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The Pharma Innovation

ISSN: 2277- 7695
TPI 2014; 3(7): 44-52

www.thepharmajournal.com
Received: 24-07-2014
Accepted: 28-08-2014

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Pancreatic lipase inhibitory screening of *Citrullus lanatus* leaves

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Abstract
Natural remedies offer an exciting opportunity and promise for the development of new therapeutic lead molecule to the treatment of obesity by inhibiting the digestion and absorption of dietary lipids which is valuable alternative to synthetic one. In this study to evaluate pancreatic lipase inhibitory activity of Methanolic extract of *Citrullus lanatus* (MECL) leaves.
Preliminary phytochemical screening of MECL was done by standard procedure. Total Phenolic and Flavonoid content, Tannin content and estimation of Vitamin C were estimated by using Gallic acid, Quercetin, tannic acid and standard Ascorbic acid calibration curve respectively. Pancreatic lipase (PL) was done by using chicken pancreas and orlistat used as a standard.
Preliminary phytochemical screening showed the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, sterols and absence of glycosides, volatile and fixed oil. Phenolic content (in terms mg GAE/g of extract), Flavonoids (mg Quercetin equivalent/g of extract) and Tannin content (mg Tannic acid/g of extract) present in the MECL were found to be 47.05±0.338, 89.99±0.30 and 290.9 ± 0.12 mg/g respectively. The amount of Vitamin C of MECL was found to be 34.00 ± 0.009mg/g. Percentage inhibition and IC_{50} of MECL and orlistat was found to be 61.77 ± 1.102 & 73.63 ± 0.553 at a concentration of 5mg/ml and 3.962 mg/ml & 3.420 mg/ml respectively.
The dose dependent pancreatic lipase inhibitory activity was observed. The extract of *Citrullus lanatus* possesses a good inhibitory activity on pancreatic lipase. It may be the presence of flavanoid, tannins, terpenes, phenols, vitamin C and saponins. MECL employed as a therapy for the management of the obesity epidemic.

Keywords: Pancreatic lipase, *Citrullus lanatus*, obesity, flavonoids.

1. Introduction
Obesity is a medical complication caused by an imbalance between energy intake and expenditure (BMI ≥ 30 kg/m^2) and is broadly recognized as a major public health problem. Obesity is a complex multi-factorial and chronic disease that can lead to variety serious diseases, including hypertension, hyperlipidemia, atherosclerosis, osteoarthritis and type II diabetes and thus indirectly leading to aging [1, 2].
The prevalence of obesity is increasing at an alarming rate, but, unfortunately, only a few drug candidates are currently available in market. Recently, newer approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase (PL) as this is the major source of excess calories [3]. New Pancreatic lipase inhibitors derived from natural sources especially from medicinal herbs are used for the treatment of obesity.
Pancreatic lipase (PL) is an enzyme, secreted from the pancreas and plays an excellent role in the absorption of triglyceride in the small intestine. Dietary fats are composed of about 95% triacylglycerols (TG). Pancreatic lipase hydrolyses the water insoluble triacylglycerols in the intestinal lumen and thereby used for the dietary fat absorption. Pancreatic lipase inhibitors are considered to be a valuable therapeutic agent for treating diet-induced obesity [4, 5].
Citrullus lanatus is well known as Watermelon plant (Family - Cucurbitaceae). Water melon is popular in indigenous system of folk medicine. It is a trailing annual plant with several herbaceous, firm and stout stems [6-8]. The leaves of *Citrullus lanatus* is used as anti-inflammatory, analgesic, gonorrhoea, mosquitocidal and anti-microbial property [9-11]. Cucurbitaceae plants are known to contain therapeutic compounds such as triterpenes, sterols, cucurbitacin and alkaloids [12].
Now-a-days it is the need of the hour to update the knowledge on the numerous natural sources that could act as inhibitors of PL in order to screen them as new potential therapeutic antiobesity agents with low secondary effects.

