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Research Paper

Metabolic Profiling, Antioxidant, and Anti-lipase Activity from Combined Leaves Extracts of *Tamarindus indica* and *Murraya paniculata*: A Simplex Lattice Design Approach

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Abstract
Tamarindus indica leaves are recognized for their potent antioxidant and hypolipidemic properties, whereas *Murraya paniculata* leaves are known for their abilities to lower lipids and glucose levels. This study aimed to assess the combined extract of both leaves against pancreatic lipase inhibition and analyze their metabolomic profiles as an initial step toward developing a polyherbal treatment for hypertriglyceridemia. The extracts were subjected to Liquid Chromatography-High Resolution Mass Spectrometer (LC-HRMS) untargeted system coupled with Compounds Discoverer software to reveal their metabolomic profile. Subsequently, both individual extracts and their combination were evaluated for anti-lipase activity using pancreatic lipase enzyme with p-nitrophenyl butyrate as the substrate. The combination of the two extracts (0-300 μg/mL, 300 μg/mL in total) was prepared following the Simplex Lattice Experimental Design with 5 different composition variations. Results: The findings indicated that *Tamarindus indica* leaf extract (TIE) predominantly exhibited lipase inhibitory activity. Interestingly, the addition of *Murraya paniculata* extract (MPE) diminished this enzyme inhibitory effect. TIE was found to be rich in polyhydroxy flavonoids followed by fatty amides, whereas MPE contained mainly polymethoxy flavonoids, fatty amides, and coumarins. The presence of fatty amides in both extracts was identified as a potential cause for this incompatibility. In summary, *Tamarindus indica* leaf extract demonstrated strong lipase inhibition; however, its effectiveness was reduced when combined with *Murraya paniculata* extract, possibly due to primary fatty amides. Further research is necessary to explore strategies for eliminating these compounds and confirming their impact *in vivo*.

Keywords
Anti-Lipase, Triglyceride Lowering, Combined Extract, Simplex-Lattice-Design

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1. INTRODUCTION

One consequence of uncontrolled hyperlipidemia (especially hypertriglyceridemia, HTG) is non-alcoholic fatty liver disease (NAFLD) (Ali et al., 2021). HTG arises from an imbalance between the production of triglyceride-rich lipoproteins (TRL) and their clearance rate. Biochemically, lipids are biomolecules (cholesterol, triglycerides, lipoproteins) in the blood that dissolve in non-polar solvents and are essential for transporting hydrophobic molecules throughout the body's tissues. However, in HTG, excessive triglycerides in plasma can induce the influx of free fatty acids into cells and lead to the production of high levels of oxygen radical species (ROS) (Bessone et al., 2019). Oxidative stress within mitochondria due to increased free fatty acids can impair organelle function.

Currently, one of strategies to lower plasma triglyceride (TG) levels is by inhibiting lipase enzymes in the intestine (e.g., orlistat). Other strategies include inhibiting the formation of TG-rich lipoproteins and promoting TG oxidation in peripheral tissues (Laufs et al., 2020). However, accelerating beta-oxidation to remove TGs may also increase reactive oxygen species (ROS) levels in cells. Therefore, developing TG-lowering agents that can inhibit lipase while counteracting ROS in clinical applications is crucial.

Current research actively explores plant-based natural products, particularly flavonoids and coumarins, as potential therapeutic agents for treating hyperlipidemia. Flavonoids such as myricetin, quercetin, apigenin, and hesperidin, abundant in fruits and vegetables like citrus fruits and onions, exhibit lipid-lowering properties by inhibiting cholesterol synthesis enzymes and enhancing low-density lipoprotein (LDL) receptor's expression (Ahmed, 2021). Coumarins like scopoletin and umbelliferone have demonstrated promising effects in reducing triglyceride levels and improving lipid profiles by modulating

lipid metabolism pathways including insulin (Gao et al., 2024; Park et al., 2023). These bioactive compounds represent a growing area of research in natural products pharmacology for managing hyperlipidemia, offering potential alternatives or adjuncts to traditional lipid-lowering therapies.

Two plants known for their richness in flavonoids and coumarins are *Tamarindus indica* and *Murraya paniculata* leaves, respectively (Menezes et al., 2017; Wiyono et al., 2022). In Indonesia, tamarind leaves are utilized in Jamu Sinom to enhance body endurance and expedite recovery after illness (Sumarni et al., 2019). Tamarind leaves reportedly contain neophytadiene, phytol, n-pentacosane, n-hexacosane, n-heptacosane, n-octacosane, squalene, n-nonacosane, methyl hexacosanoate, n-hentriacontane, alpha-tocopherol, γ-sitosterol, β-amyrone, lupenone, lupeol, 3β-hydroxystigmast-5-en-7-one, and betulinaldehyde (Aly et al., 2022). Pharmacologically, tamarind leaves are reported to exhibit hypolipidemic, hepatoprotective, anti-diabetic, and potent antioxidant activities (Amado et al., 2016; Kuddus et al., 2020; Lahamado et al., 2017; Sookying et al., 2022).

Meanwhile, *M. paniculata* leaves are empirically used by Indonesian people as an anti-obesity and lipid-lowering agent (Hasim et al., 2021). *Murraya paniculata* leaves reportedly contain coumarin compounds (murmeranzin, isopropylidine murrangatin, murralonginal, and pranferin), methyl palmitate, isospathulenol, (E,E)-geranyl linalool, benzyl benzoate, selinenol, β-karyophyllene, germacrene, and γ-elements. Pharmacologically, *M. paniculata* exhibits hypolipidemic, antioxidant, and anti-diabetic activities (Dosoky et al., 2016).

With proper knowledge, polyherbal remedies will be more beneficial than single-drug treatments. Polyherbal formulations, which combine multiple plant extracts or herbal components, have gained attention in traditional medicine and scientific research. With proper knowledge, polyherbal treatments will be more beneficial than single treatments. Some of the advantages include: (i) Synergistic Effects: these interactions can enhance therapeutic efficacy by targeting multiple pathways simultaneously; (ii) Broad Spectrum of Activity: by incorporating different plant extracts, they may target different symptoms, stages, or underlying mechanisms. This broader coverage can be especially beneficial for complex or multifactorial conditions; (iii) Reduced Side Effects: polyherbal formulations allow for lower doses of individual components, potentially minimizing side effects while maintaining therapeutic benefits; (iv) Enhanced Bioavailability: some compounds in herbal extracts may enhance or inhibit the absorption of others (Elfita et al., 2024; Karole et al., 2019).

This research represented a novel approach in the development of polyherbal traditional medicine that combined tamarind leaves and *M. paniculata* to address hypertriglyceridemia. To the best of our knowledge, there were no studies examining the hypolipidemic activity of both *T. indica* and *M. paniculata* as a combined extract. Previous researches already evaluated the anti-hyperlipidemic activity of *T. indica* (Kuddus et al., 2020; Valaparla et al., 2022; Wiyono et al., 2022) and *M. paniculata* (Menezes et al., 2017; Nuri et al., 2024; Wardani et al., 2021) as individual-extract.

