ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com
Journal DOI:10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Comparative Studies on CMC - Produced from Non- and Woody Lignocellulosic in Production of Biological Active Materials.

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Manuscript Info

Manuscript History:

Received: 12 May 2016
Final Accepted: 19 June 2016
Published Online: July 2016

Key words:
Viscose pulp, Bagasse pulp, 2-Amino-3-phenylpropanoic acid (APPA), CMC-APP conjugate, and Antibiological activity.

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Abstract

The present study deals with synthesizing novel nano-cellulose derivative from non and woody lignocelluloses, and evaluating its biological activities. The biological analysis together with conventional analysis (nitrogen content, **FT-IR**, and **TGA** analysis) were detected. Optimizing the **CMC** and **APPA** conjugate preparation conditions were studied. The **TEM** study showed that, the synthesized viscose pulp **CMC-APP** derivative was 30 to 70 nm. The antimicrobial and anticancer activities of these nano- **CMC** derivatives against four bacterial strains andsix fungal strains, as well as MCF-7 were tested. Among all the tested microorganisms and MCF-7 the synthesized nano-cellulose conjugate possible used as safety medicine for microbial infections and cancers, with **MIC** for Gram-positive bacteria 2.0µg/mL in viscose pulp conjugate, and gram-negative 4.0µg/mL in bagasse pulp. The bagasse pulp conjugate appearing has high antifungal activity *Aspergillus awamori*4.0µg/ml. Viscose pulp conjugate shows the anticancer activity with **IC50** for MCF-7 breast cancers 32 µg/ml.

Introduction

The natural biopolymers play an important role in our lives, because they are safe, available, environmental friendly, biocompatible, functional products and nontoxic in general. Cellulose and its derivatives are the most wide spread natural products in our lives. They are commonly used in pharmaceutical, medical, and food industries. Their derivatives are used in pharmaceutical industry as filler with active ingredients in the tablets **[1]** and added to formulas to increase drug bioavailability and release. For example mixing of cellulose and / or cellulosic derivatives with anticancer drugs active ingredient (**Docestal**) improves its bioavailability and stability **[2].** Carboxymethyl cellulose (**CMC**) is an excellent example for ether cellulose derivatives which is an anionic water soluble natural polymer derivative, widely used in detergents, oil exploration,food, paper, and textile industries because of its viscosity-increasing properties. **CMC** is reported to be synthesized from diverse plant biomass with monochloroacetic acid in presence of alkali. The characteristics of **CMC** such as biocompatibility, biodegradability, and nontoxicity make it the favorable material for medical applications **[3].** Also, cellulose and its derivatives are an example for natural high molecular weight bulky in which conjugate with 2-amino-3-phenylpropanoic acid (**APPA**). Bulky group inhibition systems are one of the new promising antibiological stratigies**[4].APPA** is an α-amino and essential amino acid. It is classified as a nonpolar because of the hydrophobic nature of the benzyl side chain **[5].APPA** is a precursor for tyrosine, the monoamine signaling molecules, dopamine, norepinephrine (noradrenaline), epinephrine (adrenaline), and skin pigment melanin. **APPA** is found naturally in the breast milk of mammals **[6].** It is used in the manufacture of food and drink products and sold as a nutritional supplement for its

reputedanalgesic and antidepressant effects. It is a direct precursor to the neuromodulator phenylethylamine, which is used as a commonly dietary supplement **[7].**

Cancer is the second leading causes of death after heart attack over the world, and the numbers of cancer cases are increasing gradually **[8 - 10]**. Breast cancer is the most common cancer among women, accounting for 32% of female cancers, and leading causes of cancer mortality **[11,12].** It affects about 12% of women worldwide **[13].**The development of a new class of anticancer drugs that lack the toxicity of conventional chemotherapeutic agents and are unaffected by common mechanisms of chemoresistance would be a major advance in cancer treatment. The most common form of cancer is non-invasive non-melanoma skin cancer; non-invasive cancers are generally easily cured, cause very few deaths, and are routinely excluded from cancer statistics**[14, 15].**

The traditional cancer treatments by radiotherapy and chemotherapy despite are lead to killing the cancer cells but they are toxic to the other normal cells. No drug is found to be fully effective and safe **[16]**. Some natural compounds are used as drug additive to enhance the effect of other drug substances, however itself hasn`t any activity but the combination between the natural compounds makes it biological active substance **[17].** Some of these common anticancer compounds are illustrated in Table 1.

Table 1:-The IC_{50} of some anticancer substances.

Type	Material	IC 50	Reference
Natural compounds	Extract of ***Mucunapruriens***	14 – 16 mg/ml	**[18]**
Polypeptides	Short length peptides	15 µg/ml	**[19]**
Heterocyclic	Pyridine derivative	5-300 µg/ml	**[20]**

The reason of selection these substances is referred to its biological compatibility and nontoxicity characterizes of both **CMC** and **APPA**. The blocking or disturbance of the phenylalanine makes cells deformation.

In continuation of our previous work on market **CMC** [**21**], the objective of the present work, is dealing with evaluating the source of cellulose substance as a precursor for synthesis carboxymethylcellulose-2-amino-3-phenylpropanoic acid (**CMC-APP**) derivative and consequently testing its behavior as biological and anti-cancer compound. In this respect, sugar cane bagasse and viscose pulps were used as non-wood and wood fibers substances, for preparing CMC, and synthesis **CMC-APP** derivatives.

