DEVELOPMENT OF A FIBROUS ASSEMBLY FROM ORANGE PEEL EXTRACT: CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY

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The present study focuses on the development and characterization of a fibrous assembly from orange peel extract, well known for its antioxidant and antimicrobial properties. The investigation includes the extraction of the fibrous assembly from an orange peel extract and its characterization in terms of morphological analysis, elemental analysis, chemical group identification, as well as thermal behaviour. The fibrous assembly, which is predominantly amorphous and primarily composed of about 2% lignin, 10% cellulose, 15% hemicelluloses and different polyphenolic compounds, such as flavonoids, shows excellent moisture absorbency and antimicrobial property against *E. coli* and *S. aureus* strains. The fibrous assembly has potential for applications in the wound dressing and healthcare sector.

Keywords: orange peel extract, fibrous assembly, flavonoid, lignocellulosic, antibacterial property

INTRODUCTION

Fibres of natural origin are preferred over synthetic ones due to higher biodegradability, sustainability, low cost, better interface for various industrial applications, eco-friendly, renewability and their abundant availability.[1,2] In this study, the isolation and characterization of a fibrous assembly from orange peel extract was carried out. This freshly extracted fibrous assembly obtained from orange peel extracts was yellowish-white in colour, possessing a soft handle and a pleasant fruity fragrance.

Orange is a highly poly-embryonic, even surfaced and tight skinned fruit, which has been part of human diet for ages due to its high nutritional and medicinal values. Consumption of orange fruit generates a large amount of peels as waste, which could be a potential cause of environmental pollution, if not handled properly.

Orange peel mostly consists of cellulosic fibres and pectin. Further, it has been reported that orange peel extract comprises several bioactive compounds, such as flavonoids and limonoids, which are known to act as anti-cancer and anti-oxidant agents.[3] These are also known to enhance vitamin absorption and possess a broad spectrum of medicinal properties, including anti-inflammatory, anti-allergenic, anti-tumour and anti-microbial activities.[4] For example, hesperidin is a predominant flavourless flavonoid present in the peel and membranous parts of orange, which shows such bioactivity.[5] Rutin is another major flavonoid that can chelate heavy metals like iron and also enhances vitamin absorption.[6] Limonoids are organic compounds commonly found in the peel of citrus fruits, exhibit several beneficial health effects.[7] Therefore, the isolation of a fibrous assembly from orange peel extract, preserving such bioactive compounds, will have great potential for various healthcare applications.

Only a few recent studies have reported the extraction of cellulose, pectin and flavonoids from orange peel.[8,9] Previously, the cellulose and pectin from orange peel was isolated by the use of sodium hydroxide and microwave exposure, respectively.[8,9] Similarly, in another study, pectin was isolated from orange peel using ultra-high pressure.[10] Such natural fibres made up of polysaccharides like chitosan, alginate and dextran, might have utility in different clinical applications, as wound dressings.[11] However, to

the best of our knowledge, this is the first report on the isolation of a fibrous assembly from orange peel extract by the self-assembly mechanism by keeping its bioactive compounds, and on its characterization in terms of morphology, thermal and tensile properties, moisture absorbency and antibacterial activity. The fibrous assemblies thus produced have potential for the application in wound dressings, and they represent a "green" material, without involving any chemical or radiation exposure.

EXPERIMENTAL

Preparation of fibrous assembly from orange peel extract

Fresh orange peel extract, collected by cold pressing of the peels, was used to develop the fibrous assembly. The extract collected from the peels was spread on a plastic surface and a rubbery surface was pressed onto the liquid (Fig. 1). When the two surfaces were separated, the fibrous assembly was formed between the two surfaces, which was then collected.

Roughly, about 75 to 80 mg of fibrous assembly could be generated from the peels of a single fresh orange. Initially, the process was carried out manually and, subsequently, a modified four bar slider-crank mechanism was developed for the formation of the fibrous assembly (Fig. 2).

