ANTIMICROBIAL PROPERTIES OF *MUSA PARADISIACA* AND *PIPER BETLE* AGAINST *ESCHERICHIA COLI*

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CERTIFICATION

This is to certify that we have read this research paper entitled **'Antimicrobial properties of *Musa paradisiaca* and *Piper betle* against *Escherichia coli*'** by Farhan Danial bin Muhammad Syazwan Thana, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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Thank You

DEDICATIONS

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parent, Salija, whose words of encouragement and push for tenacity ring in my ears. My brother and sister Syafiq, Liyana and Dania who have never left my side and are very special.

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Table of Contents

List of Tables

ABSTRACT

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

Colibacillosis is one of the important diseases affecting the majority of poultry industry. Colibacillosis is caused by *Escherichia coli* commonly affected all age groups of chicken and highly infectious to cause high morbidity and mortality within the infected flocks. The most prominent clinical disease is diarrhoea. There are a lots of local plants and herbs were proven to have antimicrobial properties against *Escherichia coli*. Thus, this study done to determine antimicrobial effect of local plants, *Piper betle* and *Musa paradisiaca* against *Escherichia coli*. The leaves of *P. betle* and *M. paradisiaca* were collected, extracted and further tested via antimicrobial sensitivity test (AST) to explore the antimicrobial properties of these plant.

Keywords: *Colibacillosis, Escherichia coli, Piper betle, Musa paradisiaca, antimicrobials*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Colibacillosis adalah salah satu penyakit penting yang menjejaskan sebahagian besar industri ayam. Colibacillosis yang disebabkan oleh *Escherichia coli* menjejaskan semua kumpulan umur dan sangat berjangkit hingga menyebabkan morbiditi dan kematian yang tinggi dalam kumpulan yang dijangkiti. Tanda klinikal yang paling ketara ialah cirit-birit. Terdapat banyak tumbuhan dan herba tempatan yang terbukti mempunyai sifat anti bakteria terhadap *Escherichia coli*. Justeru, kajian ini dilakukan untuk menentukan kesan anti bakteria tumbuhan tempatan, daun sireh dan daun pisang terhadap *Escherichia coli*. Daun tumbuhan sireh dan pisang dikumpul, diekstrak dan diuji selanjutnya melalui ujian sensitiviti mikrob (AST) untuk meneroka sifat anti bakteria tumbuhan ini.

Kata kunci: *Kolibasilosis, Escherichia coli, Piper betle, Musa paradisiaca,* antimikrobial

1.0 Introduction

The most common disease affecting poultry growth and development is colibacillosis inducing diarrhea caused by *Escherichia coli.* In particular, colibacillosis is prevalent, resulting in high morbidity and mortality, reduce in feed conversion ratio, not easily manage, and financial damage to the farmer. These devastating effects are severely impeding the well and fast growth of the chicken sector. Antibiotics are extensively utilised in the chicken industry to boost growth and reduce diarrhea by bacterial. But, the overuse or endless use of antibiotics results in a weakened immunity, the creation of drug-resistant strains, secondary infection, and drug residues, all of which generate a slew of food insecurity issues for humans and the habitat. (Dibner & Richards, 2005; M'Sadeq *et al*., 2015; Lekshmi *et al*., 2017). This situation encouraged the request for another treatment option that is very effective, low adverse effect, low residue, and resistance to treat and prevent bacterial diarrhea.

Alternative treatment can be done by using herbal plant extracts that have antimicrobial properties to replace the usage of microbial based antimicrobials and synthetic-based antimicrobials. Thus, this study proposes to explore the antimicrobials properties of two local plants *Musa paradisiaca* and *Piper betle. M. paradisiaca* is widely grown in many tropical countries, including Malaysia. Studies have shown presence of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, saponins, and many more, making this plant possesses numerous antibacterial properties. The ethanolic extract of *M. paradisiaca* showed antibacterial activity against *Bacillus subtilis, E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Sivasamugham *et al*., 2021). Meanwhile, the *P. betle* is a member of the Piperaceae family and is an important herbal plant with great therapeutic potential and a wide range of pharmacological activity. It is well grown throughout East Africa and the tropical regions of Asia and commonly known as *sirih*. *P. betle* leaves contain numerous beneficial bioactivities and have been used in traditional medical systems (Patra *et al*., 2016). Both of these plants are well grown in tropical climate and easily available in Malaysia.

