MODULATION OF DIGESTIVE ENZYMES AND LIPOPROTEIN METABOLISM BY ALPHA MANGOSTEEN EXTRACTED FROM MANGOSTEEN (*GARCINIA MANGOSTANA*) FRUIT PEELS

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ABSTRACT

The present work deals with the potential of *Garcinia mangostana* L. (Mangosteen, Clusiaceae) for modulation of digestive and plasma lipid transfer protein as an approach to discover novel inhibitors. *Garcinia mangostana* (in methanol, dicholromethane and hexane) extracts were screened for alpha amylase, alpha glucosidase and Cholesteryl ester transfer protein (CETP) inhibition assays. TLC, HPLC, LC-MS analysis were performed and was compared with reference standard. Alpha amylase results obtained were 39.4 µg/ml, 11.87 µg/ml and 9.048 µg/ml respectively. For CETP inhibition assay the dose response was done only for the hexane extract as others were not showing potent inhibition. Thus an IC_{50} of 10.89 µg/ml was obtained and the hexane extract was taken for further analysis to discover the compound responsible for the activity. Alpha mangosteen was found to be the active compound in *Garcinia mangostana* responsible for the potent inhibitor activity of alpha amylase and CETP enzyme in plant raw material.

Keywords: α-mangosteen, α-amylase, α-glucosidase, CETP inhibition, LC-MS

INTRODUCTION

Naturally available plant materials including herbs, food-processing by-products, contain active compounds, which have numerous health benefits. The identification of active ingredients and the mechanism of their action are very important to understand their health benefits. Some of these phytochemicals have been found to inhibit particular enzymes to treat specific disease. Bioactive molecules from plant sources having ability to inhibit the activity of digestive enzymes are targeted as preventative measures. Not only digestive enzymes but also metabolic enzymes, which catalyze the biochemical reactions taking place in body, are now the new and emerging trends to be evaluated for the specific diseases. For example, cholesteryl ester transfer protein is a metabolic enzyme, which facilitates the transfer of cholesteryl esters (CE) from high-density lipoprotein (HDL) to low-density lipoprotein (LDL) in exchange of triglycerides. Some plant-based food compounds, such as berry polyphenols are reported as having amylase inhibition activity (**McDougall *et al*., 2005**). There is still lack of research and report to prove the efficiency of compounds directly by cholesteryl ester transfer protein assay. Thus, there is need of exploring the novel inhibitors of such enzymes would definitely provide consumers a wide range of options to consume as food product and also for its disease preventing health benefits.

The present study deals with the exploration of such novel enzyme modulators, which are from natural plant sources. The phytochemicals which are responsible for the health benefits coming from plant sources are of considerable interest due to their wide range of physiological properties like antiallergenic, antiartherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic and cardio protective effects. Phytochemicals especially phenolic compounds have been reported to have many benefits on human health due to their presence in fruits and vegetables in high levels. **Zadernowski *et al*. (2009)** determined the composition of phenolic acids in various parts of mangosteen fruit (*Garcinia mangostana*) by GC-MS. Based on the traditional uses, certain plant extracts were selected which have already been reported for antioxidant, antidiabetic, antihyperlipidemic activities such as leaves of *Camellia sinensis*, *Azadirachta indica,* pericarp of *Garcinia mangostana* and rhizome of *Zingiber cassumunar* (**Ghasemzadeh *et al*. 2010, Mary *et al*. 2012**). These plant extracts were selected due to their physiological properties and were hypothesized to be potential inhibitors of digestive and metabolic enzyme. The overall objective of the present

study was to discover novel inhibitors of metabolic and digestive enzymes with the help of certain biochemical assays.