In this mechanism, the orange peel extract is spread on the surface of block A by cold pressing orange peels. Then, block A moves forward by the rotation of the crank and is pressed on the rubbery surface. When the two surfaces are separated, the fibrous assembly is formed between the two surfaces, which is then collected. The width of the fibrous assembly mainly depends on the area on which the peel extract has been spread on the plastic surface and the area on which the plastic surface is pressed on the rubbery surface (Fig. 1). The length of the fibrous assembly depends on the distance between the two surfaces when they are separated after pressing and, of course, on the velocity of block A, which is regulated by the rotation of the crank.

During the experiment, it was noticed that a maximum of about 15 cm separation distance can be achieved and after that the fibres tend to break. So, the separation is fixed at 10 cm and the width is fixed at 3 cm. Again, the revolution per minute (RPM) of the crank has been decided on the basis of the number of strokes required by block A per minute for the extraction of the fibrous assembly without breakage. A maximum of up to 30 RPM has been achieved by the crank for the successful extraction of fibres. The main factors that influence the fibre properties have been identified, *i.e.* amount of force exerted on polymer, separating distance, time of pressing and amount of polymer spread on plastic surface.

Microscopy and EDX analysis of fibrous assembly

To determine the morphology and compositional analysis, the newly developed fibrous assembly was analysed *via* scanning electron microscopy (SEM), optical microscopy (Leica) and energy dispersive X-ray (EDX) spectroscopy. Optical microscopic measurements were taken across 100 randomly selected regions of the fibrous assembly at a 20X magnification and the average diameter was calculated.

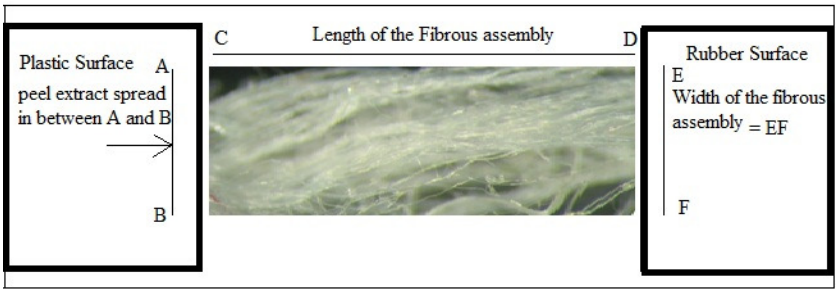


Figure 1: Diagram of manual extraction process of fibrous assembly from orange peel extract

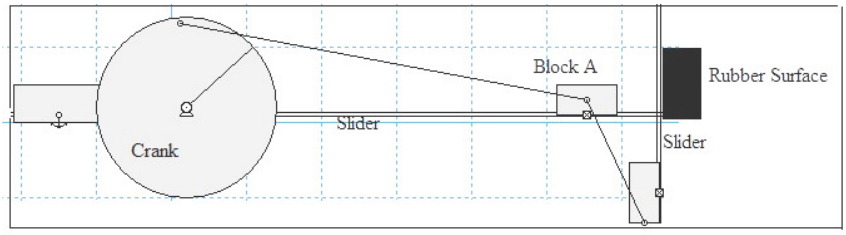


Figure 2: Schematic diagram of modified four-bar slider crank showing different parts

To investigate the surface morphology, SEM (Zeiss EVO 18) was performed using 4000 X magnification.

Elemental composition of the fibrous assembly was analysed using EDX (OXFORD INCA). The results obtained were calculated in weight %.

Analysis of chemical composition

The fibrous assembly was analysed for cellulose, hemicelluloses, lignin and ash using standard test methods, as described elsewhere.[11] Briefly, the samples were initially treated with dilute nitric acid, followed by aqueous ethanol, and extraction was carried out using NaOH, followed by H_2SO_4 treatment.

