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CHARACTERIZATION, POTENCIAL OF BAMBOO APUS CHARCOAL ACTIVATED WITH ZNCL$_2$ AS AN ANTIBACTERIALS *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Bamboo contains high cellulose so it can be used as basic ingredient for making activated charcoal. Activated charcoal has multifunctional, as an adsorbent and antibacterial. This study aims to determine the potencial inhibition of bamboo charcoal unactivation and activated with $ZnCl_2$ and the minimum inhibitory concentration (MIC) of activated bamboo charcoal $ZnCl_2$ against *Escherichia coli* and *Staphylococcus aureus*. The activated charcoal produced was characterized according to the National Standard Indonesia regarding technically activated charcoal. The functional groups possessed were analyzed by Infrared Spectroscopy. The surface reactivity was estimated by Iodine adsorption and the active site by acid-base titration. The results showed that unactivated charcoal did not has antibacterial properties. The best antibacterial properties occurred at a concentration of solution $ZnCl_2$ 35% (w/v) with an inhibitory power of 10 mm against *Escherichia coli* and *Staphylococcus aureus*. The MIC value of activated charcoal was 95% (w/v) for *Escherichia coli* and *Staphylococcus aureus* respectively, with an inhibitory power of 7 mm and 7.5 mm.

KEYWORDS: *Adsorbent, Activated Charcoal, Antibacterial, Bacteria, Bamboo*.

INTRODUCTION

Diarrhea cases in Indonesia are mostly experienced by toddlers, as many as 162 thousand children under five years die every year or about 460 children under five die every day.[1] Data from the Denpasar City Health Office in 2018 showed that there were 10,339 people with diarrhea under five years of age.[2] Diarrheal diseases is cused by pathogenic *Escherichia coli* bacteria, that trough un- healthy food,drink or contaminate materials that come into directly contact with toddlers.[3,4] Another disease that is often experienced by people in developing countries is acne. Indonesia itself has a fairly large percentage of acne sufferers, namely: around 85-100% and mostly experienced by teenagers. Acne can be caused by excessive oil gland activity and exacerbated by bacterial infection, one of the bacteria that causes acne is Staphylococcus aureus. *Staphylococcus aureus* bacteria can be found in the nose area of about 30% of healthy adults and in the skin area of about 20%.[5,6] Handling diarrhea and acne caused by bacterial infection is the use of antibacterial substances. Antibacterial is a substance that kills or suppresses the growth of bacteria. Antibacterial must have selective toxicity properties, meaning that a drug is harmful to the parasite but does not harm the host (hopses).[7,8] The use of activated charcoal as an antibacterial has been widely developed, one of them by Trogolo (2011), who said that activated charcoal is a good medium as a place for bacteria to grow and spread through water. Bacteria that grow in activated charcoal will spread into the cavities of activated charcoal (filter) and will experience growth inhibition or often called bacteriostatic.[9] Research on activated charcoal to adsorb bacteria has been carried out by Rezaee et.al, (2011) using bone waste or called Bone char as an adsorbent for gram-negative bacteria, namely *Escherichia Coli* in the air with an adsorption percentage of 99.99%.[7] Yang et.al (2009), using activated charcoal from bamboo waste modified with AgNO3 as antibacterial for *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibitory power of 11.8-14 mm and 10.8-13 mm respectively[10] Choi Mi-Suk and Kwon-Suk (2014) reported that bamboo activated charcoal can be useful as an antibacterial of *Streptococcus mutans* from teeth.[11] One of the best activators for making activated charcoal from biomaterials succsesfully is $ZnCl_2$.[12,14,15,16] Manurung, *et. al* (2019) has succeeded in making activated charcoal from bamboo *Apus* (*Gigantochloa apus*) with $ZnCl_2$ activator which was used as an adsorbent for *Remazol Yellow* FG dye and has recovery percentage of

87.18%.[15] The use of activated bamboo charcoal activated with $ZnCl_2$ as an antibacterial has not been reported, therefore the authors are interested in conducting research on activated bamboo charcoal with $ZnCl_2$ as an antibacterial, especially *Escheri- chia coli* and *Staphylococcus aureus* bacteria which are very harmful for health. Zinc ions (Zn^{2+}) are known to have anti bacterial properties, so the combination of bamboo activated charcoal with $ZnCl_2$ is thought to have excellent antibacterial against both types of bacteria.

