BACTERIAL CELLULOSE AS SEPARATING AGENT FOR USEFUL COMPOUNDS IN MEDICINAL PLANTS, A PRELIMINARY STUDY

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The present work is a part of qualitative and quantitative research about biomass from bacterial cellulose made of *nata de coco,* which found useful application in environmental and analytical chemistry area as well as pharmaceutics and biochemistry. The ability of the biomass to absorb heavy metals was already reported. Some comparison researches gave insight into the physical behavior of analyte on the surface. The sorption behavior for more complex organic compounds including natural product chemicals in medicinal plants is currently being studied. The aim is to get a best condition to extract many types of compounds, to separate and analyze the method for further applications.

The modified surface of the cellulose can also maximize the separation behavior or even combination attempts of certain compounds. For the preliminary study, the cellulose surface was used to describe the separation of complex extract from great morinda fruit (Indonesian: mengkudu, *Morinda citrifolia*). Batch and column absorption methods are going to be compared using powdered and shredded nata. Some different designs for absorption will also be tested. The result from some spectroscopic analysis including UV-VIS, AAS, GC-MS, HPLC for the compounds separation and FTIR and SEM for the biomass cellulose describe the condition obtained to optimize the desired separation or possible combination processes.

The separation of the phytochemicals was useful to widen the application possibilities to utilize the surface and the porous body of this bacterial cellulose. When the sorption behavior of classes of compounds can be well described, the use of this biomass for medicinal plants extraction can be considered further.

Key words: bacterial cellulose, separating agent, sorption, medicinal compounds from local plants.

INTRODUCTION

There are countless types of compounds in nature presents in plants. Some are beneficial, some are poisonous, some can also be used as medicinal components in traditional culture. The extraction procedure is taken to separate some target compounds, but what usually obtained after extraction is still a mixture. The extraction and separation of chemicals is the interesting topic in organic and analytical chemistry [Ramamoorthy, P.K., Bono, A., 2007]. The activity and amount of compounds would be different using different methods of extraction and different types of solvent as well as their combination.

Morinda citrifolia is a well known plant due to the richness of useful components in every part of the plant. The fruit, leaves, root of the plant are found to have many types of beneficial components [Chan-Blanco, et.al.,2007], and [Krishnaiah, et.al., 2007]. Some compunds have antioxidative properties and bioactivity. There are a lot more remarkable reviews about the biochemistry changes occuring in living organism with some particular components from the *Morinda citrifolia* in medicinal researches.

Some attempts of separating various compounds from the plants are also reported as phytochemical standard procedures. The use of various solvents and different methods were compared to obtain the target components at the best condition as well as the highest rendemen. The use of adsorption and filtration processes also appeared in some publications [Kiathevest, K., et.al., 2009], [Anonymous, 2004]. The surface modifications utilizing physical and chemical properties are interesting in analytical chemistry area. This is not including the modern separation and instrumental analytical methods to analise the compounds and their properties.

On the other hand, the use of a biomass such as *nata de coco* becomes important in many areas. This bacterial cellulose is not only produced industrially to supply the high fiber resource in food products but also for other applications in science and material science. The making of this bacterial cellulose was also reported in some studies and also interesting [Wahyudi, T., 2008]. Some researches on *nata de coco* as adsorbent for heavy metals were conducted and reported quite often [Afrizal, 2008]. In phytochemistry area the use of cellulose surface can also be the topics in future research. The application of this adsorbent to separate components in the fruit of *Morinda citrifolia* (Noni fruit) is the current investigation. The preliminary result is presented in this paper.

The surface of the biomass can be considered active since it has hydroxyl groups and also the strong hydrogen bonding with solvents. This biomass has also swelling capacity and this also be used to entrap particles. The dynamics on the surface as studied by Nuclear Magnetic Spectroscopy were reported in some certain conditions [Wonorahardjo, 2009, 1998]. When the adsorption on the surface is considered "physical" then desorption of adsorbates can be easily obtained. Especially small and simple particles can be retained only for a certain time on the surface before being desorbed back to the bulk liquid. This "strange kinetics" on the surface was analized in a thoughrough review of NMR tomography methods [Kimmich, 2002]. This physical property can be very useful in the future for separating and recovering the target components in the mixture.

The wet or dry porous media can change its surface structure according to the modification introduced to the active sites. The modification can be made using utilization of surface properties. Some trials to modify surface structure using surfactants were reported butt not for this type of cellulose biomass [Kiathevest, K., et. al., 2009]. Moreover, the biomass can be the "place" of some activity tests of the target components from the mixture. The use of *nata de coco* as well as other natas for this application will be important in the future.

