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SYNTHESIS AND CHARACTERIZATION OF NEW SCHIFF BASES FOR CHITOSAN AND STUDY THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Biopolymers and their derivatives have a wide range applications due to their biodegradability, low toxicity, and biocompatibility. The current study aimed to develop and characterize the unique Schiff 's chitosan bases by conjugation the chitosan with different aldehydes under acidic conditions to form Schiff 's chitosan bases which reacted with a plant extract derivative (capsaicin) to get new Schiff 's derivatives. The resulting compounds were diagnosed by using fourier transform infrared (FT-IR), as well as nuclear magnetic resonance(^{1}H-NMR). The biological activity of the new derivatives was studied to know its importance and its applications in the medical fields, where the antibacterial activity of positive-gram bacteria, negative- gram bacteria and fungi was tested. The results showed an increase of the substituted chitosan antibacterial activity against both positive-gram and negative- gram bacteria due to its high capsaicin content, which is highly effective against bacteria and fungi.

Keywords: Antimicrobial, Capsaicin, phthalicanhydride, Polymerization, Schiff base.

توليف وتوصيف قواعد شيف الجديدة للكيتوزان ودراسة نشاطها المضاد للميكروبات

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الخلاصة

البوليمرات الحيوية ومشتقاتها لها تطبيقات واسعة بسبب قابليتها للتحلل البيولوجي وسميتها القليلة وتوافقها الحيوي. تهدف الدراسة الحالية الى تحضير قواعد شيف مشتقة من الكيتوسان عن طريق تفاعل الكيتوسان مع الألديهايدات المختلفة والتي بدورها تتفاعل مع مشتق مستخلص نباتي (الكابسيسين) للحصول على مشتقات شيف الجديدة. تم تشخيص المركبات الناتجة باستخدام طيف الأشعة تحت الحمراء البعيدة وكذلك طيف الرنين المغناطيسي النووي. كما تمت دراسة النشاط البيولوجي للمشتقات الجديدة لمعرفة أهميتها وتطبيقاتها في المجالات الطبية، حيث تم اختبار النشاط المضاد للبكتيريا موجبة الجرام و سالبة الجرام، والفطريات. أظهرت النتائج زيادة في النشاط المضاد للبكتيريا للكيتوزان ضد البكتيريا موجبة الجرام وسالبة الجرام من خلال محتواها العالي من الكابسيسين العالي الفعالية ضد البكتيريا والفطريات.

الكلمات المفتاحية: بلمرة، قاعدة شيف، كابساسين، كيتوسان، مضادات الميكروبات.

INTRODUCTION

Capsaicin is a toxic white crystalline solid extracted from the pepper plant. Its found as cis and trans isomerisms and considered a crystalline alkaloid and lipophilic trans-8-methyl-N-vinyll-6-none-amide. Capsaicin has physical properties such as; odorless, colorless, hydrophobic, volatile alkaloid with a melting point of (62-65) ^oC with a molecular weight of 305.40 g/mol. It has the ability to dissolve in alcohol, oil and fat. Capsaicin is used as an

[*] **The research is taken from a master's thesis by the first researcher.**

analgesic in topical ointments and skin patches for pain relief, with a concentrations between 0.025% and 0.1% **(Adaszek *et al*., 2019)**, and can be used in the form of a cream for temporary relief of mild muscle and joint pain associated with arthritis, back pain, strains and sprains, often in conjunction with other reddish active ingredients**(Adhya & Sharma, 2019).**

Chitosan (CS) is a natural, biodegradable polymer that is non-toxic and biocompatible.It is Obtained by deacetylation of chitin and, it is the second most abundant natural polymer in nature. N-acetyl-D-glucosamine and D-glucosamine are two CS repeating units linked by 1,4-glycosidic bonds, where the degree of deacetylation is determined by the ratio of the former unit in the polymer intermolecular **(Agarwal *et al*., 2020**). It is noteworthy that the hydrogen bonds between chitosan molecules and other molecules are very strong. In addition to the crosslinking of the long-chain structure of chitosan, the physical and chemical properties of chitosan are weak and the solubility in common solvents is low. Chitosan isn't used widely because of it 's high viscosity **(Chang *et al*., 2022)** .To address chitosan's deficiencies, researchers degraded chitosan to obtain chitosan-oligosaccharides (COSs).

Several studies have confirmed using COS because COS is a non-toxic substance with a high level of safety **(Dragan *et al*., 2023)** .In 2016, a study in mice found that COS did not damage liver mitochondria or lung tissue function. oral doses، and another 2019 study showed that COS non- toxic to human sperm function **(Craciun *et al*., 2019)**. Literature have been reported about water-soluble chitosan derivatives, which are often pH-sensitive, while few reports are available on organic solvent-soluble chitosan derivatives. It is possible to blend organic soluble CS derivatives synthesized from natural sources with some other biocompatible commercial polymers, contributing to the development of a material with polymer additive properties **(Duceac *et al*., 2020).** Recently, chitosan has been pain attention in the pharmaceutical industry due to its unique chemical and antibacterial properties**(Gonzalez *et al*., 2021)**. However, it is combined with other biopolymers due to it's poor mechanical properties and chitosan's desirable biocompatibility attention. While, increasing the mechanical and physical properties improve the hydrogels Naturally derived biomaterials such as carbohydrates have been used to improve the mechanical properties of hydrogels**(Huang *et al*., 2018)**. Chitosan has been found to have a variety of biological activities, antibacterial, antitumor, antioxidant and Alzheimer's activities, and has been used as a drug carrier or a biopromoting material to promote human health **(Feng *et al*., 2016).** In addition, chitosan be used to remove pollutants from water because of the important role that chitosan plays in the medical field. Schiff bases were first described in 1864 when reported by Hugo Schiff and are condensation products of primary amines with carbonyl compounds, normally they could be obtained from the condensation reaction of aldehydes or ketones (cyclic or linear) with primary amines (cyclic or linear) in alcohol states **(Daoud *et al*., 2022).** Schiff base is a compound with a functional group containing a carbon-nitrogen double bond, where the nitrogen atom is attached to an aryl or alkyl group. The reaction occurs as a result of attack of the electrophilic carbonyl group of aldehydes or ketones through the nucleophilic primary amine group, presence of an acid or base or heat to catalyze the reaction. Schiff bases are excellent chelating agents because of their unique properties such as simple preparation, and structural flexibility **(Duceac *et al*., 2022)**. The research field of Schiff base coordination chemistry has greatly expanded as the importance of Schiff base complexes to organic biochemistry. It has been recognized as polymer stabilizers, dyes, organic synthesis intermediates, biomedical applications, supramolecular chemistry, catalysis and materials science, separation, encapsulation processes and the formation of compounds with unusual properties, structures, and catalysts **(Muna & Sana, 2022)**.