MATERIALS AND METHODS

The materials used in this study were bamboo *Apus* stems. The chemicals used include $ZnCl_2$, filter paper, aquades, 0.1N NaOH, 0.1N $H_2C_2O_4$, 0.1N $K_2Cr_2O_7$, phenolphtalein, 0.1N HCl,0.1NHNO$_3$, 0.1N iodine., 0.1N $Na_2S_2O_3$, KI 10%, 2N H_2SO_4, Tween 10% (v/v), starch 1%, KBr, nutrient agar powder (NA), nutrient Broth (NB). The equipment used included an analytical balance, a 200 mesh sieve (pore size = 0.074 mm), crucibles, watch glass, oven, desiccator, mortar, magnetic stirrer, pH meter, beaker glass, measuring cup, Erlenmeyer, measuring flask, pipette volume, dropper, ball filler, spatula, burette, stative, clamp, stopwatch, furnace, desiccator, analytical balance, FTIR spectrophotometer ShimadzuPrestige-1, AAS-Shimadzu AA-7000, petridish, autoclave, micropipette.

Procedures

Charcoal Making

The procedure for making charcoal from *bamboo Apus* stem use conventional method refer to Manurung et.al (2021) with a little modification[17] A total of 1000 g bamboo charcoal was produced, then ground using a mortar and sieved through a 200 mesh sieve. The collected charcoal is less than 200 mesh in size and then heated at 800^{o}C for 2 hours. The charcoal is used for futher purposes.

Chemical Activation

Activated charcoal divided into two parts, namely without chemical activation code AB_0 as blank and activated with $ZnCl_2$ code ABAZ. About 5.0 g of bamboo charcoal was put into each of five pieces of 100 mL Erlenmeyer flask and then putted 35 mL of $ZnCl_2$ solution, 20, 25, 30, 35 and 40% (w/v) respectively. The mixture was heated at a temperature of ± 80^{o}C while stirring with a magnetic stirrer until forming paste, then filtered and rinsed with distilled water until a neutral in pH. Each ABAZ charcoal divaded into two parts, ones without heated code ABAZ and heated in kiln at 700^{o}C for 2 hours code $ABAZ_T$. Activated charcoal formed was cooled to room tempe- rature. Antibacterial tests were performed on AB_0, ABAZ and $ABAZ_T$ charcoals.

Characterization

The Three charcoals were charaterized refers to the Indonesian National Standard 06-3730-1995 regarding technical activated charcoal (SNI, 1995) and Manurung et.al 2019,[13,14] namely: water content, volatile subtance, total ash, bonded carbon and Iodine number.

Determination of surface acidity

The determining of the surface acidity of ABAZ and ABo charcoals refers to Manurung. et al. (2019). Weighed each 1.0 g of activated and unactivated charcoal, then put into a 50 mL Erlenmeyer flask and added 15 mL of 0.1M NaOH solution. The mixture was stirred with a magnetic stirrer for 15 minutes. Each mixture of AB_0 and ABAZ charcoals was added 3-4 drops of 0.2% pp indicator then titrated with 0.1 M HCl. The surface acidity of the adsorbent can be determined.[15]

Determination of the Iodine Number

ABAZ and B_0 charcoal were weighed as much as 0.25 g, then each was put into 100mL an Erlenmeyer flask and 25 mL of 0.1 M iodine solution was added. The mixture was stirred for 15 minutes then covered and stored in a dark place for 2 hours. The mixture was filtered and the filtrate was pipetted as much as 5 mL, then put into a clean Erlenmeyer flask. The mixture is titrated with 0.1N $Na_2S_2O_3$.

Functional groups

The functional group ABAZ and charcoal B_0 were determined by the KBr pellets method using FTIR.

Antibacterial activity testing

Testing the antibacterial activity of activated charcoal was carried out using the agar diffusion method. Four petri dishes filled with 10 mL of nutrient agar were filled with 100 μL of bacterial suspension. Furthermore, a diffusion well with a diameter of ±6 mm was made using a 6 mm perforator. Each activated charcoal extract was made with a concentra- tion of 100% (w/v) for each activator concentration of 20, 25, 30, 35 and 40% (w/v) then 20 μL was taken and put into the well and marked then incubated for 24 hours at 37°C. The zone of inhibition is measured by diameter (mm) of inhibition in the form of a clear area around the test well.[4,6]

Determination of minimum inhibitory concentration (MIC)[8]

The method used in determining the MIC is the agar diffusion well. A petri dish that has been filled with 10 mL of nutrient agar filled with 100 μL of the test bacterial suspension was put into a diffusion well. Activated activated charcoal from the best concentration was taken as much as 20μL with various concentrations of 100, 95, 90 and 75% (w/v), ABo charcoal (negative control) and amoxicillin (positive control) was put into the well and marked and then incubated. for 24 hours at 37°C. The antibacterial activity of *Escherichia coli* and *Staphylococcus aureus* was observed by observing the presence or absence of bacteria and measuring their inhibitory power.

