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In-vitro, in-vivo, and in-silico studies of insulinotropic activity and cytotoxicity of mahogany seed extracts and its nanophytosome

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Abstract

This present study set out with the objective of evaluating the effect of ethanol–water solvent composition on extraction yield, insulinotropic activity (IA), cytotoxicity, and phytochemical profile (PP) of mahogany (*Swietenia mahagoni*) seed (MS) extracts. It also sought to study in silico and encapsulate the best extract, and to assay the antidiabetic of the extract and its nanophytosome. The extraction of MSs was achieved through the utilization of ethanol, ethanol–water (1:1), and water through ultrasound–assisted extraction. The extracts of IA and cytotoxicity were assayed in vitro using BRIN–BD11 cell lines and analyzed using LC-MS. The most effective extract was identified for its active compounds using molecular docking, encapsulation, characterization, and testing for its antidiabetic properties in vivo. The highest yield was observed in the water extract (MW), followed by the ethanol–water extract (MEW) and the ethanol extract (ME). MEW and MW demonstrated low level of cytotoxicity, with MEW exhibiting the highest level of IA. An in silico study identified four key antidiabetic compounds through the IA mechanism. The study demonstrated that MEW and its nanophytosome (250 mg/kg mouse weight) effectively reduced blood glucose level, and enhanced both erythrocyte function, and insulin secretion. This finding provides a new perspective in the development of natural-based antidiabetic agents through the nanophytosome formulation of mahogany seed extract.

Keywords: BRIN–BD11 cell lines; diabetic mouse models; liquid chromatography with tandem mass spectrometry (LCMS); molecular docking

1. Introduction

Diabetes mellitus (DM) is a degenerative disease that poses a global health challenge. At present, its global prevalence is approximately 537 million, with projections indicating an increase to 578 million by 2030 and 700 million by 2045 [1]. Indonesia has been identified as the fifth most affected country globally, with an estimated 19.5 million cases in 2021, a figure that is projected to rise to 28.6 million by 2045 [2]. DM is classified into two types, designated as Type I and Type II. Type I DM arises from elevated blood sugar levels, consequent to damage to pancreatic β–cells, resulting in a complete absence of insulin production.

Meanwhile, Type II DM arises from elevated blood sugar levels, consequent to reduced insulin secretion by the pancreas [3]. In Indonesia, Type I DM affected approximately 41,817 individuals in 2022, while Type II DM dominated with 19.5 million cases in 2021, primarily due to unhealthy lifestyles and insulin resistance [4]. The treatment of Type I DM involves the administration of insulin injection. The long-term use of this insulin therapy has been associated with the development of irritation and lipodystrophy. Conversely, therapeutic interventions for Type II DM primarily entail the administration of oral medications comprising synthetic active ingredients. These medications have been observed to induce adverse effects including hypoglycemia, digestive disorders, and an elevated risk of lactic acidosis [5]. It is evident that natural remedies for Type I DM have the capacity to complement conventional therapy through immunomodulatory effects that result in a reduction of autoimmune damage to pancreatic β-cells. These natural remedies are considered adequate with a lower potential for side effects [6]. Consequently, the development of herbal antidiabetic drugs from plants that are abundant in Indonesia, such as *Swietenia mahagoni* (L.) Jacq seeds (locally known as Mahoni) holds significant potential for further exploration.

In Indonesia, mahogany is primarily cultivated for the production of high–quality wood. Java island is home to approximately 39.99 million mahogany trees (88.36%), with a total estimated population of 45.26 million across Indonesia [7]. Furthermore, the mahogany tree produces seeds with an output of approximately 2.5–4 kg per tree per harvest, which occurs

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every six months [8]. The mahogany seed has the potential to be developed as a source of herbal antidiabetic medicine. As demonstrated by in–vitro and in–vivo studies, both ethanol and methanol extracts of mahogany seeds, obtained through the maceration extraction method, possess antidiabetic activity. The mechanism of action involves the inhibition of the α-glucosidase enzyme, leading to a reduction in blood glucose levels, an enhancement of insulin sensitivity, and an increase in insulin levels [9]. In an in vivo assay with a rat model, the ethanol extract at a dose of 500 mg/kg body weight demonstrated hypoglycemic effects and retarded the damage to pancreatic β-cells [10]. Concurrently, the methanol and 60% methanol extract of mahogany seeds at a dose of 250 mg/kg body weight increased insulin levels and supported the regeneration of pancreatic β–cells. [11]. The methanol extract demonstrated more promising results; however, it should be noted that methanol is more toxic than ethanol [12]. Therefore, there is a necessity for research to be conducted on the use of alternative solvents to methanol. Methanol has been found to exhibit a higher degree of polarity in comparison to ethanol, yet a lower polarity in relation to water [13].

The potential of mahogany seed extract can be further enhanced through the development of novel extraction techniques and the utilization of nanoencapsulation. The extraction method utilized in earlier studies was the maceration method technique [9]. The maceration method of extraction has been demonstrated to yield superior antidiabetic activity in comparison to reflux method [14]. However, the process of maceration requires more time and solvents. The ultrasound–assisted extraction (UAE) method has been proposed as a potential alternative to maceration. The extraction of sea olive wood using UAE for a duration of 45 minutes resulted in an extract that exhibited a higher yield and active ingredient concentration compared to the maceration method, which was performed over a duration of 24 hours [15]. The encapsulation of the extract with nanophytosomes has been demonstrated to enhance its effectiveness within the body. This phytosome system utilizes modified drug delivery technology to bind active compounds with phospholipid molecules. To the best of the present author's knowledge, no studies have been reported on the encapsulation of mahogany seed extract using nanophytosomes. The nanophytosome encapsulated extract has been demonstrated to enhance both bioavailability and surface penetration and maintain bioactivity over an extended timeframe [16]. Therefore, the mahogany seed extract obtained using UAE and subsequently encapsulated with nanophytosomes has the potential to enhance the antidiabetic potential of mahogany seeds.

In silico testing of mahogany seeds for antidiabetic purposes has been limited to alpha–glucosidase inhibition [17], while the evaluation of insulin secretion targets remain an unexplored area [18]. This study was conducted in silico testing on four proteins that play a crucial role in insulin secretion: The following have not been previously investigated: Glucagon–like peptide-1 1 receptor, phosphatidylinositol–4,5–bisphosphate 3 3-kinase, catalytic subunit alpha, protein kinase RNA–like endoplasmic reticulum kinase, and insulin receptor kinase. The objective of this study is to determine the effects of different solvent types on insulinotropic activity and cytotoxicity in the pancreatic β–cell line and phytochemical profile. The present study utilized BRIN–BD11 pancreatic β–cells as an in–vitro model to evaluate insulinotropic activity, because these hybrid cells, derived from the fusion of insulinoma cells and normal rat β–cells exhibit stable insulin secretion and express GLP–1 receptors, thereby closely resembling native pancreatic β–cells in function and phenotype [19]. Furthermore, this study investigates whether modifying the extract into nanophytosomes can enhance its antidiabetic efficacy in a diabetic mice model and predict bioactive compounds through a molecular docking study.

2. Materials and Methods

2.1. Extraction

The mahogany seeds were obtained from the Parung Panjang Research Forest, Bogor, West Java, Indonesia. The seeds were made into powders with a mesh size of 49 -60. The UAE methods were performed using three different solvents, including ethanol, ethanol–water (1:1), and water, with a sample–to–solvent ratio of 1:10 (w/v). 100 g of mahogany seed powder was extracted using n-hexane solvent to remove non–polar extractive substances such as oil, fat, and wax. The ratio of powder to solvent was 1:10 (w/v). The residue was treated with n, and was directly extracted using the respective solvents. Furthermore, each sample was extracted using the UAE method for 45 minutes. The filtrate obtained from the extraction process was then concentrated with a vacuum rotary evaporator and weighed to determine the extraction yield of water–dissolved extract (MW), ethanol–water dissolved extract (MEW), and ethanol dissolved extract (ME). The determination of yield refers to [15].

2.2. Cytotoxicity of BRIN–BD 11 pancreatic cells

Cytotoxicity activity was determined on mahogany seed extracts using a spectrophotometric method with the MTT (3–(4,5–dimethylthiazol–2–yl)–2,5-diphenyltetrazolium bromide) assay. The BRIN–BD11 cells were cultured at 5,000 cells in 100µL of growth medium. The extracts were added subsequent to the cells attaining 50% confluence (24 hours). The MTT assay was performed on day 3 by adding 10µL of MTT (5 mg/mL) per well and incubating for 4 hours at 37°C. The formazan crystals were dissolved in ethanol, and the degree of absorption was measured at a wavelength of 595 nm using a microplate reader. The values obtained from the measurement of the degree of absorption of light by the sample were used to calculate cell viability. A lower value of extinction coefficient at higher extract concentrations of the extract is indicative of significant cytotoxic potential [20].