In this study, the lipase inhibitory activity of the combined extracts of the two plants was tested, supported by analysis of their metabolites. The aim of this research was to determine the effectiveness of the combined extract in inhibiting lipase enzyme activity, with metabolite analysis conducted to identify potential active molecules.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used included *M. paniculata* leaves obtained from Tawangmangu, Central Java, Indonesia, and *T. indica* leaves obtained from the district of Sleman, Yogyakarta, Indonesia. Both plants were obtained in the form of fresh leaves and identified at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta. Chemicals used for testing included pancreatic lipase from porcine pancreas (L3126) and p-nitrophenyl butyrate (p-NPB; L3126) purchased from Sigma-Aldrich. Solvents and eluents were purchased from local distributors. Solvents for LC-HRMS were MS grade purchased from ThermoScientificTM. Chemicals, aside from the lipase enzyme and p-NPB, were stored at room temperature. Meanwhile, enzymes were stored at -21°C.

2.2 Methods

2.2.1 Extract Preparation

As previously described Wiyono et al. (2022), fresh leaves were dried in a tray oven at 50°C for 24 hours and then ground using a grinder. The leaf powder was macerated in 70% ethanol at room temperature (25 ± 2°C) for 24 hours. After filtration, the filtrate was evaporated to dryness using a rotary evaporator (Buchi® R-300 Rotavapor).

2.2.2 LC-HRMS Metabolic Profiling

The phytochemical content of the extracts were analyzed using the non-targeted mode of the Q-Exactive Quadrupole-Orbitrap™ Mass Spectrometer system (ThermoScientific®). The extract was dissolved in 100 μg/mL MS-grade methanol and filtered through a 0.22 μm filter. The device was configured as follows (Wiyono et al., 2022): The mobile phases were (A) 0.1% formic acid in water solution and (B) 0.1% formic acid in acetonitrile; injection volume was 5 μL; flow rate was 0.3 mL/min. The gradient system was as follows: 0 to 16 minutes (B 5% to 90%), 16 to 20 minutes (B 90%), 20.1 to 25 minutes (B 5%). The 3.62 kV ESI positive mode was used as the ion source. Subsequently, the raw chromatograms were analyzed using Compound Discoverer 3.2 software (ThermoScientific®), referencing local and online databases including MzCloud (www.mzcloud.org) and ChemSpider (www.chemspider.com).

The analytical parameters used in the metabolomics analysis were as follows: (i) candidate molecules were determined based on the similarity of fragmentation patterns and molecular weights with the MzCloud database (www.mzcloud.org);

Table 1. Experimental Design for a Mixture with Simplex Lattice Design on a Combination of *T. indica* and *M. paniculata* Leaf Extracts

Composition	Proporsion (X1)	(X2)	Concentration (μg/mL) *T. indica*	*M. paniculata*	Total
I	0	1	0	300	300
II	0.25	0.75	75	225	300
III	0.5	0.5	150	150	300
IV	0.75	0.25	225	75	300
V	1	0	300	0	300

and (ii) the deviation of molecular mass was no more than 5 ppm.

2.2.3 Antioxidant Assay

In methanol, the extracts were dissolved independently in a series of concentrations ranging from 50 to 800 μg/mL. Subsequently, the scavenging activity of DPPH was determined spectrophotometrically using a Multiskan GO Microplate Spectrophotometer (ThermoScientific®). Approximately 1400 μL of DPPH solution (0.04 mg/mL) in an amber glass tube was added to 200 μL of extract (in methanol) at various concentrations. The absorbance was measured at 515 nm after incubating for 30 minutes in the dark (Chandra et al., 2014; Susanti et al., 2023).

2.2.4 Anti-Lipase Assay

The lipase inhibitory activity of both extracts was measured as single extracts and in combination. Each extract was prepared individually at concentrations of 10, 100, and 300 μg/mL (resulting in final concentrations of 0.38, 3.8, and 11.4 μg/mL) in 2% DMSO. The combination was prepared following the Experimental Design of Simplex Lattice Design (SLD) with 2 variables (type of extract) and 5 variations (0 – 300 μg/mL each; total 300 μg/mL), as presented in Table 1.

The rate of nitrophenol production, a product of p-nitrophenylbutyrate hydrolysis by lipase, was spectrophotometrically monitored to measure the lipase inhibitory action. A final mixture concentration of 11.4 μg/mL was achieved by dissolving the combined extract in dimethyl sulfoxide (DMSO) at concentrations of 300 μg/mL. Ten microliters (10 μL) of lipase (1 mg/mL in phosphate buffered saline, pH 6.8) was added to 10 μL of extract. After 5 minutes of incubation at 37°C, 240 μL of p-nitrophenylbutyrate (0.165 mM in PBS) substrate was added. Absorbance was measured immediately (T-0) at 415 nm and again after 35 minutes (T-1). The rate of nitrophenol hydrolysis was expressed in mM nitrophenol per minute.

To determine the mode of inhibition, Km and Vmax values were calculated by reacting the enzyme with p-NPB in the presence or absence of extract (final concentrations of 3.8 μg/mL), varying substrate concentrations (1.0, 0.3, 0.2, and 0.1 mM). Standard curves were constructed using pure nitrophenol powder diluted to concentrations of 0, 1, 2, 5, 10, or 20 μg/mL, and absorbance at 415 nm was measured using a Multiskan GO Microplate Spectrophotometer (ThermoScientific®).

2.2.5 Data Analysis

The collected data were analyzed using ANOVA for mean difference at 95% significance level. In addition, SLD analysis was performed with a non-linear curve fitting in the equation, $y = a.[A] + b.[B] + c.[A][B]$. The data analysis was performed using the statistical software GraphPad Prism 7.0 and Minitab Statistical 17.0.

3. RESULTS AND DISCUSSION

3.1 Metabolites Profile

3.1.1 Metabolites of *T. indica* Leaf

Phytochemically, *T. indica* leaf extracts (TIE) contained a large amount of flavonoids, as revealed by LC-HRMS analysis shown in Figure 1. According to the analysis results (Table 2), compounds belonging to the classes of flavonoids (77.5%), fatty amides (11.3%), amines (2.1%), phthalates (1.9%), alkaloids (1.5%), phenylated sugars (0.8%), and fatty acids (0.8%) were identified in TIE. Generally, the chromatogram indicated that flavonoid compounds dominated at a retention time of 4-9 minutes, while amides and fatty acids appeared at 14-19 minutes and amines at 9-12 minutes. Based on peak areas, the top five compounds with the highest abundance were: isovitexin (32.7%), isovitexin 2'-O-arabinoside (13.9%), orientin (8.4%), hexadecanamide (8.3%), and (-)-epicatechin (6.2%) (Figure 2).

