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Characterization of Film for Medical Textiles Application

Karakterizacija filma za uporabo v medicinskih tekstilijah

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Abstract

The presented research focuses on the development and characterization of film made from lime peel extracts; well-known for its anti-oxidant and antimicrobial properties. The study includes preparation of film using the solution casting technique and characterization tests including IR spectroscopy, X-ray diffraction, and thermal behaviour through differential scanning calorimetry (DSC) and through thermogravimetric analysis (TGA). The film is also analysed for its antibacterial properties. Several functional groups are identified for the different molecules such as cellulose, hemicellulose and lignin, and some polyphenolic compounds such as flavonoids. The film shows excellent antimicrobial properties against E. Coli and S. Aureus strains.
Keywords: antibacterial, lime peel, film, FTIR, DSC, TGA, XRD

Izvleček

Raziskava se osredotoča na razvoj in karakterizacijo filma, izdelanega iz ekstrakta lupine limete, ki je dobro poznana po svojih antioksidativnih ter protimikrobnih lastnostih. Vključuje pripravo filma s pomočjo tehnike vlivanja raztopine in karakterizacijske teste IR spektroskopijo, rentgensko difrakcijo in toplotno obnašanje z dinamično kalorimetrijo (DSC) in termogravimetrično analizo (TGA). Analizirane so bile tudi antibakterijske lastnosti filma. Opredeljene so bile funkcionalne skupine celuloze, hemiceluloze in lignina ter polifenolnih spojin - flavonoidov. Film se ponaša z odlično protimikrobno lastnostjo za E. Coli in S. Aureus seve.
Ključne besede: antibakterijski, lupina limete, film, FTIR, DSC, TGA, XRD

1 Introduction

Lime is a fruit being yellowish green in colour and contains a large quantity of vitamin C. Many authors have reported that lime peel extracts have active components such as flavonoid and limonene which act as antioxidant, anti-obesity as well as anti-carcinogenic agents and also show a tendency to inhibit tumor growth [1, 2]. In addition to vitamin C, carotenoids, flavonoids, limonoids, phenolic acids are also present in the lime peels which are also beneficial to human health [3-7]. The bioactive components are responsible for different biological functions, including anti-oxidative, anti-inflammatory, antibacterial, anti-allergic, antiviral, anti-proliferative, anti-mutagenic, and anti-carcinogenic activities within the human body [8-11]. Consumption of foods rich in flavonoids is essential for preventing several degenerative pathologies, including cardiovascular diseases, atherosclerosis, cataract and several forms of cancer [12].

1.1 Structure and chemical composition of the citrus peel

The quality and scientific value of any material depends upon its structure and chemical composition. Similarly citrus lime is known for its nutritional and

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medicinal values, which are mainly associated with the various compositions of the material. Citrus peels contain several valuable compounds such as soluble sugars, insoluble carbohydrate fibres, organic acids, essential oils, flavonoids, and carotenoids [13]. Table 1 gives detailed information on the chemical composition.

Table 1: Chemical composition of citrus peel [14]

Name of constituent	Value in percentage
Simple sugar	15.0
Pectin	28.7
Protein	6.62
Flavonoids (hesperidin, querecetin etc.)	3.86
Holocellulose	7.4
Cellulose	10.4
Hemicelluloses	8.9
Lignin	1.33
Ash	2.87
Volatile oils	14.92

The flavonoid (Figure 1) molecules present in the citrus peel are mainly responsible for antibacterial and antioxidant activities and stabilising the free radicals involved in oxidative processes of various reactions taking place inside the human body [15].

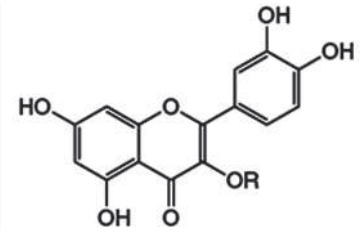

Figure 1: Structure of flavonoid

All the above studies relate to possibilities of using lime peel extracts during various healthcare-related applications. There has been no work reported so far in the direction of developing polymeric film from lime peel's extract and its behaviour. So in this paper an attempt has been made to develop the polymeric film from lime peel's extract and characterise the newly-developed film in terms of functional group identification, thermal behaviour, X-ray diffraction and antibacterial activity. This approach is of interest due to probable wound-care and tissue engineering applications where polymers having antimicrobial properties show several advantages.