2.3. Insulinotropic effects of mahogany seeds on pancreatic cell culture BRIN–BD11

The determination of the insulinotropic effect of mahogany seed extract on BRIN–BD11 cells was conducted within culture flasks (approximately 150×10^5 cells). The incubation was conducted at 37°C within a 5% CO_2 atmosphere for a duration of 24 hours on a 96–well microplate, with 5000 cells per well using RPMI 1640 medium. Following a 24-hour incubation

period, it was hypothesized that the BRIN–BD11 cells would adhere to the bottom of the wells. The cells will then be washed thrice with 1 mL of fresh Modified Krebs Ringer Buffer (KRB–1) (NaCl 115 mM, KCl 4.7 mM, $CaCl_2$ 1.3 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 24 mM, HEPES 10 mM, BSA 1 g/L, glucose 1.11 mM). The buffer will then be gassed with 5% CO_2 and 95% O_2 for 15 minutes. Prior to treatment, a 40-minute pre–incubation period was undertaken, entailing the addition of 1 mL of KRB–1 solution to an incubator maintained at 37°C with the solution gassed with 5% CO_2. The incubation was conducted for 60 minutes at 37°C and exposed to 5% CO_2 with 1 mL of mahogany seed extract solution at concentrations of 0.56, 1.125, 2.25, and 4.50 mg/L in KRB–3 (Modified Krebs Ringer Buffer with glucose concentration of 16.7 mM) and KRB–1. The media was harvested and transferred into 1.5 mL tubes, followed by centrifugation at 158 g for 5 minutes to separate the BRIN–BD11 cells that have been released into the media. The measurement of insulin levels was conducted by means of the Finetest INS Rat ELISA Kit (cat# ER113). The absorbance was measured at 450 nm employing a BioRad Model 3550 Microplate Reader. A standard insulin curve was determined using the same procedure applied to the samples [21].

2.4. Metabolite profiling

The mahogany seed extracts were identified for their compound components using liquid chromatography with tandem mass spectrometry (LC–MS). The LC–MS specifications were UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS (ThermoScientific, USA). The separation process was conducted using Accucore C18 columns. The dimensions of the specimen were 100 x 2.1 mm, with a thickness of 1.5µm (ThermoScientific, USA) at 30°C. Gradient elution was utilized as a separation system with two eluents: eluent A was H2O + 0.1% formic acid, and eluent B was acetonitrile + 0.1% formic acid (0–1 minute, 5 %B). 1–25 minutes (5–95 %B). The timeframe for the former was 25–28 minutes (95 %B), while the latter was 28–30 minutes (5 %B). The flow rate was 0.2 mL/min. Molecular fragmentation (mass spectrometry) was carried out using electrospray ionization as an ionizer (3.80 kV capillary temperature 320°C, and negative and positive ionization modes) with a resolution of 70000. Mass fragmentation readings were performed at 100–1500 m/z. Compound annotation was conducted utilizing Compound Discoverer 3.1 software (ThermoScientific, USA).

2.5. Prediction of Bioactive Metabolites in silico (molecular docking)

The Gasteiger approach was subsequently employed to apply partial charges to the structures. The Protein Data Bank (https://www.rcsb.org/) provided the receptors. The selection of these proteins as receptors was due to their physiological function, which was related to insulin production (see Table 1). AutoDockTools 1.5.7 was utilized for protein processing. AutoDock Vina was utilized to redock the co-crystallized ligands into their respective receptors. The co-crystallized ligands' coordinates served as the center of the docking grid, which had a grid box size of 22 × 22 × 22 Å along the X, Y, and Z axes, respectively. To guarantee accurate ligand posture prediction, AutoDock Vina's exhaustiveness parameter was set to 8. All receptors that were validated had RMSD values less than 2 Å, thereby satisfying the requirements (see Table 1). An RMSD value below 2 Å is considered ideal for docking validation, indicating that the predicted ligand position closely aligns with the reference or experimental position [22].

Table 1. Insulinotropic targeted protein and molecular docking validation results

Proteins	Protein code	Co–crystal ligand code	Binding coordinate (x, y, z)	RMSD (Å)	Physiological role
Glucagon–like peptide–1 receptor (GLP1–R)	6X19	UK1	130.657, 111.088, 86.069	0.6411	It regulates glucose–dependent insulin secretion, inhibits pancreatic secretion, and protects β–pancreatic cells from apoptosis [23].
Phosphatidylinositol–4,5–bisphosphate 3–kinase, catalytic subunit alpha (PI3Kα)	7TZ7	KVJ	–2.453, 9.724, –19.914	0.4273	This compound plays a role in insulin signal transduction, glucose uptake, cell survival, and proliferation [24].
Protein kinase RNA–like endoplasmic reticulum kinase (PERK)	4X7L	3Z4	27.475, 9.505, 4.166	0.2028	It regulates endoplasmic reticulum stress response through eIF2α phosphorylation to adjust protein synthesis and maintain cell survival [25].
Insulin receptor kinase (IRK)	4IBM	IR1	3.925, –9.793, 6.889	0.3582	It activates the insulin signalling pathway to enhance glucose uptake, energy metabolism, and glucose homeostasis in the body [32].

The docking protocol was validated by re–docking co-crystallized ligands to their respective receptors, and the validated protocol was then used to predict the binding behavior of phytochemical compounds from the extract. The geometry of these compounds was then optimized using the Newton–Raphson algorithm within a molecular mechanics approach implemented through the Open Babel/Obabel software [26]. The force field employed in the ligand geometry optimization process was GAFF – General Amber Force Field. Subsequently, the phytochemical compounds were subjected to docking using the AutoDock Vina algorithm [27]. Ligand efficiency (LE) was calculated by dividing the binding free energy (ΔG, in kcal/mol) obtained from docking results by the number of heavy atoms in each compound, to assess binding efficiency relative to

molecular size. The compound underwent 10 repetitions. The screening results were then subjected to a process of ranking, and the top compounds were selected for further analysis using PyMOL 3.1 and LigPlot 2.28.

2.6. Encapsulation of the best extract with nanophytosome

The most effective extract, as determined through in vitro analysis, was encapsulated using a soy lecithin–based nanophytosome. The extract was then subjected to a reaction with soy lecithin as phosphatidylcholine. The phytosome formulation was prepared at a 1:1 ratio of extract to soy lecithin, where 1 g of an extract and 1 g of soy lecithin were refluxed with 100 mL of dichloromethane at 60°C for 2 hours. The mixture was then concentrated to a 5–10 mL volume, and 20 mL of n-hexane was added. The resultant phytosome was filtered and stored in a desiccator for 12 hours, after which its yield was determined. The phytosome was then subjected to hydration with 20 mL of distilled water and homogenized using a magnetic stirrer. The nanophytosomes were formed through ultrasonication for 30 minutes, with the formulation being repeated on three occasions [17].

2.6.1. Nanophytosome characterization

The testing characteristics of nanophytosomes were analyzed using Fourier transform infrared spectroscopy (FTIR), a particle size analyzer (PSA), and a zeta potential analyzer. FTIR analysis was conducted to predict the functional group of specific compounds, thus allowing the identification of chemical bonds formed in the nanophytosome as a result of the combination of the extract and soy lecithin. The liquid sample was placed in a specialized cuvette with a window, and the analysis was performed within a wavelength range of 4000–400 cm^{-1} [28]. The occurrence of hydrogen bonding was evidenced by the shift of the hydroxyl (OH) group to a lower wavenumber in the spectrum [29]. The size of nanophytosome was determined by means of a particle size analyzer (PSA) to assess both the particle size and its distribution. Furthermore, the zeta potential value was measured using a zeta potential analyzer. The nanophytosome sediment resulting from the final centrifugation was stirred until a homogeneous consistency was achieved. A 2 mL sample of this solution was extracted and subsequently diluted to a final volume of 25 mL. The diluted solution was finally analyzed using the PSA instrument [30].