MATERIALS AND METHODS

Aldrich and Merck supplied capsaicin, maleic anhydride, Chitosan, and all other chemicals, respectively. Shimadzu spectrometer (4000-400cm^{-1}) FT-IR spectroscopy (Fourier Transform Infrared) and ^{1}H-NMR (Nuclear Magnetic Resonance) in Dimethylsulphoxide (DMSO-d6). Precision Digital Melting Point Instrument for Melting Point Recording A Varian-400 MHz spectrometer with TMS (Tetra methyl silane) as a reference was used to capture ^{1}H NMR. Compound thermograms were measured in a nitrogen environment TGA(Thermogravimetric analysis) Q50 instrument with the highest temperature set to 800 °C and the heating rate set to 10 °C/min.

1- Condensation polymerization of(hydroxy --l2-ethene - (E)-N-((3-methoxy-4-(oxo--l3--methoxy)phenyl)methyl)-9-methyldec-7-enamide)(capsaicin-g-maleic anhydride)(H1)

in a round flask with a capacity of 50 ml, equipped with a condenser equipped with a magnetic stirrer. (1 gm.)(0.0032mol) o of capsaicin was dissolved in (8) ml (0.01N) sodium hydroxide. Litmus paper was placed and its color changed from yellow to pale green at pH = (4).Then (1.3) gm. of maleic anhydride were added to the above mixture, and it was escalated for (4) hours at a temperature of (70) degrees Celsius. The thick red oily substance was collected and dried in a vacuum oven at (50) degrees Celsius, to remove any remaining components, they were washed with (10) mL of diethyl ether(**Cienfuegos *et al*., 2017)** .

2- Preparation of Chitosan Schiff base

In a 50 mL round bottom flask equipped with a magnetic stirrer, (2) grams of chitosan was placed and dissolved in (14) mL of glacial acetic acid and a yellow gelatinous substance was obtained. Prepare(0.5% of sodium hydroxide)dissolved in distilled water and take (5) ml of it and add it to the gelatinous substance above. A litmus paper was placed and its color changed from yellow to pale green at PH=4. (2) ml of benzylhyde added to the above mixture, escalated for (8) hours at a temperature of (70) degrees Celsius. The light yellow gelatinous material was collected and dried in a vacuum oven at (60) degrees Celsius, to remove any remaining components, they were washed with (10) mL of diethyl ether **(Dos *et al* .,2006).** The same procedure is used to prepare Schiff's bases from the reaction of chitosan with various aldehydes (crotonaldehyde, propanaldehyde and salicylaldehyde).

3- Preparation of Capsaicin /Chitosan Schiff base

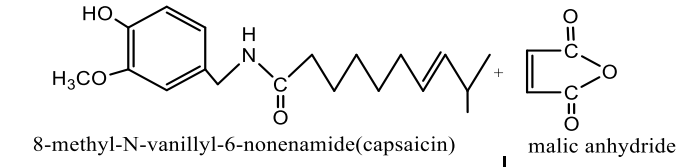
After preparing the above-mentioned capsaicin derivative, it was placed in a round flask with a capacity of 50 ml, equipped with a condenser equipped with a magnetic stirrer, and (2) gm. of Schiff bases (HCb) were dissolved in(6) mL of DMF.It was added to the above mixture, and it was escalated for a period of (6) hours at a temperature of (70) degrees Celsius. Article collected and,It was dried in a vacuum oven at (50) degrees Celsius. To remove any remaining components, they were washed with (10) mL of diethyl ether (**Duceac *et al* .,2020) .** The same method works for the rest of the Schiff bases used.

RESULTS AND DISCUSSION:

The synthesis of new derivatives began with the opening of the maleic anhydride ring by the hydroxyl group in capsaicin, whereby the anhydride ring was attacked as a nucleophile to give the maleic compounds products that interact with the chitosan-Schiff base. This reaction is illustrated in (Scheme 1). The first step، involved the preparation of a maleimic compound by cyclo-opening of anhydrous malic acid via the phenolic hydroxyl group of the capsaicin extract. The second step involved preparing Schiff bases derived from chitosan by reacting them with various aldehydes, which in turn reacted with a plant extract derivative (capsaicin). To obtain new Schiff derivatives, the resulting compounds were characterized by

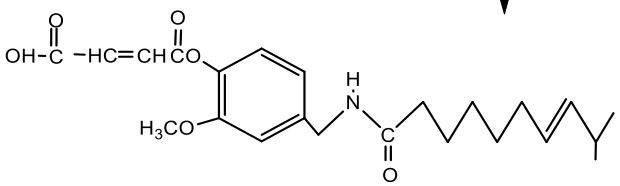
using FT-IR spectrometers listed in (Table 1), (Fig 1), (Fig 2), (Fig 3), (Fig 4), (Fig 5), (Fig 6), Fig 7).As well as 1H-NMR spectroscopy shawn in (Fig 8),(Fig 9), (Fig 10), (Fig 11). The thermal stability of the composites was studied by a thermogravimetric analyzer (TGA) and a differential calorimeter (DSC) in (Fig 12).Tthe biological activity was studied against two types of bacteria and fungi,it was shawn in (Table 2).

The results showed an increase in the antibacterial activity of chitosan against negative-gram and positive-gram bacteria through its high content of capsaicin, which is highly effective against bacteria and fungi.



