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GC-MS ANALYSIS AND *INVITRO* ENZYME INHIBITORY EFFECT OF DIFFERENT SOLVENT EXTRACTS OF *GARCINIA CAMBOGIA* FRUIT

[1]S. Swetha, *[2]V. Anuradha, [3]N. Yogananth and [3]M. Syed Ali

[1]Research Scholar, Department Of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai – 600119, Tamilnadu, India.

[2]Assistant Professor, Department Of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai – 600119, Tamilnadu, India.

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***Corresponding Author**
V. Anuradha
Assistant Professor, Department Of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai – 600119, Tamilnadu, India.

ABSTRACT

The objective of the present study was to identify bioactive compounds, quantification of preliminary secondary metabolites and to determine its anti obese and anti diabetic activity through invitro enzyme inhibitory effect on pancreatic amylase and Lipase. The presence of phytoconstituents was determined by GC-MS analysis. Thirty four compounds were identified in *Garcinia gummi-gutta* (L.) Roxb fruit extract by GC-MS analysis. The hexane extract was found to contain high amount of Phenolics, flavonoids and Tannins. The ethyl acetate extract showed potent inhibitory activity with an IC 50 value of 55.87 ± 0.60 and 83.33 ± 0.47 against both amylase and Lipase respectively.

KEYWORDS: *Garcinia cambogia*, GCMS Analysis, Pancreatic amylase and Pancreatic Lipase.

INTRODUCTION

Plants are one of the most important sources of natural medicine and number of modern drugs has been isolated from them. Over 80% of world population relied on the traditional form of medicine for their basic health care (Tinrat *et al.*, 2014). Use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research (Mohd Mazid *et al.*, 2012). Epidemiological studies on medicinal

plants support that the constituents such as phenols, flavonoids, alkaloids, tannins etc. are capable of exerting protective effect against oxidative stress (Ness and Powles, 1997). They are effective in treatment of several human diseases such as cancer, arthritis, neurodegenerative disorders, aging process and diabetes (Sowndharajan *et al.*, 2010). Medicinal plants have been investigated and reported to be useful in treatment of obesity, diabetes and other chronic diseases[Hasani Ranjbar *et al* 2009 and Hasani Ranjbar *et al* 2010]. Much research has demonstrated the effectiveness, importance and the potential use of those enzyme inhibitors in the treatment of obesity and associated comorbidities and they reinforce the need for the search of new sources of those inhibitors: amylases (Obiro *et al*., 2008; Udani *et al*., 2009), glycosidases (Kwon *et al*., 2006) and lipases (Sharma *et al*., 2005; Souza *et al*., 2010).

Garcinia is the largest genus of the *Clusiaceae* family comprising nearly 250 species. *Garcinia gummi-gutta* (L.) Roxb. (Syn.: *Garcinia cambogia* (Gaertn.) Desr; Common name: Malabar tamarind), is one of the most important members of the *Clusiaceae* family. It is a small or medium sized tree up to 12 m tall with dark green and shining leaves. The leaves are elliptic obovate, 2-5 inch long and 1-3 inch broad. Fruits are ovoid, 2 inches in diameter, yellow when ripe, with 6-8 grooves; seeds 6-8 surrounded by succulent aril (Singh, 1993). The aril and the fleshy covering encasing the seed is edible when ripe. The differentiation between male and female trees is known only at the flowering stage which takes approximately 7 to 9 years (Kalia *et al*., 2012). *Garcinia gummi-gutta* is a common species found in the Western Ghats, from the Konkan southwards to Travancore eastwards. The species has now been introduced elsewhere in the subtropical region of Asia including China, Malaysia and the Philippines (Chuah *et al*., 2013).

Earlier phytochemical reports on the plant led to the isolation of various organic acids, benzophenones and xanthones as major constituents and numerous scientific studies have indicated biological activity such as anti-obesity, hypo lipidaemic and anticancer activity amongst numerous others (Semwal *et al.*, 2015). The phytochemistry of this popular commercial medicinal plant has not been comphrehensively studied. Hence, in the present investigation, the phytochemical constituents, quantification of phenolics, flavonoids, tannins and GC-MS analysis along with invitro enzyme inhibition assays were carried out for the different solvent extracts of *Garcinia gummi-gutta* (L.) Roxb.

