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Isolation of chitosan from *Lactarus deliciousus, Cantharelles cibarius, Laccaria laccata, Hericium erinaceus* and *Pleurotus tueragium* (five wild edible Nigeria mushrooms) for drug delivery application

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ABSTRACT

Background: Nanotechnology is currently being explored as a more efficient tool in drug delivery. The production of chitosan from crustacean shells, lobsters, and shrimps, is associated with several drawbacks. The study is aimed at evaluating drug delivery potentials of chitosan from different wild edible Nigerian mushrooms.

Methods: Chitosan were prepared from the mushrooms by deproteinisation and deacetylation of chitin. Drug delivery potentials of chitosan isolated from different edible wild Nigerian mushrooms was done using standard methods.

Results: The percentage yield of the chitosan revealed that *Hericium erinaceus* had 61.11±0.01 followed by *Pleurotus tueragium* with 52.15± 0.01while Cantharelles cibarius had 11.77±0.03 and was followed by *Laccaria laccata* with 7.18±.0.01. The *In-vitro* loading capacity was 5-35% and the entrapment efficiency of the chitosan beads with ampicillin at physiological temperature and pH at concentration of 0.05 and 0.1 gave the highest value that compared favorably with each other while at other concentrations, standard and *Cantharelles cibarius* had high entrapment efficiency. The results of in-vitro releasing capacity indicated that there was an increased release of the ampicillin with time from 5 - 30 minutes and that the ampicillin-loaded formulation obtained after encapsulation exhibited a sustained release behavior in both standard and the mushroom chitosan beads with a steady rise in cumulative ampicillin.

Conclusion: Mushrooms are potential sources of chitosan and that the isolated chitosan from mushrooms could be use as a vehicle for drug delivery in biological systems.

1. Introduction

Chitin and chitosan are produced industrially from crustacean co-products, especially shrimps and crabs. However, the choice of the raw material is related to the local activity. Some geographical areas favor other resources, such as fungal sources like mushrooms, krill or pink shrimps. Fungal chitin and chitosan have the advantage to being extracted at any time of the year, avoiding seasonal fluctuations in comparison with those produced from crustaceans; the fungal mycelia can be produced in bioreactors optimizing the conditions of crops

and making it at high current research interest[1,2,3]. A moment ago, varieties of procedures were adopted to extract chitin and chitosan from fungal biomass.

Several studies show that chitin and chitosan can be extracted from different species of fungus, including *Ganoderma lucidum, Agaricus sp., Pleurotus sp., Pleurotus ostreatus, Fomes fomentarius, Boletus bovinus*, etc[4,5,6,7,8]. The extraction yield of the obtained chitin varied between 7 and 43% depending on the type of fungus. In this regard, the chemical composition of a fungus in particular the crude chitin content is not the same in different varieties of mushroom, hence the yield and the quality of the extracted chitin and/or chitosan will depend on the isolation method and its chemical composition[9,10,11]. In the present research, we have aimed to evaluate the potential of using *Lactarus deliciousus, Cantharelles cibarius, Laccaria laccata, Hericium erinaceus* and *Pleurotus tueragium* (five edible Nigeria mushrooms) as a good drug delivery agent. The study objectives are to determine the extraction yield of chitin and subsequently assess the drug delivery potentials of the isolated chitosan from different edible wild Nigerian mushrooms.

2. Materials and methods

2.1 Materials

Chitosan (Sigma Aldrich, Germany), Acetic acid (Sigma Aldrich, Germany), NaOH (Sigma Aldrich, Germany), mortar and pestle (Ayukalp UAP Pharma Pvt Ltd, India), centrifuge (Sigma Aldrich, Germany), acetone (Sigma Aldrich, Germany), sodium tripolyphosphate (Sigma Aldrich, Germany), vernier caliper (IndiaMART, India) magnetic stirrer, UV spectrophotometer, Single Beam, SP-LUV759 (Thermo Fisher Scientific), ampicillin (Jagsonpal Pharmaceuticals Ltd, India), phosphate buffer (Sigma Aldrich, Germany).

2.1.1 Collection and identification of mushrooms

Five different edible wild Nigerian mushrooms *Lactarus deliciousus, Cantharelles cibarius, Laccaria laccata, Hericium erinaceus* and *Pleurotus tueragium* were collected from different areas within the base of a thick forest located in Ugu–Uleri in Biladebia Ntezi in Ishielu Local Government Area of Ebonyi State, Nigeria and were identified by a taxonomist, Dr. E.O. Nwankwo in the Department of Applied Biology of Ebonyi State University, Abakaliki, Nigeria.