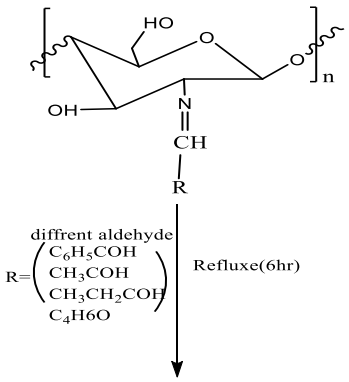
8-methyl-N-vanillyl-6-nonenamide(capsaicin) malic anhydride

Refluxe(4hr)



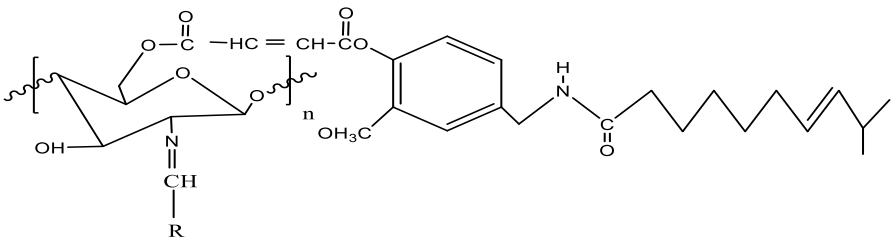
hydroxy -12-ethene - *(E)*-*N*-((3-methoxy-4-(oxo-13- methoxy)phenyl)methyl)-9-methyldec-7-enamide)(capsaicin-g-maalic anhydride)(H1)

+



diffrent aldehyde
R= C_6H_5COH, CH_3COH, CH_3CH_2COH, C_4H6O

Refluxe(6hr)



Chitosan/ Capsaicin Schiff base

Schem(1): preperation of schiff base of chitosan derivatives.

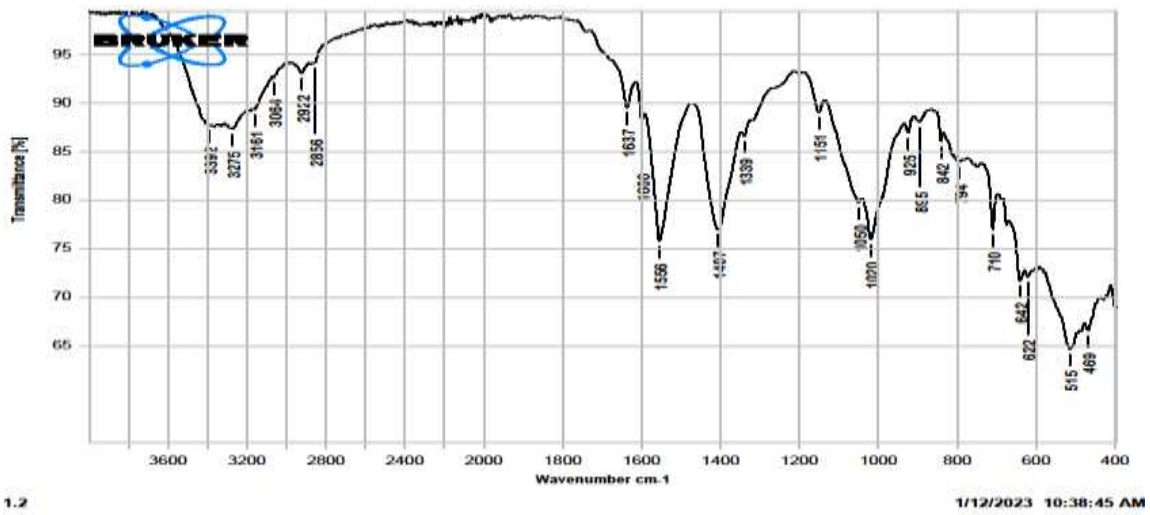
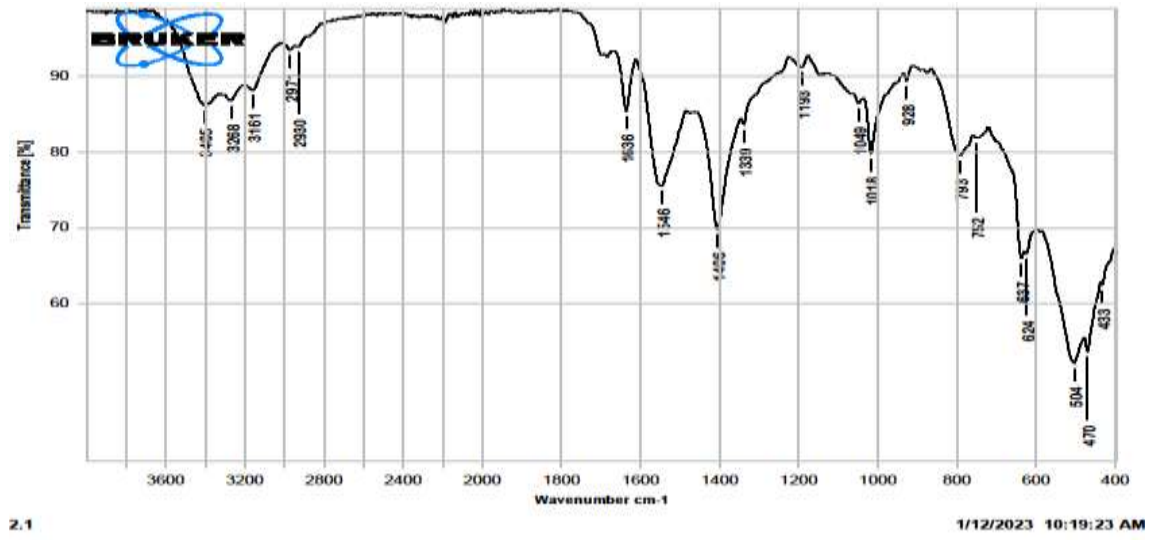
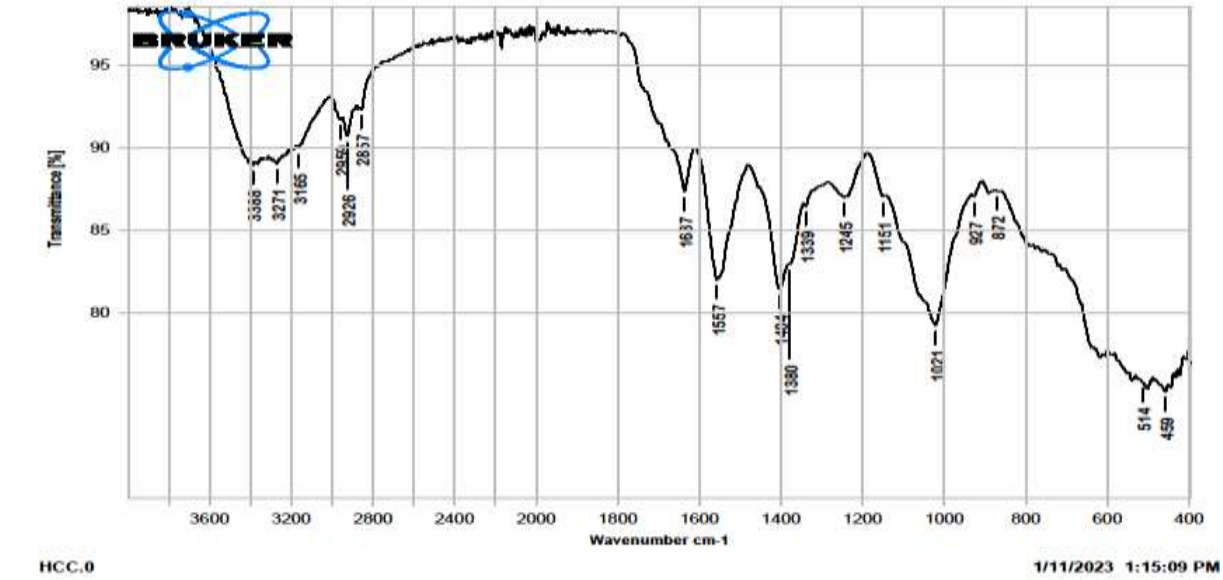


