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Original Article

Role of Broccoli Fiber for Amoxicillin Delivery and Gut Biome Survival

Gatla Venkata Amruthavalli, Arumugam Vijayalakshmi*

Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Aim: Broccoli fiber has enormous safety net as well as high pre-biotic value. Amoxicillin is although an effective drug against infections is equally harmful to gut microbiota and therefore the above side-effect impedes the use of amoxicillin. The present study deals with evaluating the dual benefits of the partially depolymerized fiber to deliver amoxicillin when the same is used as base fiber in lieu of Micro Crystalline Cellulose (MCC), as well as in protecting the gut microbiota by serving as transient refuge cum pre-biotic material. **Materials and Methods:** Broccoli was partially depolymerized by alkali treatment and subjected to pre formulation studies such as powder microscopy, ash content analysis, particle density and angle of repose. Then using the partially depolymerized fiber formulated tablet dosage form of amoxicillin (500 mg). Using XRD and FTIR the interaction of partially depolymerized fiber with amoxicillin was studied. Release pattern of amoxicillin from the fiber and selective preference and binding of various probiotic species was studied simultaneously using different simulated gut systems. **Results:** Study findings show that broccoli fiber instantaneously released amoxicillin and the space vacated by amoxicillin is being occupied rapidly by various probiotic species and thereby could survive the amoxicillin effect. The parachute effect of the fiber to probiotic is also reconfirmed by simulated gut digestion process and findings show the post gut digested fiber also exhibited significant prebiotic value. The fiber also met various physiochemical parameters that of MCC and also formulation dependent characteristics such as flow, compressibility and disintegration. Zero modification to the fiber vis-à-vis amoxicillin also established by X-ray Diffraction (XRD) and Fourier-Transform Infrared Spectroscopy (FTIR). **Conclusion:** Probiotic microbes when encounter amoxicillin shows greater preference towards the partially depolymerized fiber and seek parachute effect from the fiber. Broccoli fiber is useful to deliver amoxicillin and also protect gut microbiome.

Keywords: Amoxicillin delivery, Broccoli, Tablet excipients, Probiotic protection, Probiotics.

Correspondence:
Dr. Arumugam Vijayalakshmi
Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, INDIA.
Email: amrutha.valli4@gmail.com
ORCID id: 0000-0001-9128-7905

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INTRODUCTION

Amoxicillin is one of the important antibiotics used vastly for treating several bacterial diseases such as infection of the middle ear, throat, skin, urinary tract and the bronchiole. Amoxicillin is classified under the family of beta-lactam group of antibiotics and it shares the structure similar to that of Ampicillin but has greater systemic absorption and tissue and body fluid distribution than Ampicillin.[1] Amoxicillin attaches to the cell wall of the target bacteria which leads to the bacterial death by inhibiting cell wall biosynthesis.[2] The most common susceptible bacterial pathogens to Amoxicillin are *Streptococci, Pneumococci, Enterococci, Haemophilus influenza, Escherichia coli, Proteus mirabilis, Neisseria meningitides, Neisseria gonorrhoea, Shigella, Chlamydia trachomatis, Salmonella, Borrelia burgdorferi* and *Helicobacter pylori*.[3]

The greatest limitation of Amoxicillin is that the antibiotic also would affect several beneficial gut microbes resulting in near total elimination of the probiotic organisms in the gut system and thereby would increases the bioburden of pathogenic microbes and associated gastric irritation/complications.[4] The above unwanted side-effect on gut microbiome heavily impedes the use of Amoxicillin, otherwise the drug is very effective and safe to deal several infectious conditions.

Slow release oral dosage form of Amoxicillin has been attempted but the result has not been in desired line and so shall the coated drug formulation. Altered/modified delivery mechanism may increase the efficacy of the drug with least to minimal side-effects provided the modified method instantaneously release the drug and at the same time the base fiber material if could act as parachute to the probiotic bacteria to escape death from

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Amoxicillin, while the same base fiber also possesses prebiotic value to the gut microbes.

In the above pursuit, a suitable source material-Broccoli fiber has been identified and have developed partially de-polymerized cellulose fiber from Broccoli (*Brassica oleracea*) and studied the usefulness of the fiber in protecting the gut microbiota from drug attack along with the role of the same fiber serves as base for solid oral dosage form (tablet) for Amoxicillin in lieu of microcrystalline cellulose.

Our earlier study has shown the partially depolymerized cellulose fiber of Broccoli could be useful for timed-release of Metformin.[5] Broccoli being one of the safest vegetables used in several culinary preparations, the partially de-polymerized cellulose fiber obtained from the above vegetable automatically comes with extraordinary safety net.[6,7]

In the present study we have done the complete characterization of the partially polymerized broccoli fibre and formulated the amoxicillin with broccoli partially polymerized fibre as base. And done complete analysis of the role of the base in protecting the probiotics. This dual benefit of protecting the gut microbiome in amoxicillin formulation is the need of hour and can reduce the load of taking additional probiotic dose along with amoxicillin. Details are presented in the article.

MATERIALS AND METHODS

Method of de-polymerization process followed for Broccoli

Fresh Broccoli (*Brassica oleracea* var.) was procured from local vegetable market and got it certified by the botanist and then used for the current study. Washed the broccoli flower thoroughly using distilled water thrice, decanted the water, minced the flower to small fragments, shade dried to remove about 95% of moisture and then used.

The dried fiber material of broccoli was treated with 1% HCl for 2 hr, then neutralized the same with 1N NaOH and bleached with hydrogen peroxide, washed thoroughly and then dried to remove about 98% water, as per standard procedure.[8,9] The fiber that obtained finally was used for the further studies. Three cycle of treatment was given to understand the optimal processing required for achieving partially depolymerized fiber that would meet all most all parameters that has been set in the beginning.

For the present work, partially de-polymerized fiber prepared from Broccoli was used along with the conventional microcrystalline cellulose as reference base material/control. The pharmaceutical grade of microcrystalline cellulose was procured and used for the present study.

Similarly, the broccoli fiber not subjected to partial depolymerisation process was also used as reference for all subsequent studies.

Physical and chemical characterization of the de-polymerized cellulose fiber

Extent of depolymerisation was ascertained from initial to final weight of the fiber obtained after depolymerisation process.

Powder microscopy

The partially de-polymerized broccoli fiber was examined under light microscope to study the microscopic organization of the fiber length, width, coiling nature etc. and the above microscopic characteristics of the partially depolymerized broccoli fiber was compared with microcrystalline cellulose.

