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ORIGINAL ARTICLE

Polyphenol-Enriched Fraction and the Compounds Isolated from *Garcinia indica* Fruits Ameliorate Obesity through Suppression of Digestive Enzymes and Oxidative Stress

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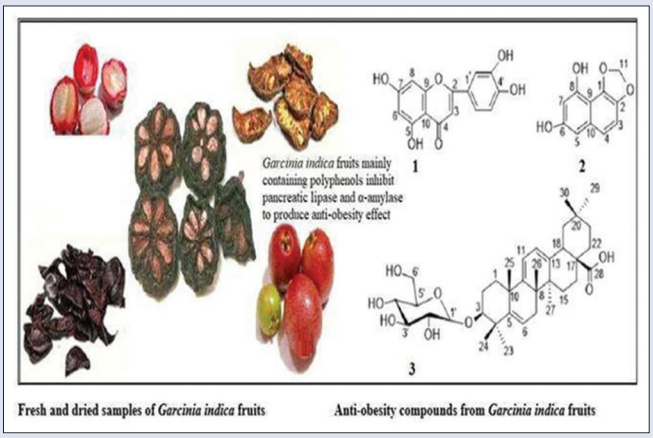
ABSTRACT

Background: The growing incidence of obesity has attracted the concern of researchers to look for effective interventions for its management. *Garcinia indica* is a widely known ethnomedicine used to treat gastritis, diabetes, and metabolic disorders. Recently, there has been a surge in the use of *G. indica* fruits in weight loss preparations. **Objective:** To explore the anti-obesity effect of polyphenol-enriched fraction and the compounds isolated from *G. indica* fruits through the inhibition of key metabolizing enzymes and oxidative stress. **Materials and Methods:** Fruits of *G. indica* were extracted with methanol that was subjected to liquid–liquid extraction to yield ethyl acetate fraction (FGIEF), chloroform, butanol, and aqueous fractions. The extract and the fractions were screened for the total polyphenols content (TPC), total flavonoids content (TFC), pancreatic lipase (PL) and α-amylase inhibition, and antioxidant activity. The effect of FGIEF on the viability of 3T3-L1 preadipocytes using 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide assay was assessed. FGIEF was subjected to normal-phase medium-pressure liquid chromatography (MPLC) to isolate compounds 1–3. Docking studies of representative polyphenols and the isolated compounds 1–3 with PL were undertaken to derive supporting evidence. **Results:** Among all the fractions, FGIEF was found to have the highest TPC (375.6 ± 4.5 gallic acid equivalent mg/g) and TFC (237.2 ± 6.2 quercetin equivalent mg/g). The extract and fractions showed concentration-dependent digestive enzyme inhibition and antioxidant effect. FGIEF inhibited PL and α-amylase (IC_{50} values 257.3 ± 3.7 and 349.7 ± 5.8 μg/mL, respectively). FGIEF did not induce any cell death up to 800 μg/mL. MPLC of FGIEF led to the isolation of luteolin (1), napthyldioxolol (2), and oleantrienoic acid glucoside (3). Preferential inhibition by polyphenols compared to other compounds was notable in the docking studies. **Conclusion:** The study suggests that the fruits of *G. indica* exhibit anti-obesity effect through the inhibition of digestive enzymes that can be mainly attributed to the presence of polyphenols.

Key words: α-amylase, *Garcinia indica*, obesity, pancreatic lipase, polyphenols

SUMMARY

- *Garcinia indica* fruits, commonly known as *Kokum*, are used in many weight loss preparation
- The fruits exhibit anti-obesity effect through the inhibition of fat and carbohydrate digestion
- The fruit, particularly its polyphenol-rich fraction, reduces oxidative stress
- This study establishes the beneficial effects of this fruit
- This edible berry is recommended to be used as a dietary supplement.



Abbreviations used: MPLC: Medium-pressure liquid chromatography; PL: Pancreatic lipase; TPC: Total polyphenols content; TFC: Total flavonoids content; TLC: Thin layer chromatography; FGIEF: Fruits of *G.indica* Ethyl acetate fraction.

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INTRODUCTION

Obesity is a common nutritional disorder which is rapidly increasing among the population worldwide. This has now become a crucial factor for developing metabolic abnormalities, including atherosclerosis, insulin resistance, cardiovascular diseases, and cancer. As per the WHO, nearly 1.9 billion people were reported to be overweight whereas 650 million of them were obese. Genetic factors responsible for obesity account for only 5%–10% of obese individuals, and the major cause lies in positive calorie intake, sedentary lifestyles, and increased urbanization.[1] The first line of prevention/treatment approach is diet, exercise, and lifestyle modifications, but these often pose a challenge to implement in practice. Their failure to adequately control the condition leads to alternative treatment options, including drug intervention. However, several of the currently used agents are limited by their mechanism of action, side effects, compliance, and

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associated complications after the cessation of the drug.[2] Among the surgical options for obesity treatment, bariatric surgery helps in sustained weight loss for severely obese individuals, but it develops mental health problems associated with post-operative outcomes.[3]

The investigational research for anti-obesity medication is often aimed at the modulation of energy homeostasis by stimulating catabolic or inhibiting anabolic pathways. Calories intake mainly include fatty components (triglycerides) and carbohydrates. Two gastrointestinal enzymes, namely pancreatic lipase (PL) and α-amylase, are involved in the metabolism of fats and carbohydrates. PL hydrolyzes the triglycerides whereas α-amylase enzyme is accountable for the digestion of carbohydrates in the human body. The therapeutic approach to control obesity lies in the inhibition of these enzymes, so as to reduce the energy intake without altering central mechanisms.[4]

Fruits of *Garcinia indica* Choisy (*Guttiferae*), commonly known as *Kokum*, have been used in Ayurveda to treat inflammatory ailments and metabolic disorders.[5] The fruit having tangy-sweet taste is a home remedy for acidity, sunstrokes, and infections.[6] It mainly contains polyphenols consisting of proanthocyanidins, anthocyanins, and flavonoids.[7] The major constituents include hydroxycitric acid (HCA); cyanidin glucoside and cyanidin sambubioside among anthocyanins;[8,9] and polyisoprenylated benzophenones – garcinol, isogarcinol, and camboginol.[10] Garcinol, isogarcinol, and HCA are exclusively present in genus *Garcinia*; however, catechins are also present in other plants. Rao *et al*., 2010 have demonstrated the appetite suppressing activity of HCA lactone.[11] Lee *et al*., 2019 reported that garcinol reduces obesity by diversifying gut microbiota constitution in high-fat diet-fed mice. In addition, garcinol also affects 5'-adenosine monophosphate-activated protein kinase pathway involved in adipogenesis and ultimately reducing cholesterol synthesis.[12] The curiosity in this remarkable fruit berry as a nutraceutical has amplified in recent years due to possible wholesome effects of its polyphenol content on health.[13] Many of therapeutic uses of *G. indica* fruits are associated to its antioxidant properties derived from phytochemicals majorly polyphenols. Polyphenolic antioxidants from dietary sources have been studied extensively for their role in lowering the incidences of cancer and cardiovascular and neurodegenerative diseases.[14] The purpose of this study was to assess the anti-obesity effect of polyphenol-enriched fraction of *G. indica* fruits by determining its effect on digestive enzymes (PL and amylase). Docking studies of the representative polyphenols as well as the compounds isolated from its fruits were undertaken to derive supporting evidence.