Materials and Methods:-

Materials:-

α-Cellulose substances: Sugar cane bagasse was kindly delivered from Quena Co. for paper production- Upper Egypt. It was subjected to different treatments, namely: pulping (12% NaOH, liquor ratio 1: 20 steamed for 30 min), bleaching (Ratio was 1: 1.2 NaOCl in presence of acetic acid for 4hrs at 75^{o}C),and alkali extraction (2% NaOH with liquor ratio 1 : 10, for 1hr at 75^{o}C).The bleached produced pulp was treated by17.5 % NaOH, 1 : 17 for 40 min at room temperature to obtain α-cellulose. Viscose pulp was delivered from Miser Viscose Co. Alex.-Egypt. Its α-cellulose was obtained, by treating directly the raw viscose pulp by alkali treatment (2% NaOH, as previously stated).The chemical constituents of these prepared cellulosic substances are presented in
Table 2:- 2-Amino-3-phenylpropanoic acid (APPA)(purity 99.5 %) was purchased from Oxford, India.

Table 2:-Chemical analyses of raw lignocelluloses materials.

Lignocellulose	Kalsonlignin,% [**22**]	Holocellulose,%	Pentosane,% [**23**]	α-cellulose,%	Ash, % [**24**]
Bagasse pulp	7.2	91.8	8	80	1.0
Viscose pulp	0	99.40	7.30	92.17	1.50

Microbial media:-

Mueller Hinton Agar medium was purchased from Mast Group Ltd., Merseyside, U.K.
Potato Dextrose Agar, ATCC Media-336 was purchased from SISCO Research Laboratories PVT. LTD. MUMBM, 400099, India.

Tissue culture media:-

RPM1 10% fetal calve serum, was purchased from Sigma-Aldrich.

MTT reagent, Thiazolyl Blue Tetrazolium Bromide (MTT), was purchased from Sigma-Aldrich.

Preparation of CMC:-

Carboxymethylcelluloses(CMCs) were synthesized from the preparedα-cellulose of sugar cane bagasse and viscose pulps. The non-aqueous methodwas used **[25].** Different monochloroacetic acid / soda ratio were used to obtain different degree of substitutionsDSs, Table 3.The constant reaction conditions were (368ml ethanol, 332ml toluene/ 6gms pulp for 30 min. at room temperature, neutralize and washed with ethanol 80%, and finally, washed with absolute alcohol and dried at 70^0C).

The prepared CMCs (2 gm) are subjected to purification through dissolving CMC in80%ethanol with ratio 1:50 at 90^0C for 0.5 hr, filtered on sintered glass (G2), the crude CMC washed several time with 80% ethanol followed by drying at 105^0C**[26].**

$$\text{Purity \%} = \frac{\text{Wt. after purification}}{2\text{g}} \text{ X } 100$$

The average degree of substitution (**DS**) of carboxymethyl cellulose was estimatedby converting the sodium CMC to acid form, adding excess sodium hydroxide solution and titrating the excess alkali**[27]**.This was repeated three times for each sample and the average value of the equivalent volume was taken for the DS calculation. The DS was calculated according to the following equation:

$$DS = \frac{162 \times n_{COOH}}{m_{ds} - 58 \times n_{COOH}}$$

where n_{COOH} (in mol) is the amount of COOH calculated from the obtained value of the equivalent volume of known molarity NaOH (1 M) and *m*ds (in g) is the mass of dry sample calculated from known sample mass *m*s (in g) and the water content, *W* (%).

$$m_{ds} = \left(1 - \frac{w_{water}}{100}\right) \times m_s$$

Modification of CMC:-

The synthesis process of CMC-APP was carried out by mixing CMC with the desired amount of **APPA**.**CMC/APPA** ratio was(1: 1.5) and heated for 1.5 hrs,at ~ 250 ^{o}C, then the product was subjected to microwave 1100 watt for ~ 30 sec/g. The resulted CMC derivative was washed several times by bi-distilled water, and air dried. Its nitrogen content was estimated, using Vario El Elemental, Germany.

Evidence of formation nano-CMC-APP derivative:-

FTIR analysis:-

Infrared spectra were recorded with a Jasco FT/IR, Nicolet, Model 670. The samples were mixed with KBr and form disks. The bands were recorded in the region from 4000 to 400 cm^{-1} with Deuterated Triglycine Sulfate DTGS. This test characterized the functional groups in the prepared nano-cellulose derivative. The crystallinity index **(Cr.I.)**was calculated according to Nelson and O'connor**[28].** The mean strength of hydrogen bonds **(MHBS)** was calculated **[29].**

Thermal analysisTGA:-

Non-isothermal thermogravimetric analyses were performed in a Perkin Elmer Thermogravimetric Analyzer TGA7. The samples were heated in pure nitrogen (flow rate 50 mL/min) at 10 °C /minute, and within the typical temperature range: 35 – 600 ^{o}C, i.e., till no additional weight loss was observed. Measurements were made using calcined alumina as reference material. Differential thermogravimetric **DTG** peaks were examined for evidencing different behaviours between the **CMC** and its modified compounds, and clarifying how the modification affected the thermal stability of modified **CMCs**. The kinetic parameters based on the weight loss data of TG curve analysis were determined according to the equations described elsewhere **[20, 31].**

Transmission electron microscopy (TEM):-

Morphological Characterization of the synthesized **CMC-APP** was carried out by using transmission electron microscopy, of type QUANTA FEG250, Japan.**(**System running at 200 keV).

