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## Optical and green catalytic properties of fenugreek water for diabetes and skincare treatments

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Fenugreek water, with its natural brilliance and dual magic, shines as a green catalyst for health and beauty. From managing diabetes to rejuvenating skin, discover the science behind this age-old remedy turned modern marvel that shows more detail study on fenugreek water. This study investigates the structural and optical properties of fenugreek water, examining its interactions with sugar solutions, anti-diabetes medications (Diapride M2 Forte), and sunscreen cream (shadow SPF50+ cream) over a period of 3 days. X-ray diffraction (XRD) of soaking fenugreek seed powder in water shows the peak intensity increased significantly from day 1 to day 3 because gel-like mucilage forms. The fenugreek seed powder exhibited broader peaks compared to the more crystalline diabetes medications, reflecting a less organized polysaccharide structure. The optical studies of fenugreek water mixed with sugar solutions (125 mg/dl, 155 mg/dl, and 185 mg/dl) showed that absorbance decreased as sugar concentration increased but increased with higher volumes of fenugreek water, revealing colloidal interactions. On day 1, the absorbance peak was around 250 nm, but days 2 and 3 introduced additional peaks at 350 nm, indicating evolving colloidal particle sizes. When mixed with anti-diabetes drugs, fenugreek water exhibited increased absorbance on day 3, suggesting enhanced interaction. Additionally, fenugreek water showed significant effects on sunscreen cream, with increased absorbance indicating improved UV protection. FTIR analysis revealed that prolonged soaking increases the colloidal formation and bioactive compound concentration, enhancing the health benefits of fenugreek water. The pH of fenugreek water remained relatively stable between 8–9 throughout the study, suggesting minimal impact on the overall effectiveness of the interactions. The findings suggest that fenugreek water plays a crucial role in green catalytic properties that have the potential to reduce diabetes levels and its promising role in enhancing the efficacy of sunscreen creams for skincare treatment, demonstrating the most effective properties for both applications. Also, the study evaluates the UV-blocking and antioxidant properties of fenugreek water, demonstrating its potential as a natural sunscreen additive and free radical scavenger. The observation shows that fenugreek water enhances UV absorption when combined with cream and exhibits peak antioxidant activity on day 2, making it suitable for skincare and pharmaceutical applications.

**Keywords** Fenugreek seed powder, X-ray diffraction, Optical properties, Sugar solutions, Anti-diabetes medications, Sunscreen cream, pH measurements

Fenugreek (*Trigonella foenum-graecum* L.) is a dicotyledonous annual medicinal plant of the Fabaceae family, ethnic to the Mediterranean, Egypt, India, North Africa, South and Central Asia, and Southern Europe. Today, fenugreek is cultivated globally due to its diverse applications in culinary and medicinal fields<sup>1</sup>. The species name, meaning “Greek hay,” highlights its historical significance, while its various names, such as “Methi” in Hindi and Nepali “Methika” in Ayurveda, and “Alholva” in Spanish, reflect its widespread use across cultures<sup>2</sup>. Fenugreek is recognized for its distinctive cylindrical stem, which can grow between 30 and 60 cm in length, and its individual pods, which are 10–15 cm long and contain 18–20 seeds. The seeds, characterized by their hard, rectangular to oval shapes and brown to golden yellow color, have a pungent odor and a taste that is slightly bitter and sweet. These seeds are extensively used as a spice in culinary dishes and are integral to various traditional

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recipes, such as the Indian condiment “curry” and the Bulgarian specialty “Sharena sol”<sup>3</sup>. The nutritional profile of fenugreek seeds includes moisture content ranging from 7.5 to 10.6%, protein content of 0.7–1.5%, fat content of 0.5–1.6%, ash content of 0.5–1.5%, and a notable galactomannan content of 73.6%<sup>4</sup>.

The plant’s historical use as both a food and medicinal crop underscores its importance. Fenugreek seeds have been utilized for their medicinal properties across different cultures for centuries<sup>2</sup>. The seeds contain a range of bioactive compounds, including non-toxic alkaloids and oil components, contributing to their characteristic bitter taste. Notably, defatted seeds do not exhibit this bitterness<sup>3</sup>. Fenugreek is rich in primary and secondary metabolites, such as flavonoids, alkaloids, steroidal saponins, tannins, and carbohydrates, particularly galactomannan. The galactomannan in fenugreek seeds, with its equal ratio of galactose and mannose residues, exhibits superior solubility in cold water compared to other polysaccharides like carob and guar gum. This property enhances its application as an excipient in pharmaceutical dosage forms and food products, affecting their texture, shelf life, and stability<sup>5</sup>.

Fenugreek’s medicinal benefits are well-documented and have been traditionally used for a range of health issues, including bone and muscle problems, respiratory issues, cardiovascular disorders, hormonal imbalances, cancer, diabetes, and reproductive health problems<sup>5</sup>. The plant’s seeds, leaves, and extracts have shown antidiabetic, antioxidant, anti-obesity, hypocholesterolemic, anticancer, and cardioprotective effects. These qualities make fenugreek a significant subject of research in health, nutrition, and nanotechnology applications<sup>6</sup>. Recent studies have also explored fenugreek’s role in food product development, demonstrating its potential to reduce insulin resistance and manage diabetes when incorporated into baked products like bread and biscuits<sup>7</sup>.

Fenugreek seeds, leaves, and extracts have been utilized as antidiabetic agents in various model systems<sup>8</sup>. In Arab countries, including Saudi Arabia<sup>9</sup> and Iraq<sup>10</sup>, fenugreek is among the most commonly used herbs for diabetes management. The antidiabetic effect of fenugreek is attributed to the formation of a colloidal-type suspension in the stomach and intestines when the mucilaginous fiber of the seeds is hydrated. This process affects gastrointestinal transit and slows glucose absorption<sup>11</sup>. However, fenugreek’s applications extend beyond medicinal and nutritional uses. The plant’s response to environmental stressors, such as UV radiation, has been investigated to understand its physiological and defensive responses. Research indicates that UV-B and UV-B + A exclusions enhance fenugreek plant growth and yield by reducing the levels of antioxidants and UV-absorbing substances<sup>12</sup>. Additionally, fenugreek is employed in the cosmetics industry, with its components used in sunscreen formulations to protect against UV radiation<sup>13</sup>. Overall, fenugreek’s versatility as a spice, food ingredient, medicinal herb, and potential cosmetic component underscores its importance.

The existing literature provides limited insight into several key aspects of fenugreek’s properties and their applications. Specifically, there has been no comprehensive study on the optimal properties of fenugreek water combined with sugar solutions, particularly concerning its effects on different sugar levels in patients with diabetes. This gap is significant, as understanding how fenugreek water interacts with various sugar concentrations could lead to more tailored and effective management strategies for diabetes. Investigating this interaction could provide valuable insights into potential synergies or conflicts between fenugreek and existing treatments, enhancing diabetes management protocols. Furthermore, in the cosmetic field, the UV protection properties of fenugreek, especially in combination with sunscreen creams, remain unexplored. Given the growing interest in natural ingredients for skin protection, understanding fenugreek’s potential to enhance UV protection in cosmetic products could offer new opportunities for developing effective sunscreen protection solutions. Addressing these gaps could not only contribute to improved diabetes management by optimizing sugar reduction strategies but also advance cosmetic formulations that better protect the skin from UV damage.

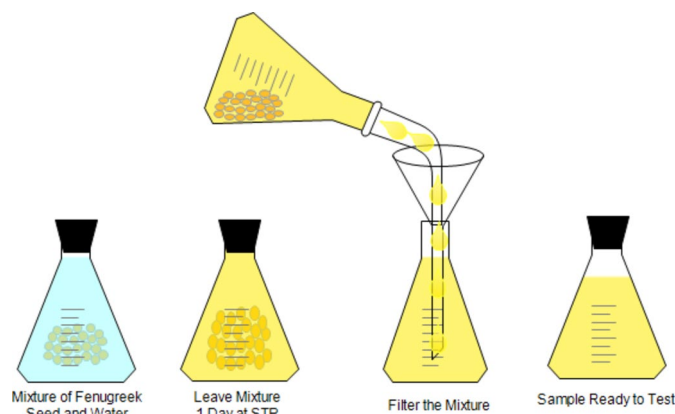
## Materials and methods

### Preparation of fenugreek water

For this experiment, fenugreek seed is bought from the local market of Kathmandu, Nepal. For the experiment, 5 g of fenugreek seeds were accurately weighed using a digital balance. After the measurement, fenugreek seeds are dried at 50 °C for 2 h in an oven, allowed to cool naturally, and then irradiated in a UV cabinet with an 8 W UV source for 5 min, maintaining a distance of 3 cm between the UV sources and the samples with wavelength 254 nm. After being irradiated with UV source the fenugreek seeds are placed in a 500 ml conical flask. Subsequently, 250 ml of distilled water was added to the seeds. The cap of the flask was covered to prevent contamination and left at room temperature (25 °C) for soaking durations of day 1, day 2, and day 3. During the soaking duration, the sample is kept at under 100–150 lux level; it is measured using a mobile app. During this soaking period, the seeds are allowed to release bioactive compounds into the water. After each specified soaking duration, the solution was carefully filtered to separate the seed residues from the water. A funnel lined with Whatman No. 3 filter paper was used for this filtration process, and the resulting filtrate was collected in a clean beaker. The filtered fenugreek water was then stored in sterile glass containers, which were labeled based on the soaking time (day 1, day 2, and day 3). The solutions were either used immediately or refrigerated for later analysis. Throughout the experiment, the appearance, clarity, and odor of the fenugreek water were observed and recorded to track any changes over time. This detailed procedure ensured consistency in the preparation of fenugreek water across different soaking durations. The details of the experiment setup are shown in Fig. 1 below.