Fourier Transform Infrared (FTIR) spectroscopy

A Perkin Elmer (Spectrum BX series MA, USA) FTIR instrument was used to identify the chemical groups present in the fibrous assembly. A total of 100 scans per sample were taken at a spectral resolution of 4 cm^{-1} in the range of 900 cm^{-1} to 4000 cm^{-1} in transmission mode.

X-ray diffraction (XRD)

X-ray diffraction of the orange peel fibre assembly was carried out using PAN analytical X'Pert PRO-DY2022 (Almelo, the Netherlands) by CuKα radiation (λ = 1.5405 Å). The scanned angle was positioned between 0^o to 60^o (2θ). Measurements were conducted at a voltage of 40 kV and 30 mA current. For calculating the degree of crystallinity, an amorphous film was produced by the solution casting method, dissolving the fibrous assembly in methylene chloride solvent.

Tensile behaviour of fibrous assembly

As the extraction of single fibres from a fibrous assembly is not possible, a bundle of fibrous assembly was prepared by taking a certain length and weight of the fibres. The tensile behaviour of the fibre bundles were measured on a Zwick/Roell universal testing machine at standard temperature and 65 ± 2% relative humidity with a gauge length of 2.5 cm. The strain rate was adjusted to give a time to break of 20 ± 2 seconds.

Thermal analysis

Thermogravimetry (DTG) and differential thermal analysis (DTA) curves were recorded on an EXSTA R6000 (DTG/DTA 6300) instrument at a heating rate of 10 °C/min from 25 °C to 700 °C.

Moisture absorbency behaviour

The moisture regains and moisture content percentage was calculated using the ASTM-2495(2001) method. For the experiment, the freshly extracted fibrous assembly was weighed at room temperature and 65 ± 2% relative humidity. Following this, the fibrous assembly was dried in the presence of phosphorus pentoxide in a desiccator and weighed again after 72 hours. Then, the dried material was moved to a different desiccator with 65% relative humidity in the presence of calcium chloride and was weighed again after it reached equilibrium. The experiment was repeated for 50 different samples of fibrous assemblies.

Antibacterial activity

The antibacterial activity of the fibrous assembly was analysed using the standard test method ASTM E2149-13a. The test culture comprising *Staphylococcus aureus* (gram positive) and *E. coli* (gram negative) incubated in a nutrient broth (composed of animal extracts supplied from Himedia Laboratories Pvt. Ltd), was diluted with 0.3 mM (sterile) phosphate buffer to obtain a working concentration of 1.5-3.0 X 10^5 CFU/ml. Following this, a specific amount of fibrous matrix was transferred to a flask containing 50 ml of the working dilution under constant stirring at 190 rpm for 1 hour. The inoculated plates were incubated for 24 hours at 37 °C and the viable cells were calculated. The antimicrobial activity was denoted as % reduction of the bacteria, obtained by comparing the total of viable bacterial cells in the test specimen to the control (working dilution without fibre assembly). The antimicrobial activity of the fibrous assembly was examined by the following Equation 1:

$$\text{Reduction \% (CFU/ml)} = [(B - A)/B]^* 100 \quad (1)$$

where A represents the total number of viable cells (CFU/ml) in the test sample after the specified exposure time and B is the zero exposure time before the addition of the specimen to determine A. The values are represented in the form of mean ±SD.

RESULTS AND DISCUSSION

Analysis of morphology of fibrous assembly

Optical microscopy and SEM micrographs (Fig. 3a, 3b) demonstrate a typical cylindrical appearance of the fibres resembling common man-made fibres.

The average diameter of individual fibres was measured to be of 6.19 ± 2.89 µm. The fibrous assembly exhibited low initial modulus and tenacity. The variation observed in the fibre diameter and inter-fibre distances could be due either to the variation in the extraction forces applied during processing of fibres or to non-uniform distribution of the orange peel extract (liquid form) during the fibre extraction process.

Analysis of chemical composition

The chemical composition of the sample was identified by EDX analysis (Fig. 4), which revealed the presence of two major elements: carbon (~91%) and oxygen (~8%).

