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Isolation, Molecular Characterization, and Antimicrobial Profiling of Fructans from *Cichorium intybus*

Ezza Shahid[1]*, Rabbia Shafqat[2]

[1]Institute of Biological Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200, Pakistan (Ezzashahid123@gmail.com)

[2]Department of Biochemistry, University of Agriculture Faisalabad, Pakistan (rabbia.shafqat123@gmail.com)

***Corresponding author:** Ezza Shahid (Ezzashahid123@gmail.com)*

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Abstract

Background: Fructans are fructose-based oligomers that function as reserve carbohydrates in several plant species, with notable abundance in the Asteraceae family. In *Cichorium intybus* (chicory), fructan biosynthesis is regulated by two key enzymes, sucrose:sucrose 1-fructosyltransferase (1-SST) and fructan:fructan 1-fructosyltransferase (1-FFT). **Objective:** The present study aimed to isolate, purify, and characterize fructans from chicory, alongside assessing their antimicrobial potential and associated genetic markers. **Methods:** Organic extracts were prepared using NaOH, HCl, ethanol, and chloroform, and their antimicrobial activity was evaluated against *Escherichia coli*, *Bacillus cereus*, and *Streptococcus pneumoniae*. Genomic DNA was isolated using the CTAB method, followed by PCR amplification with 1-SST and 1-FFT primers, sequencing, and phylogenetic analysis. **Results:** Chloroform and NaOH extracts exhibited the strongest antibacterial effects. Sequencing and phylogenetic analysis confirmed high similarity (>97%) of chicory gene sequences with related species, while revealing distinct SST and FFT sequences unique to *C. intybus*. Phylogenetic clustering linked chicory genes with members of *Taraxacum*, *Lactuca*, and *Cynara*, all known for fructan biosynthesis. **Conclusion:** These findings highlight chicory as a rich source of fructans with potential applications in functional foods, nutraceuticals, and biotechnological processes, while also providing molecular insights into its unique fructan biosynthetic genes.

Keywords: *Inulin; 1-SST; 1-FFT; PCR amplification; Phylogenetic analysis; Antimicrobial activity; Functional foods*

Highlights

- Chloroform and NaOH extracts of *Cichorium intybus* showed the strongest antibacterial activity against *Escherichia coli*, *Bacillus cereus*, and *Streptococcus pneumoniae*.
- PCR amplification and sequencing of 1-SST and 1-FFT genes confirmed high sequence similarity (>97%) with related fructan-producing species while revealing SST and FFT sequences unique to *C. intybus*.
- Phylogenetic analysis linked chicory's fructan biosynthetic genes with *Taraxacum*, *Lactuca*, and *Cynara*, supporting chicory's potential as a source of functional-food and nutraceutical fructans.

1. Introduction

Fructans are fructose-based polymers that serve as alternative reserve carbohydrates in plants, either supplementing or substituting starch and sucrose as primary energy-storage molecules. These carbohydrates occur in approximately 15% of flowering plants and are particularly abundant in the Asteraceae and Poaceae families. Beyond their role as storage compounds, fructans contribute to plant stress tolerance by stabilizing cellular membranes and regulating osmotic balance, helping plants adapt to adverse environmental conditions such as drought, cold, and salinity [1,2]. Fructans function as osmoprotectants, protecting cells against osmotic stress caused by water deficit or high salinity by maintaining cell turgor and stabilizing proteins and membranes. Under drought and cold stress, fructan accumulation increases, helping to preserve membrane integrity and enzyme activity, which are often compromised by dehydration and freezing. Fructans also scavenge reactive oxygen species generated during stress, reducing oxidative damage to cells, and may act as carbon reserves that can be mobilized rapidly to support metabolism and recovery once favorable conditions return. The degree of polymerization and branching of fructans can influence their protective capabilities, offering plants a versatile biochemical tool for coping with various stresses. Collectively, these multifunctional properties position fructans as vital molecules that enhance plant resilience and survival in fluctuating environments [1].

