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ORIGINAL ARTICLE

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

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Abstract

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). The present study aimed to isolate, purify, and characterize fructans from chicory, alongside assessing their antimicrobial potential and associated genetic markers. Organic extracts were prepared using NaOH, HCl, ethanol, and chloroform, and their antimicrobial activity was evaluated against *Escherichia coli*, *Bacillus cereus*, and *Streptococcus pneumoniae*. Chloroform and NaOH extracts exhibited the strongest antibacterial effects. Genomic DNA was isolated using the CTAB method, followed by PCR amplification with 1-SST and 1-FFT primers. 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. 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

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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 are found in approximately 15% of flowering plants, particularly abundant in members of the Asteraceae and Poaceae families. Beyond their role as storage compounds, fructans play a crucial role in plant stress tolerance by stabilizing cellular membranes and regulating osmotic balance, thereby helping plants adapt to adverse environmental conditions such as drought, cold, and salinity (1, 2). Fructans function as osmoprotectants compounds that protect 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 activities, which are often compromised by dehydration and freezing. They also scavenge reactive oxygen species generated during stress, reducing oxidative damage to cells. Additionally, fructans 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 to cope with various stresses. Collectively, these multifunctional properties highlight fructans as vital molecules that enhance plant resilience and survival in fluctuating and challenging environments (1). This comprehensive role of fructans in stress adaptation goes beyond mere carbohydrate storage, positioning them as key metabolites in plant defense and stress physiology. Their dynamic synthesis and breakdown in response to environmental cues enable plants to maintain metabolic homeostasis while mitigating stress-induced damage,

ensuring optimal growth and reproduction despite adverse conditions (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 like Bifidobacteria and Lactobacilli, which improve intestinal homeostasis and immune regulation. Additionally, fructans enhance mineral absorption, particularly calcium and magnesium, supporting skeletal health. They have also been linked to improved lipid metabolism by lowering serum cholesterol and triglycerides, thereby reducing cardiovascular disease risk. Emerging evidence suggests fructans may lower the incidence of certain gastrointestinal disorders and colorectal cancer, rendering them critical bioactive dietary components with preventative health benefits (3). These health-promoting properties have spurred widespread application of fructans in 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 utilized 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 developments (4). However, despite their commercial and biomedical importance, the molecular mechanisms underlying fructan biosynthesis in chicory are 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 to understand fructan metabolism regulation and to explore the genetic distinctiveness of chicory compared to other fructan-accumulating species (2, 5). Advancing knowledge of these genes enables targeted genetic improvements for optimized fructan yield and properties, expanding chicory's utility (2).

This study aims to isolate and characterize fructans from *C. intybus* and assess their antimicrobial activity against selected bacterial strains. Additionally, DNA extracted from chicory tissues will be amplified using primers specific to 1-SST and 1-FFT genes to investigate the molecular basis of fructan biosynthesis. Sequencing and phylogenetic analyses will elucidate evolutionary relationships of chicory with other fructan-producing species, providing insights into the unique genetic framework of its fructan biosynthetic pathway. By integrating biochemical and molecular techniques, this research 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 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 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, which involved grinding approximately 400 mg of leaf tissue in chilled conditions with 300 µL CTAB extraction buffer to lyse the cells. The homogenate was then transferred to microcentrifuge tubes, supplemented with an additional 400 µL buffer, and incubated at 65 °C for 30 minutes with intermittent mixing to enhance extraction efficiency. After cooling, samples were centrifuged at 10,000 rpm for 10 minutes 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. The 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 (CTAB extraction method with minor modifications) (7, 8).

2.4 PCR Amplification

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

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).

Table 1: List of Primers in PCR Amplifications

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)

3. Result

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

Table 2: Anti-microbial Activity of Chicory's Extract

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 high purity suitable for molecular applications. PCR amplification using 1-SST and 1-FFT primers produced distinct bands of expected sizes, as shown in Figure 1, confirming successful amplification of target genes.