The components present within the matrix included about 10 ± 0.5% of cellulose, 15 ± 0.2% of hemicelluloses, 2 ± 0.3% of lignin and about 1.5 ± 0.2% of ash. The presence of these constituents characterized by their functional groups was also confirmed by FTIR (Fig. 5).

Fourier Transform Infrared (FTIR) spectroscopy

To determine the functional groups present with the complex organic assembly, FTIR analysis of the fibrous assembly (Fig. 5) was performed. The FTIR spectra displayed a number of characteristic peaks corresponding to organic constituents of the fibrous assembly network (as determined by EDX analysis, (Fig. 4)). The majority of the bands displayed are common to those present in cellulose, hemicelluloses and lignin.[12] The high energy peak present at 3318 cm^{-1} is attributed to the stretching of –OH groups of carbohydrates, such as lignin.[13]

The peaks at 3022 and 2912 cm^{-1} are due to symmetrical and asymmetrical stretching vibration of C–H bond, indicating the presence of CH and CH_2 groups, typically present in the basic structures of cellulose and hemicelluloses components.[14]

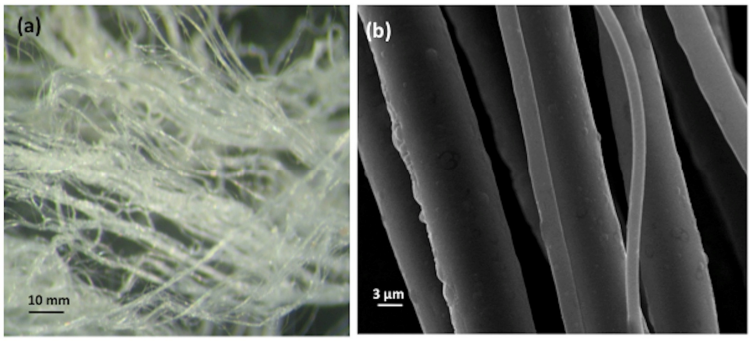


Figure 3: Optical microscopy of fibrous assembly (a) and scanning electron microscopy of fibrous assembly (b)