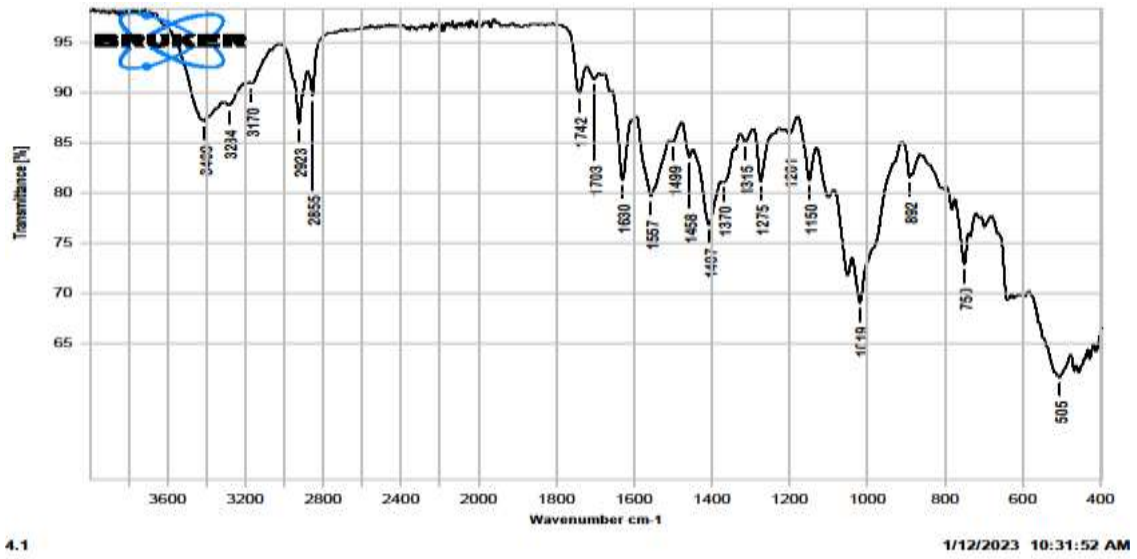
Figure (1): FTIR of (Chitosan with benzeldehyde) (HCb).



Figure(2): FTIR of (Chitosan with propanaldehyde) (HCP)



Figure(3): FTIR of (Chitosan with Crotonaaldehyde) (HCC).



Figure(4): FTIR of (Chitosan with Salcyaldehyde) (HCS)

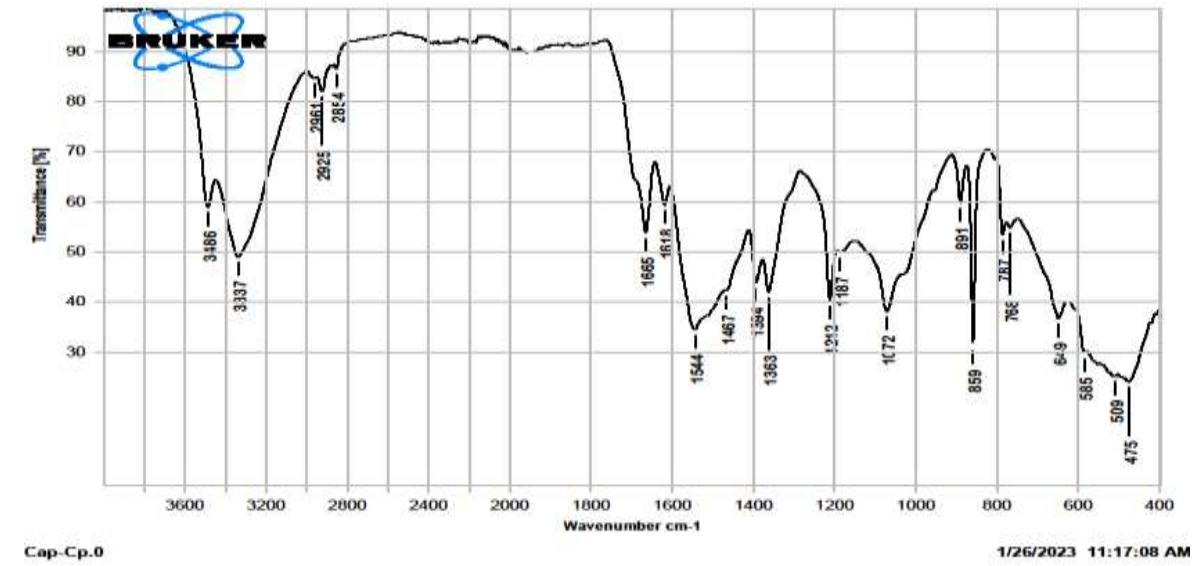


Figure (5): FTIR of (Capsaicin/ Chitosan Schiff base) (CAP-HCP).