Antibiological activity:-

Antimicrobial plate diffusion method:-

Four bacteria strains, *Staphylococcus aureus* (NCTC-7447), *Bacillus subtilis* (NCID-3610) as Gram-positive bacteria, and, *Escherichia coli* (NCTC-10416),*Pseudomonas aeruginosa* (NCID-9016) as Gram-negative bacteria were used. One colony of each bacterial strain was suspended in a physiological saline solution (NaCl 0.9% in distilled water at pH 6.5) **[32, 33].**

Anticancer activity MTT assay:-

The Microculture Tetrazolium Assay **(MTT)** was carried out as described elsewhere **[34, 35].** Cell viability was observed and cytotoxic index **(IC_{50})**was calculated. MCF7(Breast cancer) 2.5 × 104 cells/ml, was seeded in seven columns of 96 well micro-plates and incubated for 24 hrs (37 ^{0}C, 5% CO_2 air humidified). Then 20 µl of prepared concentrations (40.0, 4.0, 0.40, 0.04, and 0.0 µg/ml as negative control) of extract was added to each column and incubated for next 48 hrs in the same condition. The untreated cells incubated for 48 hrs are specified for control. Toevaluate cell survival, 20 µl of MTT solution (5 mg/ml in phosphate buffer solution) was added to each well and incubated for four hours. After four hours, carefully the supernatant was removed leaving formazan crystals. 150 µl of dimethyl sulfoxide (DMSO) was added to each well. The crystals were dissolved and the absorbance was readied at 570 nm.

Results and Discussion:-

Optimizing the synthesis of CMC conditions:-

As shown in Table 3. The**CMCs**were prepared by using different momochloroactic acid and sodium hydroxide ratio / pulps, to obtain different **DSs**.The highest**DS** was obtainedby using conditions of Expt. 1, and lowest **DS**. by using conditions of Expt. 2. The solubility of these prepared **CMCs** in water was tested.

Table 3:-CMC preparation conditions and its characterization.

α-cellulose	Sample code	Pulp : Soda : Monochloroacetic acid	Water solubility	DS	Purity, %
Soda bagasse pulp	Exp. 1	1 : 2 : 2	Transparent viscous solution	0.95	69.1
	Exp. 2	1 : 2 : 1	Turbid solution	0.30	61.7
Visose pulp	Exp. 1	1 : 2 : 2	Transparent viscous solution	0.86	72.5
	Exp. 2	1 : 2 : 1	Turbid solution	0.45	83.3

Characterization of conjugate:-

FT-IR:-

Infrared spectroscopy analysis gives some useful information about the function groups of the structural changes during modification of fibers, **CMCs**, and conjugatedone were shown inFig. 1. IR measurements were illustrated in Table 4.

