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**INTRODUCTION (Times New Roman, 11 font size, bold)**

This template explains and demonstrates how to prepare your MSTE 2024 extended abstract. The best is to read these instructions and follow the outline of this text. Page margins are formed regarding the A4 page size and are 2.54 cm wide from the right, left, top and bottom. We therefore ask that authors follow some simple guidelines. In essence, you should format your paper exactly like this document.

The full paper should be written in MS Word format, preferably as .doc file. Use 1.5 lines-spacing in 11 font size Times New Roman. Please indent the text paragraphs. Please state clearly the aim (and scope) of the study, the materials and methods, the main findings and brief analysis of the study. Extended abstracts can contain figures, tables, formulations or images. **Extended abstracts should be at least 3 pages and not exceed five (5) pages including the references** and pages should not be numbered. All references should be cited in the text by square brackets [1]. Two or more references at a time may be put in one set of brackets [2, 3]. The references are to be numbered in the order in which they are cited in the text and are to be listed at the end of the contribution under a heading References, see our example below [1, 4].

**MATERIALS AND METHODS**

All materials and methods that have been used in the work must be stated clearly and subtitles should be used when necessary.

***Subtitle***

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**RESULTS AND DISCUSSION**

***Subtitle***

All of the tables, images and figures should be centred. Figures and images should be numbered together (Figure 1) and figure definitions should be placed under the figure or image; as for the tables, they should also be numbered (Table 1) and the table header should be placed at the top of the chart. Table, image and figure headers should be written with upper case initial letters, bold and should be centred.

|  |
| --- |
| Fig |
| **Figure 1.** Example of figure for the full paper. |

***Subtitle***

A table should be inserted like the one below and referred in the text as Table 1, Table 2, and so on. Please avoid vertical rules and shading in table cells.

**Table 1.** Example of table for the extended abstract

|  |  |
| --- | --- |
| **Symbol** | **Value** |
| A |  |
| B |  |

***Equations***

Equations (refer with: (1), (2), ...) should be indented. It is recommended to have one line of space above the equation and one line of space below it before the text continues. The equations have to be numbered sequentially, and the number put in parentheses at the right-hand edge of the text. Equations should be punctuated as if they were an ordinary part of the text. Punctuation appears after the equation but before the equation number, e.g.

|  |  |
| --- | --- |
| *c*2 = *a*2 + *b*2  | (1) |

**CONCLUSION**

Please conclude your work incorporating your most important finding as well as future works (3-4 lines).

**ACKNOWLEDGEMENT**

Please acknowledge your research grant, organization, scholarship (where relevant).

**REFERENCES (Numbered, Vancouver Style)**

1. Author. Title. Journal. Year; Volume (Issue): Pages. **(Journal Article)**
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**CONTOH PENULISAN ABSTRAK LANJUTAN**

**MISAI KUCING BIO-SUSTAINABLE PERSPECTIVE:**

**EMPOWERING THE PULSE OF FOOD SAFETY**

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**ABSTRACT**

Misai kucing is widely planted in Southeast Asia as a herbal remedy known for its medicinal benefits. As one of the national-listed herb cluster, misai kucing requires proper post-harvest handling procedures for sustainable high quality raw material production. In this study, misai kucing from MARDI Pontian and MARDI Serdang plots was harvested at different maturity stages. Samples were subjected to microbiological analysis to evaluate possible source of contamination and results have shown that all stems and leaves parts of misai kucing were safe within the allowable range. Our studies also showed leaves methanolic extract of misai kucingcontained good antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Vibrio parahaemolyticus* at week 10 of maturity stages, from MARDI Pontian and Serdang plots, respectively. Subsequent investigation on dried misai kucingleaves after prolonged storage at room temperature has also shown promising results with antibacterial activity against selected pathogens. The leaves extract of misai kucingfrom MARDI Pontian and Serdang still possessed strong antibacterial activity after three years of storage with inhibition zones of 12.1 mm; 12.5 mm and MIC value 1.56 mg/mL; 1.56 mg/mL against *S. aureus*, respectively. Following that, edible seaweed films incorporated with misai kucing extract of one, five and ten times their MICs were tested for their antibacterial effect. The inhibitory effects were observed significant against *S. aureus* at higher concentrations than their MICs, and samples from MARDI Serdang gave better results in contrast to MARDI Pontian (*P* <0.05) at ten times MIC. Overall, the study has evaluated the microbiological quality of misai kucing samples from different plant parts and maturity stages. The study also indicated antibacterial properties of freshly harvested and 3-year-old stored misai kucing*.* Interestingly, misai kucing-seaweed films were also found to have antibacterial potential empowering our understanding towards synergistic food safety wonders.

Keywords: microbiology, misai kucing, antibacterial, storage, seaweed.

