Results in Chemistry 20 (2026) 103007

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

Comparative study of *Pogostemon benghalensis* extracts using different methods: chemical profiling, antibacterial, *in vitro* pancreatic lipase and diabetic enzyme inhibition activities

Udayakumar Sandhiya [a], Santosh Chokkakula [b], Selvaraju Parthibhan [c], Abdullah Alfalih [d], Ye Zhao [e,f], Azhagiya Manavalan Lakshmi Prabha [a,**], Uma Maheshwari Rajadurai [g], Joe Antony Jacob [h], Fuad Ameen [d], Bing Yang [f,*]

[a] *Department of Botany, Bharathidasan University, Tiruchirappalli 620 024, India*
[b] *Department of Microbiology, College of Medicine and Medical Research Institute, Chungbuk National University, Cheongju, Republic of Korea*
[c] *Department of Botany, Kalaignar Karunanidhi Government Arts College for Women (Autonomous), Pudukkottai, India*
[d] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[e] *Institute of Chinese Medicine, Yanting County People's Hospital, Yanting, China*
[f] *Department of Public Health, International School, Krirk University, Bangkok, Thailand*
[g] *Department of Biotechnology, Bishop Heber College (Autonomous), Tiruchirappalli 620017, India*
[h] *Department of Biomaterials, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 600077, Tamil Nadu, India*

ARTICLE INFO

Keywords:
Pogostemon benghalensis
UAE-extract
Anti-obesity

ABSTRACT

The present study aimed to compare three extraction methods for the quantity and identification of phytochemicals from *Pogostemon benghalensis* leaf extract using FTIR and GC–MS, and to evaluate their antibacterial and pancreatic lipase (PL) enzyme activity. Ultrasonication-assisted extraction (UAE) was found to be the most efficient method for extracting phenols (47.40 mg GAE/g) and flavonoids (31.78 mg RTE/g), suggesting further research on UAE techniques for phenol content. GC–MS analysis identified key bioactive compounds such as eugenol, hexadecenoic acid, and cis-vaccenic acid in all extraction methods. UAE yielded 50 compounds, microwave-assisted extraction (MAE) 39 compounds, and Soxhlet-assisted extraction (SAE) yielded 35 compounds. The UAE extract showed significant antibacterial activity and 57 % PL enzyme inhibition at 100 mg/ml, comparable to the standard drug orlistat. The extract also exhibited dose-dependent inhibition of alpha-amylase and alpha-glucosidase, indicating its potential as an alternative to synthetic drugs. This study provides a comparative analysis of extraction methods for *P. benghalensis* constituents and their anti-obesity effect *in vitro*.

1. Introduction

Plants have served as the primary source of medicine since ancient times. Herbal remedies are in high demand globally due to their cultural acceptance and minimal side effects. As a result, the nutraceutical and cosmetic industries are developing a wide range of products using plant-derived active ingredients or extracts [1]. Extraction is a chemical process used to obtain and purify intracellular bioactive chemicals from biomass (such as bacteria, plants, and animal cells) [2].

Conventional extraction methods, such as hot water bath, maceration, and soxhlet extraction, are commonly used by small–scale research and production firms, while non-conventional extraction methods such as, ultrasound, microwave, pressurized liquids, enzymatic hydrolysis, supercritical fluids, high hydrostatic pressure, and pulsed electric fields utilize modern techniques with high energy input and processing capacity, leading to enhanced extraction efficiency and selectivity [3].

Optimization of the extraction method is a critical phase in the preparation of plant products to achieve a high yield of desired chemicals [4]. Ultrasonication-assisted extraction (UAE), soxhlet-assisted

* Corresponding author at: Department of Public Health, International School, Krirk University, 3# Soi Ramintra 1, Anusawari Subdistrict, Bang Khen District, Bangkok 10220, Thailand.
** Corresponding author.
E-mail addresses: dralprabha@yahoo.com (A.M.L. Prabha), yang.bing@krirk.ac.th (B. Yang).

https://doi.org/10.1016/j.rechem.2025.103007
Received 6 July 2025; Accepted 22 December 2025
Available online 26 December 2025

extraction (SAE), and microwave-assisted extraction (MAE) are traditional methods for extracting bioactive components from plant matrices using common solvents [5]. These methods have gained popularity due to their various advantages over conventional processes, such as reduced solvent and energy consumption and increased yield [6]. UAE induces additional vibration in plant sample molecules, promoting cavitation and faster mass transfer and diffusion, which enhances chemical release [[7,8]]. MAE uses electromagnetic waves to facilitate solvent entry and improve the yield of bioactive compounds. These techniques have been shown to increase the yield of phenolic compounds, with antioxidant, antibacterial, and anti-obesity properties [[9–11]]. Higher temperatures in the UAE result in more cavitation bubbles, improving the efficiency of the extraction process [12]. Diabetes mellitus (DM) is a metabolic disease characterized by persistent hyperglycaemia and abnormalities in carbohydrate, fat, and protein metabolism due to insulin action or synthesis [13]. It is a serious illness and ranks as the third leading cause of death worldwide [14].

The global prevalence of diabetes is increasing, with type 2 diabetes becoming more common despite the availability of numerous synthetic anti-diabetic medications. The significant adverse effects associated with these treatments highlight the need for safer, more affordable, and effective management strategies. One crucial enzyme in glucose metabolism is alpha amylase, which breaks down dietary starch into trisaccharides and disaccharides, converting them into glucose. Alpha amylase inhibitors bind to mammalian alpha-amylase to regulate blood glucose levels by mitigating excessive starch hydrolysis [15]. Alpha-glucosidase, found in the human small intestine, plays a key role in converting carbohydrates into glucose. By inhibiting this enzyme, the amount of glucose entering the bloodstream can be reduced, offering a potential strategy for managing blood sugar levels [16].

Pancreatic lipase (PL) is an essential enzyme involved in the digestion of dietary fat. It breaks down dietary triglycerides into monoglycerides and free unsaturated fats. Orlistat, a medicine produced from *Streptomyces toxitricini*, is a strong inhibitor of gastric, pancreatic, and carboxyl ester lipases. Due to this property, it has shown potential as an effective treatment for obesity [17]. Natural inhibitors derived from various plant sources, such as flavonoids, polyphenols, alkaloids, saponins, and terpenoids can inhibit PL activity and reduce fat absorption into the systemic circulation [[18,19]]. Current medications for obesity, such as orlistat, have systemic side effects, including breathing difficulties, swelling of the face, throat, and tongue, oily stool, gas, stomach pain, nausea, vomiting, and diarrhoea [20]. Natural inhibitors offer a promising alternative with fewer systemic side effects.

Pogostemon benghalensis (Burm. f) Kuntze, a herbaceous plant belonging to the family Lamiaceae, has been used for medicinal purposes for centuries. This plant extract has been reported to have antibacterial, antioxidant, anti-inflammatory, and antipyretic properties, along with antifungal, antiviral, larvicidal, and anti-cancer effects [21]. Preliminary studies have identified bioactive compounds, such as flavonoids, phenolics, and terpenoids, in the plant, known for their medicinal benefits. These compounds form the basis for exploring the plant's therapeutic potential, including its antibacterial and anti-obesity effects [22]. Based on the ethnobotanical significance and emerging phytochemical profile, this plant was selected for the study.

This study aims to evaluate, *via* GC–MS profiling the *P. benghalensis* extract to identify its key bioactive compounds and provide a comprehensive chemical profile. The study also assessed its potential as a pancreatic lipase inhibitor for anti-obesity treatments and evaluated its antibacterial activity. This research is the first international comparative analysis of various extraction techniques, including UAE, MAE, and SAE, and the first report on the anti-obesity effects of these extracts *in vitro*. By integrating traditional knowledge with modern analytical techniques, this study aims to validate the efficacy of *P. benghalensis* and support its potential applications in developing novel therapeutic agents for antibacterial and anti-obesity treatments.

2. Materials and methods

2.1. Collection of plant material

The plant leaves were collected from Kolli Hills (Latitude:10°12′–11°7′N, Longitude: 76°–77°56′E) of Namakkal district, Tamil Nadu, India. The identity of the plant was confirmed at the Botanical Survey of India (BSI), Southern Regional Circle, Coimbatore, India. The voucher specimen (BSI/SRC/5/23/2024-25/Tech./603) has been deposited at Bharathidasan University's High-Altitude Plant Centre, a part of its Department of Botany. After being washed with tap water, the sample was allowed to air dry for 2 weeks under the shade. Then the dried leaves were roughly ground in an electric homogenizer and collected in an airtight container and stored in a refrigerator until further processing.

2.2. Extraction methods

Hydromethanolic solvent (aqueous: methanol at 1:1 ratio) was preferred for all the extraction methods as it employs two aqueous solvents, which helps in getting a better yield of phytochemicals [23].

2.2.1. Ultrasound-assisted extraction method (UAE)

For the UAE, Hromadkova and Ebringerova [24] protocol was followed with slight modifications. 250 ml of hydromethanolic solvent was used for 10 g of the leaf sample. The extraction was carried out for 20 min in an ultrasonicator (BRANSON Digital Sonifier, model: 102C (CE), EDP: 101-135-066R). Later, the extract was filtered through No. 1 Whatman filter paper, and the solvent was evaporated using a rotary evaporator.

2.2.2. Microwave-assisted extraction method (MAE)

For MAE, 10 g of leaf sample was mixed with 250 ml of the solvent in an extraction vessel. The extraction was performed in a microwave oven at 400 watts for 10 min (Panasonic NNSM25JB). The solvent was then evaporated using a rotary evaporator [25].

2.2.3. Soxhlet-assisted extraction method (SAE)

10 g of leaf samples was extracted using 250 ml hydromethanolic solvent for 24 h in 500 ml soxhlet apparatus equipment. Then, the solvent was evaporated at decreased pressure using a rotary evaporator [26].

