramethyl-2-hexadecen-l-ol, trans-13-octadeceboic acid) have already exhibited antidiabetic, α-glucosidase inhibitors [14-15]. 12 different compunds were found in fraction 21, out of which 3 compunds (1,2,3-propanetriol, hexadecanoic acid, octadecenoic acid) already proved as antidiabetic activity [11, 15-16].

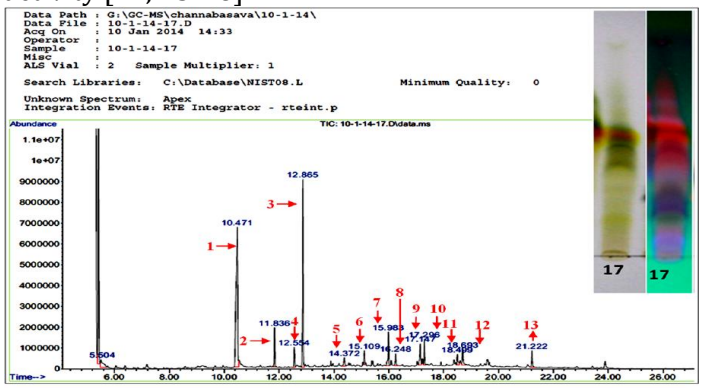

Figure 3: GC-MS Total Ion Chromatogram (TIC) of column fraction-17 of methanolic extract (*Loranthus micranthus)*

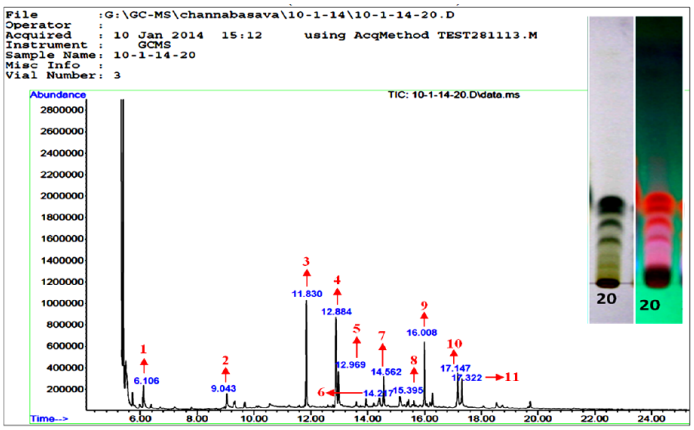

Figure 4: GC-MS Total Ion Chromatogram (TIC) of column fraction-20 of mehanolic extract (*Loranthus micranthus)*

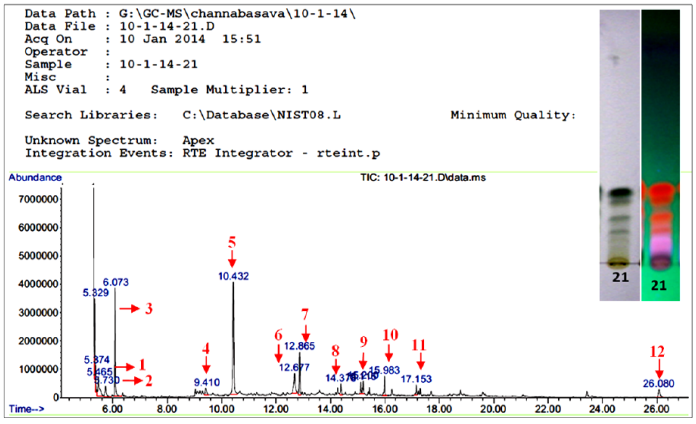

Figure 5: GC-MS Total Ion Chromatogram (TIC) of column fraction-21 of mehanolic extract (*Loranthus micranthus)*

