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EVALUATION OF ANTIDIABETIC ACTIVITY OF LYOPHILIZED TOMATO AND LEMON JUICES ALONE AND IN COMBINATION

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ABSTRACT

The present study aimed to compare the impact of lyophilization on the phytochemicals and *in-vitro* antidiabetic activity of tomato and lemon fruit juices alone and in combination, and compares their efficacy with fresh samples by using different analytical techniques. For the phytochemical analysis of samples, conventional methods like Gas Chromatography Mass Spectrometry (GC-MS), Fourier Transformed Infrared Spectroscopy (FTIR) and High-Performance Liquid Chromatography (HPLC) were used. HPLC technique was used to confirm flavonoid compounds i.e. quercetin, myricetin and kaempferol in isopropyl alcohol, methanol and acetonitrile extracts. Furthermore, *in vitro* antidiabetic activity was determined by using inhibition of alpha amylase enzyme and glucose uptake by yeast cells assays. Both lyophilized juices of lemon and tomato demonstrated high phenolic and flavonoid content than fresh samples. Lemon demonstrated higher total phenolic content of 7.15 mg/g, while tomato contained higher total flavonoid content of 73.75 mg/g, both of which are important to play critical role in glucose metabolism and antioxidant defense. When compared with fresh juices results, the lyophilized samples exhibited comparable phytochemical stability and biological activity, highlighting the effectiveness of lyophilization in preserving bioactive compounds. On the other hand, the combination extract (IC_{50} = 7.76 mg/ml) of lemon and tomato showed significant inhibition of alpha amylase enzyme activity in a concentration dependent manner ($P<0.05$) and enhanced glucose uptake in yeast cells, suggesting a complementary effect in all glucose concentration of 5, 10 and 25 mM. These findings indicate that both lemon and tomato contribute beneficially to antidiabetic activity, particularly when used in combination. Future studies should focus on validating these findings by using *in-vivo* models and exploring formulation strategies for diabetes management. Overall, this research also adds novelty of employing lyophilization as a successful preservation technique, thus offering a potential natural remedy for diabetes.

Keywords: α-Amylase, Flavonoids, Glucose uptake, Lemon, Antidiabetic, Tomato.

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INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin, or by the ineffectiveness of the produced insulin. Such deficiency results in increased glucose concentration and can cause damage to blood vessels and nerves. Type II diabetes is a serious disorder that requires more pharmacological treatment besides lifestyle changes. The global burden of diabetes is rising steadily, placing significant strain on healthcare systems (Tuobenyiere *et al.*, 2023). The second National Diabetes Survey of Pakistan (NDSP), 2016-2017, revealed that diabetes prevalence in Pakistan, increased up to 26.3% and in urban areas higher rates are observed (Basit *et al.*, 2018). By the year of 2030, it can become double which is indeed a very alarming situation (Hasan and Siddiqui, 2024).

Initially, oral agent monotherapy alone was used to treat this disorder but eventually it requires addition of more agents like biguanides, sulfonylureas, miglitol, thiazolidinedione's, acarbose and insulin. Thus, due to increased intake frequency of medications leads towards resistance and side effects. That's why, alongside conventional therapies, increasing attention has been directed toward natural dietary sources and plant-based compounds for their potential role in preventing or managing diabetes. Fruits and vegetables, rich in bioactive constituents, have attracted particular interest due to their accessibility, safety profile, and broad nutritional benefits (Zafar *et al.*, 2025). Previous evidences have confirmed that by using Chinese herbal medicines, improvement can occur through different molecular mechanisms (Ni *et al.*, 2024). In this scenario, the pharmacological effects of medicinal plants as medicine will be considered as bright future for the

management of health care in 21st century (Verma *et al.,* 2018, Timón *et al.,* 2024).

Tomato (*Solanum lycopersicum* L.) fruit belongs to the Solanaceae family. Tomato is a genus of small evergreen trees in the flowering plant. It's commonly known as tomato or garden plant. The tomato is also considered a super food for diabetes because it has much lower carbohydrate content and low-calorie content (Banihani, 2018). It also contains different phytochemicals like polyphenols, flavonoids, lycopene and carotenoids which have pharmacological effects. Tomatoes are also full of very important minerals.

Citrus limon (L.) commonly known as lemon, belong to the Rutaceae family. Lemon is fruit of the small green trees having varieties of fruits worldwide including Pakistan as the significant producer of citrus fruits, with the Punjab province contributing over 90% of the total production. Its juice is rich of vitamin C and other essential nutrients in limited quantity. The natural plant medicines have a long history of treating the diabetes and other metabolic disorders (Sitobo *et al.,* 2024). It contains tannins, flavonoids, polyphenols, citric acid, terpenes and vitamin C. Due to their flavonoids, polyphenols and vitamin C content, it is being used in diabetes and hypo-lipidemic conditions (Shahzad *et al.,* 2024).

Despite the nutritional and pharmacological potential of fruits such as tomato and lemon, a major challenge lies in preserving their bioactive compounds in a stable form suitable for long-term use. Conventional juices are prone to degradation during storage, which may compromise their efficacy (Shivendra *et al.,* 2021). Lyophilization or freeze-drying is a dehydration approach wherein the water is eliminated through sublimation to transform the liquid samples into better physically stable form. As a result of water elimination there's a reduction in deterioration processes because of decreased water activity (Martins *et al.,* 2022). For bioactive-rich juices, this technique provides a means of preserving compounds such as vitamin C, phenols, and carotenoids while maintaining consumer acceptability (Arora *et al.,* 2024).

Hence in the present study, this technique was applied to lemon and tomato juices not only for preservation purposes but also to investigate whether lyophilization influences the pharmacological activities of these extracts. After lyophilization, similarity in active compounds is the question mark. To investigate, different techniques were employed to detect the compounds and differentiate in natural and lyophilized juices which are responsible for their antidiabetic activity alone or in combination (Kamiloglu *et al.,* 2014). Therefore, this study was designed to compare the antidiabetic potential of fresh and lyophilized samples individually and in combination by using well-established *in-vitro* models.