2.7. In vivo antidiabetic assay

The study was granted ethical approval by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedicine, IPB University (No: 280/KEH/SKE/XII/2024). Male DDY-strain mice with a body weight between 25–30 grams were utilized and divided into four treatment groups as illustrated in Table 2. The experiment was conducted in several phases, including a 7-day acclimatization period, a 4-day streptozotocin (STZ) induction phase, and a 21-day drug treatment phase. The experiment was conducted in several phases, including a 7–day acclimatization period, a 4–day streptozotocin (STZ) induction phase, and a 21–day drug treatment phase.

Treatments were administered orally via gavage, utilizing a feeding tube for a period of 21 consecutive days. The effects of treatment in each group were observed throughout and at the end of the treatment period. A measurement of blood glucose levels and body weight was taken at the beginning and end of the experiment. At the end of the treatment period, the mice were euthanized using an overdose of ketamine and xylazine, in accordance with institutional ethical guidelines. Blood samples were then collected for further analysis of blood profile, glucose levels, and insulin levels.

Table 2. Mouse groups and their respective treatments

Mice Group	Diabetes Modeling	Treatment	Number of Mice
Positive Control	DM	Glimepiride	5
Negative Control	DM	Water	5
Extract	DM	Best extract (250 mg/kg BW)	5
Nanophytosome–Extract	DM	Nanophytosome–extract (250 mg/kg BW)	5

2.7.1. Hematological and biochemical profiles serum analysis

Blood collection was performed on day 21. The mice were anesthetized using ketamine (100 mg/kg BW) and xylazine (3 mg/kg BW) via intraperitoneal injection. Blood was collected intracardially, with a total volume of 4mL. 1 mL was placed into an EDTA tube for hematological analysis, while 3 mL was placed in a plain tube. Following the manufacturer's instructions, the hematological analysis was conducted using a Mindray BC–2800 hematology analyzer (PT Mindray Medical Indonesia). The assessment of hematological parameters encompassed a range of indices, including erythrocyte count, hemoglobin level, hematocrit value, total leukocyte count, and leukocyte differentials (lymphocytes, monocytes, neutrophils, eosinophils, and basophils). The biochemical composition of the blood was analyzed using a UV spectrophotometer. The biochemical parameters that were the focus of the assessment included liver function tests (Serum Glutamic Pyruvic Transaminase and Serum Glutamic Oxaloacetic Transaminase) and kidney function tests (urea and creatinine) [28].

2.7.2. In vivo insulin secretion

Serum insulin levels in mice were analyzed using the Rat INS (Insulin) ELISA Kit (Fine Test) based on an ELISA method. Blood was collected via retro–orbital bleeding or cardiac puncture, centrifuged at 1000 × g for 15–20 minutes at 2–8°C. The serum was subsequently stored at -20°C or –80°C. A 100 µL sample or standard was added to the ELISA wells, incubated, washed, and then treated with 100 µL biotinylated antibody, 100 µL HRP-Streptavidin, and 90 µL TMB substrate, with washing steps in between. The reaction was then halted with the addition of 50 µL Stop Solution, after which the degree of light absorption was measured at 450 nm. The insulin levels were calculated based on a standard curve ranging from 78.125 to 5000 pg/mL to analyze the insulin response in the diabetes model [29,30].

2.7.3. Histopathological observation of pancreatic Langerhans islets

The observation of pancreatic Langerhans islets was conducted using hematoxylin and eosin (H&E) staining. The organ slide preparations were subjected to incubation at 65°C for 3 minutes, and immersed three times in xylol, three times in absolute ethanol, and once in 95%, 90%, 80%, and 70% ethanol, each for 3 minutes. This process was followed by immersion in distilled water as a stopping point. The preparations were stained with hematoxylin for 2 minutes, followed by immersion in running tap water for 10 minutes and immersion in distilled water as a stopping point. The preparations were then stained with eosin for 1 minute, followed by rapid dehydration in 70%, 80%, 90%, and 95% ethanol, twice in 95% ethanol, and three times in absolute ethanol (with a 1-minute immersion on the third step). This was followed by rapid clearing in xylol three times (with a 1 1-minute immersion on the third step), mounting with Entellan, and covering with a cover glass. The observations were conducted using a light microscope, and images were captured using a microscope camera. The area and cell count of all Langerhans islets observed on each slide were measured using the ImageJ software (with cell nuclei appearing purple), and the average value was calculated as a single replicate per rat. The area and cell count of the Langerhans islets from five replicates per treatment group were statistically analyzed according to the method used in data analysis [31].

2.8. Data analysis

The analysis of variance (ANOVA) was utilized to identify the effect of disparate solvents or types of extracts on the yield, the inhibitory activity of insulinotropics, and the cytotoxicity of mahogany seed extract. The Duncan's multiple range test analyzed parameters that exerted a significant influence. The data was analyzed using SPSS 25 software (IBM Corporation, USA).

3. Results and Discussion

3.1. Yield of mahogany seed extract

The selection of n–hexane as the first solvent in the extraction process of mahogany seeds was aimed at purifying non–polar compounds including fats, oils, and lipophilic substances. The low polarity of n–hexane rendered it highly effective at dissolving non–polar components [32]. The extraction of mahogany seeds with n–hexane produces an extract of yellow oil. In this study, the extraction of mahogany seeds using n-hexane as a cleaning solvent did not contribute significantly to antidiabetic activity. Previous studies have also shown that the n-hexane extract of mahogany seeds is abundant in fatty acids and triglycerides, yet it does not directly contribute to the reduction of glucose levels [33].

The findings of the variance analysis demonstrated that the type of solvent affected the yield of the extract. The Duncan's test indicated that the MW exerted the highest yield, though this did not significantly differ from the MEW. However, a significant divergence from the ME was observed (Fig 1). This finding suggests that water, as a pure polar solvent, is more effective in extracting water soluble compounds, which were predominant in mahogany seeds. Furthermore, the extraction in this study was conducted using ultrasonication, which led to more optimal yields than conventional methods like maceration [32].

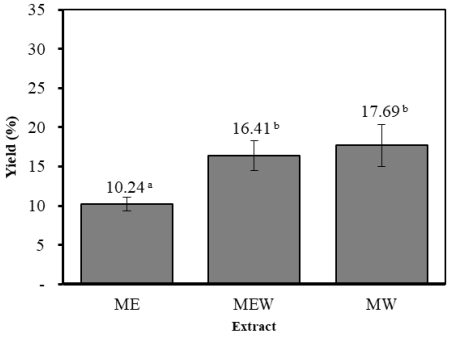

Fig. 1. Yield of mahogany seed extract. Numbers followed by different letters showed significant differences (p-value < 0.05)

As illustrated in Fig. 1, the ultrasonic extraction of mahogany seeds yielded the lowest extract yield in ME compared to MEW and ME. This phenomenon can be attributed to the divergent solubilities of bioactive compounds. MW uses a polar solvent, allowing it to hydrolyze only toxic compounds with polar properties. By contrast, MEW has a more balanced ability to hydrolyze both polar and non–polar toxic compounds [34]. The MEW yield in this study was found to be higher than in previous studies, which reported an extraction yield of approximately 18% [35]. The MW in this study demonstrated the highest yield, reaching 17.69% (Fig. 1). The ultrasonic extraction method yielded a higher yield than the maceration method, which only yielded 12.5% [36]. This finding indicates that ultrasonic extraction can enhance yields with greater efficiency, thereby corroborating previous findings that recommend using more efficient extraction methods to obtain bioactive compounds from mahogany seeds [15].

3.2. Toxicity and insulinotropic effect of mahogany seeds on BRIN–BD11

The statistical analysis demonstrated that the type of extract determined the level of cytotoxicity, as indicated by the percentage of inhibition of BRIN–BD11 cell growth (Fig. 2(a)). Duncan's post hoc test revealed that the ME at a concentration of 1000 µg/mL exerted an inhibition percentage of 63.45%, which was significantly different from the other extracts. The BRIN–BD11 cell toxicity test demonstrated that MEW exhibited a greater number of cells in the tissue, indicating lower toxicity, followed by ME. This phenomenon may be attributable to the variations in the solubility of bioactive compounds among MW, MEW, and ME (Fig 2(b)). This inhibition percentage exceeding 50% indicated that ME was considered toxic to BRIN–BD11 cells [37].

