THE EFFECT OF ETHANOLIC PLANT EXTRACT, *CENTELLA ASIATICA* AGAINST *SALMONELLA TYPHI* AND *STREPTOCOCCUS AGALACTIAE*

MOHAMAD FAKHRUL AMIN BIN YEOP ZAINUDDIN
D17A0015

A RESEARCH PAPER TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF VETERINARY MEDICINE

MAY 2022

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled **'The Effects of Ethanolic Plant Extracts, *Centella asiatica* against *Salmonella typhi* and *Streptococcus agalactiae'*** by Mohamad Fakhrul Amin binYeop Zainuddin and in our opinion it is satisfactory in terms of scope, quality and presentation as part of the requirement for the course DVT 5436 – Research Project.

Dr. Luqman Bin Abu Bakar

B. Sc (Biological Sciences), MSc. Sc, Ph.D. Biotechnology (UMT)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Supervisor)

Dr. Mohammad Sabri Bin Abdul Rahman

DVM (UPM), MVSc, (UPM)

Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-Supervisor)

ACKNOWLEDGEMENT

Special thanks to those who have given their support, guidance, advice, and aid for the completion of this project paper:

Dr. Luqman Bin Abu Bakar

Dr. Mohammad Sabri bin Abdul Rahman

Lab assistants of FPV UMK

Family

DVM 5 Class of 2017/2022

Thank You

DEDICATIONS

I wish to express my deepest gratitude to my supervisor Dr. Luqman Bin Abu Bakar for his awesome commitment and encouragement in ensuring the completion of the project. Thank you for all your invaluable help throughout the project. Special thanks to my co-supervisor, Dr. Mohammad Sabri bin Abdul Rahman for his advice and comments that I shall treasure even after the completion of my project.

I also dedicate this dissertation to many of my lecturers, and seniors, and not to forget my classmates DVM 5 2017/2022 who have supported me throughout the process. I will always appreciate all of the things they have done. Not to forget my coursemates and friends for their support and ideas that contribute directly or indirectly towards the completion of the project.

Finally, I wish to express my greatest gratitude to my parents, my sisters and te my brothers for their love and encouragement throughout my life. I wish to dedicate this project to them.

Table of Contents

List of Figures

List of Appendices

ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436 – Research Project.

Low-growing pan-tropical perennial *Centella asiatica*. It spreads and forms a dense ground cover, which is advantageous in some situations. Salmonella is one of the world's deadliest food-borne infections. *Streptococcus agalactiae* causes chronic, infectious cow mastitis, as well as invasive sickness in camels, dogs, cats, fish, and hamsters. This study examined the effect of ethanolic plant extract *on Salmonella typhi* and *Streptococcus agalactiae*. *Centella asiatica* was purchased from a wet market and stored in zip-lock bags. Allow plant samples to air dry at room temperature for three days, then grind them into a fine powder. Then, it will be soaked in ethanol with 1:100 ratio and left to rest at room temperature before filtering the extracts and concentrating the filtrate under decreasing pressure for 15 minutes at 40˚ C. *Salmonella typhi* and *Streptococcus agalactiae* have been cultivated on selective media: nutrient agar for *S. agalactiae* and Xylose Lysine Deoxycholate agar for *S. typhi*. For the antimicrobial disc diffusion test for plant extracts, the material was diluted with DMSO. The disc was placed on MHA agar already streaked with bacteria and incubated for 24 hours. The plant extract shows no inhibitory zone on bacteria. At 62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml, and 1000 mg/ml, there is no inhibitory zone on the bacteria *S. typhi* and *S. agalactiae*. No inhibition zone may be related to the morphological nature of bacteria, whose cell walls have numerous layers that can limit the extract's antibacterial activity and cause resistance to plant extracts. Antimicrobial activity was dose-dependent, and extraction solvent affected antibacterial metabolites. Antibacterial tests of *C. asiatica* showed that ethanol extracts were ineffective against

ABSTRAK

Abstrak kertas penyelidikan telah dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai keperluan separa bagi kursus DVT 5436 – Projek Penyelidikan.