MATERIALS AND METHODS

Sample procurement: The indigenous rippened tomato and lemon fruits were collected from local market, Lahore identified and authenticated by Department of Botany, Government College University, Lahore through comparison with standard herbarium specimen. After authentication, they had provided voucher number for *Solanum lycopersicum* (L.) GC. herb. bot. 3735 and for *Citrus limon* (L.) GC. herb. bot. 3736.

Preparation of Samples: Fresh fruits of tomato and lemon were washed thoroughly with water to remove impurities and dust. Firstly, cut the tomato into small pieces and blend it in laboratory blender without adding water. Then, passed the blended pulp through muslin cloth to separate the clear juice. The lemon juice was obtained by using manual juice extractor and similarly filtered through muslin cloth to remove seeds and pulp. Finally, the collected juices were centrifuged at 3000 rpm for 10 minutes to obtain a clear supernatant (Hasan *et al.,* 2022). Then until further lyophilization and analysis, filtrate was stored at –20°C (Aswathy *et al.,* 2019). The yield of the juices was determined by following formula:

$$Juice\ yield\ (\%) = (Juice\ weight/fruit\ weight) \times 100$$

Lyophilization (Freeze-Drying): The lyophilization of both fruits juices was carried out by following method described by Corrêa-Filho *et al.* (2019), but with some modifications. After lyophilization, juice of tomato was found to be reddish yellow in color having slight hard powder texture, needed crushing before processing to improve the solubility while juice of lemon was light yellowish having soft texture. Afterwards, lyophilized juices were stored separately in refrigerator for further analysis.

Phytochemical analysis

Preliminary Analysis of lyophilized tomato and lemon: Preliminary phytochemical analysis of fresh and lyophilized tomato and lemon samples was carried out to identify the presence of primary and secondary metabolites including proteins, alkaloids, glycosides, resins, tannins, flavonoids, phenols and carbohydrates (Maheshwaran *et al.,* 2024, Ibitomi *et al.,* 2024). Each qualitative test was performed in triplicates and the study followed completely randomized design (CRD) to ensure reproducibility and accuracy of results.

Gas chromatography-mass spectrometry (GCMS): Fused silica gel column 30 X 0.25mm ID, 0.25µm was selected for analysis. The detector was EI with energy of 70 electron volt. Inert helium gas was used as mobile phase; flow rate was set at 1mL / min. mass transfer line temperature was set at 220°C and injector temperature was 290°C. The temperature was set in alteration mode

through a total run time e.g. 60°C for oven for 2 minutes then increased up to 270°C at the rate of increasing order of 4°C /min, isothermal condition was sustained for 20 minutes, then gradually increased up to 300°C with the rate of 10°C/min. Injection volume was 20 µm. Run time was 25 minutes. Chromatograms were recorded and compare the highly abundant peak with molecular library in GC-MS (Agilent model 7890B, 5977B) (Pizzo *et al.*, 2024).

By Fourier Transform Infrared (FTIR) scanning: FT-IR spectral analysis was performed to` find the functional groups in the active components of lyophilized and fresh lemon and tomato samples (Subba *et al.,* 2024). This analysis provided valuable insight into the chemical nature and stability of bioactive compounds, helping to understand how lyophilization preserved key functional groups.

Total Phenol Content Determination: Total phenolic content of both fresh and lyophilized samples was estimated by Folin-Ciocalteu's method (Xiao *et al.*, 2025). First of all, 1 ml of both (fresh and lyophilized) tomato and lemon samples, solubilized in distilled water, were added to test tubes separately containing gallic acid of different concentration such as 20, 40, 60, 80 and 100 µg/ml. Then 0.5 ml of the aqueous solution of Folin Ciocalteu was vortexed and after that 2 mL of 2% (w/v) sodium carbonate/ distilled water solution was added to each test tube after incubation of 2 min at room temperature. Afterwards incubate the test tubes in dark for 2 hours at room temperature. After formation of deep blue color, UV-visible spectrophotometer was used to determine absorbance at 750 nm. Same procedure devoid of reagent was repeated in triplicates for blank, standard (Gallic acid) and samples. By using the data of calibration curve framed by using standard Gallic acid, the data of total phenolic contents of lyophilized samples were expressed as mg of Gallic acid equivalent weight / 100 g of dry mass. After absorbance, the total phenolic contents in both lyophilized samples were measured separately by using given formula;

$$C = C1 * V/m$$

Where C represents total phenolic content, C1 is concentration of Gallic acid established from the calibration curve, V is the volume of extract and m is weight of that sample extract.

Total Flavonoid Content Determination

UV-Visible Spectroscopy: For both fresh and lyophilized samples, total flavonoid content was measured with the aluminum chloride colorimetric assay (El Kamari *et al.,* 2024, Kim *et al.,* 2025). The stock solutions of both samples were prepared in distilled water. Stock solution of the standard quercetin having concentration 200, 400, 600, 800, 1000 µg/ml were also prepared. Then, 0.3 ml of 5% sodium nitrite solution was added to each test tube. After 5 minutes, 0.3 ml of 10% aluminum chloride ($AlCl_3$) was introduced, followed by addition of 2 ml of 1 M sodium hydroxide after another 6 minutes. The final volume was adjusted to 10ml using distilled water and thoroughly mixed.

The appearance of an orange-yellow color at the end of experiment indicates a positive result. Absorbance of resultant solutions of samples, standard and blank was taken at 510 nm in triplicate. The calibration curve was drawn using standard quercetin. The data of total flavonoids was expressed as mg of quercetin equivalents per100 g of dry mass. The total flavonoid contents in both of the lyophilized samples were determined by measuring absorbance separately by using this formula:

$$C = C1 * V/m$$

By RP-HPLC: Each fresh and lyophilized sample of tomato and lemon was mixed in HPLC grade methanol, acetonitrile and isopropyl alcohol (IPA) separately, filtered and stored in cool place. Flavonoid contents in each sample were determined by using standards of kaempferol, quercetin and myricetin at a wavelength of 360 nm on HPLC. Data of chromatograms was calculated by using area under the curve in different extracts of standard flavonoids (Sultana and Anwar, 2008).