The results of toxicity test of mahogany seed extracts exhibited that the type of extract determined the level of cytotoxicity against BRIN–BD11 cells (Fig 2a). The analysis revealed that ME at the concentration of 1000 µg/mL inhibited more than 50% of cell growth (Fig 3), indicating its toxic properties [33]. Conversely, the MEW and MW exhibited

reduced level of cytotoxicity, indicating their relative safety. As demonstrated in previous studies, a 50% ethanol extract at the IC50 value exhibited weak cytotoxicity against HepG2 cells at a concentration of 1000 µg/mL [39]. An extract is deemed to be of low toxicity if its IC50 value is less than 20 µg/mL [38]. The present findings are consistent with those of earlier studies, indicating that the type of extract influences the percentage of cytotoxicity, where the MEW of mahogany seed at a concentration of 1000 µg/mL exhibited a lower toxicity activity of –1.08 µg/mL compared to other extracts (Fig. 2(a)).

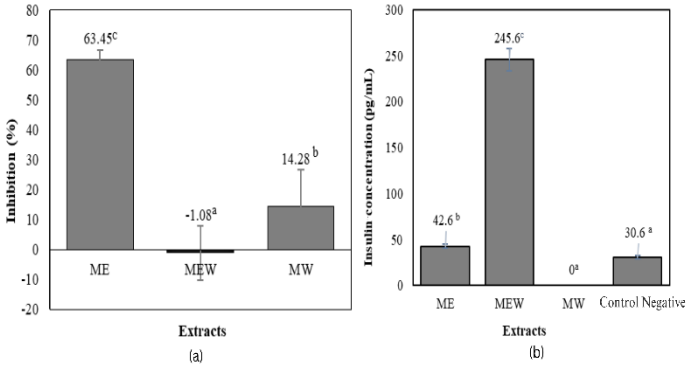

Fig. 2. Effect of mahogany seed extracts on the cytotoxicity (a) and insulin secretion (b) of BRIN–BD11 cell line. The number followed by different letters showed significant differences (p–value < 0.05)

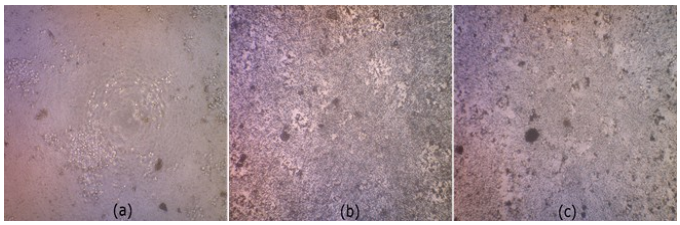

Fig. 3. Micrograph of BRIN–BD11 cultured cell treated with MEW (a), MEW (b), and MW (c)

The insulinotropic activity of mahogany seed extracts was evaluated by measuring insulin secretion in BRIN–BD11 cells. This finding suggests the presence of potent insulinotropic compounds within the MEW (Fig. 2(b)). This finding is consistent with previous research showing that methanol extracts of mahogany seeds could increase plasma insulin levels in diabetic rats with an optimal dose [39]. However, as previously mentioned, methanol extracts have been shown to be more toxic [40]. Concurrently, the study's cytotoxicity testing of the MEW revealed low toxicity against BRIN–BD11 β-pancreas cells. Mahogany seeds are rich in terpenoids and limonoids, which function as insulin secretagogues [41].

3.3. Phytochemical profile

The phytochemical profile of ME, MEW, and MW is shown by the LC–MS chromatogram, with varying retention times and relative abundances. The data indicated that each extract contained compounds with a broad range of retention times, from 1.16 minutes (citbisine C) to 24.20 minutes (Juniperic acid). The presence of certain compounds, including austalide D (RT 14.83) and austalide C (RT 16.00), was detected in all three extracts with consistent retention times observed. However, the abundances of these compounds varied, suggesting that the type and compositions of the compound might be dependent upon the solvent utilized in the extraction process. However, there were also compounds with the same retention time but different peak heights, suggesting the presence of the same compounds with varying abundances (Fig. 4) and (Table 3).

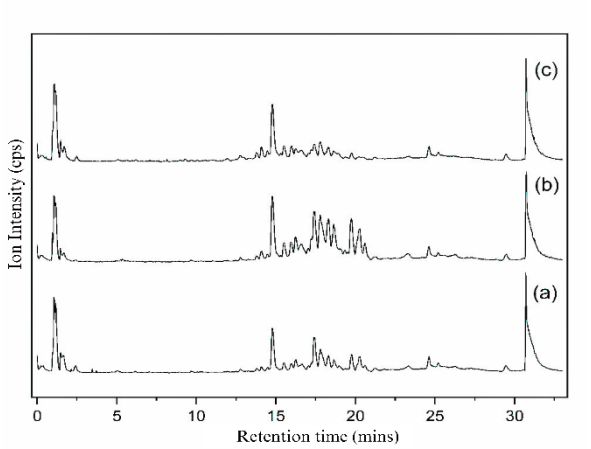

Fig. 4. Chromatogram profiles of ME (a), MEW (b) and MW (c)

As revealed by the results of the database search, nine compound groups were identified, with terpenoids being the most prevalent group across the three extracts (see Table 3). The error mass was utilized to ascertain the quality of compound identification with the match of the mass spectra being a contributing factor [42]. The compound austalide D, which appeared at a retention time of 14.83 minutes, has an error mass value close to 0 (–2.69 ppm), indicating accurate identification with the highest relative abundance in the MW (20.84%) and MEW (19.80%). Another terpenoid compound, gomisin D, at a retention time of 15.53 minutes, also exhibited a small delta mass (–2.34 ppm), thereby indicating good identification. However, its relative abundance is lower than that of austalide C, with 3.85% in the MEW. Bryotoxin A was also identified with a low error mass (–3.41 ppm), thereby supporting the validity of the data with a relative abundance of 1.14% in the MEW extract. This finding suggests that terpenoid compounds are the predominated concentration of compounds in the MEW extract. Previous studies have reported that mahogany seeds contain the significant quantities of secondary metabolites, including terpenoid compounds such as austalide D, gomisin D, and bryotoxin A. These compounds have been shown to contribute to antidiabetic activity of the seeds [43]. The results of the insulinotropic assay of the MEW extract demonstrated the highest insulin concentration and low toxicity (see Fig. 1(b) and 1(c)). Thus, it can be concluded that MEW was the most effective solvent of the three.

The analysis using LC–MS/MS revealed a diverse phytochemical profile in the ME, MEW, and MW, with varying compositions of compounds (see Table 3). Terpenoids such as austalide D, gomisin D, and bryotoxin A, known for their antidiabetic activity [43], were identified in all three extracts, with the highest concentration observed in the MEW extract. Previous studies [44] have shown that these compounds have the capacity to modulate ion channels or enhance glucose metabolism in β–pancreas cells. The results of the

insulinotropic test demonstrated that MEW exhibited high antidiabetic activity against β–pancreas cells, thereby supporting insulin secretion function. This finding is consistent with previous reports indicating that ethanol extracts can significantly reduce lower blood glucose levels, demonstrating robust antidiabetic potential [45].

Table 3. Chemical composition of mahogany seed extracts

RT (min)	Compound Name	Group	Error mass (ppm)	Relative Abundance (%)		
				MW	ME	MEW
14.83	Austalide D	Terpenoid	2.69	20.84	23.57	19.8
1.17	Fipexide	Alkaloids	4.42	4.32	5.84	3.03
16.00	Austalide C	Terpenoids	–2.37	2.01	2.27	3.85
1.31	Citric acid	Carboxylic Acid	–5.59	3.36	4.37	3.44
1.69	Methylmalonic acid	Carboxylic Acid	–10.40	2.00	0.28	1.24
15.54	Gomisin D	Terpenoids	–2.34	0.82	0.62	1.14
17.45	Bryotoxin A	Terpenoids	–3.41	0.68	0.25	1.14
17.07	Oxidized dinoflagellate luciferin	Terpenoids	–4.01	0.83	0.39	1.10
1.25	Hexaric acid	Carboxylic Acid	–4.36	1.67	0.33	1.05
24.20	Juniperic acid	Terpenoids	–2.41	1.62	0.09	0.07
1.17	Diphyllin	Lignans	–2.06	0.73	1.15	0.61
14.83	Apadenoson	Nucleoside	2.81	0.84	0.97	0.65
7.06	Diacetylphloroglucinol	Lignans	–4.3	0.22	–	0.61
22.99	13S–hydroxyoctadecadienoic acid	Fatty Acids	–2.35	0.90	0.02	0.02
14.10	Bruceantin	Alkaloids	–2.65	0.51	0.73	0.37
14.83	Limanoid obacunone	Terpenoids	–2.82	0.50	0.57	0.49
1.16	Citbismine C	Alkaloids	–1.71	0.24	0.63	0.24
1.16	Methyl–D–erythritol Phosphate	Terpenoids	–3.41	0.73	0.06	0.11
17.81	Proceranolide	Terpenoids	–2.73	0.24	0.21	0.40
1.29	Glucoheptonic acid	Gluconic Acids	–3.27	0.55	0.33	0.38
16.28	Dihydrostreptomycin 6–phosphate	Streptomycin	4.60	0.50	0.37	0.38
14.38	Verrucarin A	Terpenoids	–1.82	0.30	0.52	0.26
1.88	Isovaleryl–β–D–glucuronide	Gluconic Acids	–2.48	0.27	0.45	0.13
18.05	3′–Deoxydihydrostreptomycin 6–phosphate	Glycoamino Acids	4.77	0.37	0.14	0.31
6.25	Compound NP–003827	Polyketides	–2.22	0.27	0.42	0.03
13.94	Methylprednisolone hemisuccinate	glucocorticoid	–2.25	0.15	0.42	0.14
19.30	1–oleoylglycerophosphoinositol	Phospholipids	–1.69	–	0.38	–
18.64	1–palmitoylglycerophosphoinositol	Phospholipids	–1.93	–	0.37	–
4.44	3–Methoxy–4–hydroxyphenylglycol glucuronide	Phenolic	–1.45	0.18	0.37	0.10
1.66	N–(1–Deoxy–1–fructosyl) leucine	Glycoamino Acid	–1.78	0.44	0.02	0.01
7.08	Mannipherin	Gluconic Acids	–1.74	0.43	0.17	0.02