2 Materials and experimental procedure

The materials used for developing the film are lime peels. Lime peels are extracted from fresh limes that can be purchased from the local markets or local gardens of Jalandhar, India. Then lime peels' extract i.e. liquid from the lime peels are collected on a plastic surface by cold pressing of the fresh lime peel. The fibrous matrixes are isolated from the lime peel extracts by pressing the plastic surface on a rubbery surface through a manual mechanical process. Then 100 mg of fibrous matrix is dissolved in 10 ml of methylene chloride at room temperature with 65±2% relative humidity in a test tube (Figure 2). The time period required for the dissolution of fibrous matrix in methylene chloride is much less. Afterwards the solution is spread on a petri dish for air-drying for a minimum of 8 hours. Finally a yellowish film of 1 mm thickness being non-transparent in nature is prepared (Figure 2). In a similar way, 150 mg, 200 mg and 250 mg of fibrous matrix are each dissolved in 10 ml of methylene chloride in three different test tubes giving films of 1.5 mm, 2 mm and 2.5 mm respectively.

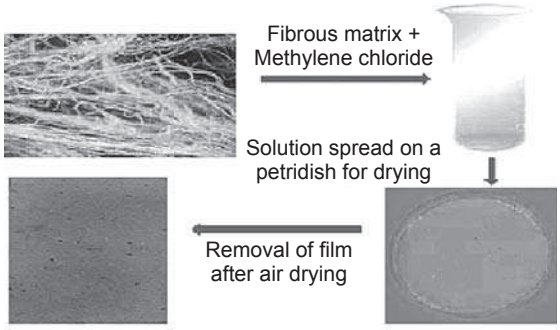

Figure 2: Solution casting technique for preparation of the film

2.1 FTIR Spectroscopy (Fourier Transformation Infrared Spectroscopy)

Perkin Elmer (Spectrum BX) FTIR was used for identifying the chemical groups within the films. A total of 100 scans per sample were taken with a resolution of 4 cm^{-1} within the frequency range of 400 cm^{-1} to 4000 cm^{-1} in the transmission mode.

2.2 Wide -Angle X-ray Diffraction

Wide-angle X-ray diffraction was carried out using the Panalytical (PW 3040/ 60 X'pert PRO) system. CuKα radiation was used with a wavelength 0.15421 nm. X ray scattering was registered on the equator between 2θ = 2° to 60°.

2.3 Thermal analysis

The TGA curves were recorded on an EXSTAR6000 (TG/DTA 6300) instrument at a heating rate of 10°C/min from 25°C to 600°C. The DSC curve was recorded on a Mettler Toledo (DSC 823^e) instrument at a heating rate 10°C/min from 25°C to 150°C.

2.4 Antibacterial activity

The antibacterial activity of the film was analysed using the standard test method using the shake flask test in accordance with GB 15979-2002 Hygienic Standard for disposable sanitary products. The test culture comprising of *E. Coli* (a gram negative) and *S. Aureus* (a gram positive) incubated in a nutrient broth (composed of animal extracts supplied from Himedia Laboratories Pvt. Ltd®) was diluted with 0.3 mM phosphate buffer (sterile) to give a working concentration of 1.5–3.0 X 105 CFU/ml. Following this, each film was transferred to a flask containing 50 ml of the working dilution under constant stirring at 190 rpm for one hour. The inoculated plates were incubated at 37°C for 24 hours and the viable cells were counted. The antimicrobial activity was expressed in % reduction of the bacteria, obtained by comparing the total of viable bacterial cells in the test specimen compared to the control (working dilution without film). The antimicrobial activity was calculated using the following equation [16]:

$$\text{Reduction \%}\left(\frac{CFU}{ml}\right) = \frac{(B-A)}{B} \times 100 \qquad (1)$$

where *A* is the total number of surviving cells (CFU/ml) in the test sample after the specified exposure time and B the zero exposure time before the addition of the specimen for determining A.