2.2 Preparation of mushrooms

The mushrooms were uprooted, destalked, washed and air-dried at room temperature for 2-6 days while turning the mushroom to avoid fungal growth. Exactly 300g of mushrooms were later pulverized using mortar and pestle to obtain the mushroom meals (MRMS) and stored in an ovum at 110^{o}C in a container for analysis.

2.3 Extraction of chitosan

Chitosan extraction was carried out by a method of Rane and Hoover[12]. The mushrooms powder was suspended with 1 M NaOH solution (1:30 w/v) and autoclaved at 126^{o}C for 15 min. Alkali-insoluble fractions (AIF) were collected after centrifugation at 12000 x 9.8m/sec^2 for 15 min, washed with distilled water to neutrality. The residues were further extracted using 2% acetic acid (1:40 w/v) at 95^{o}C for 8 h. The extracted slurry was centrifuged at 12 000 x 9.8m/sec^2 for 15 min. The pH of the supernatant fluids was adjusted to 10 with 2 ml of 1M NaOH, the solution centrifuged at 12 000 x 9.8m/sec^2 for 15 min and the precipitated chitosan was washed with distilled water, 95% ethanol (1:20 w/v) and acetone (1:20 w/v), respectively and dried at 60^{o}C to a constant weight.

2.4 Formation of chitosan beads by ionic gelation method

Chitosan beads were prepared according to the method by Garg *et al*[13]. The chitosan powder (3g) was first dissolved in 1.0% acetic acid (1:40 w/v). The solution of the chitosan powder was then introduced into a 20 mL syringe and extruded through the syringe needle into a solution of 1.5% sodium tripolyphosphate under constant stirring. The spherical beads was the picked randomly and measured using vernier caliper to get its diameter (4 - 5 mm in diameter) that were formed as a result of the ionic linkage of the sodium tripolyphosphate. The chitosan beads formed were harvested by filtration and washed with distilled water until neutrality and were stored in a distilled water at 4^{o}C until needed.

2.5 Preparation of chitosan beads for *in - vitro* drug release

Blank chitosan beads were prepared by ionic gelation method. Different concentrations of the mushroom chitosan, ranging from 0.10 - 0.5 % w/v, were dissolved in 1.5% v/v of acetic acid. Sodium tripolyphosphate solution was also prepared in distilled water in a concentrations ranging from 0.1 - 0.5% w/v. Sodium tripolyphosphate solution was added drop wise with a syringe to chitosan

solution while stirring with magnetic stirrer for 20 minutes. The resulting suspension was subsequently centrifuged at 15000 x 9.8m/sec^2 for 10 minutes. The pellets or the beads obtained were re-suspended in distilled water, stirred, centrifuged, harvested and dried at room temperature.

2.6 Determination of entrapment efficiency of chitosan obtained from mushrooms

The encapsulation efficiency and the loading capacity of chitosan beads were determined by first separating the chitosan beads formed from the aqueous medium by centrifugation at 15000 x 9.8m/sec^2 for 30 minutes. The amount of free ampicillin in the supernatant was measured by UV spectrophotometry at 257nm. The ampicillin entrapped in the nanoparticles was calculated as in equation 1 below:

Entrapment Efficiency (%) = (Tp - Tf) 100/Tp……..Equation 1

Where; T**p** is the total ampicillin trihydrate used to prepare the nanoparticles and **Tf** is the free ampicillin trihydrate in the supernatant.

2.7 Evaluation of *in - vitro* drug release

The ampicillin loaded chitosan, after separation by centrifugation, were re-dispersed in 5ml 0.2 mol/L phosphate buffer solution (PBS) at pH 7.4 and placed in a 250 mL beaker containing 150 mL of PBS. The entire system was stirred continuously at 37^{o}C with a magnetic stirrer. At pre-determined time intervals, 5mL of the released medium was removed and replaced with 5mL of fresh PBS solution. The amount of ampicillin in the released medium was evaluated by UV spectrophotometry at wavelength of 257nm.

2.8 Statistical analysis

The data was analyzed by ANOVA using SPSS and results expressed as means and standard deviation. Differences between means were regarded significant at $P<0.05$.