3.4. In silico study

The in-vitro assay (see Fig. 2(a) and 2(b)) demonstrated that the MEW was the most effective in regulating insulin secretion. Consequently, a virtual screening was conducted using AutoDock Vina by docking the 15 most effective compounds from the MEW, as identified through metabolite profiling, onto the protein structures GLP1–R, PI3Kα, PERK, F and IRK, thus yielding affinity energy data. The screening results are presented in Table 4 for the 15 compounds with the highest relative abundance. The results of binding affinity indicated that the more negative the value, the stronger the interaction. The ligand efficiency recorded in this analysis provides information on the binding efficiency of the compounds bind to the receptor relative to their molecular size [46].

The docking data (Table 4) demonstrate that the compound oxidized dinoflagellate luciferin exhibited the highest binding Affinity for GLP1–R (–9.52 kcal/mol) and PI3Kα (–8.34 kcal/mol), indicating its potential to enhance insulin secretion and modulate metabolic pathways associated with these two proteins. Furthermore, the apadenoson (CID_9805430) recorded the strongest binding Affinity against PERK (–8.15 kcal/mol), while the diphyllin (CID_100492) showed the highest binding Affinity for IRK (–8.96 kcal/mol), indicating its potential to enhance the efficacy of insulin receptor signaling. For the PERK protein, the Fipexide (CID_3351) demonstrated a binding affinity of –7.28 kcal/mol, indicating its potential to accelerate insulin secretion through the modulation of the endoplasmic reticulum stress pathway. The ligand efficiency of each compound demonstrated significant variation, indicative of the potential stability of the interactions with the target proteins. For the GLP1–R receptor, methylmalonic acid recorded the highest ligand efficiency of –0.56, thus indicating a highly stable binding, even though its binding affinity is lower than that of other compounds.

Table 4. Binding affinity and ligand efficiency of mahogany seed bioactive compounds

Compound Name	Binding Affinity (kcal/mol)				Ligand Efficiency (kcal/mol)			
	GLP1–R	PI3Kα	PERK	IRK	GLP1–R	PI3Kα	PERK	IRK
Austalide D	–8.07	–7.64	–6.23	–7.03	–0.21	–0.20	–0.16	–0.19
Fipexide	–8.75	–7.46	**–7.28**	–8.16	–0.32	–0.28	–0.26	–0.30
Austalide C	–8.31	–7.98	–6.15	–7.43	–0.20	–0.19	–0.15	–0.18
Citric acid	–5.25	–5.15	–4.49	–5.59	–0.40	–0.40	–0.34	–0.43
Methylmalonic acid	–4.52	–4.19	–3.75	–4.63	–0.56	–0.52	–0.46	–0.57
Gomisin D	–7.49	–6.34	–6.16	–6.37	–0.20	–0.17	–0.16	–0.16
Bryotoxin A	–8.89	–7.12	–6.66	–8.38	–0.20	–0.16	–0.15	–0.19
Oxidized dinoflagellate luciferin	–9.52	**–8.34**	–7.07	–8.59	–0.22	–0.19	–0.16	–0.20
Hexaric acid	–4.94	–5.22	–4.29	–5.34	–0.35	–0.37	–0.30	–0.38
Juniperic acid	–5.59	–4.72	–3.93	–5.13	–0.29	–0.52	–0.43	–0.57
Diphyllin	–8.28	–8.19	–6.69	**–8.96**	–0.30	–0.29	–0.23	–0.32
Apadenoson	**–9.75**	–8.15	–7.16	–8.00	–0.28	–0.23	–0.20	–0.22
Diacetylphloroglucinol	–6.23	–5.53	–5.23	–6.34	–0.42	–0.37	–0.34	–0.42
13S–hydroxyoctadecadienoic acid	–6.27	–6.04	–4.89	–5.84	–0.30	–0.29	–0.23	–0.27
Bruceantin	–7.77	–7.26	–6.27	–7.16	–0.20	–0.19	–0.16	–0.18

The four compounds with the highest binding affinity from the MEW demonstrated specific amino acid binding positions at the receptor site (Fig. 5). The compound fixpide and oxidized dinoflagellate luciferin exhibited substantial interactions with pivotal residues within the target protein's active site. It is evident that Fixpide formed conventional hydrogen bonds with residues LYS A:206, LYS A:211, and TYR A:154, and hydrophobic Pi–Alkyl interactions with residues such as TRP A:42 and LEU A:210. These interactions are crucial in determining ligand stability and affinity within the protein pocket. Oxidized dinoflagellate luciferin interacts with polar residues such as SER A:40, LYS A:206, and ASP A:207 via hydrogen bonds, as well as with aromatic hydrophobic residues such as TYR A:154 and TYR A:214. The compound diphyllin showed specific interactions with charged residues such as GLU A:147 and ASP A:207 through Pi–Anion interactions. Concurrently, additional stability is provided by Amide–Pi Stacked interactions with PRO A:146 and the hydrophobic residue LEU A:150. In contrast, apadenoson selectively binds with polar residues GLU A:43 and SER A:40 while also involving Pi–Pi stacked interactions with TRP A:42. These latter interactions are further strengthened by hydrophobic interactions from MET A:242 and LEU A:150, forming a stable complex [34].

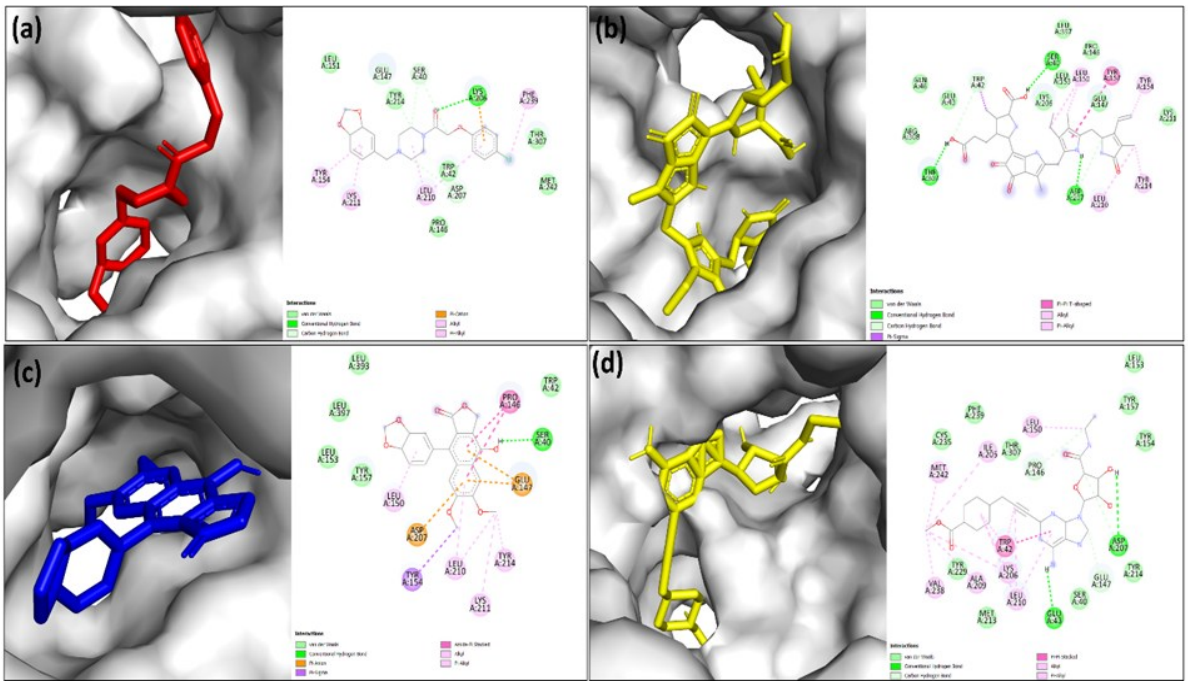

Fig. 5. Ligand–receptor interactions for ligands with the best molecular binding activity on compounds (a) fixpide, (b) oxidized dinoflagellate luciferin, (c) diphyllin, and (d) apedonoson

