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Chemical Profiling and Anti-Adipogenic Effects of *Geniotrigona thoracica* Propolis Extract in 3T3-L1 Cells

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Geniotrigona thoracica, a commonly cultivated stingless bee species in the Malaysian meliponid-culture industry, produces propolis known for various biological activities, yet its anti-adipogenic potential remains underexplored. This study evaluated the anti-adipogenic effects of ethanolic extract of *G. thoracica* propolis (EEGP) on 3T3-L1 cells. Cytotoxicity was assessed in 3T3-L1 preadipocytes across concentrations from 7.81 to 1000 µg/mL. Anti-adipogenic activity was examined using lipid accumulation assay, reactive oxygen species (ROS) measurement, and triglyceride quantification. Gene expression analysis using quantitative real-time PCR (qRT-PCR) targeted key adipogenic markers: sterol regulatory element binding protein-1 (SREBP-1), peroxisome proliferator-activated receptor-gamma (PPAR-γ), and CCAAT/enhancer-binding protein-alpha (C/EBP-α). Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify chemical constituents of the extract. EEGP demonstrated no cytotoxicity across all tested concentrations and significantly reduced lipid accumulation, ROS levels, and triglyceride content in a dose-dependent manner. Additionally, EEGP suppressed the expression of SREBP-1, PPAR-γ, and C/EBP-α during preadipocyte differentiation. GC-MS analysis revealed a diverse range of chemical compounds, including phenolics, terpenoids, aromatic compounds and sugar alcohol. These findings indicate that EEGP possesses anti-adipogenic properties without cytotoxicity, suggesting its potential as natural agent for obesity management. Nevertheless, further *in vivo* studies are warranted to confirm these effects and elucidate the underlying mechanisms.

Keywords: Adipogenesis; Anti-adipogenic; *Geniotrigona thoracica*; Preadipocyte differentiation; Stingless bee propolis

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Propolis, a complex resinous substance obtained from beehives, has been employed in traditional medicine for centuries due to its extensive array of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties [1]. Its significance transcends cultural boundaries, with its composition and therapeutic potential being influenced by geographical factors and the specific plant sources utilized by the bees [2, 3]. Among the various bee species that produce propolis, stingless bees (Meliponini) have gained particular attention for the distinctive properties of their propolis, especially in regions such as Southeast Asia and South America [4, 5, 6].

One notable species, *Geniotrigona thoracica*, is extensively domesticated within the Malaysian meliponiculture industry [7]. This industry plays a crucial role in promoting sustainable agricultural practices and supporting rural economies, while simultaneously contributing to the conservation of native bee species [8,9,10]. The propolis produced by *G. thoracica* is rich in bioactive compounds, including flavonoids, phenolic acids, and terpenes, which underpin its diverse pharmacological effects [11, 12, 13, 14]. However, despite the growing interest in propolis from stingless bees, research focusing specifically on the anti-adipogenic potential of *G. thoracica* propolis remains notably scarce. Previous studies have primarily concentrated on its antimicrobial, cytotoxicity, and antioxidant properties, leaving its potential role in obesity management largely unexplored.

Obesity represents a significant global health challenge, with its prevalence reaching epidemic levels [15]. It is primarily driven by adipogenesis, the process by which preadipocytes differentiate into mature adipocytes, leading to excessive fat accumulation [16]. The regulation of adipogenesis is governed by a complex network of transcription factors, notably peroxisome proliferator-activated receptor-gamma (PPAR-γ), sterol regulatory element-binding protein-1 (SREBP-1), and CCAAT/enhancer-binding protein-alpha (C/EBP-α) [17]. The accumulation of lipids and

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triglycerides during adipogenesis plays a critical role in the progression of obesity, with reactive oxygen species (ROS) serving as a significant contributing factor [18]. Elevated ROS levels induce oxidative stress, which not only disrupts normal cellular function but also enhances adipocyte dysfunction by interfering with insulin signaling and promoting inflammation [19]. Therefore, targeting the expression of these transcription factors and modulating lipid accumulation and ROS production presents a promising strategy for the development of anti-obesity therapies [17, 20].

Given the well-documented health benefits of propolis from various bee species, the lack of research on the anti-adipogenic effects of *G. thoracica* propolis highlights a significant gap in the literature. This study aims to investigate the anti-adipogenic effects of ethanolic extract of *G. thoracica* propolis (EEGP) on 3T3-L1 cells, a widely used *in vitro* model for studying adipogenesis. By assessing the extract's impact on lipid accumulation, ROS production, triglyceride levels, and the expression of key adipogenic genes, this research seeks to elucidate the potential of *G. thoracica* propolis as a natural agent for obesity management. Additionally, the study contributes to the understanding of the chemical composition of *G. thoracica* propolis, further enhancing its value within the Malaysian meliponiculture industry.

EXPERIMENTAL

Propolis Collection and Extraction

Raw propolis was collected in July 2022 from the previously documented location described by Idris et al. (2023) [13]. The preparation of EEGP followed the extraction protocol established by Zohdi et al. (2024) [21]. In brief, the propolis was macerated in ethanol and agitated at 250 rpm for 48 hours, maintaining a temperature range of 25-28°C. The mixture was then filtered, and the supernatant was concentrated using rotary evaporation at 40°C. The extract was kept overnight in a freezer at -18°C to eliminate wax, followed by centrifugation at 2500 rpm for 5 minutes. The final supernatant was collected, lyophilized and kept at -20°C.

Cell Culture and Differentiation

Mouse 3T3-L1 preadipocytes were sourced from the American Type Culture Collection (ATCC, CL-173, Manassas, VA, USA). The cells were cultured and differentiated according to the protocol established by Lee et al. (2011) [22] with slight modifications. The preadipocytes were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S) at 37°C in a 5% CO_2 atmosphere. Upon reaching 90% confluence, differentiation was initiated on day 0 using a hormonal induction cocktail comprising 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1.0 µM dexamethasone (DEX), and 10 µg/mL insulin. On day 2, the medium was replaced with regular DMEM containing 10 µg/mL insulin, and cells were treated with EEGP at varying concentrations. The medium was refreshed every two days, and assays were performed on day 8 after the start of differentiation [23].

