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IN-VITRO ANTIOXIDANT, ANTIDIABETIC AND ANTIOBESITY ATTRIBUTES OF OPTIMIZED METHANOLIC EXTRACT OF HELIOTROPIUM STRIGOSUM

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Abstract

The synthetic antidiabetic and antiobesity agents are associated with well-established health complications. The plants may serve the purpose being rich in antioxidants, α-glucosidase and lipase inhibitors. The in-vitro inhibition of α-glucosidase and pancreatic lipase is an expedient tool to assess the antidiabetic and antiobesity attributes which depends upon the phytochemicals. However, the extraction optimization is the most vital step to explore the full medicinal potential of plants. In current study various methanol compositions including pure (FUS-1), 80% (FUS-2), 60% (FUS-3), 40% (FUS-4), 20% (FUS-5) and aqueous (FUS-6) were used for extraction optimization from Heliotropium strigosum (H. strigosum). The extraction process was aided by freeze drying assisted ultrasonication to improve the extract yield, antioxidant activity, in-vitro α-glucosidase and pancreatic lipase inhibitions. The total phenolic contents and total flavonoid contents were also determined in this work. The highest extract yield of 22.46 ± 0.751 % was obtained for FUS-2 being statistically significant having $\rho<0.05$ as compared for employed extracts. The FUS-2 extract also exhibited highest phenolic (143.4 ± 0.794 mg Gallic acid equivalent per g of extract) and flavonoid contents (87.30 ± 1.1 mg Rutin equivalent per g of extract) . The FUS-2 exhibited highest DPPH radical scavenging % (94.94 ± 1.04) α-glucosidase inhibition % (84.50 ± 0.45) and pancreatic lipase inhibition % (44.57 ± 0.37). A strong correlation (R^2 = 0.9166) was observed between antioxidant activity and α-glucosidase inhibition % emphasizing the role of antioxidant potential and in-vitro antidiabetic capacity of H. strigosum. Conclusively, H. strigosum use to treat diabetes mellitus type-2 in traditionally practiced phytomedicine system was confirmed. The antiobesity attributes of H. strigosum remained moderate as compared to antioxidant and antidiabetic properties. However, further studies may be performed on metabolite

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fingerprinting and activity guided isolation from H. strigosum for more authentic and rational information for medicinal use and functional food feasibility.

Introduction

The reactive oxygen species (ROS) are produced by metabolic activities and human exposure to various exogenous factors like pollution, smoke, smog, chemicals and antibiotics. The sedentary life style and high caloric dietary intake also contributes significantly towards overproduction of ROS (Ozougwu, J.C., 2016).

The antioxidant defense system of body comprising of superoxide dismutase, catalase, and glutathione peroxidase with many others entities mitigates the hyper production of ROS to maintain homeostasis (Birben, *et al.*, 2012).

The disproportion between the quantum of ROS and antioxidant based defense system of body upshot the state of metabolic disturbance known as oxidative stress. The development of oxidative stress and its persistence results in many significant implications at metabolic level leading to disorders including diabetes mellitus type-2 (DM-2) and obesity. The molecular damages to biomolecules and altered metabolic process due to oxidative stress is well established to cause DM-2 (Bhatti *et al.*, 2022; Abu Khadra *et al.*, 2024).

Similarly, oxidative stress also affects the lipid metabolism and suppresses AMPK and PPAR-α to cause insulin resistance and obesity development. The phenomenon of obesity can be recognized as a condition where excessive fat accumulation occurs in body. The oxidative stress operated by ROS reduces the adipokines production to disturb lipid metabolism causing obesity development (Patil *et al.*, 2025).

The synthetic drugs used to treat diabetes and obesity are well known for their side complications and heavy cost. The alternate therapy is the use of plant extracts or products which are much safer, relatively cheaper and associated with overall boosting of antioxidant defense system of body. Medicinal plants are rich in polyphenolic compounds associated with numerous therapeutic impacts. The phytochemicals and plant based metabolites may act as good α-glucosidase inhibitors as this enzyme hydrolyses the complex carbohydrates like starches into glucose to increase the post prandial blood glucose level (Pan *et al.*, 2024).

The inhibition of α-glucosidase enzyme in intestinal brush border lines results in less conversion of carbohydrates into glucose hence inhibition of this enzyme is considered as an efficient route to assess or screen the antidiabetic impact of plant extracts (Rahman *et al.*, 2021). Similarly, pancreatic lipase enzyme converts the fats present in diet into free fatty acids and monoglycerides which are absorbed in intestine to become the part of body (Liu *et al.*, 2020). The inhibition of pancreatic lipase restricts the digestion of fats consequently less fat deposition in body leading to less chance of obesity development. The in-vitro assessment of plant extracts for antiobesity properties may be determined by measuring the pancreatic lipase inhibition (Patil *et al.*, 2022).

To explore the full pace therapeutic potential of medicinal plants, the extraction process must be optimized for better extract yields and consequently higher phytochemical amounts. The most vital aspect to deal with the extract preparation, extraction methodology must be designed comprehensively including solvent choice. In modern day extraction, freeze drying and ultrasonication assisted extraction has gained significant importance in extract preparation from plant sources as evident from previously published scientific information (William *et al.*, 2018; William *et al.*, 2019).