2.0 Research Problem

As we all know, antibiotics have been commonly used as antimicrobial growth promoter (AGP) and as treatment for bacterial diarrhea in the poultry industry. However, the abuse or long-term application of antibiotics leads to the low level of immune system, the production of drug-resistant strains, secondary infection and drug residues, which cause a series of food safety and health problems to human beings and the environment (Dibner & Richards, 2005; M'Sadeq *et al.*, 2015; Lekshmi *et al.*, 2017). Such a condition inevitably promotes the demand for valid alternatives with high efficacy, few side effects, low residue, and resistance to prevent and treat bacterial diarrhea.

To date, there are limited information regarding the antimicrobial properties from the local herbal plants being used commercially. Therefore, this study aims to explore the potential of plant extract from *M. paradisiaca* and *P. betle* as therapy of avian colibacillosis.

3.0 Research Question

3.1 Does the local plant extracts, *M. paradisiaca* and *P. betle* possess antibacterial effects against *E. coli* ?

4.0 Research Hypothesis

4.1 Plant extracts of *M. paradisiaca* and *P. betle* possess antimicrobial activity against *E. coli.*

5.0 Objective

5.1 To investigate the antimicrobial effect of plant extract of *M. paradisiaca* and *P. betle* against *E. coli.*

6.0 Literature Review

6.1 Avian colibacillosis

Avian colibacillosis is an infectious disease of birds caused by *E. coli.* It is one of the leading causes of morbidity and mortality in the poultry industry due to its relationship with a variety of disease conditions, either as a primary pathogen or as a secondary pathogen. In poultry, it causes yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicaemia, polyserositis, coligranuloma, enteritis, cellulitis, and salpingitis, among other diseases. In its acute form, colibacillosis of chicken causes septicaemia, which leads to death, and in its subacute form, pericarditis, airsacculitis, and perihepatitis (Calnek *et al.*, 1997).

Avian colibacillosis is a serious infectious disease that affects birds of all ages. This disease has a significant economic impact on the global chicken industry. The majority of the economic losses are caused by the impacted birds' mortality and decreased output (Otaki, 1995). Microbial food safety is becoming a growing public health concern around the world. According to epidemiological statistics, poultry meat is still the leading source of human food illness (Mulder, 1999). Poultry meat is more popular in the consumer market because of benefits such as easy digestion and widespread acceptance (Yashoda *et al.*, 2001). However, pathogenic and spoilage bacteria in poultry meat and meat by-products continue to be a major source of worry for suppliers, consumers, and public health officials around the world. In most nations around the world, *E. coli* and *Salmonella* have been linked to foodborne diseases (Lutful Kabir, 2010).

6.2 Pharmacological activities of the plant extract

In many countries, including Malaysia, the banana plant (*M. paradisiaca*) is widely grown (Green Herbology, 2017). Due to the presence of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, saponins, and others, this plant possesses a wide range of antibacterial properties. The ethanolic extract of *M. paradisiaca* showed antibacterial efficacy against *B. subtilis, E. coli*, *P. aeruginosa*, and *S. aureus* (Naikwade *et al.*, 2014; Alisi *et al.*, 2008). *M. paradisiaca* and *M. acuminata* ethyl actetate extracts had antibacterial efficacy against multidrug-resistant nosocomial infections (Karuppiah and Mustaffa, 2013). *M. sapientum* leaf extracts contained chemicals that are ineffective against *Staphylococci,* such as piperine, reserpine, and thymol. Gram negative bacteria such as *E. coli*, *S. typhimurium*, and *L. monocytogenes* were suppressed by terpenoids such as thymol, cinnamaldehyde, and trans-cinnamaldehyde, but not staphylococci (Barbieri *et al.*, 2017).

The *P. betle* is a member of the Piperaceae family, an important medicinal plant with a wide range of pharmacological actions. It can be found throughout East Africa and the tropical parts of Asia. The leaves of the *P. betle* exhibit wide range of bioactivities and have long been utilised in traditional medical systems (Patra *et al.*, 2016). The *P.*

betle has been found to have antibacterial properties (Syahidah *et al.*, 2017). Previous phytochemical studies found high levels of phenolic compounds in *P. betle*; nevertheless, the bioactive chemicals hydroxychavicol and eugenol were found to be substantial, and these bioactive compounds have been researched and described as powerful antibacterial agents (Atiya *et al.*, 2018; Syahidah *et al.*, 2017). Gram-negative bacteria like *E. coli* and *P. aeruginosa*, Gram-positive bacteria like *S. aureus*, and *Candida albicans* were all killed by *P. betle* leaves. Minimum bactericidal concentration/minimum inhibitory concentration values demonstrated bactericidal and bacteriostatic actions of *P. betle* leaves, whereas Minimum Fungicidal Concentration/Minimum inhibitory concentration values indicated fungicidal and fungistatic effects of *P. betle* leaves. This review also includes a list of phytochemical substances found in betel leaf extracts and essential oils, as well as safety profiles and betel leaf value-added products (Nayaka *et al.*, 2021).