Tamarindus indica leaves contained bioactive compounds such as flavonoids and phenolic compounds, contributing to their potential health benefits. Antioxidants help combat oxidative stress, which is implicated in various health conditions. A previous systematic review (Sookying et al., 2022) summarized the *in vitro* antioxidant capacities of *T. indica* leaves. These properties could be relevant in the management of hypertriglyceridemia, characterized by elevated triglyceride levels in the blood.

3.1.2 Metabolites of *M. paniculata* Leaf

Metabolomics analysis using the same method was also conducted on *M. paniculata* leaf extract (MPE) (Figure 3). Generally, MPE contained compound classes such as alkaloids, fatty amides, amines, anthracene, amino acids, phenolic acids, fatty acids, esters, flavonoids, chalcones, coumarins, steroids, and sesquiterpenes. Alkaloids appeared at a retention time of 2-16 minutes, flavonoids between 0-11 minutes, and coumarin compounds at 6-14 minutes. According to the metabolomics anal-

Table 2. Composition of Metabolites in the Hydro-Ethanolic Extract of *T. indica* Leaves. Molecules Were Detected by LC-HRMS Using 0.1% Water-Formic Acid and 0.1% Acetonitril-Formic Acid as Mobile Phases

Putative Name	Class	MW	RT (min)	Area	% Relative	Abbund. Order
Adenosine	Nucleotides	267.0967	0.776	3.1E+07	0.3	26
Hordenine	Amines	165.1152	0.779	8.1E+07	0.8	11
Prolylleusin	Amino acid	228.1472	1.077	2.6E+07	0.3	30
L-Phenylalanine	Amino acid	165.0789	1.404	1.9E+07	0.2	37
Trans-3-indoleacrylic acid	Alkaloid	187.0631	2.236	7.3E+07	0.7	13
4-o-caffeoyl quinic acid	Phenolic acid	354.0937	3.856	1.4E+07	0.1	43
7-Hydroxycoumarin	Coumarin	162.0314	3.856	2.7E+07	0.3	29
Ferulic acid	Phenolic acid	194.0575	4.286	5.6E+06	0.1	58
(-)-Epicatechin	Flavonoid	290.078	4.46	6.3E+08	6.2	5
3-[(1E,3E)-hepta-1,3-dienyl]pentanedioic acid	Fatty acid	226.1201	4.537	5.4E+06	0.1	59
6,8-di-C-glucosylapigenin	Flavonoid o-glycoside	594.1574	4.567	1.6E+08	1.6	9
5-(6-hydroxy-6-methyloctyl)-2(5H)-furanone	Furan	226.1564	4.917	7.2E+07	0.7	14
Orientin	Flavonoid c-glycoside	448.0998	4.963	8.6E+08	8.4	3
Isovitexin 2'-O-arabinoside	Flavonoid c-glycoside	564.1465	5.369	1.4E+09	13.9	2
Rutin	Flavonoid o-glycoside	610.153	5.423	2.4E+07	0.2	31
2"-O-α-L-Rhamnopyranosyl-isovitexin	Flavonoid c-glycoside	578.1622	5.445	2.7E+08	2.7	8
Isovitexin	Flavonoid c-glycoside	432.1048	5.538	3.9E+09	37.7	1
Quercetin-3β-D-glucoside	Flavonoid o-glycoside	464.0958	5.624	1.7E+07	0.2	40
Quercetin	Flavonoid	302.0423	5.626	2.1E+07	0.2	32
Vitexin	Flavonoid c-glycoside	432.1058	5.631	4.2E+08	4.1	6
Sinensin	Flavonoid o-glycoside	450.1146	5.729	8.7E+06	0.1	55
4-(4-Hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanyl β-D-glucopyranoside	Monoterpene	374.2296	6.249	6.5E+06	0.1	57
Apigenin	Flavonoid	270.0529	6.383	6.0E+07	0.6	16
Prunin	Flavonoid o-glycoside	434.1212	6.409	1.1E+07	0.1	50
Diosmetin	Flavonoid	300.0627	6.467	1.2E+07	0.1	46
Hispidulin 4'-glucoside	Flavonoid o-glycoside	462.1161	6.47	5.3E+07	0.5	17
Phloretin	Chalcone	274.0838	6.788	1.1E+07	0.1	47
5α-Androstan-3,6,17-trione	Steroid	302.188	6.901	1.1E+07	0.1	49
Daidzein	Flavonoid	254.0581	7.088	1.9E+07	0.2	36
Wogonin	Flavonoid	284.068	7.248	1.8E+07	0.2	39
Kaempferol	Flavonoid	286.0474	7.509	4.1E+07	0.4	19
Naringenin	Flavonoid	272.0684	8.449	8.2E+06	0.1	56
Nootkatone	Sesquiterpene	218.1667	8.711	3.3E+07	0.3	24
Jasmone	Cycloalcanes	164.1198	8.885	4.3E+06	0.0	61
(2R,5R,6R)-3-[(1E,3E)-1,3-Heptadien-1-yl]-5,6-dihydroxy-2-(hydroxymethyl)cyclohexanone	Cycloalcanes	254.1516	8.992	1.3E+07	0.1	45
2-Amino-1,3,4-octadecantriol	Amines	317.2917	9.704	1.1E+07	0.1	48
1-Tetradecylamine	Amines	213.2452	10.113	2.9E+07	0.3	27
Bis(methylbenzylidene)sorbitol	Phenylated sugar	386.1717	10.67	7.6E+07	0.7	12
Bis(4-ethylbenzylidene)sorbitol	Phenylated sugar	414.2032	11.647	2.0E+07	0.2	35
Bis(2-ethylhexyl) amin	Amines	241.2765	11.823	2.0E+07	0.2	34
Octadecanamine	Amines	269.3081	12.757	6.9E+07	0.7	15
α-Pyrrolidinopropiophenone	Alkaloid	203.1306	12.912	3.3E+07	0.3	25
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	Phenols	234.1617	13.056	2.8E+07	0.3	28
1-Linoleoyl gliserol	Ester gliserida	354.2759	13.101	8.8E+06	0.1	54
18-β-Glicirrhetinic acid	Triterpenoid	470.339	13.574	1.0E+07	0.1	53
Testosterone undecanoat	Steroid	456.36	13.766	1.6E+07	0.2	41
Dibutyl phtalate	Phtalate	278.1514	13.835	3.5E+07	0.3	21
Cholecalciferol	Steroid	384.3379	14.348	5.4E+06	0.1	60
Hexadecanamid	Fatty amide	255.2558	14.437	8.5E+08	8.3	4
α-Linolenic acid	Fatty acid	278.2244	14.741	3.5E+07	0.3	20
Nervonic acid	Fatty acid	366.3483	15.698	1.9E+07	0.2	38
Tridemorph	Alkaloid	297.3024	16.592	5.3E+07	0.5	18
Betulin	Triterpenoid	442.3806	16.781	1.4E+07	0.1	44
Erucamide	Fatty amide	337.3337	17.05	2.8E+08	2.7	7
Docosanamide	Fatty amide	339.3487	17.594	3.3E+07	0.3	23
Bis(2-ethylhexyl)adipate	Fatty acid	370.3077	17.686	1.0E+07	0.1	51
Di(2-ethylhexyl) phtalate	Phtalate	390.2764	17.876	1.4E+08	1.3	10
1,2-Cyclohexane dicarboxylic acid diisononil ester	Fatty ester	424.355	19.367	1.4E+07	0.1	42
Diisodecyl phtalate	Phtalate	446.3377	19.39	2.1E+07	0.2	33
Tocopherol	Phenols	430.3791	19.728	3.4E+07	0.3	22
4-Phenylbutyric acid	Fatty acid	164.0835	19.739	1.0E+07	0.1	52