From the perspective of human health, fructans - most notably inulin and fructooligosaccharides (FOS) — have gained prominence for their broad prebiotic effects. They selectively stimulate beneficial gut microbiota such as *Bifidobacteria* and *Lactobacilli*, improving intestinal homeostasis and immune regulation. Fructans also enhance mineral absorption, particularly of calcium and magnesium, supporting skeletal health, and have been linked to improved lipid metabolism through lowering of serum cholesterol and triglycerides, thereby reducing cardiovascular disease risk. Emerging evidence suggests fructans may lower the incidence of certain gastrointestinal disorders and colorectal cancer, making them notable bioactive dietary components with preventative health benefits [3]. These health-promoting properties have spurred widespread application of fructans in the food, medicine, and biotechnology industries as functional fibers and nutraceuticals [1].

Among fructan-rich plants, *Cichorium intybus* (chicory) is a principal source of inulin-type fructans. Chicory-derived inulin and oligofructose are extensively used as functional food ingredients, valued for their dietary fiber content and natural sweetness. Beyond nutrition, chicory fructans have demonstrated antimicrobial and immunomodulatory properties, including inhibition of pathogenic bacteria and modulation of host immune responses, making them promising candidates for novel nutraceutical and therapeutic development [4]. Despite their commercial and biomedical importance, however, the molecular mechanisms underlying fructan biosynthesis in chicory remain incompletely characterized [2].

Fructan biosynthesis in chicory is primarily regulated by two key enzymes. Sucrose:sucrose 1-fructosyltransferase (1-SST) catalyzes the initial transfer of fructosyl units to form the first fructan molecules, while fructan:fructan 1-fructosyltransferase (1-FFT) extends the polymer chains by transferring fructosyl residues between fructans. Characterization of these enzyme-encoding genes is essential for understanding the regulation of fructan metabolism and for exploring the genetic distinctiveness of chicory relative to other fructan-accumulating species [2,5], enabling targeted genetic improvements for optimized fructan yield and properties [2].

This study aimed to isolate and characterize fructans from *C. intybus* and to assess their antimicrobial activity against selected bacterial strains. DNA extracted from chicory tissue was amplified using primers specific to the 1-SST and 1-FFT genes to investigate the molecular basis of fructan biosynthesis, and sequencing and phylogenetic analyses were used to elucidate the evolutionary relationships of chicory with other fructan-producing species. By integrating biochemical and molecular techniques, this work reinforces the significance of chicory as a source of functional carbohydrates and deepens understanding of its fructan metabolism.

2. Materials and Methods

2.1 Plant material collection

Fresh samples of *Cichorium intybus* (chicory) were collected from cultivated fields in Punjab (Rahim Yar Khan), Pakistan. The collected plant material, primarily young leaves and other aerial parts, was thoroughly washed with distilled water to remove impurities, then air-dried under shade to preserve phytochemical integrity. Samples were stored at –10 °C until further use in organic-compound extraction, antimicrobial assays, and

molecular analyses. This standardized collection and processing protocol ensured the preservation of bioactive compounds and nucleic acids for reliable downstream experiments.

2.2 Preparation of extracts and antimicrobial activity assay

Organic compounds were extracted by dissolving 2 g of powdered plant material separately in 30 mL of 0.1 N NaOH, 30 mL of 0.1 N HCl, 50 mL of ethanol, and 30 mL of chloroform. The extracts were incubated at room temperature with occasional shaking and then filtered. Antimicrobial activity was tested against *Escherichia coli*, *Bacillus cereus*, and *Streptococcus pneumoniae* using LB agar medium. Extracts were applied to wells in agar plates inoculated with the bacterial cultures, and antimicrobial activity was recorded as growth-inhibition zones. Activity strength was categorized as weak (+), strong (++), or strongest (+++) [6].

2.3 DNA extraction

Genomic DNA was isolated from fresh young chicory leaves using a modified cetyltrimethylammonium bromide (CTAB) method. Briefly, approximately 400 mg of leaf tissue was ground under chilled conditions with 300 µL CTAB extraction buffer to lyse the cells. The homogenate was transferred to microcentrifuge tubes, supplemented with an additional 400 µL buffer, and incubated at 65 °C for 30 min with intermittent mixing to enhance extraction efficiency. After cooling, samples were centrifuged at 10,000 rpm for 10 min at 25 °C to pellet cell debris, and the supernatant was mixed with an equal volume of chloroform:isoamyl alcohol (24:1) for protein removal, followed by centrifugation. The aqueous phase containing the DNA was collected and precipitated using cold 70% ethanol and 5 M NaCl to improve purity and yield. DNA pellets were washed with 70% ethanol, air-dried, and resuspended in 50 µL TE buffer. DNA quality was assessed by agarose gel electrophoresis, and concentration was determined spectrophotometrically. The high-quality DNA was stored at –20 °C until further use, providing a reliable template for downstream molecular analyses [7,8].