Alpha amylase inhibition activity: Alpha amylase inhibition activity was determined by using slightly modified method of Tulin *et al.* (2024). This activity was performed on each fresh and lyophilized sample (tomato, lemon, in combination) in triplicates under CRD. One ml of each sample (1mg/ml stock solution) was incubated with 1ml of α-amylase solution (1% w/v) in 1 ml of sodium phosphate buffer (20 mM, 6.9 pH) at room temperature (32°C) for about 10 minutes. After incubation, 1ml of 1 % starch solution (dissolving 1 g of potato starch in 100 ml of distilled water with boiling and stirring for 15 minutes) was added and incubated at room temperature (32°C) for almost 10 minutes. Reaction mixture was vortexed, incubated for another 30 minutes at 37°C. Then, 0.5 ml of 3, 5-dinitrosalicylic acid was added as an indicator to stop the reaction. Lastly, 0.2 ml of 2N NaOH was added in reaction mixtures, then heat in water bath at 85°C for about 20 minutes and cooled down. Same procedure was repeated for control (without extract) and standard (acarbose). Absorbance of this mixture was taken at 540 nm in UV Visible spectrophotometer and the percentage inhibition of alpha amylase enzyme was calculated by applying formula:

$$\%age\ inhibition = Absorbance\ of\ control - Absorbance\ of\ Sample / Absorbance\ of\ control * 100$$

Glucose uptake by yeast cells: By following method of Qamar *et al.* (2019), this assay was performed on triplicates samples of fresh and lyophilized tomato, lemon

juices separately and in combination. Firstly, yeast powder was washed by ice cold sodium chloride solution (0.9 %) for 20 minutes centrifuged at 3000 rpm. Washed the yeast cells again by using the same procedure until the supernatant became clear. To prepare 10 % (V/V) suspension, suspend the yeast cell pellet in distilled water. 1mL of each sample (1mg/ml stock solutions) was prepared in distilled water and added in test tubes which already contains 1 mL of 5, 10 and 25 mM glucose solution. At 37 °C, test tubes were incubated for 10 minutes. On adding 100µL of the yeast, the mixture was vortexed, and further incubated at 37°C for about 60 minutes when the reaction started. Afterwards at 2500 rpm, each tube was centrifuged for 10 minutes, and the supernatant was analyzed at 620 nm against a blank., control and standard were prepared like the fresh and lyophilized samples. The activity was determined by taking metronidazole as a standard:

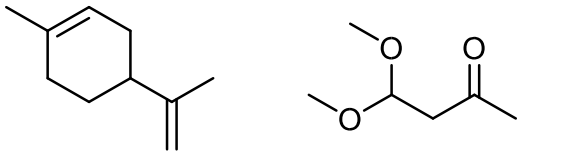
$$Glucose\ Uptake\ (\%) = Ac - As/Ac * 100$$

Where **Ac** highlight absorbance of control and **As** represents absorbance of sample. All samples were analyzed in triplicate.

Statistical analysis: The antidiabetic activity assays were conducted using a completely randomized design (CRD), with each sample tested in triplicates. Finalized data was analyzed by using the two-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, on statistic package for social sciences (SPSS 22). Results are presented as mean ± standard deviation and differences were considered significant at $p < 0.05$.

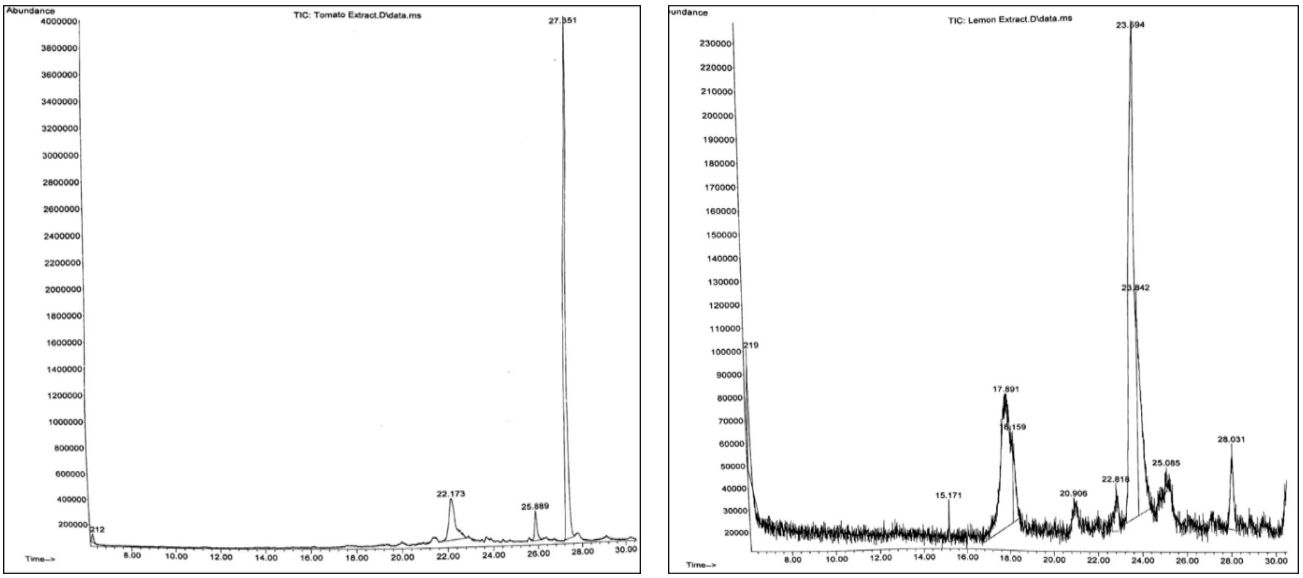
RESULTS

Gas chromatography-mass spectrometry (GCMS) analysis: The results obtained after the GC-MS analysis of lyophilized tomato extracts in methanol revealed thirteen compounds from which notably, the two groups were present in relatively high abundance. These two groups had the percentage of 17.38% and 76.54% at the retention times of 22.173 and 27.371, respectively. These compounds were identified as a combination of phenols and flavonoids, which are known to exhibit antidiabetic activity. In contrast, the lemon extract exhibited a greater diversity of compounds, with two groups accounting for 46.04% and 24.63% of the total compounds at the retention times of 23.594 and 17.891 respectively. There are a few compounds in both samples that are similar in structure and phenolic in nature as shown in Figure 1. In comparison with fresh juices of both fruits, identification of twenty-eight compounds in tomato juice has been observed.