Fraction-17					
Peak No	**Retention time (min)**	**Identified compound name**	**Biological activity**	**Source**	**Reference**
1	10.471	1,2,3-Propanetriol, diacetate	Insulin secretion	-	(Wuttke *et al.*, 2013)
2	11.836	1-Tetradecene	-	-	-
3	12.554	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	-	-	-
4	12.865	Phenol, 2,4-bis(1,1-dimethylethyl)-	-	-	-
5	14.372	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2-enyl}ethanone	-	-	-
6	15.109	Unknown compound	Unknown	-	-
7	15.983	Hexadecanoic acid, methyl ester	Anti-inflammatory activity	-	(Saeed *et al.*, 2012)
			Vasodilator	-	(Lee *et al.*, 2010)
			Release of insulin stimulation	-	(Parker *et al.*, 2003)
			Anti-diabetic activities	-	(Zuraini *et al.*,2012)
8	16.248	1,2-Benzenedicarboxylic acid, butyl octyl ester	-	-	-
9	17.147	9-Octadecenoic acid (Z)-, methyl ester	alpha-glucosidase inhibitors	-	(Artanti *et al.*, 2012)
			alpha-glucosidase inhibitors	*Oncoba spinosa*	(Balogun *et al.*, 2013)
10	17.296	Octadecanoic acid, methyl ester	alpha-glucosidase inhibitors	-	(Artanti *et al.*, 2012)
			alpha-glucosidase inhibitors	*Oncoba spinosa*	(Balogun *et al.*, 2013)
11	18.499	Eicosanoic acid, methyl ester	alpha-glucosidase inhibitors	-	(Artanti *et al.*, 2012)
			alpha-glucosidase inhibitors	*Oncoba spinosa*	(Balogun *et al.*, 2013)
12	18.693	4,8,12,16-Tetramethylheptadecan-4-olide	-	-	-
13	21.222	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- **(Squalene)**	Antioxidant effect , antimicrobial	-	(Ryszard, 2009)

Table 1: GC-MS detection of phytoconstituents of column fraction-17

Fraction-20					
Peak No	**Retention time (min)**	**Identified compound name**	**Biological activity**	**Source**	**Reference**
1	6.106	-	-	-	-
2	9.043	Nitrobenzene	Analgesic additive	-	-
3	11.830	1-Tetradecene	-	-	-
4	12.884	Phenol, 2,4-bis(1,1-dimethylethyl)-	Antifungal activity	Avocado roots	(Rangel *et al.*, 2013)
			Antioxidant		(Yoon *et al.*, 2006)
5	12.969	Dodecanoic acid, methyl ester	Osteoporosis	-	Wikipedia
6	14.217	Ar-tumerone (Terpene)	Antioxidant, antidiabetic and antiinflammatory	-	(Elmazar *et al.*, 2013)
			Antioxidant, antidiabetic	*Zingiber officinale*	(Lucia *et al.*, 2013)
7	14.562	Methyl tetradecanoate	-	-	-
8	15.395	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	Antibacterial antifungal	Ginkgo biloba	(Tao *et al.*, 2013)
			Anticonvulsant	-	(Costa *et al.*, 2012)
			Antiarthritis	-	(Hultqvist *et al.*, 2006)
			Insulin sensitizing/anti-diabetic effect	-	(Elmazar *et al.*, 2013)
9	16.008	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	-	-	-
10	17.147	trans-13-Octadecenoic acid, methyl ester	alpha-glucosidase inhibitors	-	(Artanti *et al.*, 2012)
11	17.322	Methyl 16-methyl-heptadecanoate	-	-	-

Table 2: GC-MS detection of phytoconstituents of column fraction-20

Effect of *L. micranthus* fractions on α-amylase activity

Inhibitory activities of three fractions of *L. micranthus* on α-amylase were studied using α-amylase starch model system. Inhibition of α-amylase activity of column fraction-17 (88%) was found more when compared to fraction-20 (72%) and fraction-21 (68%) (Figure 6 and Table 4). α-amylase is an enzyme that hydrolyzes alpha bonds of large, alpha-linked

polysaccharides, such as starch and glycogen. It is the major form of amylase found in humans and other mammals. Since α-amylase plays an important role in digestion of starch and glycogen, it is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity [17] to reduce postprandial glucose level. Hence α-Amylase inhibitors may be of value as novel therapeutic agents [18]. However, inhibition of α-amylase by the phytochemicals of plants could be conclusively attributed to the presence of flavonoids [19], phenols [20].

Fraction-21					
Peak No	**Retention time (min)**	**Identified compound name**	**Biological activity**	**Source**	**Reference**
1	5.465	Glycolaldehyde dimethyl acetal	-	-	
2	5.730	Acetamide, 2,2,2-trifluoro-N-methyl-	-	-	-
3	9.410	1,2,3-Propanetriol, monoacetate	Antidote	-	(Chenoweth *et al*., 1951)
4	10.432	1,2,3-Propanetriol, diacetate	Insulin secretion	-	(Wuttke *et al*., 2013)
5	12.677	1,2-Propanediol, 2-acetate	-	-	-
6	12.865	Phenol, 2,4-bis(1,1-dimethylethyl)-	Antifungal activity	Avocado roots	(Rangel *et al*., 2013)
			Antioxidant		(Yoon *et al*., 2006)
7	14.378	5-t-Butyl-4-methylimidazole	-	-	-
8	15.116	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)	-	-	-
9	15.2	p-Bromoatropine	-	-	-
10	15.983	Hexadecanoic acid, methyl ester	Anti-inflammatory activity	-	(Saeed *et al*., 2012)
			Vasodilator	-	(Lee *et al*., 2010)
			Release of insulin stimulation		(Parker *et al*., 2003)
			Anti-diabetic activities	-	(Zuraini *et al*.,2012)
11	17.153	trans-13-Octadecenoic acid, methyl ester	alpha-glucosidase inhibitors	-	(Artanti *et al*., 2012)
12	26.08	Unknown semicarbazone	-	-	