### Preparation of sugar solution

The sugar solution was prepared using white granulated sugar (purity > 99%, Zuari International, UP, India), purchased from the local market in Kathmandu, Nepal. In this experiment, three sugar solutions with concentrations of 125 mg/dl (1.25 mg/ml), 150 mg/dl (1.50 mg/ml), and 185 mg/dl (1.85 mg/ml) were prepared. To achieve this, 125 mg, 150 mg, and 185 mg of sugar were separately weighed. Each sugar sample was then dissolved in 100 ml of distilled water in a beaker. The solutions were mixed using a magnetic stirrer for 30 min to ensure complete dissolution. This process was conducted at room temperature. After stirring, the solutions were



**Fig. 1.** Experimental setup for the preparation of fenugreek water.

visually inspected for clarity to confirm that the sugar had fully dissolved. Each sugar solution was then labeled according to its concentration for further analysis.

### Preparation of sample for test

After preparing the samples of fenugreek water and sugar solutions separately, the next step involved mixing different volumes of the sugar solution with fenugreek water. These mixtures were prepared in test tubes, with the specific ratios adjusted according to the experimental design. To ensure thorough mixing, a vortex mixer (LabQuest, BOROSIL) was used to blend the contents of each test tube for 2 min. This process ensured that the sugar and fenugreek water were homogeneously combined for subsequent analysis. Then, the samples were further centrifuged at 6000 rpm (LabQuest, BOROSIL) for 2 min to separate any remaining particulates at  $25 \pm 1^\circ\text{C}$  maintained temperature. Following centrifugation, the supernatant from each sample was carefully collected and transferred into a 3 ml cuvette. The cuvette was then inserted into a UV-Vis spectrophotometer (Cary 60, Agilent Technologies) to measure the absorbance of the sample.

### Preparation of sample for XRD

The filtered and raw fenugreek seeds were dried in an oven for 6 h at  $70^\circ\text{C}$ . After the drying process, the samples were ground and then further crushed with a laboratory mortar and pestle to obtain a fine powder suitable for XRD analysis. XRD analysis of the raw and filtered finely powdered fenugreek seeds was performed on a Bruker D2 Phasor diffractometer at a scanning speed of  $2^\circ$  per minute in  $2\theta$  mode between  $10^\circ$  and  $90^\circ$  at voltage of 30 kV and current of 10 mA to investigate its crystalline properties.

### Preparation of sunscreen cream sample for UV test

To test the effect of UV exposure and compare it with sunscreen cream protection, 1.16 g of sunscreen cream was weighed and dissolved in 30 ml of distilled water. The mixture was subjected to an ultrasonic cleaner (INDOSATI) to ensure thorough dissolution of the cream in the water for 30 min. After sonication, the solution was filtered to remove any remaining undissolved particles. The filtered sample was then used to study its optical properties under different conditions (unmixed, mixed with fenugreek water: 5 min, 1 h, 2 h, and 18 h).

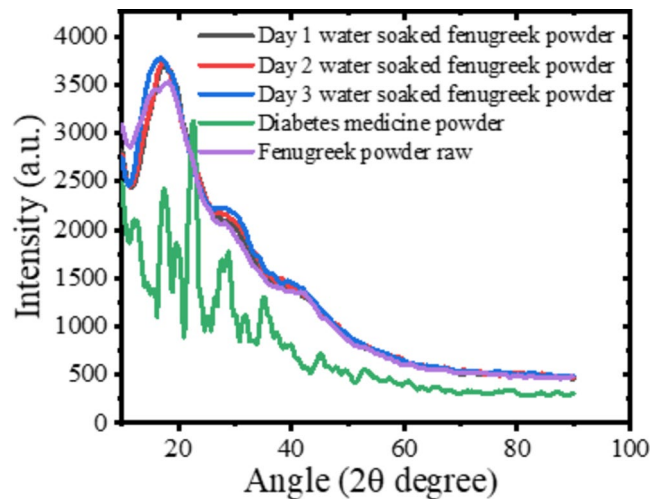
### FTIR spectroscopy

The prepared samples, including fenugreek water and fine fenugreek seed powder, were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with a SHIMADZU IR Tracer-100 spectrophotometer, covering the spectral range of  $400\text{--}4500\text{ cm}^{-1}$  to identify functional groups and chemical characteristics.

## Results and discussion

### XRD of samples

The XRD analysis of solid fenugreek seed powder, both raw and after soaking for different durations (day 1, day 2, and day 3), reveals significant insights into the changes in crystallinity and structural properties of fenugreek when exposed to water over time. The intensity of the XRD peaks increases progressively from the raw powder to day 1, day 2, and day 3, indicating that the soaking process influences the crystallinity and organization of the seed structure as shown in Fig. 2. This trend suggests that prolonged soaking leads to a more ordered structure, possibly due to the formation of a gel-like mucilage that promotes better alignment of polysaccharides within the seed. When comparing the XRD patterns of fenugreek seed powder to the diabetes medicine powder, a slight shift in the peaks is observed. This shift could indicate subtle differences in the molecular structure or crystallinity between fenugreek powder and diabetes medicine. The diabetes medicine, likely composed of more refined and crystalline compounds, may exhibit sharper and more defined peaks, whereas the fenugreek seed powder, even after soaking, shows a more gradual increase in intensity, reflecting a less crystalline, polysaccharide-rich composition.



**Fig. 2.** XRD peaks of fenugreek seed with comparison to medicine used for diabetes patients.

The XRD analysis of the diabetes medicine powder reveals a crystalline structure characterized by multiple peaks with lower intensity, indicating a complex composition with less ordered or less abundant crystalline phases. The powder's XRD pattern shows a significant match with fenugreek powder at 20° and 27°, suggesting shared crystalline components, possibly due to bioactive compounds like diosgenin or galactomannans present in fenugreek. This observation aligns with findings from Bian et al., 2019<sup>14</sup>, who reported similar multi-peak patterns between 10° and 30° for comparable materials. The similarity implies that the medicine powder may have a multi-phase crystalline structure, potentially containing natural or synthetic compounds with therapeutic properties. These findings are crucial for understanding the powder's physical and chemical properties, which influence its stability, solubility, and efficacy as a diabetes treatment. Further research could identify specific crystalline phases and their role in the powder's medicinal effects.