MATERIALS AND METHODS

SAMPLE PREPARATION

The dried fruit of *Garcinia gummi-gutta* (L.) Roxb were purchased from the local market at Chennai, Tamilnadu. Selection of plant was based on their availability and medicinal importance. The collected fruit rind material was washed with sterile double distilled water, finely cut and air dried for a week. The dried plant materials were finely powdered and stored in airtight containers for analysis. About 100 gram of fruit rind powder was mixed with 1000 ml of double distilled water and other solvents[Hexane, Ethyl Acetate and diethyl ether] and this was subjected to soxlet extraction. The obtained extract was used for the preliminary phytochemical analysis and further analysis.

PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of hexane, ethyl acetate and methanol extract of *Garcinia gummi-gutta* fruit extract was carried out to detect the phyto constituents using standard conventional protocols (Harborne, 1998). Extracts were tested for the presence of active principles such as Alkaloids, Phenols, Terpenoids, Flavonoids, Tannins, Steroids and Saponoins.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 ìMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of70 was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 ìl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was TurboMass Ver5.2.0.

QUANTIFICATION

Determination of Total Phenolic Compounds

Total soluble phenolic compounds in the extracts were determined with Folin-Ciocalteu reagent using pyrocatechol as a standard phenolic compound (Slinkard & Singleton, 1977) with some modifications. Briefly, 0.5ml of extract (500 µg/ml) in a volumetric flask was diluted with distilled water (46 ml). 5ml of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, Na_2CO_3 (4 ml, 2% w/v) was added and then allowed to stand for 15minutes incubation. The absorbance was measured at 765 nm in a spectrophotometer (Shimadzu-1700). The phenol content was expressed in mg/g.

Determination of Total Flavonoids

The total flavonoid content was determined according to Quettier-Deleu *et al*., 2000 using $AlCl_3$. This method is based on the formation of a flavonoid-aluminum complex with maximum absorbance at 435nm. 0.5ml of extract was added to the solution of 0.1ml $AlCl_3$ (10% (m/v) in water) with addition of 0.1 ml 1M potassium acetate and 2.8ml distilled water, following incubation for 30 minutes at room temperature, the absorbance of the mixture was measured at 415 nm with a UV–vis spectrophotometer type Shimadzu UV–vis 1800. The calibration curve was prepared using catechin. The flavonoid content was expressed in mg/g.

Determination of total tannins

Tannin was determined by the method of Peri and Pompei, 1971. 1ml of sample extracts of concentrations (1mg/ml) was taken in test tubes. The volume was made up to 1ml with distilled water and 1ml of water serves as the blank. To this 0.5ml of folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 minutes. Blue color was formed. The color intensity was read at 640nm. A standard graph of tannins (Gallic acid conc - 1mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content is expressed in mg/g.

ENZYME INHIBITION STUDIES

Porcine pancreatic amylase inhibitory assay

Porcine pancreatic amylase inhibitory assay was performed as per the standard method (Tamil I G *et al.*, 2010). 2 mg of starch was suspended in each of the tubes containing 0.2 ml of 0.5 M Tris-Hcl buffer (pH 6.9 and 0.01 M $CaCl_2$). The tubes containing the substrate solution were boiled for 5 min and were then incubated at 37°C for 5 min. 0.2 ml of extract was taken in each tube containing different concentrations (10, 20, 40, 60, 80, and 100 µg/ml)

of DMSO. PPA was dissolved in Tris-HCl buffer to form a concentration 2 units/ml and 0.1 ml of this enzyme solution was added to each of the above-mentioned tubes. The reaction was carried out at 37°C for 10 min and was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (Shimadzu-1700). The α-amylase inhibitory activity was calculated as follows:

α-Amylase inhibitory activity = ((Ac+) − (Ac−)) − ((As − Ab))/((Ac+) − (Ac−)) × 100

where Ac+, Ac−, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme activity), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively.