Table 3: GC-MS detection of phytoconstituents of column fraction-21

Effect of *L. micranthus* fractions on α-glucosidase activity

Three fractions of *L. micranthus* showed significant inhibition of α-glucosidase enzyme activity. Fraction-17 (83%) and fraction-20 (75%) shown comparatively of similar effectiveness and fraction-21 (54%) was shown less inhibition of enzyme activity (Figure 6 and Table 4).

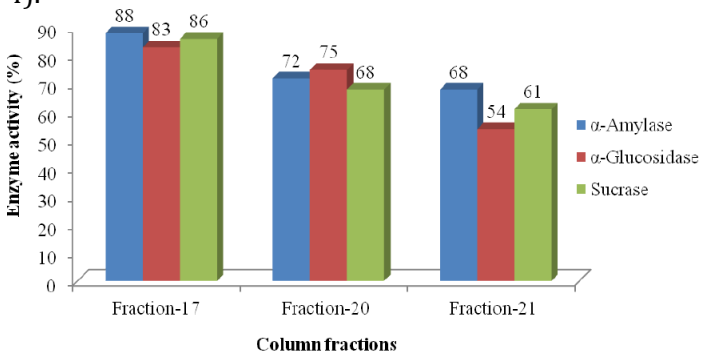

Figure 6: Effect of Loranthus Micranthus on α-amylase, α-glucosidase and sucrose activity

α-glucosidase is an enzyme located on the brush border of enterocytes of jejunum [21]. It binds to disaccharides and oligosaccharides, and cleaves terminal, non-reducing 1,4-alpha bonds and breaks down to single α-glucose molecule depending upon the substrate. It is proposed that alpha-glucosidase in the glucosidic path plays an important part in complementing phosphorolytic pathway in the liver's metabolic response to energy demands [22].

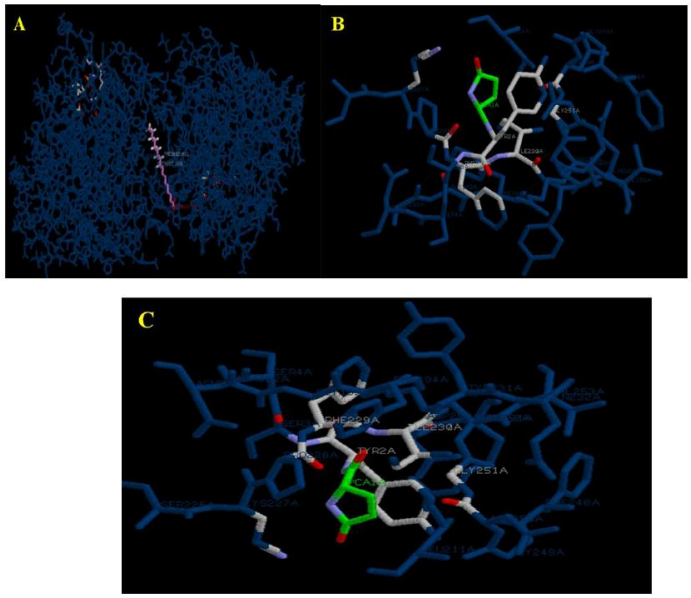

Figure 6: Effect of hexadecenoic acid on three different enzymes A. on α-glucosidase, B. amylase, C. sucrase

α-glucosidase inhibitors block the action of enzyme in the small intestine, which is rate-limiting in the conversion of oligosaccharides to monosaccharides necessary for gastrointestinal absorption. The main benefits attributed to α-glucosidase inhibitors are, reduction in both postprandial glycemic levels and the total range of postprandial glucose levels [23]. The GC-MS study of *L. micranthus* proves the presence of known α-glucosidase inhibitors, 9-octadecenoic acid (Z) - methyl ester, octadecanoic acid methyl ester, eicosanoic acid methyl ester in methanolic extracts.