Among medicinally important plants, *Helliotropium strigosum* (*H. strigosum*) is well known member of Boraginaceae plant family. The plant is well believed for its antioxidant and medicinal potential including antimicrobial, antioxidant, antibacterial, and antifungal. The plant was also reported to treat arthritis, jaundice, and also to purify blood. *H. strigosum* is widely used in local medicinal system of Pakistan to treat diabetes and obesity (Ahmad, *et al.*, 2014; Khurm *et al.*, 2016; Hussain *et al.*, 2010).

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The *H. strigosum* is locally used to treat diabetes and obesity but sound scientific evidences are lacking to support the medicinal potential of this plant. A previous work reported the hyporglycemic impact of crude methanolic extract of *H. strigosum* on blood glucose level of mice confirming its antidiabetic potential. However, the aspect of extraction optimization using varous solvents was missing (Aslam, *et al.*, 2017).

Another study explored the antidiabetic and antioxidant potential of crude methanolic extract and fractions prepared by hexane, ethyl acetate, chloroform and water. The findings explored that methanolic extract exhibited relatively better results (Qayyum *et al.*, 2016). Another report mentioned methanol as an optimal solvent choice for extraction having potential to extract higher phytochemical contents and antioxidants (Truong, *et al.*, 2019).

These studies highlighted the extraction potential of methanol as most suitable solvent choice to make extraction from plants. The polarity variation based upon composition may also influence the extraction process for better output. Various composition of methanol prepared by adding aqueous medium was reported to improve the extraction yields (Riyadi *et al.*, 2023).

Similarly, the use of freeze drying assisted ultrasonication emerged as an excellent tool to improve the extraction from various plants and their parts to get maximum extract yields for better antioxidants and medicinal properties (Mokaizh, *et al.*, 2024; Miki, *et al.*, 2024)). The use of ultrasonication in the extraction from plant materials has numerous benefits including minimize use of organic toxic solvents, maximum recovery of biologically active metabolites posing as eco-friendly technique (Shen, *et al.*, 2023).

However, the local practitioners do not use sophisticated and modern extraction technology to get maximum benefits from the plants. In this context a study was designed to optimize the extraction from *H. strigosum* using various compositions of methanol solvent and freeze drying assisted ultrasonication (FUS). The impact of extraction conditions was also studied on phenolic and flavonoids, antioxidant, *in-vitro* antidiabetic and antiobesity properties of *H. strigosum*.

Materials and Methods

Plant Sampling

The fresh and healthy *H. strigosum* plants were sampled from its natural habitat of Potohar region (Jhelum District) of Punjab, Pakistan. The material was subjected to dust removal and immediately quenched in liquid nitrogen for long term conservation of plant metabolites.

Optimized Extraction

The plant materials was ground to fine powder using mortar and pestle with continuous pouring of liquid nitrogen. The obtained powder was freeze dried for 48 hours at temperature of -68 °C for maximum removal of water contents. After freeze drying, the plant material were submerged in pure methanol (FUS-1), 80% (FUS-2), 60% (FUS-3), 40 %(FUS-4), 20 %(FUS-5) and aqueous (FUS-6)). The prepared samples were stored for 24 hours in dark, followed by orbital shaking for two hours. The samples were subjected to ultrasonication at 20 Khz for 30 min time period. The plant debris was removed from the solutions through filtration assembly and extra solvents were removed under vacuum to get extract which were stored at low temperature for further utilization. The extract yield % was calculated from following relationship by simply dividing the mass of plant material taken and amount of extract obtained.

$$Extract\ Yield\ \% = \frac{Yield\ of\ extract\ in\ mg}{Amount\ of\ plant\ material\ taken\ in\ g}$$

Total Phenolic Contents

Folin-Ciocalteu reagent based well reported method was adopted for estimation of phenolic contents. For the purpose, Folin-Ciocalteu reagent (90 µl) was added by about 20 µl of plant extract prepared previously. The 10% Na_2CO_3 was prepared and added to Folin-Ciocalteu reagent already having plant extract and stayed for a specific time period. The absorbance at 726 nm was noted using LABOMED, INC spectro UV-VIS double beam, UVD- 3000. As standard compound, the gallic acid was used and the

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observations in gallic acid equivalency (mg/g) were calculated (Siddiqui *et al.*, 2017).

Total Flavonoid Contents

An established method was used to determine TFC for *H. strigosum* extracts with little modifications. Extracts of *H. strigosum* were used to prepare reaction mixture in methanol by adding about 0.10 mL of NaNO2 (0.5M) solution. After that, $AlCl_3.6H_2O$ was added and made the volume upto 200 µL using methnaol. Kept the reaction mixture for 5 min at ambient conditions for stabilization followed by addition of NaOH. The consequent absorbance at wavelength of 510 nm was noted for each sample on spectrophotometer. Final observations were presented as milligrams of rutin equivalent for each gram of plant extract used in study. The rutin was utilized as standard flavonoid compound for spectrophotometric calculations to compute the results (Orsavova *et al.*, 2023).

Antioxidant activity

Different antioxidant assays like DPPH and FRAP were used to evaluate the antioxidant potential of *H.strigosum* extract.

DPPH Scavenging

DPPH scavenging activity was measured by using slight modifications in an already well reported method (Mensor *et al.*, 2001).

