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

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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-APPA derivative was 30 to 70 nm. The antimicrobial and anticancer activities of these nano- CMC derivatives against four bacterial strains and six 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 cellulose 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
reputed analgesic 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 *Mucunapruiriens* | 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°C), and alkali extraction (2% NaOH with liquor ratio 1 : 10, for 1hr at 75°C). The bleached produced pulp was treated by 17.5 % 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 celluloscopic 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 | Kalsom lignin, % | Holocellulose, % | Pentosane, % | α-cellulose, % | Ash, % |
|----------------|------------------|------------------|--------------|----------------|-------|
| 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 prepared α-cellulose of sugar cane bagasse and viscose pulps. The non-aqueous method was used [25]. Different monochloroacetic acid / soda ratio were used to obtain different degree of substitutions DSs, 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°C).

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

Purity % = \( \frac{\text{Wt. after purification}}{2g} \times 100 \)

The average degree of substitution (DS) of carboxymethyl cellulose was estimated by 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:

\[
DS = \frac{162 \times n_{\text{COOH}}}{m_{\text{ds}} - 58 \times n_{\text{COOH}}}
\]

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

**Modification of CMC:-**

The synthesis process of CMC-APP was carried out by mixing CMC with the desired amount of APPA. CMC/APP ratio was 1: 1.5 and heated for 1.5 hrs, at ~ 250 °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 °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 (IC₅₀) 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°C, 5% CO₂ 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. To evaluate 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 monochloroacetic acid and sodium hydroxide ratio / pulps, to obtain different DSs. The highest DS was obtained by using conditions of Expt. 1, and lowest DS, by using conditions of Expt. 2. The solubility of these prepared CMCs in water was tested.

| α-cellulose         | Sample code | Pulp : Soda : Monochloroacetic acid | Water solubility          | DS   | Purity, % |
|---------------------|-------------|-------------------------------------|--------------------------|------|-----------|
| Soda bagasse pulp   | Exp. 1      | 1 : 2 : 2                            | Transparent viscous      | 0.95 | 69.1      |
|                     | Exp. 2      | 1 : 2 : 1                            | Turbid solution          | 0.30 | 61.7      |
| Visose pulp         | Exp. 1      | 1 : 2 : 2                            | Transparent viscous      | 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 in Fig. 1. IR measurements were illustrated in Table 4.
Table 4: FT-IR measurements of α-celluloses and its conjugates compounds.

| Sample                        | MHBS \( \frac{A_{\text{OH (Str.)}}}{A_{\text{CH (Str.)}}} \) | Cr.I \( \frac{A_{\sim 1370 \text{ cm}^{-1}}}{A_{\sim 2900 \text{ cm}^{-1}}} \) | \( \nu (\text{OH}) \text{ str. Cm}^{-1} \) \( \Delta \nu (\text{cm}^{-1}) \) | 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           |
| Phenylnalanine                | 1                                               | 0.97                                          | 3404                                        | 1610.2= 0.28         |

The carboxymethylation 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 modified-α-cellulose fibers was 1.43 for bagasses and 2.83 for viscose conjugated pulps.

**TGA:**

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

![Fig. (1)-C: FT-IR spectra of α-cellulose (1), CMC (2), and conjugated (3) viscose pulp](image)
The TGA and DTG curves for bagasse and viscose pulps (fibers, CMCs, and conjugates) which were heating at rate 100°C/min are shown in Fig. 2,3 as well as thermal kinetic parameters of α-celluloses and its derivatives in Tables 5, 6.

| Cellulose samples | stage | Temp. range °C | DTG peak temp. °C | "n" | Se | Ea kJ/ mole | Wt. % |
|-------------------|-------|----------------|-------------------|-----|----|-------------|-------|
| α-cellulose fiber | 1st   | 50-102         | 63                | -   | 0.991 | 149.557    | 96.8  |
|                   | 2nd   | 206.4-333.2    | 277.1             | 0.962 | 0.1650 | 264.333    | 19.85 |
|                   | 3rd   | 333.2-443.7    | 468.1             | -   | 0.146  |           | 11.089 |
|                   | 4th   | 50-101.5       | 64                 | -   | 0.995  | 215.7227   | 88.09 |
|                   | 2nd   | 216.0          | 288.5             | 0.968 | 0.176  | 305.524    | 57.27 |
|                   | 3rd   | 332.9          | 794.8             | 0.979 | 0.1608 | 530.0308   | 39.35 |
|                   | 4th   | 669.08-821.78  | 912.4             | -   | 0.119  | 1051.277   | 29.12 |

Table 5: Thermal kinetic parameters of α-cellulose bagasse pulp and its derivatives
Fig. 2: Thermal kinetic parameters of α-cellulose bagasse pulp and its derivatives.

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

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

Table 6: Thermal kinetic parameters of α-cellulose viscose pulp and its derivative
Fig. 3:- Thermal kinetic parameters of α-cellulose viscose pulp and 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°C) represent the evaporation of the water of degraded components. The second stages of degradation peak was (up to about 291°C) 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°C and viscose fiber has its peak value at 819.29. 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].

**Table 7:** 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) |
|------------|-----------------------|---------------------------|-------------------------|------------------------|---------------------------|---------------------|
| B. subtilis | 20.00                 | 5.00                      | 7.00                    | 0.00                   | 0.00                      | 6.00                |
| S. aureus  | 4.00                  | 0.00                      | 0.00                    | 0.00                   | 0.00                      | 0.00                |
| P. aeruginosa | 2.00               | 0.00                      | 0.00                    | 0.00                   | 0.00                      | 0.00                |
| E. coli    | 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 pulp conjugate on the tested strains.

| Concentration (µg/ml) | 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                 |

| Concentration (µg/ml) | Candida albicans(NCCLS 11) | Aspergillus awamori(ATCC 22342) | Cunninghamella elegans (ATCC 36112) | Aspergillus niger (ATCC 22342) | Penicilliumsp.(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                      |

B-Fungi

| Concentration (µg/ml) | Candida albicans(NCCLS 11) | Aspergillus awamori(ATCC 22342) | Cunninghamella elegans (ATCC 36112) | Aspergillus niger (ATCC 22342) | Penicilliumsp.(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                      |

A-Bacteria

The MIC values recorded for organisms towards conjugated 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 Cunninghamella elegans (ATCC 36112).

On the other hand, the MIC values recorded for organisms towards conjugated viscose 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 conjugated bagasse pulp fungi, where Penicilliumsp.(NRRL 1889) and Cunninghamella elegans (ATCC 36112) MIC values were 4 µg/ml as well as the MIC value for Aspergillus awamori(ATCC 22342) was 20 µg/ml. Candida albicans(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.

Figure 5 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.

![Figure 5](image)

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 is suitable 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.
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