2.4 PCR amplification

PCR amplification was conducted using specific primers for the 1-SST and 1-FFT genes (Table 1). Reactions were prepared in 25 µL volumes containing template DNA, primers, dNTPs, buffer, $MgCl_2$, and Taq DNA polymerase. Thermal cycling comprised an initial denaturation at 95 °C for 10 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. PCR products were visualized on agarose gels alongside a DNA ladder. This protocol aligns with standard PCR procedures used for amplification of gene targets in plant molecular studies [9].

Table 1. Primers used for PCR amplification of the chicory 1-SST and 1-FFT genes.

Sr	Primer	Sequence (5′–3′)	Ref.
1	1-SST F	CCAACAACCATCAGGGAGGAG	[11]
2	1-SST R	AGCAACGGAGCTGTGAACGT	[11]
3	1-FFT F	CGGCTACGCAGTTGGACATAG	[11]
4	1-FFT R	CTCGTGGTGCAACCGTATTCA	[11]

2.5 Sequencing and phylogenetic analysis

Purified PCR products were sent to Macrogen Inc. (Seoul, Korea) for sequencing. Raw sequences were cleaned and aligned using JustBio software, and sequence identity was verified through BLAST analysis against the NCBI database; sequences showing ≥93% similarity were considered reliable matches. Phylogenetic trees were constructed using the neighbor-joining method, comparing chicory 1-SST and 1-FFT genes with homologous sequences from other fructan-producing plants retrieved from NCBI. This approach aligns with established methods used in recent molecular studies analyzing fructan biosynthesis genes and their evolutionary relationships [10].

3. Results

3.1 Antimicrobial activity of chicory extracts

The antimicrobial potential of *C. intybus* extracts was tested against *Escherichia coli*, *Bacillus cereus*, and *Streptococcus pneumoniae* using different solvents (HCl, NaOH, ethanol, and chloroform). As shown in Table 2, all extracts exhibited varying levels of activity, with the chloroform and ethanol extracts producing the

strongest inhibitory effects (+++). The NaOH extract also showed strong activity against all tested strains, whereas the HCl extract demonstrated comparatively weaker inhibition. Notably, *S. pneumoniae* showed strong sensitivity to the NaOH extract, while *E. coli* and *B. cereus* were most inhibited by the chloroform extract. These results confirm that chicory contains bioactive compounds with broad-spectrum antibacterial potential.

Table 2. Antimicrobial activity of Cichorium intybus extracts. +, weak; ++, strong; +++, strongest inhibition.

Bacteria	HCl	NaOH	Ethanol	Chloroform
E. coli	+	++	++	+++
Bacillus cereus	+	++	++	+++
Streptococcus pneumoniae	++	+++	+	++

3.2 Genomic DNA extraction and PCR amplification

High-quality genomic DNA was successfully isolated from chicory leaves using the CTAB method. Gel electrophoresis confirmed intact DNA with minimal degradation, and spectrophotometric analysis indicated purity suitable for molecular applications. PCR amplification using the 1-SST and 1-FFT primers produced distinct bands of the expected sizes, as shown in Figure 1, confirming successful amplification of the target genes.

3.3 Sequencing and species identification

The amplified PCR products were sequenced, and BLAST analysis revealed high similarity (94–99%) with existing *C. intybus* sequences in the NCBI database (Table 3). Specifically, the sequences amplified by the 1-SST and 1-FFT primers matched chicory reference genes with identities ranging from 94.24% to 99.53%. These results validated the accuracy of gene amplification and confirmed the species identity of the collected chicory samples.

Table 3. Percent sequence identity of 1-SST and 1-FFT amplicons relative to reference Cichorium intybus sequences (NCBI BLAST).