Cell Viability

Cell viability and cytotoxicity were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. In this assay, 3T3-L1 preadipocytes were seeded at a density of 1×10^4 cells per well in 96-well plates. Cells were then exposed to EEGP at concentrations ranging from 7.81 to 1000 µg/mL. After incubation, the cells were treated with MTT solution and incubated in the dark for 4 hours at 37°C. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a multi-well plate reader. N-Acetylcysteine (NAC) served as a positive control. The percentage of viable cells was calculated according to the following equation.

Lipid Accumulation Assay

Lipid accumulation in differentiated adipocytes was quantified using Oil Red O (ORO) staining, based on the method by Choi et al. (2018) [18]. Differentiated adipocytes treated with varying concentrations of EEGP (1000, 500, 100, 50, 10 µg/mL) were fixed in 10% formaldehyde for 1 hour after three phosphate-buffered saline (PBS) washes. The fixed cells were then stained with 0.5% ORO solution for 30 minutes at room temperature. Excess stain was removed by washing with water, and the stained lipid droplets were observed microscopically. Quantification was performed by eluting the stain with isopropanol, and the absorbance was measured at 490 nm. NAC was utilized as a positive control. Lipid accumulation was expressed as a percentage using the following formula.

$$Cell\ viability\ (\%) = \frac{(Mean\ absorbance\ of\ each\ treatment\ group)}{(Mean\ absorbance\ of\ control\ group)}\ X\ 100$$

$$Lipid\ accumulation\ (\%) = \frac{(Mean\ absorbance\ of\ each\ treatment\ group)}{(Mean\ absorbance\ of\ control\ group)}\ X\ 100$$

Nitroblue Tetrazolium (NBT) Assay

The effect of EEGP on ROS production was evaluated using the NBT assay, as outlined by Choi et al. (2018) [18]. 3T3-L1 preadipocytes were cultured to confluence and differentiated into adipocytes in the presence of EEGP at various concentrations (1000, 500, 100, 50, 10 µg/mL). On day 8 of differentiation, cells were incubated in PBS containing 0.2% NBT for 90 minutes. The formazan produced by ROS was dissolved in 50% acetic acid, and absorbance was measured at 570 nm. NAC was used as a positive control. The NBT staining solution was prepared by dissolving 20 mg of NBT powder in 10 mL of PBS. The results of the NBT assay were expressed as a percentage using the following formula:

$$ROS\ (\%) = \frac{(Mean\ absorbance\ of\ each\ treatment\ group)}{(Mean\ absorbance\ of\ control\ group)}\ X\ 100$$

Triglycerides (TG) Quantification Assay

The intracellular TG content was quantified using a commercial TG colorimetric assay kit (BioVision Inc., Milpitas, CA, USA). In brief, 3T3-L1 adipocytes treated with various concentrations of EEGP (1000, 500, 100, 50, 10 µg/mL) were washed twice with cold PBS and then lysed in a lysis buffer containing 1% Triton X-100 in PBS. The cell lysates were homogenized in isopropanol, followed by mixing with the enzyme working solution provided in the kit. The samples were incubated for 10 minutes at 37°C, and absorbance was measured at 510 nm using a microplate reader.

Quantitative Real-time PCR (qRT-PCR) Analysis

Total RNA was extracted from 3T3-L1 adipocytes treated with EEGP at different concentrations (1000, 500, 100, 50, 10 µg/mL) using a commercial RNA isolation kit (Promega, Madison, WI, USA). The extracted RNA was then reverse transcribed into cDNA using a reverse transcriptase enzyme according to the manufacturer's instructions. RT-PCR was performed by amplifying the cDNA with primers listed in Table 1. The amplification protocol involved an initial denaturation at 95°C for 10 seconds, followed by 40 cycles of 95°C for 10 seconds, 60°C for 5 seconds, and 72°C for 30 seconds. The specificity of the amplified products was confirmed by generating melting curve profiles, which involved cooling the samples to 65°C for 15 seconds and then gradually heating to 95°C while recording fluorescence. β-Actin was used as an internal control, and each experiment was conducted in triplicate.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The EEGP was subjected to silylation procedure following the method described by Bankova et al. (2019) [24]. In brief, approximately 1 mg of EEGP was mixed with 50 µL of pyridine and 75 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). The mixture was then heated at 80°C for 20 minutes. After heating, the sample was filtered through a PTFE 0.25 µm syringe filter and transferred to a vial for GC-MS analysis.

The GC-MS analysis was conducted using an Agilent G-59770 system (Agilent Technologies Inc., California, USA) equipped with an HP-5MS silica capillary column (30 m x 250 µm x 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed to start at 80°C, held for 1 minute, then gradually increased to 160°C at a rate of 5°C/minutes (held for 2 minutes), and finally elevated to 280°C at a rate of 10°C/min (held for 5 minutes). A 1 µL sample was injected in split mode, with the injector temperature set at 250°C. Compound identification was based on spectral data comparison with the National Institute of Standards and Technology (NIST14) library, using peak area and retention time as criteria.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons among experimental groups were performed using one-way Analysis of Variance (ANOVA), followed by Duncan's post hoc test. Differences were considered statistically significant at $p< 0.05$.

Table 1. Primer sets for real-time quantitative polymerase chain reaction.

Gene	Forward (5'-3')	Reverse (5'-3')
PPARγ	TTTTCAAGGGTGCCAGTTTC	AATCCTTGGCCCTCTGAGAT
C/EBPα	TTACAACAGGCCAGGTTTCC	GGCTGGCGACATACAGTACA
SREBP-1	TGTTGGCATCCTGCTATCTG	AGGGAAAGCTTTGGGGTCTA
β-actin	CTGTCCCTGTATGCCTCTG	ATGTCACGCACGATTTCC