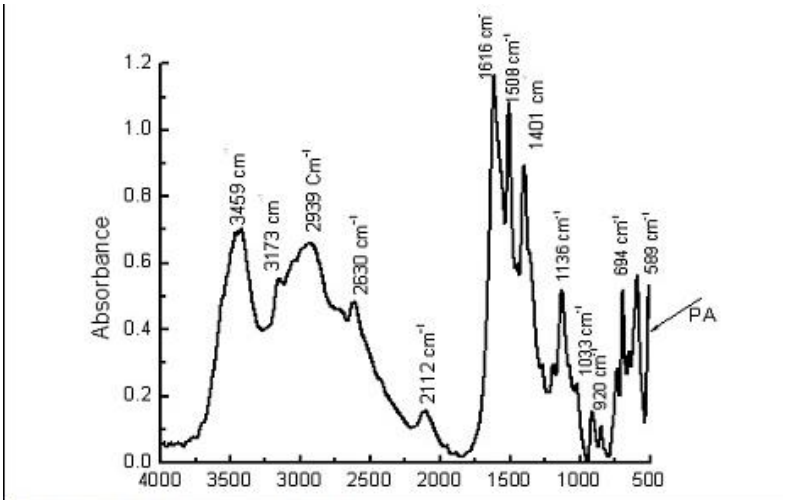

Fig. (1)-A: FT-IR spectra of APPA

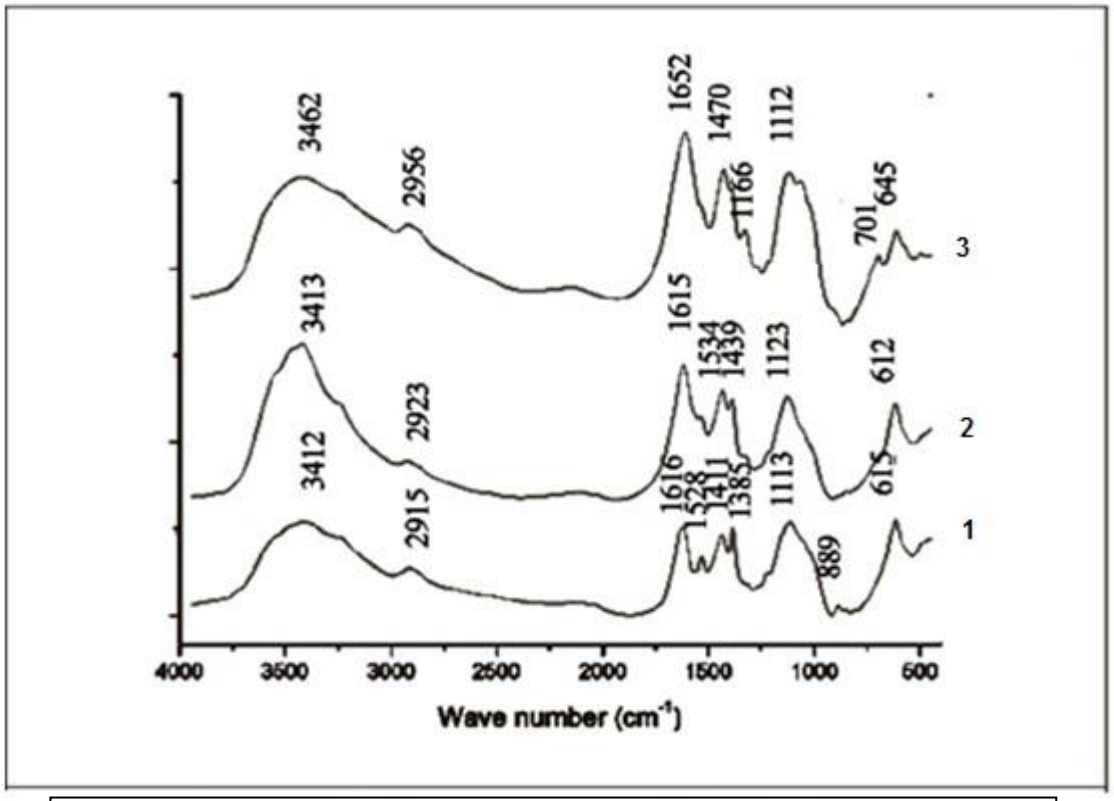

Fig. (1)-B: FT-IR spectra of α-cellulose (1), CMC (2), and conjugated (3) bagasse pulp

Table 4:-FT-IR measurements of α-celluloses and its conjugates compounds.

Sample	MHBS ($A_{OH(Str.)}$ /$A_{CH (Str.)}$)	Cr.I ($A_{\sim1370\ cm^{-1}}$ /$A_{\sim2900\ cm^{-1}}$)	ν (OH) str. Cm^{-1} (Δν, cm^{-1})	Bands of carboxylate
α-cellulose bagasse fiber	1.96	1.25	3412	1616.5=0.3
CMC bagasse pulp	2.32	1.72	3413(1)	1615= 1.1
Conj- bagasse pulp	1.45	1.01	3462 (50)	1652= 0.19
α-cellulose viscose fiber	3.93	1.87	3421	1615= 0.30
CMC viscose pulp	7.14	2.84	3405 (-16)	1611= 0.41
Conj- viscose pulp	1.26	1.35	3380 (59)	1574= 0.29
Phenylalanine	1	0.97	3404	1610.2= 0.28

Thecarboxymethylation of bagasse and viscose fibers leads to an increase in the mean strength of the hydrogen bond and the crystallinity index, in comparative with unmodified fibers. This confirms that the free unsubstituted hydroxyl groups of **CMC** (2ry alcohol) are involved in the carboxymethylation reaction, than the formation of hydrogen bond.(Inter and intra molecular hydrogen bonds). However, the modification of **CMC** by **APPA** leads to decrease in **Cr.I** in both **CMCs** cases as well as shift the carbonyl group starching position to higher frequency in both CMCs also. This probably ascribed to the 2ry alcohol which is condensed with COOH group included APPA and consequently may be lead to liberate the hydrogen bonded of 2ry hydroxyl, during synthesis processes.

Nitrogen content:-

Nitrogen content of the modifiedα-cellulose fibers was 1.43 for bagasses and 2.83 for viscose conjugated pulps.

TGA:-

Fig. (1)-C: FT-IR spectra of α-cellulose (1), CMC (2), and conjugated (3)viscose pulp.

The **TGA** and **DTG** curves for bagasse and viscose pulps (fibers, **CMCs**, and conjugates) which were heating at rate 100C/min are shown in Fig. 2,3as well as thermal kinetic parameters of α-celluloses and its derivatives in Tables 5, 6.

Table 5:- Thermal kinetic parameters of α-cellulose bagasse pulp and its derivatives

Cellulose samples	stage	Temp. range °C	DTG peak temp. °C	"n"	-r	Se	E_a kJ/ mole	Wt. %
α-cellulose fiber	1st 2nd 3rd	50-102 206.4-333.2 333.2-443.7	63 277.1 468.1		- 0.991 0.962	- 0.1650.0.146	- 119.557 144.776 ΣEa= 264.333	96.8 19.85 11.089
CMC	1st 2nd 3rd 4th	50-89 232.4-330.7 659.8-831.3 847.74-958.06	54.6 291.8 804.9 900.9		- 0.934 0.9790.953	- 0.174 0.151 0.254	- 254.8782 291.7334 664.7356 ΣEa= 1211.347	89.02 56.1 35.88 30.67
CMC-APP	1st 2nd 3rd 4th	50-101.5 216.0 - 332.9 669.08-821.78 821.78-951.49	64.0 288.5 794.8 912.4		- 0.995 0.968 0.979	- 0.119 0.1766 0.1608	- 215.7227 305.524 530.0308 ΣEa= 1051.277	88.09 57.27 39.35 29.12