Sr. No.	Common name	Scientific name	Primer	Percent identity
1	Chicory	*Cichorium intybus*	1-SST F	98.70%
2	Chicory	*Cichorium intybus*	1-SST R	94.24%
3	Chicory	*Cichorium intybus*	1-FFT F	99.53%
4	Chicory	*Cichorium intybus*	1-FFT R	96.67%

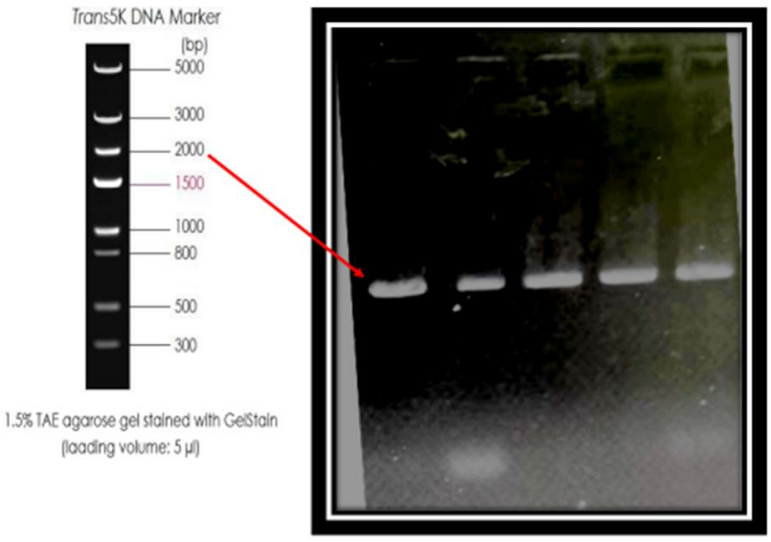

Figure 1. PCR amplification products obtained using the 1-SST and 1-FFT primers, resolved on a 1.5% TAE agarose gel. Lane M, Trans5K DNA ladder; remaining lanes, chicory genomic DNA amplicons.

3.4 Phylogenetic analysis

Phylogenetic trees were constructed to examine the evolutionary relationship of chicory SST and FFT genes with those of other fructan-producing plants. The SST phylogenetic tree (Figure 2) showed that chicory SST genes are closely related to *Taraxacum brevicorniculatum* and *Lactuca sativa*, both known to accumulate fructans. Similarly, the FFT phylogenetic tree (Figure 3) showed close clustering of chicory with *Taraxacum officinale*, *Cynara cardunculus*, *Doronicum pardalianches*, and *Lactuca sativa*. Importantly, the analysis also highlighted the distinctiveness of the chicory SST and FFT sequences, as identical matches were not found in any other plant species, suggesting that chicory possesses unique genetic determinants of fructan biosynthesis.

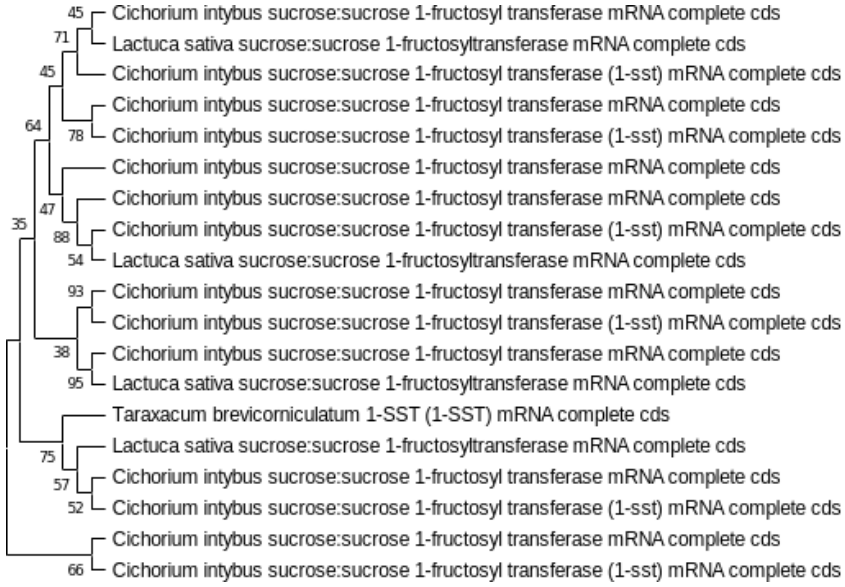

Figure 2. Phylogenetic tree of chicory 1-SST gene sequences constructed by the neighbor-joining method. Chicory SST genes cluster closely with *Taraxacum brevicorniculatum* and *Lactuca sativa*; the *Cichorium intybus* SST sequence was not matched in any other plant species. Accession numbers: *C. intybus* SST genes, U81520 and JQ346799; *L. sativa* SST gene, EU293870; *T. brevicorniculatum* SST gene, KY306453.