Garcinia mangostana, known as "Queen of fruits" is mostly found in India, Sri Lanka, Myanmar, Malaysia, Philippines and Thailand. The pericarp of the fruit contains 5.5 % (w/w) tannin resin and a yellow crystalline bitter principle compound, later named as mangostein and chemical formula is $C_{20}H_{22}O_5$ (Nadkarni & Nadkarni, 1999). Xanthones from mangosteen fruit have been found to have significant biological activities such as antibacterial, anti-inflammatory, antioxidants, antitumoral, antifungal, antiallergic, and antiviral etc. (**Suksamrarn *et al*., 2006**). Garcinone E, Alpha mangosteen, Beta mangosteen, Gamma mangosteen and gartanin are the important xanthones present in mangoteen fruit. The bioactive compounds from the fruit also positively affect lipid profile of plasma and also antioxidant activity in rats which are fed with cholesterol-containing diets (**Leontowicz *et al*., 2007**). Garcinone E, mangostinone, alpha, beta and gamma mangosteens, gartanin, 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone) and 1,5-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone were isolated from pericarps of mangosteen fruit (**Asai *et al*. 1995**). A new polyoxygenated xanthone, mangostanol and other known xanthones gartanin, α-mangostin, 8 deoxygartanin, γ-mangostin, 5,9-dihydroxy-2,2-dimethyl-8-methoxy-7-(3-methylbut-2-enyl)-2H,6H-pyrano[3,2-b]xanthen-6-one,2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3- ethoxyxanthone, garcinone E, and epicatechin were also isolated from mangosteen fruit hulls (**Chairungsrilerd *et al*., 1996**). Mangostenol, mangostenone and mangostenone B, three new xanthones along with the known xanthones α -mangosteen, trapezifolixanthone, garcinone B, tovophyllin B, beta mangosteen, mangostinone, mangostanol and flavonoid epicatechin were also isolated from mangosteen fruit hulls (**Suksamrarn *et al*., 2002**). Four new compounds garcimangosone A, garcimangosone B and garcimangosone C, also a benzophenone glucoside, garcimangosone D were also isolated (**Huang *et al*., 2001**).

MATERIALS AND METHODS

Materials

Garcinia mangostana (Mangosteen fruit) was procured from local market at Pathumthani, Thailand. Other plant materials were collected from G7 Synergon Ltd, Bangalore, India. Raw materials were cleaned; pericarp was separated from

edible pulp for Mangosteen fruit. Pericarp was dried at 50°C in hot air oven for 10-12 hrs. Dried samples (< 10 % M.C.) were ground in Tefal food processor (Hong Kong) until fine powder, stored in air tight containers in dark at room temperature (25 $\pm$ 2°C) for further analysis. Alpha amylase enzyme (Type VI B from porcine pancreas from Sigma), 2-chloro-4-nitrophenol-α-D-maltotrioside, acarbose, sodium dihydrogen orthophosphate and disodium hydrogen phosphate dihydrate (from Hi Media). Dimethyl sulphoxide was obtained from Sigma Aldrich. CETP Inhibitor Drug Screening Kit (Bio Vision) was procured from Gila Laboratories, Bangalore, India.

Preparation of crude extract

Hexane, dicholromethane and methanol were used to extract the bioactives from *Garcinia mangostana*, 20g of the powdered sample was extracted in 100mL of each of the mentioned solvents. Methanol and hexane extraction was carried out at 55°C and with dichloromethane at 37°C for 5 hours using an orbital shaker at 60 rpm. Mixture was further centrifuged at 10000 rpm, for 10 mins. Supernatant was collected and filtered using 0.45 μm membrane filters and evaporated to near dryness. Residue then collected was weighed and stored at 4 °C for further use in assay.

Preparation of rat intestinal mucosa

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India. Sprague Dawley rats (N=2) were sacrificed and their intestine was separated and chilled with ice cold phosphate buffer (80 mM, pH 7.0). Rat's intestinal mucosa was homogenized with four parts of ice-cold phosphate buffer (80 mM, pH 7.0). Tube was chilled with ice and kept for homogenization for 10 min. Large cell debris and nuclei like materials were separated by centrifugation In an eppendorf centrifuge at 10,000 rpm for 10 min maintained at 4°C and finally the supernatant was collected and stored at -20 °C until further use (**Leontowicz et al., 2007**).

Digestive and metabolic enzymes assay

α-amylase assay

Extract was dissolved in methanol and was diluted with phosphate buffer (pH 6.9) to obtain various concentrations ranging from 1-200μg/ml. The inhibitory activity of sample was assayed using procine pancreatic α-amylase, Type VI-B enzyme and 2-chloro-4-nitrophenol-α-D-maltotrioside (CNPG-3 reagent) as a substrate. Phosphate buffer (40 μl, 40 mM, pH 6.9) along with the test sample (1-200μg/ml), reference standard (0.1-10μg/ml) and 20 μl enzyme was pre incubated at 37°C for 10 mins. 80 μl substrate reagent (CNPG3) was added and incubated at 37°C for 8 mins in a 96 well plate and absorbance was read at 405 nm. A control reaction was also carried out without the test sample. The following formula was used to calculate the Inhibition %

Inhibition (%) = {Control absorbance - Test absorbance} x 100

Plot of percentage inhibition against the sample concentration was plotted and a logarithmic regression curve was established to calculate the IC_{50} value using Graph Pad Prism software. Half maximal inhibitory concentration (IC_{50}), represents the concentration of sample (μg/ml) necessary to decrease the activity of the enzyme by 50%.