**INTRODUCTION**

Misai kucing or *Orthosiphon stamineus* Benth (Lamiacea family) is widely planted in Malaysia and Southeast Asia including tropical parts of Australia and has been traditionally used in the form of dried leaves as herbal remedies [1]. Misai kucing plants have been listed as one of the High Value Herbal Products in the National Primary Economic Area (NKEA) attributed to its richness in phytochemicals [2]. In this study, misai kucing was planted and harvested at different maturity stages of 8, 10, 12 and 14 weeks by adopting Malaysian Agricultural Practices (MyGAP) at peat soil field plot (MARDI Pontian) and mineral soil field plot (MARDI Serdang), respectively. Therefore, this study was conducted to determine the microbiological quality of dried misai kucing for the development of *O. stamineus* standardized postharvest handling procedure. Comprehensive Standard Operating Procedure (SOP) based on national and international standards is upmost essential in order to complement global herbs market penetration and increase local production of high quality misai kucing. The rising popularity of natural herb-based preservatives being used in food industry has also led us to further examine the antibacterial effects of misai kucingat different plant parts and maturities on both plots. Additionally, we were interested in investigating whether misai kucing could have the same biological activity after prolonged storage and to determine the antibacterial activity of misai kucing extract incorporated into seaweed-based edible films. There are few available local published manuscripts on the study of microbiological quality and in vitro biological activity of misai kucing including fabrication into seaweed edible films hence this study was being conducted. This study helps to empower our understanding on the potential of misai kucing in natural bio-based food packaging applications and food safety interventions.

**MATERIALS AND METHODS**

***Preparation of plant materials and crude extract***

Misai kucing stems and leaves parts in this study were obtained from peat soil plantation plot at MARDI Pontian and mineral soil plantation plot at MARDI Serdang, respectively. Harvested plants were weighed, washed and air-dried in oven at 50oC until the moisture content dropped to 10%, before stored in airtight opaque bottle in room temperature (25oC ± 2oC) until needed [3]. All samples were measured at four different maturity stages from respective plots. Subsequently, 120 samples of harvested misai kucing were analyzed for Total Plate Counts, Yeast & Mould Counts, Coliform Counts, *Escherichia coli*, *Staphylococcus aureus* and Presumptive *Salmonella* based on method by Bacteriological Analytical Manual [4] with minor modifications. Then, the mean value for microbial counts (CFU/g) for samples was determined. For preparation of crude extract, misai kucing powder were mixed with 70% methanol in a proportion of 5 g: 150 ml. The extraction was performed using the method elaborated by [5]. The antibacterial activity of misai kucing extract was assayed against six bacterial pathogens using the disc diffusion method [5]. Misai kucing extract that demonstrated strong inhibition against test bacteria in the disk diffusion method were then measured with Minimum Inhibition Concentration (MIC) assay. The MIC values of the extract were determined as detailed by [5]. Further on, the 3-year-old prolonged stored plant materials consisted of misai kucing leaves parts from 10 weeks harvesting maturity stages were collected from Industrial Crop Research Centre, MARDI. Next, samples were subjected to crude extract preparation and in vitro biological analysis as [5]. After that, seaweed-based edible films were prepared by modification of the method used by [6]. Subsequently, the misai kucing extract at 1 fold (1560 mg/mL), 5 folds (7800 mg/mL) and 10 folds (15 600 mg/mL) of MIC was added and antibacterial activity analysis of the misai kucing-seaweed edible films was performed. The experimental data was next analyzed using One-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) method. Schematic layout of the whole experimental design was as illustrated as in Figure 1.



**Figure 1**. A schematic diagram of the whole experimental design of misai kucing

**RESULTS AND DISCUSSION**

***Biological activity of dried misai kucing and its extract***

Microbiological quality is one of the key criteria for Malaysian Monograph (MM) and European Herbal Infusion Association (EHIA) standard compliance. Among the investigated samples, all stems and leaf samples showed passed and satisfactory results comparable to MM and EHIA proving the postharvest SOP practiced in this study was effective. Specifically, the stems and leaves from both plots demonstrated better microbiology results in week 10 of maturity stages with l log CFU/g reduction. Antibacterial screening also showed inhibitory effects of misai kucing extract against *V. parahaemolyticus, S. aureus* and *B. cereus*, respectively from week 8 to 14 of misai kucing maturity stages. Interestingly, the highest antibacterial property was observed at week 10 maturities comparable to other weeks with inhibition zones more than 10 mm. It was thought that rosmarinic acid was at its optimum at this particular harvest week.

 Conversely, *E. coli, S. Typhimurium*, *L. monocytogenes* indicated resistance to all misai kucing extracts as no inhibitory effect was demonstrated in this study. However, strong inhibition against *S. aureus*, *B. cereus* and *V. parahaemolyticus* with inhibition zones more than 10 mm and excellent MIC values was observed from misai kucing leaves extract, better than stems extract (MARDI Pontian and Serdang), evidenced by the fact with [7], who reported the antimicrobial properties of methanol extracts of misai kucing against selected food-borne bacteria and identified leaves with the main components of pharmacologically active polyphenols and antioxidants.