The molecular docking validation in this study demonstrated that the interactions between the GLP1–R, PI3Kα, PERK, and IRK proteins with the tested ligands result in significant binding affinity values. As depicted in Table 4, the binding affinity values obtained for each protein indicate stable interactions between the ligands and the target proteins. The reduction in free binding energy indicates that the ligands have the potential to bind strongly to the proteins, thereby supporting the mechanism by which the ligands enhance insulin production [47]. This finding is consistent with previous research demonstrating that the activation of GLP1–R can enhance insulin secretion in beta–pancreatic cells, a process that is imperative in the management of type 2 diabetes [48]. The virtual screening was performed using AutoDock Vina to evaluate the interactions between 15 bioactive compounds from mahogany seeds and four target proteins. The results obtained were found to be significant. Austalide C, the most abundant compound in the MEW (see Table 4), exhibited a relatively strong interaction with insulin secretion receptors. However, when compared to four other compounds, fixpide, oxidized dinoflagellate luciferin, diphyllin, and apadenoson demonstrated a reduced binding affinity, suggesting that these four compounds possess a stronger potential to bind to insulin secretion receptors. Previous research demonstrated that terpenoid and alkaloid compounds can stimulate insulin secretion by modulating signaling pathways in beta–pancreatic cells [49].

The analysis of ligand–protein interactions revealed that compounds such as oxidized dinoflagellate luciferin and apadenoson selectively interact with key residues at the protein binding site, including LYS A:206, SER A:40, and TYR A:154 (Fig. 5). These interactions have been demonstrated to enhance the stability and affinity of the ligand–protein complex, thereby supporting their potential to increase insulin secretion [50]. Previous studies have also revealed that certain compounds, which interact with polar and aromatic residues in the GLP1–R protein can enhance insulinotropic activity [51]. The toxicity evaluation of the 15 most effective compounds from MEW revealed that most compounds were non–toxic, with some, including oxidized dinoflagellate luciferin, bryotoxin A, and austalide C, exhibiting low toxicity profiles. The bioactive compounds present at the highest concentrations of MEW have the greatest potential to support insulin regulation, given their low toxicity profile and significant activity on the PPAR–Gamma and Nrf2/ARE pathways. The findings of this study are consistent with those of relevance of previous studies indicating that effective blood glucose regulation is significantly determined by bioactive compounds that enhance insulin sensitivity and reduce oxidative stress [52].

Table depicts 5 the results of toxicity prediction evaluation for the 15 most effective compounds. These are based upon the highest concentration abundance from the MEW, which has the best insulinotropic activity and low toxicity potential. The evaluation employed five main parameters: cytotoxicity, PPAR–Gamma, Nrf2/ARE, nephrotoxicity, and cytochrome P450. Most compounds were IA regarding cytotoxicity, except for Fipexide, which demonstrated a toxicity probability of 0.55. The PPAR–Gamma parameter, which is relevant for glucose and lipid regulation, exhibited a high probability of activity for compounds such as oxidized dinoflagellate luciferin (0.99), thereby supporting its potential in insulin regulation. Compounds such as austalide C, austalide D, and gomisin D exhibited high levels of inactivity against most of the toxicity parameters tested. Austalide C demonstrated complete IA against with respect to the assessment of cytotoxicity, PPAR–Gamma, and Cytochrome P450. The inactivity probabilities recorded were 0.96 for Nrf2/ARE and 0.94 for nephrotoxicity, suggesting a favorable safety profile. Conversely, austalide D demonstrated efficacy in counteracting nephrotoxicity with a probability of 0.51, thereby suggesting the necessity for caution regarding the potential of kidney toxicity [35].

Based on the toxicity evaluation, most of the compound abundance from the MEW, predominantly oxidized dinoflagellate luciferin, bryostatin A, and fipexide, were classified as non–toxic (IA) on most of the evaluated parameters. Compounds such as oxidized dinoflagellate luciferin demonstrated a low toxicity profile and high activity in the PPAR–Gamma and Nrf2/ARE pathways, suggesting their potential for further development as therapeutic agents.

Table 5. Toxicity prediction of the 15 best test ligands

Compound Name	Cytotoxicity		PPAR–Gamma		Nrf2/ARE		Nephrotoxicity		Cytochrome P450	
	Pred.	Prob.	Pred.	Prob.	Pred.	Prob.	Pred.	Prob.	Pred.	Prob.
Austalide D	IA	0.64	IA	0.96	IA	0.94	A	0.51	IA	0.85
Fipexide	A	0.55	IA	0.99	IA	0.92	A	0.5	IA	0.65
Austalide C	IA	0.58	IA	0.96	IA	0.94	IA	0.58	IA	0.75
Citric acid	IA	0.73	IA	0.99	IA	0.99	IA	0.55	IA	0.99
Methylmalonic acid	IA	0.79	IA	0.99	IA	0.99	IA	0.60	IA	0.99
Gomisin D	IA	0.77	IA	0.95	IA	0.81	IA	0.58	IA	0.93
Bryotoxin A	IA	0.93	IA	0.99	IA	0.88	IA	0.90	IA	0.76
Oxidized dinoflagellate luciferin	IA	0.93	IA	0.99	IA	0.88	IA	0.90	IA	0.76
Hexaric acid	IA	0.74	IA	0.99	IA	0.99	A	0.53	IA	0.99
Juniperic acid	IA	0.79	IA	0.95	IA	0.97	A	0.55	IA	0.81
Diphyllin	A	0.50	IA	0.91	IA	0.89	A	0.50	IA	0.83
Diacetylphloroglucinol	IA	0.87	IA	0.97	IA	0.87	IA	0.63	IA	0.72
13S–hydroxy octadecadienoic acid	IA	0.69	IA	0.73	IA	0.61	IA	0.60	IA	0.97
Bruceantin	IA	0.79	IA	0.93	IA	0.94	A	0.53	IA	0.95

Note: Active (A); Inactive (IA)

3.5. Characteristics of extract nanophytosomes

Based on the in vitro assay results, MEW was identified as the most optimal extract (see Fig. 2(a) and 2(b)), leading to the development of nanophytosomes, employing soybean lecithin for further in vivo testing. As demonstrated by PSA analysis, the particle size distribution of NF–MEW showed that the nanoparticles formed were within the nanometer range, with homogeneous and stable particle size. It indicated their potential to influence the body's dissolution rate, absorption and drug distribution. The utilization of nanoparticles with a diameter smaller than 500 nm is considered ideal for drug delivery as they enhance penetration into cells and tissues [53]. The yield of MEW nanophytosome was 43.35±2.06% and the results of the particle size analyzer (PSA) measurement indicated the average particle size formed of 310.22 ± 12.00 nm (Fig. 6(a)). The polydispersity index of the mahogany seed nanophytosome was 0.1609 ± 8.72, indicating that the produced nanophytosomes exhibited a homogeneous and stable particle size. It has been demonstrated that the stability of nanophytosome is optimized by reducing the polydispersity index [40]. A polydispersity index below 0.5 signifies a more uniform particle size distribution within the formulation.

The formation of the mahogany seed nanophytosome complex can be confirmed through FTIR spectroscopy by comparing the spectrum of the complex with its components (mahogany seed extract and phosphatidylcholine), as demonstrated in (Fig 6b). The FTIR spectra indicated that MEW exhibited characteristic absorption at 3455 cm^{-1}, corresponding to the hydroxyl (–OH) group, 2952 cm^{-1}, indicating aliphatic C–H stretching, and 1730 cm^{-1}, associated with the carbonyl (C=O) group from flavonoid or ester compounds. In NF–MEW, minor shifts were observed in several peaks, including the –OH absorption shifting to 3457 cm^{-1} and the C=O absorption shifting to 1732 cm^{-1}.