α- Glucosidase activity of Mangosteen Extracts

Extract was dissolved in methanol and further diluted with phosphate buffer (pH 7.0) to obtain various concentrations ranging from 1-200μg/ml. The inhibitory activity of sample was assayed using α-glucosidase enzyme, isolated from rat intestinal mucosa and sucrose as a substrate. Phosphate buffer (40 μl, 80 mM, pH 7.0) along with the test sample (1-200μg/ml), reference standard (0.1-10 μg/ml) and 20 μl enzyme was pre incubated at 37°C for 30 min. The mixtures were incubated at 37°C for 20 mins after addition of sucrose solution (100 μl). The reaction was arrested by heating in a boiling water bath and cooled. Sample (50 μl) was added with 100 μl of glucose reagent from glucose reagent kit and incubated for 10 min at room temperature (25 °C), followed by measuring the absorbance at 510 nm (**Tadera et al., 2006**). A control reaction was carried out without the test sample. The following formula was used to calculate the Inhibition %

Inhibition (%) = {Control absorbance - Test absorbance} x 100

The IC_{50} values were calculated using Graph Pad Prism software as mentioned in

α-amylase assay

CETP inhibition activity of Mangosteen extracts

The assay uses a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule In the presence of CETP (rabbit serum). CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in an increase in fluorescence. Inhibitor of CETP will inhibit the lipid transfer and therefore decreases the fluorescence Intensity. Varying concentrations of the plant extracts ranging from (10-200 μg/mL) were used for the assay. Rabbit serum (1.5 μl) was added as enzyme source to the test sample and kept for pre-incubation at 37 °C for 10 min. Master mix (20 μl) containing 5 μl donor molecule, 5 μl acceptor molecule and 10 μl CETP assay buffer was added and remaining volume was made up with 20 μl of buffer and kept for incubation at 37 °C for 30 min. Blank without rabbit serum and positive control containing rabbit serum, only without test inhibitors were also conducted. The samples were analyzed by Fluorescence spectrometer (excitation wavelength 465 nm and emission wavelength of 535 nm) using a Tecan reader in a 96 well flat black microtiter plate.

Identification of active compound

Thin Layer Chromatography (TLC)

Screening of herbal products and their quality evaluations can be done easily with the help of standardized thin layer chromatographic techniques (**Mohammad et al., 2010**). According to **Mishra et al. (2009)**, quantitative estimation of alpha mangosteen can be done in fruit pericarp of *Garcinia mangostana* by using high performance thin layer chromatographic (HPTLC) method where methanol and chloroform was found to give highest and second highest recovery of alpha mangosteen, the bioactive xanthone. For our work, several solvent combinations were tried and optimized for separation of compounds in the extracts.

High Performance liquid chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LC-MS)

The separation of compounds was performed using HPLC 1200 (Agilent Technologies) with UV detector system. The reverse phase C18 Column (Phenomenex) was used, wavelength at 250 mm. The mobile phase (80 acetonitrile: 20 water) was filtered through fine membranes (0.4-0.5 micron) and samples to be injected were dissolved in HPLC grade solvent DMSO and further diluted with methanol. The injection volume for all samples was 10 μl. The flow rate was 0.6 ml/min, Total run time given was 45 min and detection was done at 210, 254, 310, 320 and 455 nm. Mass analysis was done in Agilent Triple Quad 6410 LC MS-MS instrument from Agilent Technologies. In this case, mobile phase used was Acetonitrile and 0.1% acetic acid in the ratio of 70:30 (v/v).