2.3. Determination of extraction yield

All the extracts were evaporated in a rotary evaporator at 40 °C for 6 h. The resulting extracts were then stored in the refrigerator until further use. The yield of the extracts was calculated by dividing the final weight of the extracted material by the initial weight of the leaf powder used for extraction and multiplying the result by 100. The yield of the extracts was given in percentage [27].

2.4. Quantitative analysis

2.4.1. Determination of total phenolic content

The Folin Ciocalteu reagent was used to assay total phenols with minor modifications following previous reports [[28,29]]. Folin Ciocalteu reagent (5 ml, 1,10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M) mixture was added to the dilute extract (50, 100, 150, 200, 250, 300 mg/ml) or gallic acid (standard phenolic component). After 15 mins, the total phenols were quantified using colorimetry at 765 nm. Total phenol levels are expressed in terms of gallic acid equivalent.

2.4.2. Determination of total flavonoid content

The total flavonoid content was determined using a colorimetric test

with aluminium chloride following Muhongo et al., [30], with some modifications. 1 ml (1 mg/ml) crude extract, 4 ml distilled water, and 0.3 ml of 5 % sodium nitrite were taken in 10 ml volumetric flask for the reaction. After 5 mins, 0.30 ml of 10 % aluminium chloride was added. Furthermore, after 6 mins of incubation, 2 ml of 1 M sodium hydroxide was added to the reaction mixture, and the total volume was promptly diluted to 10 ml with distilled water. The same method was used to prepare a set of standard rutin solutions (50, 100, 150, 200, 250, 300 g/ml). The absorbance of the test and standard solutions was measured at 510 nm using a reagent blank.

2.5. Fourier-transform infrared spectroscopy (FTIR) analysis

The FTIR spectrum was recorded to comprehend and identify the functional groups. The FTIR spectrum was measured between 400 and 4000 cm^{-1} using the three different extracts in a PerkinElmer Spectrum (version 0.03.09) instrument.

2.6. Gas chromatography-mass spectrometry (GC–MS)

The Clarus 680 GC instrument was employed for the investigation, which used a fused silica column packed with Elite5MS (5 % biphenyl, 95 % dimethylpolysiloxane, 30 m, 0.25 mm ID, 250 m df) to separate the components using helium as a carrier gas at a constant flow rate of 1 ml/min. During the chromatographic run, the injector temperature was set to 260 °C. The oven temperature was as follows for the 1 µl extract sample injected into the instrument: 60 °C for 2 mins, then 300 °C at a ramp rate of 10 °C per min for 6 mins. The mass detector had the following parameters: transfer line temperature of 230 °C, ion source temperature of 230 °C, ionization mode electron impact set at 70 eV, scan period of 0.2 s, and scan interval of 0.1 s. The fragments range from 40 to 600 Da. The components' spectra were compared to a database of known component spectra included in the GC-MS NIST (2008) library.

2.7. Antibacterial activity

A modified technique was used to study the antibacterial activity, adopted from the previous studies by Brinda et al. [31] and Vaghasiya et al. [32]. The typical disc diffusion method was used to work with human pathogenic bacteria such as *Salmonella typhi* (MTCC 733) and *Staphylococcus aureus* (MTCC 7443). Luria Bertani (LB) broth/agar medium was utilized to cultivate the bacteria. Fresh inoculum cultures (100 µl each) were dispensed onto LB agar plates overnight. To each plate, a sterile 5 mm paper disc loaded with 100 µl, 200 µl, and 300 µl of hydromethanolic leaf extracts and streptomycin (positive control) or kanamycin (negative control) discs were introduced. All the plates were kept at 37 °C in an incubator overnight. The diameter of the clear zone surrounding the disc was used to estimate the zone of inhibition.

2.8. In vitro pancreatic lipase enzyme assay

The study sought to assess the activity of PLs by assessing the rate of oleic acid release from an emulsified *P. benghalensis* leaf extract. This assay followed the procedure of Vadivelu et al., [33] with minor modifications. To generate the substrate emulsion, 15 mmol/L sesame oil was sonicated for 5 mins in a solution comprising 1 mmol/L NaCl, 1 mmol/L $CaCl_2$, 10 mg bovine serum albumin/ml, and phosphate buffer solution (pH 8.0). After sonication, the substrate emulsion was incubated with 50 µl of porcine pancreatic lipase and varying doses of *P. benghalensis* leaf extract (20, 40, 60, 80, 100 µg/ml) for 30 mins at 37 °C. Following the 30-mins incubation period, 3 ml of chloroform and n-heptane mixture (1,1, *v*/v) was added and extraction was done by shaking. After that, a dose-dependent inhibition effect was observed using a calorimeter.

2.9. In vitro anti-diabetic activity

2.9.1. Alpha-amylase inhibitory method

Firstly, test tubes were taken, and different concentrations (20, 40, 60, 80, 100 µl) of hydromethanol leaf extract were mixed with 250 µl of 0.02 M buffer solution (sodium phosphate), and α-amylase. 0.02 M of 1 % starch solution was taken in a buffer solution (phosphate) (pH 6.9). This reaction was terminated by the addition of 500 µl of DNS (dinitrosalicylic acid) reagent. For five mins, these test tubes were submerged in boiling water. After that, the mixture was allowed to cool to room temperature. Finally, 5 ml of distilled water was added to dilute the reaction. At 540 nm, the absorbance was measured using a spectrophotometer [34]. The percentage of α-amylase inhibition activity was calculated as follows:

$$\text{Percentage inhibition} = \left[\text{Abs control}-\frac{Abs\ Ethanol\ extract}{ABS\ \text{control}}\right] \times 100$$

2.9.2. Alpha-glucosidase inhibitory method

The method to determine the effects of the ethanolic extract on α-glucosidase was followed as described by Li et al. The solution of p-NPG (nitrophenyl glucopyranoside) was prepared in a buffered solution (phosphate-20), and pre-incubation was performed with 50 µl of different concentrations. α-glucosidase (100 µg/ml) (pH 6.9) was added to the solutions containing different concentrations (20–100 µg/ml) of hydromethanol leaf extract. The mixture was at this stage incubated at 37 °C for 10 mins. This process is stopped by adding 2 ml of sodium carbonate (0.1 M). The effects on α-glucosidase activity were measured by the intensity of yellow colour at 450 nm [[35,36]].

2.10. Statistical analysis

All the results are expressed as mean ± S.D. Statistically, all the data were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software version 26.26, and *p*-values <0.05 are considered statistically significant.

3. Results and discussion

3.1. 3.1. Extract yield of different extraction methods

The efficiency and effectiveness of the extraction process are generally influenced by various factors, such as solvent properties, temperature, particle size, pH, extraction duration, agitation, solvent-to-solute ratio, feed material characteristics, and environmental considerations [19]. Ultrasonic–assisted extraction (UAE) is a recognized novel, efficient, and environmentally friendly technique that preserves the integrity of sensitive compounds while remaining cost-effective and sustainable compared to traditional methods [37]. In this study, the UAE method yielded the highest extraction efficiency while using the hydromethanolic solvent (Table 1).

The characteristics of the UAE extraction process, such as mass transfer intensification, solvent penetration into plant material, and cell

Table 1
Effect of different extraction methods on the yield of *P.benghalensis leaf* sample (g/100 g of DW).*

Solvent	Extraction methods	Extraction of yield (100 g)
Hydromethanol (1:1)	Ultrasound-assisted extraction	45.5 ± 0.45^{a}
	Microwave-assisted extraction	41 ± 0.86^{b}
	Soxhlet-assisted extraction	40.9 ± 0.95^{b}

* Values are presented as mean ± standard deviation (n = 3); the mean in the rows bearing different superscripts are significantly different.

rupture facilitation by ultrasonics, contributed to the maximum yield [38,39]. Ultrasonic and microwave extraction methods are preferred for their energy efficiency, environmental friendliness, and production of high-quality extracts at a reasonable cost compared to the use of traditional methods like soxhlet and maceration [[40,41]]. The rationale for using 400 W in MAE is supported by earlier optimization studies showing that phenolic and antioxidant yields generally peak at this power level, while higher powers often lead to reduced yields due to thermal degradation. Previous optimization trials further confirmed that lower microwave powers resulted in insufficient extraction efficiency, whereas higher powers increased the risk of compound degradation [[42,43]]. Therefore, 400 W was selected for our study as it could provide the best balance between extraction efficiency and compound stability, ensuring consistent and reproducible results.

Aqueous solvents are considered green solvents and a viable alternative to typical organic solvents due to their non-toxic nature, low cost, and polarity, water besides their dielectric constant. The dielectric constant of water (78.5 at room temperature) decreases as the temperature rises, impacting the extraction yield significantly [44]. The polarity of the solvent significantly impacts yield, as demonstrated by numerous studies [45]. An ideal extraction technique should be user-friendly, safe, scalable, affordable, and suitable for industrial use. In this study, the UAE method displayed the highest yield compared to other methods, marking the first report on *P. benghalensis* extraction using this technique.

3.2. Quantitative analysis of phytoconstituents

In the current study, all the extracts from UAE, MAE, and SAE of *P. benghalensis* were used to assess the total phenolic and flavonoid contents. Compared to all other extraction methods, the leaf extract from UAE had the highest phenol content (47.40 mg GAE/g) and flavonoid content (31.78 mg RTE/g), followed by MAE, in which the total phenol and flavonoid contents were 22.73 mg GAE/g. and 15.89 mg RTE/g, respectively (Table 2). The extract from SAE had a relatively similar phenolic content as that of MAE, while the flavonoid content was considerably low. Sharifi-Rad et al. [46] reported that phenolic compounds can inhibit free radicals and inactive metals, scavenge oxygen, and decompose peroxides in biological systems, the processes through which they can reduce the risk of oxidative stress, aging, and other related diseases. Since both conventional and non-conventional methods have extracted the above-mentioned bioactive compounds, recent reports on *Moringa oleifera* and *Spondias purpurea* have revealed a similar higher content from UAE compared to other extraction methods [[47,48]].

