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ANTIOXIDANT AND ANTI-OBESITY ACTIVITY OF PEEL EXTRACT AND COMBINATION OF PEEL AND PULP EXTRACT OF *CITRUS MEDICA*

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ABSTRACT

Citrus Medica belongs to Rutaceae family is available all over India. The main objective of the present study was to evaluate the radical scavenging potential and antiobesity activity of aqueous extract of *Citrus medica* peel (CMP) and its combination as peel and pulp (CMPP) . The total phenol content of the extracts reported 80.48 mg GAE/g and 84.14 mg GAE/g in CMP and CMPP with gallic acid as standard, total flavonoid content showed 49.85 mg RU/g and 56.03 mg RU/g in CMP and CMPP with rutin as standard, Vitamin C content exhibited 77.19 mg AAE/g and 83.15 mg AAE/g in CMP and CMPP with ascorbic acid as standard. The radical scavenging activity by DPPH method reported 51.77 %, 56.70%, FRAP exhibited 80.55 %, 83.33 % and NO method showed 13.19 %, 14.45 % respectively for CMP and CMPP with ascorbic acid as standard. The results of antiobesity activity by pancreatic lipase inhibitory method was found to be 9.136 ±0.845 %, 15.67±1.709 %, 26.534±2.849 %, 31.491±4.462 %, and 40.855±2.586 % for CMP extract, while CMPP extract reported 5.565±0.087 %, 28.661±0.346 %, 39.677±0.087 %, 49.535±0.435 %, and 61.709±0.248 % at concentration 0.2mg -1mg using orlistat as standard. The study showed an increase in activity with an increase in concentration of extract. On the determination of IC50 standard orlistat reported the lowest value of 0.039±0.0003 mg/ml, followed by CMPP extract of 0.795±0.004

mg/ml and CMP extract of value 1.234±0.104 mg/ml. The lower the IC50 value, the higher the efficiency of inhibiting pancreatic lipase. The study demonstrated higher antioxidant activity and antiobesity activity in CMPP than CMP.

Keywords: *Citrus medica*, Antioxidant activity, Antiobesity activity, Pancreatic lipase

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the most important free radicals in biological systems (RNS). These reactive species are potentially hazardous by-products of regular biological activities **[1]**. Oxidative stress is caused by the generation of highly reactive oxygen species with a single unpaired electron is involved in the pathogenesis of a variety of physiological conditions, including cellular injury, ageing, cancer, and hepatic, neurodegenerative, cardiovascular, and renal disorders **[2, 3]**. Though endogenous antioxidant defence mechanisms in the body protect almost all organisms from free radical damage however, they may not be enough to sustain optimal cellular activities in the face of increased oxidative stress, necessitating the use of dietary antioxidants .Since synthetic antioxidants possess negative effects, such as mutagenicity, toxicity, and carcinogenesis, the search for natural antioxidants has received significant attention in the past few decades.

Obesity is a serious problem in the world and has been associated with increase in morbidity, mortality, and reduced life expectancy **[4]**. It occurs as a result of energy imbalance between energy intake and energy expenditure, leading to increased lipid concentration in the blood and enlarged fat mass **[5]**. Although fat is vital for good health, buildup of a large amount of fat is linked to a variety of health risks such as dyslipidemia, diabetes mellitus, osteoarthritis, hypertension, fatty liver disease, cancers, asthma, and obesity **[6, 7]**. The prevalence of obesity is increasing rapidly worldwide. Presently, 300 million people are medically obese while more than one billion adults are overweight **[8]**. WHO has predicted that this number might increase to 3.3 billion by the year 2030 **[9]**. In spite of the urgent need for efficient and safe therapeutics and the probable size of the market for antiobesity drugs, the current efforts for improvement of such drugs are still unsatisfactory **[10]** which is mainly due to adverse side effects and cost related to these drugs.

Pancreatic lipase is the most effective lipid digesting enzyme, generating the lipolytic product beta (β)-monoglyceride and long chain saturated and polyunsaturated fatty acids from dietary triglycerides. Pancreatic lipase inhibition is a promising targeted

method for the discovery of potent anti-obesity agents **[11]**. Over the years, many drugs have been used to treat obesity. However, due to substantial side effects, the majority of anti-obesity medications that were approved and commercialized have now been removed. Many scientists have been looking for natural anti-obesity drugs that are both safe and cost-effective **[12]**. Natural bioactive compounds including flavonoids and polyphenols have recently been shown to be involved in the treatment of obesity **[13]**. As a result, natural compounds have received significant attention in recent years in order to develop safer and more powerful pancreatic lipase inhibitors. Natural compound intake, combined with sufficient dietary changes and exercise, is gradually becoming one of the most prominent complementary and alternative medicine strategies for obesity management **[14]**.