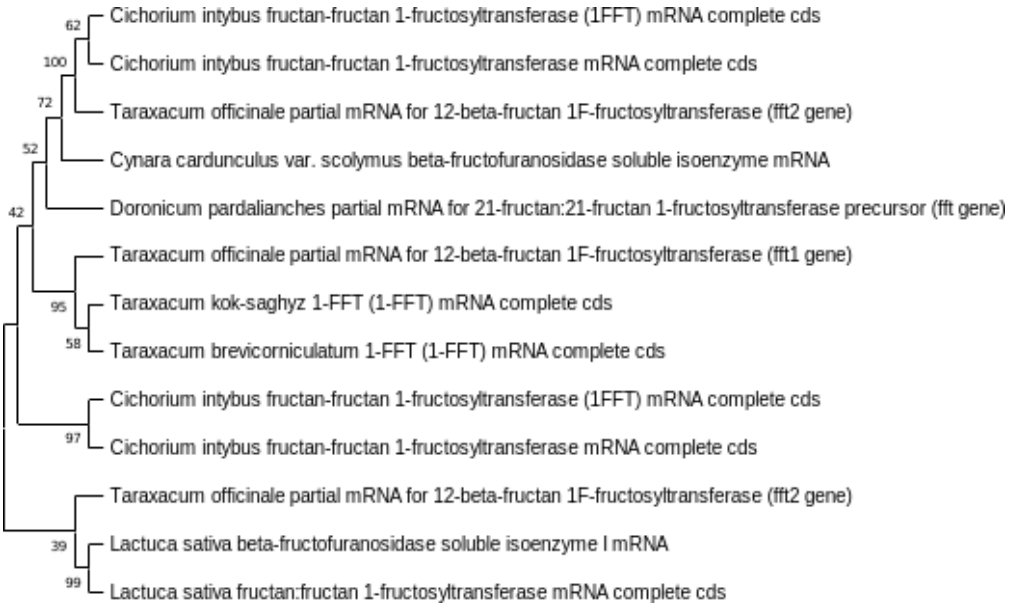

Figure 3. Phylogenetic tree of chicory 1-FFT gene sequences constructed by the neighbor-joining method. Chicory FFT genes cluster closely with Taraxacum officinale, Taraxacum kok-saghyz, Taraxacum brevicorniculatum, Cynara cardunculus var. scolymus, Doronicum pardalianches, and Lactuca sativa, all recognized for fructan biosynthesis.

4. Discussion

The present study highlights the dual biochemical and molecular significance of *Cichorium intybus*, establishing it as a promising source of fructans with both functional and genetic uniqueness. The antimicrobial assays revealed that chicory extracts prepared with chloroform, ethanol, and NaOH exhibited strong inhibitory activity

against *E. coli*, *Bacillus cereus*, and *Streptococcus pneumoniae*. These findings align with previous research demonstrating the antimicrobial potential of *C. intybus* extracts against various bacterial strains, attributed to bioactive phytochemicals such as phenolics, flavonoids, and sesquiterpene lactones [12]. The relatively higher activity of the chloroform and ethanol extracts suggests that non-polar and moderately polar compounds contribute significantly to the antibacterial effects, supporting the application of chicory-derived compounds in nutraceuticals and natural antimicrobial formulations.

Molecular analysis further underscored the biological distinctiveness of chicory. High-quality DNA extracted using the CTAB method enabled successful amplification of the 1-SST (sucrose:sucrose 1-fructosyltransferase) and 1-FFT (fructan:fructan 1-fructosyltransferase) genes, key enzymes central to fructan biosynthesis [13,14]. Sequence similarity analysis revealed 94–99% identity with existing *C. intybus* sequences, confirming molecular identification. This aligns with prior studies emphasizing the fundamental roles of 1-SST and 1-FFT in fructan metabolism, which governs inulin biosynthesis with implications for yield and polymer chain length [14]. The slight sequence divergence observed points to genetic variability within chicory populations that may influence fructan characteristics such as inulin yield and chain length [15].