3.3. Fourier-transform infrared spectroscopy (FTIR)

FTIR plays an important role in identifying the functional groups present in plant samples. It is an analytical method where infrared light is used to scan and identify the organic, polymeric, and inorganic materials that have many compounds with different bonds (C—C, C═C, C≡C, C—O, C═O, O—H and N—H) [49]. In the present study, the functional groups in the three different extracts of *P. benghalensis* were analyzed and presented (Fig. 1, supplementary Tables 1 to 3). In the UAE method, 13 characteristic peaks were present, among which the major five peaks were observed at 3332.04 cm-^{1} (N—H stretch), 1598.53 cm^{-1} (C—C stretch), 1066.17 cm^{-1} (C—N stretch), 2354.23 cm^{-1} (—C≡C— stretch) and 2926.99 cm^{-1} (C—H stretch) alongside four minor peaks at 1395.53 cm^{-1} (C—H stretch), 3777.88 cm^{-1} (O—H stretch), 3695.34 cm^{-1} (O—H stretch free hydroxyl) and 1722.43 cm^{-1} (C═O stretch). The diversity of peaks suggests that cavitation enhanced solvent penetration, liberating a wide range of polar and thermo-sensitive compounds such as phenolics, flavonoids, and carboxylic acids [50]. The MAE showed a total of 5 peaks, where the major peaks were observed at 1020.31 cm^{-1} (C—N stretch), 3327.11 cm^{-1} (O—H stretch), and 2941.20 cm-^{1} (C—H stretch). Minor peaks were observed at 2831.07 cm^{-1} (C—H stretch) and 1449.05 cm^{-1} (C—H bend). This narrower spectrum indicates selective extraction of stable polar compounds, while the absence of certain carbonyl and aromatic signals suggests possible degradation of thermo-labile groups under microwave heating [51]. The SAE revealed the presence of 10 functional groups, where 3 major peaks were observed at 1597.67 cm^{-1} (C—C stretch (in-ring), 1047.80 cm^{-1} (C—N stretch), and 3326.92 cm^{-1} (O—H stretch). Also, 4 minor peaks were observed at 672.22 cm^{-1} (C—Br stretch), 814.87 cm^{-1} (C—Cl stretch), and 1262.57 cm^{-1} (C—H wag (—CH2X)). As per the analysis, halogen-related stretches (C—Br, C—Cl) and CH2 wagging vibrations were not observed in either UAE or MAE. This reflects broader recovery of non-polar and halogenated constituents, consistent with the solvent's ability to dissolve diverse phytochemicals [52,53]. The comparative FTIR analysis demonstrates that the extraction method significantly influences the chemical composition of *P. benghalensis* extracts. UAE maximized phytochemical diversity by recovering both polar and thermo-sensitive compounds. MAE was more selective for stable polar groups but limited in diversity, and SAE displayed a wider range of non-polar and halogenated compounds. These findings highlight that UAE is the best suited method for broad phytochemical profiling, MAE for rapid recovery of stable polar constituents, and SAE for conventional extraction of both polar and non-polar groups. The functional groups observed

Table 2
Total phenol and flavonoid contents of *P. benghalensis* leaf hydromethanolic extracts obtained from UAE, MAE, and SAE.*

Method	Concentration (mg/ml)	Total phenols (mg GAE/g)	Total flavonoids (mg RTE/g)
Ultrasound-assisted extraction (UAE)	50	12.15 ± 0.03^{f}	10.82 ± 0.05^{f}
	100	19.86 ± 0.08^{e}	13.79 ± 0.03^{e}
	150	27.25 ± 0.02^{d}	17.56 ± 0.06^{d}
	200	33.78 ± 0.09^{c}	22.23 ± 0.28^{c}
	250	40.14 ± 0.07^{b}	26.55 ± 0.09^{b}
	300	47.40 ± 0.02^{a}	31.78 ± 0.07^{a}
Microwave-assisted extraction (MAE)	50	5.42 ± 0.02^{f}	5.54 ± 0.01^{f}
	100	8.83 ± 0.03^{e}	7.42 ± 0.05^{e}
	150	12.77 ± 0.04^{d}	9.05 ± 0.14^{d}
	200	16.25 ± 0.02^{c}	12.00 ± 0.09^{c}
	250	20.13 ± 0.09^{b}	14.77 ± 0.07^{b}
	300	22.73 ± 0.05^{a}	15.89 ± 0.12^{a}
Soxhlet-assisted extraction (SAE)	50	6.33 ± 0.01^{f}	2.81 ± 0.03^{e}
	100	10.60 ± 0.09^{e}	3.46 ± 0.06^{e}
	150	13.43 ± 0.04^{d}	5.13 ± 0.16^{d}
	200	17.25 ± 0.02^{c}	6.61 ± 0.07^{c}
	250	21.02 ± 0.01^{b}	8.60 ± 0.09^{b}
	300	23.60 ± 0.04^{a}	10.47 ± 0.00^{a}

* Values are presented as mean ± standard deviation (n = 3); the mean in the rows bearing different superscripts is significantly different.

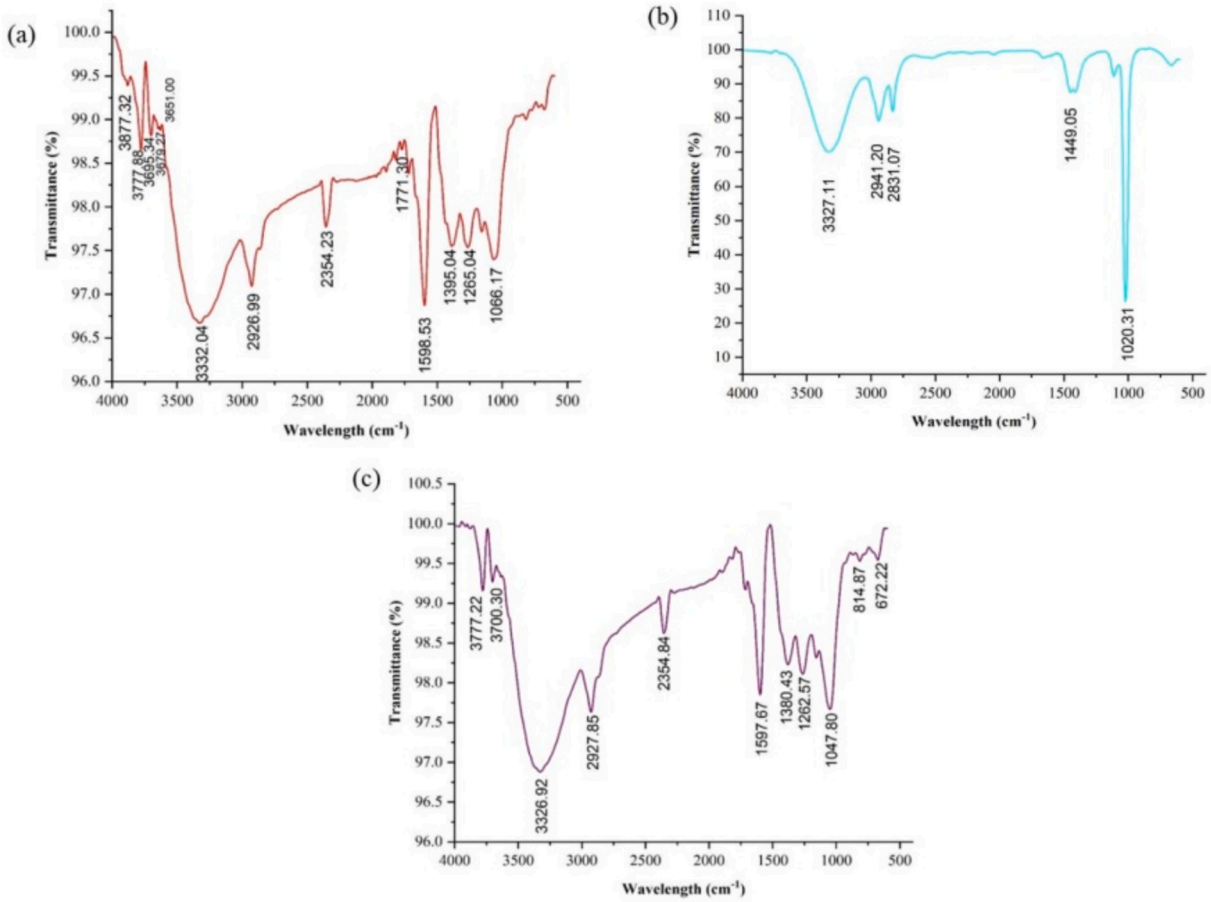

Fig. 1. The FTIR spectrum of *P. benghalensis* leaf hydromethanolic extracts obtained from (a) UAE, (b) MAE and (c) SAE methods.

from different extraction methods of *P. benghalensis*, including alcohols, phenols, aromatic amines, carboxylic acids, alkanes, ketones, aliphatic amines, and nitriles, are likely responsible for a broad range of pharmacological activities. Among the compounds under consideration, phenolic compounds characterized by hydroxyl and aromatic moieties have been extensively studied and are known to exhibit strong antioxidant, anti-microbial, anti-inflammatory, and anti-cancer properties [54]. Ketones and ketone-derived compounds, such as β-aminoketones, have been reported to possess vasodilatory, cardioprotective, and metabolic regulatory effects [55]. Additionally, plant extracts enriched with these functional groups have demonstrated radical-scavenging, enzyme-inhibitory, and antimicrobial activities in various *in vitro* studies [56]. Therefore, the diverse FTIR profiles observed in our extracts indicate that the biological activities measured, such as antioxidant, antimicrobial, and cytoprotective effects are likely attributable to the combined action of multiple bioactive constituents, potentially acting synergistically.