RESULTS AND DISCUSSION

The production of charcoal from bamboo Apus stems was carried out using a conventional method with an airtight barrel for 12 hours. This method was chosen because it can be done easily and can produce a large amount of charcoal. The reactions that occur during carbonization/pyrolysis are as follows.[15,16]

Cellulose decomposition at T = 270-310°C

$(C_6H_{10}O_5)n \rightarrow CH_3COOH + 3CO_2 + 2H_2O + CH_3OH + 5H_2 + 3CO$

Lignin decomposition at T = 310-500°C

$[(C9H10O_3)\ (CH_3O)]n \rightarrow C_{18}H_{11}CH_3(tar) + C_6H_5OH + CO + CO_2 + CH_4 + H_2$

The general reaction for the formation of carbon at T = 500-1000°C.

$(CxHyOz)n + O_2 \rightarrow C(graphite) + CO(g) + H_2O$ (g).The barrels used are tightly closed to minimize contact between the bamboo samples and O_2 directly, so that heating occurs instead of burning into CO_2 and H_2O. The bamboo charcoal that has been crushed was of 1000 g and after being sifted, the results are 324.3371 g. The sample obtained after being sifted a little because there are many bamboo fibers that are difficult to grind so they do not pass 200 mesh. This means that the pyrolysis process does not run perfectly.

Physical and chemical activation

Physical/thermal activation was carried out for 2 hours at a temperature of 800°C in a kiln, which aims to removes residual hydrocarbons that cover the pores and the surface of the charcoal.[16,17] Chemical activation was done by adding chemicals to the charcoal. The use of $ZnCl_2$ was chosen as a charcoal activator because $ZnCl_2$ is a Lewis acid that can increase the occurrence of polymerization reactions or aromatic condensation and dissolve minerals, so as to increase the surface area and adsorption capasity of activated charcoal.[9,12,15,] Chemically activated charcoal was carried out in 2 treatments, namely in a kiln at 700°C for 2 hours and without a kiln. Heating at a temperature of 700°C to enlarge the pores of the charcoal and remove the remaining impurities that were not removed in the chemical activation process. $ZnCl_2$ undergoes a reaction with water which is still tightly bound in the carbon pores which is not lost during carbonization. This water molecule is produced from the thermal cleavage of the cellulose molecule at a temperature of 240-400°C. On heating to temperature above 600°C, it is possible to form evaporation of $ZnCl_2$(g) and ZnO(s).[12] Then from these treatment obtained ABAZ and $ABAZ_T$, visually $ABAZ_T$ looks solid black, light and smooth, while ABAZ is black and thick. The same result was also reported by Manurung, et.al, (2019).

AB_0 and ABAZ characterization

Characterization carried out on AB_0 and ABAZ aims to determine the quality of AB_0 which is only thermally activated and ABAZ is thermal and chemical activated. The characterization results obtained are then compared with the standard SNI 06-3730-1995. Surface acidity and number of active sites are additional criteria in determining adsorbent (bamboo charcoal) properties through volumetric titration. The results obtained for AB_0 and ABAZ are presented in Table 1.

Table 1: Surface acidity and active site data.

Charcoals	Average surface acidity (mmol/g)	Number of active sites (molecules/g) (10^{20})
AB_0	0.5372± 0.013	3.2350
ABAZ	0.7562± 0.023	4.5538

The chemical activation process can increase the surface acidity while increasing the active site of the charcoal. This happens because the activator is able to dissolve impurities that cover pores such as tar, residual organic substances, mineral oxide, other organic compounds, so that the active sites are opened and increase the surface area of activated charcoal. Activation process able to increase surface acidity by 40.767%.[12,14] The number of the active sites of charcoal also increases automatically.

Characterisation of ABo and ABAZ Charcoals.

Qualities of charcoal determined based on Indonesian National Standard 1995 includes water, moisture, total ash, carbon content and others. The results obtained are presented in Table 2.