Phylogenetic analyses elucidated the evolutionary relationships among chicory fructan biosynthetic genes. The 1-SST sequences from chicory clustered closely with those from *Taraxacum brevicorniculatum* and *Lactuca sativa*, while 1-FFT sequences grouped with *Taraxacum officinale*, *Cynara cardunculus*, and *Doronicum pardalianches* — all members of the Asteraceae family recognized for fructan synthesis capacity [13,16]. These findings confirm the genetic relatedness of chicory to these species while highlighting distinct genetic determinants underlying its fructan biosynthetic pathway. This genetic uniqueness may explain the variability observed in fructan characteristics among chicory cultivars and provides valuable genomic resources for breeding and metabolic engineering aimed at fructan optimization [17].

Together, these findings demonstrate the multifaceted potential of chicory fructans, combining notable antimicrobial efficacy with molecular distinctiveness. Various chicory extracts, especially those obtained using ethyl acetate and supercritical-fluid extraction, exhibit strong antibacterial and antifungal activity against pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Candida albicans* biofilms [12,18]. These antimicrobial properties support chicory's emerging role as a source of functional ingredients in the food and pharmaceutical industries, while molecular characterization enhances understanding of fructan biosynthesis pathways and provides avenues for optimization. Future research should focus on quantifying gene expression across diverse environmental contexts, isolating and characterizing fructan-metabolizing enzymes, and assessing fructan chain-length variation among chicory genotypes to fully harness its biotechnological and nutritional potential [19,20].

This study provides valuable insight by integrating biochemical and molecular approaches to explore the antimicrobial and genetic potential of *Cichorium intybus*. Strong antibacterial activity of the chloroform, ethanol, and NaOH extracts confirmed the presence of bioactive compounds, while successful amplification and sequencing of the 1-SST and 1-FFT genes, together with phylogenetic analysis, highlighted chicory's distinct role in fructan biosynthesis. Despite these strengths, the study was limited to in vitro antimicrobial assays against a small number of bacterial strains, without quantitative analysis of fructan yield, gene expression under stress, or enzyme-level validation. These limitations indicate a need for broader biological testing and deeper molecular studies to fully exploit chicory's nutraceutical and biotechnological potential.

5. Conclusions

This study demonstrates that *Cichorium intybus* is a valuable source of fructans with both antimicrobial and molecular significance. Organic extracts, particularly those prepared with chloroform, ethanol, and NaOH, exhibited strong antibacterial activity against *E. coli*, *Bacillus cereus*, and *Streptococcus pneumoniae*, confirming the presence of bioactive compounds with therapeutic potential. Molecular analysis further validated the role of chicory in fructan biosynthesis, with successful amplification and sequencing of the 1-SST and 1-FFT genes. Phylogenetic analysis revealed close relationships with other Asteraceae members but also highlighted the genetic distinctiveness of chicory fructan sequences, suggesting unique determinants of inulin metabolism. Overall, these findings reinforce chicory as an important functional crop, offering dual benefits as a source of nutraceutical compounds and as a model for fructan biosynthetic research.

Future research should focus on gene expression profiling under stress conditions, purification of fructan-metabolizing enzymes, and in vivo validation of antimicrobial activity. Breeding and metabolic-engineering strategies are also recommended to enhance inulin content and diversify chicory's industrial applications. Collectively, these insights position chicory as both a functional food crop and a model plant for advancing fructan-based biotechnology; as with any single-study screen, these conclusions should be regarded as a foundation for the further validation outlined above rather than a final characterization of chicory's therapeutic potential.

Declarations

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Institutional review board statement: Not applicable. The study involved plant material and bacterial cultures and did not involve human participants or live vertebrate animals.

Informed consent statement: Not applicable.

Data availability: The data supporting the findings of this study are available in the laboratory records of the Institute of Biological Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200, Pakistan, and are available from the corresponding author upon reasonable request.

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