3. Results

The percentage yields of the chitosan isolated from five edible mushrooms indicated that *H. erinaecius* had the highest yield, followed *by P. tuberagium and L. deliciousus* had moderate yield while *C. cibarius* and *L .laccata* had lowest yield. All yields were significantly different from each other as shown in Table 1.

Table 1: percentage yields of chitosan from some edible wild Nigerian mushrooms

Species	% Yield
P. tuberagium	52.15 ± 0.01^{e}
L. deliciousus	35.78 ± 0.01^{d}
L.laccata	7.18 ± 0.01^{a}
C. cibarius	11.77 ± 0.03^{b}
H. erinaecius	61.11 ± 0.01^{f}

Data are expressed as mean ± standard deviation of triplicate determinations.

Values with different superscript alphabets are significantly different at $P<0.05$.

3.1 The effect of ampicilin concentrations on the *In-vitro* loading capacity of chitosan

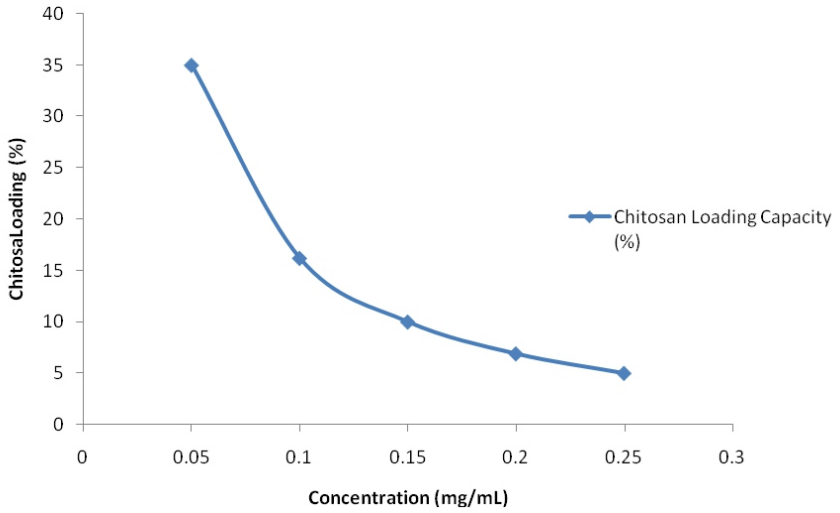

Figure 1: Loading capacity of chitosan with ampicillin.

The result indicated that the *In-vitro* loading capacity of chitosan varied with ampicillin concentrations ranging from 5 - 35% and that it increased with decreased in concentrations

3.2 The effect of concentrations of on the entrapment of ampicillin in the standard and five different mushroom chitosan beads

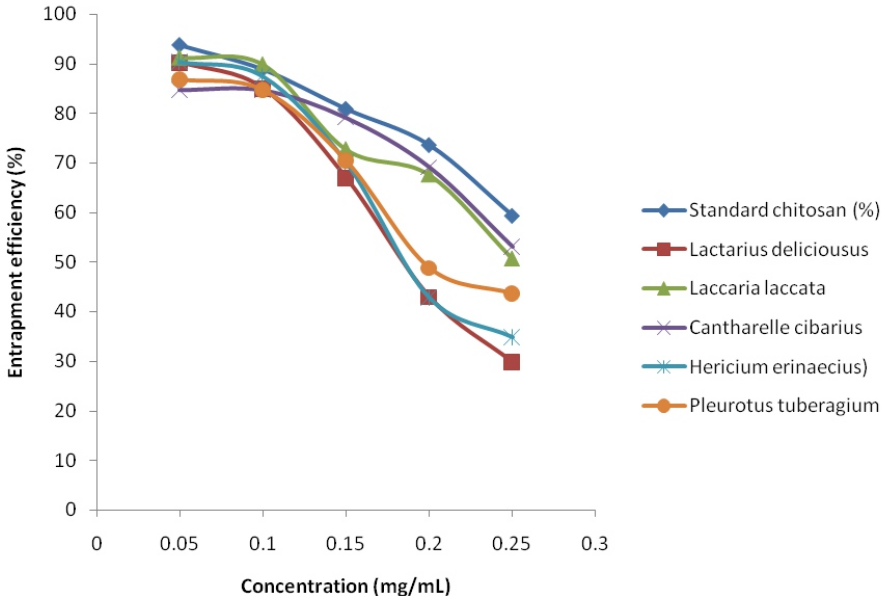

Figure 2: An entrapment efficiency of the various concentrations of Ampicillin

The result indicated that the highest entrapment efficiency (93.80%) was recorded in the standard chitosan at 0.05mg/mL followed by *L. deliciousus* chitosan beads with 91.31% at the same concentration. The *C. cibarius, H. erinaecius and P. tuberagium* chitosan beads show high entrapment that was comparable to the standard chitosan. It was observed that the increase in concentrations of ampicillin reduces the efficiency of entrapment on the chitosan beads except for *L. laccata* which reduces its efficiency at 0.15mg/ml and increases at 0.20 mg/mL.