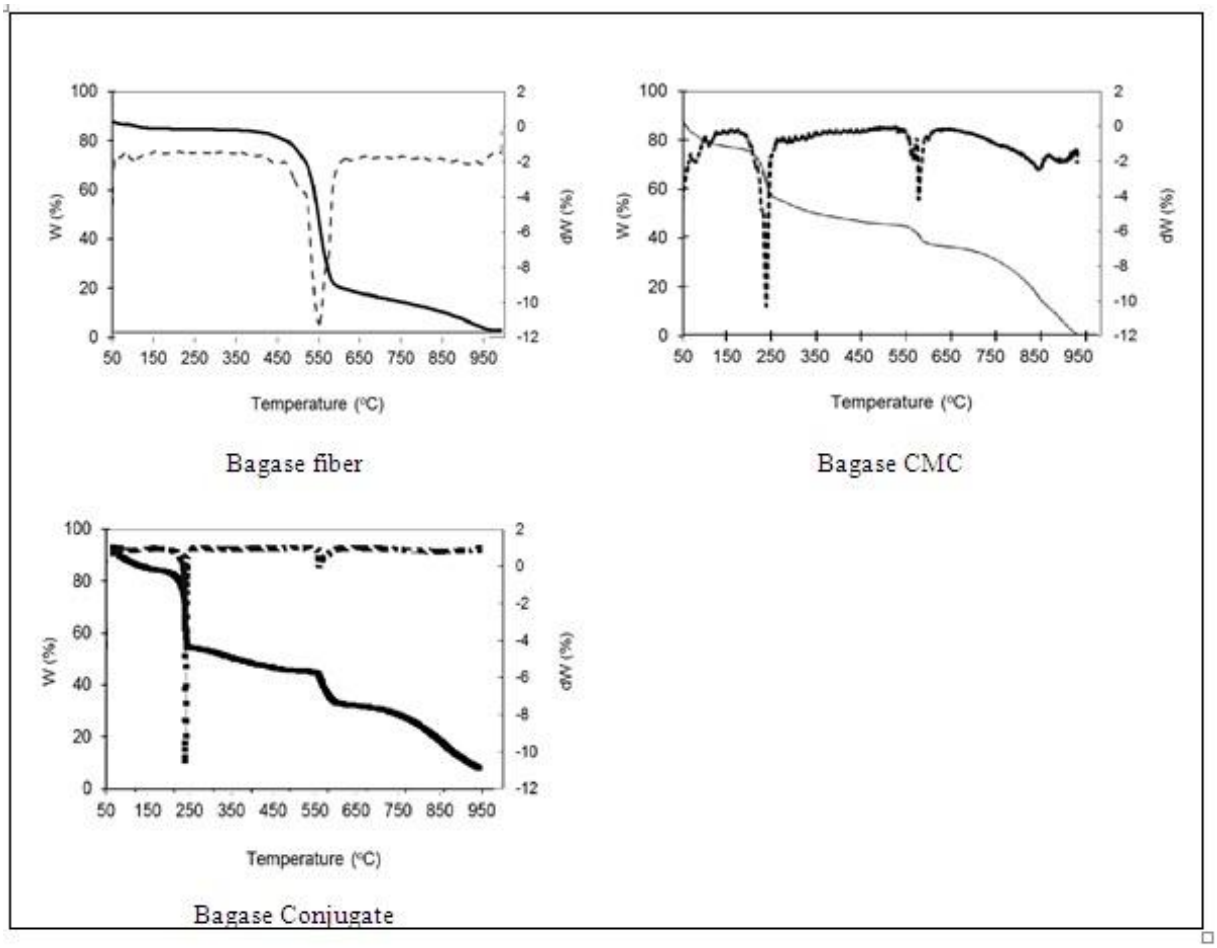

Fig. 2:-Thermal kinetic parameters of α-cellulose bagassepulp and its derivatives.

Table 6:-Thermal kinetic parameters of α-cellulose viscose pulpand its derivative

Cellulose samples	stage	Temp. range °C	DTG peak temp. °C	"n"	-r	Se	E_a kJ/ mole	Wt. %
α-cellulose fiber	1st 2nd 3rd	50-108 240.1-363.01 640.59-918.2	131.78 290.58 819.29	- 1.5 1.5	- 0.991 0.982	 0.117 0.121	- 182.727 164.7095 ΣEa= 347.436	91.87 24.93 5.8
CMC	1st 2nd 3rd 4th	50-107.21 185.15-231.04 231.2-252.01 566.4-586.73	78.3 221.9 238.8 577.6	- 1.0 1.0 1.5	- -0.971 0.960 0.982	- 0.182 0.241 0.213	- 252.024 432.20 228.7 ΣEa= 912.924	94.5 67.19 48.94 24.507
CMC-APP	1st 2nd 3rd 4th	50-102.42 137.4-264.84 264.8-338.8 591.8-699.2	62.2 235.9 292.21 716.63	- 1.0 2.0 1.5	- 0.989 -0.982 -0.915	- 0.127 - 0.209 - 0.277	- 98.9282 325.7756 324.7514 ΣEa= 749.454	94.89 51.09 31.23 23.61