The plant *Citrullus lanatus* has been selected (specially the leaves) for the present investigation on the basis of the ethnomedical information and the review of literature as the plant is widely cultivated throughout India. In this present study focused screening of pancreatic lipase activity of Methanolic extract of *Citrullus lanatus* by using chicken pancreas.

2. Materials and methods

2.1. Requirements

Chicken (*Gallus domesticus*) pancreas, Sucrose solution (0.01M), Ammonium sulphate (50% saturation), Phosphate buffer (pH 7), Olive oil, Pancreatic lipase and Orlistat (60 mg).

2.2. Instruments

Shimadzu UV Visible spectrophotometer, Model 1800.

2.3. Collection and preparation of extract

The leaves of *Citrullus lanatus* were collected in Thuvarankurichi during the month of August 2013. The plant specimen was identified and authenticated as *'Citrullus lanatus'* (Cucurbitaceae) by Dr. Stephen, Senior Lecturer in Botany and Taxonomist, Dept. of Botany, The American College, Madurai, Tamil Nadu, India. The authenticated herbarium of plant has been kept in the Department of Pharmacognosy, Madurai Medical College, Madurai.

The leaves were washed thoroughly and dried in shade. The shade dried leaves were powdered and used for further studies. Extraction of leaves of *Citrullus lanatus* was carried out by washing the plants and drying at room temperature in 14 days. After that, they were filtered with sieve analyzer to get homogeneous particles and defatted with 2.5 L of Petroleum ether (60-80 ˚C) by cold maceration method for 72 hr. The solvent was then removed by filtration and the marc was dried. The dried marc was re-soaked with 2.5 L of Methanol. The steps were performed three times and the combined filtrates were evaporated to a cohesive mass using rota vapour.

2.4. Preliminary phytochemical screening [13-15]

The preliminary phytochemical screening helps us in identifying the type of secondary metabolites present in plants. Preliminary phytochemical screening of Aqueous and Methanolic extract of *Citrullus lanatus* leaf was carried out by using standard procedure.

2.5. Quantitative estimation of phytoconstituents

2.5.1. Estimation of total Phenolic content [16-18]

Phenols are widespread in nature and are important constituents of medicinal plants. They range from simple structures with one aromatic ring to highly complex polymeric substances such as tannins, flavonoids, Anthraquinone and coumarins. Phenolic substances are water soluble and they have been reported to have multiple biological effects, including antioxidant activity.

The calibration curve of concentration versus absorbance was generated for Gallic acid at different concentrations (2, 4, 6, 8, 10 µg/ml) which was used as a standard. The amount of phenol present can be determined by linear regression analysis. The total Phenolic content of the MECL was determined by Folin-Ciocalteau reagent and it was expressed as milligram of Gallic acid equivalent (GAE) per g of extract.

2.5.2. Total Flavonoid content estimation [19-21]

Flavonoids are widely distributed in nature. It consists of one Benzene–gamma – pyrone structure. They have ability to complex with metal ions and act as an antioxidants and bind to proteins such as structural proteins and enzymes. The different classes within the groups are distinguished by additional oxygen containing heterocyclic rings and hydroxyl groups which includes Flavones, Flavanones, Flavonols, Isoflavones, Catechin, Anthocyanidins, Leucoanthocyanidins, Chalcones and Aurones.

The Aluminum chloride colorimetric technique was used for estimation of total Flavonoid content. The intensity of the colour is proportional to the amount of Flavonoids and can be estimated as Quercetin equivalent at wavelength of 415nm. The amount of Flavonoids present was determined by linear regression analysis. The total Flavonoid content in MECL was expressed as mg of Quercetin equivalents per g of extract.

2.5.3. Estimation of Tannins [22]

Tannins are naturally occurring polyphenolic compounds of varying structure. Tannins are having antioxidant and antimicrobial activities and also used as antiseptic and astringents. They are divided into two main groups namely hydrolysable and condensed. Hydrolysable tannins contain a polyhydric alcohol and Condensed tannins are mostly Flavonols.