The prepared extract samples (10 µg-100µg) were taken and suspended in DPPH solution. The mixture was mechanically shacked on shaker for at least 10 min for thorough mixing. The incubation of the reaction mixture was done for 25 min at 25°C in the dark. The value of absorbance was measured at wavelength of 517 nm as recommended protocol. Following expression was used for calculation.

$$DPPH\ Scavenging\ \% = \left(\frac{Abs\ of\ Ctr - Abs\ of\ Smp}{Abs\ of\ Ctr}\right) \times 100$$

Where, Abs of Ctr and Abs of Smp represented the absorbance of control and sample used in the study.

Ferric reducing antioxidant power (FRAP) assay

The TPTZ (2,4,6-tripyridyl-s-triazine) and $FeCl_3.6H_2O$ based reported method was used to determine the FRAP activity which is based upon the reduction of Ferric complex to Ferrous complex of blue color by plant extract (Benzie *et al.*, 1996).

An acetate buffer was prepared with pH 3.6 for suitable conditions for complex formation. The slightly acidic TPTZ (2,4,6-tripyridyl-s-triazine) solution was also prepared and further added by of 20 mM of $FeCl_3.6H_2O$ to prepare the reaction mixture. The mixture obtained was then allowed to mix with previously prepared acetate buffer. The reaction mixture was then incubated at 37°C for the completion of reaction. The plant extract and Trolox in measured quantities were added to determine the FRAP activity. The change in color indicated the formation of complex. The complex formation was checked spectrophotometrically at 593 nm. The findings were expressed as Torolox equivalent (TE mM/mL) as it was used as reference which an analogue of vitamin E.

The α-glucosidase inhibition assay

Plant extract was allowed to suspend in phosphate buffer (70 µL of 50Mm) solution. The optimal pH 6.8 was confirmed and enzyme α-glucosidase in concentration of 1 unit/mL of was added. An incubation was performed for few minutes at temperature of 37°C for homogenization. As substrate, ρ- nitrophenolglucopyranoside (5 mM) was added to mixture and reaction was carried out for half an hour time. For results computation, the absorbance was for each sample was noted at specific or recommended wavelength of 405 nm for ρ-nitrophenol freed from substrate by enzymatic cleavage (Jabeen *et al.*, 2013).

The readings on spectrophotometer were taken for the samples in triplicate manner. From the absorbance, the enzyme inhibition % were calculated through the equation given below.

$$\alpha - glucosiadse\ inhibtion\ \% = \left(\frac{Abs\ of\ Blank - Abs\ of\ Extract}{Abs\ of\ Extract}\right) \times 100$$

Acarbose was also used as reference standard. Where Abs is absorbance.

The pancreatic lipase inhibition assay

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The pancreatic lipase inhibition activity was checked by already reported protocol to assess the antiobesity properties of plant extracts (Fukumoto *et al.*, 1963). Briefly, extract was suspended in to 0.01 M Tris-HCl buffer following by supplement dose of enzyme (pancreatic lipase). The substrate was prepared by taking olive oil (as fatty acid source) and gum Arabic as dispersing agent in measured amounts and dissolved in 0.1 M Tris-HCl buffer already enriched with calcium chloride and sodium chloride.

About 100µg of plant extract was added with 0.2 mL of lipase solution. The resultant solution was stayed for a time period of 30 min at approximately 4°C. Then, 2 mL of substrate prepared in the previous step was mixed with the reactants and further incubated for 30-35 min time at most suitable temperature of 37°C. The reaction started so far needed termination, for which a mixture of pure ethanol and acetone in equal amounts was added. The free fatty acids (FFA) produced by the enzymatic action were titrated against 0.02 M NaOH with continuous monitoring of pH until it reached of 9.4 as end point. Standard drug Orlistat was used as reference anti-obesity agent. The % inhibition of enzyme was noted using following mathematical equation.

$$Pancreatic\ lipase\ inhibition\ \% = 100\% - [VS - VC] \times 100$$

The value for control enzyme was 100% which is a predetermined factor indicating activity of control where enzyme faced no restriction in performance. The Vs and Vc represented the NaOH amount by volume consumed for sample and control during acid base titration.

Results and Discussion

Extract Yield

The extract yields were calculated for each solvent composition given in Figure 1. The results elaborated that the solvent composition significantly influenced the extract yields and marked remarkable response. The FUS-2 (80% methanol) exhibited highest extract yield of 22.46±0.75% being significant in statistical term having $\rho<0.05$ when compared as indicated by one way-ANOVA. However, statistically speaking there was no difference in extract yields produced by FUS-1 and FUS-3. Trend indicated that extract yields significantly declined with further reduction in methanol content of solvent and relative increase of aqueous portion.