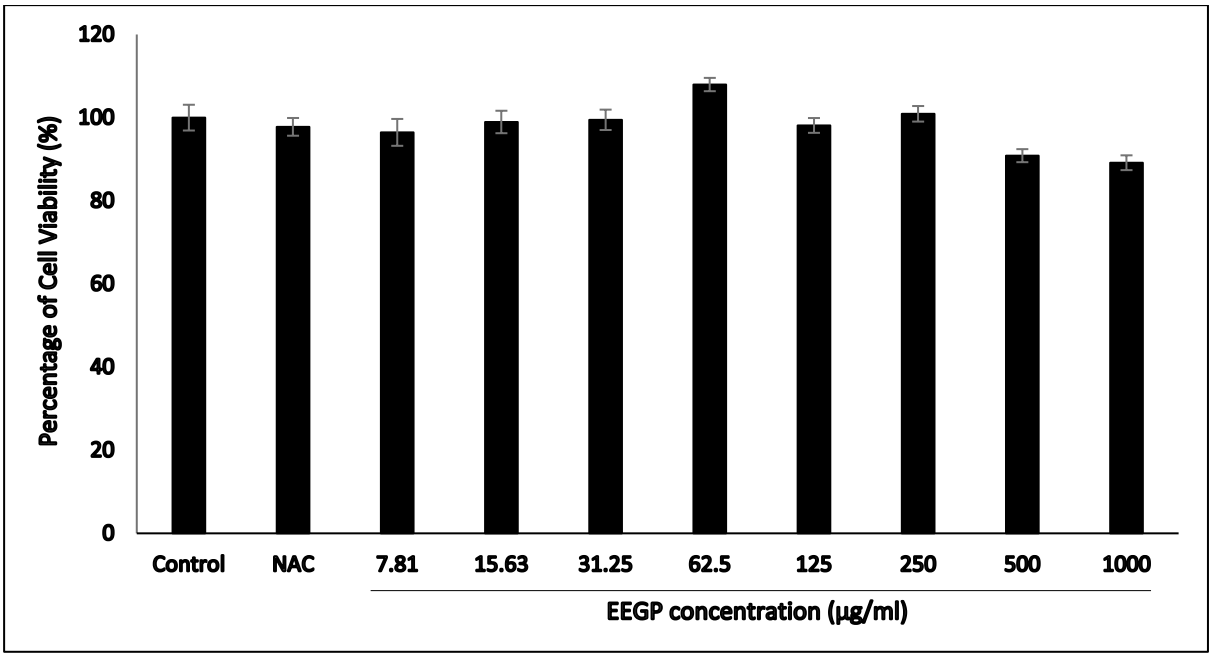

Figure 1. Effect of EEGP on the viability of 3T3-L1 adipocytes determined by MTT assay. Cells were treated with different concentration of EEGP (7.81 to 1000 µg/mL). Control group represents cells treated with 0.5% DMSO and 5 mM NAC as positive control. Results are represented as mean ± SD of three independent experiments.

RESULTS AND DISCUSSION

Effect of EEGP on Cell Viability in 3T3-L1 Cells

The MTT assay results demonstrated that EEGP, at concentrations ranging from 7.81 to 1000 µg/mL, did not exhibit cytotoxic effects on 3T3-L1 preadipocytes, as cell viability remained consistently above 80% across all tested concentrations (Figure 1). This finding suggests that EEGP is non-toxic within the tested concentration range, allowing subsequent experiments to be conducted without compromising cell integrity. Given the practical considerations of concentration range and cell population management, five non-cytotoxic doses were selected for further experimentation. Our findings align with previous research by Ikeda et al. (2011) [25], which reported no cytotoxicity in 3T3-L1 cells when Brazilian propolis extracts were tested at concentrations up to 50 µg/mL. In contrast, Iio et al. (2010) [26] observed a reduction in cell viability at 100 µg/mL when using Brazilian red propolis extract on 3T3-L1 cells. These differences may be attributed to variations in the chemical composition of propolis, which can differ based on geographic origin, botanical sources, and extraction methods. Additionally, differences in experimental conditions, such as the duration of exposure and the specific assay protocols used, could also contribute to the observed variability in cytotoxicity results.

Effect of EEGP on Lipid Accumulation in 3T3-L1 Cells

The impact of EEGP on intracellular lipid accumulation was evaluated using ORO staining. Microscopic examination revealed a gradual reduction in the number of lipid droplets with increasing concentration of EEGP (Figure 2A). Treatment with EEGP at concentrations of 10, 50, 100, 500, and 1000 µg/mL resulted in significant reductions ($p<0.05$) in lipid levels by 56.36, 41.47, 40.22, 37.34, and 36.84%, respectively, compared to the control group (Figure 2B). Notably, at concentration ranging from 50 to 1000 µg/mL, EEGP exhibited lipid reduction comparable to that observed in cells treated with NAC, the positive control (32.36%) ($p>0.05$). The reduction in lipid accumulation observed in this study suggests that EEGP effectively inhibits adipogenesis in 3T3-L1 cells. The dose-dependent nature of this inhibition, particularly at higher concentrations, indicates a potential disruption in the pathways involved in lipid synthesis and storage.

Previous studies have demonstrated that propolis extracts, rich in bioactive compounds such as phenolic acids and flavonoids, can modulate adipogenesis by interfering with key signaling pathways involved in fat cell differentiation [27]. According to Daleprane and Abdalla, (2013) [28], these compounds interfere with the differentiation of preadipocytes into mature adipocytes, thereby reducing lipid storage. For instance, quercetin, a prominent phenolic compound in propolis, has been shown to inhibit lipid accumulation throughout the adipocyte differentiation process [28]. The observed dose-dependent reduction in lipid accumulation suggests that EEGP has a significant role in regulating lipid metabolism, potentially offering therapeutic benefits in managing obesity.