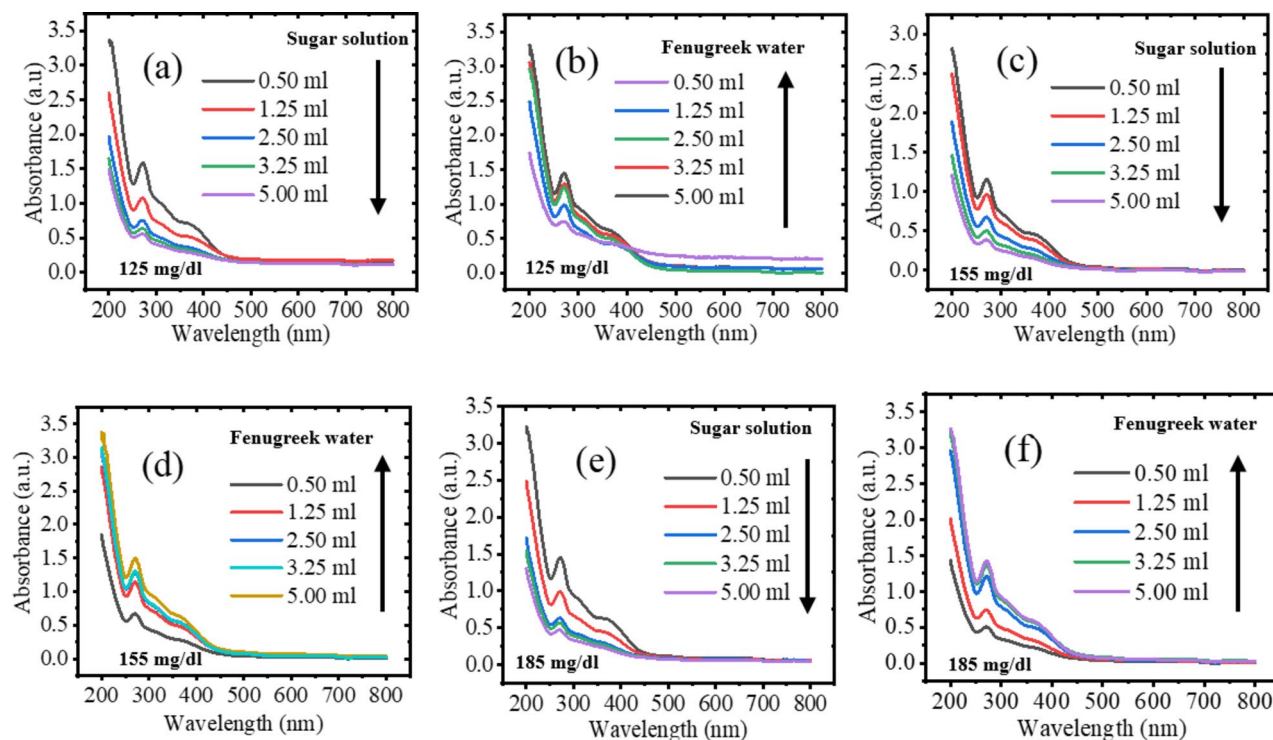
The broad peak at 2θ values of about 17–18°, along with a halo around 30° and 40°, in the fenugreek powder XRD pattern is consistent with previous studies on plant mucilage, including fenugreek mucilage<sup>15</sup> and other polysaccharides like hemicellulose xylan and cellulose<sup>16</sup>. These broad peaks are indicative of poor crystallinity, typical of complex carbohydrates and polysaccharides, as also noted in similar studies on basil seed mucilage, plantago ovata seed mucilage, and guar gum<sup>17</sup>. The low crystallinity is likely due to the presence of amorphous regions in these natural polysaccharides, which are not as well-organized as crystalline structures.

The XRD analysis of fenugreek seed powder (raw and soaked) and diabetes medicine powder reveals distinct structural and crystallinity differences. Fenugreek powder shows broad peaks at 2θ values of 17–18°, 30°, and 40°, indicative of low crystallinity due to its polysaccharide-rich, amorphous structure, consistent with studies on plant mucilage. Soaking fenugreek seeds progressively increases peak intensity, suggesting enhanced crystallinity and structural organization, likely due to gel-like mucilage formation. In contrast, the diabetes medicine powder exhibits sharper, more defined peaks with lower intensity, indicating a more refined, crystalline composition but with complex, multi-phase structures. Both materials share matching peaks around 20° and 27°, suggesting shared crystalline components, possibly bioactive compounds like diosgenin or galactomannans. However, the medicine powder's sharper peaks and slight shifts highlight its higher crystallinity and refined nature compared to fenugreek's polysaccharide-dominated, less crystalline structure. These differences underscore the medicine's potential for enhanced stability and efficacy, while fenugreek's structural modifications through soaking may improve its anti-diabetic properties by enhancing interactions with sugar.

### Optical properties of fenugreek water and sugar solution

The study focuses on the optical properties of sugar solutions with varying concentrations (125 mg/dl, 155 mg/dl, and 185 mg/dl) when mixed with fenugreek water (fenugreek seed extract) over a period of 1 day. For more information related to these please see in supplementary file<sup>18</sup>. The sugar levels represent three groups of diabetes patients: lower, middle, and high levels. The absorbance analysis was conducted using varying volumes of both sugar solutions and fenugreek water. The observation of Fig. 3(a) shows the absorbance behavior of a 125 mg/dl sugar solution when mixed with a fixed 0.50 ml of fenugreek water, and the sugar solution volume varied from 0.50 ml to 5.00 ml. The results clearly indicate that increasing the volume of sugar solution added decreased the rate of absorbance. This decrease in absorbance can be attributed to the formation of colloids from the fenugreek water, which interact with sugar particles. Fenugreek water tends to form larger colloidal particles, which absorb more light. As these particles are absorbed by the body, they interact with sugar particles, resulting in a reduction in sugar levels for the patient. Hence, a higher sugar solution volume corresponds to a reduction in light absorbance due to enhanced particle interaction. Also, the observation in Fig. 3(b) shows the absorbance behavior when the volume of fenugreek water is varied (while keeping the sugar solution volume constant). As the volume of fenugreek water increases, absorbance increases. This is due to the higher probability of colloid



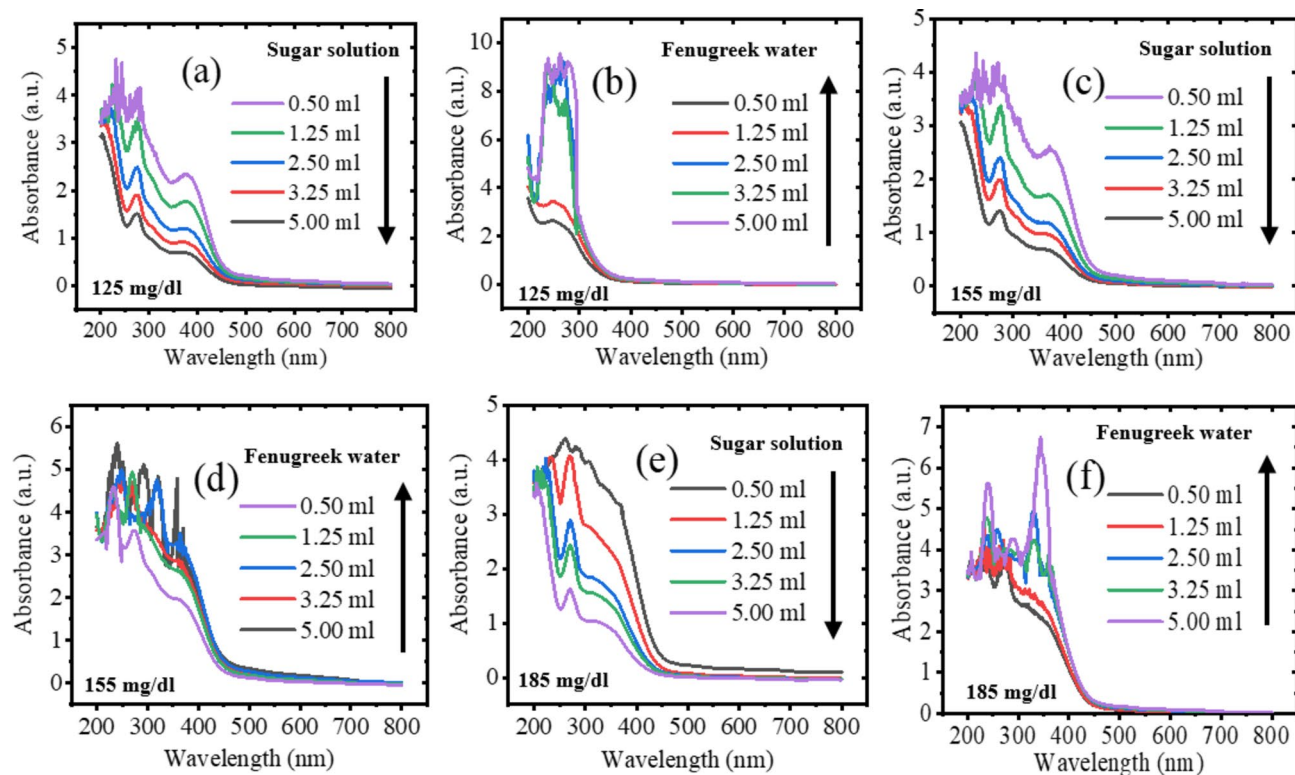


**Fig. 3.** Absorbance of day 1 fenugreek water (a) Sugar volume variation for 125 mg/dl at 0.50 ml fenugreek water, (b) Fenugreek water volume variation for 0.50 ml sugar solution at 125 mg/dl, (c) Sugar volume variation for 155 mg/dl at 0.50 ml fenugreek water, (d) Fenugreek water volume variation for 0.50 ml sugar solution at 155 mg/dl, (e) Sugar volume variation for 185 mg/dl at 0.50 ml fenugreek water, and (f) Fenugreek water volume variation for 0.50 ml sugar solution at 185 mg/dl.

formation with increasing fenugreek water. Larger particles are formed, which results in higher light absorption, supporting the idea that fenugreek water enhances the formation of colloidal particles.