MATERIALS AND METHODS

Chemicals and reagents

α-Amylase, porcine PL, orlistat, acarbose, gallic acid, ascorbic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), nitro blue tetrazolium, p-nitrophenyl palmitate (pNPP), dinitrosalicylic acid (DNS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), potassium bromide (KBr) pellets, aluminum chloride, sodium chloride, sodium nitroxide, sodium carbonate, and sodium phosphate buffer were purchased from CDH Chemicals, Delhi, India, and were of AR grade. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), streptomycin, and penicillin were purchased from Hi-Media Laboratories Ltd., Mumbai, India. AR grade methanol, chloroform, butanol, ethyl acetate, and dimethyl sulfoxide (DMSO) were obtained from Merck Ltd., Mumbai, India.

General experimental procedures

Infrared (IR) and ultraviolet (UV) spectra were measured on a Bio-Rad Fourier-Transform (FT)-IR Spectrometer (Spectra Lab Scientific Inc., Ontario, Canada) and Lambda Bio 20 Spectrometer (Perkin-Elmer, Rotkreuz, Switzerland), respectively. Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker Spectrospin Spectrometer (Bruker AXS, Karlsruhe, Germany) in CD_3OD or DMSO-*d*6 using tetramethylsilane as an internal standard. Electrospray ionization mass spectrometry (ESI-MS) was recorded on a Waters Acquity (Micromass MS Technologies, Manchester, UK) Mass Spectrometer. Column chromatographic separation was carried out on silica gel (60–120 mesh, Merck, Mumbai, Maharashtra, India). Precoated silica gel 60 F_{254} thin layer chromatography (TLC) plates (Merck, Mumbai, Maharashtra, India) were used for comparison and pooling of eluents.

Collection of plant materials

Dried fruits of *G. indica* were purchased from the local crude drug market, Khari Baoli (Delhi, India). The fruits were authenticated by Dr. H. B. Singh, Former Chief Scientist, CSIR-NISCAIR, Delhi. A voucher specimen of drug has been deposited in the Departments of Phytochemistry and Pharmacognosy at our institute with a reference number PRL-21/2015.

Fractionation and isolation of compounds

Dried *G. indica* fruits (1 kg) were dried, pulverized, and extracted with methanol (10 l) till exhaustion using a Soxhlet apparatus. The methanolic extract (FGIME) was filtered and concentrated *in vacou* using Rotavapor (Buchi, Switzerland) to get a semisolid residue (251 g). The residue was then dissolved in distilled water and partitioned sequentially with different solvents to yield ethyl acetate, chloroform, and aqueous fractions of fruits of *G. indica* that were designated as FGIEF, FGICF, and FGIAF, respectively. The fractions of fruits of *G. indica* were concentrated using Rotavapor, and the air-dried residues were kept in a refrigerator until further use.

FGIEF was subjected to normal-phase medium-pressure liquid chromatography (MPLC) for the isolation of compounds. The isolation was performed on Easy Purification System (Buchi, Flawil, Switzerland) having two pump modules (C-605), a control unit (C-615), and a UV detector (C-640). Fractionation was carried out using a 70 mm × 460 mm plastic-glass column packed with silica gel Si60 (50–60 μm). The FGIEF was homogenized, filtered, and loaded on to the column through the injector loop. Initially, the column was eluted with hexane–ethyl acetate mixtures. For running out simultaneous detection, eluents were detected by an online UV detector set at 225, 254, 277, and 330 nm. Fractions were collected manually based on the changes in absorbance. Aliquots were analyzed by on precoated TLC plates before pooling. Finally, they were processed further to obtain compounds 1–3. The purity of compounds was ascertained on Prominence High-Performance Liquid Chromatography with Diode Array Detector System (Shimadzu, Japan) fitted with a UV-visible (UV-Vis) detector (SPD-M20A), an auto-sampler (SIL-20 AC HT), and a fraction collector-10A. Characterization of the isolated compounds was done based on spectral data analysis.

Estimation of total polyphenols content

Total polyphenols content (TPC) was estimated in all the fractions using Folin–Ciocalteu method.[15] Each fraction was diluted to prepare a stock solution (10 mg/mL). Test sample solution (100 μl) of different fractions was mixed with 500 μL of Folin–Ciocalteu's reagent and 400 μL sodium carbonate (20%) and incubated at 25°C–27°C for 90 min. The absorbance was measured at 760 nm using UV-Vis spectrophotometer (JASCO V-550, Japan). TPC was expressed as milligram gallic acid equivalent (GAE) per gram of the test samples.

Estimation of total flavonoids content

The aluminum trichloride assay was performed to measure total flavonoid content (TFC) of all the fractions with slight modifications.[16] Test samples were dissolved in 10% DMSO to yield 500 µg/mL solution that was mixed with 150 µL of 5 M $NaNO_2$. After 5 min, 150 µL of 10% aqueous $AlCl_3$ was added to the mixture followed by 1 mL of 1 M NaOH. After 15 min of incubation, the absorbance was measured at 510 nm on UV-Vis spectrophotometer. All measurements were repeated thrice. A calibration curve of standard reference was assessed as quercetin equivalent in milligrams per gram of test sample.

In vitro pancreatic lipase inhibitory assay

PL inhibitory activity of FGIEF was determined by spectroscopic estimation.[17] Briefly, pNPP hydrolyzed by PL to p-nitrophenol is monitored at 410 nm. The assay mixtures composed of 1.8 ml sodium phosphate buffer (0.05 M, pH 7.6), 1.15 mg/mL sodium cholate, 0.55 mg/mL Arabic gum, 0.2 mL pNPP in isopropanol (0.01 M), and 0.02 mL of FGIEF (at different concentrations) were incubated at 37°C. Then, 0.02 ml of the PL solution in sodium phosphate buffer (50 mg/mL) was added to initiate the reaction. After 5 min of incubation at 37°C, the absorbance was recorded at 410 nm. The control reaction was carried out without adding FGIEF.