Starch Test

10 mg of the partially depolymerized broccoli fiber powder was added 90 mL water in a beaker and heated for 15 min. Then it was filtered while the mixture was hot and then allowed to cool to room temperature. To the above, 0.1 mL of iodine was added and looked for the colour change by following standard protocol.[10]

Ash content analysis

Two to three g of sample by weight was taken in a crucible and then the crucible containing the sample was ignited until grey ash inside of a Bunsen burner is attained. The ash thus obtained was weighed and % ash present in the samples was calculated.[11]

Particle Density Test

The fiber sample was weighed to 15 g in a measuring cup and then recorded the initial volume (bulk volume/Vb). Polygonal grounded glasses were placed on the bulk-tapped density tool, the tool was turned on and set to 300 beats/min and then recorded the tapped volume of the above. Bulk Density (DB) and Tapped Density (DT) were calculated with the formula;

Db=Powder weight (g)

Vb (mL)

Dt=Powder weight (g)

Vt (mL)

Angle of repose

The sample was weighed to 15 g and then poured into the flow meter funnel. The end of the funnel was opened slowly so that the powder flows down slowly. The height and diameter of the sample formed was measured.[12]

Carr's index and Hausner's Ratio (HR)

The above ratio of the sample was calculated from the bulk and tapped densities of the powder to assess the flow property of the cellulose fiber by using the equation.[13]

$$CI=\text{tapped density-bulk density}X100$$

Tapped density

HR=tapped density

Bulk density

Formulation of tablet dosage form using partially depolymerized cellulose fiber of broccoli and further analysis

Tablet dosage form using 500 mg Amoxicillin and 10% of the partially depolymerized cellulose fiber was made by direct compression in the tablet compression machine ECO IV with B tooling from Parle Elizabeth Tools Private Limited. The amoxicillin tablets were also made with microcrystalline cellulose and un-polymerized broccoli fiber and were used for comparison. The tablet formulations thus made were examined for the surface smoothness and surface evenness, devoid of cracks, capping etc., using a hand lens.

Friability testing

15 numbers of tablets from each formulation were placed in the friability drum and the drum was rotated for 100 cycles with the speed of 25 rpm. After the rotation, the tablet and the dust settled in the drum were collected separately and were accurately weighed and then the friability value was calculated using the formula.[14]

$$\text{Friability (\%)} = W1\text{-}W2/\ W1X100$$

Where, W1=weight of tablets (initial/before tumbling) and W2=weight of tablets (after tumbling or friability).

Hardness test

The above test is performed to understand how compactly the materials has been packed by the cellule fiber when compressed the same into tablet dosage form and the time and rate of deformation it suffered against a fixed unit force using a Stokes hardness tester.[15]

Disintegration test

Disintegration time of the tablet formulations was carried out using the standard procedure.[16] In brief, six tablets from each formulation were randomly selected and placed in a disintegration tester apparatus filled with 900 mL distilled water and maintained at 37±2°C. The time required for complete disintegration of the tablets with no palpable mass remaining in the apparatus was recorded as the Disintegration Time (DT).

Pepsin digestion assay of the cellulose fiber

The fiber samples, 0.1 to 0.5%/mL taken in phosphate buffer-pH ranged between 2 to 6.5 and then incubated for 15 min and 1 hr. The pepsin enzyme was then added to the above treatment systems, incubated further and then read the values using spectrophotometer.[17] The extent of digestion of the cellulose was calculated by comparing the values with control.

Details of pathogens studied

Five isolates each of the three species of the pathogenic bacteria such as *Escherichia coli, Salmonella typhi* and *Salmonella paratyphi* stored in the laboratory were used for the present study.

Details of probiotics studied

The following gut microbes such *Lactobacillus acidophilus*-ATCC 4356, *L. casei*-ATCC 393, *Bifidobactrium bifidum*-ATCC 29521, *B. longum*-ATCC 55813, *Enterococcus faecium*- ATCC 14506, *Streptococcus thermophiles*-ATCC 19258, *Saccharomyces boulardii* -ATCC 796 were used for the present study.

Antibacterial susceptibility testing of Amoxicillin

Anti-bacterial susceptibility testing was performed using the standard procedure. Agar dilution method was followed for the above purpose.[18] In brief, varying concentrations of the antibiotic was incorporated into the media (Nutrient agar and Muller and Hinton agar), such as 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL and then the media was allowed to solidify. Standardized bacterial suspension (inoculum) prepared in physiological saline (100 µL) was plated over the media and spread evenly using L-shaped glass road and then the plate was incubated at 26°C for 24 hr. After incubation, the plate was examined for the presence and confluence of bacterial growth and compared the same with concentration of the antibiotics used. Total absence of growth of the organism was taken as inhibition and presence of even a single colony of bacteria and or recovery of the organism with fresh broth when overlaid on the plate was considered as no inhibition. The above test was performed against both the pathogens and probiotics listed elsewhere in the materials and method section.

Effect of cellulose fiber base on probiotics

The present experiment was performed to understand the possible supportive/inhibitory effect of the cellulose fiber on various species of probiotics. The fiber from broccoli obtained after partial depolymerisation process was UV sterilized along with the control samples (MCC and non-depolymerized broccoli fiber) and then the sterilized fiber was divided into 7 groups with each group containing 10 g of the fiber. Then each group was treated separately with different species of probiotic suspension (100,000 CFU/mg of fiber) and then the fiber was shade dried to remove the moisture in laminar chamber.

The fiber coated with probiotics was then subjected to three different conditions such as high temperature (70°C for 15 min), vacuum treatment for 15 min and low temperature (4°C for 15 min).

Then the fiber was stored further for 24 hr at ambient temperature. After 24 hr, the fiber was plated over fresh De Man,

Rogosa and Sharpe agar (MRS) media plate to study the survival of the probiotics in the depolymerized fiber samples versus the treatment conditions.

Prebiotic effect of the cellulose fiber on probiotics, in simulated gut system

In order to understand whether the cellulose fiber base when subjected to pass through complete digestion process in simulated gut system in the laboratory still could exhibit prebiotic value, this experiment was conducted using standard procedure where the fiber was treated initially with salivary amylase enzyme to stimulate oral phase of digestion, followed by gastric phase using hydrochloric acid pH 2.5 and then with pepsin.[19] Then the fiber was treated with sodium hydroxide-pH 7 to neutralize the same and then treated the system with bile salt, pancreatin and mucin to induce intestinal phase of digestion.

The fiber thus obtained finally was incorporated into 1/10 diluted MRS broth at 100 mg/mL and then the different species of probiotics were grown in the media. Abundance of probiotics grown over the fiber was measured by comparing the result with undiluted MRS broth grown organism under the same condition.

The broth, 0.1 mL was plated over fresh MRS agar plate (with and without gut system treated fiber) and checked for the growth rate of the probiotics under both conditions.

Exposure of fiber bound probiotics to Amoxicillin to study probiotic protection offered by the fiber

The fiber pre-bounded probiotics were released into antibiotic suspension prepared in physiological saline (30 mg/mL) and allowed to stay for different time period such as 5 and 10 min and then the fiber was plated over fresh media plate to study the survival rate of probiotic microbe. The ability of organism to escape death by hiding in the fiber was studied.

Simultaneous release of fiber, probiotics and Amoxicillin to study the parachute role of fiber

The present experiment was conducted to understand whether the probiotics in the gut system would find the fiber as 'safe heaven' and use the same as parachute when antibiotic and fiber, both are introduced into the system simultaneously, a situation almost similar during antibiotic use.

For this purpose, 50 mL of physiological saline, pH 7.0 was used. The fiber was incorporated into the saline and then the antibiotic suspension and then the bacterial suspension (probiotics) were added. Stirred the mixture for 5 and 10 min and then the fiber was removed by filtration and then the fiber was washed with distilled water and then plated onto MRS media plate to check the growth of different species of probiotics attached to the fiber.