The absorbance behavior for the sugar concentrations of 155 mg/dl and 185 mg/dl (middle and high levels of diabetes) follows a similar trend to the 125 mg/dl sugar solution. When the volume of the sugar solution increases, the absorbance decreases, and when the fenugreek water volume increases, the absorbance increases. Figure 3(c) and 3(e) show the variation in absorbance for 155 mg/dl and 185 mg/dl sugar solutions, respectively, as the sugar volume increases. In both cases, a decrease in absorbance is observed as sugar volume increases, supporting the idea that sugar particles contribute to a decrease in absorbance due to colloidal interaction with fenugreek water. Figure 3(d) and 3(f) show the variation in absorbance as the fenugreek water volume increases between 155 mg/dl and 185 mg/dl sugar solutions, respectively. As the volume of fenugreek water increases, the absorbance increases, confirming the earlier observation that larger colloidal particles result in greater absorbance. This study found that the peak absorbance for the sugar solutions remains at the same wavelength, while the peak absorbance for the fenugreek water shifts slightly. This shift in absorbance peaks, particularly for the fenugreek water, suggests that the colloidal particles formed in fenugreek water influence the light interaction differently compared to the sugar solution. For day 1, a single peak was observed in the ultraviolet region for both sugar and fenugreek water variation. The peak wavelength, around 250 nm, indicates that the particle size in the sample is optimal for light absorption in this region. The high concentration of particles at this wavelength leads to increased absorbance, demonstrating that the fenugreek water particles have formed comparable-sized particles that effectively interact with light, resulting in the observed absorption pattern. The observed shift of peak absorbance towards a higher wavelength<sup>19</sup> in fenugreek water indicates a bathochromic shift, suggesting changes in the electronic environment of the colloidal particles. As fenugreek water volume increases, the absorbance increases, and the peak wavelength shifts slightly, implying enhanced colloidal interactions that alter light absorption. This red shift signifies increased particle aggregation or changes in solvent polarity, which lower the energy required for electronic transitions, thereby shifting absorption to longer wavelengths. This effect contrasts with the sugar solution, where the peak wavelength remains unchanged, highlighting the distinct optical behavior influenced by fenugreek water.

In the findings for day 1 (Fig. 3), the absorbance pattern of fenugreek water mixed with varying concentrations of sugar was observed. Similar absorbance behavior was detected for fenugreek water on day 2 (Fig. 4), with a key difference being the appearance of two distinct peaks in the UV region: one around 250 nm and another around 350 nm. Day 1 (Fig. 3) shows the absorbance pattern showing a single peak in the UV region at around 250 nm. This peak was linked to the colloidal particles formed from the interaction between fenugreek water and sugar. The particle size at this wavelength was optimal for light absorption, leading to a high absorbance,

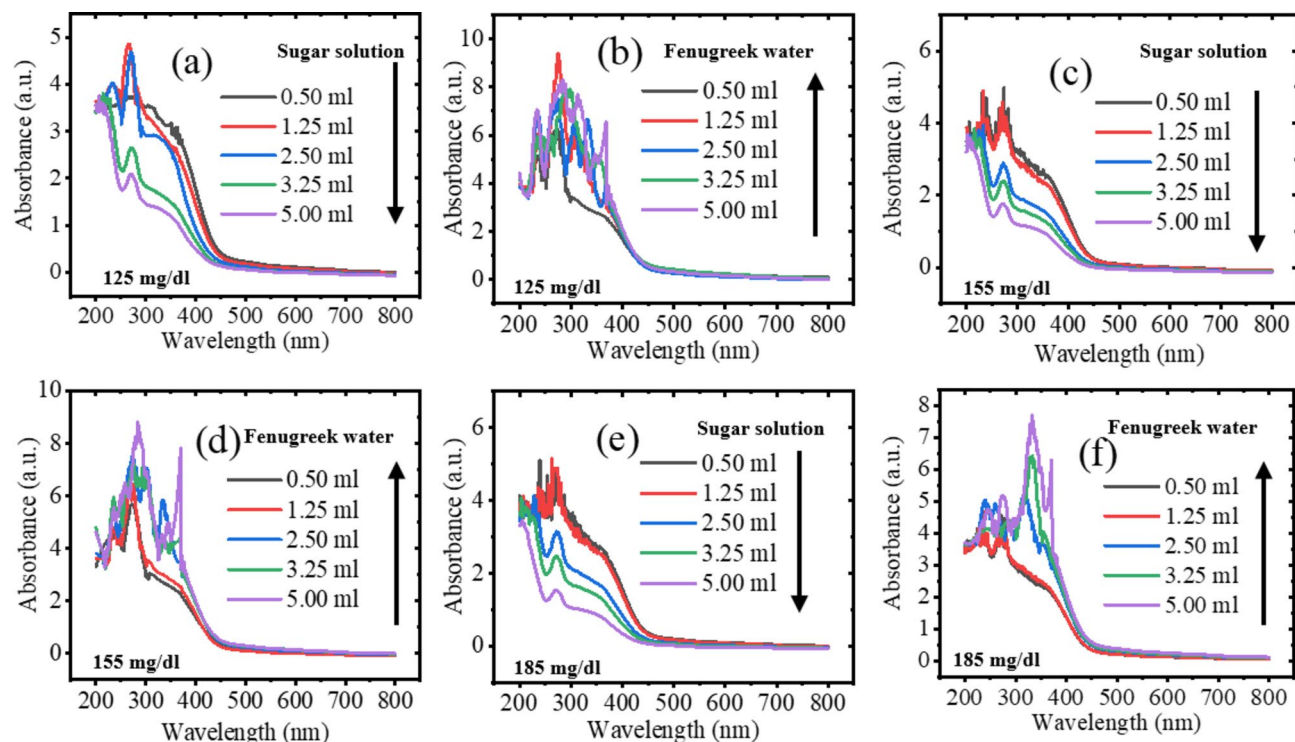


**Fig. 4.** Absorbance of day 2 fenugreek water (a) Sugar volume variation for 125 mg/dl at 0.50 ml fenugreek water, (b) Fenugreek water volume variation for 0.50 ml sugar solution at 125 mg/dl, (c) Sugar volume variation for 155 mg/dl at 0.50 ml fenugreek water, (d) Fenugreek water volume variation for 0.50 ml sugar solution at 155 mg/dl, (e) Sugar volume variation for 185 mg/dl at 0.50 ml fenugreek water, and (f) Fenugreek water volume variation for 0.50 ml sugar solution at 185 mg/dl.

indicating a concentration of particles that efficiently interacted with the light. Day 2 (Fig. 4) shows the key difference observed in day 2's results was the presence of two peaks: one at 250 nm (similar to day 1) and a new peak at around 350 nm. This additional peak suggests the formation of a second set of colloidal particles with a different size or structure, which absorbs light at a higher wavelength in the UV region. This is due bathochromic which occurs due to increased conjugation, solvent effects, pH changes, or colloidal interactions. In the case of fenugreek water in the sugar solution, the shift likely arises from colloidal particle formation, which alters light absorption by increasing particle size or modifying solvent interactions. These changes reduce the energy gap between molecular orbitals, leading to a red shift in absorbance, distinguishing fenugreek water's optical behavior from that of the sugar solution.

The peak at 250 nm likely corresponds to particles formed on day 1, which were also present on day 2. This consistency in particle size indicates that the colloids formed from fenugreek water continue to absorb light effectively at this wavelength, signifying that these particles are stable over time. The second peak at 350 nm suggests the formation of a new type of colloidal particle, possibly due to changes in the colloid structure or composition over time. The particles formed by day 2 may have undergone further aggregation or a structural transformation, which causes the system to absorb light at a higher wavelength. This new peak indicates that there is a higher concentration of particles at this wavelength, which results in stronger interaction with light. The higher absorbance observed at both 250 nm and 350 nm indicates that at these wavelengths, the colloidal particles formed in the sample have an optimal size and density for interacting with UV light. This implies that the fenugreek water, over time, leads to the formation of multiple types of particles or colloids, each interacting with light in different ways, as reflected by the distinct peaks. At 250 nm, the colloidal particles are likely similar in size to those formed on day 1, leading to similar absorbance behavior. At 350 nm, the larger or more complex particles absorb more light, possibly indicating a second stage in the colloid formation or a different mode of particle interaction with light. The shift in the absorbance peak from 250 nm to 350 nm demonstrates the synergistic interaction between fenugreek extract and sugar solutions, likely due to the formation of colloidal particles or chemical complexes. This bathochromic shift highlights fenugreek's ability to modify the optical properties of sugar solutions, which may contribute to its anti-diabetic effects by enhancing interactions with sugar molecules and potentially reducing blood sugar levels. Further studies could focus on the molecular mechanisms behind this shift and its implications for fenugreek's therapeutic applications.