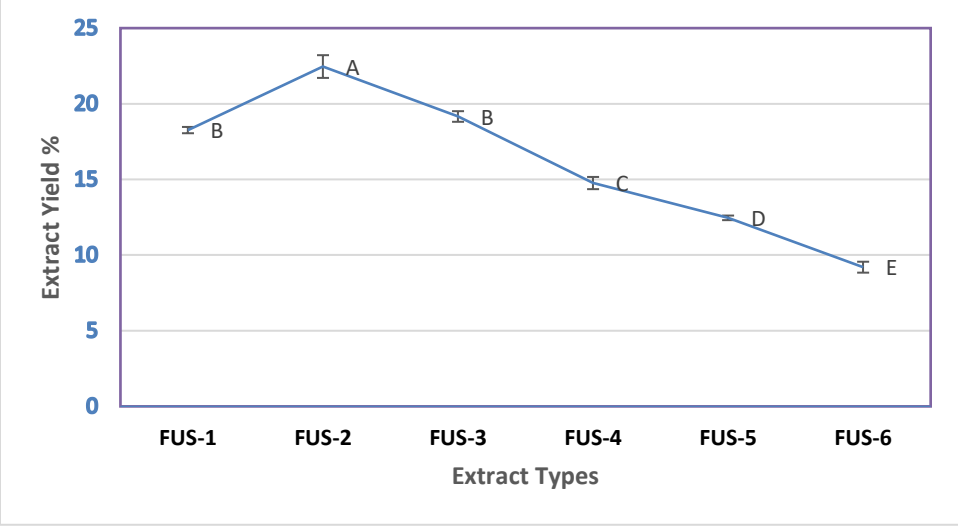

Figure 1. Extract Yields % of *H. strigosum* using various methanol concentrations. The non-sharing of alphabets (A-E) highlighted the statistical significance based on difference of means with $\rho<0.05$ and vice versa

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Most suitable reason for relatively higher yields might be the role of solvent composition which mechanistically interplayed with the plant matrix to extract maximum. The role of methanol was promising to get higher yields due to its polarity which probably enhanced the solubility of phytochemicals with similar nature. Pragmatic role of methanol or solvent polarity in improving extract yields from plant sources was reported by many researchers (Lee et al., 2024; Setiawansyah *et al.*, 2025). The significance of extract yields was well established as it might be responsible for better amounts of phytochemicals. The high contents of phytochemicals like phenolics and flavonoids consequently rendered higher antioxidant and medicinal activities Nawaz *et al.*, 2020; Raza *et al.*, 2020).

Total Phenolic and Flavonoid Contents

The values obtained for TPC and TFC were reported as Figure 2 and Figure 3, respectively.

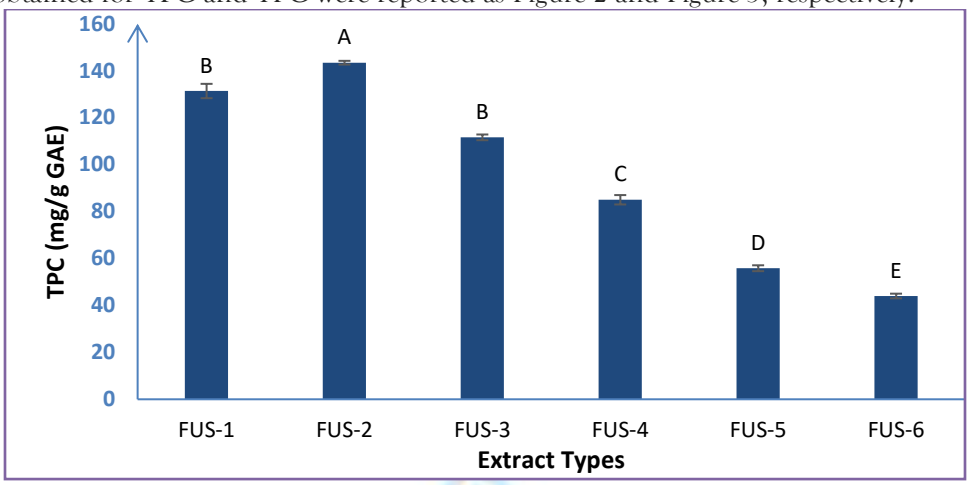

Figure 2. TPC of *H. strigosum* using various methanol concentrations. The non-sharing of alphabets (A-E) highlighted the statistical significance based on difference of means with ρ<0.05 and vice versa.

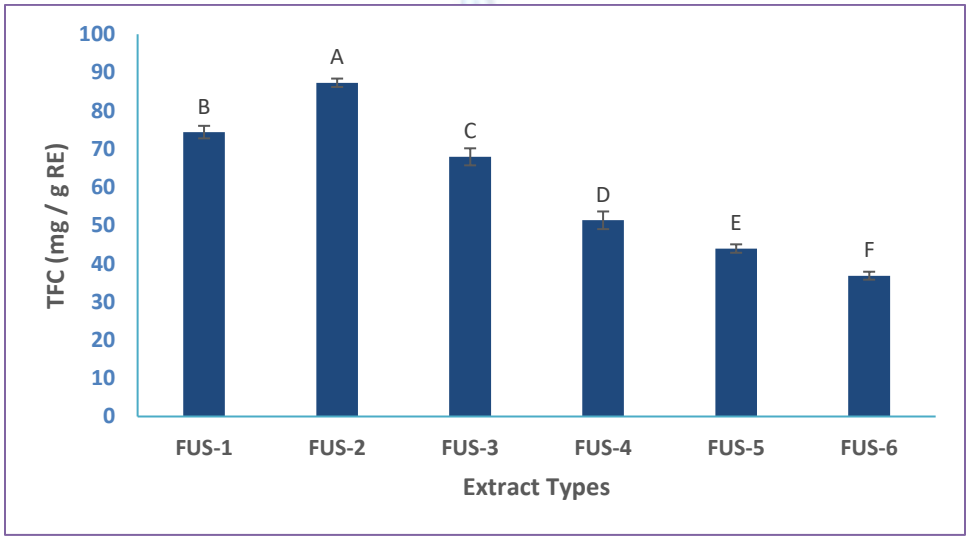

Figure 3. TFC of *H. strigosum* using various methanol concentrations. The non-sharing of alphabets (A-E) highlighted the statistical significance based on difference of means with ρ<0.05 and vice versa.