6.3 Extraction of the medicinal plants to obtain the bioactive compounds

The main process to obtain the bioactive compounds from biomass materials (medicinal plants) is through the extraction process in which this process will eventually maximize the amount of target compounds available in the plants and to get the highest biological activity of the extract. However, extraction yield and the biological activity affected by the technique used for the extraction. Not only by the technique used, the extraction yields also affected by the extraction solvent that were used in the extraction. Depends on the types of the bioactive compounds in the plants which may vary among the different types of medicinal plants, the plants may have different in solubility properties in different types of solvent and as the optimal solvent for the extraction depends on the compounds that are to be isolated. The actual and suitable extraction solvent for specific plant materials is eventually difficult (Truong *et al.*, 2019).

6.4 Colibacillosis Study Using Plant Extract

Natural additions, such as plant essential oils and extracts, have piqued the interest of researchers because of pharmacological activity, minimal toxicity, and economic viability. Secondary metabolites which are plant essential oil that are generally recognised as safe as flavouring agents for animal and human ingestion. They have been identified as another method of chemical to contain foodborne bacteria. The study provided an overview of the most prominent world medicinal herbs affecting *E.coli.* The findings revealed that 44 distinct native medicinal herbs were effective against *E.coli* and were traditionally used to treat colibacillosis. The study have showed some plants that have activity against *E. coli* effect such as *Thymus vulgaris* (Thyme*), Rosmarinus officinalis* (rosemary), *Syzygium aromaticum* (clove)*), Zataria multiflora* (Avishane shirazi), *Pluchea indica* (Indian camphorweed), *Cymbopogon martini* (Palmarosa), *Aloysia triphylla* (Lemon beebrush), *Origanum vulgare* (Oregano), *Satureja cuneifolia* (wild savory), *Eucalyptus tereticornis* (Forest Red Gum), *Aloe vera* (Aloe) and *Foeniculum vulgare* (Fennel). Results of phytochemical study has revealed that bioactive components of plants with antioxidant and antibacterial activities are a good option for synthetic pharmaceuticals in the feed and medicine industries, as well as for treating microbial infections in medicine. (Aminzare *et al.*, 2017).

7.0 Materials and Methods

7.1 Inoculum Preparation and Colony Forming Unit Counts

The inoculums used in this study were archive *E.coli* (AE) and pathogenic phylogroup *E. coli* (PPE). AE was prepared at Bacteriology Laboratory, Faculty of Veterinary medicine while , PPE is a local isolate, isolated from chicken farm located in Bachok, Kelantan and was characterized as pathogenic phylogroup *E.coli.*

7.2 Preparation of Ethanolic Extract of *M. paradisiaca* and *P. betle*

The plant leaves were purchased and collected from local market around Kota Bharu, Kelantan. Both of the plant's leaves underwent the same ethanolic extract preparation process. The leaves were dried in the oven at 37°C for three days. Then, the dried leaves were ground into fine powder by using a grinder. Briefly, ethanol was mixed with powdered of *M. paradisiaca* and *P. betle* in a flask with ratio of 100 g of sample to 1L of ethanol. After that, the mixture were left for three days at room temperature. Next, Whatman filter paper no. 1 was used to filter out the extract and then both extract were evaporated under reduced pressure by using rotary evaporator and controlled under a water bath at 30°C for 6 hours. Finally, both of the extracts were kept in a chiller for 24 hours and ready to be use for antimicrobial sensitivity test (AST). The extracts also can be kept in -20°C freezer for future used.

7.3 *In-vitro* Antibacterial Test

A colony of *E. coli* from lab was taken from the culture agar by using inoculating loop and was smeared evenly onto the surface of Mueller-Hinton agar plate. Next, a drop of *M. paradisiaca* extract was placed onto the sterile disk on the surface. Then, the plates were incubated, and as the bacteria grow on the surface of the plate, the *M. paradisiaca* extract was diffused into the agar. In positive result, the *M. paradisiaca* extract in the agar will inhibit the growth of the bacteria forming the zone of inhibition around the area of *M. paradisiaca* extract were placed. The zone was measured in millimetres and recorded. The same method was done for *P. betle* extract. The presence of inhibition indicates that the plant extracts are able to inhibit the growth of *E.coli*. All the step were repeated on the *E. coli* pathogenic group and positive control. The step were also repeated by using cork borer. Instead of sterile disk, well were made for the extract.