The *Citrus medica* (Citron) is a plant with fragrant fruit and leaves. It is a prominent member in the genus Citrus belonging to the Rutaceae or Rue family, found in the base region of Himalaya from Gadwall to Sikkim at the height of 4000 feet. It is also seen in Assam, central India and Western Ghats of India and more commonly present in the Mediterranean region and central and southern parts of America **[15]**. In India *Citrus medica* fruit pulp and peel is consumed together as pickles that offers variety of health benefits such as antioxidants, probiotics, mineral supplement etc. *Citrus medica* peel is eaten with rice in Bangladesh, India and Indonesia. In Spain, syrup made from the peel is used to flavor unpalatable medical preparations, and in Guatemala, it is used as flavouring for soft drinks **[16]**. In Brazil, the peel is used to prepare jellies and other sweets (preserves and crystallized fruit). In traditional Indian medicine ripe fruits are potent antiscorbutic, stomachic, cardiac tonic, stimulant, sedative, analgesic and used in dyspepsia, bilious vomiting, cold, fever, palpitation, sore throat, cough, asthma, thirst, cough and ear ache; peels are anthelmintic **[17-24]**. The ripe fruit juice of *Citrus medica* has been reported to possess antimutagenic and antitumorigenic activities **[25]**, analgesic, hypoglycemic and hypolipidemic effects **[26]**, while antioxidative, anti-inflammatory, anti cancer and anti viral activities of *Citrus medica* peel detected in different extracts **[27]**. Though many research investigations have documented on bioactive components and pharmacological activities, individually on peel and pulp of *Citrus medica*, this species of citrus is underutilized and only limited research has been reported on combined activity of pulp and peel of *Citrus medica*, with this regard, the current research was planned to investigate and explore the antioxidant potential and

pancreatic lipase inhibitory activity of *Citrus medica* peel (CMP) and its combination of peel and pulp (CMPP) by invitro methods.

MATERIALS AND METHODS

Collection of Materials

The research work was carried out with *Citrus medica* in the PG Department of Biochemistry, Ethiraj College for Women, Chennai.The fresh fruit of *Citrus medica* were collected from the Koyambedu Market, Chennai district, Tamil Nadu, India. The fruits taken were thoroughly washed to remove all foreign matter. The *Citrus medica* Peel and *Citrus medica* Peel and Pulp were separately cut into small pieces, and were shade dried at room temperature for 10 days. The dried samples were then grinded in a blender and the coarse powdered samples were stored in a sterile containers designated as CMP and CMPP for further study.

Preparation of aqueous extract

The extract of CMP and CMPP was prepared by maceration process by dissolving 50g of CMP and CMPP in 400ml of distilled water and allowed to stand for 24 hours. The macerated samples of CMP and CMPP were stirred occasionally to ensure proper extraction. After 24 hours, the contents of the containers were filtered through muslin cloth and the filtrate were concentrated in an incubator at 45°C, until a soft mass obtained and then preserved at 4-8ºc for further analysis. The % yield of the extract was calculated using the equation,

[Weight of Extract/weight of plant material] X100

IN VITRO ANTIOXIDANT ACTIVITY

Determination of Total Phenol Content

Total phenolic content in CMP and CMPP were determined by Folin–Ciocalteu method **[28]** using gallic acid as the standard ranging from 20 -100µg/ml.0.5ml of CMP (100µg/ml) and CMPP (100µg/ml) extract was taken into different test tubes and the volume was adjusted to 3.5 ml with distilled water. 0.125ml of Folin-Ciocaltaeu reagent was added to all the test tubes and incubated for 6 minutes in dark followed by addition of 1.25ml of 7% sodium carbonate and incubated at room temperature for 90 minutes and absorbance was read at 760nm. A calibration curve was plotted with standard to determine the levels of phenolic in the samples and the results were expressed in mg gallic acid equivalent (GAE) per gram of extract. All the experiments were performed in triplicates.