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The values of TPC (143.4 ± 0.794 mg /g GAE) and TFC (87.30 ± 1.10 mg /g RE) were reasonably higher for FUS-2 sample and significantly different from other extracts with ρ<0.05). The high values of phenolics and flavonoids for FUS-2 were apparently due to the high extract yield. A study reported that 80% methanol yielded high amount of extract from *Rhus vulgaris* which accordingly exhibited higher phenolic contents and powerful antioxidant activity confirming the role of extract yield in generating substantial concentrations of phytochemicals (Belew et al., 2025). The good extraction strategy including solvent choice might be responsible for improved extract yields with generous phytochemical output (Noorhashim *et al.*, 2025). It might be inferred from the trends in values of TPC and TFC, that higher extract yield corresponded to good amounts of polyphenols.

A previously published report on *H. strigosum* reported that its crude methanolic extract contained 84.50 ± 0.06 µg GAE/ mg phenolic contents which were very less than the findings of current work (Qayyum *et al.*, 2016). There might be many factors behind such difference in phenolic contents. The use of ultrasoniction for extraction in present work could not be ignored as ultrasonication is more often recommended for better extraction of phytochemicals. The ultrasonication based cavitation effect was reported as key mechanism for maximum rupturing and interplay within the plant matrix resulted in huge recovery of metabolites (Mokaizh *et al.*, 2024; Wang *et al.*, 2022).

Besides extraction, some other factors like habitat, environmental factors, stress, and immediate treatment after plant collections also reported to influence the extract yields and phytochemical contents for same plant (Bautista *et al.*, 2016; Zargoosh *et al.*, 2019).

Antioxidant and Medicinal Properties

The antioxidant activity was measured by DPPH radical scavenging assay and FRAP in comparison with standard synthetic antioxidant BHA (Table 1).

Table 1. Values of DPPH assay, FRAP assay, α-glucosidase and pancreartic lipase enzyme inhibition by various methanolic extracts of *H. strigosum*.

Extract	DPPH Scavenging %	FRAP Value (TE Mm/mL)	α-glucosidase inhibition %	Pancreatic lipase inhibition %
FUS-1	82.55 ± 1.25^{B}	125.44 ± 2.11^{B}	72.05 ± 0.55^{C}	43.73 ± 0.83^{B}
FUS-2	94.94 ± 1.04^{A}	156.10 ± 3.05^{A}	84.50 ± 0.45^{B}	44.57 ± 0.37^{B}
FUS-3	72.35 ± 0.85^{C}	124.28 ± 2.14^{B}	71.77 ± 0.83^{C}	33.22 ± 0.44^{C}
FUS-4	70.14 ± 0.72^{C}	88.05 ± 1.95^{C}	72.28 ± 0.74^{C}	31.18 ± 0.24^{C}
FUS-5	54.13 ± 0.66^{D}	68.91 ± 1.31^{D}	60.25 ± 0.55^{D}	17.15 ± 0.15^{D}
FUS-6	56.44 ± 0.62^{D}	66.72 ± 2.24^{D}	58.73 ± 0.33^{D}	15.61 ± 0.11^{D}
Standard Compound	BHA = 95.21± 0.30^{A}	ND	Acarbose = 96.63 ± 0.21^{A}	Orlistat = 92.97 ± 0.17^{A}

The results of DPPH assay revealed that FUS-2 showcased excellent DPPH scavenging % which was non-significant when compared to BHA (ρ>0.05) but significantly higher among all other extracts. Similarly, FRAP values were also found higher for FUS-2 extract being significantly potent. Such antioxidant potential was amazing and added significant importance to *H. strigosum* as potential source of strong antioxidants. The antioxidant activity of FUS-2 could be linked with high phenolic and flavonoid contents presents in the extract. The structural features of plant phenolics were considered as of immense importance especially when talking about antioxidant potential. The phenolics and flavonoids of plants were reported to have excellent antioxidant activities. The plant phenolics exerted antioxidant or antiradical potential by donating proton or electron transfer

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mechanism or oxygen quenching process (Kumar *et al.*, 2019).

The antiradical and antioxidant potential of plants surely depended upon the quantity and quality of these natural metabolites especially in context of structural nature as revealed by numerous investigations. The plant phenolics were given the status of best naturally available tonic for human health (Imtiaz *et al.*, 2025; Belew *et al.*, 2025; Ciupei *et al.*, 2024).

Stronger antioxidant activities are often associated with some high value compounds as many metabolite characterization and identification studies based upon modern analytical techniques like liquid chromatography equipped with mass spectrometry have unveiled the reality in real time manner (Farooq *et al.*, 2020; Raza *et al.*, 2022).