The purpose of this experiment was to study the profile of surface interaction between the beneficial compounds in *Morinda citrifolia* fruit. In the long run the utilization of the surface properties can be forced to extraction of selected compounds if the mixture compounds in the extract. Moreover, more selective preparation and extraction methods will be conducted to obtain better separation effect for the components.

MATERIALS AND METHODS

Chemicals, solvents and samples

The chemicals used for this research so far were obtained from E. Merck Germany. Solvents used were Ethyl alcohol, Methanol (HPLC grade). The ripe Noni fruit is obtained from the university garden in Malang. *Nata de coco* was made of coconut water and raw sugar from the local market. Acetic acid solution was made from Glacial Acetic Acid also from E. Merck, Germany. Ureum was also obtained from E. Merck, Germany. The starter bacteria (*Acetobacter xylinum*) was bought from Biology Department, The State University of Malang, Indonesia.

Some analytical instrumentation used for this report were UV-Vis Shimadzu Pharmaspect 1700 Spectrophotometer, Prestige 20 FT-IR Spectrophotometer, and Shimadzu GC-MS 2010 Plus Spectrometer. Additional pictures were taken from Inspect-850 FEI Scanning Electron Microscope after the *nata* sample was coated with gold as thick as 5 nm.

Preparation of *nata de coco*

Nata de coco was prepared by boiling the coconut water and sugar for around two hours. The raw material should be sterile and the making process must also be free of other microorganism. The acetic acid was poured into the mixture first before the starter bacteria, after the mixture was colled down. The final mixture was then settled in containers for some days (normally 15 days) to let the cellulose fibre to form. Some gelling time experiments were also done to see the effect of different porosity or degree of crosslinking to the sorption ability.

After being harvested the *nata* was again boiled for some hours to stop the process of biomass formation as well as to kill the remaining bacteria. The *nata* was then blended to be cut to small pieces before being used as adsorbent. The dried *nata* was obtained by treating the blended pieces on a glass plate and left for 80 minutes in 90ºC oven. Then the dry nata was sieved to 20-50 mesh in size.

Preparation of Morinda citrifolia extract

One ripe fruit of *Morinda citrifolia* was cut into very small pieces. The seeds were excluded since they must contain different types of components. As much as 5.0151 grams of the small pieces was immersed in 20 mL of Ethyl alcohol for 25 minutes. This extract was then filtered. The filtrate was taken as the "raw" extract of the fruit. The UV-Visible and GC-MS spectra of it were then obtained.

Preparation of *nata* picture and IR-spectra

A small piece of *nata* gel was taken using a sharp cutter. For the SEM picture the small piece was coated with gold since cellolose is a non-conducting material. The dried and sieved *nata* was ground with KBr to get the reflectant infrared spectrum of some functional groups present on the surface.

Adsorption of components and recovery

Into 5 mL of the extract, 3.5536 gr of blended *nata* was added. To the mixture 10 mL additional pure ethanol was given and the new mixture was then shaked for about 20 minutes. Then it was filtered again. The filtrate was called the "remains" of compounds from the raw extract.

The *nata* residue was then immersed again into 10 mL of pure ethanol and filtered again. The last filtrate was called the "recovered" of adsorbed components of the raw *Morinda citrifolia* extract from the *nata* surface.

Spectra and picture recording.

Some SEM pictures were taken from a small piece of *nata de coco*. Different magnification was explored to get the closest image possible this time. The FT-Infrared reflectant spectra were of fine ground dry *nata* in KBr were also recorded using KBr as the background.

Some UV-Visible spectra and GC chromatograms were taken for the original extract, the "remains" and the "recovered" extract. The results were compared to each other to see the profile of the surface adsorbent in gaining the compounds and recovering them afterwards.

RESULT AND DISCUSSION

From the first raw material to make *nata de coco* distinctive change was observed day by day. The solution was becoming viscous and viscous day by day until the gel became and elastic solid material following the container's form. The colour was broken white and it had high elasticity. The dried nata also showed the strength of the fibre.

When seen by SEM instrumentation some heavy crosslinked fiber was seen. Figure 1 is the *nata* surface under 2.442 times magnification. The "envelopes" formed in the fibre was one step of the mechanism of *nata de coco* formation by *Acetobacter xylinum*. The "packed" fibre can swell in presence of polar solvent. This could also be benefecial for some extraction purposes.