Rate of binding of probiotics on fiber

To understand the binding abundance of different species of probiotics on the fiber, the fiber samples from the above experiment (10 min treated fiber) was taken and the fiber was dispensed into physiological saline using the following dilutions such 1:10, 1:100, 1:1000 and 1:10000 and then the fiber was plated on fresh MRS plate to study the binding stability/abundance of probiotics.

Zero interaction of cellulose fiber with Amoxicillin

XRD Analysis

XRD analysis of the fiber before and after amoxicillin dissolution was conducted to understand the possible interaction by following the standard procedure.[20]

X-ray diffraction analysis otherwise called as XRD analysis is used in materials science to measure the crystallographic structure of the sample. XRD employ the principle of irradiating the sample with incident X-rays and then measuring the intensities and scattering angles of the X-rays that leave the sample where the diffraction patter is captured.

By doing the XRD on the same sample under different conditions, all relevant information on how the treatment has modified the fiber can be gathered. The analysis uses the Bragg's law:

$$2d\sin\theta=n\lambda$$

Where d is the spacing between diffracting planes, θ {\display style \theta} is the incident angle, n is an integer and λ is the beam wavelength. The specific directions appear as spots on the diffraction pattern called reflections. Consequently, X-ray diffraction patterns result from electromagnetic waves impinging on a regular array and the method is non-destructive one.

The following details such as lattice parameters, strain, grain size, epitaxy, phase composition etc., can be captured by XRD analysis.

FTIR analysis

FTIR analysis of the fiber before and after amoxicillin dissolution was conducted to understand the possible interaction between them by following the standard procedure.[21]

Fourier Transform Infrared Spectroscopy or FTIR analysis or FTIR spectroscopy, is one of the common analytical techniques used to identify organic, polymeric and, in some cases, inorganic materials. This technique is useful for understanding the chemical composition of smaller particles, typically 10-50 microns, as well as larger areas on the surface when the sample is treated by different conditions.

Cell surface hydrophobicity improvement in probiotics post fiber adhesion

The surface hydrophobicity assay was performed to understand the cell wall hydrophobicity of the fiber bound probiotics vis-à-vis fiber non treated cells.[22] Hydrophobicity is the critical factor that enables the probiotics to binds to gut system and escape water effect.

For the above study, PBS buffer was used as control and xylene (0.4 mL) mixed with bacteria suspension (4 mL) as test. The test samples (bacterial cells) of each probiotic culture separated from the bounded fiber as well as fiber untreated stock culture were incubated for 15 min with intermittent vortex to ensure even mixing of the solvent-xylene with every bacterial cell and then allowed to stand for 10 min to obtain the aqueous phase.

The absorption value of the sample and the control were measured at 600 nm wavelength. The surface hydrophobicity was calculated by the equation.

$$Surface\ hydrocity(\%) = \frac{OD600(control) - OD600(test)}{OD600(control)} x100$$

The data thus obtained was analysed statistically for the significance using Mann-Whitney U test as the data showed skewed pattern. The software -Social Science Statistics was used for the purpose.

Navigation and binding of probiotics from partially depolymerized broccoli fiber base to mucus membrane

Mucus adsorbed polystyrene plate assay was conducted to understand how soon the probiotics bounded on the fiber base could release and navigate the mucus membrane and adhere to the same.[23]

This experiment was essential to establish the parachute effect of the fiber base not just offer protection to the probiotics from antibiotics but also free the probiotic organism (does not imprison) once the best ecosystem is available to the probiotics to fill the niche.

Isolation of crude mucus from fish

The fishes (Guppy) were starved for 48 hr and then the intestine was removed, pooled in a sterile Petri dish. The mucus was gently scraped from the inner intestinal surface. The mucus thus obtained was homogenized in PBS. The mucus preparation in PBS was then centrifuged twice at 27000xg for 15 min at 4°C to remove the particulate and cellular materials. The mucus suspension was sterilized by UV light exposure and stored at -20°C until the use.

In vitro adhesion assay

The crystal violet method was used to determine the adhesion ability of the probiotics studied.[24]

The polystyrene plate wells were coated with 150 µL of fish intestinal mucus. After adherence, 100 µL of the probiotic organisms bounded fiber suspension was added. The quantity of inoculum was kept lower than that of mucus in order to avoid subsequent staining by crystal violet does not bind to the plate and interfere in reading of the result. The probiotic cells were allowed to get released from the fiber and adhere over the mucus at 37°C for 1 hr and the non-adherent cells were removed by washing the wells three times with 250 µL of PBS. Control was also run to understand the difference.

The adhered cells were fixed at 60°C for 20 min and stained with crystal violet (100 µL/well, 0.1% solution) for 45 min.

Wells were subsequently washed 5 times with PBS to remove the excess stain. The stain bound to the cells was released by adding 100 µL of citrate buffer (pH 4.3). After 45 min incubation at room temperature, the absorbance at 640 nm was determined using the micro titre plate reader.

Stained mucus without the cells was used as negative control.

Statistical analysis

Student's *t* test is used to compare between two groups and ANOVA is used to compare three or more groups. A significant *p* value of ANOVA test indicates for at least one pair, between which the mean difference was statistically significant. Statistical significance was tested by the Student's *t*-test, *p* values and ANOVA.[25]

In the present study the values are calculated using the standardized free software (Statistics kingdom), a free online software for the calculation of statistical data.

RESULTS

Percentage of depolymerisation based on final yield

The polymerization process employed by us (3 cycles) gave the final yield of 85% of partially depolymerized broccoli fiber suggesting the possible removal of 15% of the complex carbohydrates and the above depolymerisation process was fixed due to various fiber

Table 1: Yield details after depolymerisation.

Batch details	Initial weight of wet broccoli (g)	After shred drying (moisture level 5%)	After partial de-polymerization Gm/%
1	200	120	102 (85)
2	200	141	121 (86)

characteristics of the fiber that was obtained by the process met that of microcrystalline cellulose, (Table 1).

Powder microscopy

The partially depolymerized broccoli fiber exhibited irregularly shaped, flat fiber which accounted for nearly 30%, long thin fiber which accounts for 50% and short powdery fiber accounts for 20%.

The powder characteristics of partially depolymerized broccoli fiber were although comparable with MCC but appeared distinctly different under microscopic examination with relatively less hyaline fibers.

Starch test

Three cycle of acid treatment was required to achieve near total removal of starch from the partially depolymerized broccoli fiber to match the level of MCC, (Table 2).

Ash content analysis

The total ash content present in partially depolymerized broccoli fiber was 0.02%±0.5 as against the total ash content value of 0.17%±0.7 in MCC.

Table 2: Treatment cycle vis-à-vis starch removal.

Cycle details	Presence of starch/ blue colour formation
	Partially depolymerized broccoli fiber
Cycle 1	Light blue
Cycle 2	Fading blue
Cycle 3	No blue colour
MCC	No blue colour

Table 3: Bulk, tapped, Hausner ratio comparison.

Parameters	Partially depolymerized broccoli fiber	MCC
Bulk density	0.27	0.29
Tapped density	0.37	0.34
Hausner's ratio	1.37	1.17

Particle density test

A marginal superiority in particle density characteristics of partially depolymerized broccoli fiber was observed over MCC from the point of view of manufacturing of the tablet dosage form, (Table 3).