These terpene esters are already proven as α-glucosidase inhibitors [13, 15].

Solvent extracts	Enzymes		
	α-amylase	α-glucosidase	Sucrase
Fraction-17	88	83	86
Fraction-20	72	75	68
Fraction-21	68	54	61

Table 4: Effect of *Loranthus micranthus* (L.f) Ettingsh on α-amylase, α-glucosidase and sucrase activity (Repeated the each experiment thrice)

Effect of *L. micranthus* fractions on sucrase activity

The depicted results clearly indicate the effectiveness of *L. micranthus* extracts in sucrase inhibition. Fraction-17 (86%) has shown most effective when compared to fraction-20 (68 %) fraction-21 (61%). Sucrase is secreted in villi tips of small intestine epithelium and it`s levels increase in pregnancy, lactation and diabetes as the villi hypertrophy. Sucrase hydrolyzes disaccharide sucrose into monosaccharides glucose and fructose. The fission of the glycosidic bond takes place between the C-1 of the D-glucose ring and the glycosidic oxygen; therefore D-fructose is released as β-D-fructofuranose from sucrose in human intestine [24]. This inhibition of sucrase activity either reduces or postpones the hydrolysis preventing the formation of β-D-fructofuranose (Figure 6 and Table 4).

Effect of *L. micranthus* fractions on glucose diffusion

Glucose diffusion studies of *L. micranthus* extracts reveal significant inhibitory effects on glucose movement into external solution across the dialysis membrane. Fraction-17 (18%) has shown most effective when compared to fraction-20 (21%) and fraction-21 (27%) (Table 5).

Extracts	Glucose diffusion of out of dialysis membrane	Increase of movement (%)
Fraction 17	0.4865±0.022	18.49±0.94
Fraction 20	0.5136±0.052	21.06±0.32
Fraction 21	0.7920±0.026	27.61±0.20

Table 5: Effect of different solvent extracts of *Loranthus micranthus* on glucose diffusion from dialysis tube after 3h (Each value is the mean ± standard deviation of three replicate analyses)

Dialysis creates and maintains a concentration differential across the membrane. On liquid-to-liquid interface all the molecules will try to diffuse in either direction. Because of its close related function as that of cells, it is extensively used in *in vitro* assays as replicate to *in vivo*. Separation by dialysis membranes is based on diffusion, convection and adsorption. The adsorption is one of the important criteria options in treating diabetes. The mechanism of inhibition of glucose diffusion of plants is due to slow absorption of carbohydrates and inhibition of glucose transport [25]. Present study attributes potential evaluation of phytochemical efficiency retarding the diffusion and movement of glucose [26].

Bioinformatics tools were applied to know how our molecule is interact with ligand by using GEMDOCK method. These results confirmed how our isolated compounds are potent in inhibiting the important enzymes (Fig 6). The compunds when they interact with ligand the RMS value should be negative, we found that octadecenoic acid interact with α-glucosidase showed -1.00, with amylase RMS: -1.00 and with sucrase RMS: -1.00. This clearly indicates the selected compund octadecenoic acid have showed strong antidiabetic activity in in vitro and it is also proved in GEMDOCK.

CONCLUSION:

Present GC-MS and enzymatic investigations support the usage of *L. micranthus* as traditional antidiabetic herbal. It has been evidenced that, α-glucosidase inhibition as the possible mechanism in treating diabetes by enzyme inhibition and GC-MS identified α-glucosidase inhibitors. Inhibition of α-amylase, sucrase and *in vitro* glucose diffusion exhibit the efficacy of *L. micranthus* as antidiabetic. And further more the presence of ar-tumerone, phytol, squalene and other phenolic compounds have insulin sensitizing, hypoglycemic mechanisms predicted in the management of diabetes. Hexadeconoic acid and octadecenoic acid have already proved as strong antidibetic compounds from earlier reports. The octadecocenoic is present in all the three fractions at higher concentration. Possibly, the actadecenoic is the antidiabetic in the extract. More activity was observed in faction 17. More optimal *in vivo* studies needed in this regard and can be considered in pharmaceutical formulations, foods, nutraceuticals and an additive for the effective management of diabetes.

ACKNOWLEDGEMENT:

We thank Dr MR Hulinaykar, Managing Trustee, Sri Shridevi Charitable Trust (R.) and Dr K. Sukumaran, Principal, SIET, Tumkur, India for encouragement and suggestions during the research.

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