8.0 Results

Table 8.1 shows the results of the susceptibility test of the archive E. *coli* and pathogenic phylogroup E. *coli* against standard antibiotics. For oxytetracycline, the diameter of inhibition for AE was 3 cm while for PPE was no inhibition (resistance). For amoxicillin, the diameter of inhibition for AE was 1.7 cm while for PPE was no inhibition (resistance). For enrofloxacin, the diameter of inhibition for AE was 3.8 cm while for PPE was 0.9 cm. For neomycin, the diameter of inhibition for AE was 2 cm while for PPE was 1.2 cm. For colistin sulphate, the diameter of inhibition for AE was 2.1 cm while for PPE was 2.3 cm.

Table 8.1 Susceptibility test of the archive *E. coli* and pathogenic phylogroup *E. coli.* against five standard antibiotics.

Antibiotic (µg)	Diameter of inhibition zone in cm	
	AE	PPE
Oxytetracycline 30	3	-
Amoxicillin 10	1.7	-
Enrofloxacin 5	3.8	0.9
Neomycin 30	2	1.2
Colistin sulphate 10	2.1	2.3

AE: Archieve *E. coli.* PPE: pathogenic phylogroup *E. coli*

Table 8.2 show result of diameter of zones of inhibition of plant extracts against microorganisms at different extract concentration by using disc diffusion technique. It was observed that *P. betle* was effective against both *E. coli* while *M. paradisiaca* does not have any activity. For AE, the *P. betle* at concentration 1000 mg/ml inhibition zone was 2.6 cm, 500 mg/ml was 2.4 cm and 250 mg/ml was 2.2 cm. For PPE, the *P. betle* with concentration 1000 mg/ml inhibition zone was 1.5 cm, 500 mg/ml was 1.4 cm and 250 mg/ml was 1.1 cm.

Table 8.2 Diameter of zones of inhibition of plant extracts against microorganisms at different extract concentration by using disc diffusion technique.

Test organism	Plant extract	Diameter of inhibition zone (cm)		
		1000 mg/ml	500 mg/ml	250 mg/ml
AE	*P. betle*	2.6	2.4	2.2
	M. paradisiaca	0.0	0.0	0.0
PPE	*P. betle*	1.5	1.4	1.1
	M. paradisiaca	0.0	0.0	0.0

AE Archieve *E. coli.* PPE pathogenic phylogroup *E. coli*

Table 8.3 show result of diameter of zones of inhibition in cm of plant extracts against microorganisms at different extract concentration by using antimicrobial assay technique with cork borer. It was observed that *P. betle* was effective against both *E. coli* while *M. paradisiaca* does not have any activity. For AE, the *P. betle* with concentration 1000 mg/ml inhibition zone was 2.4 cm, 500 mg/ml was 2.3 cm and 250 mg/ml was 2.0 cm. For PPE, the *Piper betle* with concentration 1000 mg/ml inhibition zone was 2.2 cm, 500 mg/ml was 2.1 cm and 250 mg/ml was 1.8 cm.

Table 8.3 Diameter of zones of inhibition (cm) of plant extracts against microorganisms at different extract concentration by using cork-borer technique.

Test organism	Plant extract	Diameter of inhibition zone (cm)		
		1000 mg/ml	500 mg/ml	250 mg/ml
AE	*P. betle*	2.4	2.3	2.0
	M. paradisiaca	0.0	0.0	0.0
PPE	*P. betle*	2.2	2.1	1.8
	M. paradisiaca	0.0	0.0	0.0

AE Archieve *E. coli.* PPE pathogenic phylogroup *E. coli*

9.0 Discussion

In this study, *M. paradisiaca* does not have any antimicrobial activity *while Piper betle* have significant antimicrobial properties against *E.coli.* From table 8.1, PPE had resistant toward oxytetracycline and amoxicillin compare to AE. This result showed that the field *E. coli* was more virulent compared to *E. coli* obtained from the lab. The susceptibility of the organism toward other antibiotics such as enrofloxacin and neomycin is higher in AE as compared to PPE. However, only colistin sulphate can inhibit more PPE as compared to AE.

The study show that *P. betle* had antimicrobial properties as it can inhibit growth of PPE and AE. This is shown in the table 8.2 and 8.3. The plant was able to inhibit the growth of PPE which was resistance toward oxytetracycline and amoxicillin. The leaves of the *P. betle* exhibit a wide range of bioactivities and have long been utilised in traditional medical systems (Patra *et al.*, 2016).

