THE COMPARATIVE STUDY OF THE INHIBITORY POTENTIAL OF CHICKEN AND QUAIL EGG SHELLS AGAINST BACILLUS CEREUS, STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA AND ESCHERICHIA COLI USING KIRBY-BAUER TEST

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Abstract - Currently, Quail egg shells have been used to treat dermatitis. Chicken egg is one of the most consumed products in our world today. Due to the existence of researches about the antibacterial potential of the stated eggs, comparative study is made in order to know what egg shell is better in treating or inhibiting the common bacteria used in the study. This research was undertaken to identify and compare the inhibitory potential present in Quail and Chicken eggshell against the bacteria namely, Bacilluscereus, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. Thisstudy aims to identify why and which powdered eggshell show the greater zone of inhibition and aims to inhibit the growth of the tested bacteria and if the existence and activity of the zone of inhibition on these bacteria is possible. The results from the zone of inhibition shows that among the four bacteria, exhibit that Gram negative bacteria has a larger summation of zone of inhibition. Overall, the result of this experimentation showed that inhibition of Chicken egg shells is much better than of Quail eggs and concluded that chicken and quail eggshell are effective to inhibit the growth of bacteria especially S. aureus but not effective to B. cereus. Furthermore, both eggshells can help to prevent diseases caused by these bacteria that thrive in acidic environment. The researchers recommend that using of active ingredient of the egg itself and testing its antimicrobial sensitivity will provide additional knowledge to further compare and know the potential of these inhibitors of bacteria.

Keywords - Eggshells, Zone of Inhibition, bacteria, Inhibitory Potential, Anti-microbial Sensitivity, Active ingredient, inhibitors

I. INTRODUCTION

Bird eggs from a variety of different species have been a crucial component in human diet since ancient times (Hester, 2017). Eggs are the most consumed protein in the Philippines based on the food consumption survey conducted in 2008 by the Food and Nutrition Research Institute of the Department of Science and Technology (FNRI-DOST, 2017). These eggs in our modern generation are mostly used for consumption and having the egg shells usually thrown away due to its limited usage. However, a number of researchers have delved into comparing different species of bird egg shells concerningwith its structure, chemical compositions, content of fatty acids, vitamins and so much more. Eggshells are inedible and with little vendible value, however, they may contain biologically active compounds (Nakano, et.al., 2003). However, in powdered form, egg shells in scientific studies reported that it can be a source of dietary calcium (Ray, 2014). Eggshell is a valuable source material or additive for numerous branches of the industry, such as, paper manufacturers. Numerous types of collagens, hyaluronic acid, or amino acids are extracted from the egg shell membrane and purified for numerous uses such as dietary supplements and cosmetics, which makes the separated eggshell membrane a highly valuable material (Gaonkar & Chakraborty, 2016). Moreover, there are defenses that are interior to the egg shells. This includes lysozyme, ovotransferrin, and other antimicrobial proteins in the albumen and the vitelline membrane. (Guyot etal., 2016; Wellman-Labadie, Picman, &Hincke, 2008). A study in Coturnixjaponica (Japanese quail) whichstudied the usage of the quail egg of indigent people in treating dermatitis and other skin conditions (Irokanulo, Nwonuma and Chukwudi, 2017). Therefore, this study revealed the potential contribution of egg shells in the field of medicine.

Due to the existence of antimicrobial potential of these two eggs this led the team to conduct a comparative study to further investigate the possible usage of egg shells waste both for chicken and quail eggs on bacteria that thrive in our world especially concerning the Philippines and supplying the needed information about the prevention or regulation of growth on the chosen bacteria). And with the stated background studies that supports this area of research it can be expected to have unexpected occurrences or benefits upon experimentation at the observation part of the research. One of the common issues of the society regarding this is the decomposition of egg shells. A Lot of people add eggshells in their garden or compost pile. It is claimed that they add important calcium to the soil for plants. (Pavlis, 2015). It was stated that egg shells don't dissolve in water. An eggshell contains about 2,000 mg of calcium and the boiling and soaking process released 0.2% of the calcium therefore boiling water did not have much effect on the eggshell (Gillman, 2008). A research was made to see what happens to egg shells in the