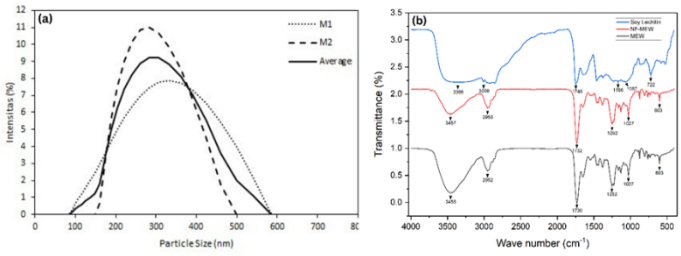

Fig. 6. Characteristics of mahogany seed extract (NF–MEW) based on particle size (a) and FTIR spectra (b)

This finding indicated the possibility of an interaction between the extract and the phospholipid from soybean lecithin. The spectrum of soybean lecithin exhibited characteristic absorption at 3366 cm^{-1} (–OH group), 3009 cm^{-1} (C–H), 1746 cm^{-1} (C=O ester from phospholipids), and other peaks such as 1166 cm^{-1} and 1067 cm^{-1}, which are associated with phosphate ($–PO_4$) groups. FTIR analysis confirmed interactions between mahogany seed extract and soybean lecithin in nanophytosome formation. This was evidenced by shifts in the absorption peaks of functional groups such as –OH and C=O in the NF–MEW spectrum compared to the individual spectra of MEW and lecithin, indicating complex formation [54]. This interaction can enhance the stability, solubility, and bioavailability of the active compounds in mahogany seed extract.

Table 6. Zeta potential of mahogany seed extract nanophytosome

Parameter	Average
Mean zeta potential (mV)	–10.74±1.59
Electrophoretic mobility (µm*cm/Vs)	–0.84±0.12
Conductivity (mS/cm)	4.74±0.01
Distribution peak (mV)	21.01±32.75

The zeta potential analysis of NF–MEW mahogany seed nanophytosomes yielded a value of –10.7412 ± 29.49, indicating that the phosphatidylcholine employed possessed a negative charge on the particle surface, rendering it susceptible to aggregation (see Table 6). The electrophoretic mobility value of –0.83715 µm·cm/Vs confirmed that the particles were negatively charged, and the solution conductivity of 4.735684 mS/cm suggested the presence of ions in the medium that may influence the charge distribution of the particles and potentially reduce system stability. The highly fluctuating peak distribution between 53.76 mV and –11.74 mV, with an average value of 21.01 mV, indicates significant variations within the system, possibly due to aggregation or measurement inconsistencies. The zeta potential analysis (Table 6) indicated that the phosphatidylcholine used carried a negative charge on the particle surface. Nanoparticles exhibiting a zeta potential greater than +30 mV or less than –30 mV are widely regarded as stable due to the robust repulsive forces between particles, thereby preventing aggregation [55].

3.6. Effectiveness of extract and extract nanophytosome in blood glucose regulation

The in vivo assay results on mice induced with Streptozotocin illustrate changes in blood glucose levels (Fig. 7(a)) and body weight in diabetic mice (Fig. 7(b)). The NF–MEW group demonstrated efficacy in the suppression of blood glucose spikes. Conversely, the MEW group exhibited a more pronounced effect on the reduction of body weight over the 21–day intervention period. Blood glucose and body weight measurements were taken at three-day interval. The NF–MEW group demonstrated the highest effectiveness in lowering blood glucose levels, with an average of 364 ± 102 mg/dL, compared to the MEW group, which reached 268 ± 68 mg/dL. On day 13, the mahogany seed extract treatment demonstrated a significant effect with $p<0.05$, as illustrated in the reduction of blood glucose levels (Fig. 7(a)). It has been demonstrated by preceding studies that the antihyperglycemic activity exhibited by mahogany seeds is attributable to the presence of β–sitosterol and fucosterol [56]. The weight loss observed in in each group was attributed to the diabetic condition, which increases free radicals in the body due to the destruction of pancreatic β–cells [57]. The inhibition of GLUT4 has been demonstrated to impede the transfer of monosaccharides from the blood for cellular maintenance and energy production, leading to excessive accumulation of reactive oxygen species within the body [58]. The ethanol extract of mahogany seeds has been found to contain flavonoids, tannins, triterpenoids, saponins, and alkaloids, which act as antioxidants to break the process of oxidative chain reaction [59].

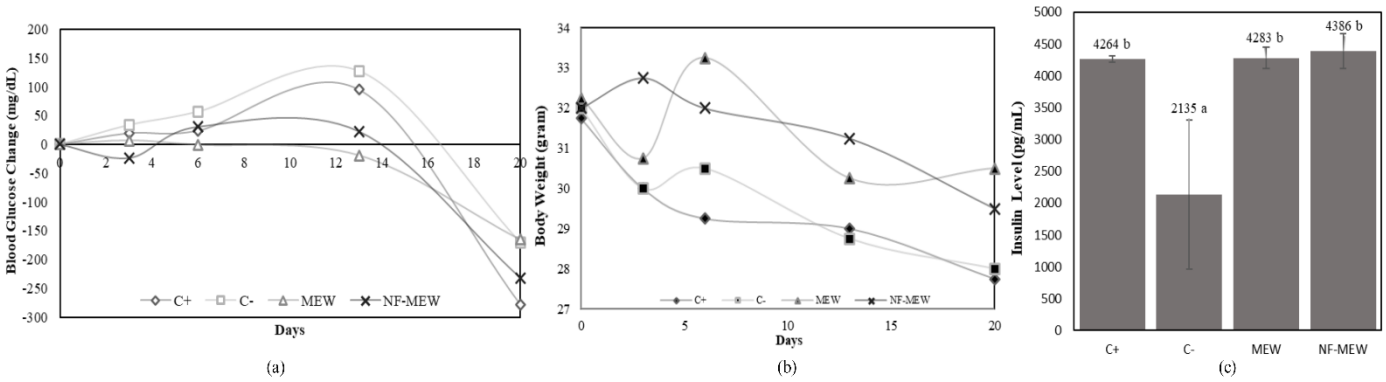

Fig. 7. Blood glucose change levels (a) and body weight (b) during treatment period and plasma insulin concentration after 20 days of treatment. Positive control (C+/Glimepiride), negative control (C–), MEW treated group, and NF–MEW treated group. Note: A significant reduction in blood glucose levels was observed in the NF–MEW group compared to the negative control group (C−) on day 13 ($p = 0.007$), indicating a highly significant result

A significant divergence in insulin secretion was observed among the treatment groups (Fig 6(c)). The negative control (C−) exhibited the lowest insulin level of 2135 pg/mL ($p < 0.05$), which was significantly lower than the level observed in the other groups. The positive control (C+) exhibited an increase in insulin secretion to 4264 pg/mL, which was comparable to the MEW group (4283 pg/mL) and the NF–MEW group (4386 pg/mL). A non-significant difference was observed among these three groups ($p > 0.05$). The findings of this study suggest that mahogany extract (MEW) has the potential to enhance insulin secretion to a significant extent, as evidenced by a comparison to the negative control. The effectiveness of MEW in this regard is comparable to that of the positive control. The results of the insulin secretion were consistent with in vitro and in–silico tests, indicating that both MEW and NF–MEW have the potential to enhance insulin sensitivity, thereby contributing to improved glycemic control as observed in diabetic animal models. This finding is consistent with previous research demonstrating that the administration of an ethanol extract of mahogany seeds at 500 mg/kg body weight resulted in a significant increase in the number of pancreatic islet cells and a reduction in blood glucose levels from >200 mg/dL to a normal range [60]. Consequently, these research findings further reinforce the hypothesis that mahogany seed extract, in both MEW and NF–MEW forms, has the potential to act as an anti–diabetic agent capable of enhancing insulin secretion.