RESULTS AND DISCUSSION

α amylase inhibition assay

Acarbose was taken as standard drug which is a prescribed α amylase inhibitor for the treatment in Type 2 diabetes (**Loo et al., 2007**). Inhibition percentage for different conc. (0.1-10 μg/ml) of Acarbose was plotted on a nonlinear regression graph using graph pad prism software. Fig 1 illustrates the dose response curve, which has log of concentration of Acarbose on X axis and % inhibition (dose response) on Y axis.
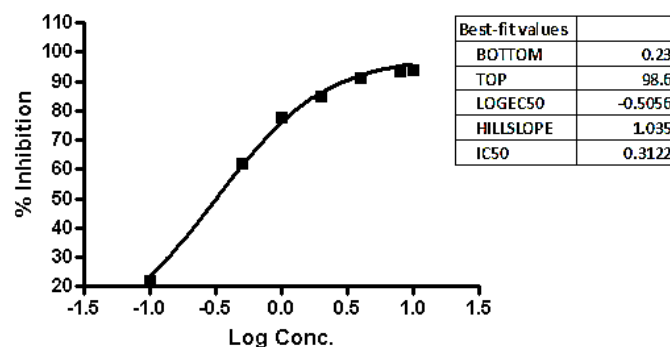
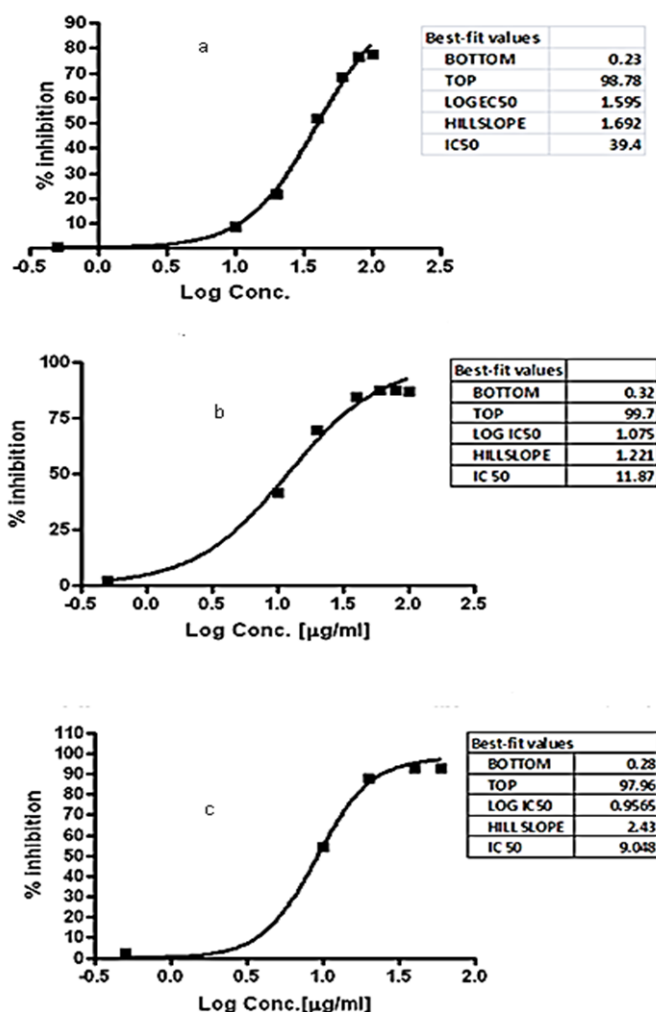**Figure 1** Dose response curve (Alpha Amylase Inhibition assay) for Reference standard Acarbose

The graph signifies the behavior of the curve at different concentrations of reference standard. An IC_{50} of 0.31 μg/ml was obtained. Acarbose has been proven as a very safe drug and a prescribed medication to treat Type 2 diabetes (**Mertes, 2001**). Thus the natural sources of alpha amylase inhibitors can be compared with this reference to prove their inhibitory activities.

Garcinia mangostana **extracts**

The methanolic, dichloromethane and hexane extract of *Garcinia mangostana* were analyzed for the alpha amylase inhibition assay at concentrations of (10-100) µg/ml. Methanol (1% v/v) was used as vehicle control. Percentage of inhibition was calculated using the equation:

$$\% \text{ Inhibition} = \{(\text{Control OD} - \text{Test OD})/\text{Control OD}\} * 100$$

The percentage inhibition plotted against log of concentration of the dose gave an IC_{50} of 39.4 µg/ml, 11.87 µg/ml and 9.05 µg/ml for methanol, dicholromethane and hexane extract respectively of *Garcinia mangostana*. Fig 2 illustrates the dose response curves for all the three extracts of *Garcinia mangostana*. The percentage inhibition was found increasing with the increased concentration of extracts. The three extracts are showing different results with the most potent activity found in the hexane extract having IC_{50} of 9.05 µg/ml which is slightly more potent than IC_{50} of dichloromethane extract which is 11.87 µg/ml. The results reflect that the active compound is of non-polar nature.