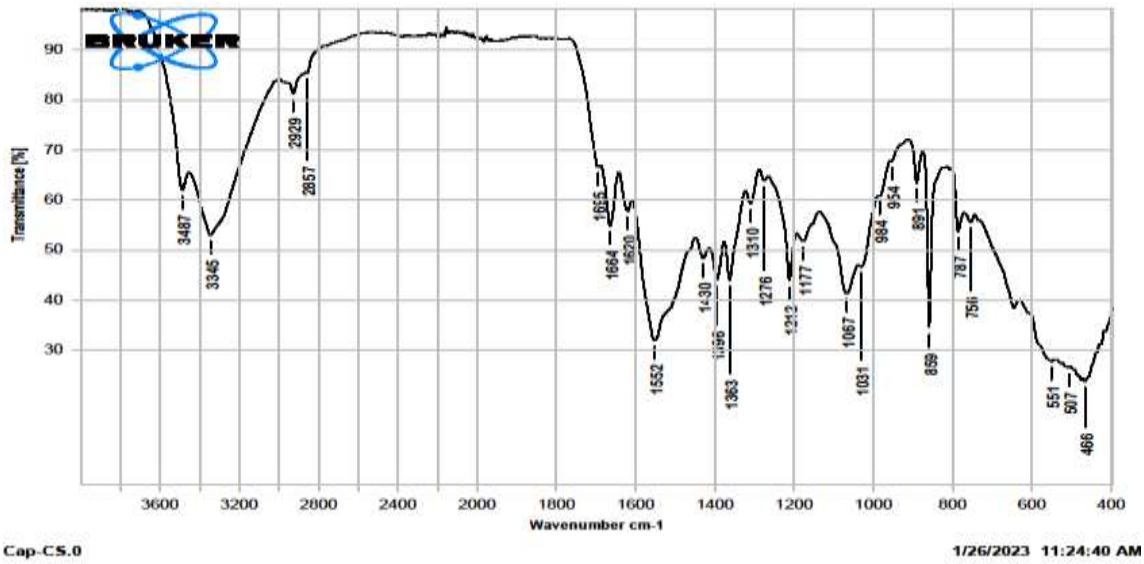


Figure (6): FTIR of (Capsaicin /Chitosan Schiff base) (CAP-HCS).

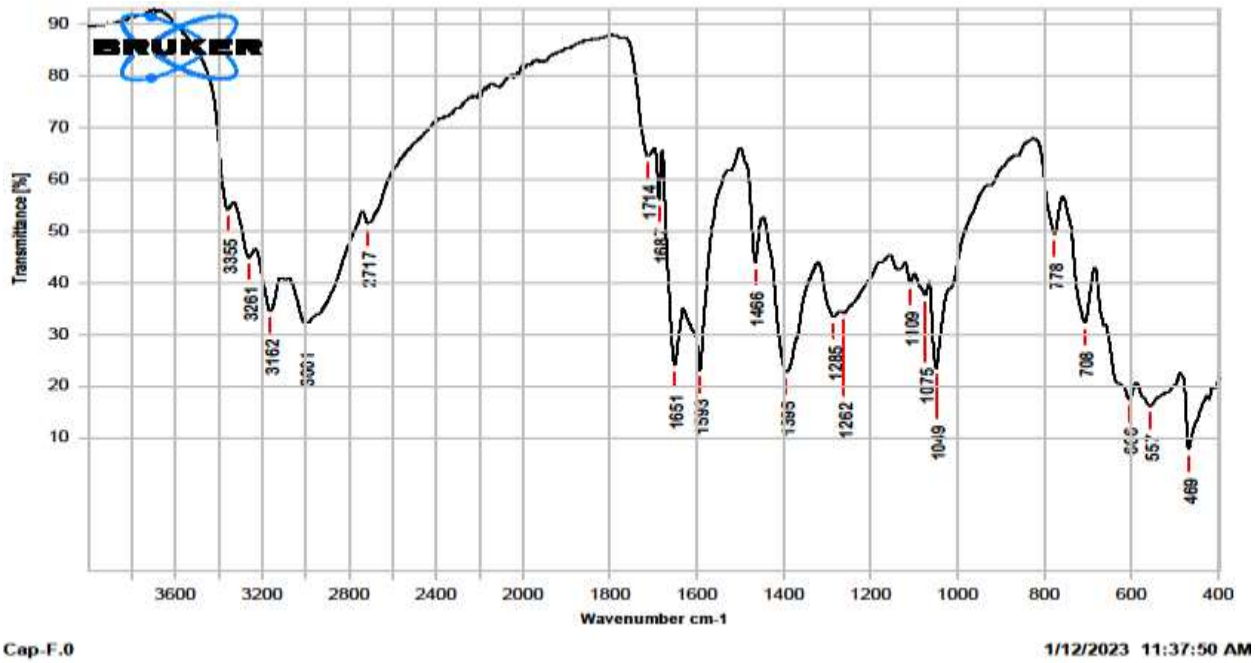


Figure (7): FTIR of (hydroxy- l2- ethene- (E)-N- ((3- methoxy-4- (oxo--l3- methoxy)phenyl) methyl)-9-methyldec-7-enamide)(capsaicin-g-maleic anhydride) (H1).