Principle: The tannins are estimated by Folin-Denis Method. This is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group. Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution. The intensity is directly proportional to the amount of tannins and measured in a spectrophotometer at 700 nm.

Procedure: About 0.2 ml of Methanolic extract of *C. lanatus* was pipetted into test tubes. To this, 0.5 ml of Folin-Denis reagent and 0.8 ml of distilled water was added. The tubes were kept aside for 15 min. To this, 1ml of Sodium carbonate solution was added and the remaining volume was made up with 7.5 ml of distilled water. Then the tubes were shaken and the absorbance was recorded at 700 nm after 30 min. Tannic acid, used as a standard was taken at different concentration i.e 2, 4, 8, 12, 16, 20 µg/ml in different test tubes and the procedure adopted above was followed. The calibration curve for Tannic acid was plotted using concentration versus absorbance. A linear regression equation was calculated and the equation was used to calculate the amount of total Tannins as Tannic acid equivalent. The amount of Tannin content is expressed in mg/g of extract.

2.5.4. Estimation of Vitamin C [23-26]

Vitamin C is also an important physiological antioxidant and has been shown to regenerate other antioxidants within the body, including α-Tocopherol (Vitamin E). Vitamin C might help to prevent or delay the development of certain cancers, cardiovascular disease, and other diseases in which oxidative stress plays a causal role. In addition to its biosynthetic and antioxidant functions, vitamin C plays an important role in immune function and improves the absorption of non-heme iron, the form of iron present in plant-based foods.

Principle: The keto group of Ascorbic acid undergoes a condensation reaction with 2, 4 Dinitro phenyl hydrazine to form a hydrazone which is orange yellow and has an absorbance of about 520 nm.

Procedure: Ascorbic acid was weighed and dissolved in water to get stock solution of 1 mg/ml. Further dilutions were made to get the concentrations ranging from 40-200 µg/ml. To 1 ml of sample 0.5 ml of Dinitro phenyl hydrazine solution was added and incubated for 3 hr at 37 ºC. After 3 hr, 2.5 ml of 85% Sulphuric acid was added and the absorbance was measured after 30 min at 520 nm. A calibration curve was constructed by plotting concentration versus absorbance of Ascorbic acid. The procedure was repeated for the plant extract as above and the absorbance was measured at 520 nm after 3 hr. The amount of Vitamin C can be determined by linear regression analysis and it was expressed as mg/g of extract.

2.5.5. Estimation of Vitamin B_2 [27, 28]

Principle: Riboflavin was treated with Potassium permanganate ($KMnO_4$) and Hydrogen peroxide (H_2O_2). Mixing of Hydrogen peroxide solution, where upon the permanganate colour is destroyed, excess oxygen is expelled and then Sodium sulphate was added and a yellow colour was obtained. The absorbance of the colour was measured at 550 nm by UV/Vis spectrophotometer.

Sample Preparation: 10 g of powdered plant material was extracted with 50% Ethanol solution and shaken for 1 hr. The plant extract was filtered and used as a sample.

Procedure: Riboflavin was weighed and dissolved in water to get stock solution of 20 mg/ml. Further dilutions were made to get the concentrations ranging from 2-10 mg/ml. To 15 ml of sample and 10 ml of 0.5% Potassium permanganate and 1ml of 30% Hydrogen peroxide were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% Sodium sulphate was added. This was made up to 5 ml. The absorbance of the chromogen was measured at 550 nm in a UV visible spectrophotometer. A calibration curve was constructed by plotting concentration versus absorbance of Riboflavin. The above procedure was repeated for the plant extract and the absorbance was measured at 550 nm. The amount of Vitamin B_2 present can be determined by linear regression analysis and it was expressed as mg/g of extract.