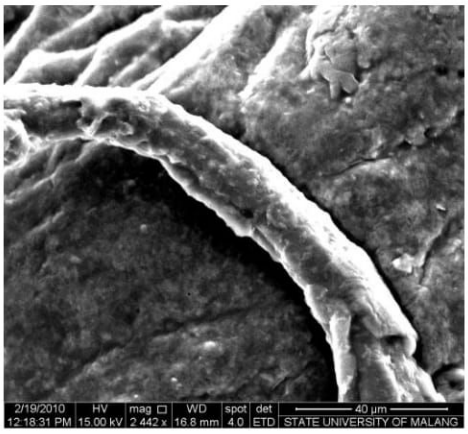

Figure 1. The SEM image of *nata* adsorbent

The further reseach will be about the possiblity of some biochemistry changes of the cellulose formation due to the presence of analyte compounds. The selected compounds would be adsorbed different way if chemical reactions occur. Biosorption is always complicated since the changes might be irreversible so that the obtained components cannot be recovered.

The bigger magnification up to 8.000 times indicated pores surface around 500 nm dimmension (Fig.2). The pore system of the surface contributes to the adsorption ability of the material towards small ions or atoms like heavy metals. Bigger organic molecules will be entangled in the surface due to hydrogen bonding or London force as well as dipole-dipole force. Bigger steric hindrance plays roles as well. Activated pore wall might also help the sorption processes. Thicker coating might help to see the real pore size more clearly. Some white dots appeared on the surface were dirt from the air.

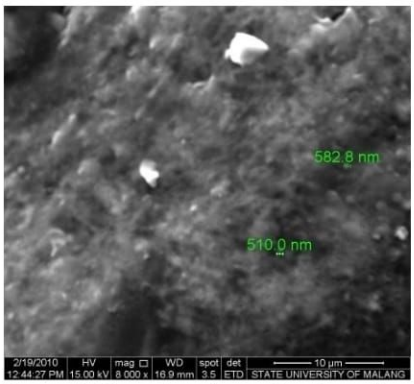

Figure 2. Pore structure of *nata* surface

The infrared spectra of dried nata described some functional groups vibration present in the surface. Figure 3 showed the domination of hydroxil groups bonded on the surface indicated by the very wide peak around 3300 cm^{-1}. Medium peak around 2900-3000 as well as in the finger print region around 1500 cm^{-1} came from the –C-H of the benzene rings of the cellulose. The duplet peaks aroun 2300-2400 cm^{-1} are from the C=C in benzene rings stretching vibration. The fingerprint region showed the vibrational modes of the cellolose.

In the future more investigation in vibrational spectroscopy would be taken into account. The spectra must be taken before and after the adsorption process to see the changes in the surface chemistry. Modification of surface would also take the benefit of the study.

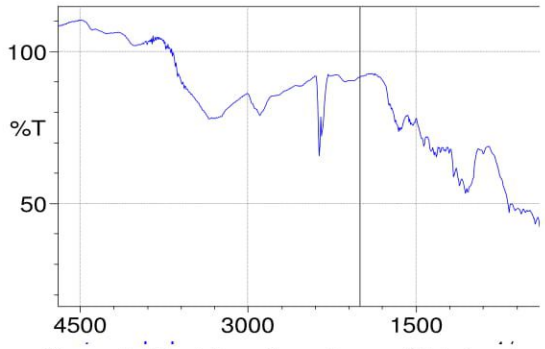

Figure 3. The infrared spectrum of dried *nata*

The "raw" extract of *Morinda citrifolia* fruit was taken from the ripe fruit by ethanol extraction method. After 25 minutes immersion the liquid obtained was clear and the colour turned yellow to brown. This extraction usually takes almost every polar component from the fruit. The phenolic and terpenoic compounds would be extracted altogether, as well as the alcaloids and nitrogen-containing compounds. More specific target compounds would be the next step for this research.

The first experiments were to force the sorption of some compunds from the extract to the *nata* surface. After being filtered, the filtrate was still clear but the colour faded. Some components were attached to the surface of nata. The Ethyl alcohol used was efficient to make the wet *nata* shrink and the molecules come into the mass quite easier. The UV-visible spectrum of the filtrate was recorded.

The actual absorbances that obey the Lambert-Beer law were not recorded since quantitative measurement was not considered.

The residue of the filtration contained the most of the components of the extract. Since the contact time was only 25 minutes there are more than one possibility. The polar components were absorbed into the surface area while the non-polar ones remained in the solution. However since every compound has its own distribution constant, not all of the polar molecules can be extracted into the polar surface. Some of them will remain in the solution. The filtrate is then contained also both of the types, the absorbed and the not-absorbed components. The colour was pale. A thourough and quantitative measurement are being considered.

