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Assessment on effect of *Mangifera* Sp microwave extraction against bacterial activity

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Extraction of bioactive compounds from plants samples have become very important for food and pharmaceutical purposed especially compounds with antimicrobial activity. Since, the usage of microwave assisted extraction without organic solvent have been suggested for Green Analytical Chemistry (GAC) optimisation should be done. Moreover, *Mangifera* sp. has been reported as a medicinal plant that possessed antibacterial, antifungal, antiseptic and astringent activity against human pathogens. In this study, the effect of the MAE was investigated based on different exposure timelines such as 5 min, 8 min, 10 min and 12 min while comparing it to the Soxhlet extraction. Standard methods of disc-diffusion assay were carried out by determination length zone of inhibition (mm) using disc-diffusion agar assay against various bacteria namely *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococus aureus*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The result obtained showed that 10 min *Mangifera sp* sample (M10) was the optimum time and possessed overall high inhibition activity compare to other timelines and soxhlet extract. Inhibition activity of mature and immature leaves extract shows different inhibition pattern due to the differences in the phytochemicals produced. Further study using different antimicrobial assays such as broth dilution and time kill assay would give a precise view on *Mangifera* sp bioactivity.

Keywords: Antimicrobial activity, *Mangifera sp*, microwave assisted extract (MAE), disc-diffusion assay, minimum inhibition concentration

INTRODUCTION

Plant based materials continue to play major role in primary health care in many developing countries. More than hundreds of plant species in Malaysia are reported to have medicinal properties. *Mangifera* sp is the one of medical plant that was tested as antibacterial, antifungal antioxidant and anti-inflammatory activities due to high phytochemical such as mangiferin, phenolic acids, flavonoids and incredibly rich with polyphenols, a large group of natural micronutrients that possess health advantages (Chaiwarit et al. 2021).

Eventhough bacterial infection has been remarkably reduce since the discovery of antibacterial drugs, there are still some of the pathogens have become resistant to many of the first discovered effective drugs. The process of drug resistance as well as occurrence of undesirable side effects of certain antibiotics (WHO, 2020) lead to the new research of antibacterial compounds from medicinal plants. High percentage resistance of microorganisms to the standard antibiotics leads to the discovery of *Mangifera* as a new potential antimicrobial agent. Previous studied conducted by Hanganu at el. (2019), stated that flavonoids are one of the secondary metabolites that are widely spread in the plants and noted for their antioxidant capacity that capable of inhibit free-radicals responsible for

cell damage. In other hand, *Mangifera* sp. also contains various classes of polyphenols, carotenoids and ascorbic acid that can act as antibacterial agents (Kanwal et al. 2009; Olasehinde et al. 2018). Moreover, *Mangifera* leaves can be used as a inexhaustible source of bioactive compounds for animal feed additive for their natural bioactive compounds such as flavonoids, tannins, glycosides, saponin and alkaloids.

Prior to this century, use of microwave for extraction of chemical compounds from plant material has shown better effect compare to conventional techniques. Conventional techniques for the plants material extraction usually are time and solvent consuming, thermally unsafe while the analysis is limited by the extraction step. Microwave assisted extraction (MAE) offer hassle free and fast extraction performance ability with less solvent consumption. In addition, MAE can improve extraction yield. The principle of heating using microwave is based on direct impact with polar solvents and is governed by two phenomena's: ionic conduction and dipole rotation, which in most cases occurs simultaneously (Letellier and Budzinski,1999). The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the rupture's cells to the surrounding solvent thus improving the yield of phytoconstituents. Higher temperature produced by microwave radiation can easily hydrolyze ether linkages of cellulose, the constituent of plant cell wall, and converting it into soluble fractions within few min. The higher temperature attained by the cell wall, during MAE, enhances the dehydration of cellulose and reduces its mechanical strength and this in turn helps solvent to access easily to compounds inside the cell (Latha C., 2006).

In this study, the optimum extraction time of MAE for *Mangifera* leaves extract will be determined and the antimicrobial activities differences between immature and mature *Mangifera* leaves extracts will be observed.