Angle of repose

The degree of angle of repose of the partially depolymerized broccoli fiber was 51.1° whereas the angle of repose of MCC was 49.7°.

Physical examination of tablet formulation

The 500 mg Amoxicillin tablet formulated with 10% partially depolymerized fiber of broccoli and MCC met all physical parameters of tablet oral dosage form while the tablet formulated by direct compression using non-depolymerized broccoli fiber did not yield acceptable result, (Table 4).

Friability

The damage resisting property of the tablet formulation was measured by way of measuring the friability which showed that the tablet formulation made with 10% of either partially depolymerized broccoli fiber or MCC were concordant with reference to friability resistance whereas the tablet formulated with non-polymerized fiber of broccoli failed completely from meeting the requirement, (Table 5).

Hardness by crushing strength

Crushing resistance of the tablet formulations by exhibiting greater hardness was observed in tablet formulation mad with 10% of either partially depolymerized broccoli fiber or MCC. The non-polymerized broccoli fiber-based formulation did not offer any appreciable hardness to the tablet formulation, (Table 6).

Disintegration test

The disintegration time of the tablet formulations made with either 10% partially depolymerized broccoli fiber or MCC was almost similar whereas the formulation made with non-polymerized broccoli fiber completely failed the test, (Table 7).

Table 4: Tablet physical test parameters.

Sl. No.	Parameters	500 mg Amoxicillin+10% partially depolymerized broccoli fiber	500 mg Amoxicillin+10% non- depolymerized broccoli fiber	500 mg Amoxicillin+10% MCC
1	Surface finish	Smooth	Rough	Smooth
2	Capping	Nil	Present	Nil
3	Hardness	Good	Poor	Good

Table 5: Friability test.

Friability in %		
500 mg Amoxicillin+10% partially depolymerized broccoli fiber	500 mg Amoxicillin+10 % non-depolymerized broccoli fiber	500 mg Amoxicillin+10% MCC
0.2	2.4	0.18

Table 6: Hardness test.

Crushing strength (kgf)		
500 mg Amoxicillin+10% partially depolymerized broccoli fiber	500 mg Amoxicillin+10% non- depolymerized broccoli fiber	500 mg Amoxicillin+10% MCC
22.4	14.3	21.8

Table 7: Disintegration test.

Disintegration time in min		
500 mg Amoxicillin+10% partially depolymerized broccoli fiber	500 mg Amoxicillin+10% non- depolymerized broccoli fiber	500 mg Amoxicillin+10% MCC
6.5	3.1	6.8

Table 8: Pepsin digestion test.

Tests	% of digestion/ time in min	
	Time in min	% of digestion
MCC 0.1%+Pepsin	10	33
MCC 0.3%+Pepsin	10	29
MCC 0.5%+Pepsin	10	30
Partially depolymerized broccoli fiber 0.1%+Pepsin	10	32
Partially depolymerized broccoli fiber 0.3%+Pepsin	10	33
Partially depolymerized broccoli fiber 5%+Pepsin	10	33

Pepsin digestion of broccoli fiber versus MCC

Almost comparable lever of susceptibility to pepsin was exhibited by both MCC and partially depolymerized fiber, (Table 8).

Antimicrobial susceptibility testing of Amoxicillin

All pathogenic microbes exhibited susceptibility to 20 µg/mL of Amoxicillin, whereas the pro-biotic organisms studied showed relatively higher resistance to amoxicillin, up to 40 µg/mL, (Table 9).

Effect of cellulose fiber bases on probiotics

The probiotic bacteria bound to partially depolymerized cellulose fiber of broccoli when subjected to different of degree of temperature and vacuum treatment, showed protective effect to the probiotic organisms as all the species probiotic bacteria could be isolated from the fiber after subjecting such treatment. Whereas MCC did not exhibit such protective effect, (Tables 10 and 11).

Exposure of fiber bound probiotics to Amoxicillin to study the parachute effect of the fiber base to avoid death.

The fiber bound probiotic bacterial species when exposed to Amoxicillin for 5 and 10 min revealed that the bacteria bound to the partially depolymerized broccoli fiber could escape death whereas the bacteria bound over MCC and non-polymerized broccoli fiber died completely during exposure to amoxicillin, (Table 12).

Simultaneous release of bacteria to partially depolymerized broccoli fiber and amoxicillin

In phosphate buffer, bacteria, fiber base and amoxicillin, all of them were released simultaneously and gently stirred and allowed to stand for 5 and 10 min. The fiber sample was then taken, gently washed with fresh phosphate butter and then inoculated onto MRS agar to check the viable probiotic bacteria present in the fiber base. The findings revealed that all probiotic species studied could rescue them from death in an ecosystem where the partially depolymerized broccoli fiber was available whereas such survivability of probiotics could not be established with non-polymerized broccoli fiber or MCC, (Table 13).

Rate of probiotic binding over different fiber base

The partially depolymerized broccoli fiber from the above experiment referred in Table 13, was collected and the fiber was dispensed into phosphate buffer under various dilutions, the fiber was gently rinsed and then the fiber was plated onto fresh MRS agar plate. The numbers of CFU's grown were counted to establish the approximate binding rate/stability of probiotics over partially depolymerized broccoli fiber. The findings show the binding of probiotics over the fiber was quite stable and substantial where the organism could be isolated even from the fiber washed with 1:10000 of phosphate buffer, (Table 14).

Interaction between partially depolymerized broccoli fiber and amoxicillin by XRD analysis

XRD analysis of the partially depolymerized broccoli fiber along with amoxicillin coated was compared with the same fiber after complete dissolution of amoxicillin. Findings show, no significant or conspicuous change in the fiber by XRD analysis after drug

Table 9: Antimicrobial testing.

Name	Inhibition value in µg/mL (+)=present (-)=Absent							
	10	20	30	40	50	60	70	80
E. coli	+	-	-	-	-	-	-	-
S. typhi	+	-	-	-	-	-	-	-
S. paratyphi	-	-	-	-	-	-	-	-
Lactobacillus acidophilus	-	-	-	-	-	-	-	-
L. casei	+	+	+	+	-	-	-	-
Bifidobactrium bifidum	+	-	-	-	-	-	-	-
B. longum	+	+	-	-	-	-	-	-
Enterococcus faecium	+	-	-	-	-	-	-	-
Streptococcus thermophiles	+	+	+	-	-	-	-	-
Saccharomyces boulardii	+	+	+	-	-	-	-	-

Table 10: Protective effect of Broccoli fiber to Probiotics.

Species	Partially depolymerized broccoli fiber bound bacterial viability/treatment		
	70°C for 15 min	Vacuum treatment for 15 min	4°C for 15 min
Lactobacillus acidophilus	+	+	+
L. casei	+	+	+
Bifidobactrium bifidum	+	+	+
B. longum	+	+	+
Enterococcus faecium	+	+	+
Streptococcus thermophiles	+	+	+
Saccharomyces boulardii	+	+	+

+=Bacterial presence by isolation.

Table 11: Protective effect of MCC to Probiotics.