Limonene 4,4-dimethoxy-2-butanone

Fig. 1. Structures of common compounds present in both lemon and tomato juices.



A B

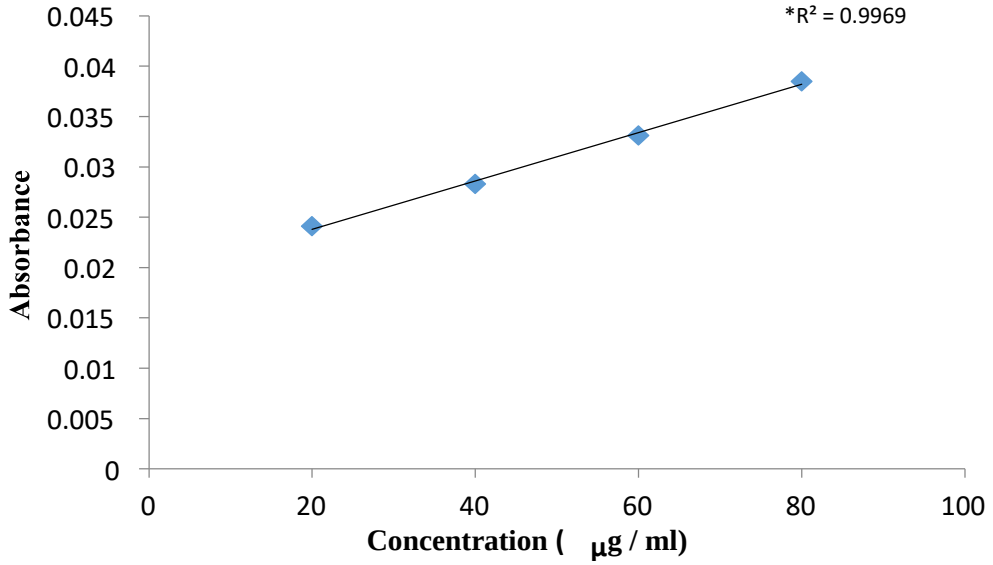
Fig. 2. GC-MS Chromatogram of A. lyophilized tomato juice, B. lyophilized lemon juice

FT-IR Spectral Analysis: The results of fresh and lyophilized tomato and lemon juice in FTIR analysis revealed different functional groups presence like phenols and flavonoids in both tomato and lemon juices (Table 1), as phenols are known to scavenge free radicals and inhibit lipid peroxidation (Untea *et al.*, 2024).

Table 1. Identification of peak values, functional groups and different classes of compounds in fresh and lyophilized tomato and lemon juice by using FTIR.

Sample Type	Peak Range (cm^{-1})	Functional Group
Tomato (Lyophilized)	3400, 900-1000, 1050	-OH (phenols), C=O (carbonyl) C-O (alcohols)
Fresh tomato juice	3350, 17515, 1045	-OH (phenols), C=O (carbonyl) C-O (alcohols)
Lemon (Lyophilized)	3200, 1190, 1230	-OH (phenols), C=O (carbonyl) C-O (alcohols)
Fresh lemon juice	3390, 1725, 1060	OH (phenols/flavonoids), C=O (carbonyl), C-O (ethers)

Total phenol content determination: The following concentrations of gallic acid solution conformed Beer's Law having R^2= 0.9969. The plot has a slope equal to 0.0002 and intercept equal to 0.019. The standard curve equation is y = 0.0002 + 0.019 as shown in Fig 3.



R² values represented mean data set of n=3

Fig. 3. Standard curve of gallic acid to determine the unknown concentration of total phenolic contents present in lyophilized lemon juice.

Table 2. Total phenolic contents of fresh and lyophilized tomato and lemon juices

Plant Form	Concentration used (mg/ml)	Phenolic content (mg of gallic acid equivalent/g dry material)
Tomato (Lyophilized)	42	4.2 ± 0.02^{b}
Tomato (Fresh)	-	2.85 ± 0.03^{c}
Lemon (Lyophilized)	71.5	7.15 ± 0.02^{a}
Lemon (Fresh)	-	4.60 ± 0.04^{b}

Values are expressed as mean ± SD (n=3). Different superscript letters within the same column indicate the statistically significant differences ($p<0.05$) between samples.

Determination of total flavonoid content

By UV-Visible Spectroscopy: The different concentrations of quercetin solution follow Beer's Law at 540 nm with R^2 = 0.9943. The plot has a slope equal to 0.0002 and intercept equal to 0.2395. The equation of standard curve is y = 0.0002x + 0.2395 as shown in Fig 4. The results have shown that more flavonoid compounds were present in lyophilized tomato extract while fresh lemon juice determined more flavonoid contents as shown in Table 3.

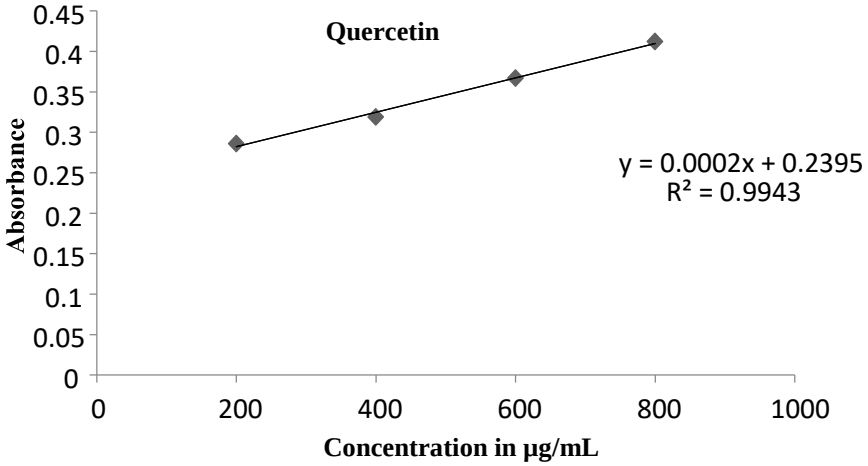


Fig. 4. Graphical presentation of different concentrations of standard quercetin to determine the unknown concentration of total flavonoid content.