3.4. Gas chromatography-mass spectrometry (GC–MS)

The GC–MS analysis has been used to examine the bioactive compounds present in the plant extract and determine the reproducibility, dynamic range, and universal mass of the bioactive compounds with a small molecular weight [57]. The GC–MS analysis of *P. benghalensis* leaf hydromethanolic extract revealed eugenol, hexadecenoic acid and cis-vaccenic acid as the major compounds from UAE, MAE, and SAE-assisted methods, respectively (Fig. 2). All these compounds showed corresponding functional groups in the FTIR spectrum. In the UAE method, a total of 50 phytocompounds were identified, with eugenol (9.72 %) being the major compound (supplementary Table 4). Eugenol is a phenolic compound known for its diverse pharmacological properties. It is predominantly found in fragrant and therapeutic plants, such as clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum spp.*), and basil (*Ocimum basilium*) [58,59]. These two studies have reported that eugenol can be readily isolated by adding bases, which form eugenolate compounds that dissolve in water, allowing for its recovery by the introduction of an acid.

Several studies have shown the biological properties of eugenol beyond its antimicrobial and anticancer effects, with additional benefits such as herbicidal activity, making it a valuable compound in both medicine and agriculture [60,61]. Other significant compounds identified in the UAE-extract include hexadecenoic acid (6.84 %), 1,3,5-Triazine-2,4,6-triamine, N (5.99 %), benzenepropanoic acid (5.76 %), octadecanoic acid (5.69 %), and coumarin (4.59 %).

MAE-extract revealed the presence of 39 phytocompounds with the major compounds being hexadecanoic acid (14.93 %), octadecanoic acid (9.13 %), and pentacosane (8.39 %) (Supplementary Table 5). Common compounds such as phenol (0.13 %), glycerin (0.23 %), propanal (0.54 %), were also detected. As per soxhlet extraction, a total of 35 phytocompounds were identified, with cis-vaccenic acid (29.94 %) being the major compound besides 3-tetradecene (10.808 %) and n-hexadecanoic acid (8.81 %) (supplementary Table 6).

The common compounds identified across all three extraction methods include hexadecanoic acid, octadecane, and n-hexadecenoic acid. These compounds have been widely documented in the literature for their therapeutic properties and are traditionally used to treat various ailments. The findings of the present study highlight *P. benghalensis* as a potential source of bioactive compounds, offering valuable insights for future research and applications.

With regard to their biological applications, hexadecenoic acid has

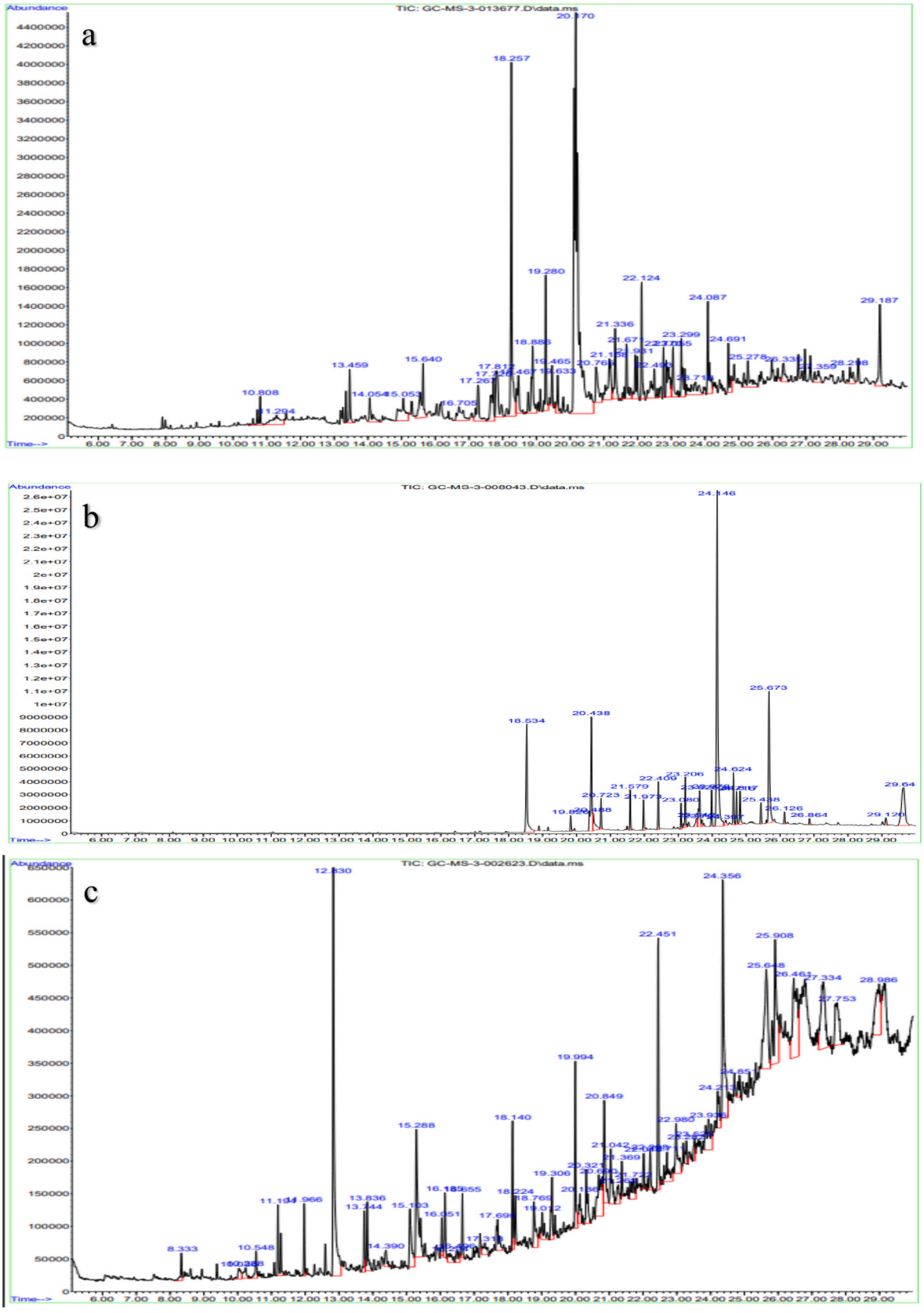

Fig. 2. Phytoconstituents of the hydromethanolic leaves extract of *P. benghalensis* (a) Ultrasound-assisted extraction method; (b) Microwave-assisted extraction method; (c) Soxhlet-assisted extraction method.

also been shown to possess antibacterial activity. Siswadi et al. reported that n-hexadecenoic acid exhibits a range of biological properties, including antioxidant, 5-alpha-reductase inhibition, and anti-fibrinolytic effects. In addition, it also demonstrates haemolytic, antimicrobial, and hypocholesterolemic activities. Moreover, This compound functions as a nematicide and pesticide, possesses antiandrogenic effects, and can be used as a flavoring agent [62]. Furthermore, it has been noted for its antioxidant and anticancer properties [63]. Yang et al. reported that, Octodecane has been shown to demonstrate considerable antimicrobial activity against a broad spectrum of microbial pathogens [64].

Also, coumarin has been reported to demonstrate antifungal, anti-inflammatory, antitumor, and antibacterial activities. It may also possess anti-obesity or hypolipidemic properties. Supporting studies have also reported the antihyperlipidemic activity of this compound [65,66]. This suggests that these compounds of the leaf could be responsible for the anti-obesity properties of the plant. In addition, benzo propanoic acid has been reported for its neuroinflammatory activity [67]. There are many phenolic compounds found in plants, vegetables, and fruits that have been reported for their antioxidant activity [68]. They play a very important and essential role in neuroprotective potential, antimicrobial, anti-inflammatory, and antioxidant effects, and as well as treatment of diseases such as obesity, certain types of cancer and diabetes [69]. *cis*-Vaccenic acid is an omega-7 fatty acid renowned for its antibacterial and hypolipidemic properties in rats [70]. Naphthalene is a compound with high biological activity. Previous literature have reported anticancer activity as well as anti-inflammatory, antibacterial, anticandidal, cytotoxic, antimicrobial and antioxidant activities besides VEGFR-2 inhibition and anti-platelet aggregation effects of the phytoconstituent [71,72]. Most of the chemical compounds identified from the leaves of *P. benghalensis* have been recorded to aid in curing several ailments both in traditional medicines and scientific research. Hence, the present study reveals the potential source of bioactive compounds and the suitable extraction methodology for the plant. However, it is advisable to identify and isolate individual components before conducting further research on their biological activity.

3.5. Antibacterial activity

Microbial infections are one of the leading causes of death and illness worldwide. Sharifi-Rad et al. have reported that gram-negative bacteria possess robust cell walls, predominantly made up of lipopolysaccharides, which contribute to their higher resistance to many antibacterial treatments [73]. On the other hand, gram-positive bacteria have uniform cell walls that lack phospholipids. It has been reported that plant extracts exhibit hydrophobic characteristics, whereas the outer membranes of gram-negative bacteria contain some hydrophilic porins. Consequently, the diffusion of plant extract compounds into gram-negative bacteria is impeded. Herbal medicines are widely employed in rural communities where medications are scarce or impossible to obtain. In westernized countries, herbalism is commonly utilized as an alternative or supplement to prescription medicine [74]. Bacterial contamination also causes agony among affected persons, reducing their productivity [75,76].