Table 3. Composition of Metabolites in the Hydro-Ethanolic Extract of *M. paniculata* Leaves. Molecules Were Detected by LC-HRMS Using 0.1% water-formic acid and 0.1% Acetonitril-Formic Acid as Mobile Phases

Putative Name	Class	MW	RT (min)	Area	% Relative	Abbund. Order
L-(+)-Arginine	Amino acid	174.1114	0.728	1.8E+07	0.1	59
Acethyl-β-methylcholin	Ester	159.1256	0.768	4.4E+07	0.3	40
6,6-dimethyl-4-piperidino-5,6-dihydro-2H-thiine-2-thion	Alkaloid	241.0949	0.771	4.9E+07	0.3	36
Adenosine	Nucleic acid	267.0965	0.774	1.5E+08	0.9	22
5-Acetamido-4-oxohexanoic acid	Fatty acid	187.0841	0.78	4.9E+07	0.3	37
Prolylleusin	Amino acid	228.147	0.78	2.4E+07	0.1	54
6,8-Di-C-glucosylapigenin	Flavonoid c-glycoside	594.1581	0.786	1.2E+07	0.1	66
Kaempferol-3-O-sophoroside	Flavonoid o-glycoside	610.1528	0.786	1.1E+07	0.1	69
L-Tyrosine	Amino acid	181.0737	1.07	4.2E+06	0.0	86
L-Phenylalanine	Amino acid	165.0787	1.414	5.3E+07	0.3	35
trans-3-Indoleacrylic acid	Alkaloid	187.0628	2.207	9.7E+07	0.6	28
Cyclomethyltriptopane	Amino acid	216.0897	3.562	3.8E+07	0.2	43
5-Hydroxy-1,3,3-trimethyl-2-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	Cycloalcanes	386.1926	4.679	3.1E+07	0.2	52
2-(3,4-Dihydroxyphenyl)-8-galactopyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one	Flavonoid c-glycoside	448.0992	4.867	2.7E+08	1.7	12
3-Feruloyl quinic acid	Ester	368.1091	4.897	2.1E+08	1.3	15
Schaftoside	Flavonoid c-glycoside	564.1467	5.623	3.6E+07	0.2	44
Ferulic acid	Phenolic acid	194.0575	5.001	2.1E+08	1.3	17
Orientin	Flavonoid c-glycoside	448.0992	5.131	7.6E+07	0.5	31
α-Piperidinobutiophenone	Alkaloid	231.1615	5.231	1.3E+07	0.1	65
Isovitexin-2-O-arabinoside	Flavonoid c-glycoside	564.1467	5.368	1.4E+08	0.9	25
3'-hydroxy Puerarin	Flavonoid c-glycoside	432.1044	5.538	1.1E+09	7.1	3
Quercetin-3β-D-glucoside	Flavonoid o-glycoside	464.0949	5.623	3.6E+07	0.2	44
Quercetin	Flavonoid	302.0416	5.623	3.2E+07	0.2	51
Cynaroside	Flavonoid o-glycoside	448.0997	5.767	1.2E+07	0.1	68
Quercetin 3-methoxy 3'-glucoside	Flavonoid o-glycoside	478.1099	6.191	4.3E+07	0.3	41
Kolumbianetin	Coumarin	246.0889	6.362	5.9E+06	0.0	80
Hispidulin 4'-O-beta-D-glucopyranoside	Flavonoid o-glycoside	462.1154	6.473	1.8E+07	0.1	58
Sentaureidin	Flavonoid	360.0828	6.48	1.0E+08	0.6	27
(1S)-4-(3-Hydroxybutyl)-3,5,5-trimethyl-3- cyclohexen-1-yl 6-O-β-L-glucopyranosyl-β-L-glucopyranoside	Flavonoid o-glycoside	536.2823	6.599	5.7E+06	0.0	82
7-Methoxy-8-senecioyl coumarin	Coumarin	258.0884	6.822	5.3E+08	3.3	8
Jacein	Flavonoid o-glycoside	522.1366	6.914	2.4E+08	1.5	14
Paprazine	Amides	283.1202	7.232	6.3E+06	0.0	77
Microminutin	Coumarin	272.068	7.251	1.7E+07	0.1	61
Sorbicillin	Phenols	232.1092	7.287	4.9E+06	0.0	83
Jaceoside	Flavonoid o-glycoside	492.1257	7.422	2.0E+08	1.2	19
3-O-Methylquercetin	Flavonoid	316.0574	7.614	1.3E+07	0.1	64
Murracarpin	Coumarin	290.1142	7.708	3.6E+08	2.2	9
Murrangatin acetate	Coumarin	318.1089	8.002	1.5E+07	0.1	62
(-)-Caryophyllene oxide	Cycloalcanes	220.1822	8.085	8.6E+06	0.1	72
Apigenin	Flavonoid	270.0526	8.297	4.2E+06	0.0	87
7-Hydroxy-3',4',5-trimethoxyflavone	Flavonoid	328.0935	8.406	7.6E+07	0.5	32
9S,13R-12-Oxophytodienoic acid	Fatty acid	292.2032	8.527	7.6E+07	0.5	30
Aurantioobtusin	Antracene	330.0727	9.294	2.8E+08	1.7	11
Nobiletin	Flavonoid	402.1299	9.423	4.1E+09	26.0	1
4-[(3E)-5-Hydroxy-3-methyl-3-penten-1-yl]-3,5,5-trimethyl-2-cyclohexenone	Cycloalcanes	236.1771	9.461	1.0E+07	0.1	70
Murrangatin	Coumarin	276.0992	9.504	1.2E+08	0.8	26
3',4',5,5',7-Pentamethoxyflavone	Flavonoid	372.1206	9.924	1.5E+08	0.9	23
Sinensetin	Flavonoid	372.1206	9.924	1.5E+08	0.9	23
19-Norandrostenedione	Steroid	272.1769	9.951	7.6E+06	0.0	75
1-Tetradecylamine	Amines	213.2452	10.116	1.8E+07	0.1	60
Casticin	Flavonoid	374.0985	10.12	6.6E+08	4.1	6
Kimcuongin	Coumarin	356.1242	10.176	1.5E+08	1.0	21
5,6,7,3',4',5'-Hexamethoxyflavone	Flavonoid	402.1304	10.179	1.7E+08	1.1	20
Decanamide	Amides	171.1618	10.249	1.4E+07	0.1	63
2-2-[5-(Ethoxycarbonyl)-2-morfolinoanilino]-2-oxoethoxy acetic acid	Alkaloid	366.143	10.255	4.6E+06	0.0	84
Scoparone	Coumarin	206.0574	10.39	3.5E+07	0.2	49