Table 3. Total flavonoid contents of fresh and lyophilized tomato and lemon juices.

Plant Form	Concentration of samples (mg/ml)	Flavonoid content (mg of quercetin equivalent / g dry material)
Tomato (Lyophilized)	737.5	7.37±0.08[a]
Tomato (Fresh)	-	5.28±0.04[c]
Lemon (Lyophilized)	312.5	3.12±0.09[b]
Lemon (Fresh)	-	2.20±0.05c

Values are expressed as mean ± SD (n=3). Different superscript letters within the same column indicate the statistically significant differences ($p<0.05$) between samples.

B) By RP- HPLC: The results of this study have shown that fresh tomato and lemon juice show higher flavonoid concentrations than their lyophilized forms while myricetin was not detected in lyophilized samples as shown in table 4. But trace amounts appear in fresh juice, which is common due to losses during lyophilization. Kaempferol shows modest increases in fresh tomato and was detectable in fresh lemon, reflecting better extraction efficiency in fresh samples.

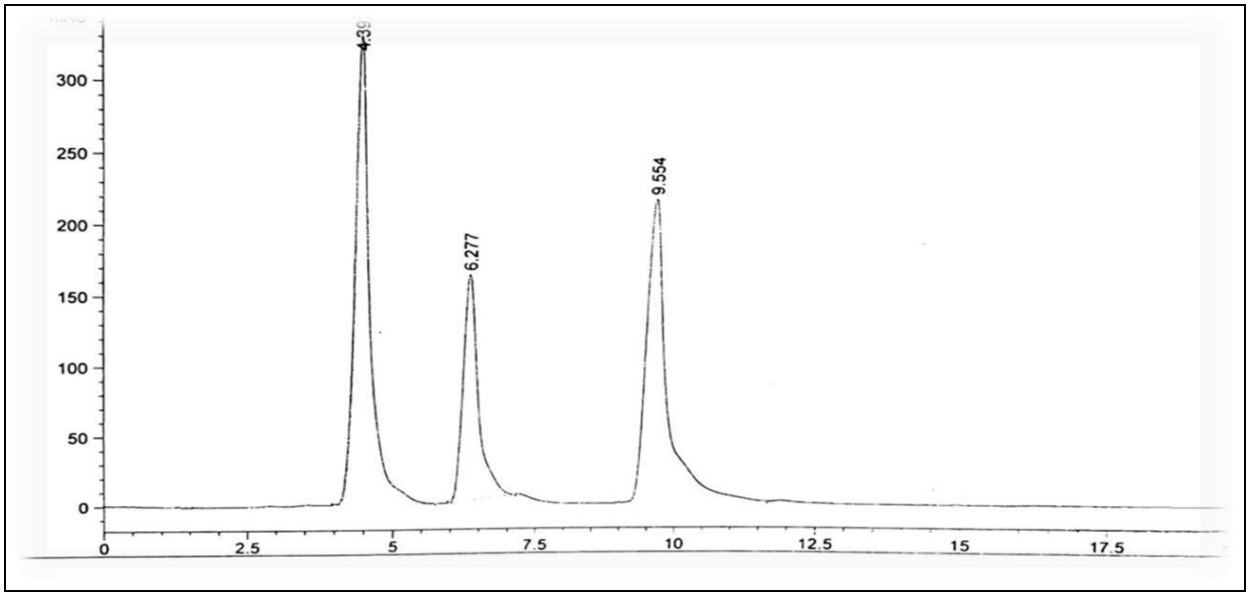


Fig. 5. HPLC chromatogram of three different standards of flavonoids a,b,c (Quercetin, Kaempferol and Myricetin)

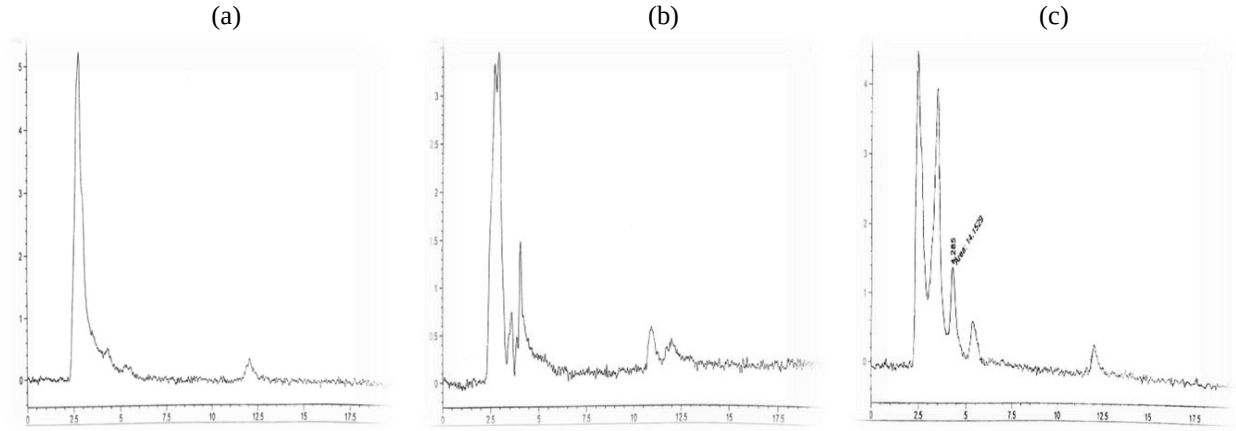


Fig. 6. HPLC chromatograms of lyophilized samples. (a) methanol extract of tomato (b) ACN extract of tomato (c) IPA extract of tomato (d) ACN extract of lemon (e) IPA extract of lemon (f) methanol extract of lemon

Table 4. Concentration of flavonoids in methanol extract of lemon and tomato by using HPLC

Flavonoids	Lyophilized Tomato (µg/ml)	Fresh Tomato (µg/ml)	Lyophilized Lemon (µg/ml)	Fresh Lemon (µg/ml)
Quercetin	0.1505	0.210 ± 0.005	0.291	0.380 ± 0.007
Myricetin	-	0.050 ± 0.003	-	0.080 ± 0.004
Kaempferol	0.136	0.190 ± 0.004	-	0.070 ± 0.003