Porcine pancreatic lipase inhibitory activity

Porcine pancreatic lipase (PPL, type II) activity was measured using *p*-nitrophenyl butyrate (*p*-NPB) as a substrate. The method used for measuring the pancreatic lipase activity was modified from that previously described by Kim *et al.*, 2010. PPL stock solutions (1 mg/mL) were prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at −20 °C. To determine the lipase inhibitory activity, the extracts (final concentrations 100, 50, 25, 10, 5, 2.5 µg/mL) or Orlistat (at same concentrations) as a positive control were pre-incubated with PPL for 1 h in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before assaying the PPL activity. The reaction was then started by adding 0.1 µL NPB as a substrate, all in a final volume of 100 µL. After incubation at 30 °C for 5 min, the amount of *p*-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer (BioTek Synergy HT, Winooski, VT, USA). The activity of the negative control was also examined with and without an inhibitor. The inhibitory activity (I) was calculated according to the following formula:

Inhibitory activity (I%) = 100 − ((B − b)/(A − a) × 100)

Where A is the activity without inhibitor; a is the negative control without inhibitor; B is the activity with inhibitor; and b is the negative control with inhibitor. DMSO was used as negative control and its activity was also examined.

RESULTS

Table 1 illustrates the qualitative analysis of various extracts of *Garcinia gummi-gutta* (L.) Roxb fruit. It is observed that the ethyl acetate, hexane and diethyl ether extracts showed high

amounts of flavonoids, phenols, tannin, Benzophenone, carbohydrates and saponins. It is also observed that Alkaloids, Coumarin and terpenoids are also present in moderate quantity. Phytosterols are present only in hexane and ethyl acetate extracts.

Table 1: Presence/Absence of Various Phytoconstituents Analysed by Biochemical Methods.

S.NO	PHYTOCONSTITUENTS	AQUEOUS	HEXANE	DIETHYL ETHER	ETHYL ACETATE
1	ALKALOIDS	----	+	++	++
2	CARBOHYDRATES	++	+++	+++	+++
3	SAPONINS	++	+++	+++	+++
4	PHYTOSTEROLS		++		+++
5	TRITERPENOIDS		+		+
6	PHENOLS		+++		+++
7	TANNINS		+++		+++
8	FLAVANOIDS	+++	+++	+++	+++
9	PROTEINS	----------	----------	----------	----------
10	LACTONES	----------	----------	----------	----------
11	GLYCOSIDES	----------	----------	----------	---------
12	CAROTENOIDS	---------	----------	----------	----------
13	COUMARINS	+++	++	++	++
14	PHLABOTANNINS	---------	----------	---------	----------
15	FLAVONONES	++	+++	+++	+++
16	ANTHRAQUIONONE [FREE]	----------	----------	----------	---------
17	CONDENSED ANTHRAQUINONES	----------	----------	----------	----------
18	CATECHIN	----------	----------	---------	----------
19	BENZOPHENONE	++	++	++	++

The reliability of medicinal plants for its usage is evaluated by correlating the phytochemical compounds with their biological activity. The phytochemical evaluation can be used for further assessment of secondary metabolites.

2. GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

The organic compounds present in the fruit extract was determined by GC-MS. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute.

Standard and Technique (NIST) having more than 62,000 patterns. The Spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight, Structure of the component of the test

material was ascertained. Thirty four compounds were identified in *Garcinia gummi-gutta* (L.) Roxb fruit extract by GC-MS analysis. The active principle, Molecular weight (MW), Concentration (%), Molecular formula (MF) and Retention Time (RT) are presented in Figure 1. The predominant compounds are 1,3-Pentadiene, 2,4-dimethyl (42.24%), Cyclohexene, 1-methyl-3-(1-methyl ethenyl)-, (27.59%), Cyclohexene, 4-methylene-1-(1-methylethyl)- (8.06%), Limonene (6.11%), Gamma Terpinene (3.21%) and alpha.-Pinene (3.07%) (Table 2).

Table 2: GC-MS analysis of *Garcinia gummi-gutta* (L.) Roxb fruit.