Best-fit values	
BOTTOM	0.23
TOP	98.78
LOGEC50	1.595
HILLSLOPE	1.692
IC50	39.4

Best-fit values	
BOTTOM	0.32
TOP	99.7
LOG IC50	1.075
HILLSLOPE	1.221
IC 50	11.87

Best-fit values	
BOTTOM	0.28
TOP	97.96
LOG IC50	0.9565
HILL SLOPE	2.43
IC 50	9.048

Figure 2 Dose response curve (α amylase inhibition assay) for a) methanolic, b) dichloromethane and c) hexane extract of *Garcinia mangostana*

In a study where different fractions of *Garcinia mangostana* plant were tested, polyphenols rich fraction was found to have highest inhibitory activity with IC_{50} of 5.4 µg/mL but total phenolic content was not having any correlation with the alpha amylase inhibitory activity. Oligomeric proanthocyanidins were the main compounds present in the fraction having high inhibitory activity (**Loo *et al.*, 2007**). In another study, the ethanol extract of the aerial parts of *Salvia Virgata* showed an Inhibitory activity on pancreatic α - amylase. Further the study led to Identification of the active flavone, chrysoeriol. (**Nickavar *et al.*, 2013**). Tea polyphenols (0.05 mg/ml) have also inhibited α amylase with 61%, which also suggested that tea polyphenol might possess antinutritional properties (**He *et al.*, 2006**). In the present study *Garcinia mangostana* has been found to have a very significant activity against alpha amylase enzyme and has already been reported to have a very good antioxidant activity. Thus the correlation between the two activities can be a good area to explore.

Alpha glucosidase inhibition assay

Methanol, dichloromethane and hexane extracts of *Garcinia mangostana* were assayed for alpha glucosidase inhibition assay with Acarbose as standard and did not show any significant inhibition. Berry extracts rich in polyphenols inhibited alpha amylase and alpha glucosidase (**Ashley *et al.*, 2012**). Anthocyanins inhibit alpha-glucosidase activity and thus, is helpful in reducing the blood glucose levels. Simultaneously, ellagitannins are found to inhibit alpha amylase enzyme. Thus intake of berries which are rich both in anthocyanins and ellagitannins can cause a cumulative effects in digestion of carbohydrates (**McDougall *et al.*, 2005**). Potent inhibitory compounds have also been investigated by quantifying the polyphenolic composition in effective and less effective extracts. Modulation of their glycemic response has been studied in humans also to prove the authenticity of their effectiveness. Polyphenols and phlorotannins (PHT) from brown seaweeds have also been found to inhibit α amylase and α glucosidase enzyme. PHT extracted from *Ascophyllum nodosum* and *Fucus vesiculosus* inhibited α amylase and α glycosidase with very low IC_{50} values which is comparable to other plant polyphenols (**Roy et al., 2011**). Orlistat induced weight loss mechanism behind which is lipid inhibition has generated interest of scientists in finding nutraceuticals that inhibit the breakdown of macromolecules such as carbohydrates and fats. Acarbose and phaseolamine have been reported to have some promising results relating to weight loss (**Tucci *et al.*, 2010**). Thus, the pericarp of *Garcinia mangostana*, which is a good source of alpha amylase inhibitors as well as have a very significant antioxidant activity, can be targeted as therapeutic drug in future. Many diseases related to macromolecules digestion can be solved by deciphering such compounds which can delay nutrient digestion and thus does not allow them to get absorbed fast in the body, helping in curing diseases associated with them.
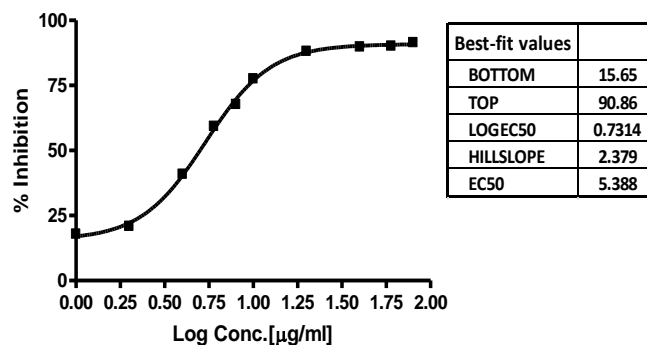
CETP inhibition assay