Anti-diabetic Assays

a) Inhibition of Alpha amylase enzyme activity: The result demonstrated that alpha amylase inhibition activity increased in a dose dependent manner for all samples, with high concentration (1.0 mg/ml) consistently, showing greater inhibition compared with lower concentrations (0.2 mg/ml). Fresh samples consistently showed significantly higher inhibition than their lyophilized counterparts ($p < 0.05$). Among the individual fruit samples, tomato exhibited stronger inhibitory activity than lemon at all concentrations, suggesting a richer or more potent alpha amylase active constituents as shown in table 5. Notably, the combination of tomato and lemon produced enhanced inhibitory effects compared with either fruit alone, with the fresh combination sample showing highest inhibition overall. This demonstrated a potential synergistic interaction between bioactive components.

b) Glucose uptake by yeast cell assay: The glucose uptake by yeast cell assay showed that fresh juice extracts had higher activity compared to their lyophilized counterparts as shown in table 6. It indicated that the bioactive compounds responsible for glucose uptake may be more active or available in the fresh form. Among the tested samples, the combination extract consistently showed the highest glucose uptake, followed by tomato, and then lemon. This trend was observed at all concentrations (0.2–1 mg/ml) and at different glucose concentrations (5 mM, 10 mM, 25 mM), suggesting a synergistic effect in the combination extract.

Table 5. Alpha amylase inhibitory activity of tomato, lemon and their combination in fresh and lyophilized forms at different concentration.

Sample	Form	0.2 mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1.0 mg/ml
Tomato	Lyophilized	12.5 ± 3.2^{c}	22.0 ± 4.5^{c}	32.6 ± 5.0^{c}	42.1 ± 6.1^{c}	48.8 ± 7.2^{c}
	Fresh	13.4 ± 3.5^{b}	23.5 ± 4.8^{b}	34.8 ± 5.4^{b}	45.0 ± 6.5^{b}	52.2 ± 7.7^{b}
Lemon	Lyophilized	14.0 ± 2.9^{c}	20.0 ± 4.0^{c}	30.0 ± 5.0^{c}	40.0 ± 6.0^{c}	42.7 ± 6.5^{c}
	Fresh	15.0 ± 3.1^{b}	21.5 ± 4.3^{b}	32.0 ± 5.3^{b}	42.5 ± 6.3^{b}	45.5 ± 7.0^{b}
Combination	Lyophilized	16.0 ± 3.5^{b}	28.0 ± 4.7^{b}	35.0 ± 5.8^{b}	44.0 ± 6.8^{b}	45.4 ± 7.5^{b}
	Fresh	17.0 ± 3.7^{a}	30.0 ± 5.0^{a}	37.5 ± 6.2^{a}	47.0 ± 7.0^{a}	48.6 ± 8.0^{a}

Values are expressed as mean ± SD (n=3). Lettering indicates a statistically significant differences between fresh and lyophilized samples at same concentration ($p<0.05$).

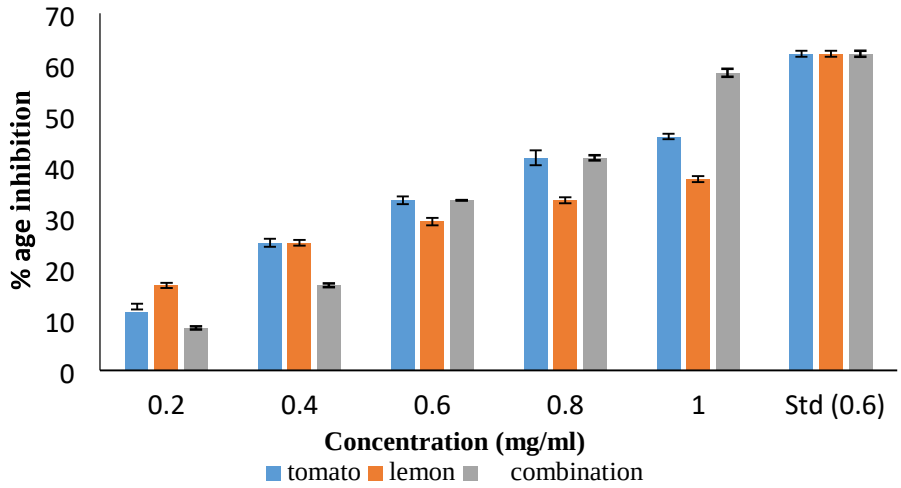


Fig. 7. Effect of lyophilized tomato and lemon juices alone and in combination on inhibition of α-amylase enzyme activity. Data are presented as mean ± SD (n = 3) using one-way ANOVA, Tukey's test analysis. Std stand for standard.

Table 6. Glucose uptake by yeast cell activity of tomato, lemon and their combination in fresh and lyophilized forms at different concentration