2.6. Extraction of Lipase from Chicken (*Gallus domesticus*) Pancreas [29]

Pancreas of freshly slaughtered chicken was collected with the guidance of a veterinary surgeon. It was washed thoroughly and pancreatic lipase was placed in ice cold sucrose solution (0.01 M). The Pancreas was homogenized in 0.01 M Sucrose and centrifuged. The supernatant solution was separated and subjected to Ammonium sulphate precipitation (50% saturation). The obtained white pellets after centrifugation was dissolved in sucrose solution and again saturated with 50% Ammonium sulphate and centrifuged. Finally pellets were used as enzyme source by dissolving in phosphate buffer (pH 7).

2.7. Determination of Chicken Pancreatic Lipase activity [30]

The chicken pancreatic lipase activity was determined by incubating an emulsion containing 8ml of Olive oil (Dietary fat), 0.4 ml of Phosphate buffer and 1ml of Chicken pancreatic lipase for an hour. The reaction was stopped by addition of 1.5 ml of a mixture containing Acetone and 95% Ethanol (1:1). The amount of liberated fatty acid was determined by titrating the emulsion against 0.02 M Sodium hydroxide (standardized by Potassium hydrogen phthalate) using phenolphthalein as an indicator. The end point is the appearance of pink colour. The volume of Sodium hydroxide consumed was taken as (A).

2.8. Pancreatic lipase inhibitory activity [30]

MECL was prepared in different concentrations such as 1-5 mg/ml. A 100 µl of each concentration of sample was mixed with 8 ml of olive oil, 0.4 ml Phosphate buffer and 1ml of chicken pancreatic lipase and it was incubated for 60 mins. The reaction was stopped by the addition of 1.5 ml of a mixture containing acetone and 95% Ethanol (1:1). Appearance of pink colour from yellow colour shows the liberated fatty acids, which was determined by titrating the solution against 0.02 M Sodium hydroxide (standardized by Potassium hydrogen phthalate) using Phenolphthalein as an indicator and the percentage inhibition of lipase activity was calculated using the following formula, Lipase inhibition = [A-B/ B] ×100, where A - Lipase activity, B - Activity of lipase when incubated with the standard and test compounds.

3. Results and Discussion

3.1. Preliminary phytochemical screening of *C.lanatus* leaves (Table 1)

Table 1: Preliminary phytochemical screening of *Citrullus lanatus* leaf

S. No.	TEST	Extract	
		Aqueous extract	Methanolic extract
1.	**Test for Carbohydrates**		
	a. Molisch's test	+	+
	c. Benedict's test	+	+
	b. Fehling's test	+	+
2.	**Test for Alkaloids**		
	a. Mayer's reagent	+	+
	b. Dragendroff's reagent	+	+
	c. Hager's reagent	+	+
	d. Wagner's reagent	+	+
3.	**Test for Phytosterols**		
	a. Salkowski's test	+	+
	b. Libermann- burchard's test	+	+
4.	**Test for Glycosides**		
	a. Anthraquinone glycosides	-	-
	i) Borntrager's test	-	-

	ii) Modified Borntrager's test	-	-
5.	**Test for Proteins**		
	a. Millon's test	+	+
	b. Biuret test	+	+
	Amino acids		
	a. Ninhydrin test	+	+
6.	**Test for Mucilage**	-	-
7.	**Test for Flavonoids**		
	a. Shinoda test	+	+
	b. Alkali test	+	+
	c. Acid test	+	+
8.	**Test for Terpenoids**	+	+
9.	**Test for Phenolic compounds**		
	a. 5% Ferric chloride solution	+	+
	b. Lead acetate solution	+	+
	c. Bromine water	+	+
	d. Acetic acid solution	+	+
	e. Dilute iodine solution	+	+
	f. Tannic acid	+	+
10.	**Test for Tannins**		
	$FeCl_3$ test	+	+
11.	**Test for Saponins**		
	Foam test	+	+
12.	**Test for Volatile oils**	-	-

(+) Present, (-) Absent

3.2: Quantitative estimation of phytoconstituents

3.2. Quantitative estimation of phytoconstituents

3.2.1. Estimation of Phenolic content

Phenolic content of MECL and calibration curve of Gallic acid was presented (Tab 2 & Fig. 1)