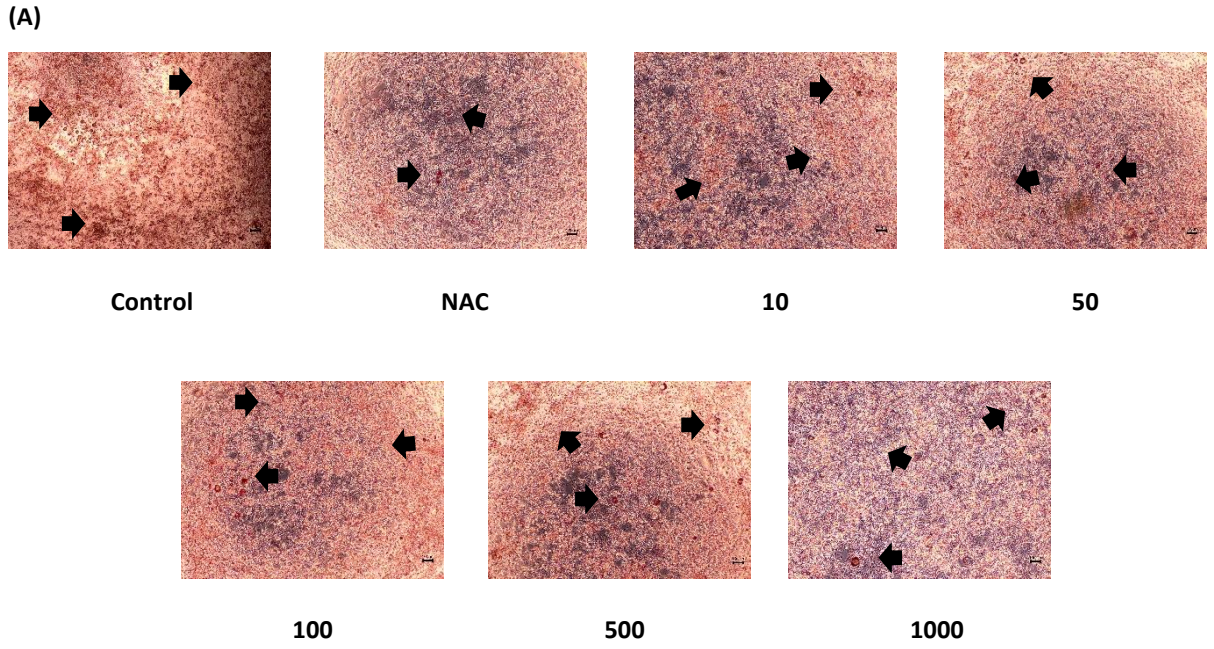

Figure 2. Effects of EEGP on lipid accumulation in 3T3-L1 adipocytes. (A) Microscopic images of adipocytes treated with EEGP at concentrations of 10, 50, 100, 500 and 1000 µg/mL, followed by Oil Red O staining (Magnification: 4×). ➧ Indicate red-stained droplets. (B) Quantification of lipid accumulation after Oil Red O elution. The control group represents cells treated with 0.5% DMSO, with5 mM NAC as the positive control. Results are represented as mean ± SD from three independent experiments. Bars with different letters indicate statistically significant differences (p<0.05).

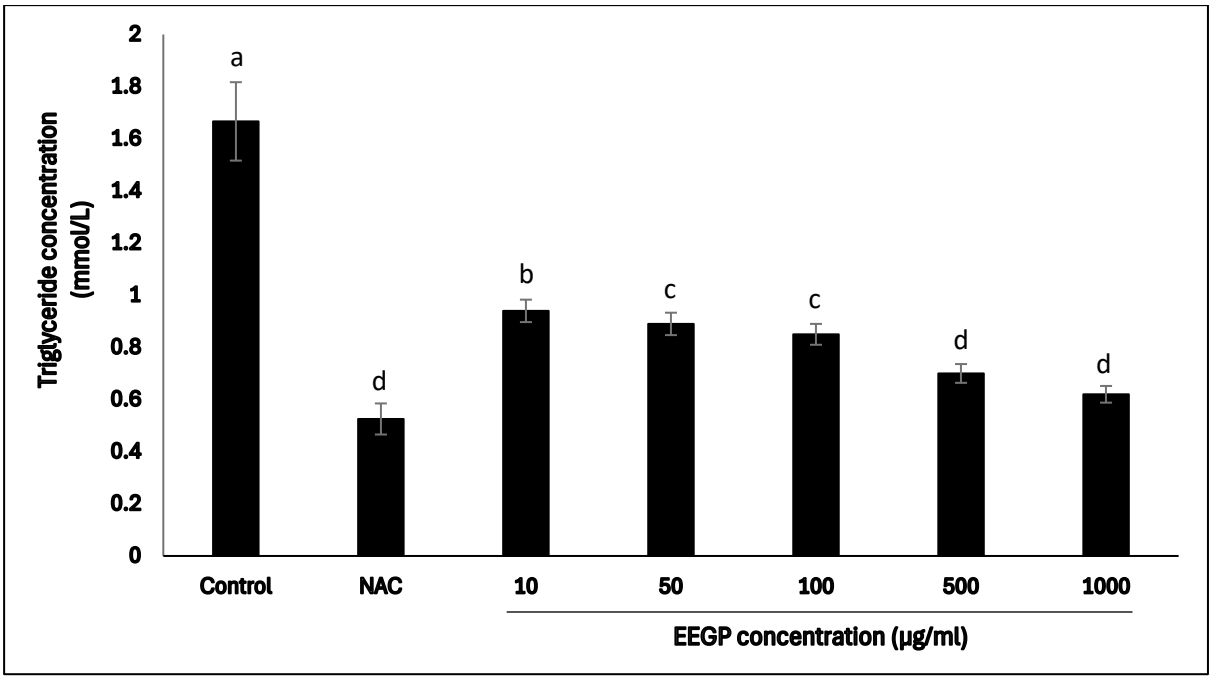

Figure 3. Effect of EEGP on triglyceride content in 3T3-L1 adipocytes. Control group represents cells treated with 0.5% DMSO and 5 mM NAC as positive control. Results are represented as mean ± SD of three independent experiments. Bars with different letters are significantly different (p<0.05).

Effect of EEGP on Triglyceride Content in 3T3-L1 Cells

The effect of EEGP on intracellular triglyceride content was evaluated in 3T3-L1 preadipocytes. Cells treated with EEGP (10, 50, 100, and 1000 µg/mL) and NAC showed significantly lower (p<0.05) TG accumulation (0.94, 0.89, 0.85, 0.70, 0.62, and 0.55 mmol/L, respectively) compared to the control (2.00 mmol/L) (Figure 3). Notably, no significant difference (p>0.05) was observed between EEGP treatment at 500 and 1000 µg/mL and NAC. The results of this study demonstrate that EEGP significantly reduces intracellular TG accumulation in 3T3-L1 preadipocytes across all tested concentrations, with reductions observed in a dose-dependent manner. The lowest TG levels were noted in cells treated with the highest concentrations of EEGP (500 and 1000 µg/mL), which exhibited similar effects to the positive control, NAC. This suggests that EEGP is effective in limiting TG accumulation, which is a key factor in the development of adipocytes and overall adiposity.

The observed decrease in TG levels with EEGP treatment is consistent with previous studies that have highlighted the potential of propolis extracts in modulating lipid metabolism. Propolis extract has been shown to inhibit TG synthesis by down-regulating the activity of key enzymes involved in lipid biosynthesis, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [29,30]. Triglycerides, the primary form of stored fat in adipocytes, serve as a crucial marker for assessing adipocyte function and overall lipid metabolism [31]. Elevated triglyceride levels are often associated with metabolic disorders, including obesity [32]. In this study, EEGP treatment led to a significant decrease in intracellular triglyceride levels, reinforcing its potential role in modulating lipid metabolism and reducing obesity-related risks.