The values of α-glucosidase and pancreatic lipase inhibition were also given in table 1. The values for inhibition of both enzymes by various methanolic and aqueous extracts showed that FUS-2 was the most potent there in. The statistical analysis also revealed that the FUS-2 extract was most effective regarding α-glucosidase inhibition % as indicated by statistical analysis ($\rho < 0.05$). Most importantly, %age inhibition of α-glucosidase activity by FUS-2 was quite comparable with standard antidiabetic agent acarbose being statistically non-significant. The finding emphasized that the FUS-2 extract of *H. strigoum* might have metabolites responsible for antidiabetic potential executed through α-glucosidase inhibition.

Reports has already established the inhibition of α-glucosidase enzyme as a potential indicator especially where *in-vitro* antidiabetic activity assessment of plant extracts involved, probably through individual or synergic mechanism (Lianza *et al.*, 2022; Dirir *et al.*, 2022). The results of present work highlighted a reasonably positive correlation between DPPH radical scavenging % and α-glucosidse was observed with R^2 value of 0.9166 and might be inferred that antioxidant activity showed strong connection with α-glucosidase activity loss.

Scientists have elaborated the mechanism for α-glucosidase enzyme activity loss or reduction by plant based phytochemicals or metabolites. The most probable mechanism was the interaction of plant metabolites with amino acid moieties of α-glucosidase to impart some structural modifications resulting in activity loss of enzyme (Sakulkeo *et al.*, 2022; Nadeem *et al.*, 2019).

However, the inhibition of pancreatic lipase by *H. strigosum* extracts used in this study was not promising as given in table 1. The FUS-2 extract displayed only 44.57 ± 0.37 % pancreatic lipase inhibition which was far less than the standard drug orlistat having 92.97 ± 0.17 % enzyme inhibition. The orlistat inhibited the pancreatic lipase to significant extent with $\rho > 0.05$.

The findings also revealed that although the FUS-2 extract exhibited highest antioxidant activity, α-glucosidase and pancreatic lipase inhibition among all other extracts. However, the *H. strigosum* was proved as workable option for diabetes mellitus management but moderate to low regarding obesity prevention. The findings confirmed the ethnomedicinal use of *H. strigosum* for diabetes mellitus treatment but not supported the reasonable antiobesity attributes. Further investigation may be performed for a rational metabolite profile based approach to explore new therapeutic agents and functional food developments.

Conclusion

The various methanol based solvent compositions were used for improved extraction from *H. strigosum* for enhanced antidiabetic and antiobesity attributes. The FUS-2 extract (80% methanol) provided much better extract yield, antioxidant activity and α-glucosidase and pancreatic lipase inhibition. However, the α-glucosidase inhibition was more pronounced as compared to pancreatic lipase and comparable with standard acarbose. The results confirmed the antidiabetic use of *H. strigosum* in traditional medicinal system of Pakistan. Further studies on metabolite profiling and activity guided route may be opted for more comprehensive outcomes to develop safe nature based antidiabetic approach. The findings of current work may also act as base line information about *H. strigosum* for interventions in functional foods.

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REFERENCES

Abu Khadra KM, Bataineh MI, Khalil A, Saleh J. Oxidative stress and type 2 diabetes: the development and the pathogenesis, Jordanian cross-sectional study. European Journal of Medical Research. 2024 Jul 17;29(1):370.

Ahmad S., Alam K., Wariss H., Anjum S., Mukhtar M. Ethnobotanical studies of plant resources of Cholistan desert, Pakistan. Int. J. Sci. Res. 2014;3:1782–1788.

Aslam, H., Khan, A.U., Ali, F., Nadeem, H. and Shah, S.M., 2017. Anti-hyperglycemic activity of Heliotropium strigosum (Boraginaecae) whole plant extract in alloxan-induced diabetic mice. *Tropical Journal of Pharmaceutical Research*, *16*(10), pp.2425-2430.

Bautista, I., Boscaiu, M., Lidón, A., Llinares, J.V., Lull, C., Donat, M.P., Mayoral, O. and Vicente, O., 2016. Environmentally induced changes in antioxidant phenolic compounds levels in wild plants. *Acta Physiologiae Plantarum*, *38*(1), p.9.

Belew, A.A. and Gebre, S.H., 2025. Comparative assessment of phenolic and flavonoid contents and antioxidant activities in methanol extracts of spices from Jigjiga market, Ethiopia. *Pharmacological Research-Natural Products*, *6*, p.100168.

Belew, A.A., Hanan, G.G.M.W., Meshesha, D.S. and Akele, M.L., 2025. Evaluation of total phenolic, flavonoid contents, antioxidant and antibacterial activity of leaf extracts from Rhus vulgaris. *Discover Plants*, *2*(1), p.141.

Benzie, I.F., Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of"antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1): 70-76.

Bhatti, J.S., Sehrawat, A., Mishra, J., Sidhu, I.S., Navik, U., Khullar, N., Kumar, S., Bhatti, G.K. and Reddy, P.H., 2022. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radical Biology and Medicine*, *184*, pp.114-134.

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World allergy organization journal. 2012 Dec;5(1):9-19.

Ciupei, D., Colişar, A., Leopold, L., Stănilă, A. and Diaconeasa, Z.M., 2024. Polyphenols: from classification to therapeutic potential and bioavailability. *Foods*, *13*(24), p.4131.

Dirir, A.M., Daou, M., Yousef, A.F. and Yousef, L.F., 2022. A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews*, *21*(4), pp.1049-1079.