MATERIALS AND METHODS

Sampling: Sample of *Mangifera* sp (leaves) were obtains from Kampung Limbongan, Besut, Terengganu.

Preparation of plant materials: Freshly collected leaves of *Mangifera* were washed under running tap water to clean from dirty substances and dried under air-dried at normal room temperature (20℃) for three days. After drying, the plant material was crushed into smaller pieces and was weighted using analytical balance. Dried plant was transferred into airtight bluecap bottles and stored at room temperature before proceeding to the next step.

Leaf extract preparation: Water was used as an extraction solvent for Soxhlet and MAE methods.

Microwave extraction: This method was referred from Kulkarni and Rathod (2016). Leaves extraction was carried out using microwave/convention oven (Panasonic NN-CD997S). 10 g of the sample leaves in beaker were soaked in 100 mL (10 g/100 mL) of distilled water and repeated three times. The extracts were exposed to microwave irradiation for 5, 8, 10, 12 min at medium-low power level (350 W) and then were filtered. Sample extracts were kept in the freezer (-80ºC) overnight and were dried using freeze dryer (72 hours) until it become pulverize form. The extracts then kept in refrigerator (-20ºC) before being used in biological assays.

Soxhlet extraction: Extraction was perform using a conventional Soxhlet apparatus. 10 g of the dried *Mangifera* sp. leaves were set in the extraction chamber, while 200 mL of distilled water was filled in the round bottom flask. The extraction in a Soxhlet apparatus take about 4h. When completed, the extract collected and quickly kept in the -80ºC for overnight. Extract then were dried using freeze dryer (72 hours) until it become a pulverized. The extracts then kept in refrigerator (-20ºC) before being used in biological assays. (Ramluckan et al. 2014).

Sample dilution: Preparations of *Mangifera sp* crude extracts for antimicrobial assay. Samples extracts were dissolved from range 200 mg/mL until 6.125 mg/ml concentration. The sample was diluted by using two-fold dilution.

Collection of the organisms: Standard bacterial organisms from the ATCC (Carter & Cole, 1990) were obtained from Department of Microbiology, UniSZA. *Klebsiella pneumoniae* (ATCC 70063), *Enterococcus faecalis* (29212), S*taphylococcus aureus* (25923), *Pseudomonas aeruginosa* (10145) *Salmonella enterica* (12011) and *Acinetobacter baumannii* (19606) were sub-cultured for 24 hours and were adjusted to 0.5 McFarland standard prior to assay.

Preparation of culture media: Muller Hilton agar (MHA) – Muller Hilton powder was weighted (19 g) and was suspended in 500 mL of distilled water, the mixture was stirred and boiled to fully dissolve. After that, the mixture was sterilized by autoclaving at 121ºC for 1 hours. After autoclaving, the liquid agar was cooled and was poured

aseptically into sterile plates. The agar allowed to cool and solidify at room temperature under UV light for 10 min. The plates were stored inside refrigerator (4ºC). The same procedures were applied for the Nutrient Agar (NA), Muller-Hilton broth and other media that will used to maintain bacteria growth for antimicrobial assay.

Disc-diffusion assay: Antimicrobial activity was carried out according to the standard methods by Bauer et al. (1966). 6 different bacteria culture plates for each timeline (M5, M8, M10 and M12) with 200 mg/mL until 6.125 mg/mL concentrations. To maintain sterile conditions, this procedure was done inside laminar flow cabinet. Before started an experiment, the apparatus that will be used were exposed under UV light (30 mins). 10 µl of microbe (1.5×108 colony-forming unit per milliliter (CFU/mL)) were pipette and spread on the agar plate using sterile cotton swab. The inoculum was spread evenly over the entire surface. Inoculated plates were allowed to dry for 15 minutes in incubator before depositing the disks. Sterile paper disc (6 mm) was impregnated with 20 µl of each sample. Paper disks were placed on the agar plate using sterile forceps. Plates were keeps in inverted state and incubated at 37ºC for 24 hours. All sample was repeated thrice to ensure accuracy of the result. A transparent ruler was used to measure the zone of inhibition (mm) and the results were recorded.