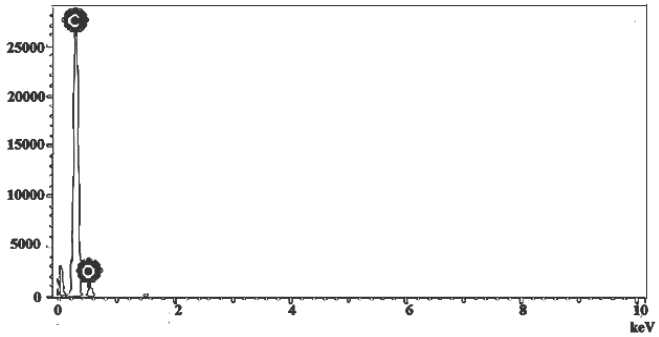


Figure 4: Elemental composition of extracted fibrous assembly by EDX

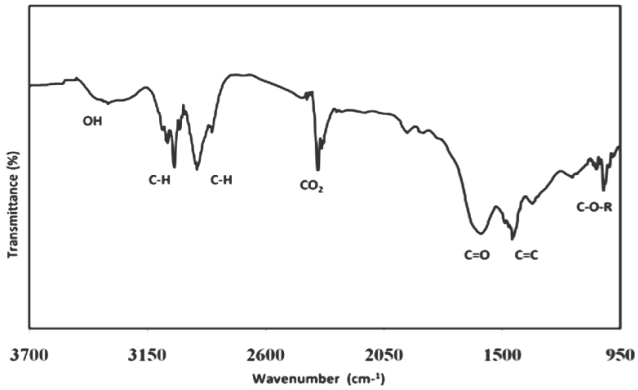


Figure 5: FTIR spectroscopy of fibrous assembly

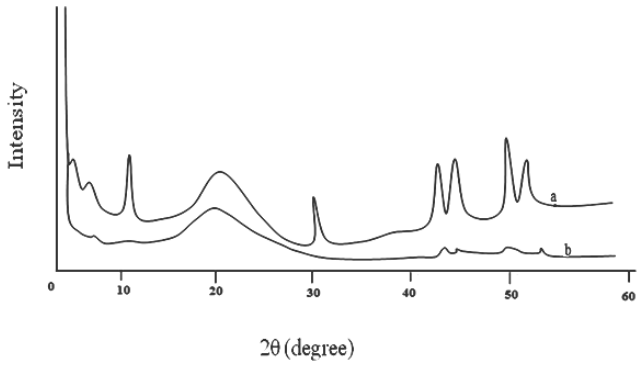


Figure 6: XRD spectra of (a) fibrous assembly and (b) film

The band at 1590 cm^{-1} indicates the presence of aromatic bond vibrations of hesperidin, while the one at 1444 cm^{-1} is assigned to aliphatic chains ($–CH_2–$ and $–CH_3$) forming the basic unit of the lignocellulosic structure.[13] The band at 1024 cm^{-1} corresponds to the C–O–R link.[15]

XRD analysis

The X-ray diffraction spectrum of the fibrous assembly (Fig. 6a) exhibits well-defined peaks between 2θ = 12° to 23° and 2θ = 30° to 52° regions. However, the spectrum of the film prepared from the fibrous assembly is devoid of any sharp peak, indicating the overall amorphous nature of the sample (Fig. 6b). Crystallinity was compared for the fibrous assembly and the film, with respect to the total amount of amorphous material, determined by the standard graphical technique. In this method, the spectrum of the fibrous assembly was overlapped by the spectrum of the film within the same plane. Thereafter, the area of the peak outside the overlapping region was calculated, which showed about 8% crystallinity. The fibre assembly sample exhibited intense peaks at 12°, 22° and 30°, which represent the characteristic peaks of a typical cellulose-I structure and flavonoid quercetin.[16-19] The peak at 22° is attributed to flavonoid hesperedin.[20] The peaks at 45°, 50° and 52° are due to the presence of hesperetin and rutin types of flavonoids.[21] The development of the molecular level order in the fibrous assembly may be due to the enhanced orientation of the polymer molecules, as a result of stretching during manual extraction of the fibres.

Tensile behaviour

The fibrous assembly thus produced from orange peel extracts shows relatively low modulus and tenacity, as observed from the stress-strain curves in Figure 7. The initial modulus of the fibrous assembly is about 0.16 cN/Tex and tenacity is of 0.068 cN/Tex. The breaking elongation lies in between 2.2-2.5% at the point where maximum stress required breaking the fibre.

The stress-strain curves of the fibrous assembly (Fig. 7) show an initial rapid rise with a marked yield point and then observed a mixed behaviour of fibre breakage and slippage due to variation in fibre length inside the fibrous bundle. This type of behaviour occurs during tensile loading because of poor fibre-fibre cohesion in the fibrous bundle. Low strength of fibre may be due to the predominantly amorphous nature of the polymer, which is confirmed by XRD results.

Thermal analysis

The TGA thermogram shown in Figure 8 reveals nearly 4% weight loss in the fibrous assembly at 190 °C, which could be attributed to the presence of volatile oils and physically adsorbed water molecules.[22] Between 190 °C and 346 °C, the total weight loss computed was of about 8%. The decomposition of hemicellulose usually occurs around 250 °C,[22] therefore, the respective peak was assigned to hemicellulose decomposition.[23] Thereafter, between 346 °C and 410 °C, the degradation of cellulose and carbon-carbon linkage in lignin resulted in approximately 28% weight loss.