S.No	Retention Time	Name of the Compound	Peak Area %
1	10.15	Methacrolein	0.03
2	14.63	Furfural	0.19
3	15.40	Bicyclo[3.1.0]hex-2-ene, 2-methyl- 5-(1-methylethyl)-	0.37
4	15.63	alpha.-Pinene	3.07
5	15.99	1,3,6-Heptatriene, 2,5,5-trimethyl	0.03
6	16.26	Cyclohexene, 4-methylene-1-(1-meth ylethyl)-	8.06
7	16.44	(1R)-2,6,6-Trimethylbicyclohept-2-ene	0.58
8	16.64	Cyclopropanecarboxylic acid, 2-pen tyl ester	0.23
9	16.71	alpha.-Phellandrene	0.19
10	16.86	2-Carene	0.79
11	17.05	D-Limonene	6.11
12	17.26	1,3-Pentadiene, 2,4-dimethyl-	42.24
13	17.40	Gamma Terpinene	3.21
14	17.64	p-Menth-8-en-1-ol, stereoisomer	0.22
15	17.82	4-Carene	1.27
16	18.65	1,6-Octadiene, 3,7-dimethyl	0.04
17	18.94	Acetic acid, decyl ester	0.08
18	19.07	Bromoacetic acid, 10-undecenyl ester	0.09
19	19.18	Terpinen-4-ol	1.18
20	19.34	alpha.-Terpineol	0.76
21	19.60	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	1.17
22	19.83	2,6-Nonadienal, (E,E)	0.19
23	19.88	2-Methyl-6-methylene-octa-1,7-dien -3-ol	0.33
24	20.25	1,6-Octadiene, 3,7-dimethyl-	0.13
25	20.75	trans-2-Ethyl-2-hexen-1-ol	0.13
26	20.95	.beta.-Myrcene	0.18
27	21.04	Cyclohexene, 1-methyl-3-(1-methyl ethenyl)-, (.+/-.)-	27.59
28	22.10	3-Cyclohexene-1-methanol, alpha.,.alpha.,4-trimethyl-, acetate	0.19
29	22.14	Isobutyl 2-(4-methylcyclohex-3-eny l)propan-2-yl carbonate	0.60
30	22.30	Cyclohexene, 1-methyl-4-(1-methyle thylidene)-	0.32
31	22.43	alpha.-Cyclogeraniol acetate	0.18
32	23.20	Cyclohexene, 3-methyl-6-(1-methyle	0.15

		thylidene)-	
33	24.78	Camphene	0.07
34	24.87	3-Buten-2-one, 4-(2,2-dimethyl-6-methylenecyclohexyl)-	0.03

3. Quantification of Total Phenolic, Flavonoids and Tannin

Table 3: Total phenolic, flavonoids and tannin content.

S.NO	Secondary metabolites	Aqueous extract Weight (mg/g dw)	Hexane extract Weight (mg/g dw)	Diethyl ether extract Weight (mg/g dw)	Ethyl acetate extract Weight (mg/g dw)
1	**Total phenolic**	3.25 ± 0.21	5.34 ± 0.48	4.21 ± 0.46	4.03 ± 0.58
2	**Total Flavonoid**	2.15 ± 0.47	2.23 ± 0.75	1.14 ± 0.79	1.11 ± 0.43
3	**Total Tannins**	1.19 ± 0.10	1.98 ± 0.61	1.46 ± 0.25	1.38 ± 0.75

From Table 3, it is detected that higher content of Total Phenolic (5.34 ± 0.48 mg/g dw), Total Flavonoids (2.23 ± 0.75 mg/g dw) and Total Tannin (1.98 ± 0.61 mg/g dw) was gained for hexane extract of *Garcinia gummi-gutta* (L.) Roxb. Moderate comparable value of secondary metabolites was obtained for both diethyl ether extract and ethyl acetate extract. *Garcinia gummi-gutta* (L.) Roxb showed phenolic content of 4.21 ± 0.46 (mg/g dw) and 4.03 ± 0.58 (mg/g dw) and flavonoid content of 1.14 ± 0.79 (mg/g dw) and 1.11 ± 0.43 (mg/g dw) for diethyl ether extract and ethyl acetate extract respectively. Total Tannin was found to be 1.46 ± 0.25 (mg/g dw) for diethyl ether extract and 1.38 ± 0.75 (mg/g dw) for ethyl acetate extract. Lower quantity was gained by aqueous extract of *Garcinia gummi-gutta* (L.) Roxb which showed 3.25 ± 0.21 mg/g dw of Total Phenolics, 2.15 ± 0.47 mg/g dw of Total Flavonoid and 1.19 ± 0.10 mg/g dw of Total Tannin.