When the residue was washed again with the same solvent, the adsorbed molecules were then redissolved to the solution. However, since the separated components have also their distribution coefficients, the recovery cannot be 100 procent sucessful. The filtrate content was the "difference" between the first "raw" extract and the "remains" of the extract. This indicated the recovery of the components from the surface adsorbent.

The spectra of the three filtrates can be seen in the figure 5 below. The original spectrum from *Morinda citrifolia* extract was the biggest in intensity. This absorption came from the electronic transitions in the ultra-violet region. The organic compounds must have bonding sigma and phi electrons that contributed a lot to the complex absorbances in the region (220-350 nm). The absorption in the visible area was due to the yellow-brown colour of the solution. The intensity is smaller compared to the UV-absorption. This overall absorption arised from a lot of compounds present in the extract.

After the *nata* sorption was done, some of the compounds were trapped in the cellulose network and retained there for some time. The filtrate indicated the remaining compounds which could be the non-polar or semi-polar compounds that did not prefer the polar surface. There was an indication that the reduced absorption at around 300 nm was huge compared to those in the UV region. That was the contribution of the polar components of the extract.

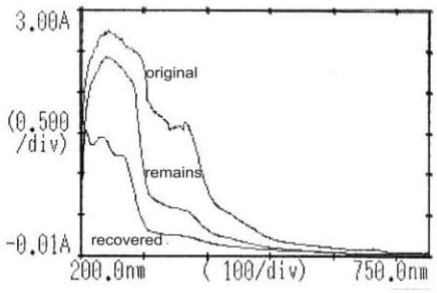

Figure 4. The UV-VIS absorption spectra of the original (raw) extract, the remains and the recovered components in the extract.

On the other hand, the recovered spectrum showed that not all of the adsorbed components were released from the surface. Some absorbance at around 300 nm dissapeared and not recovered at the same amount. There was still difference in the absorbance, in a qualitative point of view. Only a part of the absorbed compounds was washed by the same solvent.

The spectra also indicated the adsorption process for most of the components was only physisorption. There was little portion of the chemisorption or even biosorption occuring in the *nata de coco* and ethanol system.

One step further was the chromatographyc separation that was conducted to see the profile of adsorption in more details. The profile of each extract, before and after *nata* sorption as well as the solvent washed components can be seen in the figure 5. The three chromatograms had very similar profiles at a glance. The recovered extract gave the smallest intensity compared to the previous ones. This also showed the same compounds were recovered from the surface adsorbent. However, real calculations of the peak areas were not done yet to see the recovery percentage of the components from the *nata* surface.

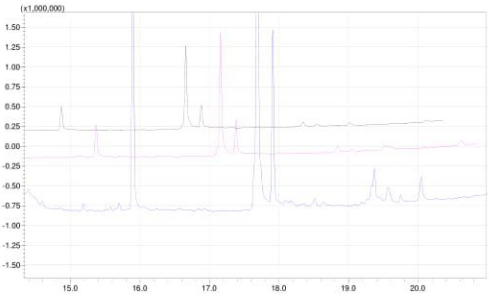

Figure 5. GC-MS chromatograms of the original (raw) extract of *Morinda citrifolia* (in front) compared with the "remains" solution (in the middle) and the "recovered" components solution (at the back)

The chromatograms also indicated physisorption occurred during adsorption. The difference between each component due to different activity towards the cellulose surface was not really taken into consideration this time. The future of the research would treat some targetted compounds from various types of *Morinda citrifolia* extracts. The surface modification would be also done to get benefit of the activity in extracting those desired compounds. It would show the possibility of *nata* to be the probe material to separate beneficial natural products which usually present in form of mixtures. High Performance Liquid Chromatography (HPLC) would be the main instrumentation used to extract information about the adsorption processes.

CONCLUSION

Some preliminary study about adsorbing behavior of some beneficial compounds in *Morinda citrifolia* fruit onto the surface of bacterial cellulose *nata de coco* was conducted. The fact that most of the compounds were adsorbed in the surface using Ethyl alcohol solvent by physisorption and this was indicated from the Uv-visible spectrum and GC chromatograms. The surface activities and its modification possibility has been considered for further research. The use of ethanol as solvent can be combined with other solvent with different polarity to probe more of the surface dynamics. At the end the application of this surface as separationg agent was taken into account.

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