In vitro α-amylase inhibitory assay

α-Amylase inhibition assay was carried out as per the method described by Dong *et al.*, 2012.[18] α-Amylase (40 µL of 5U/mL) in 0.36 mL of sodium phosphate buffer (0.02 M, pH 6.9 containing 0.006 M NaCl) was mixed with 0.2 mL of FGIEF or acarbose. The mixture was incubated for 20 min at 25°C and 300 µL of starch solution (1% in 0.02 M sodium phosphate buffer) was added, the mixture was incubated again for 20 min at 25°C. The reaction was stopped by addition of 0.2 mL of DNS, the contents were kept in a boiling water bath for 5 min, and the absorbance was recorded at 540 nm The control reaction was carried out without adding FGIEF.

The percentage of enzyme inhibition was determined as:

$$\%\text{Enzyme Inhibition} = \frac{A_{control}-A_{test}}{A_{control}} \times 100$$

Where A_{test} and $A_{control}$ represent the absorbance of reaction mixture with and without test sample, respectively. IC_{50} values represented concentration of samples required for inhibiting 50% of enzyme activity under the assay conditions.

Estimation of antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl radical method

DPPH method was used to determine the antioxidant activity of test samples (extract as well as its fractions) taking ascorbic acid as control with minor modifications.[19] Precisely, 1 mL from methanolic solution of DPPH (0.3 mM) was added to 2.5 mL of test samples (100–500 µg/mL). The resultant mixtures were kept in the dark for 30 min, and the absorbance was measured at 517 nm against blank that did not contain DPPH. A decrease in absorbance recorded for DPPH solution indicated an increase of DPPH radical scavenging activity. The measurements were repeated thrice and the scavenging activity was calculated as:

$$\%\text{DPPH inhibition} = \frac{A_{control}-A_{test}}{A_{control}} \times 100$$

Where A_{test} and $A_{control}$ represent the absorbance of the reaction mixture with and without test sample, respectively. The percentage inhibition was plotted against log concentration for the calculation of IC_{50}.

Cell viability 3-(4, 5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide assay

3T3-L1 preadipocyte cells were obtained from the National Centre for Cell Science, Pune. Cells were maintained in DMEM containing 10% FBS and 1% penicillin and streptomycin in a humidified 5% CO_2 atmosphere at 37°C. The cells were cultured at in a 96-well plate (5 × 104 cells/mL) to evaluate the cytotoxicity of FGIEF. The cells were treated with different concentrations ranging from 100 to 2000 mg/mL for 48 h. It was followed by the incubation of cells with MTT solution for 3 h at 37°C under a humidified 5% CO_2 atmosphere. The supernatants were aspirated, DMSO was added to each well, and the absorbance was measured at 570 nm using a microwell plate reader. The cytotoxicity of the test samples was estimated by comparing their absorbance values with the untreated control cells.

Docking study for representative polyphenols

Representative polyphenols, namely cyanidin, proanthocyanidin, and flavanoyl flavone, and the compounds isolated from *G. indica* were docked with PL enzyme to assess their interaction and binding modes with the target enzyme using Glide extra precision (XP) Maestro 10.1 Schrodinger, running on Linux 64 Operating System (Schrodinger, Version 10.1, 2016, LLC, New York, USA). PL is a validated target for anti-obesity drug, and the crystal structure was downloaded from protein data bank (PDB 1 LPB). Two-dimensional (2D) structures for the test compounds were converted to their respective 3-dimensional (3D) structures using LigPlot. Protein preparation wizard in Maestro 10.5 was used to complete the protein preparation through preprocess, review, and refinement. Methoxyundecyl phosphinic acid was used as co-crystal ligand for the grid. This followed replacing all the water molecules by hydrogen atoms. The energy of the structures and ligands was minimized using Optimized Potential for Liquid Simulations (OPLS) 2005 force field. The ligand was docked into the grid generated from the protein and the glide score was recorded.

Prime molecular mechanics/generalized born surface area (MM-GBSA) was calculated using Maestro 10.5. The test compounds and orlistat were used against PL enzyme (PDB ID-1 LPB). Protein preparation and the ligand preparation were followed by deleting all the water molecules. The free binding energy calculation was undertaken using model prime MM-GBSA. Alternatively, the results were procured by running the program directly from the file generated by running the docking protocol.

Statistical analysis

All data were presented as an average of triplicate determinations ± standard deviation (SD). Statistical analysis was performed by one-way analysis of variance followed by Dunnett's multiple comparison test (GraphPad® InStat version 3.06, San Diego California, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Total polyphenols content and total flavonoids content of *Garcinia indica* fruits

The results of TPC and TFC for the methanolic extract of the fruits of *G. indica* and its fractions are mentioned in Table 1. Highest values for TPC (375.6 ± 4.5 GAE mg/g) and TFC (235.5 ± 6.2 QE mg/g) were found in ethyl acetate fraction of the fruits followed by chloroform, butanol, and aqueous fraction [Table 1].

Table 1: Total phenols and total flavonoids content of methanolic extract and fractions of the fruits of *Garcinia indica*

Sample	TPC (GAE mg/g)	TFC (QE mg/g)
Methanolic extract of dried fruits	178.7±2.5	112.4±9.7
Ethyl acetate fraction	375.6±4.5	237.2±6.2
Chloroform fraction	214.7±8.5	103.2±5.6
Butanol fraction	93.3±5.5	58.3±3.0
Aqueous fraction	61.6±9.4	41.6±9.4

Data are represented as mean±SD of triplicate determinations. GAE: Gallic acid equivalent; QE: Quercetin equivalent; SD: Standard deviation; TPC: Total polyphenols content; TFC: Total flavonoids content

Table 2: Inhibitory concentration$_{50}$ values of the methanolic extract and fractions of *Garcinia indica* fruits against pancreatic lipase, α-amylase, and 1,1-diphenyl-2-picrylhydrazyl radical

Sample	IC_{50} (μg/ml)		
	Pancreatic lipase	α-Amylase	DPPH
Methanolic extract of dried fruits	359.7±4.6	367.4±3.6	514.5±5.1
Ethyl acetate fraction	257.3±3.7	349.7±5.8	294.3±2.3
Chloroform fraction	370.3±5.9	556.6±4.2	318.5±3.3
Butanol fraction	722.7±8.4	980.0±7.1	329.8±4.0
Aqueous fraction	905.8±6.1	925.8±6.6	495.1±2.4
Orlistat	100.8±2.6	-	-
Acarbose	-	113.7±2.3	-
Ascorbic acid	-	-	97.5±3.2
Luteolin (1)	68.7±1.3	ND	ND
Napthyldioxolol (2)	61.5±0.9	ND	ND
Oleantrienoic acid glucoside (3)	73.5±1.2	ND	ND