Centella asiatica pan-tropika yang tumbuh rendah. Ia merebak dan membentuk penutup tanah yang padat, yang berfaedah dalam sesetengah keadaan. Salmonella adalah salah satu jangkitan bawaan makanan yang paling mematikan di dunia. *Streptococcus agalactiae* menyebabkan mastitis lembu yang kronik dan berjangkit, serta penyakit invasif pada unta, anjing, kucing, ikan dan hamster. Kajian ini mengkaji kesan ekstrak tumbuhan etanol terhadap *Salmonella typhi* dan *Streptococcus agalactiae*. *Centella asiatica* dibeli dari pasar basah dan disimpan dalam beg berkunci zip. Biarkan sampel tumbuhan kering di udara pada suhu bilik selama tiga hari, kemudian kisar menjadi serbuk halus. Kemudian, ia akan direndam dalam etanol dengan nisbah 1:100 dan dibiarkan berehat pada suhu bilik sebelum menapis ekstrak dan menumpukan turasan di bawah tekanan menurun selama 15 minit pada suhu 40˚ C. *Salmonella typhi* dan *Streptococcus agalactiae* telah ditanam pada media terpilih. : agar nutrien untuk *S. agalactiae* dan agar Xylose Lysine Deoxycholate untuk *S. typhi*. Untuk ujian resapan cakera antimikrob untuk ekstrak tumbuhan, bahan tersebut telah dicairkan dengan DMSO. Cakera diletakkan di atas agar-agar MHA yang sudah berbelang bakteria dan diinkubasi selama 24 jam. Ekstrak tumbuhan tidak menunjukkan zon perencatan pada bakteria. Pada 62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml dan 1000 mg/ml, tiada zon perencatan. Tiada zon perencatan mungkin berkaitan dengan sifat morfologi bakteria, yang dinding selnya mempunyai banyak lapisan yang boleh mengehadkan aktiviti antibakteria ekstrak dan menyebabkan ketahanan terhadap ekstrak tumbuhan. Aktiviti antimikrob adalah bergantung kepada

dos, dan pelarut pengekstrakan menjejaskan metabolit antibakteria. Ujian antibakteria *C. asiatica* menunjukkan bahawa ekstrak etanol tidak berkesan terhadap *S. typhi* dan *S. agalactiae*. Kesan antibakteria *C. asiatica* tidak berkaitan dengan komposisinya.

Kata kunci *: Centella asiatica, ekstrak tumbuhan, S. typhi, S, agalactiae, antibakteria*

1.0 INTRODUCTION

Plants have long been a source of traditional medicine, and traditional treatments integrating plants have been utilised to treat a wide range of human problems for centuries. Many of these plants have recently been found to have a wide variety of bioactive phytochemicals, which explains their traditional use (Saranraj *et al.,* 2014). *Centella asiatica*, often known as pegaga, is a perennial with a pan-tropical distribution. In some settings, its propensity to spread and form a dense ground cover is advantageous; in others, it is not. Although it is not especially competitive in crops, it may have an effect on natural vegetation and biodiversity. *C. asiatica* is also one of the invasive species discovered in China's Dongting Lake marshes in Hunan Province (Hou *et al.,* 2011).

Salmonella species are frequently regarded as one of the most severe food-borne infections in the world. Many of the serotypes that are most frequent in people (such as *S. typhimurium* and *S. enteritidis*) are also often found in poultry, showing the significance of poultry as a reservoir for the transmission of salmonellae to humans (Martelli *et al.,* 2012). *Streptococcus agalactiae* causes the chronic, infectious mastitis of cows. In rare cases, it also causes mastitis and invasive disease in camels, as well as in dogs, cats, fish, and hamsters. Its presence in milk is typically associated with elevated somatic cell counts and decreased milk supply (Jain *et al.,* 2012). This study examined the antibacterial activity of a plant extract against the pathogenic microorganisms *Salmonella typhi* and *Streptococcus agalactiae*.