Table 2: Total Phenolic content of MECL in terms of Gallic acid equivalents

S. No.	Conc. of Gallic acid in µg/ml	Absorbance at 760 nm	Conc. of extract in µg/ml	Absorbance at 760 nm*	Amount of total Phenolic content in terms mg GAE/g of extract*
1	2	0.229 ± 0.010	50	0.256±0.004	43.90±0.304
2	4	0.452 ± 0.006	100	0.578±0.004	50.20±0.373
3	6	0.695 ± 0.005	**Average 47.05 ± 0.338**		
4	8	0.918 ± 0.031			
5	10	1.162 ± 0.028			

* mean of three readings ±SEM

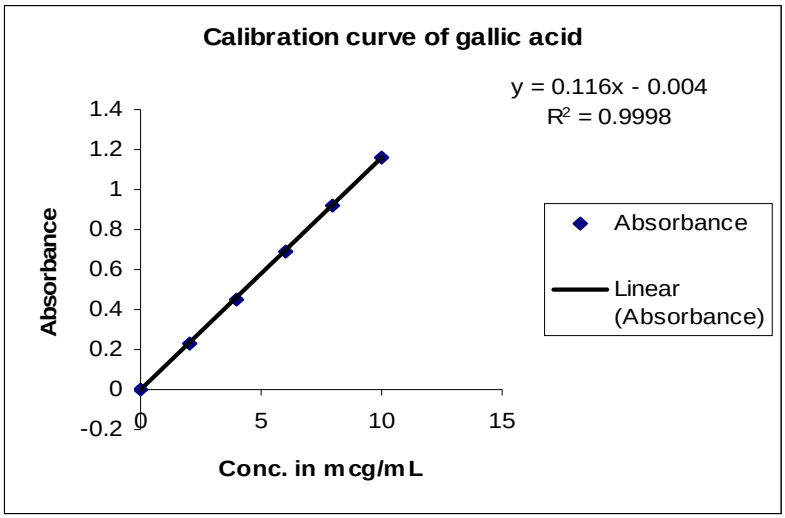

Fig 1: Calibration curve of Gallic acid for estimation of total Phenolic content

The linear regression equation was found to be y=0.116x-0.004 while the correlation coefficient was found to be 0.9998. The amount of Phenolic content present in the extract in terms mg GAE/g of extract was found to be 47.05±0.338 by using the above linear regression equation.

Polyphenols are naturally occurring compounds largely found in the herbals and medicinal plants. Phenolic compound may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress [31]. Phenolic compounds have some potential efficacy for preventing obesity. They inhibit enzymes related to fat metabolism including PL, lipoprotein lipase, and glycerophosphate dehydrogenase [32]. Polyphenol extracts are able to decrease the blood levels of glucose, triglycerides, and LDL cholesterol, increase energy expenditure and fat oxidation, and reduce body weight and adiposity [33, 34]. Gallic acid (3, 4, 5-trihydroxybenzoic acid; GA) is a naturally abundant phenolic compound in most of the vegetables [35]. A number of studies have demonstrated that antioxidants may act as a regulator of obesity in mice or rats with high fat-diets [36, 37].

3.2.2. Estimation of total Flavonoids

Total Flavonoid estimation MECL & Calibration curve of Quercetin was presented (Tab 3 & Fig. 2).

