Characterization and Antimicrobial Characteristics of Chitosan Modified *Hibiscus sabdariffa*L.extract

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Abstract

Samples of Chitosan (Cs) natural polymer containing successive amount of *Hibiscus sabdariffa*Lextract were succsesfuly synthesized using traditional simple casting route. Prepared thin films were characterized using fourier transform infrared (FTIR). FT-IR of synthesized thin films reveals maintenance of the characteristic bands of chitosan in addition to the appearance of two new sharp intense bands at 1782 and 954 cm^{-1} intensified with increasing plant extract content and assigned to the interaction between NH2 of polymer skeleton with falvanoids present in the extract. Obtained data poit out to a formation of homogenous composite structure. X-ray diffraction data (XRD) reval no prounounced band indicating the amorphous structure of synthesized final polymeric product. In vitro antimicrobial studies were performed using both gram negative and positive bacteria in addition to Fungul and Yeast activity using simple minimum inhibition zone (MIZ) standeredroutain.

Keywords: Chitosan; *Hibiscus sabdariffa L.*extract; FTIR; Antimicrobial Activity.

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Introduction

Recently biodegradable, biocompatible polymers modified with different fillers are studied by different researchers [1-3] for their versatile uses in different fields of application. Chitosan is one of the most important and low cost natural biodegradable polymers used as a carrier for pharacuticals active ingredients as stabilizing agent to enhance their characteristics.

Chitosan represent a class of linear polysaccharide prepared through acetylation process of chitin and their monomer consists of a combination of N-acetyle-D-glucosamine (acetyled) and D-glucosamine (deacetyled) groups whose percent depends on the deactylation ratio [4]. Chitosan usually obtained from the hard outer skeleton of shellfish and owe a huge medical uses especially in the obesity and high cholesterol disease treatments [5].

Chitosan shows a promising biological activities against pathogenic grams so its film is unique in the application of food preservation through extension of time interval and reduction of the growth rate of microorganisms as reported by Han [6] in addition to their uses as chelating agent and heavy metal trapper and also in water treatment [7, 8].

*Hibiscus sabdariffa*L. usually used in foods,wines while their leaves and tender stem can be added to salads and chutney. As all plants, the compositions of *Hibiscus sabdariffa*L, contains polyphenolic acids, flavonoids, and anthocyanins [9] which owes as manymedicinal applications including treatment of hypertension, pyrexia, liver damage and leukaemia due to its high content of protocatechuic acid [10].In addition to these characteristics *Hibiscus sabdariffa*L. extract can also usedas an antioxidant for protection against low density lipoprotein (LDL)-oxidation and has hypolipidemic effects in vivo [11].

Present work aims to evaluate the role of *Hibiscus sabdariffa L.* addatives for anatural degradable polymer (chitosan) in the antimicrobial activity and to correlate change in the activity index with the concentration and type of interaction between composite constituent material.

Materials and Methods

Chitosan of low molecular weight supplied by Sigma Aldrich Co. *Hibiscus sabdariffa L. obtained from Egyptian markets*. The studied pristinechitosan thin films and other samples containing successive amount of plant extract were synthesized via casting technique with labled in Table (1). Bi-distiled water containing 2% acetic acid were used a common solvent. Starting materials was vigorously stirred at room temperature until a clear transparent viscous liquid is obtaine. Calculated amounts of extract were added to the polymer solution and poured in a plastic Petri dishes. Dishes are incubated in an oven at 50° up to two days for drying. Obtained films was peeled from the Petri dishes and kept in desiccator until use.

Table (1) Sample notation and composition

Sample	Cs	H1	H2	H3	H4	H5	H6
Chitosan	100	99	98	97	96	95	94
Extract ml added	0	1	2	3	4	5	6

XRD diffraction (XRD) was used to identifythe degree of crystallinity throughout the samples and to investigate complexation behavior between polymeric matrices and *Hibiscus sabdariffaL.*.XRD diffraction data plotted as Bragg's angle (2θ) versus intensity using PANalyticalX`Pert PRO adopting Cu Kαtargetusing wavelength λ = 1.540 Å and tube operating at 45 kV-40 mA within the Bragg's angle (2θ) ranging between 5°-80°. FT-IR spectral data collected using Nicolet iS10 spectrophotometer adopted 32 runs at room temperature

within the spectral range 4000-400 cm^{-1}. UV/vis. spectral data collected using Jasco 570 double beam spectrometer within the range 190-650 nm.