***Biological activity of prolonged stored misai kucing and antibacterial study of misai kucing extract incorporated into seaweed edible films***

Our present study demonstrated strong inhibition activity of misai kucing extract against *S. aureus* (MARDI Pontian and Serdang) and *B. cereus* (MARDI Serdang only). Among the investigated extract, *S. aureus* exhibited promising antibacterial activity with inhibition zones of more than 10 mm from both field plots (p>0.05) however *B. cereus* posed strong inhibitory activity only from MARDI Serdang with inhibition zones more than 10 mm to leaves extract but showed significant difference (p<0.05) from MARDI Pontian with modest degree of inhibitory activity. In contrast, *E. coli*, *S. Typhimurium*, *L. monocytogenes* and *V. parahaemolyticus* were observed to be resistant to all misai kucing extract as no inhibitory effect was demonstrated in this study. These findings corresponded well to previous studies by [1] that indicated *S. aureus* and *B. cereus* were more sensitive to misai kucing methanolic leaves extract. The results of this study also showed interesting findings that the biological activity in misai kucing leaves over the storage period of three years at room temperature still fairly stable comparison to the fresh samples [5] [8]. Based on the lowest MIC values given, *S. aureus* shown to be the most susceptible with prolonged stored misai kucing leaves with MIC values of 1.56 mg/mL for MARDI Pontian and Serdang, respectively. Moderate antibacterial activity was shown by the same methanolic leaves extract against *B. cereus* with MIC values of 3.13 mg/mL for MARDI Serdang. Observation of the present study was supported by the previous work by [9]. This present data showed it is essential to indicate the stability of antibacterial agents during storage when studying their MICs. In term of antibacterial efficacy of misai kucing-seaweed edible films, the strongest zone of inhibition was observed at 10 times MIC (S10) against *S. aureus* (p<0.05). The results showed the zone of inhibition subjected to *S. aureus* increased from 10.8 to 20.2 mm diameter for MARDI Serdang samples during MIC values increased from 5 times to 10 times. Hence, the promising results were similar to previous observations [10]. The results (Table 1) showed the antibacterial activity of the misai kucing-seaweed films were enhanced against *S. aureus* with an increase in misai kucing extract concentration from MARDI Serdang samples.

**Table 1.** Antibacterial activity of misai kucing leaves extract incorporated into edible seaweed films against *S. aureus* and *B. cereus*

|  |  |  |
| --- | --- | --- |
| **Bacteria types area** | **Misai kucing*-*seaweed extract (mg/L)** | **Observation at 24 h** |
| **Zone of inhibition\*** **(mm)** | **Contact** |
| *Staphylococcus aureus* | P1 (Pontian 1) | 0.0 ± 0.0C | - |
| P5 (Pontian 5) | 0.0 ± 0.0C | - |
| P10 (Pontian 10) | 0.0 ± 0.0C | - |
| S1 (Serdang 1) | 0.0 ± 0.0C | - |
| S5 (Serdang 5) | 10.8 ± 0.2B | +++ |
| S10 (Serdang 10) | 20.2 ± 0.4A | +++ |
| Sw (Control) | 0.0 ± 0.0 | - |
| Chl (Control) | 25.4 ± 0.5 | +++ |
| Te (Control) | 20.9 ± 0.3 | +++ |
| *Bacillus cereus* | P1 (Pontian 1) | 0.0 ± 0.0 | - |
| P5 (Pontian 5) | 0.0 ± 0.0 | - |
| P10 (Pontian 10) | 0.0 ± 0.0 | - |
| S1 (Serdang 1) | 0.0 ± 0.0 | - |
| S5 (Serdang 5) | 0.0 ± 0.0 | - |
| S10 (Serdang 10) | 0.0 ± 0.0 | - |
| Sw (Control) | 0.0 ± 0.0 | - |
| Chl (Control) | 25.8 ± 0.4 | +++ |
| Te (Control) | 10.8 ± 0.4 | +++ |

**CONCLUSION**

In summary, this study concluded that the postharvest quality of misai kucing was in satisfactory condition at all maturity stages and plant parts from MARDI Pontian and Serdang plots are in accordance to the recommended microbiological specifications. The experiment findings also observed misai kucing extract demonstrated highest inhibitory activity at week 10 maturity stages from the leaves parts. Further studies proved that storage of dried misai kucing at room temperature for three years still exhibited antibacterial activity against *S. aureus* with 1.56 mg/mL of MIC values. In addition, misai kucing leaves extract incorporated into seaweed-edible films at levels of 10 times of its MIC led to a significant effective antibacterial activity of *S. aureus*. Overall, our results warrant new understanding on misai kucing due to its economic potential as natural food preservatives and sustainable food packaging.

**ACKNOWLEDGEMENT**

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