Table 2: Characteristic data of ABo and ABAZ Charcoals.

Parameters	ABAZ	ABo	SNI
Water Content (%)	5.83±0.2887	8.50±0.00	max 15
Volatilesubstances(%)	2.12±0.1050	4.19±0.0346	max 25
Total Ash content(%)	8.70±0.00	21.50±0.00	max 10
Carbon content (%)	83.47±0.00	65.85±0.00	min 65
Iodine Number (mg/g)	1012± 37.76	1062.78± 37.76	min 750

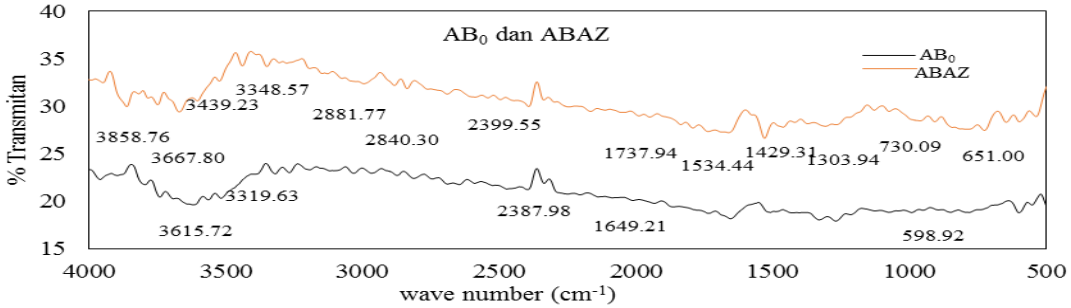

Figure 1: FTIR Spectra of the ABo and ABAZ charcoals.

Base on the characteristic data shown Table 2 that ABAZ better than ABo charcoal for all parameters. The reactivity of activated charcoal can be seen from adsorption capasity to Iodine which is called Iodine Number. Iodine number can describe the ability of charcoal to adsorb material or substances with molecular size less than 1 nm(10 Å). At the same time shows the pore size of charcoal is microporous and mesoporous. Iodine number ABAZ less than ABo because ABAZ only dried in an oven for 5 hours at a temperature 105^{o}C and it is suspected that there is still water present binds to ABAZ so that it covers the pores of the charcoal and inhibits the adsorption of iodine. ABAZ meets SNI standards but ABo is not completely, for example total ash content as much of 21.50 % greater than standard maximum 10%. Carbon content is 65%, while ABAZ more than 83%.

Functional group analysis

The FTIR spectra for ABAZ and AB_0 charcoal are illustrated in Figure 1 and the absorption peaks of the functional groups belong to the two types of charcoal. There is a shift in the wave number that occurs in ABAZ as a result of the addition of an activator, namely $ZnCl_2$, and also the appearance of ZnO vibrations at wave numbers of 400-600 cm^{-1} region, this is also supported by the opinion of Yuvakkumar et al., (2015) and Ma, Y., et.al, (2015) which states that the Zn-O group is present at wavenumbers 500-600 cm^{-1}.[12,17] From the results of the analysis, it can be concluded that AB_0 charcoal is thought to have free OH, C≡C, C=O carbonyl and CH functional groups outside the field, while the functional groups in ABAZ are OH, CH3, CH2, CH, C≡C, COO., C=O carbonyl, C=C aliphatic, OH, CO, CH alkenes and CH out of bounds. In general the main functioanal groups possessed by both bamboo charcoals are almost the same. Function of the presence of this functional group is to bind $ZnCl_2$ and the target bacterial wall, where after the target bacterial wall is bound by $ZnCl_2$ it can cause inhibition of bacterial growth. Anti-bacterial test results from charcoal are presented in Table 3. Antibacterial activity testing was carried out on ABo, ABAZ and $ABAZ_T$ bamboo charcoals against *E. coli* and *S. aureus* bacteria ABo as negative controls (-), while those amocillin, norit are acting as positive controls (+) including 35% ZnCl2 solution. The Zn^{2+} ions adsorpted on the surface of the charcoal form coordinating covalent bonds with functional groups that have free electrons (-OH, C=O and COOH), then enter the bacterial wall to form complex bonds with Zn^{2+} ions as the the central of the compleks.[18] Zn-O which has been bound with charcoal then enters the walls of bacteria, where these have different mechanisms because the compositi.

Table 3: Antibacterial test data.