Figure 5 shows absorbance patterns of fenugreek water for day 3 with variations in sugar concentration and fenugreek water volumes. The absorbance behavior for day 2 and day 3 fenugreek water mixed with sugar solutions is quite similar. However, one significant difference is that the concentration of larger-sized particles in



**Fig. 5.** Absorbance of day 3 fenugreek water (a) Sugar volume variation for 125 mg/dl at 0.5 ml fenugreek water, (b) Fenugreek water volume variation for 0.50 ml sugar solution at 125 mg/dl, (c) Sugar volume variation for 155 mg/dl at 0.50 ml fenugreek water, (d) Fenugreek water volume variation for 0.50 ml sugar solution at 155 mg/dl, (e) Sugar volume variation for 185 mg/dl at 0.50 ml fenugreek water, and (f) Fenugreek water volume variation for 0.50 ml sugar solution at 185 mg/dl.

day 3 fenugreek water is higher than on day 2 and substantially higher than on day 1. As fenugreek water ages, the colloidal particles appear to aggregate or grow larger. As colloids remain in solution, they tend to aggregate due to vander Waals forces or possibly London dispersion interactions. Larger particles tend to scatter and absorb more light, leading to increased absorbance compared to day 2. This increase in particle size is consistent with the observed increase in absorbance peaks as the colloidal particles evolve from day 1 to day 3. Despite the increase in particle size, the overall nature of the absorbance pattern on day 3 remains similar to day 2. This suggests that the colloidal particles formed from fenugreek water are stable and follow a predictable pattern of growth over time. The fundamental properties of the colloidal system remain unchanged, but the increasing particle size enhances the system's ability to absorb light.

The study presents important findings regarding the relationship between fenugreek water (across different days) and sugar concentration, highlighting its potential benefits for diabetes management. This finding is particularly relevant for understanding how fenugreek water interacts with sugar molecules in the context of diabetes management. This suggests that when there is more sugar present in the solution, the colloidal particles in fenugreek water become less effective for absorbing light. This is likely due to the fact that a higher concentration of sugar limits the interaction between fenugreek particles and sugar molecules, thereby reducing the formation of colloids.

On day 1, the colloidal particles in fenugreek water are finer and better distributed, which leads to a higher probability of interaction between fenugreek particles and sugar molecules. The finer colloids can easily gather sugar molecules, forming a colloidal network that helps reduce sugar levels. This makes day 1 fenugreek water highly effective in managing blood sugar without the need for medication. As fenugreek water ages, the concentration of larger particles increases. On day 2 and day 3, the colloidal particles formed are larger and less effective at interacting with sugar molecules. Larger particles create more space in the colloid structure, allowing sugar molecules to “escape” from interaction, thus reducing the ability of fenugreek water to lower sugar levels. Nevertheless, day 2 and day 3 fenugreek water can still have some impact on sugar levels, although less effective compared to day 1. The findings suggest that fenugreek water can play a role in reducing blood sugar levels for diabetic patients, effectively on day 1. The fine colloidal particles present in fresh fenugreek water have a higher probability of forming effective colloids, which interact with and gather sugar molecules, lowering blood sugar levels. This offers a potential alternative to medication for managing diabetes, especially in the early stages. For patients looking to use fenugreek water as a natural treatment for lowering blood sugar, the day 1 solution appears to offer the best result. This achievement could be because of the bathochromic effect (red shift) observed in the absorbance spectra, where the peak shifts to a higher wavelength, suggests that the interaction between fenugreek extract and sugar molecules alters the electronic environment of the system. This



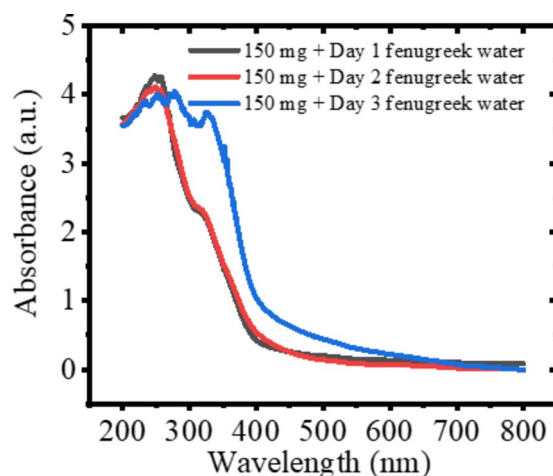
shift indicates that the formation of colloidal complexes or chemical interactions between fenugreek components (e.g., polysaccharides, diosgenin) and sugar molecules stabilizes the excited states of the system, lowering the energy gap for light absorption. This phenomenon is particularly evident in day 1 fenugreek water, where finer colloidal particles are more effective at interacting with sugar molecules, leading to higher absorbance and greater potential for reducing blood sugar levels. As fenugreek water ages (day 2 and day 3), the larger particle sizes reduce its effectiveness in interacting with sugar molecules, highlighting the importance of using fresh fenugreek water for optimal anti-diabetic effects. The bathochromic shift, along with the observed absorbance trends<sup>20</sup>, underscores the synergistic role of fenugreek extract in modifying the optical and structural properties of sugar solutions, which could enhance its therapeutic potential for diabetes management. These findings align with previous studies on fenugreek's hypoglycemic properties and provide a novel, optical-based approach for assessing its efficacy in reducing blood sugar levels, offering a potential alternative to traditional chemical methods.

In this research, the effectiveness of fenugreek water as a potential treatment for diabetes patients was examined using an optical technique. Building upon existing studies that have demonstrated fenugreek's antidiabetic effects in vitro and animal models<sup>21,22</sup>, the current work aimed to investigate the relationship between fenugreek water and blood sugar levels in human samples, particularly those with varying degrees of diabetes. Different concentrations of sugar (125 mg/dl, 155 mg/dl, and 185 mg/dl) were considered to simulate conditions commonly found in diabetic patients. The findings revealed that as the concentration of fenugreek water increased, the absorbance of the solution also increased for any fixed concentration of sugar. This increase in absorbance is indicative of a decrease in blood sugar levels, as higher absorbance correlates with a more significant reduction in sugar due to the presence of fenugreek water. These results suggest that fenugreek water actively reduces glucose concentration in the blood, aligning with its purported hypoglycemic properties. Importantly, the use of an optical technique to assess sugar levels presents a novel approach compared to traditional chemical methods. This method offers a more straightforward, non-invasive means of monitoring fenugreek's effects on blood glucose, which could potentially lead to more practical applications in diabetes management. Therefore, this study not only confirms the antidiabetic potential of fenugreek water but also provides an innovative, optical-based approach to assessing its efficacy, which could be more efficient for clinical and home use.