Data are represented as mean±SD values of triplicate determinations. DPPH: 1,1-diphenyl-2-picrylhydrazyl radical; SD: Standard deviation; ND: Not determined; IC_{50}: Inhibitory concentration$_{50}$

Inhibition of digestive enzymes

The results of PL and α-amylase inhibition assay are presented in Figure 1 and Table 2. As shown in Figure 1, all the fractions of the methanolic extract of *G. indica* fruits exhibited a concentration-dependent inhibition of PL and α-amylase. All tested fractions were stronger PL inhibitors than the α-amylase inhibitors [Table 2]. Highest ability to reduce PL activity was demonstrated by the ethyl acetate fraction, FGIEF (72.7% at 500 μg/mL) with the IC_{50} value of 257.3 ± 3.7 μg/mL. Similarly, FGIEF possessed the highest inhibition of α-amylase enzyme (71.7% at 500 μg/mL) with the IC_{50} value of 349.7 ± 5.8 μg/mL. The aqueous fraction exhibited the lowest inhibitory activity for both the enzymes. However, all the tested extract and fractions were less active than the standard positive controls, PL inhibitor orlistat (IC_{50} value 100.8 ± 2.6 μg/mL) and α-amylase inhibitor acarbose (113.7 ± 2.3 μg/mL). These results correlated well with the results of TPC and TFC estimation.

In vitro antioxidant activity

The results of DPPH inhibition assay for the methanolic extract and fractions of fruits of *G. indica* are demonstrated in Figure 2. Among the fractions, FGIEF showed the highest antioxidant activity (68.8% at 500 μg/mL) with an IC_{50} value of 294.3 ± 2.3 μg/mL. Other fractions showed relatively lesser DPPH inhibition in the order of FGICF (318.5 ± 3.3 μg/mL), FGIBF (329.8 ± 4.0 μg/mL), and FGIAF (495.1 ± 2.4 μg/mL). The IC_{50} value of FGIME was reported to be 514.5 ± 5.1 μg/mL while that of the standard ascorbic acid was 97.5 ± 3.2 μg/mL. These results were in agreement with the results of TPC and TFC estimation.

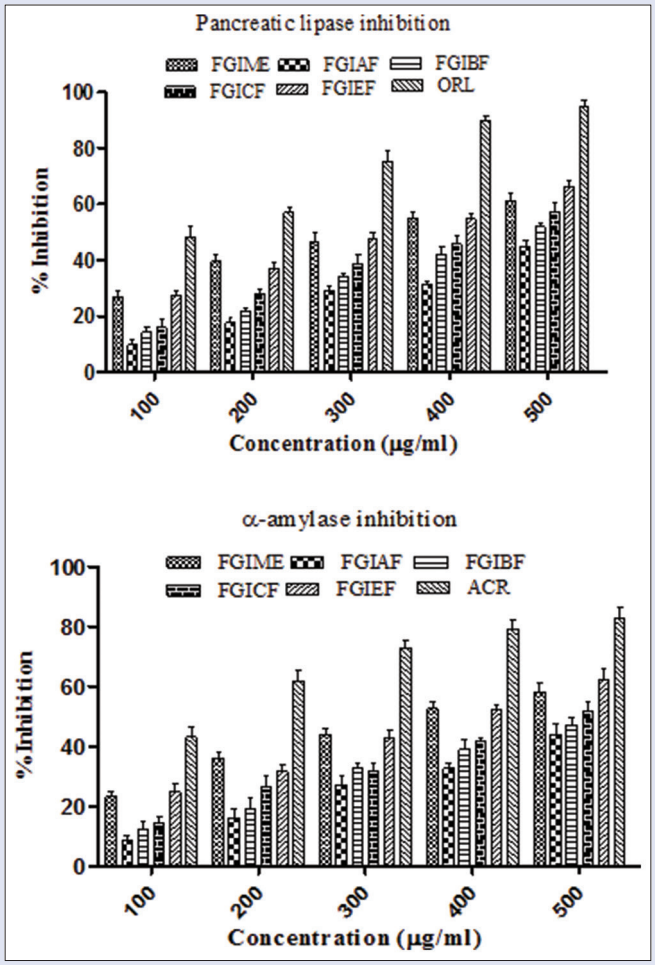


Figure 1: *In vitro* pancreatic lipase and α-amylase inhibitory assays of the extract and fractions of *Garcinia indica* fruits. Data are represented as mean ± standard deviation values of triplicate determinations. FGIME: Methanol extract; FGIAF: Aqueous fraction; FGIBF: Butanol fraction; FGICF: Chloroform fraction; FGIEF: Ethyl acetate fraction of *Garcinia indica* fruits; ORL: Orlistat; ACR: Acarbose; SD: Standard deviation

Effect of fractions on 3T3-L1 cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The effect of FGIEF on cell viability was evaluated by MTT assay using 3T3-L1 preadipocytes. FGIEF did not induce any cell death up to the dose of 800 mg/mL beyond which the cell viability decreased in a dose-dependent manner [Figure 3]. Accordingly, the concentrations of FGIEF up to 500 mg/mL were used for further studies.

Characterization of compounds isolated from *Garcinica indica* fruits

MPLC of FGIEF resulted in the isolation of three compounds 1–3. The structures of the isolated compounds [Table 3] were elucidated on the basis of spectral data analysis.

Luteolin (1)

Eluent: 25% ethyl acetate in hexane; FTIR ν_{max} (KBr): 3396, 2925, 2810, 1664, 1610, 1569, 1443, 1259, 1031 cm^{-1}; ^1H NMR (CD_3OD): δ

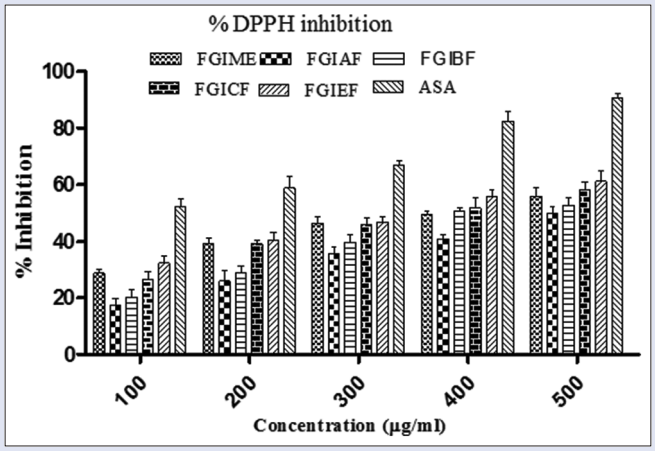


Figure 2: *In vitro* 1,1-diphenyl-2-picrylhydrazyl radical antioxidant assay of the extract and fractions of *Garcinia indica* fruits. Data are represented as mean ± standard deviation values of triplicate determinations. FGIME: Methanol extract; FGIAF: Aqueous fraction; FGIBF: Butanol fraction; FGICF: Chloroform fraction; FGIEF: Ethyl acetate fraction of *Garcinica indica* fruits; ASA: Ascorbic acid; SD: Standard deviation