Previous literatures have reported that the *P. benghalensis* aerial portions have antibacterial activity properties against *Bacillus subtilis* and *Staphylococcus aureus* [77]. Culture medium is composed of water and nutrients that provide a vital source of energy for bacterial growth and maintenance [78]. The current research evaluated the antibacterial activity of *P. benghalensis* leaf hydromethanolic extract derived using different extraction methods against two bacterial strains grown on nutrient agar media. The results obtained using the disc diffusion method revealed a dose-dependent inhibitory activity of all the extracts (Table 3 and Fig. 3). The extract from UAE showed the highest zone of inhibition against *Staphylococcus aureus* (18 mm) and *Salmonella typhi* (11 mm) at 300 µl. The hydromethanolic leaf extract showed antibacterial activity against the both gram-positive and gram-negative bacteria after extraction using all the extraction methods. The enhanced antibacterial activity of *P. benghalensis* leaf extracted using UAE method over other extraction methods could possibly be credited mainly to the major phenolic compound eugenol. The role of eugenol in altering the permeability of bacterial membrane and ATPase inhibition activity presumed by Gill and Holly [79] and Jeyakumar and Lawrence [80] may be responsible for the positive effect in the study. Obesity-associated comorbidities such as diabetes and inflammation are due to an impaired immune system, leading to hospitalization, nosocomial infection, and bacteria-infested urinary tract infections [81,82].

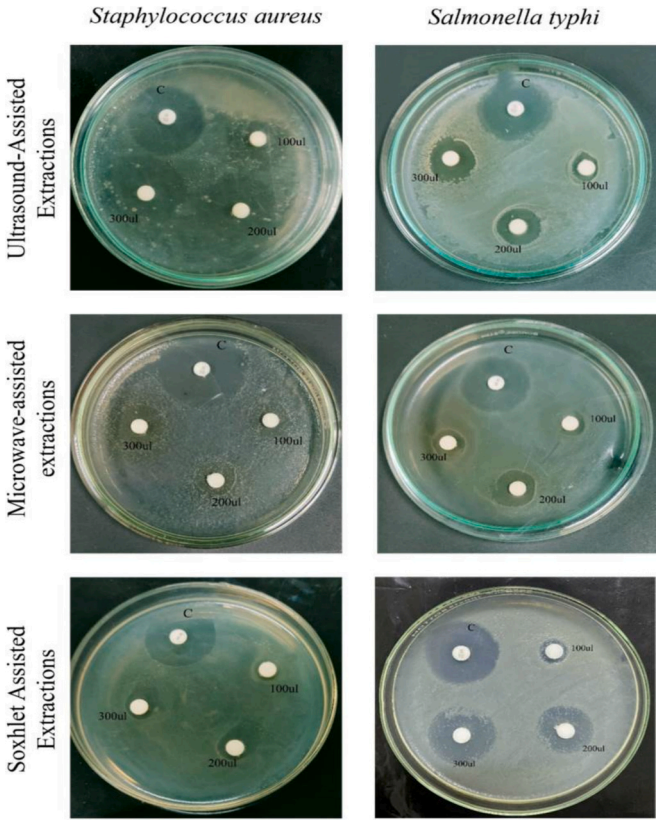

Fig. 3. Antibacterial activity of *P. benghalensis* leaf extract.

Table 3
Antibacterial activity of *P. benghalensis* leaf extract obtained from different extraction methods by disc method.

Extraction methods	Pathogens							
	Staphylococcus aureus				*Salmonella typhi*			
	100 µl	200 µl	300 µl	Antibiotic Disc (mm) Standard	100 µl	200 µl	300 µl	Antibiotic Disc (mm) Standard
Ultrasound-assisted extractions	10	14	18	23	7	10	11	23
Microwave-assisted extractions	5	7	8	23	6	9	8	22
Soxhlet assisted extraction	5	8	9	24	6	10	10	23

Staphylococcus aureus is a frequent bacterial pathogen causing infections in humans, causing various clinical signs. Both community and hospital-acquired infections are common, and treating them is difficult due to the rise in multidrug-resistant strains such as MRSA (Methicillin-resistant *Staphylococcus aureus*), which can cause a variety of potentially deadly diseases [83]. Numerous researches have looked into the relationship between particular bacterial species and human obesity at the species level. In both pregnant women and children, a correlation between *S. aureus* and being overweight has been shown [84]. Therefore, the use of this extract is a viable therapeutic strategy for thwarting and treating obesity and related diseases or in combination with existing drugs.

3.6. In vitro pancreatic lipase enzyme assay

The PL enzyme activity was performed with UAE extract based on its highest phenol, and flavonoid content, and antibacterial activity (Fig. 4). The phenolic compounds are plant secondary metabolites responsible for a wide range of bioactivities, as it may act on the enzyme's active site, inhibiting and blocking lipid binding [85]. A dose-dependent inhibitory effect was observed in the current study. At 100 mg/ml dosage, the extract showed 57 % inhibition, roughly equivalent to the activity of the standard drug, orlistat which displayed 62.3 % inhibition. The IC_{50} value of the UAE hydromethanolic leaf extract was 86.2 µg/ml, which indicates its solid inhibitory potential.

Previous works of literature on Lamiaceae members such as *Origanum onites, Salvia sclarea* (leaves) and *S. sclarea* (flowers) had a similar inhibitory effect on pancreatic lipase enzyme [86]. However, this is the first report of inhibiting the PL enzyme activity of *P. benghalensis* leaf extract using a hydromethanolic solvent. Previous reports stand support for the effectiveness of phenolic compounds identified in the present study obtained using UAE extract, suggesting their possible role in the observed PL enzyme inhibitory activity [87]. Eugenol compound can donate H^+ to a free radical. As an active compound in cloves (89 %), it offers a wide range of health benefits, such as reducing the risk of diabetes, relieving stress, and acting as an antimicrobial agent [88,89]. Mnafgui et al. have also reported the considerable effect of eugenol in reducing pancreatic lipase enzyme activity along with pancreatic a-amylase and angiotensin-converting enzyme activity in diabetic rats [90]. The study did also record an increase of high-density lipoprotein-cholesterol and restoration of transaminases, alkaline phosphatase, creatine phosphokinase and gamma-glutamyl transpeptidase activities, total bilirubin, creatinine, urea, and uric acid levels.

Oroojan et al. have reported that glucose homeostasis is crucial in regulating insulin secretion whereas phenolic compounds have been shown to enhance glucose–stimulated insulin secretion (GSIS). It is expected that phenolics-rich UAE extract might have conferred the lipase inhibitory activity and can be used as supplementary therapy with orlistat for effective anti-obesity effect [91].

3.7. In vitro anti-diabetic inhibitory assay

Alpha-amylase, along with another intestinal glycosidase, plays a key role in starch hydrolysis into simple sugars like glucose, enabling its absorption by the body. This enzyme primarily targets 1,4-connected polysaccharides such as starch inter-linked by alpha-1,4 glycosidic bonds [92,93], which are subsequently broken down into glucose by α-glucosidase for absorption through the intestinal epithelium. Inhibition of these enzymes can slow the release of glucose into the bloodstream, helping to prevent rapid postprandial spikes in blood sugar levels [94,95]. Inhibiting alpha-glucosidase and alpha-amylase, the two essential enzymes in carbohydrate digestion, is a well-established strategy for managing blood glucose levels in individuals with type 2 diabetes and those at risk of developing the condition. This approach significantly reduces post-meal spikes in blood glucose [96]. Additionally, the FDA-approved medication acarbose can be used to treat type 2 diabetes by modifying blood glucose levels *via* blocking alpha-amylase [97]. Voglibose, Acarbose, Metformin, and Miglitol are the most widely used alpha-glucosidase inhibitors [98–100]. Previous literature has reported that the methanol extract of *P. benghalensis* aerial part exhibits (IC_{50} value 106.3) alpha-amylase inhibitory activity in comparison to the standard acarbose [101]. Therefore, the doses for the current study were selected based on standard protocols to ensure measurable inhibition without substrate saturation based on a previous report [102].

The hydromethanolic extract of *P. benghalensis* leaves exhibited a dose-dependent inhibition of alpha-amylase and alpha-glucosidase, with mean inhibition rates of 56.32 % and 43.72 % at extract concentrations of 20, 40, 60, 80, and 100 µg/ml. In comparison, acarbose showed IC_{50} values of 42.18 µg/ml for alpha-amylase and 23.51 µg/ml for alpha-glucosidase. The extract demonstrated a stronger inhibitory effect against alpha-amylase than alpha-glucosidase. These current results indicate that the hydromethanolic leaf extract possesses significant *in vitro* alpha-amylase and alpha-glucosidase inhibitory activity (Tables 4 & 5).

The UAE-assisted hydromethanolic extract may act as a better alpha-amylase and alpha-glucosidase inhibitor, and can be considered as an alternative for the existing synthetic drugs like acarbose. Our *in vitro* studies demonstrated appreciable alpha-amylase and alpha-glucosidase inhibition activities. Further experiments at the preclinical level are warranted on animal models to confirm the anti-obesity activity and the effects on glycemic index.

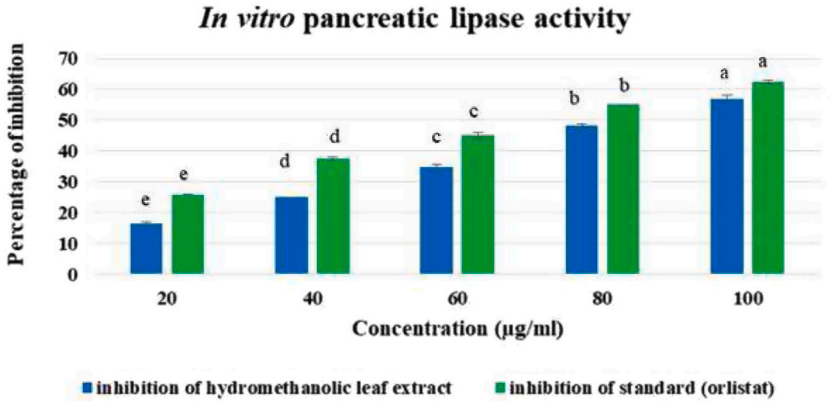

Fig. 4. *In vitro* PL enzyme inhibition activity of *P. benghalensis* leaf hydromethanolic extract and standard drug (Orlistat). All values are presented as mean ± standard deviation ($n = 3$) and the statistical significance has been mentioned as alphabets in superscripts.