### Optical properties of fenugreek water and sugar solution with medicine

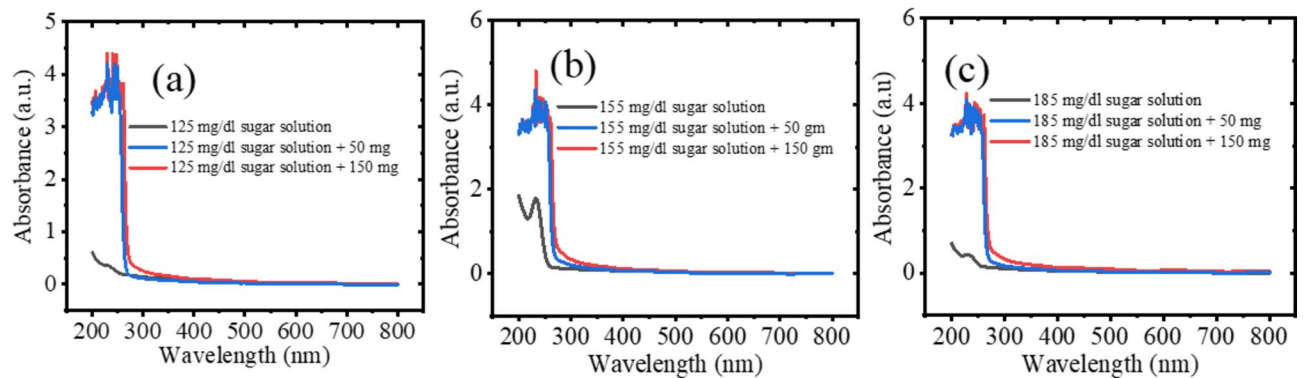
Figure 6 illustrates the absorbance of fenugreek water prepared on different days (day 1, day 2, and day 3) with the addition of 150 mg of anti-diabetes drugs as Diapride M2 Forte (Metformin Hydrochloride Prolonged Release and Glimepiride Tablets IP). This medicine is used in the treatment of type 2 diabetes mellitus. It improves blood glucose levels in adults when taken along with a proper diet and regular exercise. To study the absorbance of the anti-diabetes drugs, they were ground using a mortar and pestle. Figure 6 reveals that day 3 fenugreek water along with medicine exhibits a higher absorbance compared to day 1 and day 2. This suggests that when a diabetic person consumes both anti-diabetes drugs and day 3 fenugreek water, there is a notable reduction in sugar levels. The increased absorbance on day 3 implies that the larger colloidal particles formed in the fenugreek water enhance its interaction with the anti-diabetes drug, possibly boosting the efficacy of both in reducing blood sugar. This observation highlights fenugreek's potential as a natural anti-diabetic agent, as its components can synergize with conventional medication to further lower blood sugar levels. Fenugreek's ability to form colloidal structures with varying particle sizes over time (with larger particles observed on day 3) appears to contribute to its effectiveness. Thus, fenugreek water, particularly from day 3, can complement or even substitute anti-diabetes drugs for managing blood sugar levels, showcasing its valuable role in diabetes management.

Figure 7 demonstrates the absorbance behavior of sugar solutions at different concentrations of anti-diabetes drugs, which were administered to patients categorized by their sugar levels according to medical guidelines: 125 mg/dl for lower-level diabetes, 155 mg/dl for medium diabetes, and 185 mg/dl for higher-level diabetes. The

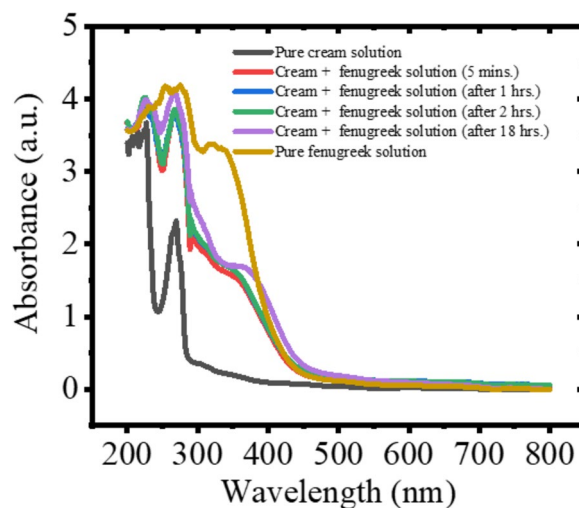


**Fig. 6.** Absorbance of medicine within 5.00 ml fenugreek water.





**Fig. 7.** Absorbance of (a) 125 mg/dl, (b) 155 mg/dl, and (c) 185 mg/dl sugar solutions with 150 mg and 50 mg of anti-diabetes drugs.



**Fig. 8.** Anti-UV properties of Fenugreek water.

study observed the effects of mixing 150 mg and 50 mg of anti-diabetes drugs into 5.00 ml of sugar solution for each category. The results reveal that absorbance increases as the concentration of anti-diabetes drugs increases, indicating that when the drugs interact with the sugar solution, they form larger particles. These larger particles are capable of absorbing more light, resulting in higher absorbance readings.

Across all sugar concentration levels, this pattern of increasing absorbance with drug concentration is consistent. Notably, the peak absorbance at around 250 nm is most prominent for the 155 mg/dl sugar solution, whereas it decreases for the 125 mg/dl and 185 mg/dl solutions. This difference can be attributed to the concentration of sugar and the dissolution of sugar particles within the solution. The middle concentration (155 mg/dl) likely allows for optimal interaction between the sugar and the anti-diabetes drugs, leading to a clearer peak, while at lower (125 mg/dl) or higher (185 mg/dl) concentrations, the particle formation and light absorption efficiency are reduced, resulting in lower absorbance values.

### Optical properties of fenugreek water with sunscreen cream

The findings in Fig. 8 highlight the potential of fenugreek water as a natural alternative to commercial anti-UV sunscreen creams (shadow SPF50 + cream). The sunscreen used is Shadow Silicone Matte Cream SPF 50, which provides broad-spectrum protection against UVA (320–400 nm), UVB (280–320 nm), and other harmful rays. It is enriched with zinc oxide and titanium dioxide, offering sun protection with an SPF of 50. The cream has a non-greasy, oil-free formula that does not leave a white cast, making it 100% safe for the skin. It is fragrance-free, non-comedogenic, and water-resistant. Key ingredients include Cyclopentasiloxane, Dimethicone Crosspolymer, Caprylic/Capric Triglyceride, Dicaprylyl Carbonate, Titanium Dioxide, Zinc Oxide, and others<sup>23</sup>. The comparative study analyzed the UV absorption properties of fenugreek water and a standard sunscreen cream solution, revealing that fenugreek water has significantly higher UV light absorbance. This suggests that fenugreek water could offer superior UV protection, making it an effective option for safeguarding the skin from harmful rays. A key observation from the study is that as the concentration of fenugreek water increases when mixed with a pure cream solution, the absorbance of UV light also rises. This indicates that

fenugreek water enhances the sunscreen cream’s ability to block UV radiation, particularly in the ultraviolet region of the spectrum, where protection is critical. The enhanced absorption in the UV range could be due to the natural compounds in fenugreek that form particles capable of interacting more effectively with UV rays, thereby providing stronger protection. Interestingly, the study found that in the visible light region, there was no significant increase in absorbance, underscoring that fenugreek water’s benefits are specific to UV light rather than visible light. This distinction is important, as it indicates that fenugreek water is primarily effective as a UV protecting agent without altering the appearance or visibility of the skin. These findings open up possibilities for fenugreek water to be developed as a natural ingredient in sunscreen protection products, offering both an eco-friendly and effective alternative to chemical-based sunscreen creams.

Furthermore, the study explored the impact of the contact time between fenugreek water and the sunscreen cream solution on UV absorption. The data, represented in Fig. 8, shows that contact times of 5 min, 1 h, 2 h, and 18 h were tested. The results indicate that UV absorption increased with longer contact times, suggesting that fenugreek water’s UV-protective properties become more effective with prolonged exposure. After a certain period, however, the effect plateaus, meaning that beyond a certain time, the UV absorbance remains constant, showing no further increase. This finding is crucial for practical applications, as it suggests that using fenugreek water either as a standalone ingredient or as a supplement in sunscreen creams could offer long-lasting UV protection, potentially protecting the skin during extended sunscreen exposure: fenugreek water appears to offer consistent UV protection even after long exposure times, making it a promising ingredient for natural sun protection formulations. This study indicates that fenugreek water could be a superior and natural alternative to commercial chemical-based sun creams for UV protection. Its ability to absorb harmful UV rays, especially in the ultraviolet region, makes it a valuable ingredient for skin care products aimed at protecting against UV radiation. Additionally, its effectiveness over time suggests that fenugreek water can provide sustained protection against prolonged sunlight exposure, further enhancing its potential as a key component in UV protection solutions.

The UVA protecting percentages based on absorbance values, where higher absorbance indicates greater UV protecting, are as follows. Pure fenugreek with an absorbance of 3.37 a.u. represents 100% UV protecting. After 18 h with sunscreen, the absorbance is 1.89 a.u. corresponding to 56.08% UV protecting. For the 5 min, 1 h, and 2 h samples, the absorbance is around 1.71 a.u. giving 50.74% UV protecting. Lastly, pure cream with an absorbance of 0.29 a.u. results in 8.61% UV protecting. The UVB protecting percentages at 282.00 nm, where higher absorbance indicates greater UV reduction, are calculated as follows: Pure fenugreek, with an absorbance of 4.10 a.u., represents 100% UV protecting. After 5 min to 18 hr with sunscreen, the absorbance is 3.57 a.u. corresponding to 87.07% UV protecting. For pure cream, with an absorbance of 1.22 a.u., the UV protecting is 29.76%. The SPF is calculated using methodology<sup>24,25</sup>. The value of SPF is quite comparable to the result obtained by Mouffouk et al., and more detail of the present work is shown in Table 1 below.