Putative Name	Class	MW	RT (min)	Area	% Relative	Abbund. Order
Isoliquiritigenin	Chalcone	256.0729	10.45	8.5E+06	0.1	73
β-Lapachone	Cycloalcanes	242.094	10.511	1.9E+07	0.1	57
Bis(methylbenzylidene)sorbitol	Phenylated sugar	386.1719	10.665	1.2E+07	0.1	67
Xanthiletin	Coumarin	228.0782	10.856	7.1E+06	0.0	76
(4S,4aS,8aS)-4-(Hydroxymethyl)-3,4a,8,8-tetramethyl-4a,5,6,7, 8,8a-hexahydro-1(4H)-naftalinon	Cycloalcanes	236.1771	10.972	4.7E+07	0.3	39
5,2'-Dihydroxy-6,7,8,6'-tetramethoxyflavone	Flavonoid	374.0985	11.004	3.5E+07	0.2	47
4,7-dimethoxyfenalen-1-one	Naphtalene	240.078	11.375	5.7E+06	0.0	81
Isoliquiritigenin	Chalcone	256.0733	11.821	9.4E+06	0.1	71
Nootkatone	Sesquiterpene	218.1669	12.249	2.1E+08	1.3	16
Psoralen	Coumarin	186.0313	12.433	6.2E+06	0.0	78
Monoolein	Ester	356.2911	12.552	4.0E+06	0.0	88
α-Eleostearic acid	Fatty acid	278.2239	12.741	7.3E+07	0.5	33
Octadecanamine	Amines	269.308	12.763	4.9E+07	0.3	38
α-Pirrolidinopropiofenone	Alkaloid	203.1307	5.623	3.6E+07	0.2	44
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	Phenols	234.161	13.06	2.1E+07	0.1	56
Scopoletin	Coumarin	192.0419	13.429	2.1E+08	1.3	18
Linoleoyl etanolamide	Fatty amide	323.281	13.625	8.4E+06	0.1	74
Palmitoyl etanolamide	Fatty amide	299.2815	13.788	4.4E+06	0.0	85
Dibutyl phtalate	Phtalate	278.1512	13.831	3.5E+07	0.2	48
7-Hydroxycoumarin	Coumarin	162.0311	14.09	5.6E+08	3.5	7
Auraptene	Coumarin	298.1554	14.09	2.5E+08	1.6	13
5α-Dihydrotestosterone	Steroid	290.2237	14.117	2.2E+07	0.1	55
5.7-Dihydroxy-4-methylcoumarin	Coumarin	192.0419	14.539	2.6E+07	0.2	53
α-Linolenic acid	Fatty acid	278.2239	14.739	6.7E+07	0.4	34
Oleamide	fatty Amide	281.2707	15.373	7.9E+08	4.9	5
Hexadecanamide	Fatty amide	255.2554	16.053	1.5E+09	9.1	2
Tridemorph	Alkaloid	297.3018	16.59	3.9E+07	0.2	42
Methyl eleostearate	Ester	292.2392	16.638	3.2E+07	0.2	50
Erucamide	Fatty amide	337.3332	17.05	3.0E+08	1.9	10
Stearamide	Fatty amide	283.2864	17.058	1.1E+09	6.9	4
Di(2-ethylhexyl) phtalate	Phtalate	390.2758	17.874	9.0E+07	0.6	29

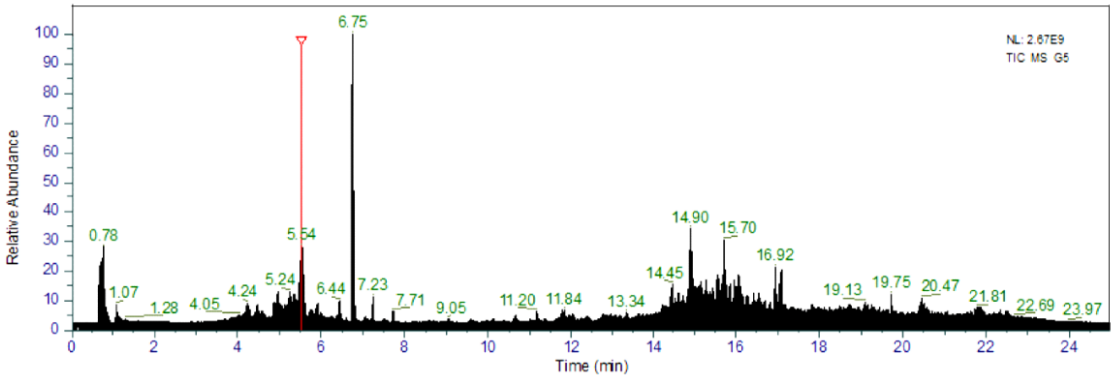

Figure 1. Non-Targeted LC-HRMS Chromatogram of Tamarind Leaf Extract. The Extract Was Eluted with the Mobile Phase (A) 0.1% Water-Formic Acid, (B) 0.1% Acetonitril-Formic Acid. Gradient System 0-16 Minutes (B 5-90 %); 16-20 Minutes (B 90%); 20-20.1 Minutes (B 90-5 %); 20.1-25 Minutes (B 5%)

ysis results (Table 3), the top five compounds with high abundance in *M. paniculata* were: nobiletin (26%), hexadecanamide (9.1%), 3'-hydroxy puerarin (7.1%), stearamide (6.9%), and oleamide (4.9%) (Figure 4). Based on compound class, the highest abundances were flavonoids (48.8%), fatty amides (23%), coumarins (14.4%), esters (1.8%), and anthracene (1.7%).