Extract	Form	Conc (mg/ml)	5mM (% ± SD)	10mM (% ± SD)	25mM (% ± SD)
Combination	Lyophilized	0.2	15.21 ± 1.2^{I}	23.87 ± 1.8^{I}	20.37 ± 1.5^{I}
		0.4	31.04 ± 2.0^{h}	39.04 ± 2.5^{h}	33.33 ± 2.1^{h}
		0.6	41.66 ± 2.5^{g}	57.12 ± 3.0^{g}	54.81 ± 3.0^{g}
		0.8	60.87 ± 3.5^{f}	67.76 ± 3.8^{f}	61.98 ± 3.2^{f}
		1	69.08 ± 4.0^{e}	76 ± 4.2^{e}	76 ± 4.0^{e}
	Fresh	0.2	20.0 ± 1.5^{h}	28.0 ± 2.0^{h}	25.0 ± 1.8^{h}
		0.4	38.0 ± 2.2^{g}	50.0 ± 2.5^{g}	45.0 ± 2.3^{g}
		0.6	50.0 ± 2.5^{f}	70.0 ± 3.0^{f}	65.0 ± 3.0^{f}
		0.8	70.0 ± 3.5^{d}	82.0 ± 3.8^{d}	75.0 ± 3.2^{d}
		1	80.0 ± 4.0^{c}	90.0 ± 4.2^{c}	88.0 ± 4.0^{c}
Tomato	Lyophilized	0.2	10.61 ± 1.0^{j}	20.0 ± 1.5^{j}	8.33 ± 0.8^{j}
		0.4	29.45 ± 2.0^{i}	25.0 ± 1.8^{i}	20.83 ± 1.5^{i}
		0.6	36.07 ± 2.2^{h}	50.0 ± 2.5^{h}	35.41 ± 2.0^{h}
		0.8	48.14 ± 2.8^{g}	53.33 ± 3.0^{g}	56.25 ± 3.2^{g}
		1	61.11 ± 3.0^{f}	66.21 ± 3.2^{f}	64.58 ± 3.0^{f}
	Fresh	0.2	15.0 ± 1.2^{i}	25.0 ± 1.8^{i}	15.0 ± 1.2^{i}
		0.4	35.0 ± 2.0^{h}	33.0 ± 2.2^{h}	30.0 ± 2.0^{h}
		0.6	45.0 ± 2.5^{g}	55.0 ± 2.8^{g}	45.0 ± 2.5^{g}
		0.8	60.0 ± 3.0^{f}	65.0 ± 3.2^{f}	65.0 ± 3.0^{f}
		1	70.0 ± 3.5^{e}	75.0 ± 3.8^{e}	72.0 ± 3.5^{e}
Lemon	Lyophilized	0.2	11.11 ± 1.0^{j}	22.02 ± 1.5^{j}	18.23 ± 1.2^{j}
		0.4	12.96 ± 1.2^{i}	25.52 ± 1.8^{i}	30.04 ± 2.0^{i}
		0.6	35.18 ± 2.2^{h}	39.02 ± 2.5^{h}	48.92 ± 2.8^{h}
		0.8	51.85 ± 2.8^{g}	47.87 ± 3.0^{g}	55.87 ± 3.2^{g}
		1	55.50 ± 3.0^{f}	56.33 ± 3.2^{f}	67.43 ± 3.5^{f}
	Fresh	0.2	15.0 ± 1.2^{i}	28.0 ± 1.8^{i}	22.0 ± 1.5^{i}
		0.4	18.0 ± 1.5^{h}	32.0 ± 2.0^{h}	35.0 ± 2.2^{h}
		0.6	40.0 ± 2.5^{g}	45.0 ± 2.8^{g}	55.0 ± 3.0^{g}
		0.8	58.0 ± 3.0^{f}	55.0 ± 3.2^{f}	65.0 ± 3.5^{f}
		1	63.0 ± 3.5^{e}	63.0 ± 3.8^{e}	70.0 ± 3.5^{e}

Values are expressed as mean ± SD (n=3). Lettering indicates a statistically significant differences ($p<0.05$) between fresh and lyophilized samples at same concentration

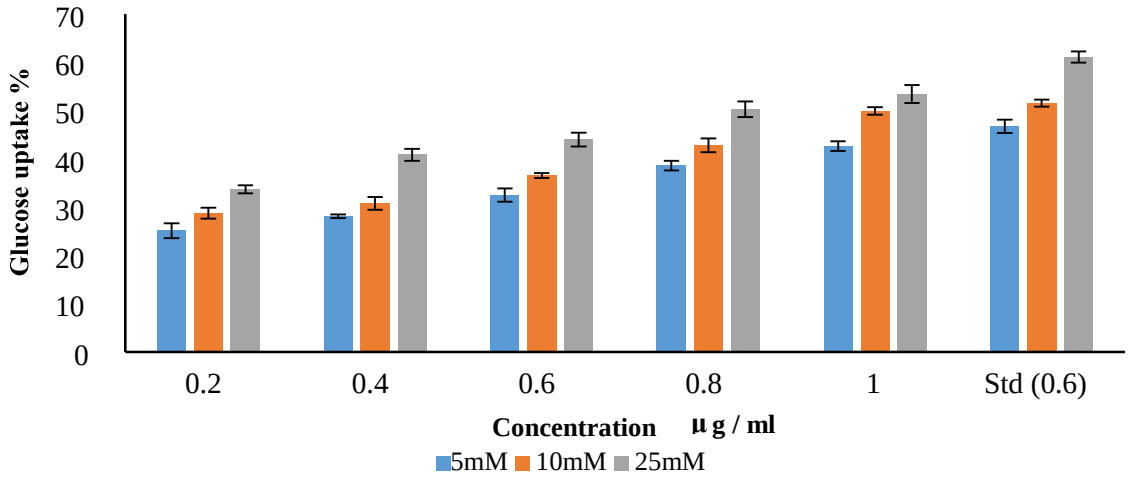


Fig. 8. Effect of lyophilized tomato juice on glucose uptake by yeast cell assay. Data is presented as mean ± SD (n = 3). Std stand for standard.

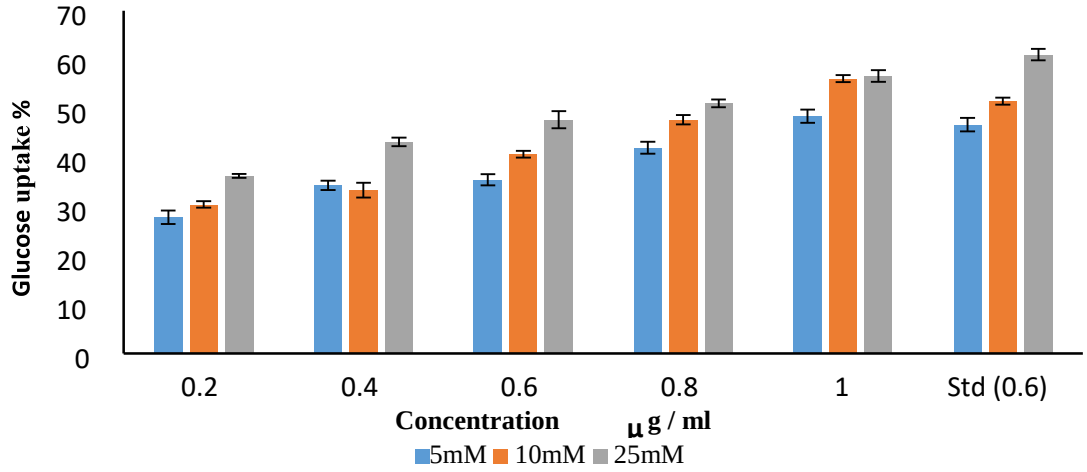


Fig. 9. Effect of lyophilized lemon juice on glucose uptake by yeast cell assay. Data is presented as mean ± SD (n = 3). Std stand for standard.