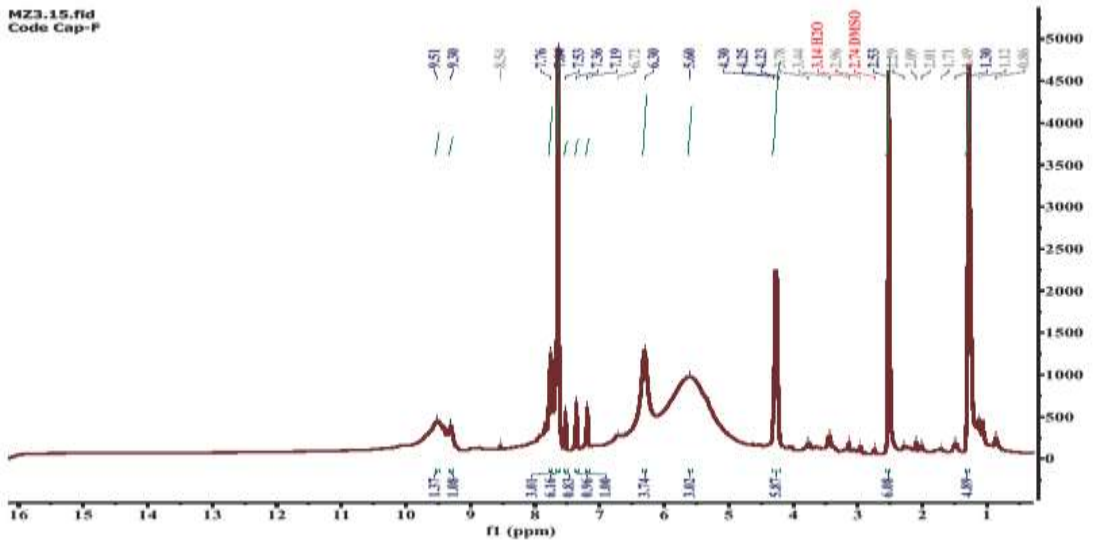


Figure (8): ^{1}H-NMR of (hydroxy -l2-ethene- (E)-N-((3- methoxy-4- (oxo-l3- methoxy) phenyl)methyl)-9-methyldec-7-enamide)(capsaicin-g-maleic anhydride) (H1)**.**

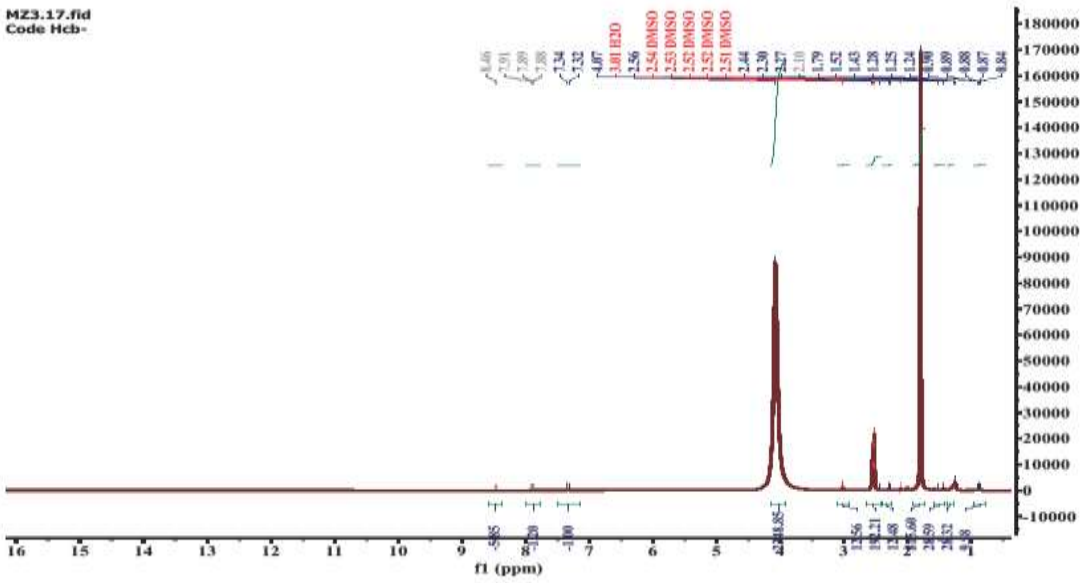


Figure (9): ^{1}H-NMR of (Chitosan with benzeldehyde) (HCb).

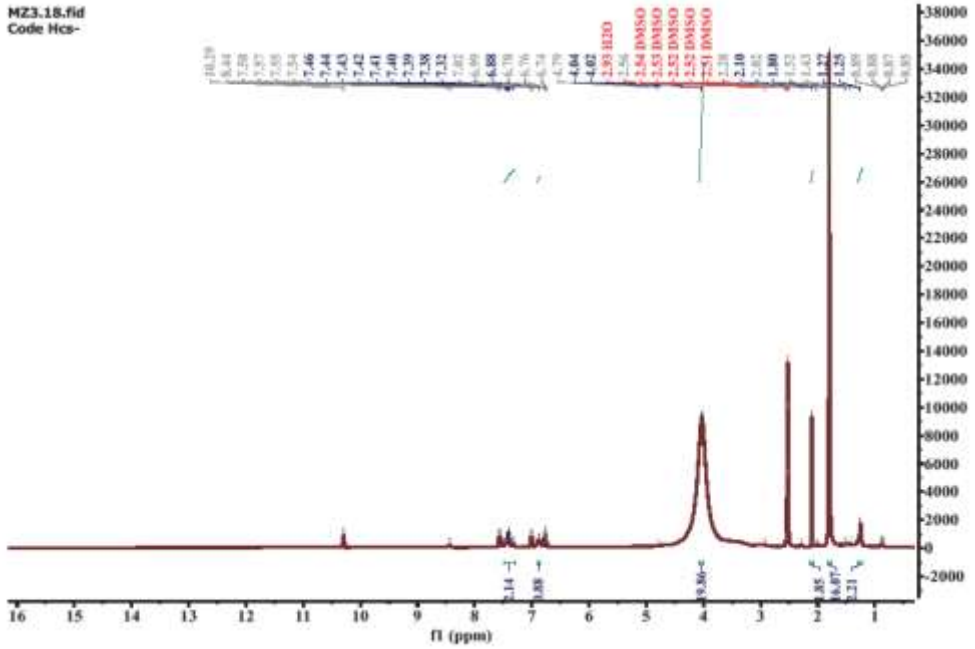


Figure (10): ^{1}H-NMR of (Chitosan with Salcyaldehyde) (HCS)