Charcoals	$ZnCl_2$ (%)	Inhibitory (mm)		Category	
		E.coli	***E.aureus***	***E.coli***	***E.aureus***
ABo (-)	0	-	-	-	-
ABAZ	20	*4*	-	*W*	-
	25	*5*	-	*M*	-
	30	*6*	-	*M*	-
	35	*10*	*10*	*M*	*M*
	40	*7*	*9*	*M*	*M*
$ABAZ_T$	20	-	-	-	-
	25	-	-	-	-
	30	-	-	-	-
	35	-	-	-	-
	40	-	-	-	-
Amoxcillin(+)		*34*	*24*	*S*	*S*
Norit (+)		-	-	-	-
ZnCl2 (+)	35	*40*	*35*	*VS*	*VS*

Description: *W = Weak; M= Medium; VS=Very Strong; - = not detected*

On of the cell walls of *S. aureus* bacteria is different from *E. coli*. *Staphylococcus aureus* have a thick layer of peptidoglycan and contain *teichoic acid*. This *teichoic acid* is only owned by gram positive (+) bacteria, which functions as a chelating agent and gives strength to cells. *Escherichia coli* bacteria have three layers of peptidoglycan in the cell wall and there are porins in the outer layer. Porins function as ion channels in peptidoglycans, besides that porins also play a role in nutrient absorption by cells and interactions between cells.[18] Zn^{2+} that enters bacterial cells then inhibits protein and nucleic acid (DNA/RNA) synthesis as a result of misreading of mRNA to synthesize vital proteins that support the bacterial growth process as well as inhibition of transcription and replication of these bacteria. Decreased drag on *S.aureus* and *E.coli* in addition, the decrease in inhibition can also be influenced by the rate of diffusion of antibacterial materials.[8,18,19]

The minimum inhibitory concentration (MIC) of ABAZ

The determination of MIC is to determine the lowest concentration that can inhibit bacterial growth. This study used 4 concentration variations, namely 75%, 90%, 95% and 100% (w/v) which are presented in Table 4.

Table 4: The Data of determination of MIC for *E.coli and S.aureus bacteria.*

Concentration (%) (w/v)	Inhibition(mm)	
	S. aureus	***E. coli***
100	11	10
95	7	7.5
90	-	-
75	-	-

Description: - = *Not detected*

The minimum inhibitory concentration possessed by ABAZ was at a concentration of 95% (w/v) with 7.5 mm against *E.coli* bacteria and 7mm against *S. aureus* bacteria. The composition of the 95%(w/v) concen tration was 0.19 g ABAZ and 0.2 mL Tween 10%(v/v). Tween is used as a solvent because Tween is a non-ionic surfactant group that is often used to increase the solubility of drugs that are poorly soluble in oral preparations, besides that surfactants in general have the function of reducing surface/ interface tension, increasing dispersion and forming emulsions.[6,8] Based on the these research obtained, that the inhibitory power directly proportional to the concen tration of $ZnCl_2$ adsorpted by the activated charcoal. Then it was influenced by several factors including the rate of diffusion of antibacterial com- pounds on agar media, type of bacteria and others supporting conditions.[7,8,19]

CONCLUSION

Bamboo charcoal with thermal activation (ABo) and chemical activation with $ZnCl_2$ (35%) continous heating in furnice 700^{o}C for 2 hours($ABAZ_T$) have not inhibition against *E. coli* and *S. aureus* bacteria. While activated bamboo charcoal, ABAZ, without heating has antibacterial properties that inhibitory power 10 mm and 11 mm respectively. The MIC concentration, ABAZ, of activated charcoal $ZnCl_2$ against *E. coli* and *S. aureus* was 95% (w/v) with an inhibitory power of 7.5 mm and 7 mm, respectively. At the lower concentration become undetectable.