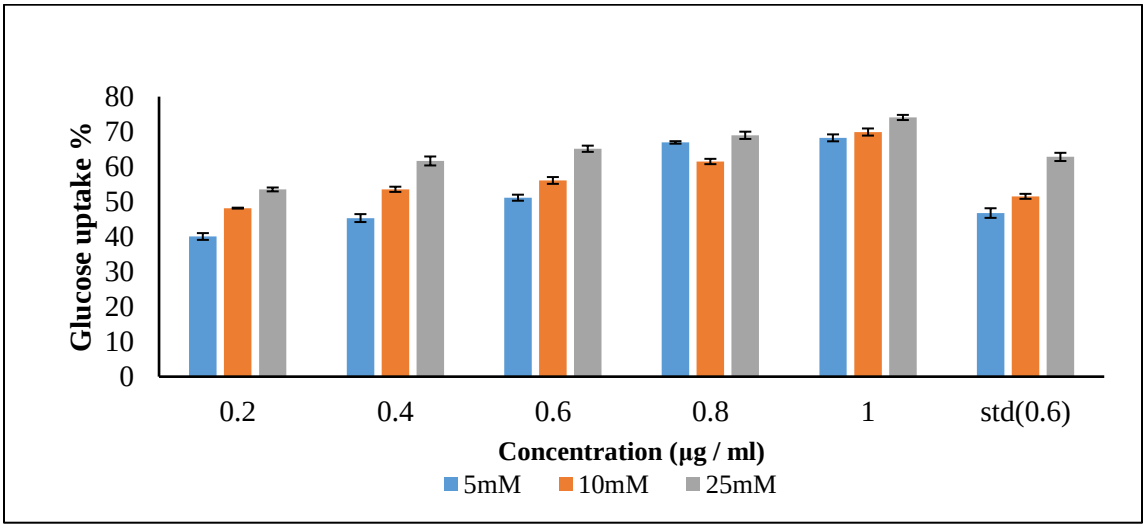


Fig. 10. Effect of lyophilized tomato and lemon juices alone and in combination on glucose uptake by yeast cell assay. Data is presented as mean ± SD (n = 3). Std stand for standard.

DISCUSSION

The present study was designed to evaluate and compare the antidiabetic potential of tomato and lemon juices in their fresh and lyophilized forms. The management of diabetes mellitus traditionally relies on pharmacological agents such as insulin and oral hypoglycemic drugs. However, these therapies may cause adverse effects and are often costly, prompting growing interest in plant-based treatments (Wang *et al.,* 2024). Earlier research has focused mainly on the antidiabetic properties of fresh tomato and lemon juices, but little attention has been given to their combined activity and lyophilized forms, to determine whether lyophilization affects the phytochemical bioactivity and integrity of these fruits (Uscanga *et al.,* 2021).

Phytochemical screening of the lyophilized samples confirmed the presence of major bioactive classes such as phenols, flavonoids, alkaloids, tannins, and organic acids and they are known contributors to antioxidant and antidiabetic effects (Saini *et al.,* 2022). This profile was further validated and refined using GC–MS, FTIR, and HPLC to understand the qualitative and functional chemical differences between fresh and lyophilized samples (Mathew *et al.,* 2012) but having only minor variations, which likely due to the loss or alteration of heat and temperature sensitive compounds during processing. GC-MS analysis results suggest that the number of compounds differences may be attributed to the lyophilization and solvent selection procedures employed (Encinas-Basurto *et al.,* 2017, Kashaninejad *et al.,* 2021). As lyophilization can selectively affect highly volatile components without altering core phenolic and flavonoid structures (Gomez-Gaete *et al.,* 2024).

In vitro antidiabetic assays demonstrated meaningful alpha amylase inhibition and glucose uptake enhancement in all samples, confirming the functional relevance of the detected phytochemicals. The combination of tomato and lemon extracts consistently produced the strongest activity, supporting the concept of synergistic interactions among flavonoids, phenolic acids, and alkaloids. In alpha amylase enzyme inhibition assay, when both extracts were tested in combination, they showed greater inhibitory activity, highlighting the synergistic interaction between the phytochemical constituents. The IC_{50} value of the combination lyophilized extract (7.76 mg/ml) was lower than that of the individual lyophilized extracts, indicating enhanced potency. However, the inhibition level was still lowered than the standard drug, acarbose, which further proved that natural plants inhibitor showed mild activity (Kashtoh *et al.,* 2023).

On the other hand, in glucose uptake by yeast cell assay, the fresh samples showed higher results than lyophilized samples. This difference can be logically attributed to the presence of heat and oxygen sensitive volatile compounds that are naturally abundant in fresh juices but partially lost during lyophilization and solvent extraction (Conrado *et al.,* 2024, Patil *et al.,* 2022). Fresh samples also contain active enzymes, natural cofactors, and intact matrices that may enhance bioavailability and synergistic interactions, contributing to higher biological potency (Benayad *et al.,* 2021). Nevertheless, lyophilized extracts demonstrated strong and reproducible activity, indicating that the core antidiabetic phytochemicals remain stable and therapeutically relevant. Likewise, at an equivalent dose of 1 mg/mL, the combination lyophilized extract achieved uptake levels above 70%, which were comparable to the standard drug metronidazole.

Overall, these findings indicate that lyophilized tomato and lemon extracts, particularly in combination, maintain their phytochemical integrity and antidiabetic potential. Fresh samples provide slightly superior bioactivity, but lyophilized forms offer a stable, concentrated, and practical alternative for future natural antidiabetic product development. The study underscores the importance of synergistic interactions among phytochemicals and confirms that lyophilization process does not compromise the therapeutic potential of tomato and lemon.

Conclusions: The present study demonstrated that lyophilization process effectively preserve and concentrate the natural bioactive compounds (phenols, flavonoids) in tomato and lemon juices especially when both fruits are used in combination, they exhibit synergistic effect in diabetes management.

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