3 Results and Discussion

3.1 Functional Group Identification

From Figure 3, the most intense band at 3435 cm^{-1} was assigned to the stretching of OH groups of the carbohydrates and those of lignin [17]. The signal at 2901 cm^{-1} is caused by asymmetrical and symmetrical stretching vibrations of the C–H groups. The band at 1803 cm^{-1} was assigned to the carbonyl (C=O) stretching. It follows from analysis of the literature [18, 19] on the IR spectra of film containing flavonoids that the carbonyl group bands had the following vibration frequencies such as 1603 and 1631 cm^{-1} being the characteristic peaks of the hesperidin types of flavonoid.

The C–H bending is available at wave number 1454 cm^{-1} for querecetin type of flavonoid. Other typical absorption bands [20] characteristics of pure cellulose are those at wave numbers 1030, 907, 3029–3059 cm^{-1}.

3.2 XRD Analysis

The XRD spectrum of the film is shown in Figure 4. The X-ray diffraction spectrum exhibited a number of well-defined crystalline peaks as expected for

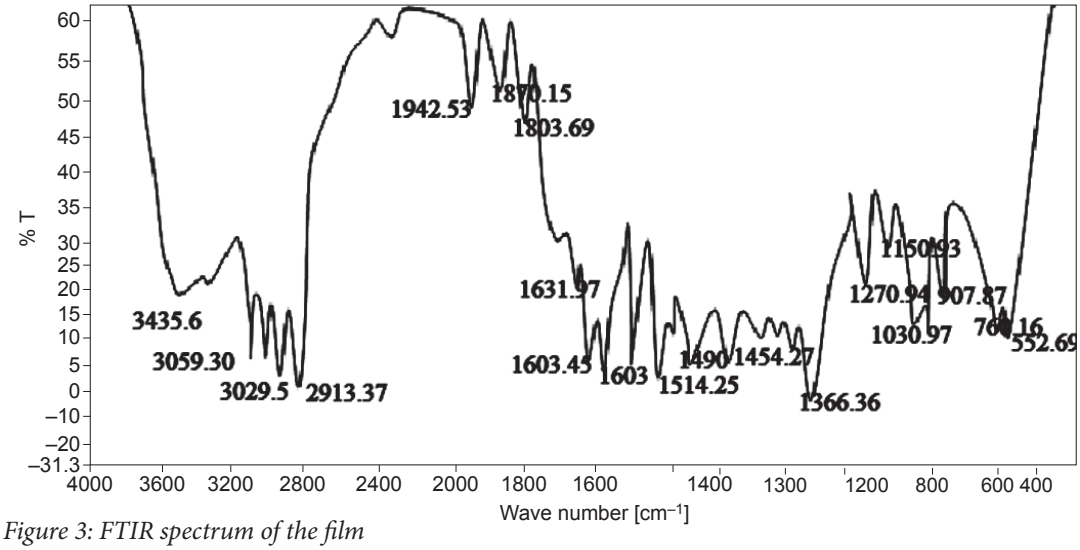

Figure 3: FTIR spectrum of the film

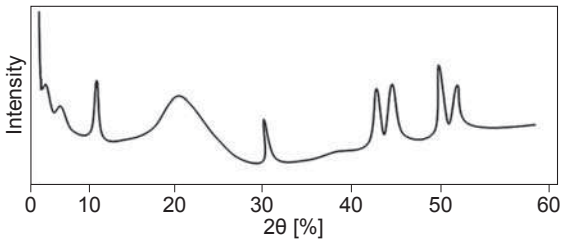

Figure 4: XRD spectrum of the film

natural organic materials [21, 22]. Crystallinity is a measure of the amount of crystalline material with respect to the entire amount of polymer material. A crystallinity parameter that was determined using the standard method [23] was 16%. Using the equation [24], $L = (k\lambda)/\beta \cos\theta$, the crystallite size (L) of the cellulose-I crystal present in the film was found to be 7 nm at $2\theta = 23^{\circ}$. Where, k is a constant (0.89), β is the peak's full width at half-maximum and λ is the wavelength of radiation.

However, the presented film is a mixture of cellulose and different polyphenolic compounds. As seen from Figure 5, the crystalline peaks are noticeable between $2\theta = 12°$ to $2\theta = 23°$ which corresponds to the crystallographic plane family of cellulose- I and another set of peaks appear between $2\theta = 30°$ to $2\theta = 52°$ which may be due to the presence of quercetin types of flavonoid [25, 26]. According to Chen Run, who described in his PhD thesis [27, 28] that the second set of peaks may be due to the presence of hesperetin, rutin types of flavonoids [28].