Determination of Total Flavonoid Content

The total flavonoid content of CMP and CMPP extract was determined by aluminum chloride method **[29]** with some modifications. To 2ml of CMP (200µg/ml) and CMPP (200µg/ml) extract, 75µl of 5% sodium nitrite was added and incubated for 5minutes.To this 150µl of 10% AlCl3 was

added and incubated for 6 min at room temperature followed by addition of 0.5 ml of 1 M sodium hydroxide. The absorbance was determined at 510nm versus blank that contained all reagents except standard rutin. Rutinat concentration ranging from 40 - 280µg/ml was used as standardand calibration curve was plotted to determine the amount of flavonoids in the samples and the result were expressed in mg rutin equivalent (RU) per gram of extract. All the experiments were performed in triplicates.

Estimation of Ascorbic Acid Content

The amount of Vitamin C content of CMP and CMPP extract was determined by 2,4 DNPH method, **[30]** with slight modifications using ascorbic acid as standard at concentration ranging from 10 – 100µg/ml.0.5ml of CMP (100µg/ml) and CMPP (100µg/ml) was taken and the volume was adjusted to 3.0ml of with distilled water. 0.05ml of 4% thiourea and 1ml of 2,4- DNPH solution was added and kept in water bath for 30 mins followed by addition of 5ml of H_2SO_4 to all test tubes and the absorbance was measured at 540nmversus a blank containing all reagents except standard ascorbic acid.A calibration curve was plotted to determine the amount of Vitamin C in the samples and the result were expressed in mg ascorbic acid (AAE) equivalent per gram of extract. All the samples were prepared in triplets for the average value of absorbance.

DPPH (2,2-Diphenyl-1- picrylhydrazyl) Radical Scavenging Assay

In vitro antioxidant activities of the extracts of CMP and CMPP were determined using the DPPH free radical scavenging activity assay described by Nithianantham *et al* **[31]**. 0.5 ml of DPPH solution was added in tubes labelled T1 and T2followed by 2ml of CMP and CMPP extracts (100µg/ml) in labelled tubes. 0.5ml of DPPH and 2 ml of ethanol served as control C, 2.5 ml of ethanol alone served as a blank and 0.5 ml of DPPH and 2.0ml of ascorbic acid served as positive control. The reaction mixture in the tubes were vortexed for 10 seconds and allowed to stand at room temperature for 30 minutes. The absorbance was recorded at 517 nm by using UV-Spectrometer. All the samples were prepared in triplets for the average value of absorbance. The DPPH radicals scavenging activity was calculated according to the following equation,

[(Ac-As)/Ac] X 100

Where, Ac – Absorbance of control, As – Absorbance of Sample

Nitric Oxide Radical Scavenging Assay

Nitric oxide scavenging activity was determined by Garrat *et al* **[32]**, with slight modifications. 2ml of Sodium nitroprusside was added to the tubes labelled T1, T2, C and positive control.0.5ml of CMP and CMPP extract (100µg/ml) was added to the tubes T1, T2, 0.5ml of ascorbic acid was added to the tube positive control and

0.5mL of phosphate buffer saline was added to tube C. 2.5ml of phosphate buffer saline served as a blank. The contents of the tubes were mixed well and incubated at 37°C for 4 hours. After 4 hours of incubation, 0.5ml of Griess reagent was added to all the tubes. The contents were mixed well and absorbance of chromophore formed was read at 546nmAll the samples were prepared in triplets for the average value of absorbance and the percentage of nitric acid inhibition was calculated by the equation,

[(Ac-As)/Ac] X 100

Where, Ac - Absorbance of control, As - Absorbance of Sample

Ferric Ion Reducing Antioxidant Power Assay

In vitro reducing power of the extracts were determined using FRAP assay described by K.I.Berker *et al* **[33]**. This assay involves reduction of Fe3+ to Fe2+ by electron transfer reaction. The clean dry test tubes were labelled as T1, T2, C and Positive control. 0.1ml of CMP and CMPP extract (100µg/ml) was added to the test tubes T1, T2 and 0.1ml of ascorbic acid was added to the Positive control tube. 0.9ml of ethanol was added to all the tubes followed by 5ml of distilled water. To the reaction mixture 0.5ml of 1M HCL, 0.5ml 1% potassium ferric cyanide solution, 0.5 ml of 1% sodium dodecyl sulphate, and 0.2ml of 0.2% ferric chloride solution was added and boiled at 50^0C for 30 minutes. The absorbance of each mixture was measured at 750 nm using spectrophotometer. All the samples were prepared in triplets for the average value of absorbance and the ferric ion reducing power of extract was compared with ascorbic acid applying the formula,

