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Extraction of Galacto Oligosaccharides from Plant Sources and Their Potential Use in Enhancing Gut Health

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Abstract: *Foods and supplements with prebiotic or probiotic qualities have most certainly been available for years, if not millennia, and have been utilized empirically in health maintenance as well as the therapy of gastrointestinal symptoms and illnesses. Prebiotics are fibers that enable the majority of the gut microbiota develop and, beyond a certain limit, boost the placebo level, which aids in psychological effects. Human milk oligosaccharides (HMO) are prebiotics which are naturally derived complex sugars that significantly promote the growth of gut microbes in infants. Galacto-oligosaccharides (GOS) are type of oligosaccharides, a derivative of lactose is a group of prebiotics which are not digested in small intestine meaning they enter large intestine intact and serve as a source of nutrition for beneficial gut microbes. GOS is found in a variety of plants, fruits, and roots, and has qualities comparable to HOS generated by beta-galactidase. Overall, both HMOs and GOS are types of oligosaccharides that can promote gut health by selectively stimulating the growth of beneficial gut bacteria. While HMOs are specific to breast milk, GOS can be added to formula and other food products as a prebiotic. Although GOS promotes the formation of the gut microbiota and only moderately inhibits pathogens in the gut. Biofilm can spawn widespread bloodstream infections (candidemia), which can result in invasive systemic infections of tissues and organs. It can also operate as a reservoir for pathogenic cells, is extremely resistant to medications and the host immune system, and can spread to other parts of the body. By blending GOS with caprylic acid, the combined treatment can enhance the efficacy in combating candida overgrowth. This approach offers a promising alternative to antibiotics, as it targets the biofilm formation and reduces the need for conventional antimicrobial medications.*

Keywords: Biofilm, Chickpea, Galacto Oligosaccharides, Gut Health, Prebiotics, Soyabean

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1. Introduction

Glenn Gibson and Marcel Roberfroid proposed the prebiotics idea for the first time in 1995. Prebiotics, as defined by definitions, are "a non-digestible food ingredient that advantageously affects the host by specifically stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thereby improves host health." For more than 15 years, this definition remained mostly constant. Dietary prebiotics are defined as "selectively fermented ingredients that cause specific modifications to the composition and/or activity of the gastrointestinal microbiota, resulting in benefit(s) to host health" by the International Scientific Association of Probiotics and Prebiotics (ISAPP) at its 6th Meeting in 2008."(Davani-Davari, D. et al., 2019). Without being used by other intestinal bacteria, prebiotics move through the small intestine and into the lower gut where they are available to probiotic bacteria. Inulin and its hydrolysates, malto-oligosaccharides, resistant starch, Galacto-oligosaccharides, fructo-oligosaccharides, lactulose, and fructo-oligosaccharides are prebiotics that are typically utilized in human diets. Short-chain fatty acids, in particular acetic acid, propionic acid, and butyric acid, are crucial end products of the metabolism of carbohydrates and are used as an energy source by the host organism. They are also present in a variety of plants, including chicory, tomatoes, onions, leeks, artichokes, asparagus, and many more (Al-Sheraji, S.H. et al., 2013).

Galacto-oligosaccharides (GOS) were the primary focus of the investigation because they are relatively stable prebiotics, functionally comparable to oligosaccharides found in human milk, and have antibacterial qualities that can combat certain pathogens. Galacto-oligosaccharides (GOS) are a significant class of food-grade oligosaccharides that are indigestible by humans and function as prebiotics because they specifically boost the gut microbiota's beneficial activity by promoting the growth of beneficial microorganisms like *Bifidobacterium* and *Lactobacillus.* The main advantages of consuming GOS are the bifidogenic effect, the suppression of pathogenic bacterial activity, the decrease in colon infections and the production of toxic