Species	Partially depolymerized MCC bound with bacterial viability/treatment		
	70°C for 15 min	Vacuum treatment for 15 min	4°C for 25 min
Lactobacillus acidophilus	-	-	-
L. casei	-	-	-
Bifidobactrium bifidum	-	-	-
B. longum	-	-	-
Enterococcus faecium	-	-	-
Streptococcus thermophiles	-	-	-
Saccharomyces boulardii	-	-	-

dissolution when the data was compared with drug uncoated fiber, (Figure 1a-1g).

FTIR analysis

FTIR analysis of the partially depolymerized fiber along with amoxicillin coated one was compared with the same fiber after complete dissolution of amoxicillin. Findings show, no significant or conspicuous change in the fiber by FTIR analysis after drug dissolution when the data was compared with drug uncoated fiber. The functional groups of the fiber have not been altered by the drug and vice versa, (Figure 2a-2g).

Cell surface hydrophobicity modification in probiotics, post fiber adhesion

Hydrophobicity of the probiotic bacteria after exposure to partially depolymerized broccoli fiber (i.e., the fiber bound bacteria were regrown in MRS medium) showed significant increase in hydrophobicity suggesting the positive aspect, especially the gut

Table 12: Probiotic adhesion time.

Species	Exposure time in min /viability (+), No viability (-)					
	Polymerized broccoli fiber		Non polymerized broccoli fiber		MCC	
	5	10	5	10	5	10
Lactobacillus acidophilus	+	+	-	-	-	-
L. casei	+	+	-	-	-	-
Bifidobactrium bifidum	+	+	-	-	-	-
B. longum	+	+	-	-	-	-
Enterococcus faecium	+	+	-	-	-	-
Streptococcus thermophiles	+	+	-	-	-	-
Saccharomyces boulardii	+	+	-	-	-	-
Lactobacillus acidophilus	+	+	-	-	-	-
L. casei	+	+	-	-	-	-

Table 13: Probiotic adhesion time vs Simultaneous release of amoxicillin.

Species	Exposure time in min /viability (+), No viability (-)					
	Polymerized broccoli fiber		Non polymerized broccoli fiber		MCC	
	5	10	5	10	5	10
Lactobacillus acidophilus	+	+	-	-	-	-
L. casei	+	+	-	-	-	-
Bifidobactrium bifidum	+	+	-	-	-	-
B. longum	+	+	-	-	-	-
Enterococcus faecium	+	+	-	-	-	-
Streptococcus thermophiles	+	+	-	-	-	-
Saccharomyces boulardii	+	+	-	-	-	-
Lactobacillus acidophilus	+	+	-	-	-	-
L. casei	+	+	-	-	-	-

Table 14: Stability and selective preference of probiotics to fiber.

Species	Number of CFU's on MRS plate			
	1:10	1:100	1:1000	1:10000
Lactobacillus acidophilus	30	12	8	5
L. casei	20	11	9	4
Bifidobactrium bifidum	23	9	8	5
B. longum	15	10	9	4
Enterococcus faecium	22	11	8	5
Streptococcus thermophiles	32	12	7	5
Saccharomyces boulardii	25	12	9	4
Lactobacillus acidophilus	17	14	10	6
L. casei	28	11	9	3

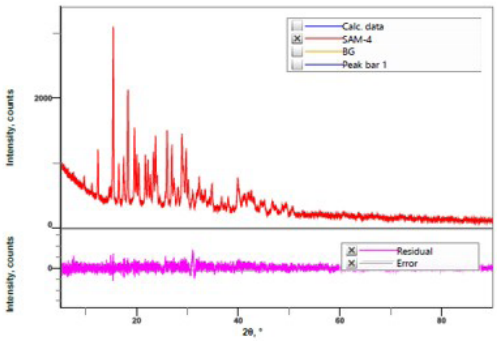

Figure I a: Amoxicillin

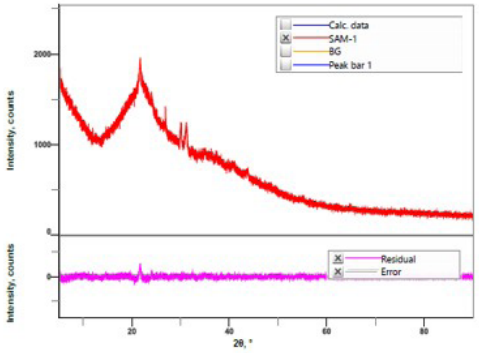

Figure I b: Partially depolymerized broccoli fiber

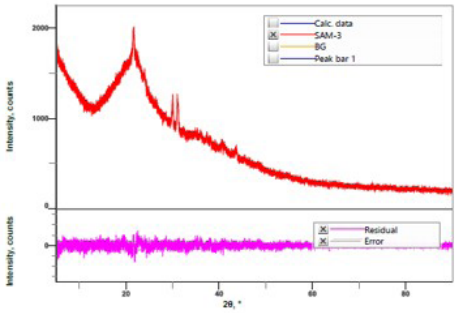

Figure I c: Partially depolymerized broccoli fiber +Amoxicillin

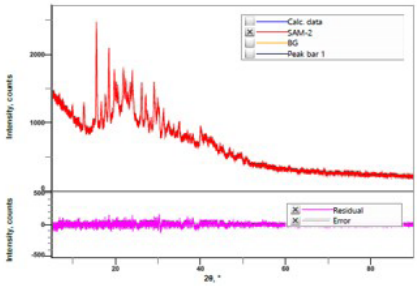

Figure I d: Partially depolymerized broccoli fiber +Amoxicillin after dissolution

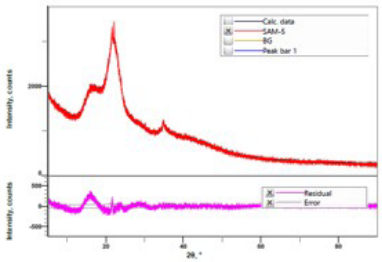

Figure I e: Polymerized broccoli

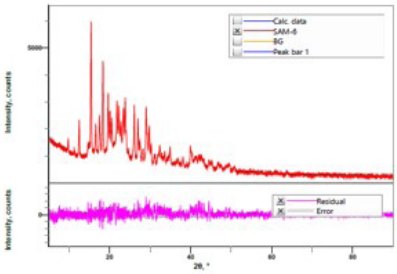

Figure I f: Polymerized broccoli +Amoxicillin

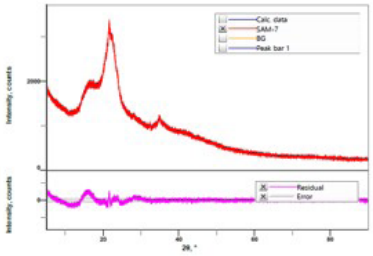

Figure I g: Polymerized broccoli +Amoxicillin after dissolution

Figure 1a-1g: The crystalline structure of the fiber has not been altered by the drug and vice versa.

mucosal membrane adhesion and water-resistant property of the bacteria post fiber treatment. Result is summarised below, (Tables 15-18).

The probiotic bacteria that got exposed to partially depolymerized broccoli fiber showed significantly greater degree of hydrophobicity over the untreated probiotic bacterial cells.