3.3 Thermal analysis

The occurrence of chain scission is clearly demonstrated by TGA. If the polymer undergoes degradation, its weight will decrease. TGA and DSC curves of the film are shown in Figure 5. Corresponding to the weight loss on TGA curves, at least four main thermal events can be clearly distinguished up to 600°C.

In the cases of TGA curves [29], the first weight loss step below 105°C refers to the volatile components and physically adsorbed water molecules within the samples. The main mass losses are associated with the biomass decomposition, essentially to its three main components (hemicelluloses, cellulose and lignin). The second step from 150 to 265°C can be attributed to decomposition of hemicelluloses. The third decomposition process between 265 and 372°C was associated with the degradation of cellulose [30]. Finally, the carbon-carbon linkage [31] between lignin structural units was cleaved within the temperature range from

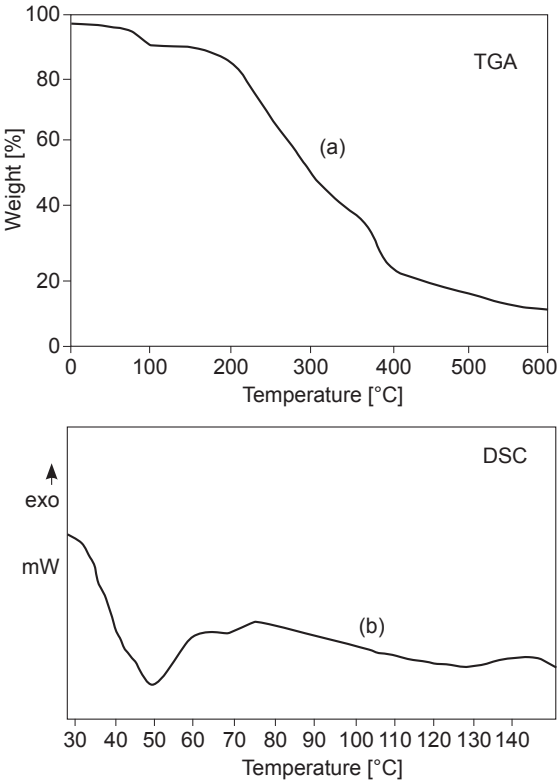

Figure 5: TGA (a) and DSC curves of the film (b)

372 to 570°C. The endothermic peak at 10°C/min rate of heating (Figure 5b) was observed perhaps due to the presence of various volatile oils and physically adsorbed moisture in the sample. The film is predominantly amorphous which indicates a lesser number of crystals present in the film. So there is no significant melting peak, as shown in the DSC curve.

3.4 Antibacterial behaviour

The antibacterial effect of film prepared from lime peels' extract with different bacterial species in terms of reduction of bacterial count is shown in Table 2.

Table 2: Antibacterial activity of the film

No.	Sample thickness [mm]	*E. Coli* reduction* [%]	*S. aureus* reduction* [%]
1	1	83.75 ± 2.5 SD	94.71 ± 4.35 SD
2	1.5	90.00 ± 4.0 SD	95.21 ± 3.54 SD
3	2	93.00 ± 4.3 SD	96.00 ± 2.7 SD
4	2.5	95.22 ± 3.3 SD	96.25 ± 2.1 SD

*Mean value of three replicates.

All the samples were very effective against both test bacteria with a reduction of over 83% for *E. Coli* and 94% for *S. aureus*, thus indicating excellent antibacterial properties. The reason behind the antibacterial property may be due to the presence of different flavonoids and other polyphenolic compounds present in the film which is also identified in the FTIR and XRD results.

4 Conclusions

The film was successfully produced from lime peels' extract. The various active ingredients such as flavonoids of rutin, hesperitin and qurecetin were identified using the FTIR method and XRD technique. All the flavonoid molecules had very good antioxidant and antibacterial activities. The XRD technique revealed the crystalinity index of the material as 16%. The thermal behaviour of the material was studied by DSC and TG-DTA techniques. The thermal stability was established with respect to the decompositions of the various constituent elements. The antibacterial activities of the films were found to be excellent against *E. Coli* and *S. aureus*. This work could yield beneficial outcomes in terms of the development of wound dressing and healthcare materials, and it could act as an effective method for recycling solid residual wastes, a potential cause of environmental pollution.

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