The plant extract can also avoid the resistance toward the organism such as PPE on the oxytetracycline and amoxicillin. This is because of the properties and chemical of the plant in which the antibiotic does not have. The bioactive chemicals hydroxychavicol and eugenol were found to be substantial, and these bioactive compounds have been researched and described as powerful antibacterial agents (Atiya *et al.*, 2018; Syahidah *et al.*, 2017). Gram-negative bacteria like *E. coli* and *P. aeruginosa*, gram-positive bacteria like *S. aureus*, *and C. albicans* were all killed by *P. betle* leaves (Nayaka *et al.*, 2021).

In this study, *M. paradisiaca* showed no antimicrobial activity. However, other study such as the ethanolic extract of *Musa paradisiaca* showed antibacterial efficacy against *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Naikwade et al., 2014; Alisi et al., 2008) had shown the antimicrobial effect of the plant. Presence of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, saponins, and others, this plant possesses a wide range of antibacterial properties. The ethanolic extract of *M. paradisiaca* showed antibacterial efficacy against *B. subtilis, E. coli*, *P. aeruginosa*, and *S. aureus* (Naikwade *et al*., 2014; Alisi *et al*., 2008).

10.0 Conclusion

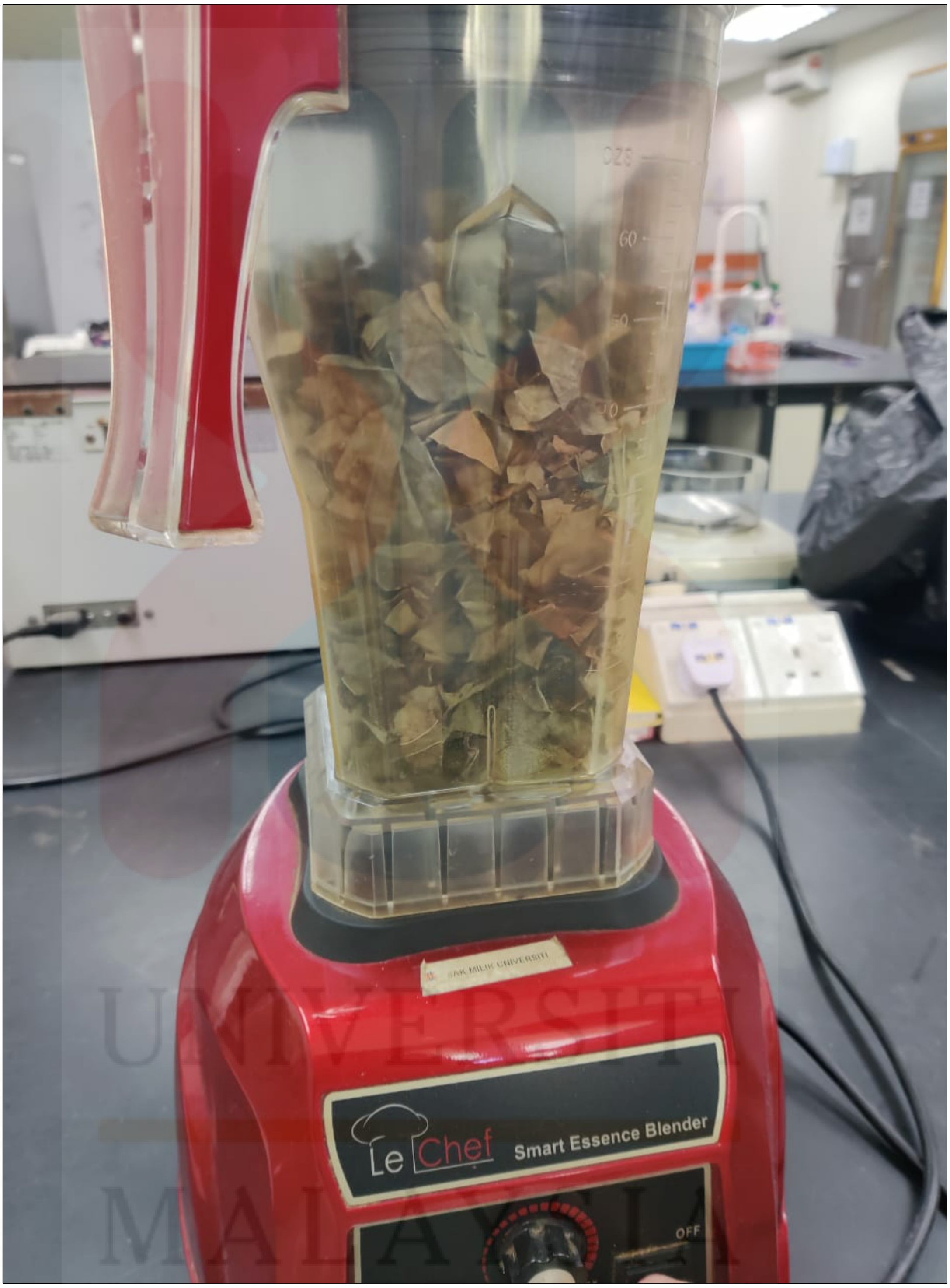
In conclusion, the application of local herbal for an alternative to antimicrobial treatment especially for virulent strain in poultry industry is a must. We can see that the antibiotic resistant is developing rapidly and this is the reason to think over and use effective herb as antimicrobial for therapeutic. The study had potential to become herbal antibacterial agent against resistant and susceptible bacteria and provide information for further studies which may support the use of the plant extract in treating colibacillosis and act as alternative to antibiotic agents in poultry industry.