metabolites, the increase in mineral absorption, the decrease in colon cancer risk, and the amelioration of existing diseases like obesity and diabetes (Souza, A. F. C. E. et al., 2022). They are typically made up of two to eight sugar units, including galactose and glucose, which are produced by microbial-galactosidase from the substrate lactose. Numerous GOS structures with various levels of polymerization, glycosidic linkage, and branching have been found as a result of improvements in contemporary analytical methods. Following consumption, GOS modifies the gut flora, which generates short chain fatty acids and has great biological functions. They relieve gastrointestinal, neurological, metabolic, and allergy problems, modify the formation of metabolites, and alter ion storage and absorption. They specifically boost the proliferation of probiotics. Additionally, GOS are also commonly utilized as food additives due to their great solubility, stability, safety, and clean taste. GOS can enhance food items' visual appeal, flavour, texture, viscosity, rheological qualities, shelf life, and health benefits (Wang, K. et al., 2023). α-GOS may be extracted from plants, primarily from the seeds of legumes including chickpeas, lentils, cowpeas, lupins, and soybeans. The only oligosaccharides from legumes on the market are those from soybeans, and Japan is their primary supplier (Martins, G. N. et al., 2019). When the immune system of the host is compromised by the use of antibiotics, steroids, or being hospitalized owing to certain conditions, Candida albicans, an opportunistic pathogen, produces biofilm. It is highly challenging to stop these biofilms since they are resistant to antimicrobial medications and other physiological conditions in our gut. The study largely concentrated on the use of Galacto-oligosaccharides, which aid in the growth of probiotics, which in turn offer immunity, coupled with coconut oil, which contains caprylic acid and lauric acid, both of which have antibacterial qualities that break down and prevent the formation of biofilm. The combination of both Galacto-oligosaccharides and coconut oil promotes the growth of prebiotics, which creates a healthy microbiota in the gut and lowers the consumption of antibiotics.

2. Materials and Methods

Sample Collection: various specimens were gathered from distinct locations to encompass a diverse range of microbial entities and organic materials. The first sample, identified as Yakult containing *Lactobacillus casei strain Shirota*, was obtained from the Shop and Save store in K.S Layout.100µl of Yakult was mixed with 100mL of autoclaved MRS (deManRogosa Sharpe) Broth and incubated for 24 hours at 37°C. Every week, subculture was performed in 10ml of MRS Broth. The second sample consisted of *Enterobacter aerogenes* (MTCC2822), while the third sample comprised *Staphylococcus aureus* (MTCC7443). Additionally, the fourth sample encompassed *Candida albicans* (MTCC4748) were collected from Microbial Type Culture Collection and Gene Bank (MTCC). Each organism was cultured in 100mL of Nutrient Broth for 24hours at 37°C. Subculture was done every week to maintain the strains. To introduce a botanical component, the fifth and sixth samples were sourced from the local market in K.S Layout, representing Soy Bean and Chickpea, respectively.

Extraction of GOS from Plant Sources: By using an ethanol extraction process, GOS were extracted from plant sources (soybeans and chickpeas). Soybean and chickpea were soaked overnight and were dried in hot air oven at 80°C. The dried samples were grinded and made into fine powder. The fine powder (2gm) of plant sources were mixed with ethanol (70% of 30ml) and homogenized with the help of homogenizer. In order to break the cells sonification process was done at regular interval of 15 minutes at 90°C for two hours. Centrifuge both samples at 3500rpm for 10 minutes. After collecting the supernatant, add 30 ml of ethanol to the pellet; centrifuge again to ensure that the extraction is complete. Later, gather the supernatant and discard the pellet. To get crystalline form, the collected samples were heated to 130°C in a hot air oven or hot plate and collected samples were stored in a vial.

Germ Tube Test (Identification of *Candida albicans):* The germ tube test is done to identify the *Candida albicans;* it can be done using yeast broth, sodium bicarbonate and bovine serum. The cultured sample of 2ul is added to yeast broth, bovine serum and sodium bicarbonate and incubate it for 2 to 4 hours to allow the growth of *Candida albicans,* after incubation add drop of inoculation from the previously incubated samples on to the slides and observe under the microscope. Germ tube formation was identified by the presence of long, slender, hyphal-like extensions that emerged from the yeast cells.