Effect of EEGP on ROS Production in 3T3-L1 Cells

The NBT assay was performed to evaluate the effect of EEGP on ROS generation during adipogenic differentiation. Treatment with EEGP at concentrations of 10, 50, 100, 500, and 1000 µg/mL resulted in significant inhibition of ROS generation by 51.56, 48.37, 43.05, 42.06, and 41.06%, respectively, compared to the control group (Figure 4). Notably, at concentrations ranging from 100 to 1000 µg/mL, EEGP reduced ROS formation to levels comparable to those observed with NAC treatment (33.57%), with no significant difference (p>0.05). These findings indicate that EEGP significantly inhibits ROS generation during adipogenic differentiation of 3T3-L1 cells in a dose-dependent manner.

Elevated ROS levels have been shown to promote adipocyte differentiation and lipid accumulation by activating redox-sensitive transcription factors and signaling pathways [33]. The ability of EEGP to suppress ROS formation suggests that it may interfere with these oxidative stress-mediated pathways, thereby inhibiting adipocyte differentiation and reducing lipid accumulation. These findings align with previous research that has demonstrated the antioxidant capacity of propolis and its active constituents, such as flavonoids and phenolic acids, in reducing ROS levels and protecting against oxidative damage [34].

Furthermore, Zullkiflee et al. (2022) [35] reported that propolis exhibits potent antioxidant activity, neutralizing ROS and exerting anti-inflammatory effects against oxidative stress. By maintaining cellular redox balance and preventing oxidative damage, EEGP may offer protective effects in the context of obesity and related metabolic disorders.

Effect of EEGP on the Expression of Adipogenic Transcription Factors in 3T3-L1 Cells

The expression levels of key adipogenic transcription factors, including PPAR-γ, C/EBP-α, and SREBP-1, were assessed using qRT-PCR to evaluate the effect of EEGP on target genes involved in adipogenesis. The results demonstrated that EEGP treatment significantly suppressed the expression of these transcription factors in a dose-dependent manner compared to the control group (Figure 5). Notably, at higher concentrations (500 and 1000 µg/mL), the inhibitory effects of EEGP were comparable to those of NAC, with no significant difference observed (p>0.05). These findings suggest that EEGP effectively reduces the expression of adipogenic transcription factors that are essential for the differentiation of preadipocytes into mature adipocytes.

Adipogenesis is a tightly regulated process governed by transcription factors such as C/EBP-α, and PPAR-γ, which play a critical role in adipocyte differentiation and the regulation of genes involved in lipid metabolism [19]. The observed decrease in these transcription factors by EEGP aligns with prior studies that have highlighted the potential of propolis and its bioactive constituents, such as flavonoids and phenolic acids, to modulate adipogenic signaling pathways [36]. For instance, caffeic acid phenethyl ester (CAPE), a well-known component of propolis, has been reported to significantly inhibit the expression of PPAR-γ and C/EBP-α in adipocytes, leading to reduced triglyceride accumulation in 3T3-L1 cells [28, 37].

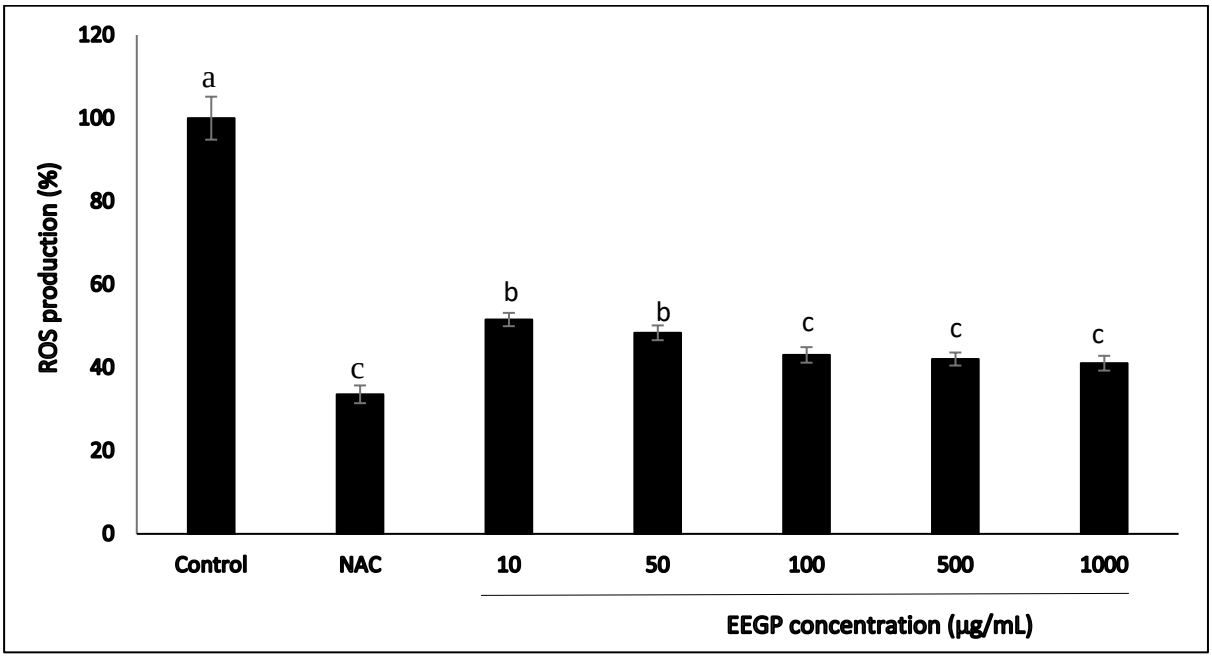

Figure 4. Effect of the EEGP on ROS production in 3T3-L1 adipocytes. Control group represents cells treated with 0.5% DMSO and 5 mM NAC as positive control. Results are represented as mean ± SD of three independent experiments. Bars with different letters are significantly different (p<0.05).