Table 3: Total Flavonoid content of MECL in terms of Quercetin equivalents

S. No.	Conc. of Quercetin in µg/ml	Absorbance at 415 nm	Conc. of Methanolic extract in µg/ml	Absorbance at 415nm*	Amount of total Flavonoid content in terms mg Quercetin equivalent/ g of extract*
1	20	0.589 ± 0.01	50	0.090±0.001	86.55±0.21
2	40	1.151 ± 0.04	100	0.243±0.003	93.44±0.39
3	60	1.710 ± 0.09	**Average 89.99±0.30**		
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03			

* mean of three readings ±SEM

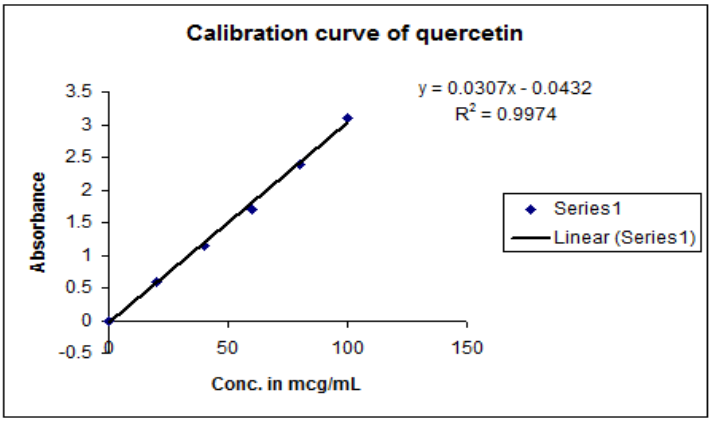

Fig 2: Calibration curve of Quercetin

The linear regression equation was found to be y=0.0307x-0.0432 while the correlation was found to be 0.9974. The amount of Flavonoid content present in the extract in terms mg Quercetin equivalent/g of extract was found to be 89.99 ± 0.30 by using the above linear regression equation.

More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the therapeutic activity in humans. Quercetin, Myricetin, Catechin etc., are some most common flavonoids [38]. Molecular studies showed flavonoids act as viable and promising therapeutic agents to treat the dysregulation of lipid homeostasis, metabolic disease, and its cardiovascular complications [39].

An inverse relationship between flavonoid consumption and many risk factors including improved weight management [40], and improved dyslipidemia [41, 42] has also been established, suggesting that flavonoids have multiple targets.

Quercetin decreased the expression of Sterol Regulatory Element-Binding Proteins (SREBP)-1 and Fatty Acid Synthase (FAS), and by increasing Acetyl-CoA Carboxilasa (ACC) phosphorylation [43]. Quercetin to be a potent inhibitor of the stimulating effect of vanadate on lipoprotein lipase (LPL) activity. Vanadate shows insulinmimetic effects, such as increases in LPL and suppression of hormone-dependent lipolysis, in isolated rat adipocytes [44]. Quercetin induced a dose- and time-dependent increase in lipolysis, which was synergic with epinephrine-induced lipolysis. This flavonoid produces a competitive phosphodiesterase (PDE) inhibition [45]. In addition, this flavonoid can also reduce the number of adipocytes, either by decreasing adipogenesis or increasing apoptosis [46].

3.2.3. Total Tannin estimation

Total Tannin estimation of MECL & calibration curve of Tannic acid was presented (Tab 4 & Fig 3).

Table 4: Total Tannin content in MECL in terms of Tannic acid equivalents

S. No.	Conc. of Tannic acid in µg/ml	Absorbance at 760nm	Conc. of Methanolic extract in µg/ml	Absorbance at 760nm*	Amount of total Tannin content in terms mg Tannic acid/g of extract*
1	4	0.098 ± 0.020	10	0.060±0.03	260.60±1.51
2	8	0.183 ± 0.010	20	0.131±0.07	292.42±2.00
3	12	0.203 ± 0.010	**Average 276.51±1.75**		
4	16	0.361 ± 0.200			
5	20	0.451 ± 0.100			