2.0 PROBLEM STATEMENT

One of the greatest challenges affecting human and animal welfare is the growth of super bacteria. Super bacteria mean bacteria that are resistance to most of antibiotic and other medication that commonly used to treat the infectious they cause. Antibiotics are continuously administered to commercial livestock such as poultry as antimicrobial growth promoters which enhanced selection of resistant bacteria livestock. The extract of the plant for the antimicrobial resistance of bacteria needs to be study for alternative medication as common antibiotic in laboratory.

3.0 RESEARCH QUESTION

3.1 How the extraction of the plant can be used in pathogenic bacteria such as *Salmonella typhi* and *Streptococcus agalactiae*?

3.2 Are the plant extract effective as antimicrobial against *Salmonella typhi* and *Streptococcus agalactiae?*

3.3 Does the different concentration of plant extract affect the antimicrobial activity against the tested bacteria *?*

4.0 RESEARCH HYPOTHESIS

4.1 Plant extract can be used as anti-microbial against *Salmonella typhi* and *Streptococcus agalactiae.*

4.2 Most of the *Salmonella* spp. and *Streptococcus agalactiae* can be less resistance based on the antimicrobial mechanism of the extraction of *C. asiatica.*

5.0 RESEARCH OBJECTIVES

5.1 To investigate the effect of the plant extract to the pathogenic *Salmonella typhi* and *Streptococcus agalactiae.*

6.0 LITERATURE REVIEW

6.1 Experimental Plant Description

Centella asiatica, sometimes known as pegaga, is a perennial of pan-tropical distribution with a sluggish growth rate. In some settings, its propensity to spread and form a dense ground cover is advantageous; in others, it is not. On a number of Pacific islands where it has been introduced, it has been categorised as invasive and assigned a High Risk (scoring 7) rating; however, the circumstances under which it is causing problems are unknown. Although it is not especially competitive in crops, it may have an effect on natural vegetation and biodiversity. *C. asiatica* is also one of the invasive species discovered in China's Dongting Lake marshes in Hunan Province (Hou *et al.,* 2011).

Scientific name	Family	Common name	Local name	Parts used	Traditional use
Centella asiatica	Umbelliferae	Asiatic Pennywort, Gotu Kola	Pegaga	Aerial part, roots	Treating bronchitis, asthma, dysentery, lowering blood pressure

(Ali,2008; Abas *et al.,* 2003)

6.2 *Centella asiatica* as an alternative medicine.

Centella asiatica (Pegaga) has reportedly proven effective in treating inflammations, diarrhoea, and other skin lesions (Dash & Faruquee,2011). *C. asiatica* is an essential plant with antibacterial properties against intestinal infections. *C. asiatica* plant extracts possess antibacterial properties against five gram-positive and eight gram-negative bacteria (Ullah *et al.,* 2009).

6.3 Salmonella as food-borne bacteria

Salmonella is reportedly the most prevalent cause of foodborne illness in the chicken industry. Salmonella enterica serovar Thyphimurium is the most important serotype that causes salmonellosis in poultry, while Salmonella enterica serotype Enteriditis is the most common source of illness outbreak in poultry farms (Wessels et al., 2021). Salmonellae are nonselective bacteria that can proliferate in a range of non-host settings. They do not require sodium chloride to grow, but can thrive in concentrations between 0.4 and 4%. Others can grow as low as 2 to 4°C or as high as 54°C (Gray and Fedorka-Cray, 2002).

Vertical (transovarian) transmission of the disease is conceivable in birds, but the disease can also be transmitted through direct or indirect contact with infected birds via the respiratory route, faeces, or contaminated feed, water, or litter. Antimicrobials such furazolidone, gentamycin sulphate, and antimetabolites are used to treat pullorum disease (sulfadimethoxine, sulfamethazine, and sulamerazine) (Msoffe et al.,2009)

6.4 *Streptococcus agalactiae* as etiological agents of mastitis in cattle.