One-way ANOVA was used to analyse the effect of extraction time on the maximum inhibition zone. All statistical analyses were conducted with SPSS software (v.20, SPSS, USA) at a significance level of 0.05.

RESULTS

Disc-diffusion assay

Antimicrobial activity of *Mangifera* leaf extract assay against 6 human pathogenic microorganisms showed a great potential in inhibit the growth of microorganisms. This is probably due to the presence of active phytochemicals substances in *Mangifera* leaves such as tannins, saponins and alkaloids that able to protect plants from infections.

Table 1 showed the result of antimicrobial activity of mature *Mangifera* leaves aqueous extract against *Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

The result obtained shows that M12 shows maximum inhibition against *E. faecalis* (15±3.2 mm), *S. aureus* (12±0.5 mm), *P. aeruginosa* (16±0.5 mm) with minimum inhibition concentration of 25 mg/mL (*E. faecalis*), 100 mg/mL (*S. aureus*) and 25 mg/mL (*P. aeruginosa*) respectively. M10 sample observed to have maximum inhibition against *E. faecalis* (15.3±0.5 mm), followed by *S. aureus* (15±0.5 mm), *S. enterica* (12±0.5 mm), *P. aeruginosa* (12±0.5 mm) and *A. baumannii* (18±2.9) with minimum inhibition concentration of 25 mg/mL (*E. faecalis*), 50 mg/mL (*S. aureus*), 200 mg/mL (*S. enterica*), 25 mg/mL (P. aeruginosa) and 6.125 mg/mL (*A. baumannii*) respectively. Sample M08 only show good inhibition activity against *A. baumannii* (13.5±1.7 mm), *E. faecalis* (12±1.8 mm) *P. aeruginosa* (12±0.5 mm) with minimum inhibition concentration of 100 mg/mL (*A. baumannii*), 50 mg/mL against *E. faecalis* and 100mg/mL against *P. aeruginosa*. Sample M05 only show moderate inhibition activity against *A. baumannii* and *P. aeruginosa* while all sample does not inhibit the growth of *K.pneumoniae* as can be seen in Table 1. Table 2 above showed the result of antimicrobial activity of immature *Mangifera* leaves aqueous extract against *K. pneumoniae*, *E. faecalis*, *S. aureus*, *S. enterica*, *P. aeruginosa* and *A. baumannii*.

Compare to mature leaves extract, the result obtained shows that immature leaves extract did have inhibition activity against *K. pneumoniae* with M10, M08 and Soxhlet sample show maximum inhibition zone of 18±1.2 mm, 10±0.0 mm and 9±0.5 mm, respectively. The minimum inhibition concentration observed were at 25 mg/mL (M10), 50 mg/mL (M08) and 200 mg/mL (Soxhlet) respectively. A high inhibition activity can be observed against *E. faecalis* by all samples tested as in Table 2. Only sample M10 and M08 show inhibition activity against *S. aureus* with maximum inhibition zone of 19±0.5 mm and 14±0.5 mm, respectively and minimum inhibition concentration of 25 mg/mL and 50 mg/mL respectively. Only M05 show low inhibition activity against *S enterica* compare to other samples. M12 show the highest inhibition zone at 16±0.5 mm followed by M10 (14.7±2.3 mm), M08 (13±1.8 mm) and Soxhlet (11.7±2.6 mm) while the minimum inhibition concentration of Soxhlet at 12.5 mg/mL compare to M12, M10 and M08 at 25mg/mL. No inhibition can be observed against *P. aeruginosa* and *A. baumannii*. All statistical test shown less than 0.01 significant level thus, can be concluded that extraction time did have effect on the inhibition activity of the test microbes.