The data in Table 1 illustrates the SPF variation with fenugreek water over time across different wavelengths (290–320 nm). The SPF values increase significantly when fenugreek water is combined with cream, indicating enhanced UV protection. Over time, the SPF continues to rise, with the highest values observed at 18 h, suggesting a prolonged stabilization effect of fenugreek on the cream. The peak SPF is observed at 305 nm and 300 nm, confirming optimal UV absorption at these wavelengths. This trend highlights fenugreek’s potential as a natural UV-protecting agent when incorporated into skincare formulations.

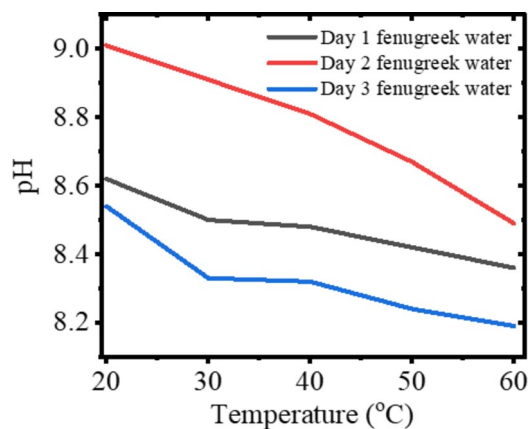
pH value of fenugreek water

The pH measurements of the three fenugreek water samples revealed a decreasing trend with increasing temperature, as depicted in Fig. 9. Despite this variation, all the samples maintained a basic pH, ranging from 8.20 to 9.00, indicating that fenugreek water exhibits basic properties. Among the samples, the fenugreek water from day 2 exhibited the highest basicity, with the pH value closer to 9. In contrast, the day 3 sample displayed a lower pH, still basic but closer to 8.20, while the day 1 sample had an intermediate pH between the day 3 and day 2 samples. Poonam and Sonika reported the pH of fenugreek gum from the HM-57 variety as 6.86 ± 0.05 and Kasuri Methi as 7.03 ± 0.11<sup>26</sup>. In the present research, it is shown that the sample is more basicity than those reported by Poonam and Sonika.

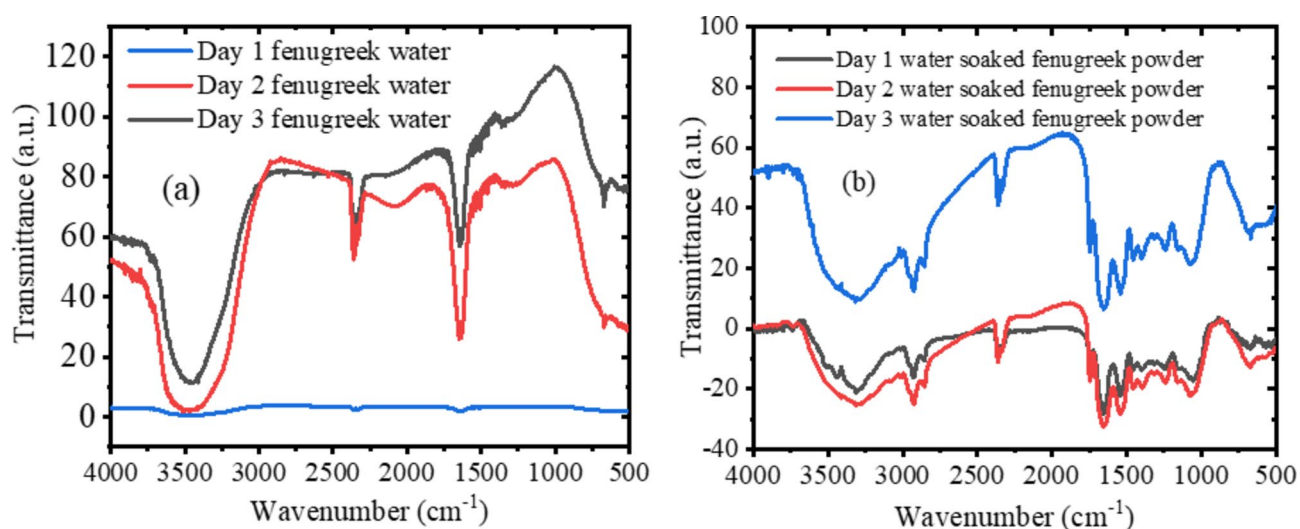
These observations suggest that the basicity of the fenugreek water changes over time, likely due to the continued release of bioactive compounds and ions during the soaking process. The higher basicity of the day 2 sample indicates a more pronounced basic nature, which may have specific health benefits depending on the required pH levels for consumption. Understanding the pH range of fenugreek water is crucial for determining its suitability for health purposes, as basic water is often considered beneficial for neutralizing acidity in the body. This research helps identify the appropriate level of basicity for preparing fenugreek water that meets health

| Wavelength (nm) | Cream | Fenugreek and Cream Instant | Fenugreek and Cream 1 h | Fenugreek and Cream 2 h | Fenugreek and Cream 18 h |
|-----------------|-------|-----------------------------|-------------------------|-------------------------|--------------------------|
| 290             | 0.06  | 0.36                        | 0.38                    | 0.38                    | 0.44                     |
| 295             | 0.33  | 1.84                        | 1.96                    | 1.97                    | 2.28                     |
| 300             | 1.14  | 6.28                        | 6.64                    | 6.71                    | 7.59                     |
| 305             | 1.26  | 6.95                        | 7.31                    | 7.35                    | 8.35                     |
| 310             | 0.67  | 3.86                        | 4.07                    | 4.09                    | 4.52                     |
| 315             | 0.28  | 1.70                        | 1.76                    | 1.79                    | 1.93                     |
| 320             | 0.06  | 0.35                        | 0.37                    | 0.37                    | 0.39                     |

Table 1. Sun protection factor (SPF) with Fenugreek water with time.



**Fig. 9.** Variation of pH with temperature of fenugreek water.



**Fig. 10.** FTIR of fenugreek (a) water and (b) powder.

standards. The different pH values measured in this study can guide future formulations of fenugreek water for optimal consumption. The degree of basicity can affect the efficacy of water in promoting health benefits, such as improved digestion, detoxification, and pH balance in the body. Therefore, pH analysis plays an essential role in establishing the potential of fenugreek water as a healthy drink.

#### FTIR analysis of fenugreek water and powder

This analysis investigates the transformation of organic compounds in fenugreek seeds over time, observing the transmittance patterns in Fourier-Transform Infrared Spectroscopy (FTIR) for both fenugreek water and powder across multiple days, as illustrated in Fig. 10(a) and (b). On day 1, fenugreek water shows a higher transmittance compared to days 2 and 3. This suggests that, initially, fewer organic compounds have dissolved into the water, allowing more infrared (IR) light to pass through. Over time, as fenugreek seeds are soaked, organic compounds increasingly dissolve and form colloids in the water, reducing transmittance as these compounds absorb more IR radiation. The colloid formation intensifies from day 1 to day 3, indicating an accumulation of organic compounds. This build-up creates denser colloidal structures that scatter and absorb IR, lowering the transmittance on subsequent days. Hence, the transformation of fenugreek compounds into the water medium becomes more effective over time, which can be beneficial due to the nutritional and skin health benefits these compounds provide.

The presence of the broad peak around  $3411\text{ cm}^{-1}$  in fenugreek seed is due to the presence of organic moieties like carboxylic acid functional groups and O-H stretching vibrations along with N-H stretching vibrations (Deshmukh et al., 2019). Such observation was also observed in the present work, as shown in Fig. 10 (a) and (b). For fenugreek powder [Fig. 10 (b)], day 3 displays the highest transmittance, contrary to the water samples. This could be because the organic compounds initially present in the powder may be released or altered over time, possibly due to environmental exposure or chemical changes that reduce their capacity to absorb IR. Thus, the powder's transmittance is highest on day 3, indicating fewer functional compounds compared to earlier

days. To validate these findings, fenugreek seeds soaked in water for days 1, 2, and 3 were also analyzed. The transmittance pattern mirrors that of fenugreek water samples. On day 1, transmittance is high, implying minimal transformation of organic compounds from seeds to water. As days progress, transmittance decreases due to the continuous release of organic compounds from seeds into the water. By day 3, the transmittance for soaked seeds is significantly lower, aligning with increased organic matter transformation in the water, confirming that longer soaking times lead to a greater release of beneficial compounds.

Table 2 shows that the current study’s FTIR findings on fenugreek align well with previous studies, confirming key functional groups: -COOH, O-H, and N-H (3500 cm<sup>-1</sup>), C-H stretching (2922 and 2887 cm<sup>-1</sup>), acetate (2357 cm<sup>-1</sup>), C=O amide I (1643 cm<sup>-1</sup>), C-O stretching (1394 cm<sup>-1</sup>), and β-D-manopyranose units (871 cm<sup>-1</sup>). These groups highlight fenugreek’s rich composition of bioactive compounds and its alignment with established profiles in the literature.