Growth Substrate (Galacto-oligosaccharides) Effect on Microbes: The pour plate approach allows for more sensitivity, so is ideal for samples with lower bacterial numbers. It's is also the best method for organisms requiring a microaerophilic environment. MRS &MHA media was prepared and GOS was added

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to media and autoclaved at 121°C for 15 min at 15lbs.The bacterial strains (*Lactobacillus casei strain Shirota*, *Enterobacter aerogenes, Staphylococcus aureus, Candida albicans)* were poured into the Petri dish and Autoclaved media were transferred into plates and let it to solidify and then incubated at 37°C for 24 hours with various concentrations of GOS extracted from soybean and chickpea (0%, 1%, 2%, 3%, and 4% w/v).

McFarland Number: McFarland turbidity standards are used as a reference standard to approximate the number of microbial cells in a liquid suspension (Davani-Davari, D. et al.,2019) McFarland number is done by using dilution by maintain the turbidity at 0.08-0.12, where the sub cultured bacterial strains are diluted with the MRS and MHA broth the diluted sample with media is agitated vigorously in a vortex and check the OD value for every dilution and store in deep freezer to maintain the turbidity until using it.

Antibiotic Assay along with GOS (Effectof Antibiotics along with GOS on Microbes): The microbiological test of an antibiotic is based on a comparison of the suppression of micro organism growth caused by known concentrations of a standard preparation of the antibiotic with a known activity with that produced by measured concentrations of the antibiotic under evaluation. MHA and MRS media was prepared and GOS was added to media and autoclaved at 121°C for 15 min at 15lbs. Autoclaved media were poured in sterilized plates and let it to solidify. Astandardizedfourdiluted0.8-0.12McFarland bacterial cultures are *Enterobacter aerogenes* (MTCC2822), *Staphylococcus aureus* (MTCC7443), *Lactobacillus casei strain Shirota,* and *Candida albicans* (MTCC4748) 2µL were spread over the entire surface with the help of L-Loop and incubate it for 24hrs at 37oC in incubator, After incubation, observe the plates for any zone of inhibition, which are clear areas around the filter paper disc or well where no growth of the micro organism has occurred.

Antimicrobial Activity: The 24 well plate is a technique used for antimicrobial activity, an antimicrobial is a substance that either eliminates or inhibits the development of bacteria. Antimicrobial drugs can be categorized based on the bacteria they predominantly combat**.** The MRS and MHA media was prepared and GOS was added to media and sterilized by autoclave at 121°C at 15lbs for 15 minutes, and transfer the media to the microtiter plate in accordance with the positive and negative control. A standardized four diluted 0.8-0.12 McFarland bacterial cultures are *Enterobacter aerogenes* (MTCC2822), *Staphylococcus aureus* (MTCC7443), *Lactobacilluscasei strain Shirota,* and *Candida albicans* (MTCC4748) 2ulwereadded to respective well and different concentrations of coconut oil (caprylicacid/lauric acid) was added to each well. Add resazurin (40 -60) micro liters in each well. The color change determines the growth of microorganisms.

Biofilm Inhibition: Biofilms are complex communities of microorganisms that attach to surfaces and form a protective matrix. In the gut, biofilms can form on the lining of the intestinal tract and contribute to a number of health problems, such as inflammatory bowel disease, colorectal cancer, and antibiotic-resistant infections. To treat biofilms, there are no licensed antimicrobials. A biofilm can only be treated by physically removing it from the body. But through the recent studies that the caprylic acid and lauric acid from the coconut can inhibit the biofilm in the gut and act as antimicrobial compound. Test tube turbidity method was performed with 2µL of inoculum (*Candida albicans*) in each tube and different concentrations (2,6,8 µL) of coconut oil was added. The tubes were incubated at 37°C for4-6 hours. The turbidity was measured by colorimeter at 420nm.