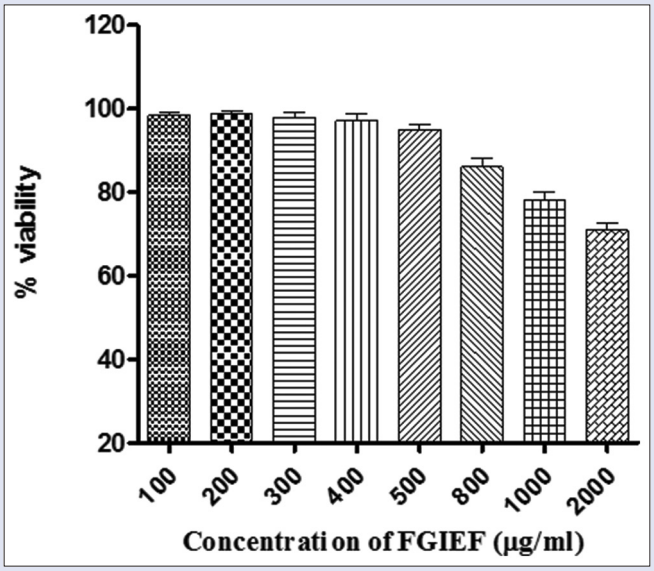


Figure 3: Effect of ethyl acetate fraction of *Garcinia indica* fruits on 3T3-L1 cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data are represented as mean ± standard deviation values of triplicate determinations. FGIEF: Ethyl acetate fraction of *Garcinia indica* fruits; SD: Standard deviation

7.35 (d, *J* = 2.0 Hz, H-8), 7.36 (d, *J* = 8.0 Hz, H-6'), 6.88 (d, *J* = 8.0 Hz, H-5'), 6.53 (s, H-3), 6.43 (d, *J* = 2.5 Hz, H-2'), 6.19 (d, *J* = 2.0 Hz, H-6); ^{13}C NMR (CD_3OD): δ (C2-10) 166.0, 100.1, 183.8, 163.2, 100.1, 166.3, 94.9, 159.4, 103.8; (C1'-6') 123.6, 114.0, 147.0, 150.9, 116.7, 120.2; +ve ESI-MS: 287 [M + H] + $C_{15}H_{11}O_6$; HR-ESI-MS: $C_{15}H_{11}O_6$ [M + H] +287.0537 (observed), 287.0511 (calculated).

Napthyldioxolol (2)

Eluent: 80% ethyl acetate in hexane; FTIR ν_{max} (KBr): 3488, 3274, 1669, 1623, 1427, 1229, 1160 cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.78 (d, *J* = 2.5 Hz, H-5), 7.26 (d, *J* = 8.5 Hz, H-3), 7.12 (dd, *J* = 3.0, 8.5 Hz, H-4), 7.07 (s, H-7), 4.84 (br s, H_2-11); ^{13}C NMR (CD_3OD): δ (C1-11) 145.2, 133.2, 112.5, 114.7, 108.7, 134.1, 108.7, 158.7, 109.7, 124.5, 101.9;-ve ESI-MS: 204 $[M]^-C_{11}H_8O_4$; HR-ESI-MS: $C_{11}H_8O_4$ $[M]^-$204.0284 (observed), 204.0423 (calculated).

Oleantrienoic acid glucoside (3)

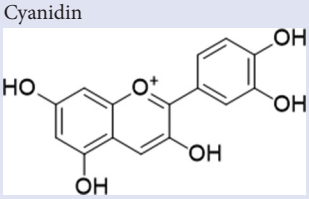
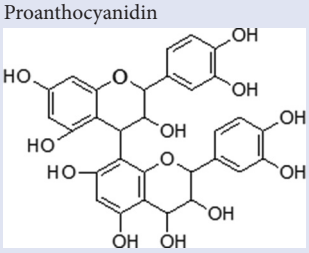
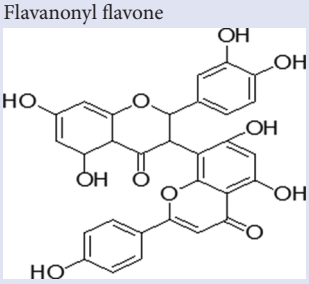
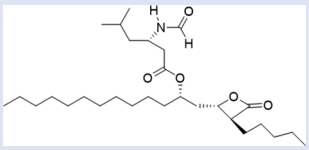
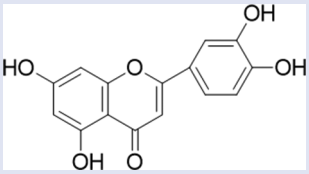
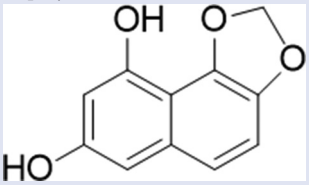
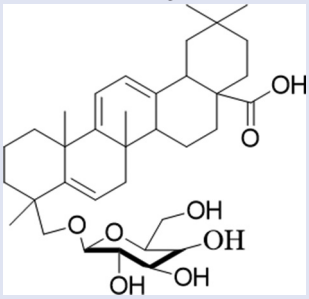
Eluent: 95% ethyl acetate in hexane; FTIR ν_{max} (KBr): 3422, 2932, 2841, 1740, 1637, 1459, 1072 cm^{-1}; ^{1}H NMR (DMSO-*d6*): δ 5.33 (d, *J* = 5.0 Hz, H-11), 4.85 (d, *J* = 5.0 Hz, H-12), 4.80 (br d, H-6), 4.41 (br s, H-3), 4.22 (d, *J* = 8.0 Hz, H-1'), 3.40 (dd, *J* = 4.0, 12.0 Hz, H_2-6'), 1.43 (s, H_3-26), 1.02 (s, H_3-24), 0.98 (s, H_3-23), 0.90 (s, H_3-30, H_3-27), 0.86 (s, H_3-25), 0.65 (s, H_3-29); ^{13}C NMR (DMSO-*d6*): δ (C1-29) 39.0, 18.9, 76.7, 36.8, 140.4, 116.0, 35.9, 41.8, 163.9, 44.2, 116.5, 121., 156.4, 43.5, 28.7, 22.6, 46.2, 41.2, 45.4, 31.4, 33.4, 36.5, 11.6, 22.6, 23.6, 24.5, 27.6, 182.7, 32.0), 21.5; (C1'-6') 100.7, 73.4, 76.7, 70.1, 81.4, 61.1; +ve ESI-MS: 615 [M + H] + $C_{36}H_{55}O_8$; HR-ESI-MS: $C_{36}H_{55}O_8$ [M + H] +615.3973 (observed), 615.3852 (calculated).