Biological activities were evaluated against *Candida albicans* fungi, *Pseudomonas aeuroginosa, Escherichia coli gram negative bacteria and Bacillus subtilis, Staphylococcus aureus*gram positive bacteria via minimum inhibition zone (MIZ) route previously discuused [12].Antibacterial activity of a known standard antibiotic (ampicillin) and antifungal (Colitrimazole) was also measured to calculate the percent activity index of synthesized samples using the formula:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diametre)}}{\text{Zone of inhibition by standard (diametre)}} \times 100$$

Results and Discussion

X-ray diffraction data

X-ray diffraction experimental data measured in the Bragg angle (2θ) range 5-80° shown in Figure (1) reveals the presence of a single hump at about 20° characterized the amorphous nature of prepared thin films. It was notices also that the intensity of this hump decreases with increasing filler content indicating an increase of the amorphous nature of prepared film with increasing *Hibiscus sabdariffa L.* extract content and pointing to the homogeneity and complexation behaviour between the two component.

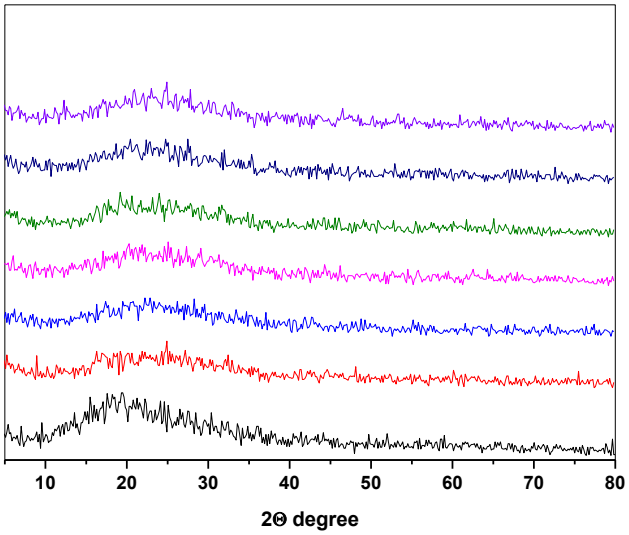

Figure (1): XRD spectra of pure and doped chitosan samples

FTIR Absorption Spectra

FTIR Spectrum of Hibiscus extract Figure (2) revels the presence of many bands characterize several plant extract including a broad band located between 3350-3500 cm^{-1} assigned for water molecule or OH bond,. A sharp intense band observed at about 1180 and 2360 cm^{-1} related to double bond C=C and C-H of the aromatic ring respectively. In addition, the band of carbonyl group located at about 1782 cm^{-1} was also observed combined with that of ether group at about 1070 cm^{-1}. Such bands characterize flavonoid compounds in *Hibiscus sabdariffa L.* havingthe chemical structure shown in Figure (3).