3. Results

Extraction of GOS from Plant Sources: Using ethanol extraction and sonification to break down the cell wall and centrifugation to eliminate all unwanted chemicals such as proteins and amino acids, the crude extract of GOS from plant sources is the initial step towards further research. After full extraction was completed, crystalline samples were kept in vials for later analysis, and GOS (crude extract) was kept in glass tubes.

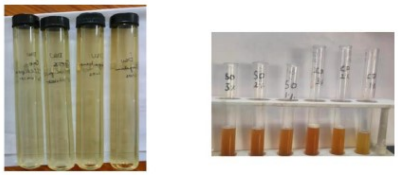

Figure 1

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Figure1: GOS Crude extract from plant sources (soyabean and chickpea) (left) and Crystalline GOS diluted in distilled water at different concentration 1%, 2%, 3% w/v (soyabean and chickpea) (right)

Germ Tube Test (*Candida Albicans* Identification): Yeast broth, bovine serum, and sodium bicarbonate are among the media used in the germ tube test to identify candida albicans. Long, thin, hyphal-like extensions that protruded from the yeast cells were indicative of germ tube production; *Candida* has been seen in yeast broth and sodium bicarbonate but not in bovine serum.

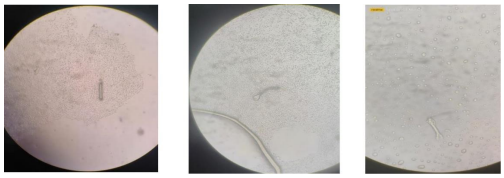

Figure 2

Figure 2: *Candida albicans* negative in bovine serum and positive in sodium bicarbonate and in yeast broth respectively

GOS Effect on Beneficial Microbes and Pathogens: According to the results obtained as the concentration increases from 1% - 3 % the growth of beneficial microbes have seen along with those the growth of pathogens also seen but not as much compared to beneficial microbes, hence3% of GOS have been selected for the further process.

Figure 3

Figure 3: GOS effect on *Lactobacillus, Candida albicans, Enterobacter aerogenes,* mix *(lactobacillus and S. aureus) and S. aureus* respectively at different concentrations

GOS – Antibiotic Assay Along With GOS: The *Lactobacillus casei* shows resistant to tetracycline and streptomycin and in remaining all microbes the penicillin, ampicillin and chloramphenicol showing resistant expect *lactobacillus.*

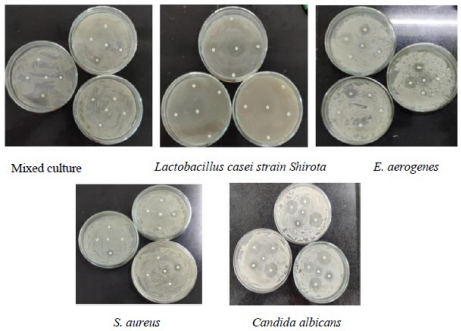

Figure 4

Figure 4: Antibiotic assay along with GOS concentrations

3% GOS	Penicillin-g	Ampicillin	Chloramphenicol	Tetracycline	Streptomycin
Mix	R	R	R	10mm	10mm
L.casei shirota	22mm	24mm	23mm	R	R
C.albicans	R	R	R	15mm	13mm
s.aureus	R	R	R	15mm	12mm
E.aerogenes	R	R	R	16mm	12mm

Table 1: Antibiotic activity along with-GOS (3%) effect, R-Resistance

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4% GOS	Penicillin-g	Ampicillin	Chloramphenicol	Tetracycline	Streptomycin
Mix	R	R	R	10mm	11mm
L.casei shirota	30mm	26mm	25mm	R	R
C.albicans	R	R	R	10mm	12mm
s.aureus	R	R	R	11mm	11mm
E.aerogenes	R	R	R	13mm	11mm

Table 2: Antibiotic activity along with-GOS (4%) effect

Antimicrobial Activity: The concentration of coconut oil improves the inhibition of pathogens *(S. aureus, C. albicans, and E. aerogenes*), which has also been reported to promote the growth of beneficial microorganisms (*Lactobacillus casei strain Shirota*) in 3% and 4% of GOS cases. Resazurin functions as a reagent in this situation, blue color indicating when there is no growth, and when it is diminished, the oxygen utilized by the microorganisms is indicated by a pink color, which indicates the growth of both beneficial microbes and pathogens.