soil, yet, as a result, the hand crushed eggshells did not change the soil pH level, and did not increase the level of calcium in the soil after being in the soil for three weeks (Mitchell, 2005). Hence, it shows that throwing away used chicken egg shells or quail eggs shells only adds up to the waste in our environment if not used as fodder or fertilizers specially to our soil basing that its decomposition time is long. Furthermore, another is the issue of which will be a better antimicrobial inhibitor to the bacteria that thrives in non/local areas, such as, P. aeruginosa that thrives in moist environment, S.aureus that is a natural humanmicroflora are present in the environment except in places with extreme temperature, B. cereus that are commonly found in soil and food and E.coli which is naturally found in warm-blooded animal's intestine.

These disease-causing bacteria are common to the environment, thus, carrying potential diseases such as STEC infections caused by E. coli, skin infections induced by S. aureus, gastrointestinal disorder resulted from B.cereus and Pneumonia caused by P. aeruginosa. The objective of the study is to test and compare the inhibitory potential of egg shells against B. cereus, S. aureus, P.aeruginosa, and E. coli by using Kirby-Bauer test. This is to prove if scientific basis on the medicinal practices of indigenous people exist.

The research was conducted in order to know the potential of the chosen variables and to compare it having an independent variable which is the type of bacteria used to mainly contribute to the environment in terms of using the expected waste against the regulation and survival of bacterial growth in places which it can possibly thrive and infect locals. It does not only serve as a recycle of waste but seeing and knowing the effect of the everyday egg shells potential against these thriving bacteria in which antibacterial activity may exist in this field the researchers are delving.

It can also contribute to the further research in eggs shells usage against potential harmful bacteria which can be used for possibly treating diseases. Not only it can help in preventing diseases, powdered eggshells can also be used in diet because of its high calcium content and it is an effective calcium supplement. Hence, eggshell is not actually a waste in our economy but can be a known treatment (Arnarson, 2017).

II. METHODOLOGY

2.1 Collection of Samples

The 15 chicken (refer to Figure A.) and 25 Quail (refer to Figure B) eggs were acquired from the public market of Wakas, Bocaue, Bulacan (14.7980° N, 120.9261° E)

2.2 Preparation of Pulverized Egg Shells

The outside of the eggshells was cleaned using two Purified Drinking water (Aquafina which is Purified using Hydro-7TM process that is indicated on the label) to wash the undesired dirt or waste on the shells. After the cleaning of eggs, then proceeds to the opening of the eggs to separate the egg shells and its contents. Which is then placed on a platform (cardboard box) and left to sun dry having a climatic condition of mostly sunny in Francisco Street, Taal, Bocaue Bulacan (14.811999° N, 120.935504° E) and has a 32°CI o F (Refer to Figure C.). The Dried eggshells were pulverized using a mortar into a fine powder and then the powdered eggshells were stored in a sterile glass jar (refer to Figure D. 1 for Chicken egg shells and Figure D. 2 for Quail egg shells.)After the collection and preparation of the Pulverized shell, it is directly sent to the biology laboratory in De La Salle University, Dasmariñas (DLSUD). And the bacteria were bought at the National Institute of Molecular Biology and Biotechnology (BIOTECH), UPLB for the preparation of the culture media.

2.3 Preparation of Culture Media

"The Procedure for the preparation starts by Suspending 38g of the powder in 1 liter of purified water then is mixed thoroughly. Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder then autoclaved at 1210C for 15 minutes. Then is cooled to 45-500C and dispensed into petri dishes and poured 15-20 ml of the ready media on to petri dishes. Left standing for thirty minutes to solidify and is labeled on the bottom of each plate with date of preparation and batch number." The standard procedures used were from Becton Dickinson, U.S.A.