ANOVA *t* test

It means the fiber bound probiotics could navigate and adhere to mucus as efficiently as the untreated cells; the parachute effect of the fiber does not seem to imprison the organism.

DISCUSSION

The present investigation has unambiguously proved the fact that partially depolymerized broccoli fiber has definite 'benefit dualism' in both delivering the antibiotics-Amoxicillin as an ideal base material for making tablet drug dosage form and also acts like 'parachute' for the probiotics to escape death from the same antibiotics. The term 'parachute' in drug delivery mechanism has been already used and practised.[26,27]

Prebiotic benefit of broccoli fiber, many other cellulose fiber and several nutritional supplements are well known and therefore our

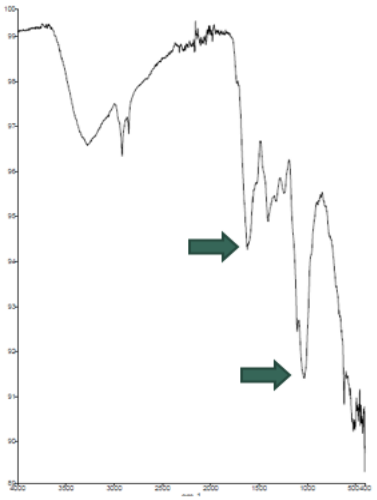

Figure 2a: Depolymerized broccoli fiber

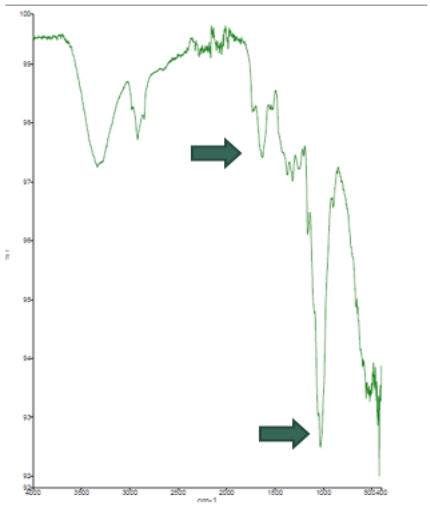

Figure 2b: Polymerized broccoli fiber

Figure 2a & 2b shows the mild variation in peaks gives clear differentiation in the process

Figure 2a and 2b: Shows the mild variation in peaks gives clear differentiation in the process.

Table 15: Hydrophobicity assay.

Probiotics	Fiber untreated isolates	Isolates from partially depolymerized broccoli fiber treatment
	% hydrophobicity	
Lactobacillus acidophilus	11	32
L. casei	15	27
Bifidobactrium bifidum	13	18
B. longum	14	22
Enterococcus faecium	18	32
Streptococcus thermophiles	12	36
Saccharomyces boulardii	11	29

present finding is not an attempt to re-confirm the knowledge that already exist but instead to report the base that could also serve as base material for amoxicillin delivery besides offering refuge to gut microbiome.[28] The same prebiotic material also would serve as base or vehicle for drug delivery while exhibiting prebiotic value by belonging to the same system has so far not been explored or established. The reason being, the base fiber material must completely release the antibiotics rapidly as well the same fiber material also must exhibit high prebiotic value. Besides that, the prebiotic fiber base also must attract or show special affinity for the probiotic species so that the bacteria would come towards the fiber to seek refuge and later would re-navigate its original habitat.

Amoxicillin, although an effective drug against wide spectrum of bacterial infections, its use is reasonably restricted as most patients would prefer to avoid the drug due to mild to severe gastric complications, especially due to increased acidity and acid reflux. The reason being, amoxicillin also would destroy effectively the gut commensal microbes besides the pathogens. When the gut microbial biome is severely altered, the intestinal mucosal space vacated due to the removal of probiotic bacteria is easily occupied by the pathogen or other undesired microbes and would induce further complications. It is not just the space availability that offer advantage to the pathogens to colonize in the colon space, the probiotic bacteria also would produce lactic acid, propionic acid, acetic acid etc., which would in turn limit the success of pathogens in the colon region.[29]

Therefore, always when antibiotic therapy is followed especially when the course of treatment is, a bit longer, often probiotic supplementation is recommended besides the use of antacids.[30]

Our present study, at least, at concept level, has demonstrated, partially depolymerized broccoli fiber while dispensing the amoxicillin also would give refuge to the gut biome when used as base material in lieu of conventional microcrystalline cellulose.

The binding affinity of amoxicillin over partially depolymerized broccoli fiber was poor but the fiber has high compressibility and plasticity and therefore, through direct compression, it could be used as base material for formulating amoxicillin as tablet dosage form.

The yield of partially depolymerized fiber from broccoli was quite substantial (85%) when acid treatment was used for cracking the polymeric structure of the sugar molecules in the cellulose fiber. Relatively high yield of the fiber after depolymerisation process suggest the economic fitness the fiber with considerable health benefit and amoxicillin delivery.

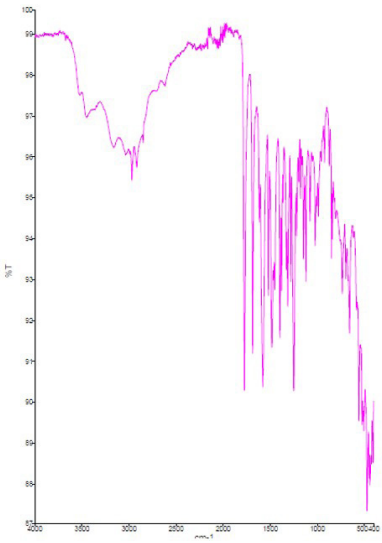

Figure 2 c: Amoxicillin

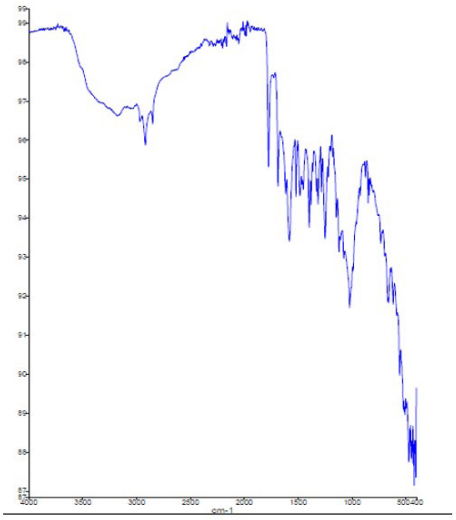

Figure 2 d: Depolymerized broccoli +Amoxicillin

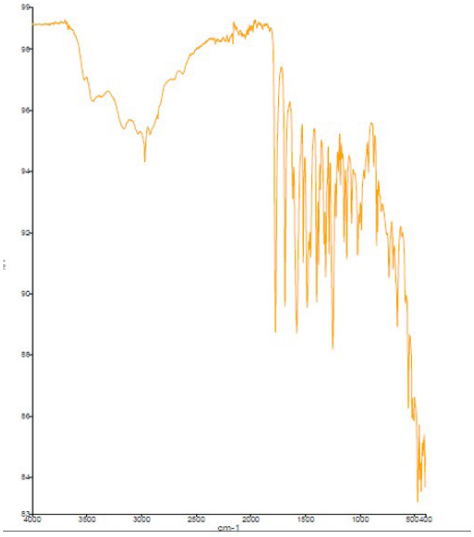

Figure 2 e: Polymerized broccoli +Amoxicillin

Figure 2c-2e: Shows polymerized broccoli got interacted with amoxicillin whereas the depolymerized one does not have any interaction.