REFERENCES

1. Adisasmito, W. Faktor Resiko Diare Pada Bayi dan Balita Indonesia. *Jurnal Makara*, 2007; 11(1): 1-10.
2. Dinas Kesehatan Kota Denpasar. *Profil Dinas Kesehatan Kota Denpasar Tahun 2018.* Dinas Kesehatan Kota Denpasar, 2019.
3. Deenandayalan, K.G, Nandhini, V., Yogesan, M, Kremasavel, K Adherence Patterns of Escherichia coli in the Intestine and its role in pathogenesis *Medicine in Microecology*, 2020; 5. https: //doi.or/10.1016/ j.medmic.2020. 100025.
4. Wahyu Zikra, Arni Amir, Andani Eka Putra. Jurnal Kesehatan Andalas (*Andalas Journal of Health*), 2018; 7(2). http:// jurnal.fk. unand.ac.id.DOI:http://doi.org/ 10.25077/ jka.v7i2.804
5. Datta, P., Neelam G. Nidhi S., Hena R. V., Kiran B. Jagdish C. dan Varsha G. Evaluation of Various Methods for the Detection of Meticillinresistant *Staphylo coccus Aureus* Strains And Susceptibility Paterns. *Journal of Medical Microbiology*, 2011; 60: 1613-1616.
6. Carrol, K.C., Butel, J.S., Morse, S.A., Mietz ner, T., Jawetz, Melnick & Adelberg's *Medical Microbiology*, 27th ed. The Mc Graw -Hill Companies, Inc. United State of America, 2016.
7. Rezaee, A., Maryam R., Ghader G. dan Afshin N. Adsorptionof *Escherichia coli* Using Bone Char. *J.Appl.Sci. Environ. Manage*, 2011; 15(1): 57-62.
8. Talaro, K. P. *Foundation in Micro- biology: BasicPrinciples. 6thed.* McGraw Hill, New York, 2008.
9. Trogolo, J.A. Activated Carbon: Modern Advances for ancient Technolo gy, Coditioning & Purification, Magazine Tucson, 2011.
10. Yang, Fu-Chu., Kuo-Hui W., Wen-Po L., Ming-Kuan H, Preparation and AntibacterialEffect of Bamboo Charcoal /Ag on Staphylococcus aureus and Pseudomonas aeruginosa. *Journal of the Chinese Chemical Society*, 2009; 56(2): 327-334.
11. Choi, Mi-Suk and Kwon-Suk Ahn Antibacterial effect of bamboo charcoal on Streptococcus mutans, *Journal of Korean Society of Dental Hygiene*, 2014; 14*(1): 95-100*.
12. Ma, Y., Wang Q., Wang X., Sun X. A Comprehensive Study on Activated Carbon Prepared from Spent Shiitake Substrate Via Pyrolysis with $ZnCl_2$. *J. Porous Mater*, 2015; 22(1): 157–169.
13. SNI-1995, SNI 06-37370. *Arang* Aktif Teknis. Badan Standarisasi Nasional, Jakarta, 1995.

14. Manurung.M., Putra, A.A.B and Octavia, I. Karakterisasi Arang Bambu yang dibuat dengan Metode Konvensional dan Aktivasi Termal serta Aplikasinya sebagai Adsorben Logam Pb(II) dan Cr(III). *Jurnal Kimia(Journal of chemistry)*, 15*(1).* Program Studi Kimia, Universitas Udayana Bali. DOI:https://doi.org/10.24843 /JCHEM .2021.v.15 .i01.p08, 2021.
15. Manurung, M., Oka R. dan Rizgyandhaka A. P. Aktivasi Arang Limbah Bambu dengan $ZnCl_2$ dan Penggunaan nya sebagai Adsorben Zat Warna *Remazol Yellow FG*. *Cakra Kimia [Indonesian E-Journal of Applied Chemistry]*, 2019; 7(1). ISSN 2302-7274.
16. Demiral, I., Samdan C.A and Demiral H., Production and Characterization of Activated Carbons from Pumpkin Seed Shell by Chemical Activation with $ZnCl_2$. *Journal Desalination and Water Treatment*, 2016; 57(6): 2446-2454.
17. Yuvakkumar R, Suresh J, Saravanakumar B, Nathanaeld, AJ, Honga, SI, Rajendran Rambutan peels promoted biomimetic synthesis of bioinspired zinc oxide nanochains for biomedical applications. Spectrochimica Acta Part A: *Molecular and Biomolecular Spectros copy*, 2015; 137: 250-258. Doi:10.1016/j.saa .2014 .08 .022.
18. Agrawal, H., Soumya M.S., Venkat K and Rajeshkumar, S, Mechanistic Study On Antibacterial Action Of Zinc Oxide Nanoparticles Synthesized Using Green Route, *Chemico-Biological Interaction*, 2018; 286: 60-70.
19. Ong, Y.K, Mark, J.W and Alastair, G.M, Zinc Disrupts Central Carbon Metabolis and Capsule Biosyinthesis in Streptococcus pyogenesis, *Scientific Reports*, 2015; 5(1): 10799.