2.4 Maintenance and Preparation of Bacterial Cultures

The bacterial cultures were obtained from the National Institute of Molecular Biology and Biotechnology (BIOTECH), UPLB. The cultures were cultivated in nutrient agar (NA) at 37°C for 24 hrs. After 24hrs, they were stored at 10°C until further use. Prior to antimicrobial sensitivity testing, bacterial strains were cultivated in NA at 37°C for 24 hrs. After 24hrs, the cells were harvested by centrifugation at 5,000 rpm for 5 mins and were resuspend in 0.85% sterile saline solution to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard or approximately 1 to 2 x 108 cfu/mL.

2.5 Extraction of Antimicrobial Substance using Rotary Evaporator

The egg shell powder was mixed with 95% ethanol in 50% w/v. It was mixed thoroughly and set aside for 12 hrs. After 12 hrs., the sample was subjected to rotary evaporator set at 60°C. The resulting volume was placed in a micro tube and refrigerated until further use. (Refer to figure E.)

2.6 Antibacterial Activity using Agar Well Diffusion Method

A Mueller-Hinton Agar was seeded with 1 X 108 cfu/mL of B. cereus, P.aeruginosa, S. aureus and E. coli. Usinga Cork borer, wells measuring 5 mm in diameter were prepared. Using a Pipettor, 200 µL extract was placed in the well. The plates were incubated at 37°C for 24 hrs.

III. RESULTS AND DISCUSSION

Upon knowing the possibility of the existence of zone of inhibition in the Agar plate from the extract against the said bacteria, it is evident that the certain outcomes the researchers got vary from one bacterium to another.

3.1 Antibacterial Activity

The results from the Zones of inhibition among the four: gram positive Bacteria (S. aureus and the B. cereus), and the Gram negative bacteria (P. aeruginosa and E. coli) showed a difference in which the Gram negative bacteria have a larger zone of inhibition and transparency compared to the Gram positive bacteria. Ex 1 – (Chicken egg shells) finely ground, Ex 2 – (Quail egg shells) coarsely ground, C1 – Amoxicillin, C2 – Ethanol.

3.1.1B. cereus

Extract 1 (Ex 1) which showed the slight change in transparency together with the (small) zone surrounding the Ex 1. Extract 2 (Ex 2) showed the surrounding zone a bit bigger than Ex 1 however the zones transparency is higher than the Ex 1. Control 1(C1) showed no zone. Control 2 (C2) showed a larger in Zone however its transparency is lower than of the two extracts.

3.1.2 S. aureus

Ex 1 showed a small surrounding zone which the transparency of it is visible but not clear. Ex 2 showed a zone that is larger but a slight difference in transparency which is lower than of the Ex 1. C1 showed a zone which has the same transparency as the Ex 1 also having the area of the zone similarly close to the Ex 1. C2 showed a clear in transparency and a visible zone which is smaller than the two extract and one controlled but the zone of inhibition appeared to be stable in its circular surrounding.

3.1.3 P. aeruginosa

Ex 1 showed its zone of inhibition in which the transparency of it is high but the size of the zone is small. Ex 2 showed a smaller zone of inhibition from the Ex 1 but the same transparency. However, the inner surrounding of the extract has been surrounded by sediments in which in the picture showed an encircling white region on it. C1 showed a larger zone of inhibition than Ex 1 in which its transparency is lower than Ex 1. C2 showed similarly the same zone of inhibition of Ex 2 and the same sediment occurrence.

3.1.4 E. coli

Ex 1 showed the zone of inhibition in which its transparency is average but the area of the zone is small. Ex 2 have its zone of inhibition irregular in shape and the irregularity of it shows the size is larger than of the Ex 1. C1 among the zone of inhibition in the petri dish, this is the largest and transparent. C2 showed its zone of inhibition however the transparency of it is similar of Ex 2. The size of the zone is smaller than of C1 but bigger than Ex 1. The shape is circular but not clearly stable in accordance to its shape.