Streptococcus agalactiae and *Staphylococcus aureus* are two primary infectious bacteria that cause bovine subclinical mastitis worldwide. *S. agalactiae*-induced subclinical mastitis may have a significant impact on the amount and quality of milk produced. *S. agalactiae* can live in the mammary gland for lengthy periods of time, and unidentified affected animals that are not treated may serve as infection reservoirs (Estuningsih *et al*., 2002). Chronic, infectious cow mastitis is caused by *Streptococcus agalactiae*. It also causes mastitis and invasive illness in camels, as well as disease in dogs, cats, fish, and hamsters on rare occasions. Its presence in milk is commonly linked to high somatic cell counts and lower milk output (Jain *et al*., 2012).

7.0 MATERIAL AND METHODOLOGY

7.1 Sample collection

Centella asiatica was bought from the wet market in Kota Bahru. The plants were sample properly into the zip lock plastic bag to prevent any form of contamination.

7.2 Preparation of plant extracts

Collected sample was washed under running tap water to remove any dirt and allow them to air dry for three days at room temperature (27 °C) with a relative humidity of 70%. Mechanical grinder was used to make the dried plant will be ground to a fine powder. Each 100 g of ground sample was soaked in ethanol with ratio 1:100 and allowed to sit at room temperature for one days. Whatman filter paper was used to filter the extracts, and the residues were employed for the second and third extractions. To get crude extract, the filtrate will be concentrated under decreased pressure for 15 minutes in a rotary evaporator at 40°C. The crude extract was kept at -20°C.

7.3 Microbial culture

In this study, pathogenic *Salmonella typhi and Streptococcus agalactiae* strains were employed from preserve bacteria of glycerol stock from bacteriology laboratory. Nutrient Agar and Xylose Lysine Deoxycholate are the media agars used to culture the microorganisms (XLD) which *Salmonella* spp. and *S. agalactiae* was cultured on nutrient agar. To prevent overgrowth and contamination, streak the bacteria onto the agar plate, incubate the agar at 37°C overnight, and then cool the agar.

7.4 Antimicrobial assay of plant extracts via disc diffusion test

The sample from the plant extract was prepare in which been diluted with DMSO solution based on its concentration. The concentration are 6.25 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml. Then, the was out on the small discs. The bacteria strain had been streaked on Mueller-Hinton agar which are *S. typhi* and *S. agalactiae.* Next, the discs were put on the agar and then incubate for 24 hours. After that, the inhibition zone was measured for each sample.

8.0 RESULTS

8.1 Antimicrobial activities

Agar diffusion method are used to determine the antimicrobial activity and the result was shown on the table below.

Table 8: Antibacterial activity on *Salmonella typhi* and *Streptococcus agalactiae.*

Bacteria strains / Concentration (mg/ml)	Inhibition zone (cm)				
	62.5	125	250	500	1000
S. typhi	-	-	-	-	-
S. agalactiae	-	-	-	-	-

There is no inhibition zone observed on both bacteria. For *S. typhi* there is no inhibition zone observed on the concentration of the plant extract on 62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml. For *S. agalactiae* also there are no inhibition zone observed on the concentration of the plant extract on 62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml.