Farooq, M.U., Mumtaz, M.W., Mukhtar, H., Rashid, U., Akhtar, M.T., Raza, S.A. and Nadeem, M., 2020. UHPLC-QTOF-MS/MS based phytochemical characterization and anti-hyperglycemic prospective of hydro-ethanolic leaf extract of Butea monosperma. *Scientific Reports*, *10*(1), p.3530.

Fukumoto, J., Tsujisaka,Y., Iwai, M. (1963). Studies on lipase. *The Journal of General and Applied Microbiology*, 9(3): 353-361.

Hussain, S., Jamil, M., Ullah, F., Khan, A., Ullah, F., Arfan, M., Ahmad, S. and Khatoon, L., 2010. Antimicrobial and antioxidant activities of the plant Heliotropium strigosum. *African Journal of Biotechnology*, *9*(45), pp.7738-7743.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Imtiaz, F., Ahmed, D., Mohammed, O.A., Younas, U. and Iqbal, M., 2025. Optimized recovery of phenolic and flavonoid compounds from medicinal plant extracts for enhanced antioxidant activity: A mixture design approach. *Results in Chemistry*, *13*, p.101960.

Jabeen. B., Riaz, N., Saleem, M., Naveed, M.A., Ashraf, M., Alam, U., Rafiq, H.M., Tareen, R.B., Jabbar, A. (2013). Isolation of natural compounds from *Phlomisstewartii* showing α- glucosidase inhibitory activity. *Phytochemistry*, 96: 443-448.

Khurm, M., Chaudhry, B.A., Uzair, M. and Janbaz, K.H., 2016. Antimicrobial, Cytotoxic, Phytotoxic and Antioxidant Potential of Heliotropium strigosum Willd. *Medicines*, *3*(3), p.20.

Kumar, N. and Goel, N., 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology reports*, *24*, p.e00370.

Lee, J.E., Jayakody, J.T.M., Kim, J.I., Jeong, J.W., Choi, K.M., Kim, T.S., Seo, C., Azimi, I., Hyun, J. and Ryu, B., 2024. The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. *Foods*, *13*(19), p.3151.

Lianza, M., Poli, F., Nascimento, A.M.D., Soares da Silva, A., da Fonseca, T.S., Toledo, M.V., Simas, R.C., Chaves, A.R., Leitao, G.G. and Leitão, S.G., 2022. In vitro α-glucosidase inhibition by Brazilian medicinal plant extracts characterised by ultra-high performance liquid chromatography coupled to mass spectrometry. *Journal of enzyme inhibition and medicinal chemistry*, *37*(1), pp.554-562.

Liu, T.T., Liu, X.T., Chen, Q.X. and Shi, Y., 2020. Lipase inhibitors for obesity: A review. *Biomedicine & Pharmacotherapy*, *128*, p.110314.

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C.D., Coube, C.S., Leitão, S.G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15(2): 127-130.

Miki, K.S.L., Dresch, A.P., Cavali, M., da Silva, A.P., Marafon, F., Fogolari, O., Mibielli, G.M., Bagatini, M.D. and Bender, J.P., 2024. Influence of drying methods in the ultrasound-assisted extraction of bioactive compounds from Byrsonima crassifolia to evaluate their potential antitumor activity. *Food and Humanity*, *2*, p.100242.

Mokaizh, A.A.B., Nour, A.H. and Kerboua, K., 2024. Ultrasonic-assisted extraction to enhance the recovery of bioactive phenolic compounds from Commiphora gileadensis leaves. *Ultrasonics Sonochemistry*, *105*, p.106852.

Mokaizh, A.A.B., Nour, A.H. and Kerboua, K., 2024. Ultrasonic-assisted extraction to enhance the recovery of bioactive phenolic compounds from Commiphora gileadensis leaves. *Ultrasonics Sonochemistry*, *105*, p.106852.

Nadeem, M., Mumtaz, M.W., Danish, M., Rashid, U., Mukhtar, H., Anwar, F. and Raza, S.A., 2019. Calotropis procera: UHPLC-QTOF-MS/MS based profiling of bioactives, antioxidant and anti-diabetic potential of leaf extracts and an insight into molecular docking. *Journal of Food Measurement and Characterization*, *13*(4), pp.3206-3220.

Nawaz, H., Shad, M.A., Rehman, N., Andaleeb, H. and Ullah, N., 2020. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. *Brazilian Journal of Pharmaceutical Sciences*, *56*, p.e17129.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Noorhashim, N.S., Azar, S.N.I., Azlan, A., Razali, N.A., Li, A.R. and Abidin, N.H.K.Z., 2025. Total phenolic contents, total flavonoid contents and antioxidant activities of endocarp and mesocarp of Malaysian local Durio Zibethinus (Musang King and Durian Kampung Peel). *Journal of Food Measurement and Characterization*, pp.1-11.

Orsavová, J., Juríková, T., Bednaříková, R., Mlček, J. (2023). Total phenolic and total flavonoid content, individual phenolic compounds and antioxidant activity in sweet *rowanberry cultivars*. *Antioxidants*, 12(4):913.

Ozougwu, J.C., 2016. The role of reactive oxygen species and antioxidants in oxidative stress. *International Journal of Research*, *1*(8), pp.1-8.