Docking studies of representative polyphenols and compounds isolated from *Garcinica indica* fruits

The docking study was carried out to know the binding mode of the representative polyphenols (cyanidin, proanthocyanidin, and flavanoyl flavone) and the compounds isolated from *G. indica* fruits inside the PL receptor binding pocket. Orlistat was used as the standard for comparison. The molecular docking studies were undertaken using PL as a target protein. This was done to assess the binding ability of the compounds at the PL-colipase complex binding site. The docking scores of the tested compounds and co-crystal ligand methoxyundecyl phosphinic acid are presented in Table 3. Tested polyphenols showed hydrogen bond interaction with different residues and were compared with orlistat [Figure 4a-d]. The results revealed that proanthocyanidin shows key hydrogen bond interaction with Asp 79, Phe77, Leu 153, and Ser152, similar to the hydrogen binding interaction shown by the standard drug orlistat. Proanthocyanidin shows additional hydrogen bond with Glu179 and Tyr114 that may be the reason for more strong binding with the receptors. The docking score of the proanthocyanidin was found to be −6.545, which is higher than the orlistat (−5.402). Cyanidin and flavanonyl flavone also possessed higher docking score than orlistat showing more binding affinity. The results of free binding energy showed that the polyphenols fit into the PL binding domain (PDB ID-1 LPB). The binding energy was in the range of −36.49 to −49.95 Kcal/mol. The binding energy of proanthocyanidin was found to be −49.95 Kcal/mol, which is higher than the standard drug orlistat (−47.41 Kcal/mol). Other two docked polyphenols, named cyanidin and flavanonyl flavone, possessed low binding energy than proanthocyanidin and standard drug.

The isolated compounds were found to strongly inhibit PL by completely occupying the active sites in the target protein. All inhibitors showed good docking scores than the co-crystal ligand, taken as the standard. Among all the titled compounds for the receptor, oleantrienoic acid glucoside was found to be most potent and has high docking score of −10.11. This ligand also assumes favorable orientation within the PL-colipase complex binding site. The binding mode of oleantrienoic acid glucoside is exactly the same as the co-crystal ligand. The 2D docked pose of oleantrienoic acid glucoside possesses two hydrogen bond interactions, among which one of the hydroxyl group attached at 3-glycoside formed H-bond with a backbone residue PHE 77 and other 6-methyl hydroxy of glycoside group formed a hydrogen-bond with a side chain residue HIE 151. Strong interaction with a backbone residue of oleantrienoic acid glucoside is the reason for its high affinity. All hydrogen interactions of receptor-oleantrienoic acid glucoside complex in 3D-image are represented in Figure 4e. The docked pose of second

Table 3: *In silico* molecular docking analysis of the representative polyphenols and compounds isolated from *Garcinia indica* fruits with pancreatic lipase

Sample name and structure	Docking score	Hydrogen bond interactions	π-π stacking	Binding energy (Kcal/mol)
Cyanidin 	−8.222	ASP 79 GLY 76 PRO180 SER152	PHE 215 HIE151 HIS 263	−38.0386
Proanthocyanidin 	−6.545	ASP 79 PHE 77 LEU 153 GLU 179 TYR 114 SER 152	-	−49.9582
Flavanonyl flavone 	−6.529	ASP 79 GLY 76 PHE 77 HIS 263 ALA 259	HIS 263	−36.4929
Orlistat 	−5.402	Asp 79, Leu153, and Phe77		−47.4155
Luteolin (1) 	−9.46	HIE 151 GLY 76	HIE 151 PHE 77 HIP 263	−44.021
Napthyldioxolol (2) 	−8.19	SER 152	-	−38.500
Oleantrienoic acid glucoside (3) 	−10.11	PHE 77 HIE 151	-	−35.000