[(As-Ac)/As] X 100

Where, Ac – Absorbance of control, As – Absorbance of Sample

IN VITRO PANCREATIC ANTI-LIPASE ACTIVITY

Lipase inhibitory activity of samples aqueous extract of CMP and CMPP was carried out as described by Etoundi *et al* **[34]**, with slight modifications. Briefly, a suspension containing 1% (v/v) triolein and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. The assay was then initiated by adding 1600 µl of the triolein emulsion to 200 µl of porcine pancreatic lipase (0.5 gm pancreatin in 15ml of 0.1 M phosphate buffer at pH 8.0) and 200µl of the sample at different concentrations. The test tubes containing the reaction mixture were incubated at 37°C for 30 min and then the absorbance was recorded at 450 nm. All the samples were prepared in triplets and the variation in absorbance was calculated for both control and test groups and the % inhibition was calculated using the following formula,

[(Ac-As)/Ac)]X100

Where, Ac – Absorbance of control, As-Absorbance of sample

RESULTS and DISCUSSION

Percentage Yield of Extract

The percentage yield of aqueous extract of CMP and CMPP is given in Table 1. The results demonstrated greater yield of 45.34% for CMPP while CMP recorded a yield of 29.53% this could be possibly due to the mass of peel and pulp in CMPP extarct

Determination of Total Phenolic content

Phenolic compounds, as natural antioxidant, are a kind of plant metabolites with a various numbers of phenol rings. Flavonoids, anthocyanins and tannins are the most important of these compounds which act as potent radical terminators. The high potential of polyphenols to scavenge free radicals may be due to the presence of multiple hydroxyl groups **[35]**. The absorbance values obtained at different concentrations of gallic acid were used for the construction of calibration curve. Total phenolic content of the extracts was calculated from the regression equation of calibration curve ($y = 9E\text{-}06x + 4E\text{-}05$; $R^2 = 0.9878$) and expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g) and presented in Table 1.Total phenolic content of CMPP recorded greater value of 84.14 ± 10.97 mg GAE/g than CMP which recorded 80.48 ± 16.76 mg GAE/g. Total phenolic content was observed higher in CMPP than CMP. The survey of past literature have reported that citrus peel contained greatest phenol content **[36-40]** and in contrast Munwar *et al* **[41]**, reported citrus medica pulp extract contained higher phenol content than peel and root. The value of phenolic content in this current study showed highest phenolic in CMPP than CMP and this may be attributed to the combination of both peel and pulp in CMPP extract attributing for greater activity.

Determination of Total flavonoid content

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depend on the number and position of free OH groups **[42]**. Flavonoids are the most diverse group of natural compounds commonly found in plants and have been reported to have multiple biological and pharmacological activities including antioxidative, cytotoxic, anticancer, antimicrobial, antiviral, and anti-inflammatory activities **[43]**. The total flavonoid content of the present investigation recorded 49.85 ± 9.27mg RU/g for CMP and 56.03 ± 14.16 mg RU/g for CMPP. The results demonstrated higher flavonoid content in CMPP than CMP Table 1.The works of previous literature, stated that total flavonoid content was found higher in citrus peel **[44, 45]**, While, Pallavi *et al* **[46]** observed greater

flavonoid values in combined form of citron peel and pulp extract which is in accordance to the current study. The high total flavonoid content may be due to the amalgamation of both peel and pulp in the extract of CMPP that comparatively accounts for more bioactive compounds than CMP extract that consisted of peel.