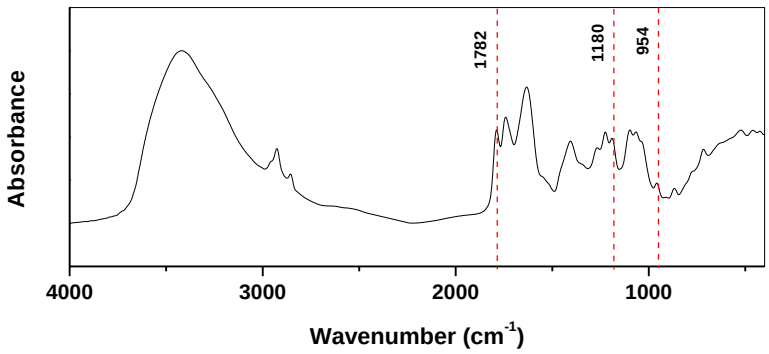

Figure (2) FT-IR spectrum of *Hibiscus sabdariffa L.*extract

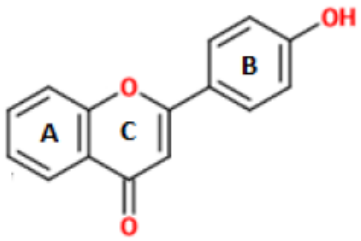

Figure (3) Functional groups in the structure of flavonoids

Chitosan represents a class of natural polymers that are mainly consists three functional groups, amino grous, primary and secondary alcohol. Figure (3) indicates FTIR spectral data of pure chitosan and other samples that contain variable amounts of the Hibiscus extract. All samples show the maintenance of the main constituent material (chitosan) with an obvious change in peak position and intensity with increasing filler content indicating notable change in their physicochemical characteristics. Table (2) list the observed band position and their assignments.

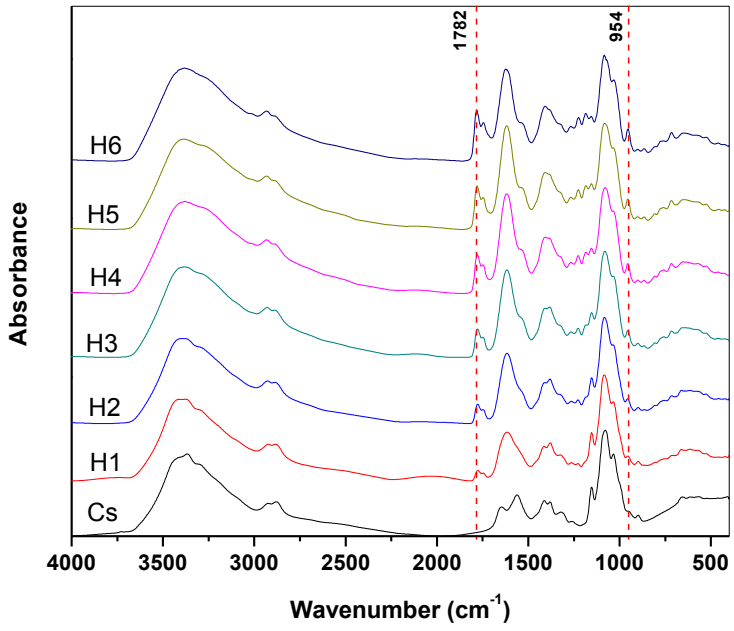

Figure (4): FTIR optical absorption spectra of pure and doped chitosan samples

Table (2): FTIR band assignment and peak position for pure chitosan

Wavenumber (cm^{-1})	Band assignment	Reference
3000-3500	Stretching vibration of(O-H) overlapped with (N-H)	
2927-2882	Asymmetric stretching vibration of aliphatic CH, CH2 and CH3	
1665	C=O bond stretching	
1561	N–H stretching of amide II	
1411	Bend vibration of OH and CH	
1342	Amide III (NH3)	
1151, 1081,1034 and 656	Characteristic to saccharide structure	

Antibacterial Studies

In vitro antimicrobial studies were performed using both gram negative and positive bacteria in addition to Yeast activity using simple minimum inhibition zone (MIZ) standered routain. Biological activities were evaluated against *Pseudomonas aeuroginosa, Escherichia coli gram negative bacteria and Bacillus subtilis, Staphylococcus aureus*gram positive bacteria in addition to *Candida albicans* yeast. Obtained data was also compared with a standard antibiotic to calculate the activity index.

Table (3) shows the variation of diameter zone and calculated activity index with pristine sample of chitosan (Cs) and samples of chitosan containing different amount of *Hibiscus sabdariffa L.*. It was observed that increasing of *Hibiscus sabdariffa L.* content result in increase of the diameter zone and activity index in all cases as shown in Figure (5). Such effect can be attributed to the presence of OH group in the falvonoids structure (Figure 2) which may react with free ions released during the interaction between NH_2 group of chitosan and other constituent of the extract.