Table 1: Antimicrobial activity of aqueous *Mangifera* leaf extracts (mature)

Test Organism	Zone of Inhibition (mm) *Mangifera sp.* extract (mg/ml)						Gentamycin (ug)
	200	100	50	25	12.5	6.125	10
K. pneumoniae							
M12	-	-	-	-	-	-	11±0.5
M10	-	-	-	-	-	-	10±0.5
M08	-	-	-	-	-	-	10±0.5
M05	-	-	-	-	-	-	10±0.5
Soxhlet	-	-	-	-	-	-	12±0.5
E. faecalis							
M12	15±3.2	11.3±0.5	9.3±0.5	7.0±3.9	-	-	13.3±2.7
M10	15.3±0.5	11.7±0.5	9.7±0.5	7.3±4.1	-	-	15±2.4
M08	12±1.8	10.3±1.03	9.0±0.5	-	-	-	13.7±2.25
M05	9±1.1	-	-	-	-	-	14.3±2.9
Soxhlet	8±0.5	-	-	-	-	-	16±0.5
S. aureus							
M12	12±0.5	9±0.5	-	-	-	-	10±0.5
M10	15±0.5	13±0.5	9±0.5	-	-	-	14±0.5
M08	9±0.5	-	-	-	-	-	11±0.5
M05	7±0.5	-	-	-	-	-	10±0.5
Soxhlet	-	-	-	-	-	-	18±0.5
S. enterica							
M12	-	-	-	-	-	-	12±0.5
M10	12±0.5	-	-	-	-	-	14±0.5
M08	9±0.5	-	-	-	-	-	14±0.5
M05	7±0.5	-	-	-	-	-	13±0.5
Soxhlet	-	-	-	-	-	-	16±0.5
P. aeruginosa							
M12	16±0.5	13±0.5	9±0.5	9±0.5	-	-	14±0.5
M10	12±0.5	9±0.5	8±0.5	8±0.5	-	-	16±0.5
M08	12±0.5	7±0.5	-	-	-	-	19±0.5
M05	10±0.5	-	-	-	-	-	15±0.5
Soxhlet	9±0.5	-	-	-	-	-	19±0.5
A. baumannii							
M12	-	-	-	-	-	-	18±2.3
M10	18±2.9	13.5±1.7	13±0.5	10±0.5	8±0.5	7±0.5	15±3.5
M08	13.5±1.7	9.5±0.6	-	-	-	-	17.5±1.7
M05	10.5±0.6	9±0.0	-	-	-	-	15±0.0
Soxhlet	-	-	-	-	-	-	13±0.5

- : No zone of inhibition observed.

Table 2: Antimicrobial activity of aqueous *Mangifera* leaf extracts (Immature)

Test Organism	Zone of Inhibition (mm) *Mangifera sp.* extract (mg/ml)						Gentamycin (ug)
	200	100	50	25	12.5	6.125	10
K. pneumoniae							
M12	-	-	-	-	-	-	15±0.5
M10	18±1.2	15±0.6	12±0.6	10±0.6	-	-	14.7±0.5
M08	10±0.0	9±0.5	8±0.5	-	-	-	14.7±1.37
M05	-	-	-	-	-	-	16±0.5
Soxhlet	9±0.5	-	-	-	-	-	14.5±2.2
E. faecalis							
M12	15±0.5	12±0.5	11±0.5	9±0.5	7±0.5	-	30±0.5
M10	14±1.8	11.3±1.9	10.7±1.0	9.3±1.2	7±2.3	-	23.3±2.7
M08	13±1.6	11±0.9	9±1.5	10±0.5	9±0.5	-	21±3.2
M05	15±1.1	13.3±1.4	10±1.2	10±0.5	8±0.5	-	27.5±2.3
Soxhlet	14.7±1.4	14.3±1.0	10.7±1.0	10±0.5	10±0.5	-	17±.1.6
S.aureus							
M12	-	-	-	-	-	-	17±0.5
M10	19±0.5	15±0.5	10±0.5	8±0.5	-	-	19±0.5
M08	14±0.5	11±0.5	9±0.5	-	-	-	19±0.5
M05	-	-	-	-	-	-	20±0.5
Soxhlet	-	-	-	-	-	-	20±0.5
S. enterica							
M12	16±0.5	11±0.5	9±0.5	9±0.5	-	-	28±0.5
M10	14.7±2.3	12±1.8	9.7±0.5	8.7±0.5	-	-	24.7±2.3
M08	13±1.8	10.7±1.0	8.3±0.5	9±0.5	-	-	21.3±3.7
M05	8±3.3	-	-	-	-	-	24.5±2.5
Soxhlet	11.7±2.6	10.3±2.1	5.3±2.3	9±0.5	8±0.5	-	24±3.2
P. aeruginosa							
M12	-	-	-	-	-	-	15±0.5
M10	-	-	-	-	-	-	15±0.5
M08	-	-	-	-	-	-	17±0.5
M05	-	-	-	-	-	-	16±0.5
Soxhlet	-	-	-	-	-	-	17±0.5
A. baumannii							
M12	-	-	-	-	-	-	10±0.5
M10	-	-	-	-	-	-	12±0.5
M08	-	-	-	-	-	-	11±0.5
M05	-	-	-	-	-	-	11±0.5
Soxhlet	-	-	-	-	-	-	9±0.5