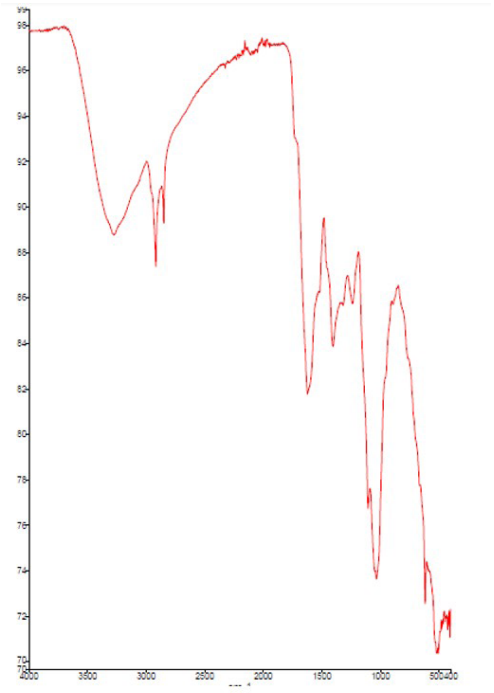

Figure 2 f: Depolymerized broccoli +Amoxicillin after dissolution

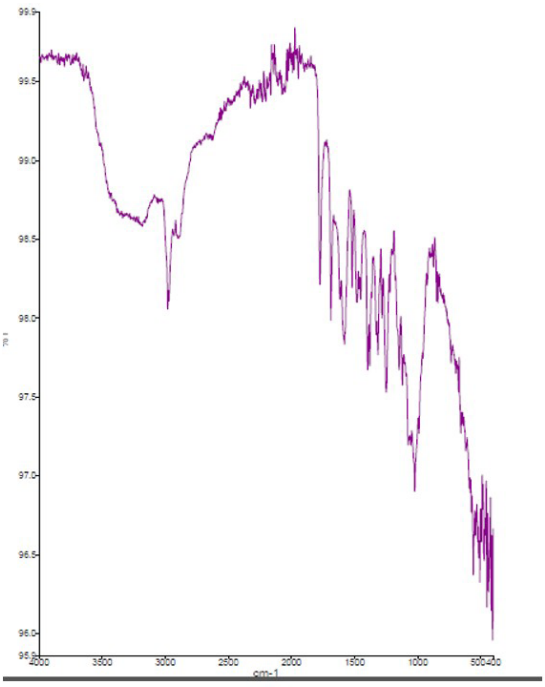

Figure 2 g: Polymerized broccoli +Amoxicillin after dissolution

Figure 2f and 2g: Even after dissolution process depolymerized broccoli fiber remains unaltered unlike polymerized one.

Table 16: Mann-Whitney U test.

Sample 1	Sample 2	S1 values	S1 ranks	S2 values	S2 ranks
11	32	11	1.5	18	7.5
15	27	11	1.5	22	9
13	18	12	3	27	10
14	22	13	4	29	11
18	32	14	5	32	12.5
12	36	15	6	32	12.5
11	29	18	7.5	36	14

Table 17: Level of significance=0.05.

Sample 1	Sample 2
Sum of ranks-28.5	Sum of ranks-76.5
Mean of ranks-4.07	Mean of ranks-10.93
Expected sum of ranks- 52.5	Expected sum of ranks- 52.5
Expected mean of ranks-7.5	Expected mean of ranks-7.5
U- value-48.5	U- value-0.5
Expected U-value-24.5	Expected U-value-24.5

The U value is 0.5. Critical value of U at $p<0.05$ is 8. Result is significant at $p<0.05$.The Z score is -3.00272. The p-value is 0.0027. Result is significant at $p<0.05$.

Table 18: Navigation and binding of probiotics from partially depolymerized broccoli fiber base.

Probiotics	depolymerized broccoli fiber bound isolates (100,000 CFU/mg fiber)	Isolates from grown in MRS broth 100,000 CFU/ mL
	% of bacterial binding on mucus	
Lactobacillus acidophilus	29	36
L. casei	33	41
Bifidobactrium bifidum	38	41
B. longum	41	39
Enterococcus faecium	28	30
Streptococcus thermophiles	39	41
Saccharomyces boulardii	35	39

Initially the partially depolymerized fiber was compared with MCC on all possible aspects widely considered in the pharmaceutical realm such as powder characteristics, bulk and tap density, flow property, starch content and resistance to pepsin enzyme digestion and compared with the values of MCC. The partially depolymerized broccoli fiber perfectly met the norms set for MCC to be used as the base material for formulating tablet dosage form.

Five hundred milligram of amoxicillin tablet formulated with 10% of the partially depolymerized broccoli fiber by direct compression passed all merit tests such as friability, hardness, disintegration and other physical parameters, unequivocally to that of the formulation made with MCC.

After confirming the usefulness of partially depolymerized broccoli fiber for formulating tablet dosage form through simple direct compression, the focus was turned towards the special beneficial effect of the above fiber to the probiotics. For establishing such benefit, the antimicrobial effect of amoxicillin

Treatment 1 (X)	$Diff\,(X - M)$	$Sq.\ Diff\,(X - M)^2$
29	-5.71	32.65
33	-1.71	2.94
38	3.29	10.80
41	6.29	39.51
28	-6.71	45.08
39	4.29	18.37
35	0.29	0.08
	M: 34.71	SS: 149.43

Treatment 2 (X)	$Diff\,(X - M)$	$Sq.\ Diff\,(X - M)^2$
36	-2.14	4.59
41	2.86	8.16
41	2.86	8.16
39	0.86	0.73
30	-8.14	66.31
41	2.86	8.16
39	0.86	0.73
	M: 38.14	SS: 96.86

Difference Scores Calculations

Treatment 1

N_1: 7
$df_1 = N - 1 = 7 - 1 = 6$
M_1: 34.71
SS_1: 149.43
$s^2_1 = SS_1/(N - 1) = 149.43/(7-1) = 24.9$

Treatment 2

N_2: 7
$df_2 = N - 1 = 7 - 1 = 6$
M_2: 38.14
SS_2: 96.86
$s^2_2 = SS_2/(N - 1) = 96.86/(7-1) = 16.14$

The *t*-value is -1.41585. The *p*-value is .091123. The result is not significant at *p*< .05.

was studied against three species of pathogens as well as 7 species of probiotic bacteria viz., *E. coli, S. typhi, S. paratyphi, Lactobacillus acidophilus, L. casei, Bifidobactrium bifidum, B. longum, Enterococcus faecium, Streptococcus thermophiles* and the yeast *Saccharomyces boulardii*.

The data was required to explore how the partially depolymerized broccoli fiber base protect the probiotic bacteria when exposed to the same concentration amoxicillin that exhibited strong antimicrobial activity.