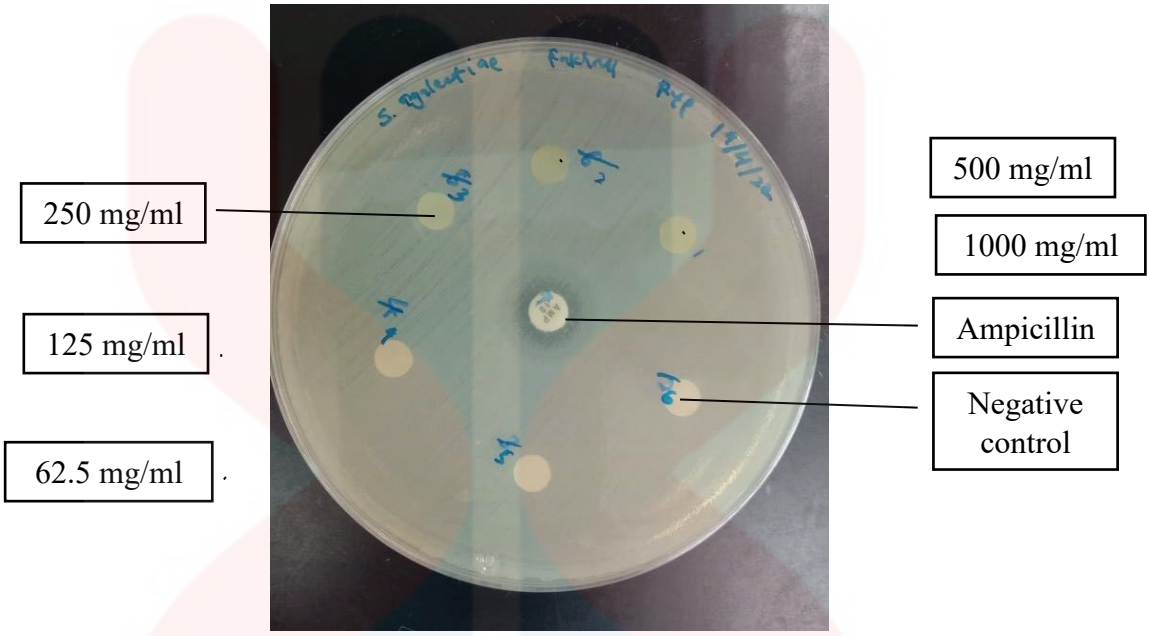

Figure 8.1 : Ethanol extract concentration of *C.asiatica* which shows no zone of inhibition against *S.agalactiae.*

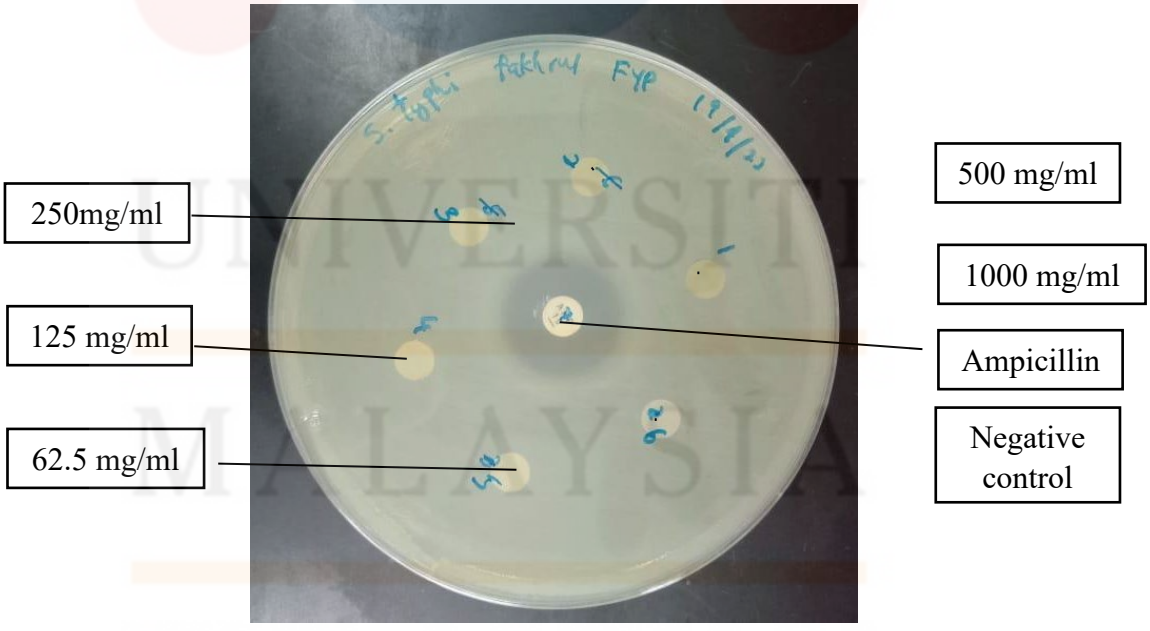

Figure 8.2 : Ethanol extract concentration of *C. asiatica* which show no zone of inhibition against *S. typhi*.

