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Antibacterial activity test of mixture seed extracts of *Moringa oleifera* Lamk and *Schleichera oleosa* L. against *Staphylococcus epidermidis* and *Shigella dysenteriae*

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ABSTRACT

Moringa (*M. oleifera* Lamk.) is known to have antibacterial ability, because the seeds contain tannin, flavonoids, alkaloids, and saponins. Kesambi or Malay lac tree (*S. oleosa* L.) has been used to fight skin inflammation, ulcers, itching, acne and skin infections. Its seeds contain oil, which is very good for the skin. The ability of a mixture of the seed of moringa and kesambi has not been widely reported. This study aims to determine the inhibitory power resulting from a mixture seed extracts of Moringa and kesambi against *S. epidermidis* dan *S. dysenteriae*. The method used is experimental using CDR (Completely Randomized Design) with 5 treatments and 3 replicates which are P1 = 10 g Moringa + 0 g Kesambi, P2 = 2.5 g Moringa + 7.5 g Kesambi, P3 = 5 g Moringa + 5 g Kesambi, P4 = 7.5 g Moringa + 2.5 g Kesambi, P5 = 0 g Moringa + 10 g Kesambi. One way Anova and Tukey test were performed for statistical justification. The inhibitory test of the mixture seeds extract of moringa and kesambi showed that there was antibacterial activity against *S. epidermidis* and *S. dysenteriae*. The mixed extract showed the maximum effect on Gram (+) *S. epidermidis*, namely P3 with inhibitory power of 25.33±0.76 mm**.** While on the Gram (-) bacteria, *S. Dysenteriae,* the P4 had the highest inhibition (9.17±1.15 mm). The mixture of Moringa seed extract and kesambi seed has a higher effect on gram positive bacteria.

Key words : *Antibacterial Activity, Moringa seeds, Kesambi seeds, S. dysenteriae, S. epidermidis*

Introduction

The need for new medicine is currently an emerging research topic. Currently, there has been increasing research on that the use of medicinal plants in alternative medicine is one way to cure cases of non-severe infectious diseases. Medicinal plants can also serve as source of new and inexpensive antibiotics for it pathogenic strains are not resistant. Several works have provided a scientific basis for popular use of plants against infectious diseases (Kitula, 2007). The usage of medicinal plants is well-known for the community of people in East Nusa Tenggara, Indonesia. Some medicinal plants grow wild and some are cultivated.

Moringa (*M. oleifera* Lamk.) is a plant that has many benefits and also known to have antibacterial properties. In Agustie and Samsuharto's research (2013), Moringa leaves can inhibit the growth of *S. aureus* bacteria. Research by Sari *et al.,* (2017) shows

the effectiveness of *Moringa* seeds (*M. oleifera* Lamk.) against *E. coli* bacteria in an effort to prevent diarrhea. This is because moringa seeds contain tannins, flavonoids, alkaloids, and saponins (Kheir *et al.*, 2014).

Schleichera oleosa L. or known in local name *Kesambi* or Malay lac tree includes one of the medicinal plants that have versatile benefits and great potential to be developed such as the essential oil, and also known that the plant (Sapindales family) is widely used to fight skin inflammation, ulcers, itching, acne and other skin infections (Thatavong, 2015). According to Sari *et al.,* (2019), the benefits of *kesambi* are to treat skin inflammation, ulcers, malaria, as an anticancer, natural antioxidant and natural antimicrobial. Kesambi seeds contain oil. The use of the two medicinal plants, especially moringa seeds and *kesambi* seeds, into a mixture of medicinal ingredients has not been widely used. Many of the society have not used in a mixture of moringa seeds and *kesambi* seeds to their full potential as medicinal material.

Staphylococcus epidermidis is a species of bacteria known to cause opportunistic infections that attack the immune systems of weak individuals. *Shigella dysentriae* is a very serious cause of disease because it produces exotoxins that have neurotoxic and enterotoxic properties. Exotoxin is a antigenic protein, which stimulates the production of antitoxin so that it can kill the patient.

The objective of this research was to determine the antibacterial activity of a mixture of extracts of Moringa seeds (*M. oleifera* Lamk.) and *Kesambi* or Malay lac (*S. oleosa* L.) seeds against *S. epidermidis* and *S. dysenteriae.*

Materials and Methods

Collection and Maintenance of Test Organisms

The pure cultures of *S. epidermidis* and *S. dysenteriae* were obtained from the National Agency of Drug and Food Control, Kupang City, East Nusa Tenggara Province, Indonesia. The isolates were maintained on nutrient agar slants at 4 °C until required for use.

Plant sample collection

The seeds of *Moringa oleifera* and *M. oleifera* Lamk were collected along Kupang City, East Nusa Tenggara Province. The sample was transported to the laboratory for preparation and analysis.