The low and high temperature treatment and vacuum treatment of the partially depolymerized broccoli fiber bound probiotics in desiccated form did not suffer casualty whereas the probiotic bacteria bound to MCC and un-polymerized broccoli fiber could

not survive the treatment condition. The above findings clearly led us to believe the possibility of the fiber having 'some' special advantage feature for the probiotics. The fiber bound probiotics was then released into the buffer containing amoxicillin to understand the survival rate of probiotics in the fiber. Interestingly the probiotics bound to partially depolymerised fiber survived death as the organism could be isolated from the fiber base after antibiotic exposure whereas such possibility was not found with MCC and non-depolymerized broccoli fiber.

The fiber bound probiotic bacteria gaining protection during antibiotic exposure may be due to the poor permeation of antibiotics into the fiber and therefore such same benefit cannot automatically be assumed in the gut system where the antibiotic formulation is made with such fiber, when released. In order to answer the above question, simultaneous release of the bacteria, fiber and antibiotics into a buffer and stirred for a while. Surprisingly the probiotic binding over the fiber was observed as well as their survival. It means, during stirring, the bacteria that comes in contact with the fiber may be getting attracted to it or in other word, the fiber is offering the parachute benefit to the probiotic bacteria during antibiotic encounter and thereby they could escape death.

While analysing the above result, a new question was raised. In the laboratory condition, the fiber could be studied without causing much modification or alteration to the fiber but in the gut system scenario is different, therefore translating and extrapolating the laboratory findings require further studies.

To answer the above question, the fiber was subjected to simulated oral and gut phase of digestion by treating the fiber with salivary amylase, then with HCl treatment, neutralization of the above, pepsin treatment, bile salt treatment and finally pancretin and mucin treatment. The fiber that had passed through all such treatment was tested for its prebiotic value and found that the simulated gut system treatment did not alter the special prebiotic value of the fiber. It was found that the probiotic binding either during antibiotic exposure or otherwise was quite rigid and intentional and such binding is not a chance occurrence or accidental. Therefore, to know further whether such stable/firm biding would make the probiotic species 'prisoner' to the fiber and or the probiotic bacteria could easily get back to its original habitat only after the fiber is totally disintegrated or the fiber would simple act just like parachute and the probiotic bacteria would simply walk back to the habitat easily when gut condition turns to conducive from hostile.

Before finding an answer to the above question, attempt was made to understand the possible mechanism of action of the partially depolymerized broccoli fiber in inducing special affinity in probiotic species. With limited facility available with us, another experiment was conducted with the hypothesis that the fiber base may be increasing the hydrophobicity of the probiotic cells and such benefit may be offering transient escape scope for the probiotic bacteria from antibiotics as well to bind quickly to the fiber. Our study findings clearly show that the fiber treated probiotic bacterial species had greater cellular hydrophobicity when compared to the untreated cells and the finding was statistically significant by Mann-Whitney U test.

The selective obsession of probiotic bacteria to the partially depolymerized fiber and associated increased hydrophobicity whether could impede their rapid re-navigation of the colon mucosa and if so, the very purpose of the prebiotic value and parachute effect of partially depolymerized fiber is lost while the same when serve as base binder to the tablet formulation.

For the present study, the mucus was isolated from inner wall of fish intestine and coated the mucus over polystyrene micro well plate. The fiber bound and untreated probiotics were incubated with mucus coated well. Then the plate was washed and stained and read the result. Findings show that probiotic bacteria studied show blemish-free rebinding and colonization ability over mucus coated polystyrene plate from the fiber and the result obtained between treated versus control showed no statistical significance.

It was established that no interaction occurred between amoxicillin and the partially depolymerized fiber base that may lead to some modification or alteration to either, by XRD and FTIR. The findings suggest that partially depolymerized broccoli fiber base is quite safe for formulating at least amoxicillin from physio-chemical per se.

Our present investigation appears to be the first detailed study to establish how the fiber base alteration in antibiotic tablet formulation could offer some special additional benefit which is mandated heavily by the same drug, in the present case, amoxicillin.

CONCLUSION

The present study has revealed that the partially depolymerized broccoli fiber is useful for both delivering amoxicillin and also for gut microbiota protection. Amoxicillin instantaneously released from the fiber when used as tablet dosage form and the space thus vacated in the fiber base is immediately occupied by probiotics to achieve refuge from amoxicillin. The above findings were reconfirmed by series of experiments. The fiber also retained its pre-biotic value after passing through the entire gut digestion process. The fiber appears to improve the hydrophobicity of probiotics which help the probiotic to escape anti-biotic attack and binds to the fiber firmly. Statistical analysis has clearly confirmed the 'Parachute' benefit of the broccoli fiber to gut microbes while the same being used as fiber base for amoxicillin tablet dosage form. No interaction between the fiber and amoxicillin was also established by XRD and FTIR analysis.

The study findings clearly show that the partially depolymerized fiber of Broccoli provided definite sheltering benefit possibly

by acting like a parachute to the following probiotics such as *Lactobacillus acidophilus, L. casei, Bifidobactrium bifidum, B. longum, Enterococcus faecium, Streptococcus thermophiles* and *Saccharomyces boulardii* when the same base material was used for delivering amoxicillin.

Our study findings clearly indicate the scope for the use of partially de-polymerized fiber from Broccoli for the simultaneous delivery of Amoxicillin and protection of gut-microbiota.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MCC: Micro crystalline cellulose; **Mg:** Milligram; **FTIR:** Fourier-transform infrared spectroscopy; **XRD:** X-Ray diffraction; **HCl:** Hydro chloric acid; **NaOH:** Sodium hydroxide; **Vb:** Bulk volume; **DB:** Bulk Density; **DT:** Tapped Density; **g:** Grams; **mL:** Milli liters; **CI:** Carr's index; **HR:** Hausner's ratio; **DT:** Disintegration test; **µL:** Micro litre; **MRS:** De Man, Rogosa and Sharpe agar; **CFU:** Colony forming unit; **ANOVA:** Analysis of Variance; **PBS:** Phosphate buffer saline.

LIMITATIONS AND FUTURE DIRECTION

The scope of present study is *in vitro* and is complete with the data as worked in probiotics but can extend to *in vivo* and bioequivalence in the future.

SUMMARY

Oral dosage forms are widely used and the formulation of it needs an excipient especially fillers and binders. But these are non-digestible and in long term usage of medication may have side-effects or not have any value addition to formulation. Amoxicillin is widely used anti-biotic but comes with limitation of inhibiting pro-biotics along with pathogens. To overcome this problem, in the current study depolymerized cellulose fiber from *Brassica oleracea* (Broccoli) flower was isolated and formulated with Amoxicillin. The fiber isolated is subjected to IR, XRD to confirm the non-interaction of it with drug. The formulation developed with the isolated depolymerized fiber is subjected to various evaluation methods to identify its release and its prebiotic nature. The study results showed the developed fiber has characteristics of an excipient and the formulation showed the drug release. The formulation developed with brassica depolymerized fiber also showed protective action towards probiotic microbes. Hence proves depolymerized broccoli fiber is an value based excipient.

SOURCE OF SUPPORT

Vels Institute of Science, Technology and Advanced Studies (VISTAS).

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