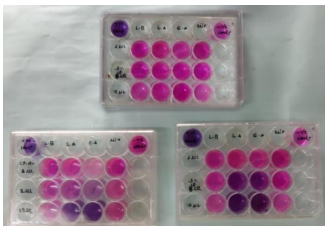

Figure 5

Figure 5: Antimicrobial activity (GOS3and4%+coconut oil at different concentrations) on gut microbes

Biofilm Inhibition: As the concentration of coconut oil from 2-8µl the turbidity decreases (OD value decreases) that states that the coconut oil inhibit the biofilm.

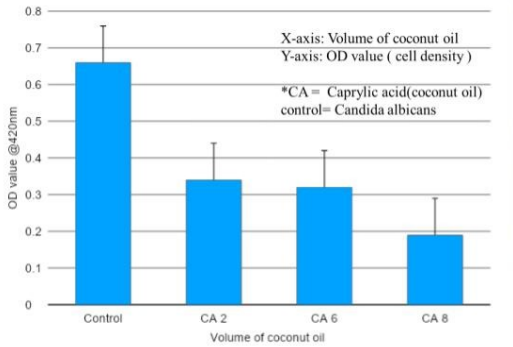

Figure 6

Figure 6: Biofilm inhibition bar graph (as the concentration increases the growth of *Candida albicans* decreases)

4. Discussion

The quality of food has deteriorated recently, and consumption of excessive amount of processed foods has changed the composition of the gut microbiota and contributed to a number of disorders, including neurodegenerative diseases (NDs) brought on by chronic metabolic disease (Leo, E. E. M. et al.,2020). None the less, eating foods enriched with natural prebiotics enhances gut health and replenishes the probiotic population in the gut. Vegetables, grains, fruits, and beans, among other foods that contain various prebiotics, are a natural supply of prebiotics (Iqbal, M. W. et al., 2022). Galacto-oligosaccharides, a type of prebiotic found naturally in foods like dry beans, chickpeas, soybeans, artichokes, and others, were given particular attention in this study due to their similarity to human milk oligosaccharides and better effect on gut health. Hence, the extraction of Galacto-oligosaccharides (GOS) from chickpea and soybean and fortifying them with food improves the quality of the food. GOS is secreted and stored in the plant cell's vacuoles. Ethanol extraction with sonication, which breaks down the cellular components, was selected for

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the extraction of GOS from chickpeas and soybeans. Crude GOS was produced, dried, and crystallized using a hot-air oven since GOS has a high degree of stability and can survive temperatures of upto 170°C. The naturally extracted GOS does not have any side effects and can be consumed by lactose-intolerant individuals, compared to the synthetically produced GOS, which contains lactose. The effect of GOS was checked in vivo by growing both beneficiary probiotics (*Lactobacillus spp*.) as well as pathogens (*Candidaalbicans, Enterobacter aerogenes,* and *Staphylococcus aureus*) on nutrient media containing different concentrations (1%, 2%, and 3% w/v) of extracted GOS from both chickpeas and soybeans. It was observed that both beneficiaries and pathogens grew with an increase in concentration, and at 3% w/v of chickpea extract, the growth of pathogens was comparatively less. Hence, 3% w/v of chickpea extract was used for further study as it helped in the growth of the beneficiary microbe and slightly inhibited the pathogens. This may be because different plant sources contain different types of prebiotics. Higher concentrations of GOS are not recommended since they have a laxative impact. The experiment also focused on applying coconut oil's inherent antibacterial properties to prevent *Candida albicans* from forming biofilms. *Candida albicans* were taken from MTCC cultures and identified using a variety of techniques, including yeast broth, bovine serum albumin solution, and sodium bicarbonate solution. The formation of germ tubes, which are lengthy projections from yeast cells, is one of *Candida albicans'* key properties. The existence of *Candida albicans* in the culture that was utilized in the experiment was confirmed when the *Candida albicans* culture was suspended in various media and the germ tube was seen in yeast broth and sodium bicarbonate medium.