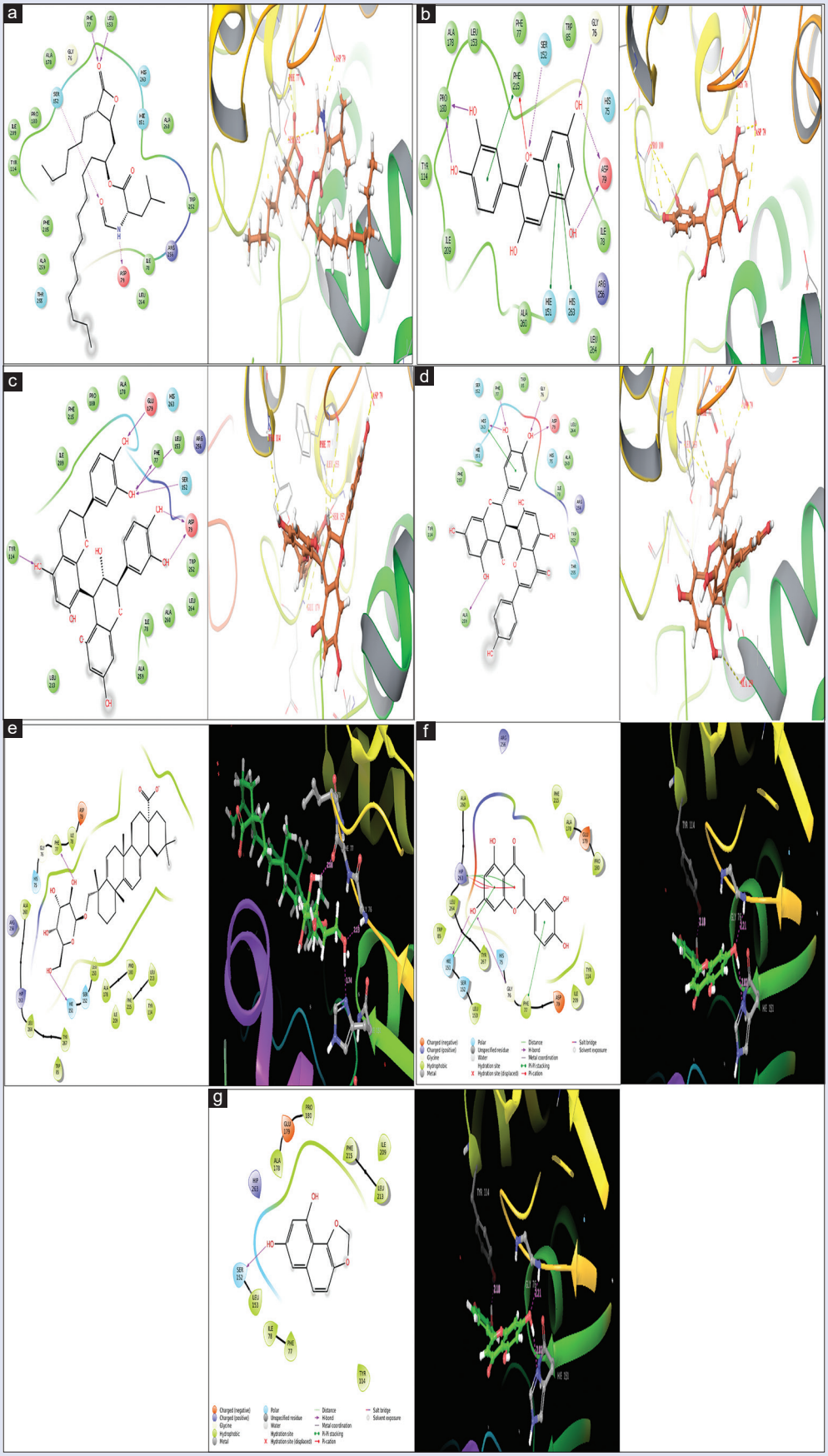


Figure 4: Docking studies of orlistat (a), representative polyphenols (b-d) and compounds isolated (e-g) from *Garcinia indica* fruits with pancreatic lipase

most dock ranked ligand luteolin (docking score of −9.46), the hydroxyl group of dihydroxy chromen-4-one forms hydrogen bond with both HIE 151 (side chain residue) and GLY 76 (backbone residue) as exhibited by oleantrienoic acid glucoside. One more similar pi–pi interaction was obtained by phenyl ring of dihydroxy phenyl ring at the second position of luteolin with backbone residue PHE 77. Both rings of chromen-4-one form pi-cation with HIP 263 which fills the compounds' account with penalties, and hence, it fell on the second position with docking score less than oleantrienoic acid glucoside [Table 3]. The interactions exhibited by the luteolin are shown in Figure 4f. The third ligand napthyldioxolol possess only one hydrogen bond of hydroxyl group with SER 152, which is a side chain residue and hence possesses least score of −8.19 among all three ligands. The 3D docked pose of napthyldioxolol is shown in Figure 4g.

The results related to the free binding energy showed that all the compounds fitted well in the PL binding domain [Table 3]. The best one among them, i.e., oleantrienoic acid glucoside, has more suitable conformation to fit into the domain. The binding energy ranged between −35.000 and −44.021 Kcal/mol. The binding energy of the test compound luteolin was found to be −44.021 which is comparable to orlistat (−47.41 Kcal/mol).

In vitro pancreatic lipase inhibitory activity of the isolated compounds

As a follow of docking studies, the isolated compounds were tested for PL inhibitory activity *in vitro*. The results were not found to be in complete agreement with the results of docking study of the three compounds. Napthyldioxolol (2) possessed the lowest IC_{50} value followed by luteolin (1) and oleantrienoic acid glucoside (3). The results are presented in Table 3.

DISCUSSION

In the last few decades, obesity has emerged as a pandemic medical condition and its effective management has caught the attention of biomedical researchers and dieticians and to the end users. Obesity results from an altered metabolism of fat and carbohydrate besides oxidative stress. Currently, the management of obesity is through lifestyle modifications, pharmacotherapy, and surgical interventions. The lifestyle modifications fail due to poor compliance by the patients on long-term basis. Orlistat, a PL inhibitor, is the only Food and Drug Administration-approved drug used clinically for obesity. It suffers from various unpleasant gastrointestinal adverse reactions such as bloating and oily stools together with decrease in fat-soluble vitamin absorption. Surgical interventions include restricted and malabsorptive procedures such as bariatric surgeries. These procedures are usually twinned with side effects on human health.[20] Thus, the hunt for improved pharmacotherapies for obesity continues till date.

In the quest for the alternatives to anti-obesity therapeutics, nutraceuticals are fast emerging as an alternative approach. Development of functional foods by incorporating bioactive plant components in the food is the new trend to control body weight gain in obese population or those who are susceptible to obesity. This becomes possible by supplementation of food with substances that inhibit fat and carbohydrate metabolism. Their consumption may also induce satiety or feeling of fullness, thereby deterring the patient from overeating. Polyphenols have been found to be capable of lowering plasma free fatty acid levels, hepatic lipid accumulation, and body weight by increasing lipolysis, lowering food intake, and inhibiting adipocyte differentiation.[21] Dietary polyphenols exert their protective action against oxidative stress by neutralizing free radicals and decreasing oxidative inflammatory status associated with the weight gain.[22] Digestive enzyme (PL and α-amylase) inhibitors from natural food sources play an essential role in weight loss process. PL inhibitors from natural products act peripherally by inhibiting fat digestion and absorption and decrease the chances of systemic side effects. Inhibition of α-amylase enzyme increases the amount of undigested food in the intestine and delays gastric emptying and food intake.[23] Some clinical trials also demonstrated the beneficial role of polyphenols in reducing body weight.[24,25]

In this study, we evaluated the role of polyphenol-rich fraction from *G. indica* fruits in the inhibition of digestive enzymes and oxidative stress that are considered to be responsible for obesity. The results of TPC and TFC for the methanolic extract and the fractions of *G. indica* of fruits presented in Table 1 indicated the highest values for TPC (375.6 ± 4.5 GAE mg/g) and TFC (235.5 ± 6.2 QE mg/g) for ethyl acetate fraction of the fruits. Ethyl acetate fractions from plants have been reported to be enriched with a number of polyphenols.[26]

High phenolic content of the fruit supports antioxidant potential of *G. indica* fruits as depicted by DPPH activity. Among the fractions, FGIEF again showed highest antioxidant activity (68.8% at 500 μg/mL) with an IC_{50} value of 294.3 ± 2.3 μg/mL. The other fractions showed relatively lesser DPPH inhibition. These results were in good agreement with the results of TPC and TFC estimation. Therefore, the potent antioxidant effect of the fruit might contribute to its anti-obesity effect which has been indicated by the inhibition of PL and α-amylase enzymes. Similar anti-obesity potential has been demonstrated by antioxidant fractions of the Saskatoon berry.[27] The results of the MTT assay clearly established the safety of FGIEF as it did not affect preadipocytes up to the dose of 800 mg/mL beyond which the cell viability decreased in a dose-dependent manner [Figure 3]. Accordingly, concentrations of FGIEF up to 500 mg/mL were used for further studies.