Methanol, dichloromethane and hexane extracts of *Garcinia mangostana* were analyzed for CETP inhibition assay at various concentrations (10-100 µg/ml) by using CETP inhibition drug screening kit. Similar to α amylase inhibition results, it was found that hexane extract gave a remarkable inhibition of 85.67%. Due to this significant result, the extract was taken for dose response study and an IC_{50} of 10.89 µg/ml was obtained (Fig 3).

CETP inhhibition assay for GMH Extract

Best-fit values	
BOTTOM	10.51
TOP	86.76
LOGEC50	1.037
HILLSLOPE	1.714
EC50	10.89
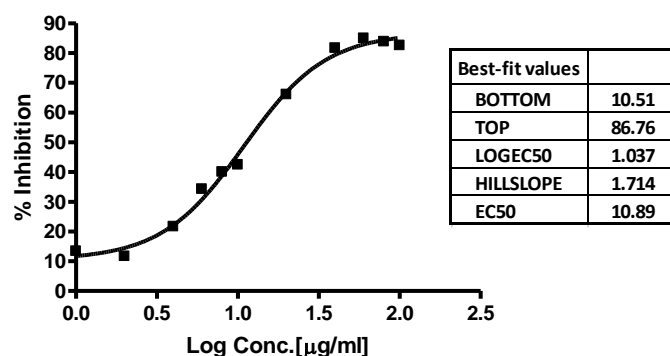
Figure 3 *Dose response curve (CETP Inhibition assay) for hexane extract of* Garcinia mangostana

CETP Inhibition assay for alpha mangoteen

Best-fit values	
BOTTOM	15.65
TOP	90.86
LOGEC50	0.7314
HILLSLOPE	2.379
EC50	5.388

Figure 4 Dose response curve (CETP Inhibition assay) for reference standard α mangosteen

In order to confirm that α mangosteen is the potent compound, CETP inhibition assay was again performed with the reference standard and below is the dose

response curve (Fig 4) obtained for the different concentrations of α mangosteen. A significant IC_{50} of 5.388 µg/ml was obtained which confirmed that the potent active compound responsible for CETP inhibition is α mangosteen. The IC_{50} for hexane extract is 10.89 µg/ml which is in the range of two fold and thus acceptable. Till date, the most active natural CETP inhibitor, and [10] - dehydrogingerdione, has been found from the rhizomes of *Zingiber officinale* Roscoe. This active compound inhibited cholesteryl ester transfer protein with IC_{50} values of 35 µM and it was compared against a positive control D-Sphingosine that gave an IC_{50} of 11.5 µM. Very few compounds isolated from the natural sources have been identified as CETP inhibitors among which dehydrogingeridione was found to have the strongest inhibitory activity against cholesteryl ester transfer protein (**Choi *et al.*, 2011**). Four ceramides which have been isolated from the gorgonian *Acabaria undulata* and N, N-dimethylsphingosine found to inhibit cholesteryl transfer protein with IC_{50} values of 46.8, 57.3, 86.3, 65.6 and 6.3 µm (**Jeong *et al.*, 1997**). Xanthohumol, a prenylated chalcone also showed the highest inhibition against cholesteryl ester transfer protein. Naringenin chalcone showed weak cholesteryl ester transfer protein inhibition compared with xanthohumol. Isoxanthohumol and Naringenin significantly decreased the inhibitory activity. According to these results, it was concluded that the prenyl group and chalcone structure of xanthohumol were responsible for the cholesteryl ester transfer protein inhibitory activity. Xanthohumol had a potent cholesteryl ester transfer protein inhibitory property with an IC_{50} of 31.2 µg/ml (88.0 µm). The mechanism behind was noncompetitive and chalcone structure and prenyl group were found to be essential for its inhibitory action (**Hirata *et al.*, 2012**). The observation from Japanese populations with CETP deficiency has led to the conclusion that cholesteryl ester transfer protein inhibiting drugs may elevate concentration of high density lipoprotein and thus decrease cardiovascular risk and atherosclerosis (**Barter *et al.*, 2006 and 2007**).Thus from the present study, where alpha mangosteen has been found as a novel inhibitor of cholesteryl ester transfer protein. As the screening was performed by biochemical assay, further if taken up for analysis in human plasma, the results then can be translated into the possibility of a drug as cholesteryl ester transfer protein inhibitor derived from a natural source and can be a promising cure for certain diseases.