11.0 Recommendations and future work

Several limitations were noted in this study. For the future study, it is wise to increase the sample size (e.g., 5kg) to produce meaningful results to calculate the inhibition zone of plant extract. Secondly, the study can be improved by using different solvent for the plant extract formulation. After that, it is suggest to do triplicate for each of the plant extract and antibiotic so that a statistical analysis can be done. Next , is to perform study at *in-vivo* level as the metabolism and physiology of live animal may produce different results.

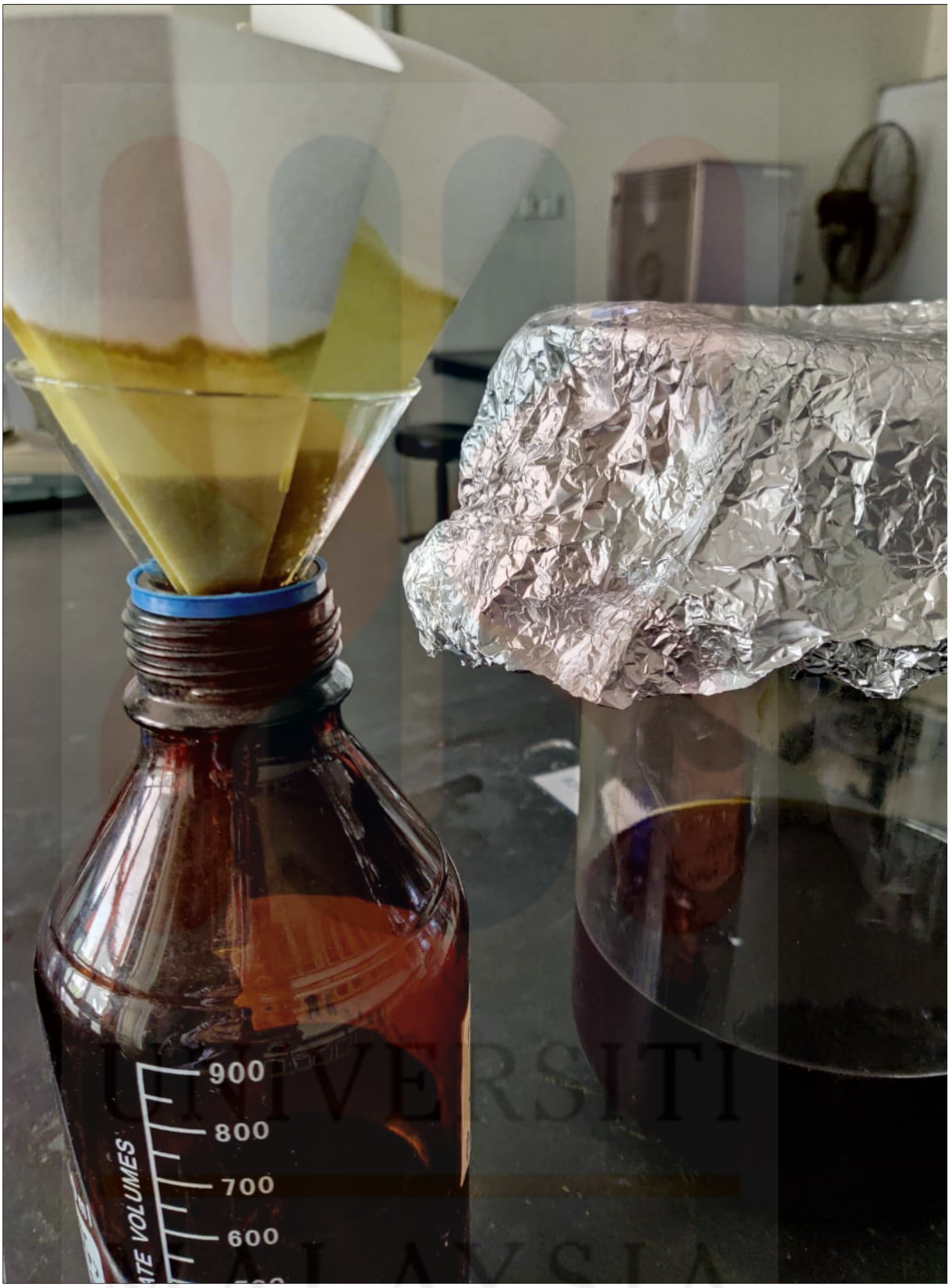
Appendix A

Appendix A-1 Drying of the plant



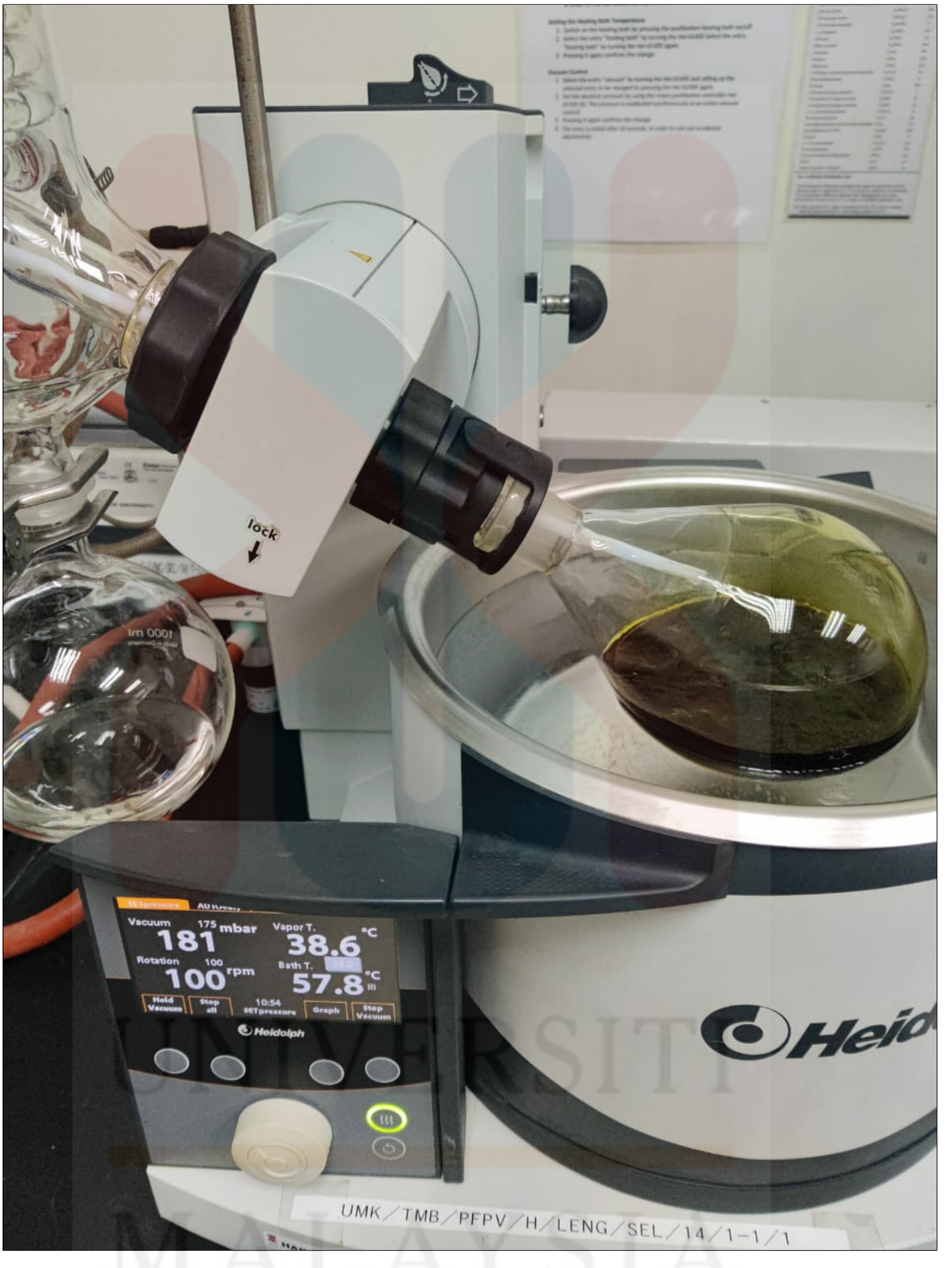
Appendix A-2 Grinding of the plant