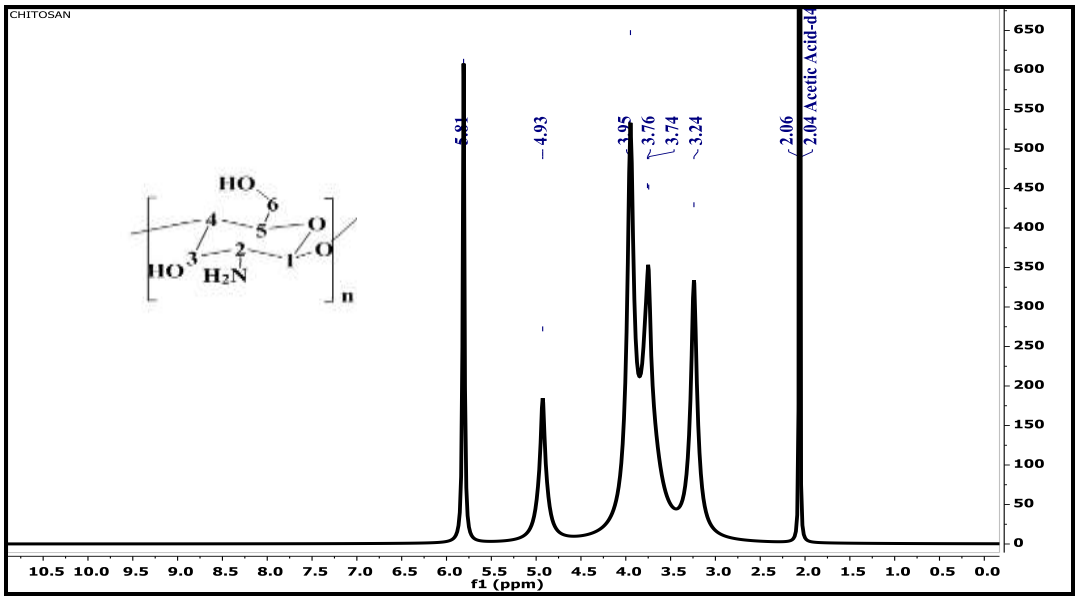


Figure (11): ^{1}H-NMR of Chitosan.

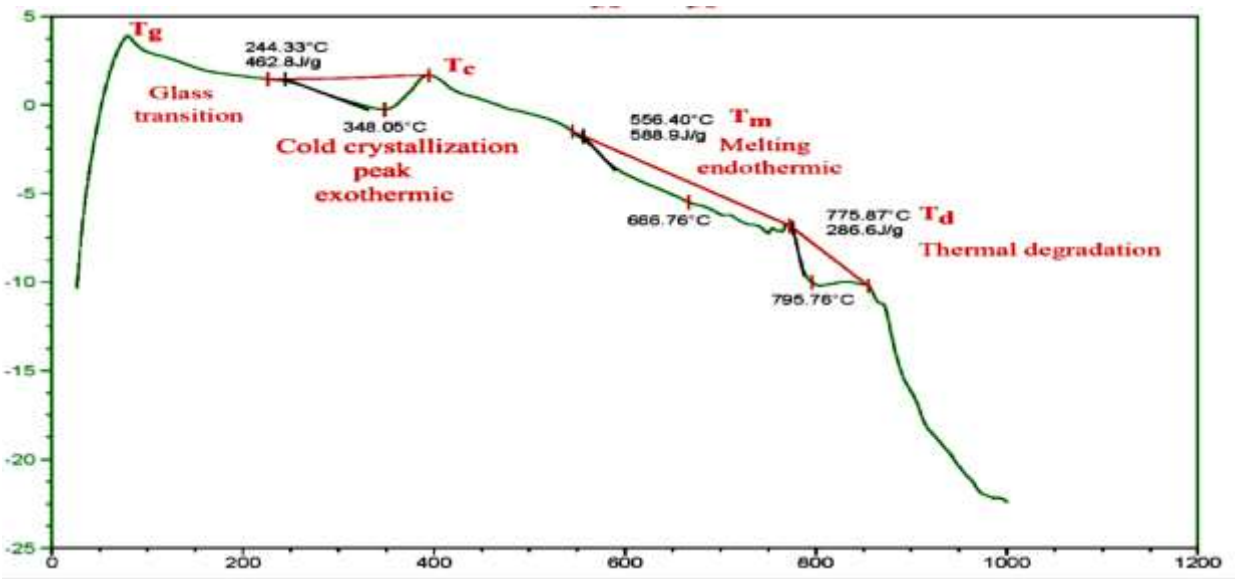


Figure (12): Thermal analysis of (hydroxy -l2-ethene- (E)-N-((3- methoxy-4-(oxo-l3-methoxy) phenyl)methyl)-9-methyldec-7-enamide)(capsaicin-g-maleic anhydride) (H1)**.**

Table (1): FTIR data for compounds [Hcb-H1].

com.	(O-H) cm^{-1}	(N-H) cm^{-1}	(C-H) cm-1 Aro.	(C-H) cm-1 Ali.	(C=O) cm^{-1} amide	(C=O) cm^{-1} ester	(C=O) cm^{-1} carboxylic	(C-N) cm^{-1}	(C=C) cm^{-1}
HCb	3295	3410	3064	2880-2922	1600	1637	-	1339	1407
HCp	3279	3405	3161	2880-2979	1636	1685	-	1339	1406
HCC	3365	3322	3271	2872-2926	1637	-	-	1339	1404
HCS	3284	3485	3161	2855-2923	1630	1662	1742	1339	1407
CAP-HCP	3337	3486	3001	2854-2925	1618	1665	1700	1336	1467
CAP-HCS	3345	3487	3001	2857-2929	1620	1664	1700	1396	1430
H1	3355	3230	3001	2880-2971	1651	1687	1714	1395	1466

Proton Nuclear Magnetic Resonance (^{1}H-NMR):

^{1}H-NMR of Chitosan : Nuclear magnetic resonance spectrum of chitosan has been shawn in (Figure, 11), a broad signal at (4.93 ppm), indicating a ^{1} H proton. In addition spectrum showed multiplet signals in the range of(3.24–3.95 ppm), corresponding to protons (H_3-H_6). The spectrum also showed a relatively broad singlet signal belonging to the H_2 protons at(3.24 ppm) (Lundquist, 1992). It is worth noting that this spectrum agrees exactly with the literature, with only minor changes in chemical shift values **(Kasaai *et al.*, 2010).** T NH protons do not appear to be overlapped with the solvent signal at 2.06 ppm **(Sieval *et al.*, 1998)**.