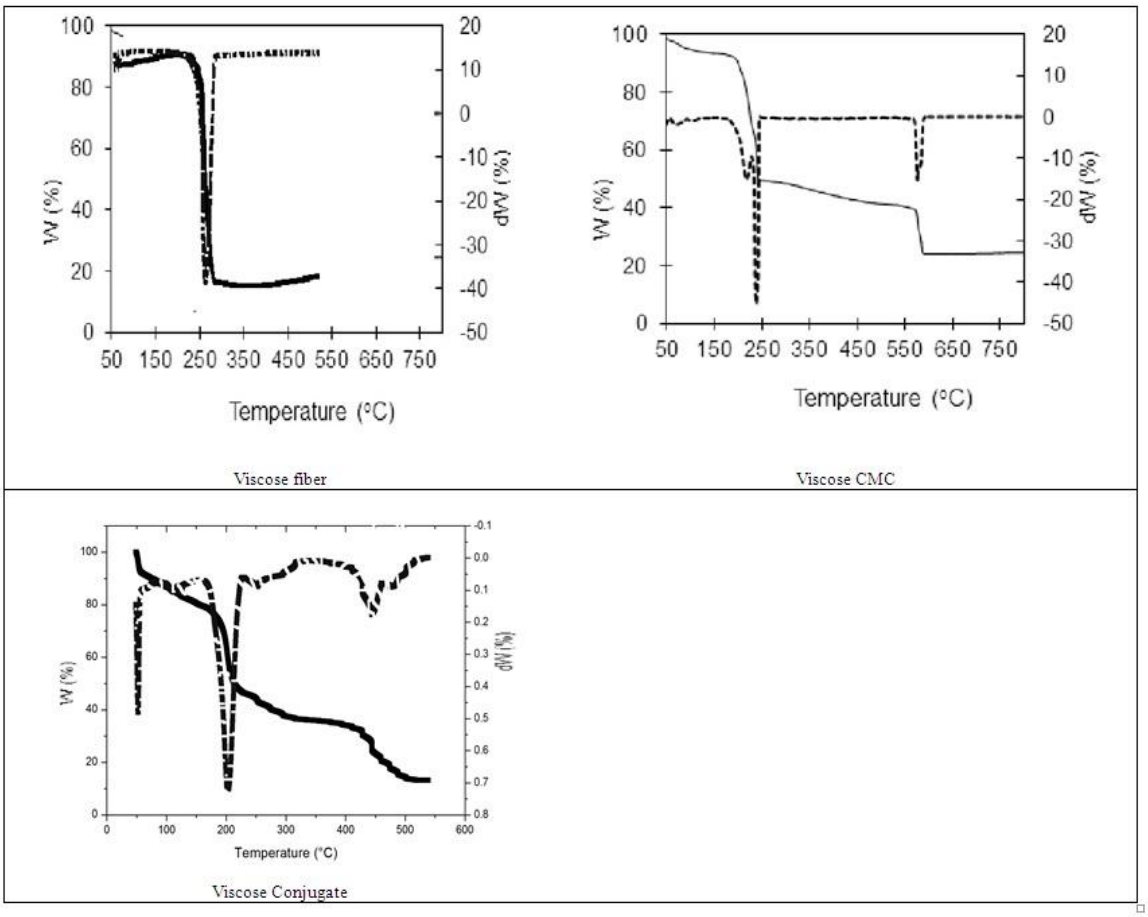

Fig. 3:-Thermal kinetic parameters of α-cellulose viscose pulpand its derivatives.

The DTG curves show the degradation rate of the samples. It can be seen that both non- and wood fiber exhibited a three-step degradation pattern. As samples contain a small quantity of water (water of free), the first stages of degradation (up to about 108^0C) represent the evaporation of the water of degraded components. The second stages of degradation peak was (up to about 291^0C) represent the volatilization of the easily degraded components**[36]**. The major difference between two fibers took place in the third step, while the bagasse fiber has its peak value at 443.7 ^{0}C and viscose fiber has its peak value at819.29. The total activation energy also shows the big difference between the both fibers which is 264.33 kj/mole for bagasse fiber and 347.44 kj/mole for viscose fiber.

In contrast to the CMCs from the both fibers origins. The bagasse CMC shown the great third and fourth peaks than viscose CMC as well as the total activation energy, 1211.34 and 912.92 kj/mole, respectively. The thermal degradation rate in CMC-APP was carried out in the same CMCs case. Overall, the thermal stability of viscose CMC and its conjugate is lower than the bagasse one.

Transmission electron microscope (TEM):-

Nano-size plays an important role in biologically active substance, The TEM photograph of our investigated CMCs derivatives show that the particle size sited between 30 – 70 nm in viscose CMC-APP case and between 70 – 100 nm in bagasse pulp CMC-APP. These nano forms confirm the visualized of viscose pulp nano-CMC-APP to easier moves than bagasse pulpnano-CMC-APP. Their affects and behaves their biological active material are shown in Fig. 4.