* mean of three readings ±SEM

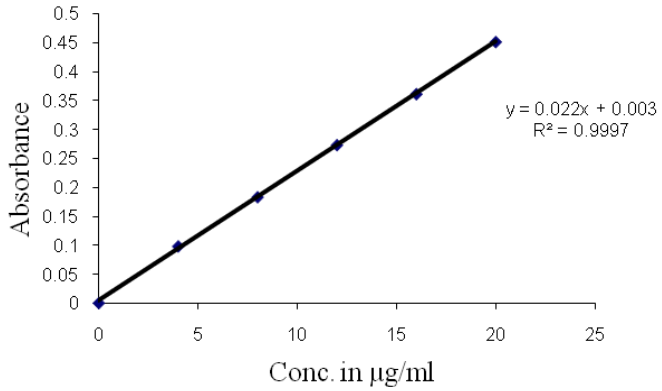

Fig 3: Calibration curve of Tannic acid

The linear regression equation was found to be y = 0.022x + 0.003 while the correlation was found to be 0.9997. The amount of Tannin content present in the MECL in terms of mg Tannic acid/g of extract was found to be 276.51 ± 1.75 by using the above linear regression equation.

These protective effects are related to their capacity to: (a) act as free radical scavengers; (b) activate antioxidant enzymes. Capacity of tannins to enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of non-insulin dependent diabetes mellitus & obesity [47].

3.2.4. Estimation of Vitamin C

Vitamin C content of MECL & calibration curve of standard Ascorbic acid was presented (Tab 5 & Fig 4).

Table 5: Estimation of Vitamin C of MECL

S. No.	Conc. of Ascorbic acid in µg/ml	Absorbance at 520nm	Conc. of Methanolic ext in µg/ml	Absorbance at 520nm	Amt of Vitamin C present / g of extract
1	40	0.135 ± 0.000	100	0.076 ± 0.004	237.03 ± 0.006
2	80	0.265 ± 0.015	200	0.137 ± 0.007	253.70 ± 0.006
3	120	0.346 ± 0.010	**Average 245.37 ± 0.006**		
4	160	0.468 ± 0.011			
5	200	0.525 ± 0.010			

*mean of three readings ± SEM

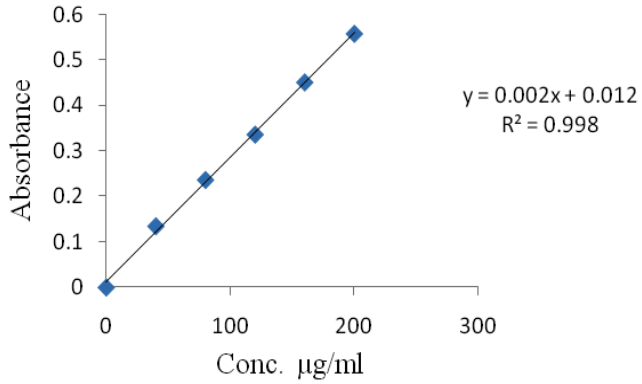

Fig 4: Calibration curve of Ascorbic acid

The linear regression equation was found to be y = 0.0027x + 0.012 and a correlation coefficient of 0.9982. The amount of Vitamin C content present in the MECL was found to be 245.37 ± 0.006 mg/g by using the above linear regression equation.

Oxidative stress may be linked to tissue damage and the development of regenerative disorders. By transformation into coenzymes, vit C are involved in fatty acid synthesis and oxidation reactions. By its involvement in the microsomal respiratory chain, vitamin C promotes cholesterol transformation into bile acids [48].

3.2.5. Estimation of Vitamin B_2

Vitamin B_2 content of MECL & Calibration curve for standard Riboflavin was presented (Tab 6 & Fig. 5)

Table 6: Estimation of Vitamin B_2 of MECL

S. No.	Conc. of Riboflavin in mg/ml	Absorbance at 360nm	Conc. of Methanolic ext. in mg/ml	Absorbance at 360nm*	Amt of Vitamin B_2 present mg/ g of extract*
1	2	0.161 ± 0.006	2	0.047 ± 0.004	31.99 ± 0.011
2	4	0.377 ± 0.012	4	0.111 ± 0.007	32.50 ± 0.006
3	6	0.555 ± 0.002	6	0.203 ± 0.003	37.29 ± 0.010
4	8	0.766 ± 0.005	**Average 34.00 ± 0.009**		
5	10	0.958 ± 0.004			