3.3 Sequencing and Species Identification

The amplified PCR products were sequenced, and BLAST analysis revealed a high similarity (94–99%) with existing *C. intybus* sequences in the NCBI database (Table 3). Specifically, the

sequences amplified by 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 Identity of 1-SST and 1-FFT Primers Used for *Cichorium intybus* (Chicory) Amplification

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%

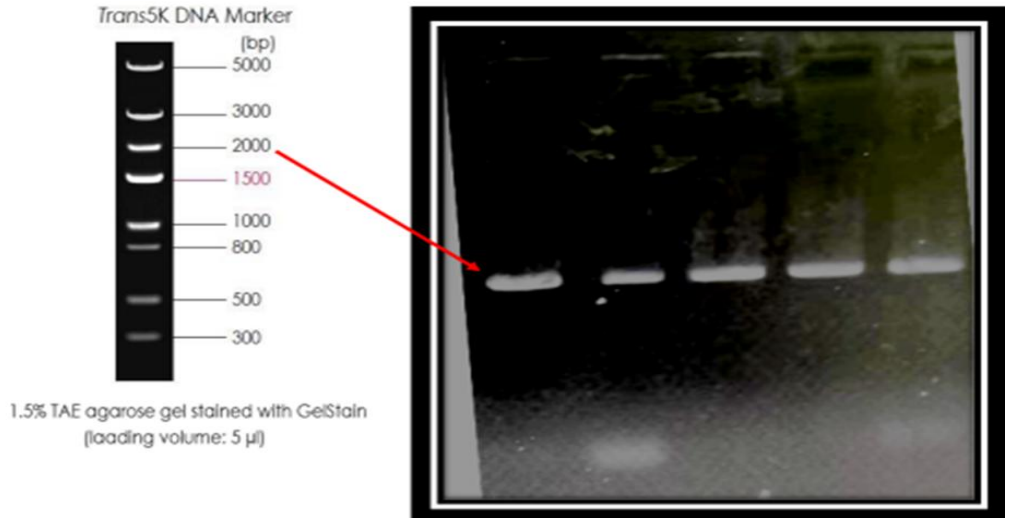

Figure 1. PCR amplifies products by 1-SST and 1-FFT primers. M DNA ladder, remaining are DNA of Chicory plant

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) revealed 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 chicory SST and FFT sequences, as identical matches were not found in any other plant species. This suggests that chicory possesses unique genetic determinants of 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

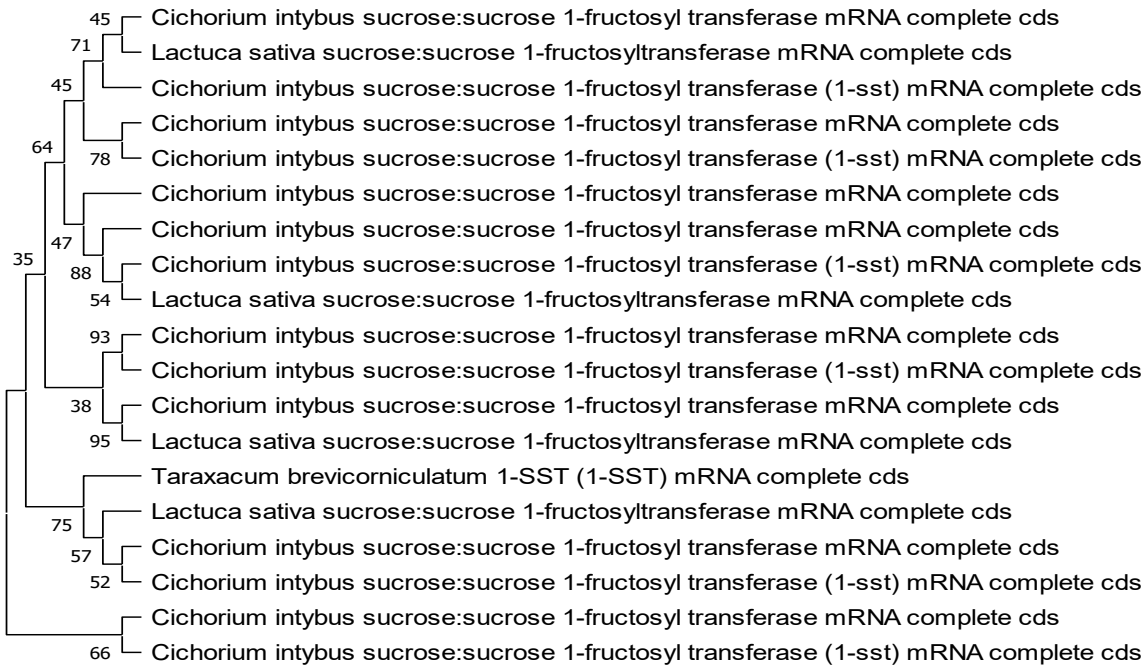

Figure 2. Phylogenetic Tree of Chicory Plants with 1-SST Primer. The phylogenetic relationship of five chicory SST genes closely related to Taraxacum brevicorniculatum and Lactuca sativa, both of which are involved in fructan synthesis, is illustrated. The exact SST gene sequence of Cichorium intybus is not found in any other plant species, as depicted in the above phylogenetic tree. The accession numbers of Cichorium intybus SST genes are (U81520) and (JQ346799); Lactuca sativa SST gene: (EU293870); Taraxacum Breviconiculatum SST gene: (KY306453)