The results of PL and α-amylase inhibition assay presented in Figure 1 and Table 2 indicated that the ethyl acetate fraction (FGIEF) had the highest ability to reduce the PL activity with the IC_{50} value 257.3 ± 3.7 μg/mL. Similarly, FGIEF possessed the highest inhibition of α-amylase enzyme with the IC_{50} value of 349.7 ± 5.8 μg/mL. Our study proved a direct correlation between the total polyphenols and flavonoids contents and the inhibition of digestive enzymes. Low antilipase and α-amylase activities exhibited by the methanolic extract of the fruit can be due to the presence of nonphenolic plant components, such as sugars, pigments, and acids. Remarkably, all tested samples possessed higher PL inhibition than the α-amylase inhibition. This is in accordance with the previous reports that showed polyphenol-enriched compositions as potent inhibitor of PL than the α-amylase.[28] However, the tested samples were lesser potent than orlistat. The weaker effect of extract and its fractions may be due to the complexity of composition and lesser binding affinity of non-phenolic components. Thus, it can be safely concluded that the anti-obesity effect of *G. indica* fruits may be partly linked to its inhibitory activity against PL and α-amylase enzymes, leading to the attenuation of dietary fat and carbohydrate absorption in the gastrointestinal tract.

After this bioactivity-guided fractionation of methanolic extract of *G. indica* fruits, FGIEF was subjected to MPLC for the isolation of active compounds. Three compounds (1–3) were isolated and their chemical structures of compounds were elucidated based on spectral data. Compound 1 was found to be luteolin on the basis of comparison of spectral data reported earlier.[29] Compound 2, named as napthyldioxolol, consisted of cream-colored crystals from 80% ethyl acetate in hexane eluents. FTIR spectrum exhibited absorption bands for hydroxyl (3488, 3274 cm^{-1}) and aromatic (1623, 1427 cm^{-1}) functionalities. Based on ^{13}C NMR and mass spectral data, the molecular mass of 2 was established at 204 that was consistent with the molecular formula $C_{11}H_8O_4$. The mass spectrum displayed a base peak at *m/z*

160 corresponding to ($C_{10}H_{10}O_4$) that arose due to fission of dioxole ring. ^{1}H NMR spectrum of **2** exhibited signals for aromatic protons at δ 7.78 (d, *J* = 2.5 Hz, H-5), 7.26 (d, *J* = 8.5 Hz, H-3), 7.12 (dd, *J* = 3.0, 8.5 Hz, H-4), and 7.07 (br s, H-7), indicating the presence of an ABX system. It also displayed a two-proton singlet at δ 4.84 attributed to dioxymethylene protons (H_2-11). The ^{13}C NMR spectrum of two displayed signals for 11 carbons that consisted of four oxygenated aromatic carbons, six aromatic carbons, and a dioxymethylene carbon. Therefore, compound 2 was characterized as naphtha [1, 2-d] [1, 3] dioxole-6, 8-diol.

Compound 3, named as oleantrienoic acid glucoside, consisted of a light green waxy mass from 95% ethyl acetate in hexane eluents. FTIR spectrum displayed bands for the presence of hyrdoxyl (3422 cm^{-1}), carboxyl (1740 cm^{-1}), and vinylic (1670 cm^{-1}) functionalities. Its molecular mass was established at *m/z* 614 based on NMR and mass spectrum. It was consistent with the molecular formula $C_{36}H_{54}O_8$. The fragment ion peaks at *m/z* 433 ($C_{30}H_{41}O_2$) + arouse due to glycosodic fission. The fragment ion peak at *m/z* 206 and 228 appeared due to retro-Diels Alder fission. ^{1}H NMR spectrum of **3** exhibited two one-proton doublets at δ 5.33 and 4.85 (*J* = 5.0 Hz) and a broad signal at δ 5.39 (1H) ascribed correspondingly to H-11, H-12, and H-6 vinylic protons. A broad signal at δ 4.41 integrating for one proton was assigned to H-3 carbinol proton. A doublet at δ 4.22 (*J* = 8.0 Hz) was accounted to anomeric protons H-1'. The other sugar protons appeared from δ 4.04 to 3.40. Five three-proton singlets at δ 1.43, 1.02, 0.98, 0.86, and 0.65 were ascribed to Me-26, Me-24, Me-23, Me-25, and Me-29 protons, respectively. A six-proton singlet at δ 0.90 was attributed to Me-27 and Me-30 methyl protons. ^{13}C NMR spectrum of **3** displayed important signals for carboxyl carbon at δ 182.7 (C-28); vinylic carbons from δ 156.3 to 116.0; and anomeric carbon at δ 100.7 (C-1). The methyl carbons resonated between δ 32.0 and 11.6. On acid hydrolysis, compound 3 yielded D-glucose. Thus, compound 3 was elucidated to be olean-5, 9 (11), 12-trien-28-oic acid-3-ol-3-*O*-β-D-glucopyranose.

Nowadays, *in silico* studies are pursued to simulate the interaction of test substance with its target. We also carried out docking modeling to study the interaction of representative polyphenols and isolated compounds with PL. The results presented in Table 3 showed strongest interaction of oleantrienoic acid glucoside among the tested substances as it assumed favorable orientation within the PL-colipase complex binding site. The polyphenols exhibited significant antilipase activities but weaker than the orlistat. Our results are in line with previous reports on PL inhibition by polyphenol-rich ethyl acetate fraction from different plants.[30,31] Polyphenol-rich grape seed extract has been reported to reduce dietary fat absorption through PL inhibition and slow down the accumulation of fat in adipose tissue.[32] Similarly, ethyl acetate fraction of *Terminalia chebula* diminishes obesity by reducing lipid accumulation through suppression of digestive enzymes such as PL and amylase and 3T3-L1 adipocyte differentiation.[33] Recently, phenolic acid-rich fruit extracts of *Cornus mas* and *Cornus alba* have been shown to have antiobesogenic effects by inhibiting PL and α-amylase enzymes.[28] The results of the docking studies involving isolated compounds were not in complete concordance with their *in vitro* PL inhibitory activity. This observation also highlights the need to follow-up *in silico* studies with *in vitro* tests.

CONCLUSION

The fruits of *G. indica* exhibit anti-obesity effect through the inhibition of digestive enzymes that can be mainly attributed to the presence of polyphenols. *G. indica* fruit berries, particularly its polyphenol-enriched fraction, causes the amelioration of obesogenic and oxidative stress. The study also emphasized the potential of this edible fruit as a novel food ingredient to be pursued as a nutraceutical or a dietary supplement. Further, in depth studies on the experimental animals and humans can pave its way to market as an anti-obesity agent.

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Conflicts of interest

There are no conflicts of interest.

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