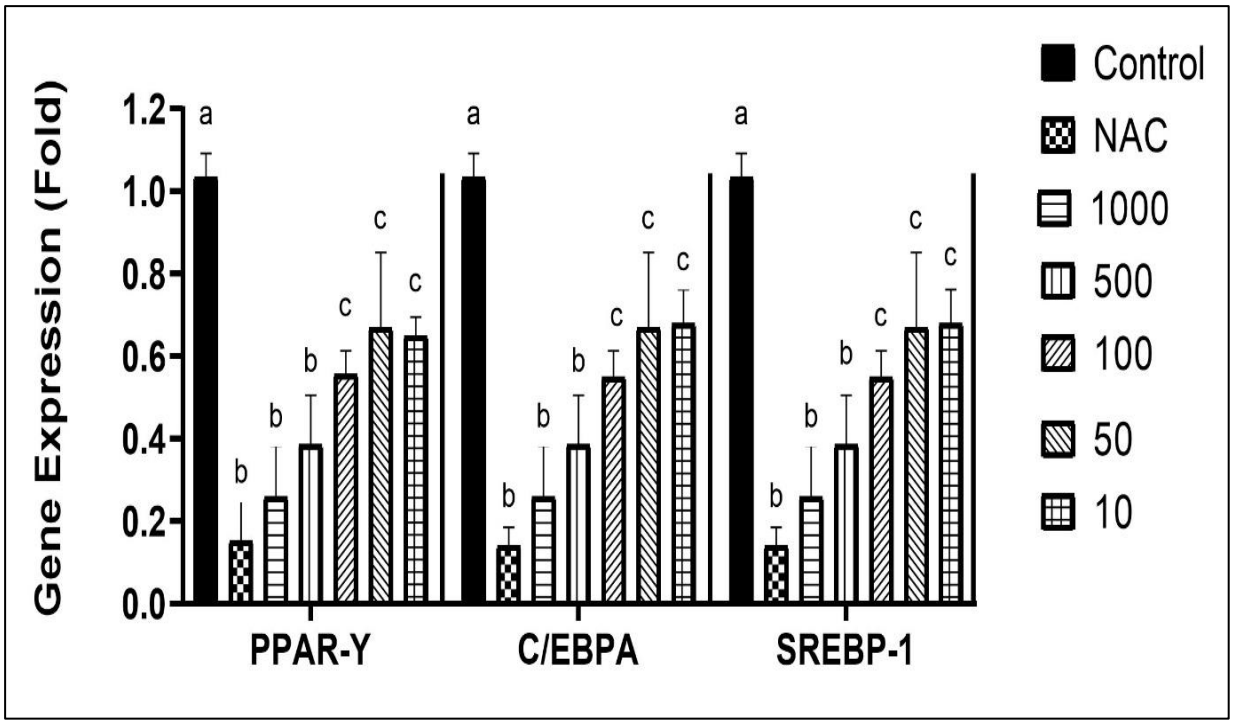

Figure 5. Effect of EEGP on the expression of adipogenic transcription factors in 3T3-L1 adipocytes. Control group represents cells treated with 0.5% DMSO and 5 mM NAC as positive control. Results are represented as mean ± SD of three independent experiments. Bars with different letters are significantly different (p<0.05).

Table 2. Phytochemical compounds identified in EEGP using GC-MS.

Retention Time (min)	Compound Name	Area (%)
	Phenolic Compounds	
10.7627	2-Methoxy-4-vinylphenol	0.31
8.2062	Catechol, TMS derivative	0.11
24.5433	2,4-Dihydroxybenzoic acid, 3TMS derivative	0.58
23.4618	2,6-Dihydroxyacetophenone, 2TMS derivative	1.46
28.2602	5-Methylsalicylic acid, 2TMS derivative	1.46
30.8877	Cardanol C17:1 (TMS)	0.87
	Terpenoids	
13.2863	Caryophyllene	0.18
14.0843	1,4,7, -Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	0.18
17.0865	(-)-Globulol	0.11
28.142	Taraxasterol	0.21
	Sugar Alcohols	
22.4733	Myo-Inositol, 6TMS derivative	0.94
24.727	Scyllo-Inositol, 6TMS derivative	1.36
	Aromatic Compounds	
24.3949	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	0.22
34.121	6H-Benzo[b]naphtho[2,3-h] carbazole	0.39
6.2831	5-Methyl-2-phenylindolizine	0.18

* TMS derivative: trimethylsilyl derivative

GC-MS Analysis

The GC-MS analysis of EEGP identified a total of 15 phytochemical compounds, with phenolic compounds being the predominant class, followed by terpenoids, sugar alcohols, and aromatic compounds (Table 2). Among these, 2,6-dihydroxyacetophenone and 5-methylsalicylic acid were the most abundant phenolics, which are known for their antioxidant and anti-inflammatory properties [38, 39, 40]. The

presence of taraxasterol, a significant terpenoid, further highlights the potential therapeutic properties of EEGP due to its reported anti-adipogenic and anti-inflammatory effects [41, 42]. The identification of myo-inositol, a sugar alcohol, is particularly noteworthy given its roles in lipid metabolism and insulin sensitivity [43, 44]. This diverse chemical profile suggests that the anti-adipogenic effects observed in the study may be attributed to the synergistic action of these bioactive compounds, warranting further investigation into their individual and combined mechanisms of action.

CONCLUSION

In conclusion, EEGP demonstrated anti-adipogenic effects without inducing cytotoxicity, suggesting its potential as a natural agent for obesity management. These findings provide a foundation for further exploration of *G. thoracica* propolis in the context of natural product research and its potential applications in addressing obesity. Additionally, this highlights the importance of stingless bee products within the Malaysian meliponiculture industry, encouraging their sustainable use and further investigation into their diverse applications. Future studies could focus on elucidating the specific bioactive compounds responsible for the observed effects, as well as their mechanisms of action at the molecular level. Additionally, *in vivo* studies are recommended to assess the efficacy and safety of *G. thoracica* propolis in animal models of obesity. This could be complemented by research into its effects on metabolic parameters such as glucose and lipid profiles.

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REFERENCES

1. Belmehdi, O., Menyiy, N. E., Bouyahya, A., Baaboua, A. E., Omari, N. E., Gallo, M., Montesano, D., Naviglio, D., Zengin, G., Senhaji, N. S., Goh, B. H. and Abrini, J. (2022) Recent advances in the chemical composition and biological activities of propolis. *Food Reviews International*, **39(9)**, 6078–6128.