4. ENZYME INHIBITORY STUDIES

Porcine pancreatic amylase inhibitory assay

Acarbose the standard drug (at a concentrations 100 µg/mL) showed 56.45% inhibitory effects on the α-amylase activity with an IC_{50} value 88.77 ± 0.71 µg/mL (Table 4). The aqueous extracts of *Garcinia gummi-gutta* (L.) Roxb fruit (at a concentration 100 µg/mL) exhibited 80.57% of α-amylase inhibitory activity with an IC_{50} values 42.77 ± 0.16 µg/mL and the hexane extracts of *Garcinia gummi-gutta* (L.) Roxb fruit (at a concentration 100 µg/mL) exhibited 90.46% of α-amylase inhibitory activity with an IC_{50} values 40.74 ± 0.75 µg/mL (Table 5). However, the diethyl ether extract shows α-amylase inhibitory activity of 81.18% (at a concentration 100 µg/mL) with IC_{50} values 52.93 ± 0.53 µg/mL which is similar to hexane extract. Ethyl acetate extract shows 79.36% of α-amylase inhibitory activity with

IC_{50} values 55.87 ± 0.60 µg/mL. Moreover all the extracts of *Garcinia gummi-gutta* (L.) Roxb fruit showed higher α-amylase inhibitory effects when compared with standard drug acarbose.

Table 4: Alpha-amylase inhibitory effects of Acarbose (standard α-amylase inhibitor).

Drug	Concentration (µg/ml)	% of Inhibition	IC 50 value (µg/ml)
Acarbose	10	16.27	88.77 ± 0.71
	20	20.15	
	40	27.91	
	60	36.24	
	80	45.25	
	100	56.45	

Table 5: Alpha-amylase inhibitory effects of Water, Hexane, Diethyl ether and Ethyl acetate extracts of *Garcinia gummi-gutta* (L.) Roxb fruit.

Extract	Concentration (µg/ml)	% of Inhibition	IC 50 value (µg/ml)
Water	10	18.62	42.77 ± 0.16
	20	32.46	
	40	61.42	
	60	68.27	
	80	74.16	
	100	80.57	
Hexane	10	28.25	40.74 ± 0.75
	20	42.18	
	40	70.52	
	60	76.45	
	80	81.45	
	100	90.46	
Diethyl ether	10	25.20	52.93 ± 0.53
	20	40.48	
	40	68.46	
	60	73.20	
	80	78.89	
	100	81.18	
Ethyl acetate	10	23.79	55.87 ± 0.60
	20	38.35	
	40	52.72	
	60	63.03	
	80	71.08	
	100	79.36	

Porcine pancreatic lipase inhibitory activity

Table 6: Lipase inhibitory effects of Orlistat.

Drug	Concentration (µg/ml)	% of Inhibition	IC 50 value (µg/ml)
Orlistat	2.5	09.12	84.79 ± 0.45
	5	16.45	
	10	21.12	
	25	30.75	
	50	44.26	
	100	50.92	

Table 7: Lipase inhibitory effects of Water, Hexane, Diethyl ether and Ethyl acetate extracts of *Garcinia gummi-gutta* (L.) Roxb fruit.