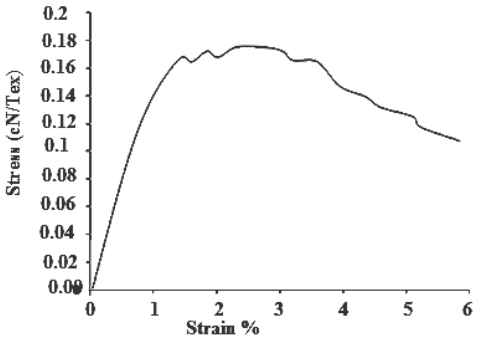
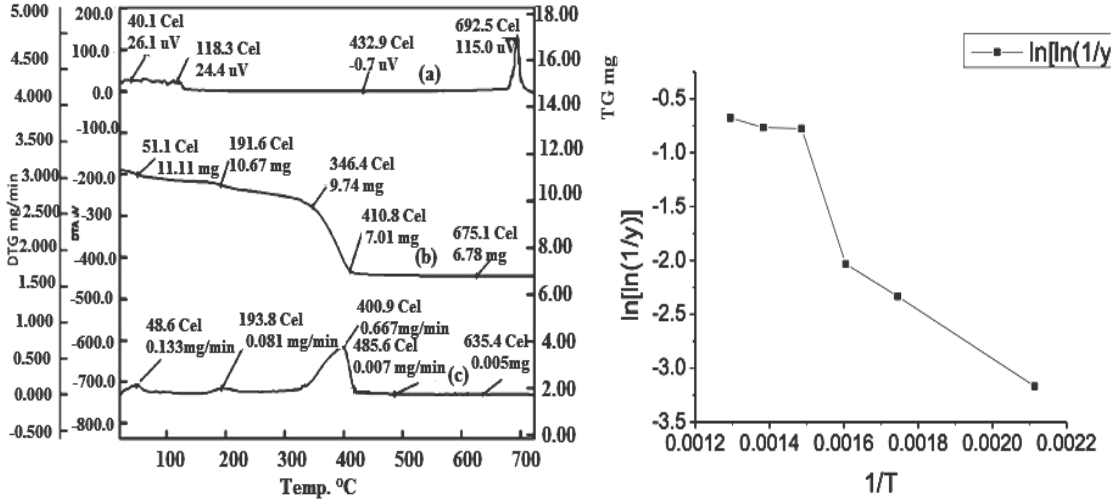


Figure 7: Stress-strain curve of fibrous assembly



(A) (B)

Figure 8: (A) Thermal analysis of obtained fibrous assembly: (a) DTA curve, (b) TGA curve and (c) DTG curve, and (B) graphical representation of ln[ln(1/y)] *vs*. 1/T using Broido's equation

Above 410 °C, the process became stable with a resultant weight loss of nearly 39%.[23,12] Thermal degradation of cellulose within the fibrous assembly could be due to the depolymerization event. The phenomenon occurs when the cellulose molecules absorb energy, resulting in the cleavage of the glycosidic linkages between oligosaccharides and glucose. The decomposition of hemicellulose usually occurs around 250 °C.[24] The long molecules of cellulose are bonded with each other by hydrogen bonds and undergo degradation at about 350 °C.[25] It has been reported that there is usually no decomposition observed up to 160 °C, however, when the temperature exceeds this value, the thermal stability decreases.[23,12] In summary, the thermal stabililty was established in the following order with respect to the decomposition of the constituent elements: hemicellulose > cellulose > lignin, with one endothermic peak (between 190-346 °C) and two exothermic peaks (corresponding to depolymerization and cleavage of glycosidic linkages).

The activation energy of thermal degradation was measured by Broido's equation (Eq. 2)[26] in the temperature range of 200 °C-600 °C:

$$\ln[\ln(1/y)] = - E/R \{(1/T)\} + K \quad (2)$$

where T is the temperature in Kelvin, R is the gas constant, K is a constant, y denotes the normalized weight (w_t/w_0), w_t is defined as weight of samples at any temperature t, whereas w_0 stands for their respective initial weights.

The energy of activation (E) was calculated by the plot of ln[ln(1/y)] *vs* 1/T, as shown in Figure 8. The activation energy of about 17 kJ/mol was interpolated from the plot obtained at 400 °C temperature. Therefore, it was evident from the

above values that a lower amount of crystaline cellulose was present in the fibrous matrix, as already depicted in XRD analysis.