2. Bobiş, O. (2022) Plants: Sources of diversity in propolis properties. *Plants*, **11(17)**, 2298.

3. Sarıkahya, N. B., Gören, A. C., Okkalı, G. S., Çöven, F. O., Orman, B., Kırcı, D., Yücel, B., Kışla, D., Demirci, B., Altun, M., Önem, A. N. and Nalbantsoy, A. (2021) Chemical composition and biological activities of propolis samples from different geographical regions of Turkey. *Phytochemistry Letters*, **44**, 129–136.

4. Zulhendri, F., Perera, C. O., Chandrasekaran, K., Ghosh, A., Tandean, S., Abdulah, R., Herman, H. and Lesmana, R. (2021) Propolis of stingless bees for the development of novel functional food and nutraceutical ingredients: A systematic scoping review of the experimental evidence. *Journal of Functional Foods*, **88**, 104902.

5. Popova, M., Trusheva, B. and Bankova, V. (2021) Propolis of stingless bees: A phytochemist's guide through the jungle of tropical biodiversity. *Phytomedicine*, **86**, 153098.

6. Salatino, A., Pereira, L. D. L. and Salatino, M. L. F. (2019) The emerging market of propolis of stingless bees in tropical countries. *MOJ Food Processing and Technology*, **7(2)**, 27–29.

7. Jaapar, M. F., Halim, M., Mispan, M. R., Jajuli, R., Saranum, M. M., Zainuddin, M. Y., Ghazi, R. and Ghani, I. A. (2016) The diversity and abundance of stingless bee (Hymenoptera: Meliponini) in Peninsular Malaysia. *Advances in Environmental Biology*, **10(9)**, 1–8.

8. Nurdiansyah, M. A., Indira, T. I., Burhan, K. H., Maulani, R. R., Supriyadi, A., Permana, A. D. and Abduh, M. Y. (2024) Effects of meliponiculture Tetragonula laeviceps on productivity, physical, and chemical characteristics of tomatoes (*Solanum lycopersicum*). *Journal of Research in Agriculture and Food Sciences*, **1(1)**, 14–14.

9. Rattanawannee, A. and Duangphakdee, O. (2020) Southeast Asian meliponiculture for sustainable livelihood. *In Modern Beekeeping: Bases for Sustainable Production*, 173–190.

10. Jalil, A. H. (2014) *Beescape for meliponines: Conservation of Indo-Malayan stingless bees*, *Partridge Publishing: Singapore.*

11. Badiazaman, A. A. M., Aziz, A. N., Mohamad, H. and Suryati Mohd, K. (2024) Bioassay-guided isolation of cycloartane-type triterpenes, their cytotoxic properties, and identification of potential chemical markers from *Geniotrigona thoracica* propolis. *Natural Product Research*, 1–6.

12. Tandean, S., Japardi, I., Rusda, M., Indharty, R. S., Lelo, A., Aman, R. A., Amin, M. M., Siahaan, A. M. P., Eyanoer, P. C., D'Prinzessin, C. A., Lesmana, R., Popova, M., Trusheva, B., Bankova, V. and Zulhendri, F. (2024) Chemical composition and neuroprotective properties of Indonesian stingless bee (*Geniotrigona thoracica*) propolis extract in an in-vivo model of intracerebral hemorrhage (ICH). *Nutrients*, **16(12)**, 1880.

13. Idris, L., Adli, A., Yaacop, N. and Zohdi, R. M. (2023) Phytochemical screening and antioxidant activities of *Geniotrigona thoracica* propolis extracts derived from different locations in Malaysia. *Malaysian Journal of Fundamental and Applied Sciences*, **19**, 1023–1032.

14. Maroof, K., Chen, K. F., Lee, R. F., Goh, B. H., Mahendra, C. K., Siow, L. F. and Gan, S. H. (2023) A preliminary study on phenolics, antioxidant, and antibacterial activities of *Acacia mangium* and *Garcinia mangostana* propolis collected by *Geniotrigona thoracica*. *Food Chemistry Advances*, **2**, 100255.

15. Benton, D. (2024) The obesity epidemic, in *Tackling the obesity crisis: Beyond failed approaches to lasting solutions*, *Springer International Publishing: Cham, Switzerland*.

16. Jakab, J., Miškić, B., Mikšić, Š., Juranić, B., Ćosić, V., Schwarz, D. and Včev, A. (2021) Adipogenesis as a potential anti-obesity target: A review of pharmacological treatment and natural products. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, **14**, 67–83.

17. Moseti, D., Regassa, A. and Kim, W. K. (2016) Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. *International Journal of Molecular Sciences*, **17(1)**, 124.

18. Choi, S. -I., Lee, J. S., Lee, S., Lee, J. -H., Yang, H. -S., Yeo, J., Kim, J. -Y., Lee, B. -Y., Kang, I. -J. and Lee, O. -H. (2018) Radical scavenging-linked anti-adipogenic activity of *Alnus firma* extracts. *International Journal of Molecular Medicine*, **41(1)**, 119–128.

19. Čolak, E. and Pap, D. (2021) The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. *Journal of Medical Biochemistry*, **40(1)**, 1–11.

20. Guru, A., Issac, P. K., Velayutham, M., Saraswathi, N. T., Arshad, A. and Arockiaraj, J. (2021) Molecular mechanism of down-regulating adipogenic transcription factors in 3T3-L1 adipocyte cells by bioactive anti-adipogenic compounds. *Molecular Biology Reports*, **48(1)**, 743–761.

21. Zohdi, R. M., Yaacob, N. N., Hasif, N. A. M., Adli, M. A., Payaban, M., James, R. J. and Jaapar, F. (2024) Comparative study of different Malaysian stingless bee propolis: Physicochemical characterization, phytochemical contents, and anti-bacterial activity. *Research Journal of Pharmacy and Technology*, **17(3)**, 1021–1028.

22. Lee, O. H., Kwon, Y. I., Apostolidis, E., Shetty, K. and Kim, Y. C. (2011) Rhodiola-induced inhibition of adipogenesis involves antioxidant enzyme response associated with pentose phosphate pathway. *Phytotherapy Research*, **25(1)**, 106–115.

23. Tacherfiout, M., Petrov, P. D., Mattonai, M., Ribechini, E., Ribot, J., Bonet, M. L. and Khettal, B. (2018) Antihyperlipidemic effect of a *Rhamnus alaternus* leaf extract in Triton-induced hyperlipidemic rats and human HepG2 cells. *Biomedicine & Pharmacotherapy*, **101**, 501–509.