Table 4
Anti-diabetic activity as per alpha-amylase inhibitory activity.

S.no	Concentrations (µg//ml)	Alpha amylase (%)	
		Hydromethanol leaf extract	Acarbose
1.	20	30.35	39.28
2.	40	42.03	50.00
3.	60	53.57	56.25
4.	80	62.21	69.64
5.	100	71.42	80.35
	IC_{50} Value	**56.32**	**42.18**

Table 5
Anti-diabetic activity as per alpha-glucosidase inhibitory activity.

S.no	Concentrations (µg//ml)	Alpha-glucosidase (%)	
		Hydromethanol leaf extract	Acarbose
1.	20	38.18	49.09
2.	40	47.27	56.36
3.	60	61.81	65.45
4.	80	69.09	74.54
5.	100	72.72	81.81
	IC_{50} VALUE	**43.72**	**23.51**

4. Conclusions

This study evaluated various extraction methods (UAE, MAE, and SAE) to extract bioactive compounds from the leaves of *P. benghalensis*. Among the methods tested, UAE showed the highest extraction efficiency and yielded extracts with significant biological effects. It was also shown to possess higher phenolic and flavonoid contents, and has been proven to be the most effective method for extracting bioactive compounds. The FTIR analysis confirmed the presence of functional groups, corresponding to the major compounds, and GC–MS identified diverse bioactive compounds, mainly in the UAE extract. All the extracts exhibited significant antibacterial activity against *S. aureus* and *S. typhi.* The UAE leaf extract was found to be having effective inhibitory activity on pancreatic lipase (PL), enzyme, owing to its higher phenolic content, and also significant inhibitory activity on alpha-amylase and alpha-glucosidase. The UAE-assisted hydromethanolic extract may act as a better alpha-amylase and alpha-glucosidase inhibitor, and could serve as an alternative to existing synthetic drugs like acarbose for inhibiting alpha-amylase and alpha-glucosidase.

All these findings suggest that the UAE is an optimal method for extracting phenolic and flavonoid bioactive compounds from *P. benghalensis* leaves. This research not only advances our understanding of the chemical properties of *P. benghalensis* but also opens up opportunities for utilizing its derivatives and suggests potential applications in anti-obesity treatments and other medicinal uses. This attempt can consequently lead to bridging applications, integrating modern scientific knowledge with traditional practices. The limitations of the current study pertain to the shortage of *in vivo* experiments besides the isolation and biological effects of the major compounds present in all three extracts identified in this study. Further, we intend to conduct the anti-obesity and anti-diabetic effects of the extracts and the mechanisms involved through studies in the future.

CRediT authorship contribution statement

Udayakumar Sandhiya: Writing – original draft, Software, Methodology, Conceptualization. **Santosh Chokkakula:** Writing – review & editing, Formal analysis. **Selvaraju Parthibhan:** Visualization, Software. **Abdullah Alfalih:** Writing – review & editing, Formal analysis. **Ye Zhao:** Writing – review & editing, Formal analysis. **Azhagiya Manavalan Lakshmi Prabha:** Visualization, Validation, Supervision, Data curation, Conceptualization. **Uma Maheshwari Rajadurai:** Writing – review & editing. **Joe Antony Jacob:** Writing – review & editing. **Fuad Ameen:** Writing – review & editing. **Bing Yang:** Writing – review & editing, Visualization, Validation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Research Foundation of Bharathidasan University for the University Research Fellowship (URF) and the facility to carry out the research. Also, the authors extend their appreciation to the ongoing research funding program, (ORF-2025-364), King Saud University, Riyadh, Saudi Arabia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2025.103007.

Data availability

No data was used for the research described in the article.

References

[1] G. Mustafa, R. Arif, A. Atta, S. Sharif, A. Jamil, Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan, Matrix Sci. Pharma 1 (2017) 17–26.
[2] Y.P. Didion, T.G. Tjalsma, Z. Su, M. Malankowska, M. Pinelo, What is next? The greener future of solid liquid extraction of biobased compounds: novel techniques and solvents overpower traditional ones, Sep. Purif. Technol. 320 (2023) 124147.
[3] S. Oubannin, L. Bijla, M.N. Ahmed, M. Ibourki, Y. El Kharrassi, K. Devkota, et al., Recent advances in the extraction of bioactive compounds from plant matrices and their use as potential antioxidants for vegetable oils enrichment, J. Food Compos. Anal. 128 (2024) 105995.
[4] C. Bitwell, S.S. Indra, C. Luke, M.K. Kakoma, A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants, Sci. Afr. 19 (2023) e01585.
[5] D. Zheleva-Dimitrova, K.I. Sinan, O.K. Etienne, G. Zengin, R. Gevrenova, M. F. Mahomoodally, et al., Chemical composition and biological properties of *Synedrella nodiflora* (L.) Gaertn: a comparative investigation of different extraction methods, Process Biochem. 96 (2020) 202–212.
[6] E. Brglez Mojzer, M. Knez Hrnčič, M. Škerget, Ž. Knez, U. Bren, Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects, Molecules 21 (2016) 901.
[7] K. Kumar, S. Srivastav, V.S. Sharanagat, Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: a review, Ultrason. Sonochem. 70 (2021) 105325.
[8] W. Ashraf, A. Rehman, A. Hussain, A. Karim, H.R. Sharif, M. Siddiquy, et al., Optimization of extraction process and estimation of flavonoids from fenugreek using green extracting deep eutectic solvents coupled with ultrasonication, Food Bioproc. Tech. 17 (2024) 887–903.
[9] N. Azwanida, A review on the extraction methods use in medicinal plants, principle, strength and limitation, Med. Aromat. Plants 4 (2015), 2167-0412.
[10] T. Seal, Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India, J. Appl. Pharm. Sci. 6 (2016) 157–166.
[11] M.G. Pereira, F. Hamerski, E.F. Andrade, AdP Scheer, M.L. Corazza, Assessment of subcritical propane, ultrasound-assisted and Soxhlet extraction of oil from sweet passion fruit (*Passiflora alata Curtis*) seeds, J. Supercrit. Fluids 128 (2017) 338–348.
[12] A. Bampouli, K. Kyriakopoulou, G. Papaefstathiou, V. Louli, M. Krokida, K. Magoulas, Comparison of different extraction methods of *Pistacia lentiscus* var. chia leaves: yield, antioxidant activity and essential oil chemical composition, J. Appl. Res. Med. Arom. Plants 1 (2014) 81–91.
[13] X. Meng, X. Liu, J. Tan, Q. Sheng, D. Zhang, B. Li, et al., From Xiaoke to diabetes mellitus: a review of the research progress in traditional Chinese medicine for diabetes mellitus treatment, Chin. Med. 18 (2023) 75.