- : No zone of inhibition observed.

DISCUSSION

Based on experiment conducted, M10 sample showed a great outcome of antimicrobial activities if compared to others sample. So, it can be concluded that 10 min extraction time was the optimum time point of *Mangifera* sample. Interestingly, M12 sample shows inhibition activity on a few microbes while M08 and M05 does not have good overall inhibition activity This can be concluded that the time of microwave exposed play an important role in determine the effectiveness of samples. Longer time exposure has been proven to be able to destroy some nutritional value in plants and make it less effective while less exposure time might not have ample time to totally extract bioactive compounds. This explains why there is depletion in microbial activities on M12 samples. Microwave temperature is functioning to softening plant tissue that make it easier to remove *Mangifera* leaves phytochemicals. However, with the excessive time and temperature exposed, it was able to destroy some phytochemicals in *Mangifera* leaves.

This finding almost in agreement with the previous studies by Taamali et al. (2012) that

determined the optimum phenolics compounds extraction time of 6 min around 80 ℃ using methanol :water (8:2 v/v) and Qing Niu et al. (2019) determined that the optimum extraction time of flavonoids at 10 min using 300W and 60% ethanol.

The strains that were not susceptible to the *Mangifera* sp extracts was *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*. These could be due to a distinctive feature of gram-negative bacteria, which is the presence of double membrane cells (encapsulated). Gram-negative bacteria have a special outer membrane that excludes certain drugs and antibiotics from penetrating the cell, most probably answered for why gram-negative bacteria are generally more resistant to antibiotics than other gram-positive bacteria and these bacterial were known as pandrug-resistant (PDR) Gram-negative bacterial (Falagas et al. 2008).

Though, with higher samples concentration used, the better effect of antibacterial can be observed is supported with the earlier study that *Mangifera* extract was required in high concentration, at least 500 mg/ml to act as good antimicrobial agents (Olasehinde, et al. 2018) this water extract sample produce good inhibition activity even at lower concentration. It can be concluded that the used of MAE and water extract can infused more phytochemicals and that water extract is a safer choice to use for example as feed enhancer for small ruminants.

CONCLUSION

The result obtained revealed that the water extract of *Mangifera* sp. possess good antimicrobial effects in against all tested microbes with optimum MAE time of 10 min, using 350 W compare to the Soxhlet extraction sample. Mature and immature *Mangifera* leaves extracts show different inhibition pattern due to the differences of the phytochemical produced in the leaves. This interesting finding will be further study against gut microbes of small ruminant towards producing feed additive. More optimization factor can be studied in this interesting extraction methods.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AAA designed and perform lead the experiments and also wrote the manuscript. NBS and SRR performed extraction procedures, preparing maintaining cultures and repeating the experiments. All authors read and approved the final version.

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