9.0 DISCUSSION

The finding in Figure 8.1 and Figure 8.2 revealed that the bacteria samples tested with different concentration of extracts of *C. asiatica* showed no inhibition zone in extracts. There are no antibacterial activities in both extract against *S. typhi and S. agalactiae.* This is because the structure of the microorganisms itself also play role in susceptibilities towards the antibacterial agent. Based on the study by Madigan & Martinko,2006 suggested that because of morphological structure of bacteria which bacteria cell wall contain many layers which can inhibit the antibacterial effect from the extract that cause resistance towards the antibacterial effects of the plant extracts. Other than that, there is studies by Marino *et al.,* 2011 reported about gram negative bacteria demonstrated more resistancy toward plant extract due to the characteristics of the outer membrane layer surrounding their cell wall that are very rich with lipopolysaccharide in which may restrict the diffusion of the hydrophobic and hydrophilic through alteration of porin and permeability thus it will lead in resistancy towards antibacterial properties.

According to (Manikkuwadura *et al*., 2019) the existence of insufficient quantities of active constituent or constituents in the extract to demonstrate antimicrobial activity, could be the cause of the poor results. The absence of antimicrobial activity does not imply that the plant lacks bioactive chemicals or that it has no antibacterial effect against pathogens. The current study found that antimicrobial activity was dose dependent, and that the type of solvent used for extraction influenced the extraction of metabolites required for antibacterial activity.

As a result, the availability of plant crude extracts is influenced by the type of solvent used for extraction and the concentration of the plant material. Various parameters, such as the rotary evaporator employed during crude extraction, have an impact on the activities of crudes based on the boiling point of the extraction solvent, according to this study. For example, the temperature used for ethanol and water is different (i.e., the temperature used during the rotary evaporator is 45 °C for both solvents; because water requires a higher temperature than ethanol, it requires a longer extraction time), and this can have an adverse effect on plant material activity.

According to Figure 8.1 and Figure 8.2, plant extract concentrations ranged from 62.5 mg/ml to 125 mg/ml, 250 mg/ml, 500 mg/ml, and 1000 mg/ml, but no antibacterial activity was seen on the selected bacterial strains. Nanasombat, 2009 suggested that the occurrence of an antagonist effect could be attributed to the various chemicals. Furthermore, the plant extract used in this study contains a mixture of secondary metabolites as phenolic acids, flavonoids, alkaloids, and terpenoids, which can inhibit *C.asiatica* extracts' antibacterial properties.

According to Nassar *et al.,* 2019 every possible combination of antimicrobial agent and reference organism using NA was shown to be inadequate when compared to MHA. Each antibiotic-organism combination yielded more than three incorrect readings over a thirty-day period. In light of CLSI's guidelines, none of the results were deemed acceptable. It was evident from testing clinical isolates that there were considerable disparities between the susceptibility data acquired by NA and the standard MHA, with total errors of 27.76 percent, 22.4 percent, and 3.6 percent for *P. aerginosa, S. aureus*, and *Enterobacteriaceae*, respectively.

10.0 CONCLUSION

Present preliminary of *in vitro* antibacterial testing of *C. asiatica* revealed ethanol extracts of *C. asiatica* were found to be ineffective for growth control of *S. typhi* and *S. agalactiae.* In conclusion, the content of the C. asiatica does not show any relationship towards antibacterial activity.

11.0 RECOMMENDATION AND FUTURE WORK

In the future, it is suggested that sequential solvent extraction be used to lessen the antagonistic effect of additional secondary metabolites that can reduce antibacterial activity. Determine the best solvent concentration for extracting the most plant extract. Additionally, experimental research on the antagonistic effects of these secondary metabolites in plants should be conducted. Future studies should separate all plant chemicals to determine which ingredient contributes to antibacterial activity. Besides, we can change the agar from Mueller-Hinton agar to nutrient agar so that it may show the positive results of the inhibition zone. Finally, with this unique study of ethnopharmacology, there is promise for combating antibiotic resistance through the use of phytochemicals.