Tablee (3) Diameter of inhibition zone and activity index of tested samples

No.	Compound	*E. coli*		*Pseudomonas aeuroginosa*		*S. aureus*		*Bacillus subtilis*		*C. Albicans*	
		(mg/ml)									
		R	A	R	A	R	A	R	A	R	A
1	Cs	8.0	30.8	5.0	21.7	6.0	25.0	8.0	33.3	2.0	7.4
2	H1	8.5	32.7	6.0	26.1	8.0	33.3	8.8	36.7	2.7	10.0
3	H2	11.0	42.3	7.2	31.3	9.2	38.3	9.6	40.0	3.1	11.5
4	H3	12.5	48.1	8.4	36.5	10.1	42.1	10.0	41.7	5.2	19.3
5	H4	13.5	51.9	9.9	43.0	11.9	49.6	11.0	45.8	6.9	25.6
6	H5	14.0	53.8	11.0	47.8	12.1	50.4	12.1	50.4	7.9	29.3

7	H6	15.5	59.6	12.6	54.8	14.6	60.8	14.2	59.2	9.2	34.1
Ampicillin		26	100	23	100	24	100	24	100	---	NA
Colitrimazole		NA	----	NA	----	NA	----	NA	----	27	100

R Diameter of inhibition zone (mm), A Activity index %

Figure (5) represent a bar representation the in vitro activity index test for a different pathogenic grams and Yeast. It was obvious that the activity index generally increases with increasing concentration of the extract. It was also clear that E-Coli and S. arures are most effected by the extract concentration while P. aeuroginosa nd C. Albicans is the lowst.

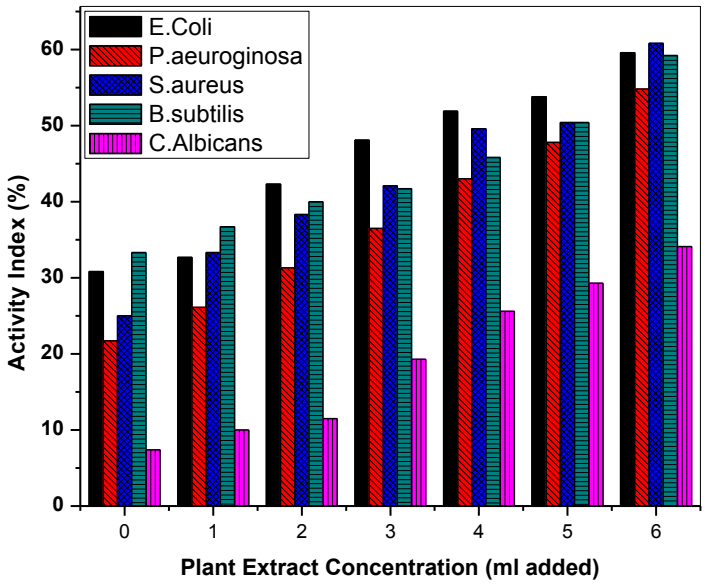

Figure (5): Activity index as a function of *Hibiscus sabdariffa L.* content

Conclusions

Chitosan doped *Hibiscus sabdariffa* L extract were preperead via traditional solution casting route. FT-IR of synthesized reveals maintenance of the characteristic bands in addition to the appearance of two new bands at 1782 and 954 cm^{-1} whose intensity increases with further addition of plant extract indicating a formation of new vibrational mode resulting from interaction between NH2 of polymer skeleton with falvanoids present in the extract. All samples were tested for their in vitro antimicrobial behavior, their activity index calculated and compared to a standared drug via simple minimum inhibition zone (MIZ) standered routain. It was observed that increasing of *Hibiscus sabdariffa L.* content result in increase of the diameter zone and activity index in all cases as shown in Figure (5). Such effect can be attributed to the presence of OH group in the falvonoids structure which may react with free ions released during the interaction between NH_2 group of chitosan and other constituent of the extract.

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