Estimation of Ascorbic acid content

Vitamin C is defined as hexuronic acid, cevitamin acid or xiloascorbic acid. The term vitamin C is commonly used to describe all these compounds even though the representative of which is ascorbic acid **[47]**. Ascorbic acid is also known as Vitamin C or L-ascorbic acid or antiscorbutic vitamin. Morethan 90% of the vitamin C in Human diets is supplied by fruits and vegetables **[48]**. Vitamin C content of the extracts was calculated from the regression equation of calibration curve ($y = 0.0011x + 0.0372$; $R^2 = 0.9974$) and expressed as mg ascorbic acid equivalents per gram of sample (mg/g). Vitamin C content of CMP was 77.19 ± 13.66 mg AAE/g and CMPP was 83.15 ± 8.94 mg AAE/g, presented in **Table 1**. The obtained results indicates that CMPP contained higher amount of Vitamin C than that of CMP. Our results are in agreement with Moraes Barros **[49]**, who reported that the pulp of different commercial citrus fruits from brazil contained higher amount of ascorbic acid than in the peels. The higher Vitamin C contents in CMPP could be related to the combination of pulp and peel of *Citrus medica* in the extract than CMP extract, obtained from peel.

DPPH radical scavenging activity

DPPH is a stable organic free radical, which loses its absorption spectrum band at 515–528 nm when it accepts an electron or a free radical species **[50]**. The DPPH assay is a simple, acceptable and most widely used technique to evaluate the radical scavenging potency of plant extracts **[51]**. The odd electron of nitrogen atom in DPPH is reduced by the antioxidants which are hydrogen donors or electron donors present in the extracts. The antioxidants in the CMP and CMPP extracts can donate hydrogen atoms, which convert 1,1-diphenyl-2- picrylhydrazyl (free radical) to its reduced form 1,1-diphenyl-2-picrylhydrazine resulting in the colour change from purple to pale yellow colour .The results of the present study reported 51.77% inhibition of free radicals by CMP and 56.70% inhibition by CMPP, **Table 2**. The study revealed better radical scavenging activity in CMPP compared to CMP. Previous studies have reported that *Citrus medica* pulp extract showed the highest antioxidant activity by DPPH method, followed by juice and peel **[52]**. The high antioxidant activity in CMPP extract than CMP could be possibly

attributed to the high total phenol and flavonoid contents.

Nitric oxide scavenging activity

Nitric oxide (NO) is an important pleiotropic mediator generated by endothelial cells, macrophages, neurons, etc. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer, and other pathological conditions (Moncada *et al*., 1991). The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that is detrimental to human health. The assay procedure is based on the principle that sodium nitroprusside, in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. In the present study, the extract of CMP and CMPP compete with oxygen to react with NO and thus inhibits generation of anions and there by showed a moderate nitric oxide scavenging activity. The results of the current study reported 13.19% scavenging activity by CMP and 14.45% activity by CMPP **Table 2**. The study showed that the CMPP showed better scavenging activity than CMP. The works of past literature, have stated that nitric oxide scavenging activity of orange peel extract increased as the concentration of extract increases, while lime peel and mandarin peel extract showed no concentration dependence of nitric oxide scavenging activity **[39]**, in contrary highest nitric oxide scavenging activity was also reported in *Citrus medica* peel, followed by juice, root and pulp **[41]**. The result of the present study showed better nitric oxide scavenging in CMPP extract than CMP extract, this could be possibly due to the higher phenol values and synergistic activity of peel and pulp in CMPP extract

Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power (FRAP) assay signifies the power of antioxidants as reductants in the redox linked colorimetric reaction. This assay is also used to quantify the capability of antioxidants in a sample to reduce ferric (III) ions to ferrous (II) ions at a low pH **[53]**. The transformation ability of compounds from Fe3+/ferricyanide complex to Fe2+/ferrous form acts as a potential indicator for antioxidant activity **[54]**. The presence of reductants in the test solution reduces Fe3+ to Fe2+, which can be monitored by measurement of Prussian

blue colour at 700 nm **[55]**. The current study recorded reducing power of 83.33 % for CMPP and 80.55 % for CMP **Table 2**. The study revealed that CMPP showed better reducing antioxidant activity than CMP. The earlier reports have stated that reducing power of citrus aurantium fruit peel and pulp increased with increasing concentration **[56]**, besides few literatures determined increased reducing power of *Citrus unshiu* peel, lime peel as concentration was increased **[57, 39]**. The highest reducing power was reported in CMPP extract than CMP, which may be attributed to the synergistic activity of bioactive components present in peel and pulp of CMPP extract

ANTILIPASE ACTIVITY:

Pancreatic lipase inhibitors have emerged as a new player in the fight against obesity. The inhibition of pancreatic lipase activities decreases the digestion of fat-rich foods, resulting in a lower calorie intake **[58]**. One of the most well-studied mechanisms in evaluating the possible effectiveness of natural products as anti-obesity agents is the anti-lipase effect **[59]**. The result of pancreatic lipase inhibitory activity of CMP extracts was found to be 9.136±0.845, 15.67±1.709, 26.534±2.849, 31.491±4.462, and 40.855±2.586, while CMPP extract reported 5.565±0.087, 28.661±0.346, 39.677±0.087, 49.535±0.435, and 61.709±0.248 at concentration 0.2mg -1mg. Positive control, orlistat reported 51.358±5.594, 64.520±1.052, 72.327±2.730, 78.291±1.122, and 86.914±1.414 at the concentration 0.02mg – 0.1mg (Table 3). The study showed an increase in activity with an increase in the concentration of extract. On the determination of IC50, positive control orlistat reported the lowest value of 0.039±0.0003, followed by CMPP extract of 0.795±0.004 and CMP extract of value 1.234±0.104. The lower the IC50 value, the higher the efficiency of inhibiting pancreatic lipase. Over all, the study disclosed best anti lipase potential in CMPP with IC50 values of 0.795±0.004 mg/ml than CMP extract(IC50 1.234±0.104 mg/ml).Many previous studies, have shown that flavonoids and other phenolic compounds act as pancreatic lipase enzyme inhibitors by binding to the enzyme-substrate complex, reducing the lipid absorption **[60, 61]**. The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin **[62, 63]**. The aqueous extract of CMP and CMPP exhibited antioxidant potential and CMPP recorded higher activity than CMP however less than the standard antioxidant used according to the DPPH, NO and FRAP assays. Our findings indicated that the whole fruit could be used as subsidiary treatment or to replace orlistat in the treatment of obesity.

Table 1: Antioxidant activity of aqueous extract of *Citrus medica* peel (CMP) and peel and pulp (CMPP)

Extract	Extraction Yield (%)	Total Phenol (mg GAE/g)	Total Flavonoid (mg RU/g)	Vitamin C (mg AAE/g)
CMP	29.53 ± 6.36	80.48 ± 16.76	49.85 ± 9.27	77.19 ± 13.66
CMPP	45.34 ± 6.96	84.14 ± 10.97	56.03 ± 14.16	83.15 ± 8.94

The values are presented as mean ± standard deviation (n = 3)

Table 2: Radical scavenging activity of aqueous extract of *Citrus medica* peel (CMP) and peel and pulp (CMPP)

Extract	DPPH assay (%)	Nitric Oxide assay (%)	FRAP assay (%)
CMP	51.77 ± 1.22	13.19 ± 5.66	80.55 ± 9.61
CMPP	56.70 ± 1.20	14.45 ± 9.68	83.33 ± 8.33

The values are presented as mean ± standard deviation (n = 3)

Table 3: Pancreatic lipase inhibition and IC50of Positive control Orlistat, CMP and CMPP

S. No.	Concentration	RESULTS	
		% Inhibition	IC50 mg/ml
ORLISTAT	0 mg	0.00±0.00	0.039±0.0003
	0.02 mg	51.358±5.594	
	0.04 mg	64.520±1.052	
	0.06 mg	72.327±2.730	
	0.08 mg	78.291±1.122	
	0.1 mg	86.914±1.414	
CMP	0 mg	0.00±0.00	1.234±0.104
	0.2 mg	9.136±0.845	
	0.4 mg	15.67±1.709	
	0.6 mg	26.534±2.849	
	0.8 mg	31.491±4.462	
	1 mg	40.855±2.586	
CMPP	0 mg	0.00±0.000	0.795±0.004
	0.2 mg	5.565±0.087	
	0.4 mg	28.661±0.346	
	0.6 mg	39.677±0.087	
	0.8 mg	49.535±0.435	
	1 mg	61.709±0.248	

The values are presented as mean ± standard deviation (n = 3)

CONCLUSION

The present study indicates that *Citrus medica* peel extract and its combination of peel and pulp extract possessed phenol, flavonoid, vitamin C and exhibited antioxidant activity and antiobesity activity, however the study revealed that the combined form of peel and pulp extract demonstrated higher activity than the peel extract. This study suggests that the whole fruit of *Citrus medica* can thus be considered as function foods that possess phytochemicals to scavenge free radicals and prevent obesity. Furthermore pharmacological in vivo studies are needed to confirm these findings and to identify the main chemical compounds responsible for these pharmacological effects.

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