Sample preparation and Extraction

The seeds were dried in the shade for 7 days and mashed using an electric blender, sterile and clean until powder form. The powder seeds were then differentiated into 5 treatment groups, namely: P1 (10g Moringa powder only), P2 (2.5g Moringa and 7.5 *Kesambi* powders), P3 (5.5g Moringa and 5.5g *Kesambi*), P4 (7.5g Moringa and 2.5g *Kesambi*) and P5 (10g *Kesambi* powder only). Each treatments were soaked in ethanol (1:3, w:v) and was left in a shaker for overnight. All experiments were conducted in triplicate. The extract was filtered using sterile filter paper (Whattman No 1). The filtrate was evaporated to dryness using *rotary vacuum evaporator*. The extract was scrapped into a sterile sample bottle using a sterile spatula (Kurniawan, 2015; Mashiar *et al.,* 2009).

Standardization of the inoculums

All bacterial strains were inoculated in Nutrient Agar for about 16 h. The concentration of the suspensions was adjusted to 0.5 (optical density) with the help of spectrophotometer.

Antibacterial assay using Disc diffusion method

The antibacterial test of 5 mixture extracts of Moringa seeds and *Kesambi* seeds was individually tested against the studied bacteria. The antibacterial activity was the carried out *in vitro* by disc diffusion method. Agar plates were inoculated with 0.1 ml of 0.5 McFarland standardized inoculums of test microorganisms and were spread all over the surface of the agar. Then, 6 mm filter paper discs were soaked into each concentrations of the test compound, are placed on the agar surface. The plates were incubated for 24 hours at 37 °C (Bauer *et al.,* 1966).

Inhibition zone essay

Isolates showing the diameter of the inhibition zone corresponding to the upper or lower limits of a distribution were examined by visual re-evaluation of the recorded inhibition zone diameter and by cross-examination with clinical laboratory reports. The zone of clearance was measured using a meter rule (Vandepitte *et al.*, 2003). Diameters of the zone of inhibition were compared with standard antibiotics tetracycline.

Statistical analysis

One Way Analysis of Variance (ANOVA) and a post-hoc Tukey's test were performed for statistical justification.

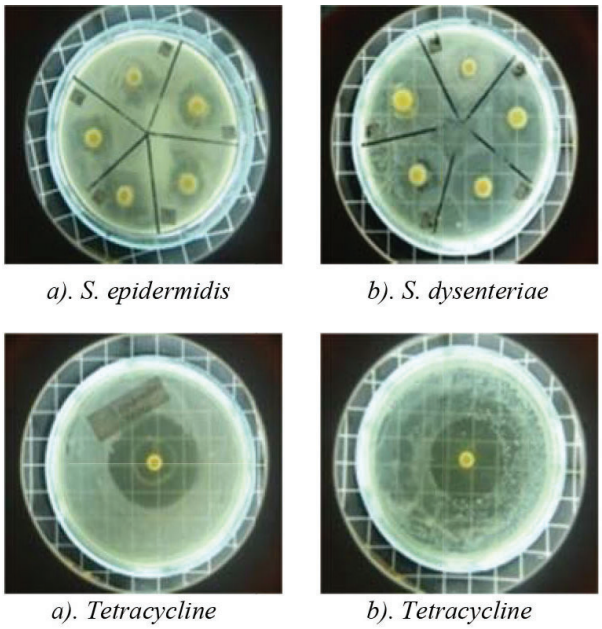
Results and Discussion

Table 1 shows the inhibition zone test of mixture extracts of moringa seeds and *kesambi* seeds against all tested of gram-positive and gram-negative bacteria. The result indicated that all combination showed different mean value zone of inhibition, the mixture extracts' antibacterial activity ability was depending on the concentrations of the seed extracts used. The highest antibacterial effect against a gram negative bacterial *S. epidermidis* (25.33±0.76 mm) at the 5g + 5g concentration of mixture extract of moringa seed and *kesambi* seed when compare to other combinations. While, the highest effect showed at gram-negative bacteria *S. dysenteriae* (9.17±1.15 mm), found at 5g moringa + 2.5g *kesambi* combination. The antibacterial activity is lower than tetracycline as positif control.

The statistical test for both *S. epidermidis* and *S. dysenteriae* bacteria showed a sig value of 0,000 <p value 0.01, which means that the average 6 treatments of the mixture of Moringa and *kesambi* seed extracts had a significant effect on the antibacterial activity of the tested bacteria. Based on the Tukey test, it was found that the antibacterial ability of the extract mixture in the P3 treatment was effective against *S. epidermidis* bacteria. Meanwhile, the GP *S. dysenteriae* test bacteria showed that between treatments had no significant effect. Test of tetracycline positive control on the two tested bacteria showed a very significant effect.