[14] L.K. Pandey, K.R. Sharma, Analysis of phenolic and flavonoid content, α-amylase inhibitory and free radical scavenging activities of some medicinal plants, Sci. World J. 2022 (2022) 4000707.
[15] Pmd Sales, Pmd Souza, L.A. Simeoni, PdOMD Batista, D. Silveira, α-Amylase inhibitors: a review of raw material and isolated compounds from plant source, J. Pharm. Pharm. Sci. 15 (2012) 141–183.
[16] A.K. Oyebamiji, E.A. Soetan, S.A. Akintelu, A.O. Ayeleso, E. Mukwevho, Alpha-glucosidase activity of phytochemicals from *Phyllanthus amarus* leaves via in-silico approaches, Pharmacol. Res.-Mod. Chinese Med. 2 (2022) 100054.
[17] N. Akhlaghi, G. Najafpour-Darzi, Phytochemical analysis, antioxidant activity, and pancreatic lipase inhibitory effect of ethanolic extract of *Trigonella foenumgraceum* L. leaves, Biocatal. Agric. Biotechnol. 32 (2021) 101961.
[18] P.-K. Liu, Z.-M. Weng, G.-B. Ge, H.-L. Li, L.-L. Ding, Z.-R. Dai, et al., Biflavones from *Ginkgo biloba* as novel pancreatic lipase inhibitors: inhibition potentials and mechanism, Int. J. Biol. Macromol. 118 (2018) 2216–2223.
[19] T.-T. Liu, X.-T. Liu, Q.-X. Chen, Y. Shi, Lipase inhibitors for obesity: a review, Biomed. Pharmacother. 128 (2020) 110314.
[20] S.V. Mhatre, A.A. Bhagit, R.P. Yadav, Pancreatic lipase inhibitor from food plant: potential molecule for development of safe anti-obesity drug, MGM J. Med. Sci. 3 (2016) 34–41.
[21] S. Dahiya, D.R. Batish, H.P. Singh, Ethnobotanical, phytochemical and pharmacological aspects of Bengal Pogostemon (Pogostemon benghalensis), J. Herbmed. Pharmacol. 9 (2020) 318–327.
[22] Y. Liu, W. Zhe, R. Zhang, Z. Peng, Y. Wang, H. Gao, et al., Ultrasonic-assisted extraction of polyphenolic compounds from *Paederia scandens* (Lour.) Merr. Using deep eutectic solvent: optimization, identification, and comparison with traditional methods, Ultrason. Sonochem. 86 (2022) 106005.
[23] M.R. Venkateswaran, S. Hemaiswarya, S. Jayabal, T. Erusappan, A. Shanmugam, M. Doble, Mehani formulation is rich in bioactive compounds and ameliorates diabetes and associated inflammatory condition-in vitro and in vivo studies, S. Afr. J. Bot. 154 (2023) 56–66.
[24] Z. Hromadkova, A. Ebringerová, Ultrasonic extraction of plant materials—investigation of hemicellulose release from buckwheat hulls, Ultrason. Sonochem. 10 (2003) 127–133.
[25] M.H. Al Rashid, S. Majumder, V. Mandal, S.C. Mandal, R.A. Thandavarayan, In search of suitable extraction technique for large scale commercial production of bioactive fraction for the treatment of diabetes: the case *Diospyros melanoxylon* Roxb, J. Tradit. Complement. Med. 9 (2019) 106–118.
[26] S.A. Heleno, P. Diz, M. Prieto, L. Barros, A. Rodrigues, M.F. Barreiro, et al., Optimization of ultrasound-assisted extraction to obtain mycosterols from *Agaricus bisporus* L. by response surface methodology and comparison with conventional Soxhlet extraction, Food Chem. 197 (2016) 1054–1063.
[27] S.A.S. Chatha, F. Anwar, M. Manzoor, Bajwa J-u-R, Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays, Grasas Aceites 57 (2006) 328–335.
[28] S. McDonald, P.D. Prenzler, M. Antolovich, K. Robards, Phenolic content and antioxidant activity of olive extracts, Food Chem. 73 (2001) 73–84.
[29] A. Mokrani, L. Chaouch, K. Messaoudene, Selection of the suitable extraction solvent to improve phenolic content and antioxidant activity in some Algerian black olive varieties, Chem. Pap. (2025) 1–13.
[30] M.N. Muhongo, M. Kangogo, C. Bii, Qualitative and quantitative phytochemical profiling of crude fractions of Pechuel-Loeschea leubnitziae leaves, J. Med. Plants Res. 15 (2021) 64–72.
[31] P. Brinda, P. Sasikala, K. Purushothaman, Pharmacognostic studies on Merugan kizhangu, Bull. Med. Eth. Bot. Res. 3 (1981) 84–96.
[32] Y. Vaghasiya, R. Nair, S. Chanda, Antibacterial and preliminary phytochemical and physico-chemical analysis of *Eucalyptus citriodora* Hk leaf, Nat. Prod. Res. 22 (2008) 754–762.
[33] B. Vadivelu, V.A. Arumugam, S. Subbarayan, A.A. Alshatwi, R. Krishnamoorthy, Effect of *Macrotyloma uniflorum* on antiobesity in rats fed with a high fat diet, Saudi J. Biol. Sci. 26 (2019) 1772–1778.
[34] S.A. Jaber, In vitro alpha-amylase and alpha-glucosidase inhibitory activity and in vivo antidiabetic activity of *Quercus coccifera* (oak tree) leaves extracts, Saudi J. Biol. Sci. 30 (2023) 103688.
[35] P.-H. Li, Y.-W. Lin, W.-C. Lu, J.-M. Hu, D.-W. Huang, In vitro hypoglycemic activity of the phenolic compounds in longan fruit (*Dimocarpus Longan* var. Fen ke) shell against α-glucosidase and β-galactosidase, Int. J. Food Prop. 19 (2016) 1786–1797.
[36] P.-F. Zheng, Z. Xiong, C.-y. Liao, X. Zhang, M. Feng, X.-Z. Wu, et al., In vitro and in silico studies of bis (indol-3-yl) methane derivatives as potential α-glucosidase and α-amylase inhibitors, J. Enzyme Inhib. Med. Chem. 36 (2021) 1938–1951.
[37] H.S. MacTavish, Factors Affecting Yield and Composition of Floral Extract from *Boronia megastigma* Nees, University of Tasmania, 1995.
[38] I.T. Karabegović, S.S. Stojičević, D.T. Veličković, Z.B. Todorović, N.Č. Nikolić, M. L. Lazić, The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (*Prunus laurocerasus*) leaf and fruit extracts, Ind. Crop. Prod. 54 (2014) 142–148.
[39] F.L. Garcia-Larez, J. Esquer, H. Guzmán, D.S. Zepeda-Quintana, M.J. Moreno-Vásquez, F. Rodríguez-Félix, et al., Effect of ultrasound-assisted extraction (UAE) parameters on the recovery of polyphenols from pecan nutshell waste biomass and its antioxidant activity, Biomass Convers. Biorefinery (2024) 1–19.
[40] L. Wang, C.L. Weller, Recent advances in extraction of nutraceuticals from plants, Trends Food Sci. Technol. 17 (2006) 300–312.
[41] B. Soldo, T. Bilušić, J. Giacometti, I. Ljubenkov, V. Čikeš Čulić, A. Bratanić, et al., A comparative study of oleuropein extraction from wild olive leaves (*Olea europea* subsp. oleaster, Hoffmanns. & link), its gastrointestinal stability, and biological potential, Appl. Sci. 14 (2024) 869.
[42] N.W. Ismail-Suhaimy, S.S.A. Gani, U.H. Zaidan, M.I.E. Halmi, P. Bawon, Optimizing conditions for microwave-assisted extraction of polyphenolic content and antioxidant activity of *Barleria lupulina* Lindl, Plants 10 (2021) 682.
[43] C.-H. Chan, R. Yusoff, G.-C. Ngoh, F.W.-L. Kung, Microwave-assisted extractions of active ingredients from plants, J. Chromatogr. A 1218 (2011) 6213–6225.
[44] K. Hartonen, J. Parshintsev, K. Sandberg, E. Bergelin, L. Nisula, M.-L. Riekkola, Isolation of flavonoids from aspen knotwood by pressurized hot water extraction and comparison with other extraction techniques, Talanta 74 (2007) 32–38.
[45] D. Yang, Q. Wang, L. Ke, J. Jiang, Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn) rhizome, Asia Pac. J. Clin. Nutr. 16 (2007) 158.
[46] M. Sharifi-Rad, P. Pohl, F. Epifano, G. Zengin, N. Jaradat, M. Messaoudi, *Teucrium polium* (L.): phytochemical screening and biological activities at different phenological stages, Molecules 27 (2022) 1561.
[47] G. Rocchetti, F. Blasi, D. Montesano, S. Ghisoni, M.C. Marcotullio, S. Sabatini, et al., Impact of conventional/non-conventional extraction methods on the untargeted phenolic profile of *Moringa oleifera* leaves, Food Res. Int. 115 (2019) 319–327.
[48] M.E.S. Júnior, M.V.R. Araujo, A.A. Santana, F.L.H. Silva, M.I.S. Maciel, Ultrasound-assisted extraction of bioactive compounds from ciriguela (*Spondias purpurea* L.) peel: optimization and comparison with conventional extraction and microwave, Arab. J. Chem. 14 (2021) 103260.
[49] S. Kale, A. Dhabe, Qualitative phytochemical screening and FTIR spectroscopic analysis of *Thalictrum dalzelli* hook leaf extract, J. Pharmacogn. Phytochem. 12 (2023) 272–274.
[50] S.A. Siddiqui, A. Ali Redha, M. Salauddin, I.A. Harahap, H.V. Rupasinghe, Factors affecting the extraction of (poly) phenols from natural resources using deep eutectic solvents combined with ultrasound-assisted extraction, Crit. Rev. Anal. Chem. 55 (2025) 139–160.
[51] R. Singh, P. Singh, V.K. Pandey, K.K. Dash, Mukarram S.A. Ashish, et al., Microwave-assisted phytochemical extraction from walnut hull and process optimization using Box–Behnken design (BBD), Processes 11 (2023) 1243.
[52] V. Mandal, Y. Mohan, S. Hemalatha, Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research, Pharmacogn. Rev. 1 (2007) 7–18.
[53] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: a review, Anal. Chim. Acta 703 (2011) 8–18.
[54] W. Ji, F. Chen, Z. Chen, H. Jiang, Research in advances in the bioactivity of plant polyphenols, Int. J. Food Sci. Technol. 59 (2024) 8037–8044.
[55] M.M. Hammouda, K.M. Elattar, Recent progress in the chemistry of β-aminoketones, RSC Adv. 12 (2022) 24681–24712.
[56] D. Sarma, B.K. Datta, Qualitative and quantitative phytochemical analysis and antioxidant activities of *Aphanomixic polystachya* leaf, J. Med. Plants Stud. 13 (2025) 222–231.
[57] O.R. Alara, N.H. Abdurahman, C.I. Ukaegbu, N.A. Kabbashi, Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques, J. Taibah Univ. Sci. 13 (2019) 414–422.
[58] M.F. Nisar, M. Khadim, M. Rafiq, J. Chen, Y. Yang, C.C. Wan, Pharmacological properties and health benefits of eugenol: a comprehensive review, Oxid. Med. Cell. Longev. 2021 (2021) 2497354.
[59] A. Abdou, A. Elmakssoudi, A. El Amrani, J. JamalEddine, M. Dakir, Recent advances in chemical reactivity and biological activities of eugenol derivatives, Med. Chem. Res. 30 (2021) 1011–1030.
[60] A. Hussain, K. Brahmbhatt, A. Priyani, M. Ahmed, T.A. Rizvi, C. Sharma, Eugenol enhances the chemotherapeutic potential of gemcitabine and induces anticarcinogenic and anti-inflammatory activity in human cervical cancer cells, Cancer Biother. Radiopharm. 26 (2011) 519–527.
[61] Z. Pu, Z. Yin, R. Jia, X. Song, J. Xu, X. Wang, et al., Preliminary isolation and antibacterial activity of the ethyl acetate extract of *Prinsepia utilis* Royle in vitro, Agric. Sci. 5 (2014) 540–545.
[62] S. Siswadi, G.S. Saragih, Phytochemical analysis of bioactive compounds in ethanolic extract of Sterculia quadrifida R. Br, in: AIP Conference Proceedings, AIP Publishing, 2021.
[63] B. Bharath, K. Perinbam, S. Devanesan, M.S. AlSalhi, M. Saravanan, Evaluation of the anticancer potential of Hexadecanoic acid from brown algae *Turbinaria ornata* on HT–29 colon cancer cells, J. Mol. Struct. 1235 (2021) 130229.
[64] L. Yang, J. Zhang, S. Zheng, A. Hou, S. Wang, H. Yu, et al., The phytochemistry, pharmacology and traditional medicinal use of Glechomae Herba–a systematic review, RSC Adv. 11 (2021) 19221–19237.
[65] M. Al-Haiza, M. Mostafa, M. El-Kady, Preparation of some new coumarin derivatives with biological activity, Sci. J. King Faisal Univ. (Basic Appl. Sci.) 6 (2005) 1426.
[66] O.M. Tsivileva, O.V. Koftin, N.V. Evseeva, Coumarins as fungal metabolites with potential medicinal properties, Antibiotics 11 (2022) 1156.
[67] J. Li, M. Duan, X. Yao, D. Tian, J. Tang, Prenylated benzenepropanoic acid analogues from the *Citrus grandis* (L.) Osbeck and their anti-neuroinflammatory activity, Fitoterapia 139 (2019) 104410.
[68] W. Li, M. Zhang, R. Zhang, F. Huang, L. Dong, X. Jia, et al., Structural elucidation, binding sites exploration and biological activities of bound phenolics from Radix Puerariae Thomsonii, Food Chem. 450 (2024) 139323.
[69] Y. Zhang, P. Cai, G. Cheng, Y. Zhang, A brief review of phenolic compounds identified from plants: their extraction, analysis, and biological activity, Nat. Prod. Commun. 17 (2022), 1934578X211069721.