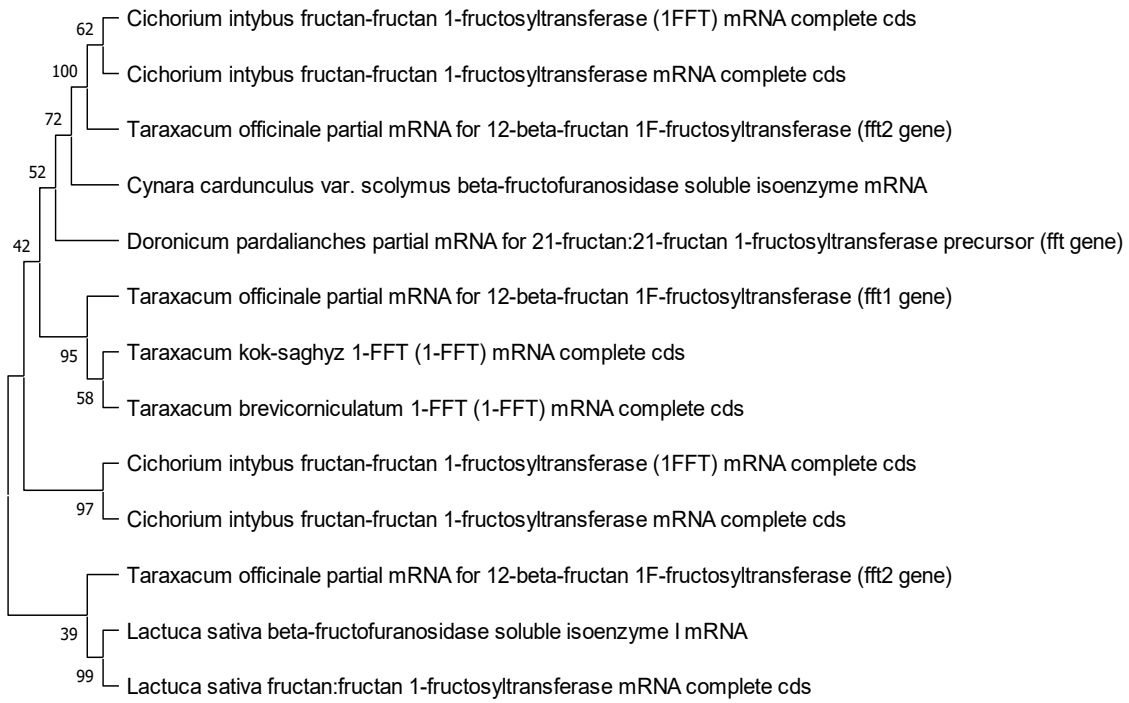

Figure 3. Phylogenetic tree of the chicory plant with 1-FFT Primer. The phylogenetic relationship of four chicory FFT genes closely relates to Taraxacum officinale, Kok-saghyz, brevicorniculatum; Cynara; Doronicum, and Lactuca, which are involved in fructan synthesis

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 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 (*Cichorium intybus*). 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, which are 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 regulates inulin biosynthesis with implications on yield and polymer chain length (14). The observed slight sequence divergence 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 gene 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. The 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 activities against pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Candida albicans* biofilms (12, 18). The antimicrobial properties support chicory's emerging role as a source of functional ingredients in food and pharmaceutical industries. Concurrently, molecular characterization enhances understanding of fructan biosynthesis pathways, providing avenues for optimization. Future research should focus on quantifying gene expression in 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 insights by integrating biochemical and molecular approaches to explore the antimicrobial and genetic potential of *Cichorium intybus*. Strong antibacterial activity of chloroform, ethanol, and NaOH extracts confirmed the presence of bioactive compounds, while successful amplification and sequencing of 1-SST and 1-FFT genes, along 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 few bacterial strains, without quantitative analysis of fructan yield, gene expression under stress, or enzyme-level validation. These limitations suggest the need for broader biological testing and deeper molecular studies to fully exploit chicory's nutraceutical and biotechnological potential.

Conclusion

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 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. The combined biochemical and molecular insights gained here provide a foundation for future studies on enzyme purification, gene expression profiling, and metabolic engineering, aimed at enhancing the nutritional and industrial applications of chicory fructans.

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.

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Conflicts of Interest: Authors declare there is no conflict of interest.

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