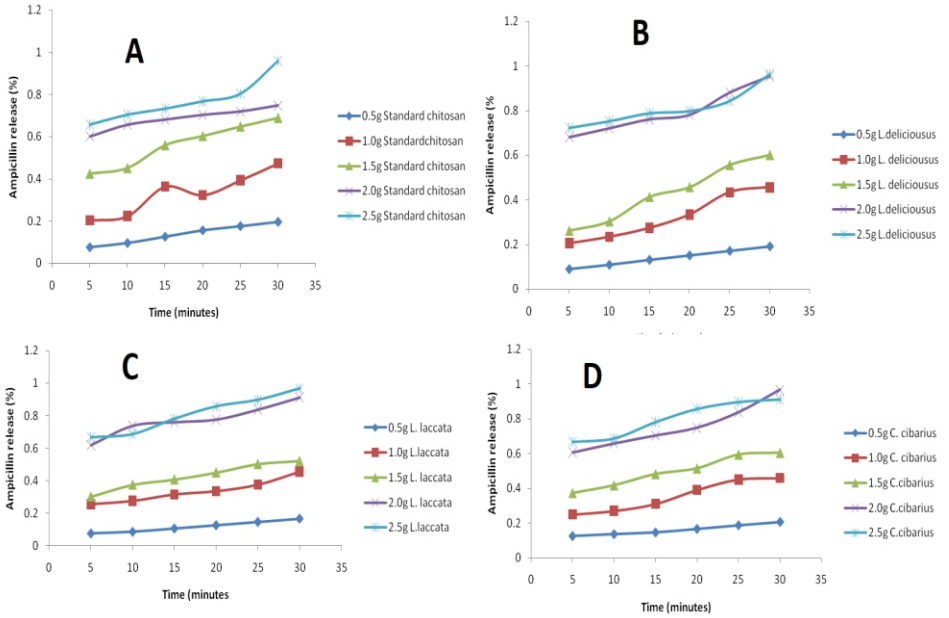

Figure 3(A) *In-vitro* release of ampicillin with time in standard chitosan and different mushroom chitosan beads (B) In-*vitro* release of Ampicillin with time in *L. deliciousus* chitosan beads (C) *In-vitro* ampicillin release with time in *L. laccata* chitosan beads (D) *In-vitro* release of ampicillin with time in *C. cibarius* chitosan beads

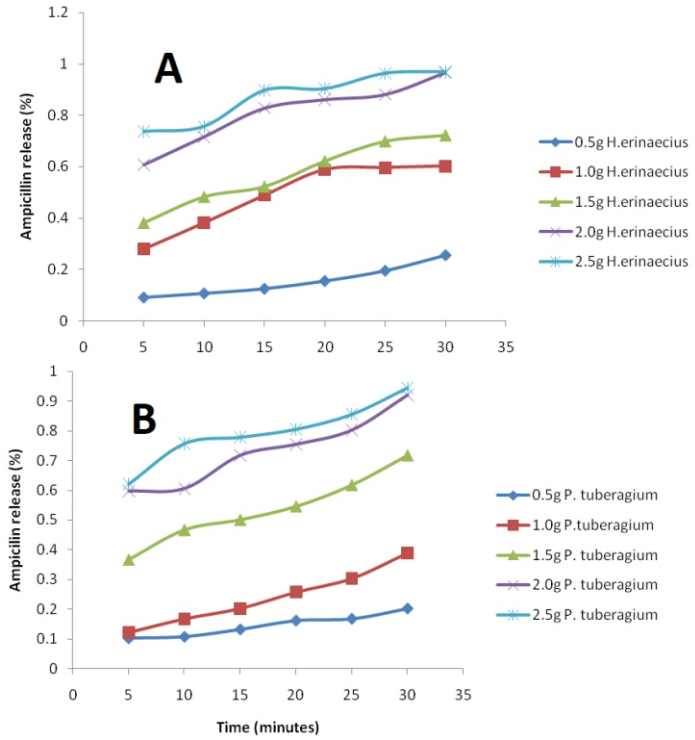

Figure 4 (A) *In-vitro* release of ampicillin with Time in *H. erinaecius* Chitosan beads (B) *In-vitro* release of ampicillin with time in *P. tuberagium* chitosan beads