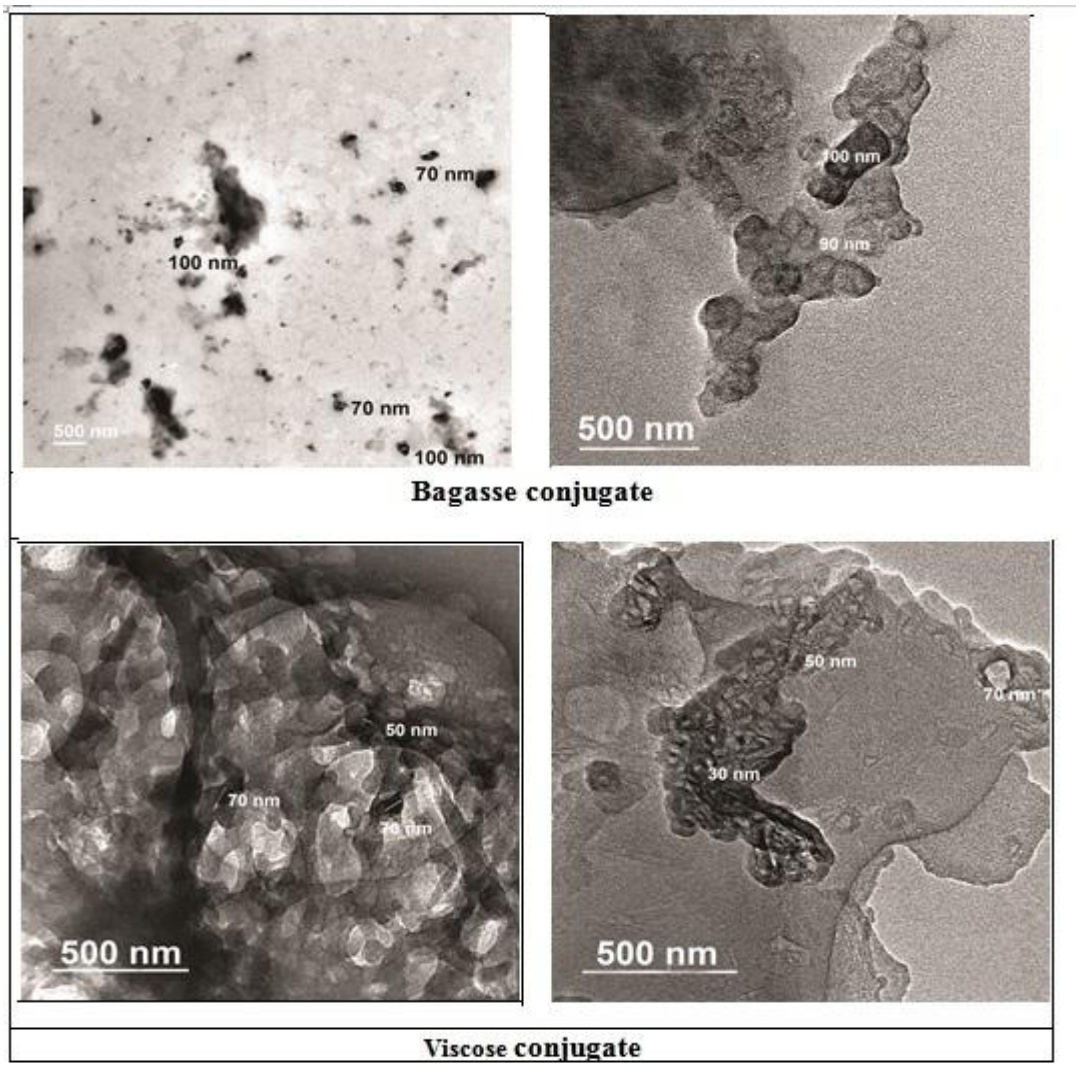

Fig. 4:-TEM photographs of α-cellulose and its derivatives from bagasse and viscose pulps.

Biological Activity:-

Antimicrobial activity:-

The Minimal Inhibitory Concentration **(MICs)**was calculated from Tables 7, 8**.** Different concentrations (20, 4, 2, 1, 0.5, and 0 as negative control) of the tested substances were prepared via serial dilution method **[32, 33]**.

Table7:-MIC of the bagasse pulp conjugate on the tested strains.

A-Bacteria

Concentration (µg/ml)	Clear zone diameter (mm)			
	B.subtilis (NCID-3610)	*S. aureus* (NCTC-7447)	*P. aeruginosa* (NCID-9016)	*E.coli* (NCTC-10416)
20.00	5.00	7.00	0.00	6.00
4.00	0.00	0.00	0.00	0.00
2.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00
0.50	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00

B-Fungi

Concentration (µg/ml)	Clear zone diameter (mm)				
	Candida albicans(NCCLS 11)	*Aspergillus awamori*(ATCC 22342)	*Cunninghamellaelegans* (ATCC 36112)	*Aspergillus niger* (ATCC 22342)	*Penicillium*sp.(NRRL 1889)
20.00	4.00	3.00	5.00	0.00	0.00
4.00	0.00	0.00	0.00	0.00	0.00
2.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00	0.00
0.50	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00

Table 8:- MIC of the viscose pulpconjugate on the tested strains.

A-Bacteria

Concentration (µg/ml)	Clear zone diameter (mm)			
	B. subtilis (NCID-3610)	*S. aureus* (NCTC-7447)	*P. aeruginosa* (NCID-9016)	*E.coli* (NCTC-10416)
20.00	9.00	6.00	4.00	12.00
4.00	4.00	4.00	0.00	7.00
2.00	1.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00
0.50	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00

B-Fungi

Concentration (µg/ml)	Clear zone diameter (mm)				
	Candida albicans(NCCLS 11)	*Aspergillus awamori*(ATCC 22342)	*Cunninghamellaelegans* (ATCC 36112)	*Aspergillus niger* (ATCC 22342)	*Penicillium*sp.(NRRL 1889)
20.00	14.00	5.00	7.00	0.00	8.00
4.00	8.00	0.00	3.00	0.00	3.00
2.00	5.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00	0.00
0.50	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00

The **MIC** values recorded for organisms towardsconjugated bagasse pulp were 20 µg/ml for (*Bacillus subtilis* (NCID-3610), *Staphylococcus aureus* (NCTC-7447), *and Escherichia coli* (NCTC-10416)) as well as fungi **MIC** values were 20 µg/ml for *Candida albicans*(NCCLS 11), *Aspergillus awamori*(ATCC 22342), and *Cunninghamellaelegans* (ATCC 36112).