The measurement of the zone of inhibition of the 2 extract and 2 controlled per bacteria in the agar plate are shown in the (Table 1). The zone of inhibition (ZOH) of the B. cereus measured about 5 millimeter (mm) which did not exhibit zone of inhibition.

Table 1. Zone of inhibition (ZOH) (mm)

Bacteria	Control antibiotic (amoxicillin)	Control antibiotic (Ethanol)	Chicken Egg shells	Quail Egg shells
P. aeruginosa	11	7	9	7
E. coli	13	11	9	7
S. aureus	10	7	11	10
B. cereus	5	5	5	5

Table 1. shows the measurements of the zone of inhibition using Amoxicillin and Ethanol as the Control Antibiotic and having the Chicken egg shell ZOH greater than of the Quail egg shells. The 5 mm shows no zone of inhibition (B. cereus) and is the size of the agar well.

Quail eggs are believed to treat an individual with a number of health conditions. According to (Ronjit et al., 2017) Chicken egg shells are used for preparation as ash which Indians use in order to treat food poisoning. However, numbers of claims are still yet to be proven in scientific research to further understand and know why such beliefs exist concerning health treatment.

Based on the findings or results of the inhibitory activity of both egg shells shows that both extracts inhibit two Gram negative and one Gram positive which is the S. aureus bacteria based on our Kirby Bauer Antibiotic Test.

Out of the 4 bacteria used in the study, 3 only showed zone of inhibition and the bacteria that showed no zone of inhibition is the B. cereus. Because of this, the hypothesis of the study is not fully achieved for which the Extracts of the egg shells shown its inhibitory potential for which the existence of zone of

inhibition is met except for the B.cereus but all shows a clear comparison of ZOH.

B. cereus is a Gram-positive aerobic orfacultative anaerobic. It is being associated with food poisoning and stated as the cause of serious and potentially fatal non-gastrointestinal-tract infections given that it causes foodborne illnesses. (Bottone, 2010) In the said bacteria, as the researchers' test for the zone of inhibition of the 2nd extract, which is the quail egg shells, it can be observed that it has no zone of inhibition at all. Based on research, B.cereus can be treated by vancomycin,which is an antibiotic and a natural treatment for the infection of the intestines. Since the extract that we have used that came from the quail egg shell has no presence of the said antibiotic or any type that can be considered as substitute, the bacteria was able to resist the quail egg shell extract. As when it is to be compared to the reaction of the bacteria if the amoxicillin was used that it has no zone of inhibition as well, the researcher can conclude that the quail egg shells extract does not support the hypothesis of the study for the potential antibacterial property.

In our findings, the bigger the size of the diameter of the zone of inhibition provides us the knowledge that it is better. S. aureus (staph) today are usually found in the lower extremities and drugs like Amoxicillin (C1) that is not being relied upon treatment for lower extremities because of its beta-lactamase susceptible (Kosinski, 2003). And in the (Table 1 included in the results part) shows that Amoxicillin has the third highest diameter of zone of inhibition which behind E. coli and P. aeruginosa. Amoxicillin though is still considered an appropriate antibiotic against Staphylococcal infection or together with clavulanate (Stanway, 2015). The inhibition against Staph of the Ex 1 and Ex 2 records the highest zone of inhibition in all extracts against four bacteria which Ex 1 has a higher zone of inhibition than the drug amoxicillin that shows a higher inhibitory of the growth of S. aureus. The zone of inhibition of Ex 2 is the same of the amoxicillin which has the same inhibitory effect against this Gram Positive bacterium. And the ethanol shows the least of inhibiting this bacterium in growth of the susceptible thriving environment.

Madappa (2019) states that E. coli is one of the most habitual causes of many usual bacterial infection comprehending cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as Neonatal meningitis. Therefore, it thrivesin warm blooded animals including humans specifically in the lower intestine. The results showed that E. colihave a larger zone of inhibition with the extracts 1 (chicken egg shells) but Ex 2 (Quail egg shells) falls 2 diameters short than of Ex 1 however these extracts are smaller in diameter compared to Amoxicillin and ethanol having amoxicillin the largest zone of inhibition followed by the ethanol and the Ex 1. Because of these amoxicillin is the recommended inhibitor of E. coli and Ex 2 (Quail egg shells) as the least but Ex 1 when choosing between the better inhibitor of the extracts.