Extract	Concentration (µg/ml)	% of Inhibition	IC 50 value (µg/ml)
Water	2.5	10.15	75.67 ± 0.10
	5	17.42	
	10	24.20	
	25	32.18	
	50	47.84	
	100	55.07	
Hexane	2.5	18.21	67.30 ± 0.78
	5	23.56	
	10	32.75	
	25	43.15	
	50	49.22	
	100	57.03	
Diethyl ether	2.5	16.30	72.30 ± 0.16
	5	21.98	
	10	30.14	
	25	40.56	
	50	47.12	
	100	55.61	
Ethyl acetate	2.5	12.21	83.33 ± 0.47
	5	17.21	
	10	26.02	
	25	36.02	
	50	43.03	
	100	51.65	

Table 6 and Table 7 shows, the inhibitory activities of Orlistat and various solvent extract of *Garcinia gummi-gutta* (L.) Roxb fruit against porcine pancreatic lipase. The aqueous, Hexane, Diethyl ether and ethyl acetate extract inhibited pancreatic lipase activity by 55.07%, 57.03%, 55.61% and 51.65% respectively at concentration of 100µg/ml. Orlistat, a pancreatic lipase inhibitor used as an anti-obesity agent, inhibited the enzyme activity by 50.92% at concentration 100µg/ml respectively which indicates that all the test samples shows higher

inhibitory activity than the standard Orlistat. The IC_{50} values of 75.67 ± 0.10 (µg/ml), 67.30 ± 0.78 (µg/ml), 72.30 ± 0.16 (µg/ml) & 83.33 ± 0.47 (µg/ml) respectively was obtained by Water, Hexane, Diethyl ether and ethyl acetate extract of *Garcinia gummi-gutta* (L.) Roxb fruit and the IC_{50} values of standard Orlistat was found to be 84.79 ± 0.45 (µg/ml).

DISCUSSION

In this *in vitro* study, the porcine pancreatic α-amylase and lipase inhibitory activity of *Garcinia gummi-gutta* (L.) Roxb fruit were evaluated and compared with the positive drug acarbose and orlistat respectively against amylase and lipase. Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide to mono and disaccharide (Subramanian *et al*., 2008). In our study, the standard drug Acarbose is a microbial pseudo-tetrasaccharide, an inhibitor to both α-amylase and α-glucosidase that was widely used as an oral hypoglycaemic agent (Asano, 2009; Mogale *et al*., 2011). The aqueous, Hexane, Diethyl ether and ethyl acetate extract of *Garcinia gummi-gutta* fruit showed the activity in inhibiting α-amylase up to 90%. The hexane extract registered the maximum level of inhibition. The standard Acarbose gave 56.45 at the concentration of 100 µg/ml. The variation in inhibitory effect of the different solvent extracts on α-amylase could be as a result of the phytochemicals present in them, which are flavonoids, steroids, saponins and tannins. Flavonoids have been reported to preserve β-cell integrity and function by scavenging free radicals in the system and therefore protect against the progression of diabetes mellitus (Kazeem et al., 2016). Tannins have also been reported to induce phosphorylation of the insulin receptors as well as translocation of glucose transporter 4, a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis. It also helps in the repression of the key gene responsible for adipogenesis thereby helping to reduce blood glucose level without increasing the adiposity (Song et al., 2005).

Pancreatic lipase is the key enzymes in lipid absorption that hydrolyses triacylglycerols in the gastrointestinal tract (Gholamhoseinian *et al*., 2010). Pancreatic lipase inhibitor which help in limiting intestinal fat absorption at initial stage have been proved as useful medications for the treatment of hyperlipidaemia and a great promise as anti-obesity agents (Rahimi *et al*., 2010). The inhibition of Pancreatic lipase is one of the most widely studied mechanisms used to determine the potential efficiency of natural products as antiobesity drugs (Ramos et al.,

2005). The standard drug orlistat is the most active compound in inhibiting gastric, pancreatic and carboxyl ester lipase and has been proved to be effective for the human obesity treatment (Scarpace *et al*., 2002; Mantzoros *et al*., 1998; Soderberg *et al*., 1999). In the present study, the significant inhibition of Pancreatic lipase was observed upto 57% with *Garcinia gummi-gutta* fruit. The Hexane extract gave the maximum amount of inhibition (57%) when compared the others solvents used. The presence of flavonoids, tannins, polyphenol, phytosterol and saponin in *Garcinia gummi-gutta* fruit extract could be responsible for its pancreatic lipase inhibitory activity although exact mode of action is still unclear (Zeng *et al*., 2012).