*mean of three readings ± SEM

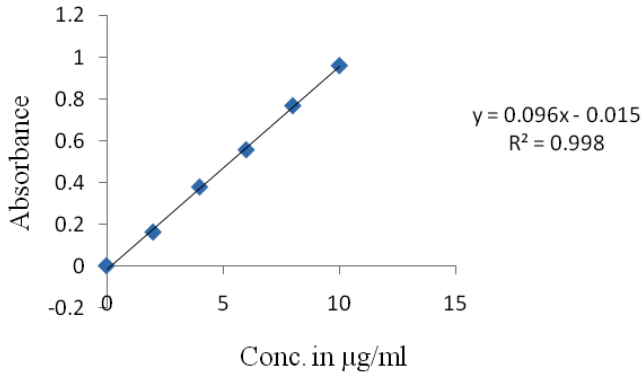

Fig 5: Calibration curve of Standard Riboflavin

The linear regression equation was found to be y = 0.0969x - 0.015 and a correlation coefficient of 0.9989. The amount of Vitamin B_2 content present in the MECL was found to be 34.00 ± 0.009 mg/g by using the above linear regression equation.

Steps in the cyclical β oxidation of fatty acids are also dependent on flavins as electron acceptors. An effect on the β oxidation of fatty acids is thought to be responsible for the altered fatty acid profile in hepatic lipids in severely riboflavin-deficient rats, which seems to be independent of the dietary source of lipid [49, 50].

Pancreatic lipase inhibition assay

The results obtained for pancreatic lipase inhibition assay and the graphical representation were presented (Table 7 & Fig.6).

Table 7: Pancreatic lipase inhibition of MECL and Orlisat

S. No	Conc. in mg/ml	% Inhibition of Orlistat	% Inhibition of MECL
1	1	20.91 ± 0.293	18.20 ± 0.306
2	2	30.93 ± 0.583	26.40 ± 0.363
3	3	40.23 ± 0.666	35.04 ± 0.416
4	4	57.47 ± 0.490	52.90 ± 0.523
5	5	73.63 ± 0.553	61.77 ± 1.102
IC_{50}		3.420 mg/ml	3.962 mg/ml

From the table 7, it can be seen that the MECL showed a percentage inhibition 61.77 ± 1.102 at a concentration of 5mg/ml. The IC_{50} values calculated using the linear regression analysis was found to be 3.962mg/ml for MECL. The Orlistat showed a percentage inhibition 73.63 ± 0.553 at a concentration of 5mg/ml, IC_{50} value was found to be 3.420 mg/ml. The extract of *Citrullus lanatus* possesses a good inhibitory activity on pancreatic lipase. The dose dependent pancreatic lipase inhibitory activity was observed. i.e inhibition of enzyme was increased on increasing concentration of extract.

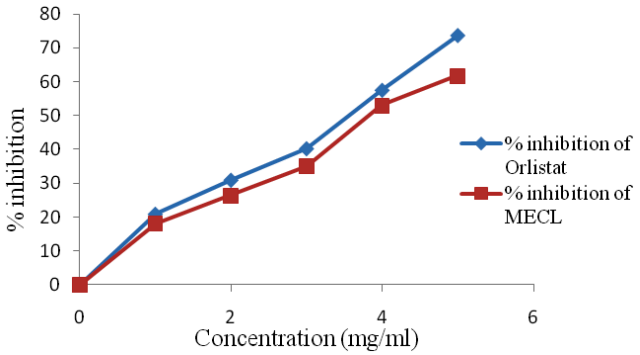

Fig 6: Determination Pancreatic lipase inhibition of MECL and Orlisat

Conclusion

The extract of *Citrullus lanatus* possesses a good inhibitory activity (dose dependent) on pancreatic lipase. It may be the presence of flavanoid, tannins, phenols, vitamin C and saponins. MECL employed as a therapy for the management of the obesity epidemic. Furthermore *in vivo* studies needed to confirm this activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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