Thin layer chromatography

Chloroform and ethyl acetate (80:20) solvent system gave the best result in silica plates. It showed one major band with the separation of one particular compound which made it easier for further analysis of HPLC. One prominent band in Chloroform: ethyl acetate (80:20) solvent system indicated one major compound in the hexane extract of *Garcinia mangostana* pericarp. Along with this majorly present compound some other compounds were also suspected to be present in small quantity.

High performance Liquid Chromatography (HPLC)

HPLC was further used for analyzing the compounds of the hexane extract of *Garcinia mangostana* pericarp. Result showed one major peak prominent at RT 16.28 min along with some other minor compounds (Fig 5).
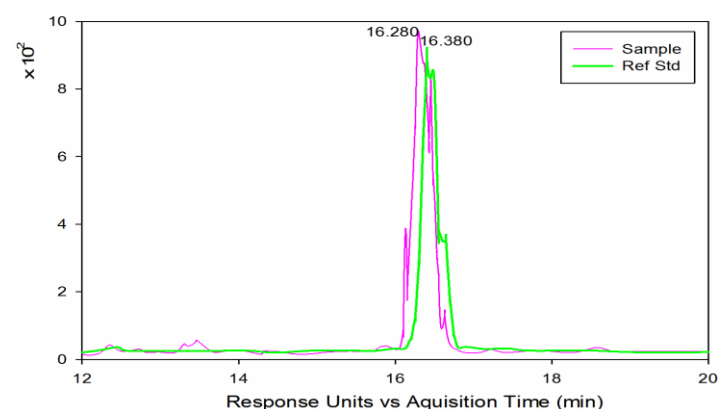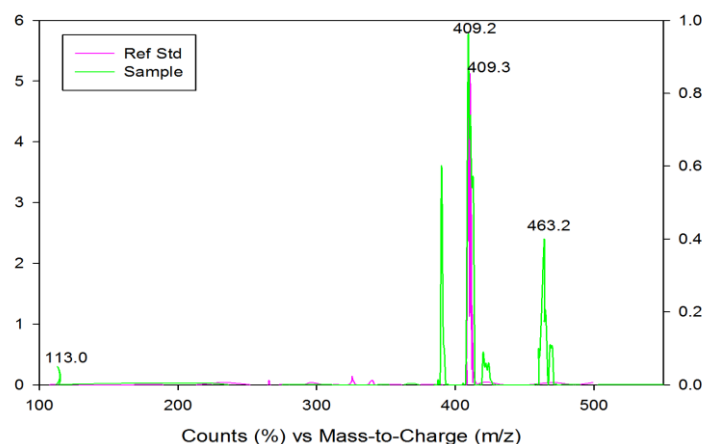

Figure 5 Comparison between HPLC results *of Garcinia mangostana* hexane extract and α mangosteen

Method for detection and quantification of α mangosteen in plasma consists of reversed phase high performance liquid chromatography (C18 reversed phase column) with ultraviolet detection. The mobile phase consisted methanol and water (95:5 % v/v) at a flow rate of 1.0 ml/min. Under these conditions, the retention time for α mangosteen was 10.84 min (**Syamsudin *et al.*, 2010**). In another study, chromatographic separation was done on a Hypersil BDS C-18 column using a gradient mobile phase (70-80 % acetonitrile in 0.1 % v/v ortho phosphoric acid). The flow rate used was 1 ml/min with UV detection at 320 nm where two extracts showed similar pattern and a major peak of α mangosteen was