The gradual increase in colloid formation as fenugreek seeds are soaked longer is important. Colloids in fenugreek water are likely to contain bioactive compounds, which enhance the health and skin benefits when used for topical or dietary applications. Day 1 fenugreek water, having fewer colloidal particles, may serve specific applications requiring clearer solutions, whereas day 3 fenugreek water would be richer in bioactive compounds due to extensive colloid formation. The data from FTIR analysis reflects the dynamic transformation of fenugreek seed compounds into water over time. The higher transmittance on day 1 for both fenugreek water and seeds implies an initial state with fewer dissolved compounds. Over subsequent days, organic compounds accumulate in the water, reducing IR transmittance and increasing potential health benefits due to higher concentrations of beneficial compounds. This study demonstrates the significance of soaking time in releasing bioactive compounds, potentially maximizing health benefits in fenugreek applications.

Antioxidant properties of fenugreek waters with DPPH

The antioxidant activity of fenugreek water was assessed using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. To prepare the stock solution, 6 mg of DPPH was dissolved in 25 mL of methanol. A 3 mL aliquot of the working DPPH solution was then mixed with 100 μL of fenugreek water from day 1, day 2, and day 3 in separate test tubes. These tubes were incubated in complete darkness for 30 min, after which absorbance was measured at 517 nm. The antioxidant activity percentage was calculated using the formula shown in below Eq. (1)<sup>33,34,20</sup>:

% Antioxidant Activity = ( (A<sub>c</sub> - A<sub>s</sub>) / A<sub>c</sub> ) × 100 (1)

where A<sub>c</sub> represents the absorbance of the control sample and A<sub>s</sub> denotes the absorbance of the test sample. The absorbance of pure and mixed DPPH is shown in Fig. 11.

The antioxidant activity of fenugreek water was assessed using the DPPH assay, where lower absorbance values indicate higher antioxidant potential. The control sample (DPPH alone) had an absorbance of 0.85. Day 1 fenugreek water showed an absorbance of 0.93, resulting in negative antioxidant activity (-9.43%), suggesting interference with DPPH reduction. Day 2 exhibited the highest antioxidant activity (17.54%) with the lowest absorbance (0.70), indicating a peak in bioactive compounds, while day 3 showed 0.85828 absorbance and -0.59% activity, suggesting a decline in effectiveness. These results highlight the time-dependent nature of fenugreek water’s antioxidant potential, possibly due to degradation or transformation of active compounds. Radical scavengers, including antioxidants, help prevent lipid peroxidation, which affects food stability and quality<sup>35</sup>. The findings confirm that day 2 fenugreek water is the most effective in inhibiting lipid peroxidation, making it a promising natural antioxidant for food and pharmaceutical applications.

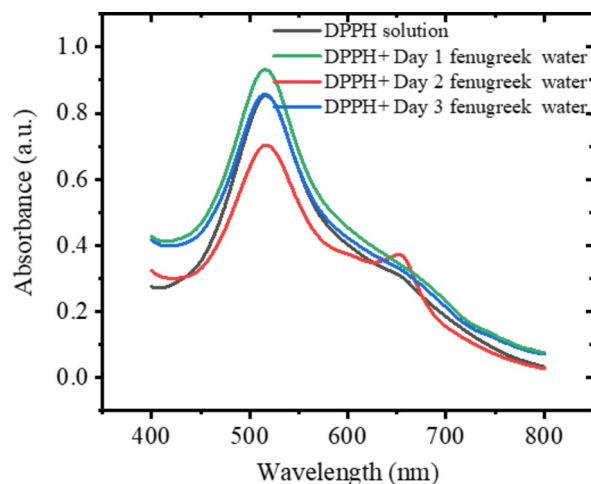
Table 3 presents the antioxidant properties of fenugreek water using the DPPH assay, where lower absorbance indicates higher antioxidant activity. The control sample (DPPH alone) shows the highest absorbance (0.85 a.u.), while the test samples exhibit varying absorbance values over three days. On day 1, the absorbance increases (0.93 a.u.), suggesting a lower initial antioxidant effect. However, on day 2, the absorbance drops significantly (0.70 a.u.), indicating an enhanced antioxidant activity due to the possible formation of bioactive compounds. On day 3, the absorbance slightly rises (0.86 a.u.), suggesting a decline in antioxidant effectiveness over time. These variations highlight the time-dependent behavior of fenugreek water’s antioxidant properties and its potential as a natural antioxidant source.

Future research could explore the optimization of fenugreek water’s antioxidant properties by examining various extraction methods and concentrations over extended periods. Further studies could investigate the synergistic effects of fenugreek water when combined with other natural antioxidants, assessing its stability and

| Present work                   | Previous works                 |   | References |
|--------------------------------|--------------------------------|---|------------|
| Wavenumber (cm <sup>-1</sup> ) | Wavenumber (cm <sup>-1</sup> ) | Functional group  |            |
| 3500                           | Broad peak around 3411         | -COOH and O-H stretching vibrations along with N-H stretching vibration | 27         |
| 2922                           | 2927.94                        | C-H   | 28         |
| 2357                           | 2307                           | Acetate   | 29         |
| 1643                           | 1657                           | C=O, amide I  | 30         |
| 1394                           | Around 1315                    | C-O stretch   | 31         |
| 871                            | 887 and 891                    | β-D-manopyranose units  | 32         |

Table 2. Functional group present in Fenugreek sample.





**Fig. 11.** Impact of DPPH with fenugreek water for antioxidant properties.

| Testing Compositions         | Absorbance (a.u.) |
|------------------------------|-------------------|
| DPPH (Control)               | 0.85              |
| DPPH + Day 1 Fenugreek Water | 0.93              |
| DPPH + Day 2 Fenugreek Water | 0.70              |
| DPPH + Day 3 Fenugreek Water | 0.86              |

**Table 3.** Antioxidants properties using DPPH assay of Fenugreek water.

effectiveness in different formulations such as food preservatives, pharmaceuticals, and cosmetics. Additionally, exploring the potential for fenugreek water to improve the efficacy of other active ingredients in topical applications like sunscreen creams and anti-diabetic medications would be valuable. Practical applications could extend to developing eco-friendly and bioactive preservatives, as well as enhancing the performance of existing health and beauty products, with a focus on sustainability and natural alternatives. However, limitations of the study include the relatively short duration of testing, which may not fully capture the long-term stability and effectiveness of fenugreek water in various applications. More comprehensive studies are needed to address these factors and confirm the findings across different conditions and formulations.

## Conclusion

The study reveals that fenugreek seed significantly improves the absorbance of sugar solutions, indicating its potential as a preventive in anti-diabetes medications. XRD analysis confirms the structural integrity of fenugreek powder when incorporated into sugar solutions, ensuring no adverse changes in its composition. The absorbance of fenugreek water decreases with increasing concentration of sugar solution, and vice versa. In addition, the inclusion of fenugreek water enhances the stability and effectiveness of sunscreen creams, likely due to its beneficial interactions with other ingredients. FTIR analysis indicates that as fenugreek seeds are soaked longer, more organic compounds transfer into the water, progressively lowering transmittance due to colloidal formation, which suggests that extended soaking enhances the water's potential health benefits. The pH levels of the fenugreek water decrease with an increase in temperature. These findings suggest that fenugreek water can serve as an effective component in both pharmaceutical and cosmetic products, offering improvements in product effectiveness and stability. This study opens the use of fenugreek in the development of enhanced therapeutic and cosmetic formulations, leveraging its natural properties for improved performance. Also, the results confirm that fenugreek water significantly improves UV protection over time, with the highest SPF observed at 305 nm and 300 nm after 18 h. Additionally, its antioxidant activity peaks on day 2, highlighting its potential as a natural bioactive compound for cosmetic and health applications.

## Data availability

All the raw data is freely accessible. The URL of data: <http://dx.doi.org/10.13140/RG.2.2.26949.36322> and Contact person of data is first author of the manuscript Saddam Husain Dhobi (saddamdhobe@gmail.com). The supplementary materials for this article contain additional data of experimental results, as referenced in<sup>18</sup>.

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## Author contributions

Saddam Husain Dhobi, Pramod Subedi and Bishnu Neupane all contribute equally (†). Dikshya Mulmi: Review, conceptual frame and formal analysis. Deependra Das Mulmi: Supervised, validation and Review draft. Note: Primary contact author is Saddam Husain Dhobi.

## Declarations

### Competing interests

The authors declare no competing interests.

### Additional information

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