Appendix A – Plant Material

Figure A: The dried plant material of *Centella asiatica*

Appendix B

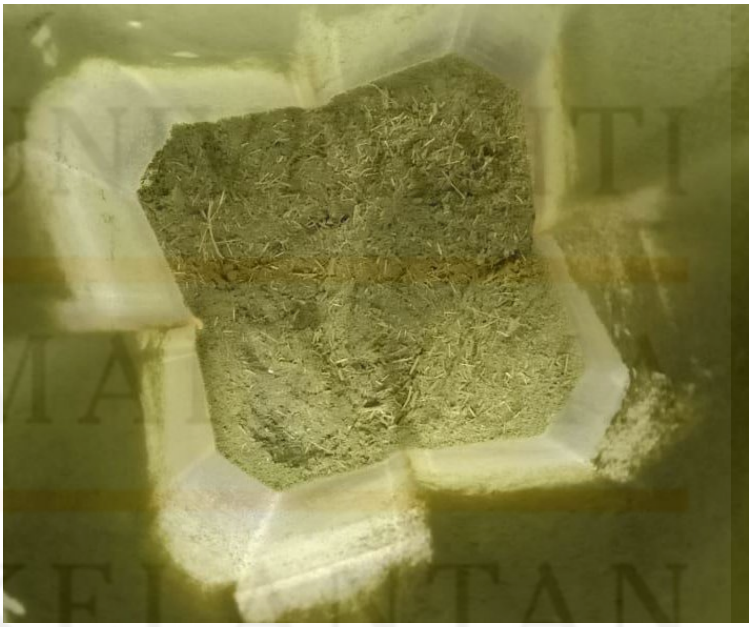

Figure B : The grind material of the *C.asiatica*

Appendix C

Figure C : The rotatory evaporation process before become crude extract.

REFERENCES

Bean, N.H., Griffin, P.M.: Food-borne disease outbreaks in the United States, 1973-1987: pathogens, vehicles and trends. J.Food Prot., 1990; 53:804-817

Dash BK, Faruquee HM, Biswas SK, Alam MK, Sisir SM and Prodhan UK: Antibacterial and Antifungal Activities of Several Extracts of Centella asiatica against L. against Some Human pathogenic Microbes. Life Scineces 2011: 1-5.

EFSA Scientific opinion on a quantitative estimate of the public health impact of setting a new target for the reduction of Salmonella in laying hens. EFSA J 2010; 8: 1546

Estuningsih, S., Soedarmanto, I., Fink, K., Lammler, C., & wibawan, I. W. T. (2002). Studies on Streptococcus agalactiae Isolated from Bovine Mastitis in Indonesia. *Journal of Veterinary Medicine Series B*, *49*(4), 185–187. https://doi.org/10.1046/j.1439-0450.2002.00482.x

Ferreira, V.; Cardoso, M.J.; Magalh£es, R.; Maia, R.; Neagu, C.; Dumitra Åcu, L.; Nicolau, A.I.; Teixeira, P. (2020). Occurrence of Salmonella spp. in eggs from backyard chicken flocks in Portugal and Romania - Results of a preliminary study. Food Control, 113(), 107180–. doi: 10.1016/j.foodcont.2020.107180

Gray, J. T. and Fedorka-Cray, P. J. 2002. Salmonella. In Cliver, D. O. and Riemann, H. P. (Eds.). Foodborne diseases, p. 55-68. San Diego: Academic Press.

Hamidun, B. (2014). Cosmos Caudatus Kunth: A Traditional Medicinal Herb. Global Journal of Pharmacology, 8(3), 420–426. https://doi.org/0.5829/idosi.gjp.2014.8.3.8424

Hou ZY; Xie YH; Chen XS; Li X; Li F; Pan Y; Deng ZM, 2011. Study on invasive plants in Dongting Lake wetlands. Research of Agricultural Modernization, 32(6):744-747.

Jain B, Tewari A, Bhandari BB and Jhala MK.2012. Antibiotic resistance and virulence genes in Streptococcus agalactiae isolated from cases of bovine subclinical mastitis. Veterinarski Arhiv 82(5): 423-432.