From our study, it has been proved that the *Garcinia gummi-gutta* fruit extract has an anti-obesity potential through pancreatic lipase and α-amylase inhibitory activity. This inhibitory activity may due to their active phytochemical content including phenol, terpenoid, saponin, flavonoid, tannin and steroid.

CONCLUSION

Inhibition of α-amylase is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases. Pancreatic lipase (triacylglycerol acyl hydrolase) is a common target for anti-obesity drug research which plays an essential role in the digestion of triacylglycerols. In the present study, various solvent extracts of *Garcinia cambogia* fruit was found to inhibit both Pancreatic amylase and Lipase when compared with standard drugs. Hence, the bioactive phytocompounds can be explored for therapeutic uses against diabetes and associated obesity.

REFERENCES

1. Tinrat S, Akkarachaneeyakorn and Inghapol C. Evaluation of antioxidant and antimicrobial activities of *Momordica cochinchinensis* spreng (gac fruit) ethanolic extract. International Journal of Pharmaceutical Sciences and Research, 2014; 5(8): 3163-3169.
2. Mohd Mazid, Taqi Ahmed Khanb and Firoz Mohammada. Medicinal plants of rural India: A Review of use by Indian Folks. Indo Global Journal of Pharmaceutical Sciences, 2012; 2(3): 286-304.
3. Ness AR and Powles JW. A review: Fruit and vegetables and cardiovascular disease. International Journal of Epidemiology, 1997; 26(1): 1-13.

4. Sowndharajan K, Jince MJ, Arunachalam K and Manian S. Evaluation of *Merremia tridentates* (L.) Hallier f. for *in-vitro* antioxidant activity. Food Science and Biotechnology, 20l0; 19(3): 663-669.
5. Hasani-Ranjbar S, Nayebi N, Larijani B and Abdollahi M. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. World J. Gastroenterol, 2009; 15: 3073-3085.
6. Hasani-Ranjbar S, Nayebi N, Larijani B and Abdollahi M. A systematic review of the efficacy and safety of *Teucrium* species; from anti-oxidant to anti-diabetic effects. Int. J. Pharmacol, 2010; 6: 315-325.
7. Obiro WC, Zhang T and Jiang B. The nutraceutical role of the Phaseolus vulgaris α-amylase inhibitor. Br. J. Nutr, 2008; 100(1): 1-12.
8. Udani JK, Singh BB, Barret ML, Preuss HG). Lowering the glycemic index of white bread using a white bean extract. Nutr, 2009; 8: 1-5.
9. Y.-I. Kwon, E. Apostolidis, and K. Shetty. Evaluation of pepper (Capsicum annuum) for management of diabetes and hypertension. Journal of Food Biochemistry, 2007; 31(3): 370–385.
10. Sharma N, Mogra R, and Upadhyay B. Effect of stevia extract intervention on lipid profile. Ethno-Med, 2009; 3: 137–140.
11. Souza SP, Pereira LLS, Souza AA and Santos CD. Inhibition of pancreatic lipase by extracts of Baccharis trimera (Less.) DC. Asteraceae: evaluation of antinutrients and effect on glycosidases. Rev. Bras. Farmacogn, 2010; 21(3).
12. Singh N P. Clusiaceae (Guttiferae *nom. alt*.) *In*: Sharma, BD and Balakrishnan NP (eds.), Flora of India. Botanical Survey of India, Kolkatta, 1993; 86-151.
13. Kalia RK, Malik SK and Chaudhury R. *In vitro* morphogenetic studies on three species of *Garcinia*. Journal of Plant Biochemistry and Biotechnology. 2012; 21: 279-85.
14. Chuah LO, Ho WY, Beh BK and Yeap SK. Updates on Antiobesity effect of *Garcinia* origin (−)-HCA. Evidence Based Complementary and Alternative Medicine, 2013; 2013.
15. Semwal RB, Semwal DK, Vermakk I and Viljoen A. A comprehensive scientific overview of *Garcinia cambogia*. Fitoterapia, 2015; 102: 134-148.
16. Harborne JB. Phytochemical methods. London: Chapman and Hall, 1998; 60.
17. Slinkard K and Singleton VL. Total Phenol Analysis: Automation and Comparison with Manual Methods. American Journal of Enology and Viticulture, 1977; 28: 49-55.
18. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet J, Luyck M, Cazin M, Cazin JC, Bailleul F and Trotin F. Phenolic compounds and antioxidant activities of buckwheat

(Fagopyrum esculentum Moench) hulls and flour. Journal of Ethnopharmacology, 2000; 72: 35-40.