Murraya paniculata extract contained a significant amount of methoxylated flavonoids, followed by fatty amides, coumarins, esters, anthracene, and fatty acids. These findings aligned with research by Liang et al. (2020), which reported that *M. paniculata* leaves are rich in methoxylated flavones. The pharmacological activities associated with these methoxylated flavone compounds include anti-cancer (anti-angiogenic) and anti-inflammatory effects, reduction of triglyceride accumulation in adipose tissue, and promotion of hepatic fatty acid oxidation (Berim and Gang, 2016). One of the methoxylated flavones in *M. paniculata* leaf extract is nobiletin. According to previous studies Yuk et al. (2018), nobiletin exhibits anti-lipogenesis

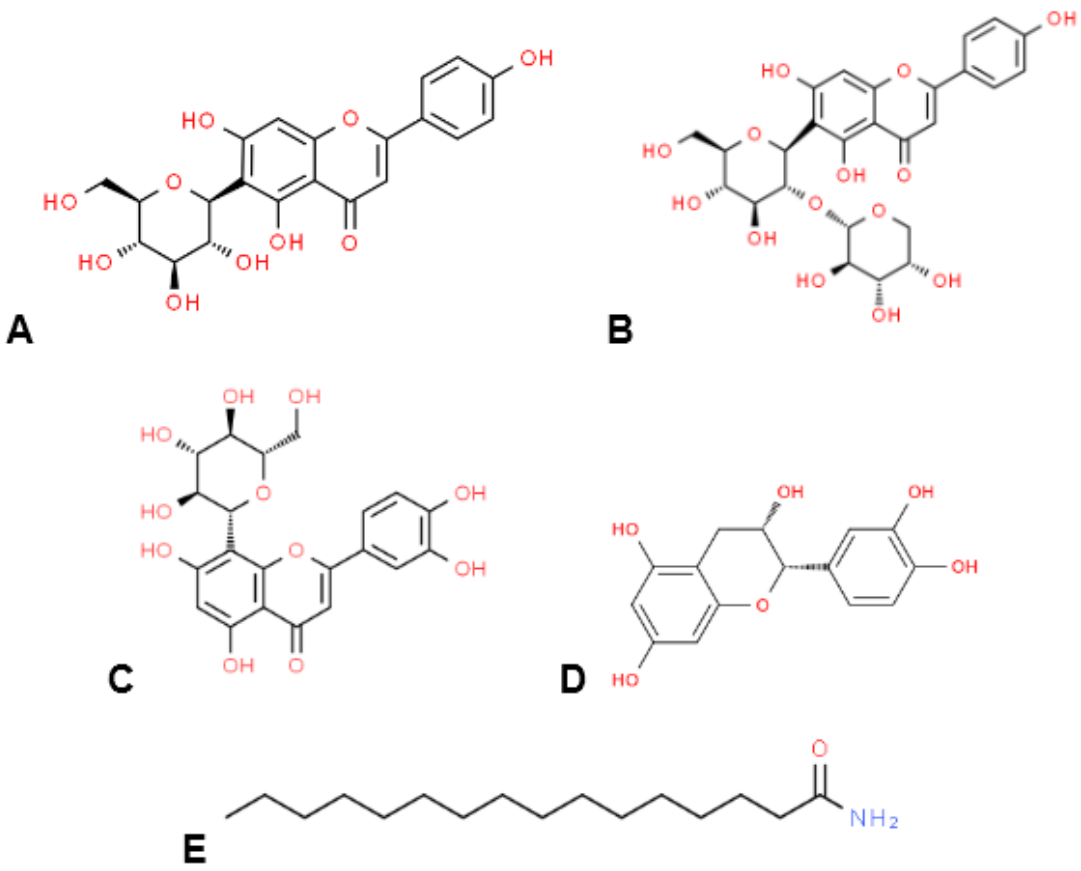

Figure 2. Chemical Structure of Major Molecules in *T. indica* Leaf Extract. (A: Isovitexin, B: Isovitexin 2'-O-Arabinoside, C: Orientin, D: (-)-Epicatechin, E: Hexadecanamide)

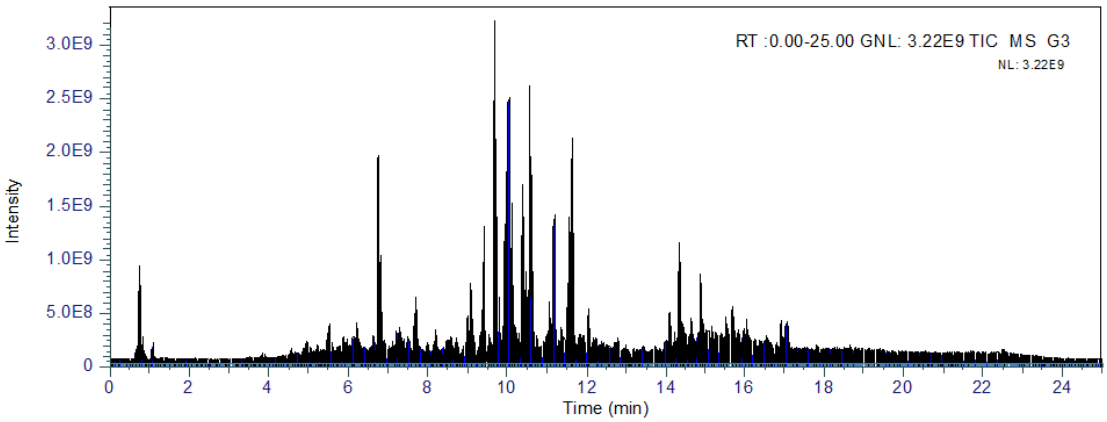

Figure 3. LC-HRMS Chromatogram of *M. paniculata* Leaf Extract. The Extract Was Eluted with the Mobile Phase (A) 0.1% Water-Formic Acid, (B) 0.1% Acetonitril-Formic Acid, Gradient System 0-16 Minutes (B 5-90 %); 16-20 Minutes (B 90%); 20-20.1 Minutes (B 90-5 %); 20.1-25 Minutes (B 5%)

activity by activating AMPK and acetyl-CoA carboxylase.

3.2 Antioxidant Activity (DPPH Assay)

To assess the antioxidant activity against free radicals of both extracts, the DPPH scavenging assay was performed. Figure