The results of hematological profile analysis (see Table 7) demonstrated significant differences between the treatment and control groups in several blood parameters. The MEW group exhibited the highest hemoglobin (HGB) level (15.5±10.2 g/dL), which was significantly higher than both the positive control (K+) and negative control (K–) groups. This finding suggests that the treatment administered in the MEW group may have the capacity to enhance red blood cell production or improve oxygen transport efficiency in the body. The Mean Corpuscular Volume (MCV) values demonstrated no significant differences among the groups, ranging from 43.2 to 47.5fL. This finding indicates that, despite fluctuations in hemoglobin and blood cell count, red blood cell size maintained relative stability. Consequently, the treatments administered in the MEW and NF–MEW groups may improve blood quality by increasing hemoglobin and erythrocyte levels while concomitantly reducing signs of inflammation. As depicted in Table 7, the levels of hemoglobin in the MEW group were found to be the highest, thus demonstrating a robust correlation with its protective effect against oxidative stress induced by diabetes. This finding aligns with previous studies reporting that treatment with certain bioactive compounds can enhance hemoglobin production in diabetic conditions associated with anemia [61]. NF–MEW demonstrated the highest MCV values (see Table 7), indicating that an increase in MCV may signify enhanced erythrocyte maturation and increased red blood cell volume. These parameters are frequently affected in diabetes due to impaired red blood cell metabolism [62].

Table 7. Hematological profile of mice after induction for 21 days

Sample	HGB (g/dL)	WBC (10^3mm^3)	Neu (10^6/mm^3)	RBC (10^6/mm^3)	MCV (fL)
K (+)	11.9±4.7a	8.5±3.2c	3.9±1.3b	8.4±0.4a	43.2±0.9a
K (–)	12.2±7.4a	6.0+0.9bc	1.8±0.4a	8.7±1.0ab	43.9±3.8a
MEW	15.5±10.2b	4.0±0.4bc	1.2±0.3a	10.1±0.6ab	45.4±0.4a
N–MEW	13.9±3.2a	5.0±0.8c	1.1±0.4a	8.7±0.1b	47.5±1.7a

Note: HGB: Hemoglobin, WBC: White Blood Cell, NEU: Neutrophil, RBC: Red Blood Cell, MCV: Mean Corpuscular Volume. Different superscripts within the same row indicate significant differences at a 95% confidence interval ($p<0.05$).

3.7. Evaluation of the effectiveness of extract and extract nanophytosome on the structure of pancreactic β-Cells through histopathological analysis

The profile of beta cell count in the Langerhans islets of mice is presented in Fig. 8. The detection of beta cells was accomplished through the utilization of the Hematoxylin and Eosin (HE) staining technique. These cells demonstrated a positive reaction, manifesting as a purple coloration within the Langerhans islets. The purple coloration was produced by the application of hematoxylin, which stains pancreatic cells [31]. The hematoxylin and Eosin in pancreatic beta cells from the treated groups can be observed in Fig. 8(a). The number of beta cells in the Langerhans islets of pancreatic tissue in treated mice, observed at 400× magnification, is presented in Fig. 8(b).

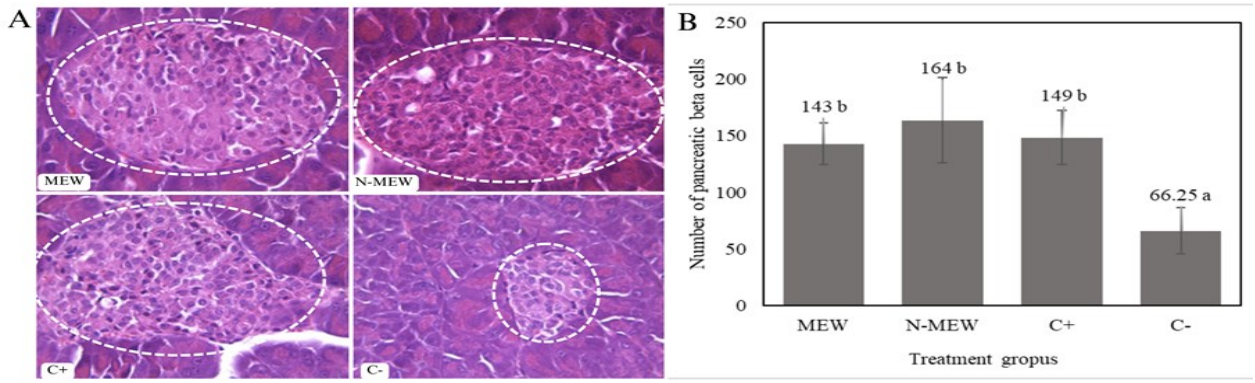

Fig. 8. Photomicrograph of beta cells stained with hematoxylin–eosin (HE) within the islets of Langerhans (A) and the profile of the beta cell count in the pancreas of treated mice (B). The white dashed lines represent beta cells within the islets of Langerhans. The MEW and N–MEW groups showed a significantly higher number of beta cells ($P \leq 0.05$) compared to the C– group

The N–MEW treatment group exhibited the highest number of pancreatic beta cells, while the C– treatment group exhibited the lowest. The MEW, N–MEW, and C+ groups demonstrated a substantial increase ($P<0.05$) in beta cell count compared to the C– group. Previous studies have shown that terpenoid compounds can enhance insulin secretion by stimulating beta–cell regeneration and exerting antioxidant effects [63]. Consistent with these findings, MEW and N–MEW contain the highest abundance of terpenoid and alkaloid compounds. A molecular docking analysis was conducted on four insulin secretion target proteins, which revealed strong binding interactions with bioactive compounds. Terpenoids present in mahogany seeds have been observed to activate the PI3K/Akt pathway, a process that plays a role in beta–cell proliferation and protection. In addition, alkaloids can enhance cellular sensitivity to insulin, thereby optimizing blood glucose regulation [64].

4. Conclusion

The type of solvent utilized exerted an influence on the extraction yield and the antidiabetic activity through insulinotropic mechanisms as well as the cytotoxicity of the resulting extract on BRIN–BD11 β–pancreatic cells. The highest extraction yield of mahogany seed extract was obtained using the UAE method for 45 minutes (three extractions after clean–up with n–hexane) was obtained from the ethanol–water (50%) dissolved extract (MEW), reaching 16.41%. At a concentration of 1000 µg/mL, MEW exhibited the lowest level of cytotoxicity towards BRIN–BD11 β–pancreatic cells, indicating its safety. The study revealed that MEW demonstrated the most pronounced insulinotropic effect, with an insulin concentration of 245.6 pg/mL. Phytochemical profiling using LC–MS/MS identified nine compound classes, predominantly terpenoids. The molecular docking validation of 15 compounds with the highest concentration abundance from MEW successfully evaluated their potential interactions with four key target proteins: GLP1–R, PI3Kα, PERK, and IRK. The analysis indicated that fixpide, oxidized dinoflagellate luciferin, diphylin, and apedonoson exhibited the highest binding affinity, demonstrating the greatest potential in influencing insulin secretion activity. The evaluation of the toxicity profile indicated that most compounds from MEW exhibited low toxicity. Oxidized dinoflagellate luciferin displayed high activity in the PPAR–Gamma and Nrf2/ARE pathways, thus rendering it a potential candidate for diabetes therapy. The MEW was insulinotropic without toxicity and was formulated into nanophytosomes (NF–MEW) using soybean lecithin. NF–MEW exhibited homogeneous and stable particles, thereby enhancing the dissolution and bioavailability of active compounds. FTIR analysis was employed to confirm extract–lecithin interactions, thus reinforcing complex stability. In vivo tests on diabetic mice demonstrated that MEW has the potential to reduce blood glucose levels, while NF–MEW also has the potential to rectify erythrocyte metabolism disorders in diabetic conditions and enhance insulin secretion with an efficacy comparable to glimepiride.

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Ethic Declarations

This analysis has passed ethical review by the Animal Ethics Commission of the School of Veterinary Medicine and Biomedicine, Institut Pertanian Bogor, with the document number 280/KEH/SKE/XII/2024.

Author Contributions

Conceptualization and Project Administration: Rita Kartika Sari (RKS), Yanico Hadi Prayogo (YHP), Wasrin Syafii (WS), Eva Harlina (EH) and Bayu Febram Prasetyo (BFP); Resources: RKS; Methodology: RKS, YHP; Setyanto Tri Wahyudi (STW); Validation: RKS, STW, YHP, Zikri Hamidi (ZH), Adinda Zahra Marissa Kasmi (AZMK); Formal Analysis: RKS, STW, YHP, ZH; Investigation: RKS, STW, YHP; Data Curation: RKS, STW, YHP, ZH; Writing Original Draft Preparation: RKS, YHP, ZH; Writing Review and Editing: RKS, YHP,

STW, ZH; Visualization: STW and ZH; All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest

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