19. Peri C and Pompei C. Estimation of different phenolic groups in vegetable extracts. Phytochemistry, 1971; 10: 2187-2189.
20. Kim YS, Lee YM, Kim H, Kim J, Jang DS, Kim JH, Kim JS. Anti-obesity effect of *Morus bombycis* root extract: anti-lipase activity and lipolytic effect. Journal of Ethnopharmacology, 2010; 130: 621-624.
21. Tamil I G, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. *In vitro* study on α-amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus.* Indian Journal of Pharmacology, 2010; 42(5): 280-282.
22. Asano N. Sugar-mimicking glycosides inhibitors:bioactivity and application: A Review. Cell Mol Life Sci, 2009; 66(9): 1479-1492.
23. Mogale M, Lebelo S, Thovhogi N, de Freitas A, Shai L. Alpha-amylase and alpha-glucosidase inhibitory effects of *Sclerocarya birrea*[(A.Rich.) Hochst.] subspecies caffra (Sond) Kokwaro (Anacardiaceae) stem-bark extract. Afr J Biotechnol., 2011; 10(66): 15033-39.
24. Subramanian R, Asmawi M, Sadikun A. In vitro alphaglucosidse and alpha-amylase enzyme enzyme inhibitory effect of Andrographis paniculata extract and andrographolide. Acta Biochim Pol, 2008; 55(2): 391- 398.
25. Song Y, Manson JE, Buring JE, Howard D, Liu S. Association of dietary flavonoids with risk of type 2 diabetes and markers of insulin resistance and systemic inflammation in women: A prospective study and cross-sectional analysis. *J. Am. Coll. Nut,* 2005; 24: 376-384.
26. Kazeem, MI, Mayaki, AM., Ogungbe, BF, Ojekale, AB. *In-vitro* Studies on *Calotropis procera* Leaf Extracts as Inhibitors of Key Enzymes Linked to Diabetes Mellitus. Iranian Journal of Pharmaceutical Research, 2016; 15(Special issue): 37-44.
27. Gholamhoseinian A, Shahouzehi B, Sharifi-far F. Inhhibitory effect of some plant extracts on pancreatic lipase. I J Pharmacol, 2010; 6(1): 18-24.
28. Rahimi R, Ghiasi S, Azimi H, Fakhari S, Abdollahi M. A review of the herbal phosphodiesterase inhibitors; future perspective of new drugs. Cytokine, 2010; 9: 123–129.
29. Ramos S, Aliäa M, Bravo L, Goya L. Comparative Effects of Food-Derived Polyphenols on the Viability and Apoptosis of a Human Hepatoma Cell Line (HepG2). J Agric Food Chem, 2005; 53(4): 1271-80.

30. Soderberg S, Ahren B, Jansson JH: Leptin is associated with increased risk of myocardial infarction. J Intern Med, 1999; 246: 409-418.
31. Scarpace PJ, Matheny M, Zhang Y, Tumer N, Frase CD, Shek EW. Central leptin gene delivery evokes persistent leptin signal transduction in young and aged-obese rats but physiological responses become attenuated over time in aged-obese rats. Neuropharmacol, 2002; 42(4): 548-61.
32. Mantzoros CS, Liolios AD, Tritos NA. Circulating insulin concentrations, smoking, and alcohol intake are important independent predictors of leptin in young healthy men. Obes Res, 1998; 6: 179-186.
33. Zeng WC, Zhang Z, Gao H, Jia LR, He Q. Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (Cedrus deodara). J Food Sci, 2012; 77(7): C824-9.