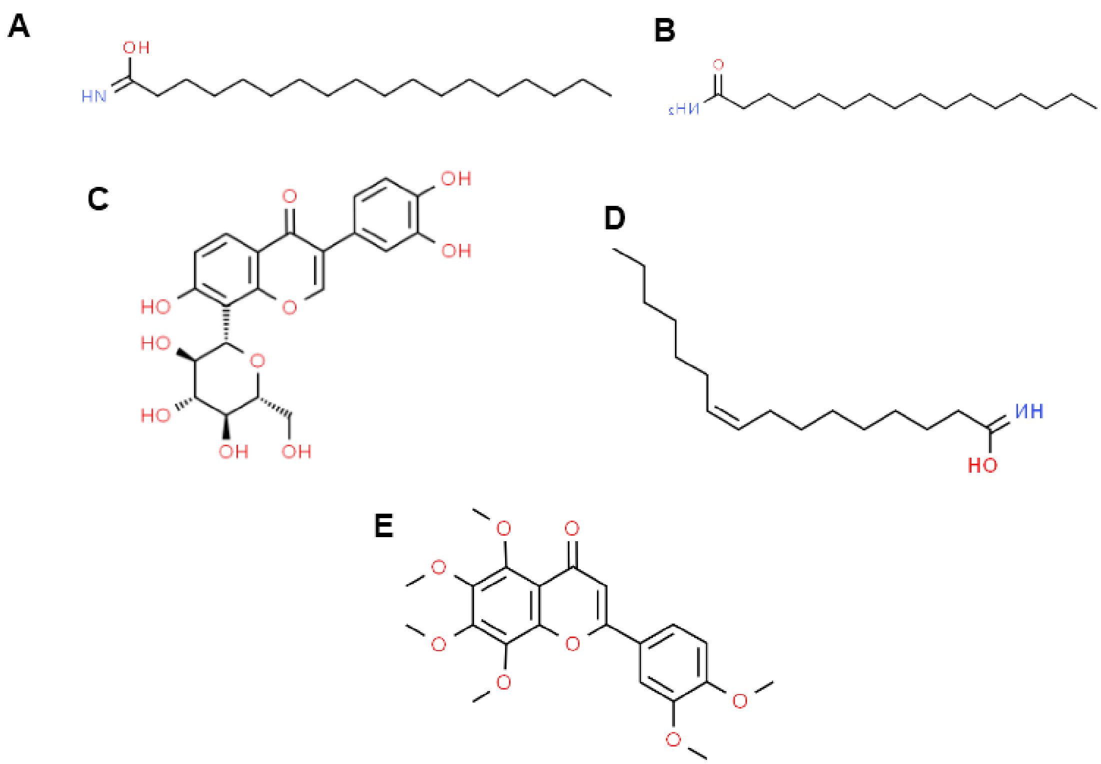

Figure 4. Major Compound in *M. paniculata* Extract (A: stearamide, B: Hexadecanamide, C: 3'-Hydroxy Puerarin, D: Oleamide, E: Nobiletin

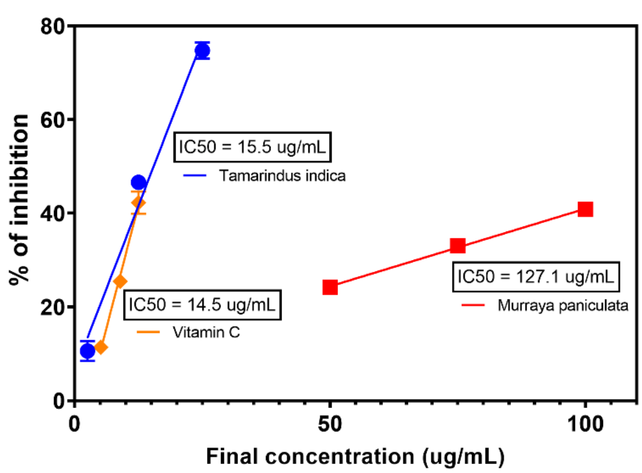

Figure 5. Antioxidant Activity (DPPH Radical Scavenging) of *T. indica* and *M. paniculata* Leaf Extracts

5 illustrates that the TIE extract (IC_{50} = 15.5 μg/mL) exhibited scavenging power comparable to vitamin C (IC_{50} = 14.5 μg/mL), significantly stronger than the MPE extract (IC_{50} = 127 μg/mL). Physiologically, fat metabolism, particularly fat oxidation, is closely linked to redox reactions. An imbalance in the production of radicals and their scavenging enzymes can lead to harmful oxidative stress. In this study, the DPPH radical scavenging activity of the two extracts was evaluated. The findings indicated that the antioxidant properties of TIE extracts were markedly stronger compared to those of MPE extracts (approximately 8 times stronger). The robust antioxidant activity of TIE was attributed to its high levels of polyhydroxy flavonoid molecules. These results corroborate the findings of Vo et al. (2019), which highlighted the role of hydroxy groups in flavonoids for antioxidant activity. In contrast, in the MPE extract, the hydroxyl groups of flavonoids were substituted with methoxyl groups, resulting in lower measured antioxidant capacity.

3.3 Pancreatic Lipase Inhibitory Activity

3.3.1 *Tamarindus indica* Versus *Murraya paniculata* (Individual Extract)

Furthermore, apart from antioxidant activity which has an indirect effect on fat metabolism, the activity of the two extracts was also measured for its direct effect on this metabolism. One of the enzymes involved in fat absorption is pancreatic lipase which converts triglycerides in the intestine into free fatty acids. Measurements of lipase inhibitory activity showed that the TIE extract at 3.8 and 11.4 μg/mL exhibited significant inhibitory activity (58.3% and 67%, $p < 0.05$) compared to the solvent control (Figure 6). Meanwhile, MPE extract at doses of 0.38 – 11.4 μg/mL provided 25 - 34% lipase inhibition ($p < 0.05$). From LC-HRMS analysis, it was determined that the major

Table 4. Km and V_{max} Values of Pancreatic Lipase Enzyme with/without *T. indica* or *M. paniculata* Leaf Extract

Extract	Km	V_{max}	$Km/_{Vmax}$	R^2
DMSO	0.809 ± 0.451	3.83 ± 1.19	0.211	0.950
T. indica (TIE)	0.580 ± 0.150	2.26 ± 0.294	0.256	0.982
M. paniculata (MPE)	0.860 ± 0.227	2.84 ± 0.424	0.302	0.988

*Units: Km (mM), V_{max} (μM/min)

Table 5. Analysis of Variance for Inhibitory (Component Proportions) of Combined Extract of *T. indica* and *M. paniculata* Leaves

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	*p*-Value	Coef. (ammount)
Regression	2	4151.6	4151.6	2075.81	20.05	0.000	
Linear	1	2263.0	2263.0	2263.01	21.86	0.001	
Quadratic	1	1888.6	1888.6	1888.61	18.25	0.001	
TIE*MPE	1	1888.6	1888.6	1888.61	18.25	0.001	
Residual Error	12	1242.1	1242.1	103.51			
Lack-of-Fit	2	283.5	283.5	141.73	1.48	0.274	
Pure Error	10	958.6	958.6	95.86			
Total	14	5393.7					
Term Coef.							
TIE						0.001	0.216027
MPE						0.001	0.100223
TIE*MPE						0.001	-0.001192

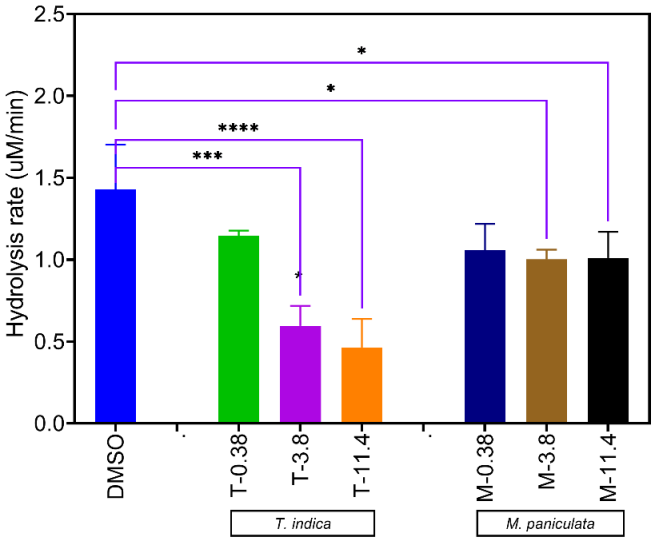

Figure 6. Pancreatic Lipase Enzyme Inhibitory Activity by Standardized Extracts of *T. indica* and *M. paniculata* Leaves Expressed in a Decrease in Hydrolysis Rate. Measurements Were Made at Concentrations of 10. 100, 300 μg/mL (0.38; 3.8; 11.4 μg/mL final), Asterisks Indicate Significance Compared to Solvent Control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.001$)