Figures 3-4 showed *In-vitro* release of ampicillin in standard chitosan and chitosan from *L. deliciousus, L. laccata. C. cibarius, H. erinaecius and P. tuberagium.* The results indicated that there was an increased release of the ampicillin with time from 5 to 30 minutes and that the ampicillin-loaded formulation obtained after encapsulation exhibited a sustained release behavior in both standard and the mushroom chitosan beads with a steady rise in cumulative ampicillin. Thereafter, there was no further release of the ampicillin.

4. Discussion

The percentage yield of chitosan from different mushrooms showed high amounts in *H. erinaecius*, *P. tuberagium* and *L. deliciousus* with low amounts in *C. cibarius* and *L. laccata* (Table 1). The present observation indicates that mushroom could be a better source of chitosan production. The result of this study is in consonance with the study carried out by Elem and Uraku[14].

The *In-vitro* loading capacity of chitosan varied with ampicillin concentrations between 5 to 35% and that the chitosan loading capacity raised with decreased in concentration of ampicillin (Figure 1).

The concentration of ampicillin for optimal entrapment in the chitosan from mushroom was at the range of 0.05 - 0.25 mg/mL (Figure 2). The result revealed that the increased in concentration of ampicillin leads to low entrapment efficiency (Figure 2). The outcome of this study agrees with the report of Dhanasekaran *et al*.[15]. This could be due to its diminutive surface area of chitosan and its cationic interactions. Mushroom chitosan is a biocompatible, biodegradable, and natural polymer. Therefore, it appeared to have good compatibility with the entrapped ampicillin as there was no clear evidence of interaction between the two compounds. Thus, mushroom chitosan prepared by ionic gelation method using mushroom chitosan as a polymer and sodium tripolyphosphate as a cross linking agent produced particles of good stability. Entrapment of the ampicillin within the network of the mushroom chitosan matrix may have made the formulation more stable. It seems that the relatively lower viscosity of the mushroom chitosan with concentration low as 0.05-0.25 mg/ml and appropriate concentration of TPP (1.5% w/v) prompted the formation of encapsulates. A fixed sonication time of 30 minutes yielded smaller, and more uniform encapsulates. This may be attributed to the fact at this concentration; viscosity was attained by chitosan solution for encapsulates formulation. Sonication time was also an important parameter for nanoparticle formation probably because sonication helped to reduce particle size.

The *in vitro* release of ampicillin was carried out at 37^{o}C for 30 minutes in phosphate buffer saline (pH 7). The results of the *In-vitro* release of ampicillin in standard chitosan and chitosan from *L. deliciousus, L. laccata. C. cibarius, H. erinaecius and P. tuberagium were shown in figures 3-4.*These results correspond to the amount of ampicillin released over time which implies that ampicillin is continuously released from the encapsulated chitosan for 30 minutes. The continuous release observed in the standard and the mushroom chitosan beads were found to be dependent on the concentration of the ampicillin due to the facile separation, large surface areas and numerous active sites available in the immobilize enzymes. The higher the concentration of the ampicillin, the faster the release and vice versa. The observations provided some insights on the mechanism of its release. Under the present observations, the chitosan is not expected to degrade. Thus, the release of the associated ampicillin might depend also on encapsulation efficiency. The result is in divergence with the report of Vila *et al*.[16] who reported that *tetanus toxoid* from chitosan was release very slowly which they attributed to the large size of the *tetanus toxoid* and its interaction forces with chitosan. Herdiana and Muchtaridi[17] reported bi-phasic release; initial burst release drug in a particular time followed controlled manner release in achieving the expected release which is in consonance with the results of this study. Also, report of Dhanasekaran *et al*.[15] agreed with obtained results.

5. Conclusion

Based on the results, the study concludes that chitosan from edible mushrooms are potential vehicles for drug delivery and could be used as a better drug releasing agents in the system.

Competing interests: The authors declare that they have no competing interests

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