Jameel, Khairi & Isa, Jawad. (2020). Isolation and Identification of Salmonella from Marketing Eggs Isolation and Identification of Salmonella from Marketing Eggs.

J.L.S. Taylor, T. Rabe, L.J. McGaw, A.K. Jäger, J. Van Staden Towards the scientific validation of traditional medicinal plants Plant Growth Regul., 34 (1) (2001), pp. 23-37

Karen O'Hanlon Cohrt. (2017). Crash Course in Microbial Identification. Bitesize Bio.https://bitesizebio.com/36644/methods-microbial identification/:///files/2341/methods-microbial-identification.html

Kosaka Y; Xayvongsa L; Vilayphone A; Chanthavong H; Takeda S; Kato M, 2013. Wild edible herbs in paddy fields and their sale in a mixture in Houaphan Province, the Lao People's Democratic Republic. Economic Botany, 67(4):335-349. http://rd.springer.com/article/10.1007/s12231-013-9251-6

Madigan M.T. and J.M. Martinko. 2006b. Endospore forming, low GC, gram-positive bacteria: Bacillus, Clostridium and relatives, p. 379. In: Brock Biology of Microorganisms. 11th ed. Pearson Prentice Hall, USA.

Manikkuwadura, Hasara Nethmini De Zoysa, Hasanga, Rathnayake, Ruwani, Punyakanthi Hewawasam, Weerasinghe, Mudiyanselage Dilip Gaya Bandara Wijayaratne, 2019. Determination of In Vitro Antimicrobial Activity of five Sri Lankan medicinal plants against selected human pathogenic bacteria. Int. J. Microbiol. 2019, 8.

Manning, J., Gole, V., & Chousalkar, K. (2015). Screening for Salmonella in backyard chickens. Preventive Veterinary Medicine, 120(2), 241–245. https://doi.org/10.1016/j.prevetmed.2015.03.019

Martelli F, Davies RH. Salmonella serovars isolated from table eggs: An overview. Food Res Int 2012; 45: 745– 754

Marino, M., N., Abdollahi-Zivech, B., Salamatdoustnobar, R., Ahmadzadeh, A., Aghajanzadeh-Golshani, A. and Mohebbizadeh, M. 2011. Dtermining nutritive value of soybean straw for ruminant using nylon bags technique. Pak. J. Nutr. 10, 838-841

Msoffe PL, Aning KG, Byarugaba DK, Mbuthia PG, Sourou S, Cardona C, Bunn DA, Nyaga PN, Njagi LW, Maina AN, Kiama SG. Handbook of Poultry Diseases Important in Africa. CRSP: A Project of the Global Livestock; 2009. p. 83

Nanasombat, S. and Teckchuen, N. 2009. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. Journal of Medical Plants Research 3(5): 443-33

Nasser MS, Hazzah WA.and Bakr MK. Validation of AST results on NA medium as a substitute for MHA by some Microbiology laboratories in Alexandria, Egypt. Journal Egypt Public Health Association, 2019; 94 (1):4-10.

Qamar, A., Ismail, T., & Akhtar, S. (n.d.). Prevalence and antibiotic resistance of salmonella spp. in south Punjab-pakistan. PLOS ONE. Retrieved December 25, 2021, from https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0232382

Saranraj, P. and S. Sivasakthi, 2014. Medicinal Plants and its Antimicrobial Properties: A Review. Global Journal of Pharmacology, 8(3): 316-327.

Ullah, M.O.; sultana, S.; hauw, A.; and Tasmin, S. 2009. Antimicrobial, cytotoxic and antioxidant activity of Centella asiatica. European J.Sci. Rs. 30: 260-4.

Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, et al. Host adapted serotypes of Salmonella enterica. Epidemiol Infect 2000; 125: 229– 2

Wessels, K., Rip, D., & Gouws, P. (2021). Salmonella in Chicken Meat: Consumption, Outbreaks, Characteristics, Current Control Methods and the Potential of Bacteriophage Use. Foods, 10(8), 1742. https://doi.org/10.3390/foods100817