24. Bankova, V., Bertelli, D., Borba, R., Conti, B. J., da Silva Cunha, I. B., Danert, C., Eberlin, M. N., I Falcão, S., Isla, M. I., Moreno, M. I. N., Papotti, G., Popova, M., Santiago, K. B., Salas, A., Sawaya, A. C. H. F., Schwab, N. V., Sforcin, J. M., Simone-Finstrom, M., Spivak, M. and Zampini, C. (2019) Standard methods for *Apis mellifera* propolis research. *Journal of Apicultural Research*, **58(2)**, 1–49.

25. Ikeda, R., Yanagisawa, M., Takahashi, N., Kawada, T., Kumazawa, S., Yamaotsu, N., Nakagome, I., Hirono, S. and Tsuda, T. (2011) Brazilian propolis-derived components inhibit TNF-α-mediated down-regulation of adiponectin expression via different mechanisms in 3T3-L1 adipocytes. *Biochimica et Biophysica Acta (BBA) - General Subjects*, **1810(7)**, 695–703.

26. Iio, A., Ohguchi, K., Inoue, H., Maruyama, H., Araki, Y., Nozawa, Y. and Ito, M. (2010) Ethanolic extracts of Brazilian red propolis promote adipocyte differentiation through PPARγ activation. *Phytomedicine*, **17(12)**, 974–979.

27. Zhu, L., Zhang, J., Yang, H., Li, G., Li, H., Deng, Z. and Zhang, B. (2023) Propolis polyphenols: A review on the composition and anti-obesity mechanism of different types of propolis polyphenols. *Frontiers in Nutrition*, **10**, 1066789.

28. Daleprane, J. B. and Abdalla, D. S. (2013) Emerging roles of propolis: Antioxidant, cardioprotective, and antiangiogenic actions. *Evidence-Based Complementary and Alternative Medicine*, 1–13.

29. Koya-Miyata, S., Arai, N., Mizote, A., Taniguchi, Y., Ushio, S., Iwaki, K. and Fukuda, S. (2009). Propolis prevents diet-induced hyperlipidemia and mitigates weight gain in diet-induced obesity in mice. *Biological and Pharmaceutical Bulletin*, **32(12)**, 2022–2028.

30. Xie, Q. Y., Shu, X., Liu, T., Chen, L. L. and Luo, L. P. (2024) Ethanol extract of propolis attenuates liver lipid metabolism disorder in high-fat diet-fed SAMP8 mice. *Molecular Nutrition & Food Research*, 2400297.

31. Bays, H. E., Kirkpatrick, C., Maki, K. C., Toth, P. P., Morgan, R. T., Tondt, J., Christensen, S.

M., Dixon, D. and Jacobson, T. A. (2024) Obesity, dyslipidemia, and cardiovascular disease: A joint expert review from the Obesity Medicine Association and the National Lipid Association. *Obesity Pillars*, **10,** 100108.

32. Klop, B., Elte, J. W. F. and Cabezas, M. C. (2013) Dyslipidemia in obesity: Mechanisms and potential targets. *Nutrients*, **5(4)**, 1218–1240.

33. Čolak, E. and Pap, D. (2021) The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. *Journal of Medical Biochemistry*, **40(1)**, 1–9.

34. Attia, A. A., ElMazoudy, R. H. and El-Shenawy, N. S. (2012) Antioxidant role of propolis extract against oxidative damage of testicular tissue induced by insecticide chlorpyrifos in rats. *Pesticide Biochemistry and Physiology*, **103(2)**, 87–93.

35. Zullkiflee, N., Taha, H. and Usman, A. (2022) Propolis: Its role and efficacy in human health and diseases. *Molecules*, **27(18)**.

36. Kim, H. -Y., Jang, H. -J., Muthamil, S., Shin, U. C., Lyu, J. -H., Kim, S. -W., Go, Y., Park, S. -H., Lee, H. G., and Park, J. H. (2024) Novel insights into regulators and functional modulators of adipogenesis. *Biomedicine & Pharmacotherapy*, **177**, 117073.

37. Chen, Y. -W., Chien, Y. -H. and Yu, Y. -H. (2020) Taiwanese green propolis ethanol extract promotes adipocyte differentiation and alleviates TNF-α-mediated downregulation of adiponectin expression. *Journal of Functional Foods*, **73**, 104135.

38. Bentes, A. L., Borges, R. S., Monteiro, W. R., de Macedo, L. G. and Alves, C. N. (2011) Structure of dihydrochalcones and related derivatives and their scavenging and antioxidant activity against oxygen and nitrogen radical species. *Molecules*, **16(2)**, 1749–1760.

39. Dao, T. T., Chi, Y. S., Kim, J., Kim, H. P., Kim, S. and Park, H. (2004) Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives. *Bioorganic & Medicinal Chemistry Letters*, **14(5)**, 1165–1167.

40. Borges, R. S. and Castle, S. L. (2015) The anti-oxidant properties of salicylate derivatives: A possible new mechanism of anti-inflammatory activity. *Bioorganic & Medicinal Chemistry Letters*, **25(21)**, 4808–4811.

41. Sener, S. O., Ozgen, U., Kanbolat, S., Korkmaz, N., Badem, M., Hanci, H., Dirmenci, T., Arabaci, T., Aliyazicioglu, R., Yenilmez, E. and Iscan, G. S. (2021) Investigation of therapeutic potential of three endemic Cirsium species for global health problem obesity. *South African Journal of Botany*, **141**, 243–254.

42. Ammendola, S. and d'Abusco, A. S. (2022) Nutraceuticals and the network of obesity modulators. *Nutrients*, **14(23)**, 5099.

43. Bu, X., Wang, X., Lin, Z., Wang, C., Li, L., Liu, S., Shi, Q., Qin, J. G. and Chen, L. (2022) Myo-inositol improves growth performance and regulates lipid metabolism of juvenile Chinese mitten crab (*Eriocheir sinensis*) fed different percentage of lipid. *British Journal of Nutrition*, **127(5)**, 666–678.

44. Croze, M. L., Vella, R. E., Pillon, N. J., Soula, H. A., Hadji, L., Guichardant, M. and Soulage, C. O. (2013) Chronic treatment with myo-inositol reduces white adipose tissue accretion and improves insulin sensitivity in female mice. *Journal of Nutritional Biochemistry*, **24(2)**, 457–766.