On the other hand, the MIC values recorded for organisms towards conjugatedviscose pulp showed wide range; additionally all bacterial strains were sensitive. MIC value for *Bacillus subtilis* (NCID-3610) was 2µg/ml and for *Staphylococcus aureus* (NCTC-7447),and*Escherichia coli* (NCTC-10416) were 4µg/ml, as well as *Pseudomonas aeruginosa* (NCID-9016) MIC value was20µg/ml.

In contrast, the fungal strains showed an increase in **MIC** values in comparison with conjugatedbagasse pulpon fungi, where*Penicillium*sp.(NRRL 1889)and *Cunninghamellaelegans* (ATCC 36112) MIC values were 4 µg/ml as well as the MIC value for*Aspergillus awamori*(ATCC 22342) was 20 µg/ml.*Candidaalbicans*(NCCLS 11) shows the great **MIC** value 2.0µg/ml however *Aspergillus niger* (ATCC 22342) was resist.

Anti tumor activity:-

The present study was carried out to examine the capability of the synthesized nano-**CMC-APP** in treating human breast cancer cells, MCF-7 in vitro. The inhibition effect was determined by using **MTT** bioassays. It is noticed that

this nano derivative inhibits cell growth and decreased cell survival through induction of cell death by dose-dependent manner. The data obtained revealed that the inhibition percentage of MCF-7 increased with increasing concentration of investigated nano-**CMC** derivative and it was more sensitive at 40µg/ml viscose pulp-**CMC-APP**, where it provides 85% cell death Fig.4. While, the concentration value that can inhibit proliferation of MCF-7 breast cancer cells by 50%, is ~ 32µg/ml.

Figure5.Illustrates the breast cancer cell line behavior after treatment with different concentrations of conjugated, compared with untreated plate. After 24 hrs incubation period, the examined cells shape (without treatment) by OLYMPUS light inverted microscope was deformed to spherical shape.This deformation takes place from basic epithelial shape, as shown in control well. The observed cells deformation is indication to initial apoptotic phase.

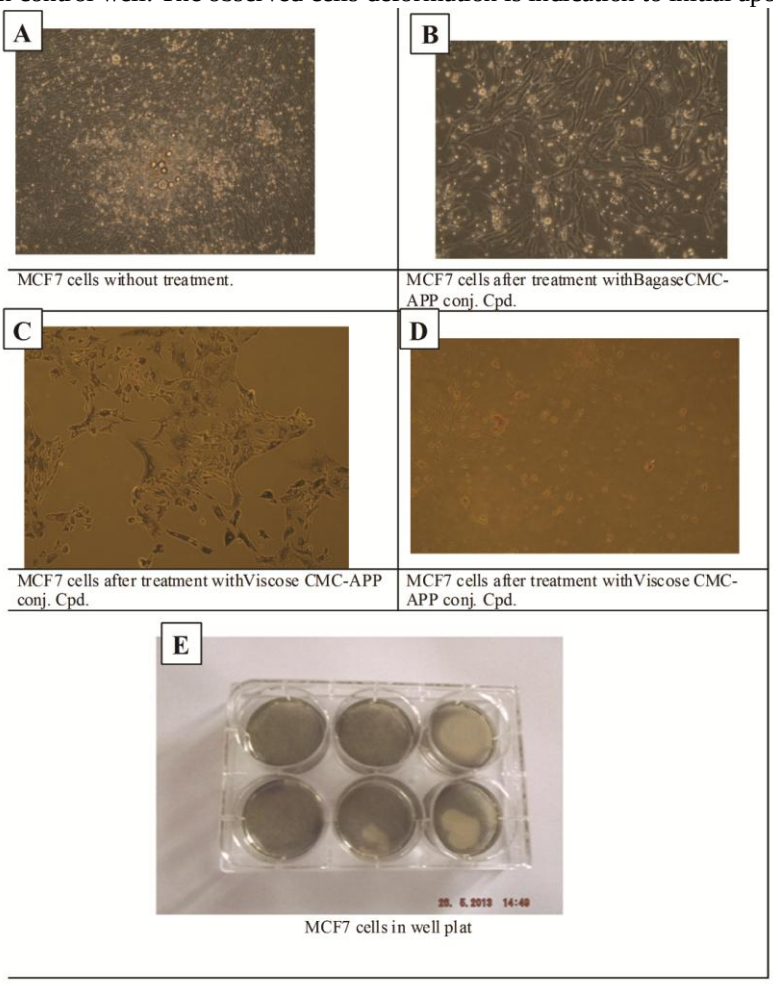

Fig. 5:-The breast cancer cell line behavior after treatment with different concentrations of conjugated.

However, for treated cell well, the microscopic examination shows that, treated with conjugated compound provides deactivation of metabolic pathway, because it makes blocking to the essential amino acid, which plays an important role in proteins building up and other protein derivative. On the contrary, the bagasse pulp conjugated lacks to any anticancer activity, this may be attributed to the particles size which can`t penetrate cells and deactivate cells growth.

Conclusion:-

The current study demonstrated the synthesis of nano-**CMC-APP** derivative via conjugation, of **CMC** from different cellulosic pulps.The nitrogen content emphasized that the**CMC** produced from viscose pulp issuitable to react with **APPA**more than in case of bagasse pulp**CMC**.The viscose pulp conjugate gives the smaller nano sizing particles, than bagasse pulp conjugate. The small particles size leads to the highest biological activity for viscose pulp conjugate in both antimicrobial and antitumor via facilitated the particles penetration the cells.

Acknowledgment:-
This work was supported by the National Research Centre in Cairo, Egypt.

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