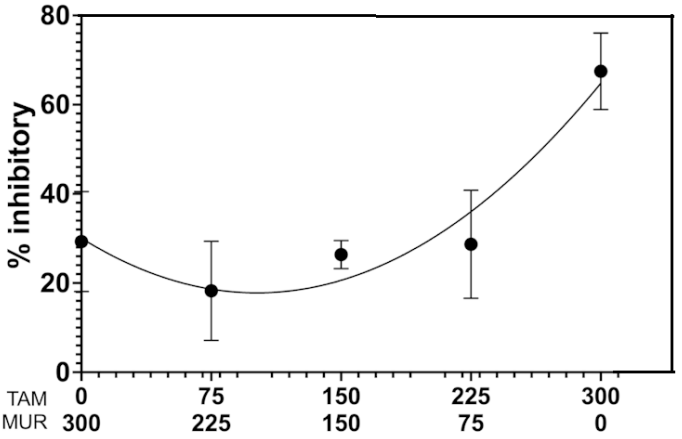

Figure 7. Percentage of Pancreatic Lipase Inhibition by Various Combinations of Standardized Extracts of *T. indica* (TAM) and *M. paniculata* (MUR) Leaves. The Concentration of the Prepared Extract = 300 μg/mL (Final Concentration = 11.4 μg/mL)

compounds in TIE were vitexin, isovitexin glycoside, and orientin. Several previous studies have reported lipase inhibition activity by vitexin or vitexin-rich extracts (Li et al., 2021). Isovitexin and orientin have also been reported to possess anti-lipase activity (Guo et al., 2020). Meanwhile, the major compound (non-fatty amide) in MPE was nobiletin. In vitro and in silico tests have reported nobiletin to be a potent lipase inhibitor (Zeng et al., 2018).

3.3.2 The Effect of *T. indica* and *M. paniculata* Leaf Extracts on Km and V_{max} of Lipase

In this study, the mode of inhibition of the two extracts was also determined by varying the substrate concentration to construct the Michaelis-Menten curve. The inhibition mode was discerned by comparing the maximum reaction rate (V_{max}), Michaelis constant (Km), and the slope value of the Lineweaver-Burk plot (LB) in the presence and absence of inhibitors. Based on the data obtained (Table 4), compared to the DMSO control profile (V_{max} = 3.83 μM/min; Km = 0.809 mM), the results indicated that the TIE extract slightly decreased V_{max} (2.26 μM/min) and Km (0.58 mM), though not significantly ($p > 0.05$), while increasing the LB slope, as presented in Table 5. Meanwhile, the MPE extract appeared to reduce the Vmax value (2.84 μM/min) without affecting the Km value (0.86 mM).

According to enzyme inhibition criteria Palmer and Bonner (2011), competitive inhibition is characterized by a constant V_{max} with an increased Km. Non-competitive inhibition is marked by a decreased Vmax with a constant Km, whereas uncompetitive inhibition involves changes in both Km and Vmax values while maintaining a constant LB slope. Therefore, the TIE extract demonstrated mixed-mode inhibition on lipase activity, whereas the MPE extract exhibited non-competitive inhibition.

3.3.3 Anti-Lipase Activity of Combined Extract (SLD Analysis)

The lipase inhibition assay of the two extracts in combination was conducted using a Simplex Lattice Design with five variations (0-300 μg/mL, total mixture 300 μg/mL). The percent inhibition values (y-axis) were then fitted using non-linear regression against concentration (x-axis), as depicted in Figure 7. According to ANOVA analysis (Table 5), it was evident that there was a significant negative interaction between the two extracts ($p < 0.05$). This resulted in the following fitted Equation (1):

$$Y = 0.216[\text{TIE}] + 0.100[\text{MPE}] - 0.001[TIE][\text{MPE}] \quad (1)$$

Here, Y represents the inhibitory activity, [TIE] and [MPE] denote the concentrations of TIE and MPE extracts, respectively. These findings indicate that TIE (coefficient value = 0.216, $p < 0.05$) exerted a stronger influence on lipase inhibition compared to MPE (coefficient value = 0.100, $p < 0.05$), while the combined proportion of the two extracts significantly decreased inhibition (coefficient value = -0.001, $p < 0.05$).

The non-linear regression curve demonstrated a decline in enzyme inhibitory activity when TIE concentrations ranged from 75 to 150 μg/mL, forming a quadratic relationship. TIE as a single extract exhibited the highest inhibition towards lipase. However, when combined with MPE, the enzyme inhibitory activity was attenuated. This suggests a potential incompatibility between the two extracts in inhibiting the lipase enzyme. Further investigation is necessary to determine whether this negative interaction (incompatibility) is due to chemical or biological factors.

The TIE extract was believed to contain a blend of compounds exhibiting both anti-lipase and pro-lipase activities. In a study by Hofmann and Borgström (1963), which examined the effect of solvents on pancreatic lipase enzyme activity, it was noted that the enzyme's hydrolysis activity increased significantly with the addition of bipolar substances like bile salts to the solvent. A similar effect was observed with the addition of small amounts of fatty acids or a two-phase heptane buffer system. These bipolar molecules form micelles that enhance the exposure of triglycerides to enzymes.

In the present study, metabolomic testing using LC-HRMS revealed that both TIE and MPE extracts contained elevated levels of primary fatty acid amides (e.g., hexadecanamide, stearamide, oleamide). These primary fatty amide molecules exhibit bipolar characteristics. Their presence in the extracts is believed to alter the solution environment by forming micelles, thereby enhancing hydrolysis by lipase. Thus, the primary fatty acid amide compounds act as pro-lipase agents, in addition to other fatty acids present in the extracts. It is speculated that the anti-lipase activity of both extracts may be overshadowed by the enhancing effects of these pro-lipase compounds.

Therefore, the development of polyherbal drugs targeting hypertriglyceridemia (HTG) using *M. paniculata* and *T. indica* leaf extracts still faces challenges, particularly regarding the presence of lipase-enhancing substances that need careful consideration. However, the implications of such interactions should be validated at the in vivo level.

4. CONCLUSION

The extract from *T. indica* leaves was found to be highly effective in inhibiting lipase enzyme activity. However, when mixed with *M. paniculata* extract, the effectiveness of lipase inhibition was actually reduced. This decrease in anti-lipase activity was suspected to be attributed to primary fatty amides. Further research was deemed necessary, particularly to explore methods for removing these lipase-enhancing substances and to verify their effects at the in vivo level.

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