To assess the activity of antibiotics in the presence of GOS, the impact of common antibiotics (penicillin, ampicillin, chloramphenicol, tetracycline, and streptomycin) on the microorganisms isolated was examined. Various microorganisms have also demonstrated resistance to various antibiotics, including *Lactobacillus*, which has shown resistance to tetracycline and streptomycin. The zone of inhibition suggests that GOS may affect how well antibiotics work. After determining the impact of GOS on conventional antibiotics, it was tested with naturally antibacterial virgin coconut oil to see if isolated microorganism growth was occurring. The test was carried out using a 24-well plate and resazurin dye, which is nontoxic, cell-permeable, and redox-sensitive. Resazurin dye, which is initially blue in color, diffuses into cells, where it is irreversibly reduced by diaphorase enzymes to a highly fluorescent compound called resorufin, which is pink in color. Resorufin may then be further reduced to colorless, non-fluorescent hydro resorufin (Uzarski, J. S. et al.,2017), which suggests that the dye is blue in its oxidized form, indicating that there is no microbial growth in the medium, but when the microbial growth occurs, the dye is reduced, turning pink as the oxygen content in the medium decreases. Using varying concentrations of coconut oil and GOS, the resazurin test showed that *Candida albicans* growth was inhibited while *Lactobacillus* growth was encouraged. This discovery implies that the two chemicals can be utilized to treat gut biofilm and to feed probiotics. *Candida albicans* development was slowed down as the concentration of coconut oil increased. In a separate study, the impact of coconut oil on *Candida albicans* growth was examined. The graph showed that as coconut oil concentration increased, *Candida albicans* growth decreased. This is due to the presence of medium-chain fatty acids like lauric acid and caprylic acid, which may rupture the cell membrane of several harmful bacteria and imitate and actively prevent the formation of biofilms. According to the experiment, increasing the concentration of coconut oil inhibits *Candida albicans* growth (Nitbani, F. O. et al., 2022).

The future component is the purification and analysis of extracted GOS, which may then be combined with coconut oil to make a product (chewing gum). Consuming it will destroy the biofilm in the mouth as well as in the gut lining because coconut oil and GOS, which are stable at high temperatures and low PH, reach the probiotics in the lower gut and get metabolized, forming short-chain fatty acids, which have many physiological effects on our body and fight against certain diseases. Combining GOS, which has antimicrobial action against specific pathogens, with coconut oil, a natural antibacterial, may reduce the need for antibiotics, which have detrimental effects on the body by destroying beneficial microorganisms. Since GOS is durable and versatile, it can also be used with other antimicrobials to maximize its effectiveness. GOS can improve food quality by improving gut health, which in turn boosts immunity and enables people to live healthy lives free from opportunistic diseases.

5. Conclusion

In this study, a natural antibiotic and nutrient supplement to beneficial microbes was formulated where it inhibits the biofilm. Isolation of microbes was carried from different sources and crude GOS extracted from plant sources (soybean and chickpea) by ethanol extraction, by antibiotic assay and antibacterial activity and GOS effect on microbes ,the GOS of 3% has shown good results by improving the growth of beneficial bacteria (lactobacillus)and inhibition of biofilm and pathogens along with coconut oil (caprylic acid/lauric acid), which were incorporated into the product, as a result the product (GOS + COCONUT OIL) were formulated with enhanced growth of beneficial microbes and improve the gut health in both direct and indirect ways.

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Appendix: The appendix is an additional section that can include details and data that are supplemental to the main text. For instance, understandings of experimental details that would disrupt the main text's flow but are still essential to comprehending and replicating the research displayed; figures of replicates for experiments for which representative data is shown in the main text can be added here if brief, or as Supplementary data.