P. aeruginosa is an aerobic gram-negative bacterium, motile, non-spore forming rods that are oxidase positive and lactose non-fermenters (fujitani et al., nd) that able to thrive in highly diverse ecological niches (Valot et al., 2015). It is associated with nosocomial infections, often severe and life-threatening, especially in immunocompromised hosts (Fujitani et al. nd). Larger diameter zone of inhibition of Ex1 shows it can be an alternate to C1 that shows amoxicillin who has the larger diameter zone means it is still the better antimicrobial to use against P. aeruginosa. Ex 2 and Ethanol both shows the least zone of inhibition.

Overall, when comparing the Extracts used in the study, chicken egg shell Extract is the better inhibitor of the three bacteria. While Quail egg shell Extract is close to the size of the zone of inhibition of the chicken egg shell extract against S. aureus.

The study in the in vitro antimicrobial potential of Coturnix japonica egg shell and membrane powder, achieved zone of inhibitions in which certain amount of concentration of Quail egg shells are used and showed that in a 5 ml of Egg shell powder concentration will result to a zone of inhibition of 9 mm and in our study against S. aureus of Quail egg shell powdered extract shown of a zone of inhibition of 10 mm which is higher than the results of the in vitro study because of this occurrence, the researchers found out that a higher zone of inhibition is met when you use the extract of quail egg shell powder rather than the crude state. Existence of inhibitory activity is met in our study which is similar of the antibacterial and fungal property existence. (Irokanulo, Nwonuma and Chukwudi, 2017).

CONCLUSION

The results of the study indicate that inhibitory potential of Chicken egg shell and Quail egg shells will inhibit bacteria which met the hypothesis of the study and shows that Chicken egg shells is the better extract that can be used against dealing with these bacteria thriving in our local environment. This concludes that it can also be of help to the antibacterial activity of Ovotransferrin ("which has an iron-binding property that aids the membrane of the egg shell to deprive bacteria of free iron for their metabolism and replication". (Irokanulo, Nwonuma and Chukwudi, 2017)) which Pseudomonas sp. and E. coli are one of the most sensitive species to it while S. aureus is considered as one of the mostresistant to it yet in our findings, both chicken and quail egg shells the was used showed the highest inhibitory in all bacteria used. Furthermore, the researcher conclude that the chicken and quail eggs are effective to inhibit the growth of bacteria (specially, S. aureus) but not effective to B. Cereus.Both eggs can help the society

to prevent number of victims which get diseases cause by these bacteria by the means of inhibiting their growth in the environment they thrive in and also prevention of accumulation of shell waste in the soil that do not decompose quickly in the soil that can only breaks if an acidity is met and powdered form are used which the study can make use of inhibiting the growth of bacteria that thrives in acidic environment. And to use Chicken egg shells based on the bacteria being inhibited for it to further become effective inhibitor of growth of the tested bacteria.

RECOMMENDATION

The researchers would like to further assess the antimicrobial property of the egg shell and its effectiveness to biological entities through the usage of different methods to be done with the egg shells itself. Through the results that our study has attained, it can be concluded that our subject can possibly be a source and addition to some antimicrobials if further be developed. Aside from extracting the egg shells, it is advisable for it to be heated and then be pulverized (Oshima et al., 2015). In addition, the heated egg shell powder can be tested with bacterial vegetative cells, fungi and bacterial spores and examine its results for the zone of inhibition it has created. Furthermore, the results of this study can be improved through the use of various kinds of egg shells since this study focuses on only two kinds of shells that can be easily found in your local supermarket. It is highly possible that other kinds of egg shells have several more componentsthat is feasible in producing a zone of inhibition to a few or chosen bacteria. We researchers recommend the usage of active components or ingredients and subjected to antimicrobial sensitivity.

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