Appendix A-3 Sample in powder form, before soak in ethanol



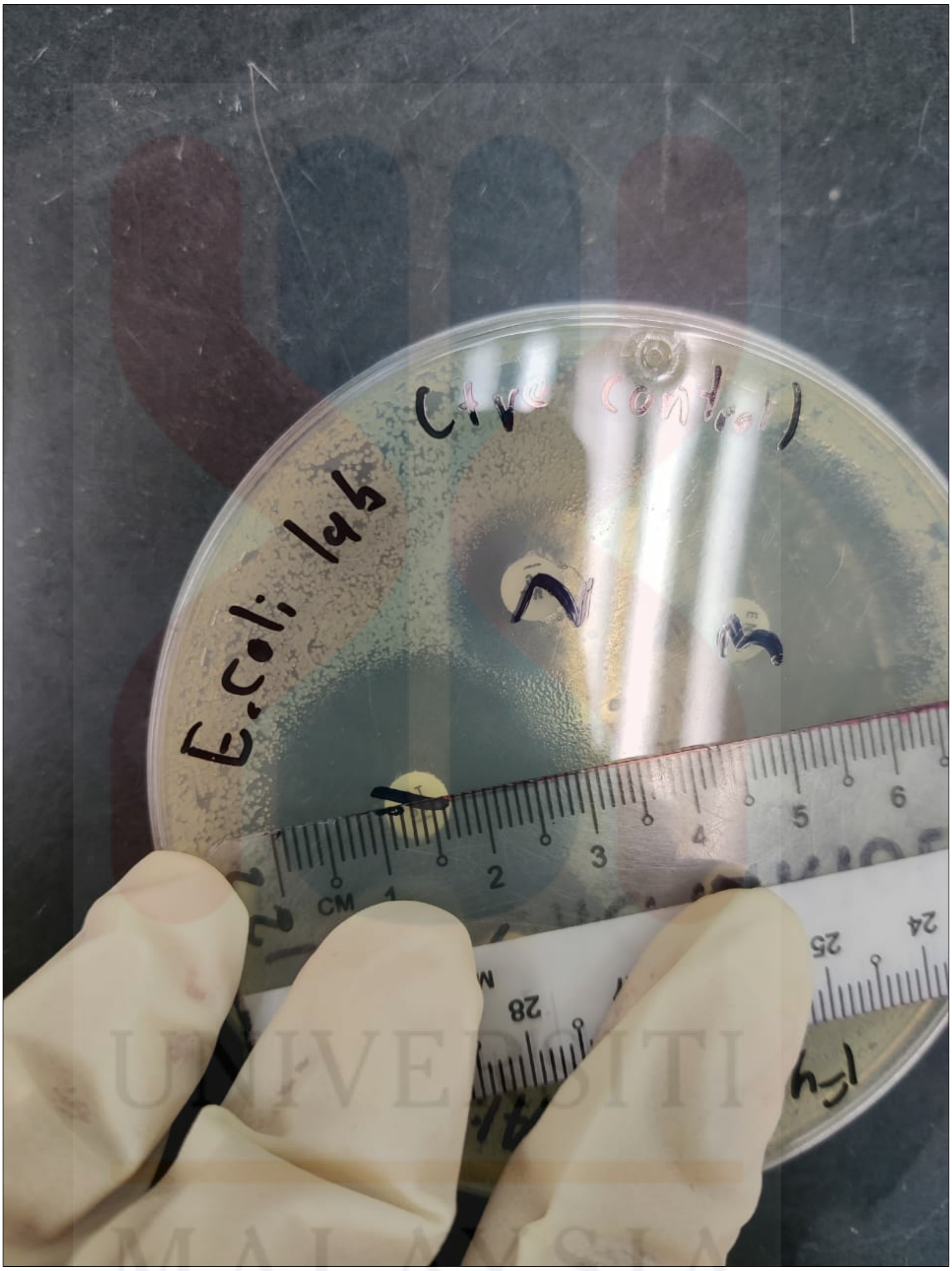
Appendix A-4 Filtration of the extract by using filter paper

Appendix B



Appendix B-1 Concentrating extract by using rotary evaporator

Appendix B-2 AST for the plant extract



Appendix B-3 Measuring the diameter of inhibition zone

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