^{1}H-NMR of HCb: The^1 H-NMR of chitosan benzaldehyde has been shawn in (Figure, 9), it showed a broad signal at (4.76 ppm), indicative of the H_1 proton. In addition, the spectrum showed multiplet signals in the range of (3.85–4.04 ppm) corresponding to protons (H_3-H_6). The spectrum also showed a relatively broad singlet signal associated with the H_2 protons at(3.54 ppm). In addition, the spectrum shows the characteristic signals of the added molecule (benzaldehyde) as two multiplies in the range of (7.80-7.89 ppm), and (7.20-7.26 ppm), which are assigned to the HCb protons of the aromatic ring, respectively. In addition, the spectrum showed a singlet signal at 8.46 ppm, which is assigned to the CH proton of the azomethine group. The high-intensity signals at 2.50 and 3.37 ppm represent the protons of DMSO and H_2O, respectively **(Kasaai *et al.*, 2010)**.

^{1}H-NMR of HCS: The ^{1} H NMR of chitosan salicylaldehyde has been shawn in (Figure 10),it showed a broad signal at 4.79 ppm assigned to the H1 proton. In addition, the spectrum showed multiplet signals in the range of (3.71-3.79 ppm) corresponding to protons (H_3-H_6). The spectrum also showed a broad singlet signal belonging to the H_2 proton at 3.60 ppm. In addition, the spectrum shows the characteristic signals of the added molecule (salicylaldehyde) as multiplate signals in the range of (6.76–7.47 ppm), which are assigned to the protons of the aromatic ring (CH(a,b,c)). The protons of the OH group (aromatic ring) appeared at (10.02 ppm) as a singlet signal. The proton of the azomethine group appeared at 8.45 ppm. **(Kasaai *et al.*, 2010).**

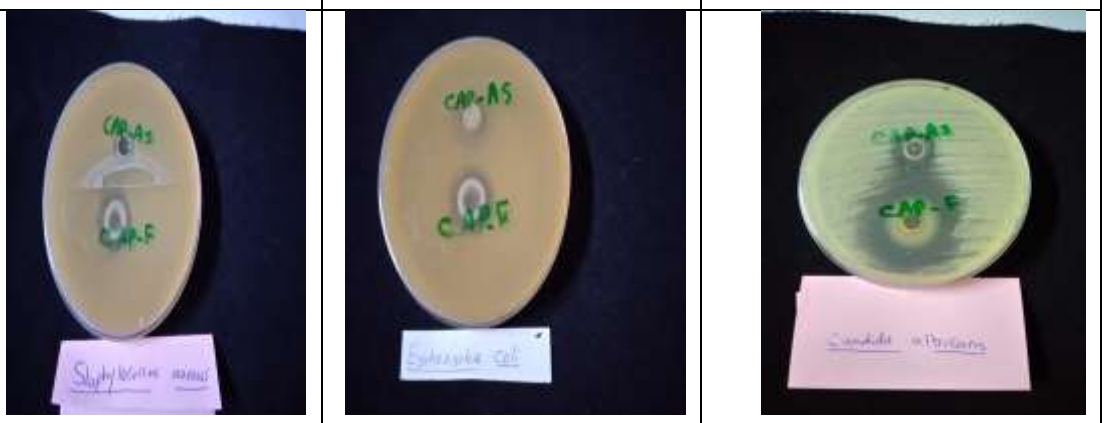
Thermal analysis:

The heat emission peaks at a temperature of 348 C^0. The enthalpy is (463 J/g) which is the cold crystallization phase CT as shown in Fig.8 (H1) followed by a broad peak. of heat absorption at about (667 ^oC) for the (H1) enthalpy. The fusion in this case is (589 J/g), which can be attributed to the Tm fusion phase that occurs on the crystalline chain-linked polymer of capsaicin-malic anhydride, which chain fusion is thought to be random chain fusion of polymer chains or what known as attachment therapy, the luxury of absorbing heat and continuing the heating cycle, polymer degradation occurs at (796 °C), which is the highest temperature that observed.

Biological activity:

Candida albicans is caused by human fungal pathogens that cause candidiasis. *Candida albicans*، A pathogenic yeast that is part of the human gut microbiota. It is also able to live outside of the human organism. This study includes the evaluation of the antifungal activities against *Candida albicans* in three different concentrations of the polymers and showed moderate to good antifungal activity, (Table, 2).The polymers and their antibacterial activity were tested against *Escherichia coli* and *Staphylococcus aureus* (in vitro). DMSO was used as a reference by the agar diffusion technique at a concentration of (1 mg) since there was no visible change in bacterial growth and the plates were incubated (24 hrs) at (37°C). The inhibition area is measured in (mm) and the results are shown in (Table, 2). It was found that all polymers had a larger diameter of the growth inhibition zone and showed low activity against *Escherichia coli* **(Agarwal *et al*., 2020).**

Table (2) : Inhibition zone(mm) for tested bacteria using prepared treatments.

			
			
Comp.	***Staphylococcus***	***E. Coli***	***Candida***
HCb	10	8	10
HCS	12	8	12
Cap-M	25	24	32

CONCLUSION

Schiff's chitosan/capsaicin base was successfully prepared by coupling the carbonyl group of chitosan to the carbonyl group of capsaicin. The thermal stability (TGA) was measured and the compounds prepared were determined by FT-IR and ^{1}H-NMR measurements. The antimicrobial activity of chitosan/capsaicin derivatives of Schiff's bases was studied. The materials prepared showed activity against gram-positive and gram-negative bacteria and fungi. The biological activity of the copolymers was determined to determine if these materials have medical applications.

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