Moisture absorbency behaviour

The freshly extracted fibrous assembly from orange peel absorbed moisture with an increase in time and achieved equilibrium after 1 hour (Fig. 9). The average moisture regain and moisture content values of the fibrous assembly calculated were 12% and 10%, respectively. This property of the fibrous assembly was also upheld by its moisture retention capability. This could be due to the amorphous structure of the fibre assembly, as revealed by XRD analysis, as well as the presence of polar chemical groups, such as hydroxyl, carboxyl and carbonyl *etc*., as revealed by FTIR analysis.[27]

Antibacterial activity

All the samples were highly efficient against both test bacteria with a reduction rate of 83.75 ± 2.5 for *E. coli* (n=5) and 94.71 ± 4.35 for *S. aureus* (n=5), which indicated the excellent antibacterial property of the fibrous assembly, as depicted in Table 1. The percentage reduction of bacteria increases with the increase of fibrous material in the samples. A possible reason for this antibacterial nature is the presence of different flavonoids and other poly-phenolic compounds within the fibrous assembly. Further work is warranted to generate insight about the specific antibacterial mechanism.

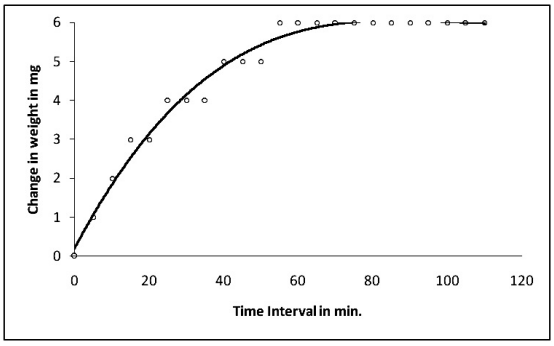


Figure 9: Weight *vs*. time curve depicting moisture absorbency behaviour

Table 1
Antibacterial activity of newly developed fibrous assembly

Sample weight in grams	*E. coli* (% reduction of bacteria) Mean value of 3 replicates	*S. aureus* (% reduction of bacteria) Mean value of 3 replicates
1	83.75 ± 2.5	94.71 ± 4.35
2	90.00 ± 4.0	95.21 ± 3.54
3	93.00 ± 4.3	96.00 ± 2.7
4	95.22 ± 3.3	96.25 ± 2.1

CONCLUSION

The fibrous assembly prepared from orange peel residues amounted to 90% carbon and oxygen, with a predominantly amorphous structure, with 8% crystalline regions. Morphologically, the assembly exhibited cylindrical glass rod-type structure, akin to melt spun man-made fibres. Analytical data revealed the presence of 10% cellulose, 15% hemicelluloses and 2% lignin within the fibrous matrix. Like all plant constituents, treatment in polar solvents resulted in complete dissolution of the matrix, suggesting the presence of abundant phenolic compounds, such as bioactive flavonoids, carbohydrates, cellulose and hemicelluloses, rendering the structure with high antibacterial properties against *E. coli* and *S. aureus* bacterial cultures.

Four main factors have been found to influence fibre properties, *i.e.* amount of force exerted on the polymer, separating distance, time of pressing and amount of polymer spread on the plastic surface. As far as the tensile property is concerned, the maximum amount of force required for the production of the fibrous assembly is 0.16 cN/Tex, to spread the peel

extract uniformly. Non-uniform spreading of the liquid resulted in variation of fibre diameter.

Clearly, the method of fibrous assembly extraction proposed herein is exciting and intriguing, but also requires in-depth research and investigation to explore the technical and economic aspects. After this initial successful study, the work could yield beneficial outcomes in terms of development of wound dressing and healthcare materials, introducing an innovative method for producing antibacterial drugs, alongside the standard industrial production; secondly, it can act as an effective method to recycle solid residual wastes, a potential cause of environmental pollution.

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