obtained for which retention time was 16.32 min (**Pothitirat and Gritsanapan, 2009**). It has been found that acetone and water (80:20) is an efficient solvent system to extract a wide variety of xanthones. Subsequent to which HPLC analysis was done using C-18 reverse phase column and a 30 min gradient of 65–90% methanol in 0.1% formic acid solvent system was found effective to separate different xanthones with UV detection at 254 nm. Various xanthones and 9 hydroxycalabaxanthone, α mangosteen, β mangosteen, gartanin, 8 desoxygartanin and 3 mangosteen were identified (**Edward, 2007**). Thus this method proved to be a good method for analysis of xanthones in the pericarp of *Garcinia mangostana*. Fig 5 illustrates the comparative HPLC results of hexane extract of *Garcinia mangostana* and α mangosteen where the peak in pink colour represents the hexane extract and green color represents alpha mangosteen. At 310 nm, the retention time of α mangosteen was found to be at 16.38 min which is very much near to the result obtained for the sample i.e. 16.28 min. In this case, under the same chromatographic conditions similar retention time has been achieved.

Liquid Chromatography Mass Spectrometry (LC-MS)

Fig 6 shows the LC-MS results for *Garcinia mangostana* hexane extract in negative mode. The chromatogram clearly indicated the presence of α mangosteen in the sample along with other xanthones such as Garcinone E etc. Result illustrated green color peak for hexane extract of *Garcinia mangostana* and pink color for standard α mangosteen. LC-MS results for the sample were found comparable with reference standard results. *M/z* value for sample was found to be 409.2 compared to α mangosteen M.W. 409.3 (**Zarena *et al.*, 2009**). Thus it can be concluded that α mangostin was present as major compound in the sample and might be responsible for potent activity.

Figure 6 Comparison between LC-MS results of *Garcinia mangostana* hexane extract and α **mangosteen**

To determine the concentration of alpha mangosteen for toxicological investigation of a nutraceutical formulation, the LC separation was developed on a Phenomenex Gemini C-18 column using a 12 min. gradient consisting of 1% formic acid and methanol: acetonitrile (20:80, v/v). In a single 35 min chromatographic run, ethyl acetate, acetone and hexane extracts of mangostin peel were analysed by HPLC (**Xiuhong *et al.*, 2007**). The polarity of the solvents played a major role in the extraction and purification of a compound from a complex mixture. This requires a comprehensive study about the type of compounds present in the raw material. Seven xanthones 3-isomangostin-*m/z* 427, 8-desoxygartanin- *m/z* 379, gartanin-*m/z* 395, α-mangostin- *m/z* 409, garcinone E - *m/z* 463, 9-hydroxycalabaxanthone- *m/z* 407 and β-mangostin - *m/z* 423 were detected with α mangostin being the predominant compound in all the extracts. α mangostin has *m/z* value of 409 in the negative mode and it shows m/z value of 411 in the positive mode which shows that it is a kind of neutral compound which gives reading in both the modes (**Zarena *et al.*, 2009**).

CONCLUSION

The focus of the study was to discover novel natural inhibitors of digestive and metabolic enzymes which can be taken up for further clinical trials and can be targeted as therapeutic drugs in future. Among many plant extracts tested for the inhibitory activity, not many have been found to show the potent activity. In the current study, the methanolic, dichloromethane and hexane extracts of *Garcinia mangostana* was found to be effective against alpha amylase enzyme and thus can be targeted as a therapeutic drug for Diabetes. The hexane extract of *Garcinia mangostana* pericarp has shown potent activity against cholesteryl ester transfer protein. The findings of the study conclude that the pericarp of *Garcinia mangostana* is a remarkable inhibitor of α amylase enzyme with the most effective hexane extract of IC_{50} 9.048 µg/ml. The pericarp of *Garcinia mangostana* is also a remarkable inhibitor of cholesteryl ester transfer protein

with the hexane extract of IC$_{50}$ 10.89 µg/ml. The most bioactive compound present in the pericarp of *Garcinia mangostana* is responsible for its potent activity to inhibit cholesteryl ester transfer protein with an IC$_{50}$ of 5.388 µg/ml which is also comparable to the hexane extract of *Garcinia mangostana* pericarp. The pericarp of *Garcinia mangostana* is a rich source of xanthones having antidiabetic and anticholesterol activity. The pericarp of *Garcinia mangostana* can be further purified to identify the active compound responsible for its inhibitory activity against alpha amylase enzyme. α mangosteen can be taken for further clinical trials to prove the results for CETP inhibition in animal studies. Application of alpha mangosteen in food products and beverages can be done to develop functional foods.

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