Pan, G., Lu, Y., Wei, Z., Li, Y., Li, L. and Pan, X., 2024. A review on the in vitro and in vivo screening of α-glucosidase inhibitors. *Heliyon*, *10*(18).

Patil BS, Patil JK, Chaudhari HS, Patil BS. Oxidative Stress, Inflammation, and Obesity: Insights into Mechanism and Therapeutic Targets. InProceedings 2025 Jun 27 (Vol. 119, No. 1, p. 6). MDPI.

Patil, S., Patil, M., Maheshwari, V.L. and Patil, R.H., 2022. Pancreatic lipase (PL) inhibitors from medicinal plants and their potential applications in the Management of Obesity. In *Natural Products as Enzyme Inhibitors: An Industrial Perspective* (pp. 153-167). Singapore: Springer Nature Singapore.

Qayyum, A., Sarfraz, R.A., Ashraf, A. and Adil, S., 2016. Phenolic composition and biological (anti diabetic and antioxidant) activities of different solvent extracts of an endemic plant (Heliotropium strigosum). *Journal of the Chilean chemical society*, *61*(2), pp.2903-2906.

Qayyum, A., Sarfraz, R.A., Ashraf, A. and Adil, S., 2016. Phenolic composition and biological (anti diabetic and antioxidant) activities of different solvent extracts of an endemic plant (Heliotropium strigosum). *Journal of the Chilean chemical society*, *61*(2), pp.2903-2906.

Rahman, N., Muhammad, I., Afridi, S.G., Khan, A., Nayab, G.E., Ahmad, I., Khan, H., Belwal, T., Devkota, H.P. and Milella, L., 2021. Inhibitory effects of plant extracts and in Silico screening of the bioactive compounds against α-glucosidase. *South African journal of botany*, *143*, pp.330-343.

Raza, S.A., Chaudhary, A.R., Mumtaz, M.W., Adnan, A., Mukhtar, H. and Akhtar, M.T., 2020. Metabolite profiling and antidiabetic attributes of ultrasonicated leaf extracts of Conocarpus lancifolius. *Asian Pacific Journal of Tropical Biomedicine*, *10*(8), pp.353-360.

Raza, S.A., Rashid Ch, A., Mumtaz, M.W., Bashir, S., Ahmad, M., Touqeer, T., Mukhtar, H., Akhtar, M.T., Ali, S. and William, J., 2022. Metabolites in Conocarpus erectus leaves attenuate α-amylase activity by modulating amino acid residues of α-amylase: an in vitro and docking study. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *21*(3).

Riyadi, P.H., Susanto, E., Anggo, A.D., Arifin, M.H. and Rizki, L., 2023. Effect of methanol solvent concentration on the extraction of bioactive compounds using ultrasonic-assisted extraction (UAE) from Spirulina platensis. *Food Research*, *7*(3), pp.59-66.

Sakulkeo, O., Wattanapiromsakul, C., Pitakbut, T. and Dej-Adisai, S., 2022. Alpha-Glucosidase Inhibition and Molecular Docking of Isolated Compounds from Traditional Thai Medicinal Plant, Neuropeltis racemosa Wall. *Molecules*, *27*(3), p.639.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Setiawansyah, A., Fiolita, B., Agatha, S., Sitindaon, R.S., Reynaldi, M.A., Hidayat, L.H., Hadi, I., Permatasari, L., Hidayati, N., Arsul, M.I. and Dirgantara, S., 2025. Impact of Post-Harvest Process and Methanol Polarity on The Content of Niazirin, Flavonoids, Phenols, And Antioxidant Activity Index of Moringa oleifera Lam Leaves. *Food and Humanity*, p.100767.

Shen, L., Pang, S., Zhong, M., Sun, Y., Qayum, A., Liu, Y., Rashid, A., Xu, B., Liang, Q., Ma, H. and Ren, X., 2023. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrasonics Sonochemistry*, *101*, p.106646.

Siddiqui, N., Rauf, A., Latif, A., Mahmood, Z. (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*NepetabracteataBenth*). *Journal of Taibah university medical sciences*, 12(4):360-3.

Truong, D.H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H. and Nguyen, H.C., 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. *Journal of food quality*, *2019*(1), p.8178294.

Wang, N., Shi, N., Fei, H., Liu, Y., Zhang, Y., Li, Z., Ruan, C. and Zhang, D., 2022. Physicochemical, structural, and digestive properties of pea starch obtained via ultrasonic-assisted alkali extraction. *Ultrasonics Sonochemistry*, *89*, p.106136.

William, J., John, P., Mumtaz, M.W., Ch, A.R., Adnan, A., Mukhtar, H., Sharif, S. and Raza, S.A., 2018. Antioxidant activity, hypoglycemic potential and metabolite profiling of Hyophorbe indica leaf extract. *Pak. J. Pharm. Sci*, *31*(6), pp.2737-2742.

William, J., John, P., Mumtaz, M.W., Ch, A.R., Adnan, A., Mukhtar, H., Sharif, S., Raza, S.A. and Akhtar, M.T., 2019. Antioxidant activity, α-glucosidase inhibition and phytochemical profiling of Hyophorbe lagenicaulis leaf extracts. *PeerJ*, *7*, p.e7022.

Zargoosh, Z., Ghavam, M., Bacchetta, G. and Tavili, A., 2019. Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. *Scientific reports*, *9*(1), p.16021.