[70] P. Semwal, S. Painuli, H. Badoni, R.K. Bacheti, Screening of phytoconstituents and antibacterial activity of leaves and bark of Quercus leucotrichophora *A. Camus* from Uttarakhand Himalaya, Clin. Phytosci. 4 (2018) 1–6.
[71] D. Osmaniye, Sağlık BmN, N. Khalilova, S. Levent, G. Bayazıt, U.D. Gul, et al., Design, synthesis, and biological evaluation studies of novel naphthalene-chalcone hybrids as antimicrobial, anticandidal, anticancer, and VEGFR-2 inhibitors, ACS Omega 8 (2023) 6669–6678.
[72] S.R. Ibrahim, G.A. Mohamed, Naturally occurring naphthalenes: chemistry, biosynthesis, structural elucidation, and biological activities, Phytochem. Rev. 15 (2016) 279–295.
[73] M. Sharifi-Rad, Y.K. Mohanta, P. Pohl, N. Jaradat, M.A. Aboul-Soud, G. Zengin, Variation of phytochemical constituents, antioxidant, antibacterial, antifungal, and anti-inflammatory properties of *Grantia aucheri* (Boiss.) at different growth stages, Microb. Pathog. 172 (2022) 105805.
[74] P. Ochieng Nyalo, G. Isanda Omwenga, Ngugi M. Piero, GC-MS analysis, antibacterial and antioxidant potential of ethyl acetate leaf extract of *Senna singueana* (Delile) grown in Kenya, Evid. Based Complement. Alternat. Med. 2022 (2022) 5436476.
[75] C. Proestos, N. Chorianopoulos, G.-J. Nychas, M. Komaitis, RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity, J. Agric. Food Chem. 53 (2005) 1190–1195.
[76] E.M. Abdallah, A.M.E. Sulieman, Z.A. Saleh, New discoveries in toxins from gram-positive bacteria *Staphylococcus aureus*, in: Microbial Toxins in Food Systems: Causes, Mechanisms, Complications, and Metabolism, Springer, 2024, pp. 235–252.
[77] R. Taylor, N. Manandhar, G. Towers, Screening of selected medicinal plants of Nepal for antimicrobial activities, J. Ethnopharmacol. 46 (1995) 153–159.
[78] M. Bonnet, J.C. Lagier, D. Raoult, S. Khelaifia, Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology, New Microbes New Infect. 34 (2020) 100622.
[79] A. Gill, R. Holley, Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics, Int. J. Food Microbiol. 108 (2006) 1–9.
[80] G.E. Jeyakumar, R. Lawrence, Mechanisms of bactericidal action of eugenol against *Escherichia coli*, J. Herb. Med. 26 (2021) 100406.
[81] W. Wang, Q. An, K. Huang, Y. Dai, Q. Meng, Y. Zhang, Unlocking the power of lactoferrin: exploring its role in early life and its preventive potential for adult chronic diseases, Food Res. Int. 182 (2024) 114143.
[82] S. Togayeva Gulnora, J. Umarov, O. Tursunov, X. Shakarov, Obesity, classification, etiology, pathogenesis, clinics, diagnosis and treatment, Лучшие интеллектуальные исследования 17 (2024) 116–122.
[83] F.D. Lowy, Staphylococcus aureus infections, N. Engl. J. Med. 339 (1998) 520–532.
[84] M. Million, J.-C. Lagier, D. Yahav, M. Paul, Gut bacterial microbiota and obesity, Clin. Microbiol. Infect. 19 (2013) 305–313.
[85] W. Liu, X. Cui, Y. Zhong, R. Ma, B. Liu, Y. Xia, Phenolic metabolites as therapeutic in inflammation and neoplasms: molecular pathways explaining their efficacy, Pharmacol. Res. 193 (2023) 106812.
[86] V. Spínola, E.J. Llorent-Martínez, P.C. Castilho, Inhibition of α-amylase, α-glucosidase and pancreatic lipase by phenolic compounds of Rumex maderensis (Madeira sorrel). Influence of simulated gastrointestinal digestion on hyperglycaemia-related damage linked with aldose reductase activity and protein glycation, Lwt 118 (2020) 108727.
[87] H. Ekin, Orhan D. Delİorman, İ. Erdocan Orhan, N. Orhan, M. Aslan, Evaluation of enzyme inhibitory and antioxidant activity of some Lamiaceae plants, J. Res. Pharm. (2019) 23.
[88] M.J. Rahman, P. Ambigaipalan, F. Shahidi, Biological activities of camelina and sophia seeds phenolics: inhibition of LDL oxidation, DNA damage, and pancreatic lipase and α-glucosidase activities, J. Food Sci. 83 (2018) 237–245.
[89] R. Indiarto, J.A. Herwanto, F. Filianty, E. Lembong, E. Subroto, D.R. A. Muhammad, Total phenolic and flavonoid content, antioxidant activity and characteristics of a chocolate beverage incorporated with encapsulated clove bud extract, CyTA-J. Food 22 (2024) 2329144.
[90] K. Mnafgui, F. Kaanich, A. Derbali, K. Hamden, F. Derbali, S. Slama, et al., Inhibition of key enzymes related to diabetes and hypertension by Eugenol in vitro and in alloxan-induced diabetic rats, Arch. Physiol. Biochem. 119 (2013) 225–233.
[91] A.A. Oroojan, Eugenol improves insulin secretion and content of pancreatic islets from male mouse, Int. J. Endocrinol. 2020 (2020) 7416529.
[92] V. Shilpa, S. Lekshmi, T. Swapna, In vitro antidiabetic potential of *Euphorbia hirta* Linn.: a nutritionally significant plant, J. Pharmacogn. Phytochem. 9 (2020) 01–04.
[93] R. Khator, V. Monga, Recent advances in the synthesis and medicinal perspective of pyrazole-based α-amylase inhibitors as antidiabetic agents, Future Med. Chem. 16 (2024) 173–195.
[94] R. Jha, K. Goyal, S. Mehan, G. Singh, Dual α-amylase and α-glucosidase inhibitors: recent progress from natural and synthetic resources, Bioorg. Chem. 163 (2025) 108762.
[95] G. Pan, Y. Lu, Z. Wei, Y. Li, L. Li, X. Pan, A review on the in vitro and in vivo screening of α-glucosidase inhibitors, Heliyon 10 (2024) e37467.
[96] A.S. Hassan, N.M. Morsy, W.M. Aboulthana, A. Ragab, Exploring novel derivatives of isatin-based Schiff bases as multi-target agents: design, synthesis, in vitro biological evaluation, and in silico ADMET analysis with molecular modeling simulations, RSC Adv. 13 (2023) 9281–9303.
[97] L.K. Williams, X. Zhang, S. Caner, C. Tysoe, N.T. Nguyen, J. Wicki, et al., The amylase inhibitor montbretin a reveals a new glycosidase inhibition motif, Nat. Chem. Biol. 11 (2015) 691–696.
[98] S. Riaz, I.U. Khan, M. Bajda, M. Ashraf, A. Shaukat, T.U. Rehman, et al., Pyridine sulfonamide as a small key organic molecule for the potential treatment of type-II diabetes mellitus and Alzheimer's disease: in vitro studies against yeast α-glucosidase, acetylcholinesterase and butyrylcholinesterase, Bioorg. Chem. 63 (2015) 64–71.
[99] S. Kumari, R. Saini, A. Bhatnagar, A. Mishra, Exploring plant-based alpha-glucosidase inhibitors: promising contenders for combatting type-2 diabetes, Arch. Physiol. Biochem. 130 (2024) 694–709.
[100] Z.A. Awan, H.A. Khan, A. Jamal, S. Shams, G. Zheng, A. Wadood, et al., In silico exploration of the potential inhibitory activities of in-house and ZINC database lead compounds against alpha-glucosidase using structure-based virtual screening and molecular dynamics simulation approach, J. Biomol. Struct. Dyn. 43 (2025) 2412–2422.
[101] A. Khadka, A. Budha Magar, K.R. Sharma, Chemical profiling and biological activities on Nepalese medicinal plant extracts and isolation of active fraction of Nyctanthes arbor-tristis, Scientific World Journal 2024 (2024) 5080176.
[102] S. Das, S. Das, B. De, In vitro inhibition of key enzymes related to diabetes by the aqueous extracts of some fruits of West Bengal, India, Curr. Nutr. Food Sci. 8 (2012) 19–24.