From the statistical analysis and zone of inhibition values it showed that the mixture extract show lower ability to the standard antibiotics, it can be concluded that the extracts of moringa seed and *kesambi* or lac seed are considered to have a broad spectrum antibacterial activity against both gram positive and gram negative bacteria (Burman *et al.*, 2018).

The largest clear zone formed in the test against the growth of *S. epidermidis*, a gram negative bacteria, was 25.33±0.76 mm (P3) (categorized as very strong) is higher than on the gram-positive bacteria, *S. dysenteriae* which was the P4 extract treatment with average 9.17 ± 1.15 mm (P4) categorized medium (David and Stout, 2009). The moringa seed contain of tannins, saponins, steroid, that might have antimicrobial properties (Bello and Jamiu, 2017; Walter *et al.*, 2011), and *kesambi* contain alkaloids, terpenoids and phenolic (Krishnaiah, 2007).



a). S. epidermidis *b). S. dysenteriae*

a). Tetracycline *b). Tetracycline*

Fig. 1. Antioxidant activity of a mixture of Moringa seeds and kesambi seeds against bacteria tested (a) *S. epidermidis* – Tetracycline (Control), (b) *S. dysenteriae* – Tetracycline (Control).

Table 1. Antibacterial activity of mixture extracts of moringa seeds and kesambi seeds against *S. epidermidis* and *S. dysenteriae*

Moringa seeds + Kesambi seeds		Average ± S.E.MInhibition zone (mm)	
		S. epidermidis	*S. dysenteriae*
P_1	10 g + 0 g	$14.50±1^{b}$	$8.33±1.04^{bc}$
P_2	2.5 g + 7.5 g	$13.67±0.29^{ab}$	$4.33±0.76^{a}$
P_3	5 g + 5 g	$25.33±0.76^{c}$	$7.33±0.76^{abc}$
P_4	7.5 g + 2.5 g	$10.50±1^{a}$	$9.17±1.15^{c}$
P_5	0 g + 10 g	$12.17±1.04^{ab}$	$5.33±0.76^{ab}$
P_6	Tetracycline	$39.67±1.61^{d}$	$29.17±0.76^{d}$

The different responses of the two groups of tested bacteria to the antibacterial mixture of moringa seeds and kesambi seeds were due to the different sensitivity because of major role in the cell membrane of gram-positive and Gram-negative bacteria. The resistance ability is refers to the gene of bacteria. GN bacteria are more resistant because they have pre plasmic so when the antibiotic is reaching to bacteria can be harder and easier to identify by bacteria and can fight back better. Other reason is the second layer of phospholipids that play role as another barrier compared to GP bacteria (Fardiaz, 1992).

Extracts from kesambi seeds and extract mixtures of the two ingredients are called indifferent if the effect of the mixture is the same as the effect of each single extract (Blesson *et al.*, 2015). The effect of the two extract ingredients has the same binding site of the action target so that it overlaps and produces indifference, the second possibility can be due to the molecular interaction between the two extracts. The dose or concentration of the mixture of the two antibacterial ingredients contained in the mixture of moringa seeds and *kesambi* seeds cannot indirectly show a synergistic effect in the inhibition of *S. epidermidis* and *S. dysenteriae*, but rather an antagonistic effect as a response of the secondary metabolite including terpenes, phenylpropenoids and N- and S-containing compounds. When the major group are present, the interactions between those components may lead to antagonistic, while additive or synergistic effects to phenolic and alcohol compounds are available on the plant (Bassolé and Juliani, 2012).

Conclusion

The mixture of moringa seeds and kesambi seeds extracts has antibacterial activity against *S. epidermidis* and *S. dysenteriae*. In the extract mixture (P3) of 5 g moringa seeds + 5 g *kesambi* seeds showed the greatest inhibitory power in the S. epidermidis test bacteria while the extract mixture (P4) 2.5 g of moringa seeds + 7.5 g of kesambi seeds showed the greatest inhibitory power in bacteria. *S. dysenteriae* test. Statistically, the mixture of moringa seed and kesambi seed extracts had a significant effect on *S. epidermidis* GN bacteria.

Conflict of Interest

The authors declare that there is no conflict interest for this article.

References

Aguestie, A.W.D. and Samsumaharto, R.A. 2013. Uji aktivitas antibakteri ekstrak maserasi daun kelor (*Moringa oleifera* Lamk) terhadap bakteri *Staphylococcus aureus*. *Jurnal Biomedika* 6(2) : 14-19.

Bassolé, I. H. and Juliani, H. R. 2012. Essential oils in combination and their antimicrobial properties. *Journal Molecules* (Basel, Switzerland). 17(4): 3989–4006. https://doi.org/10.3390/molecules17043989

Bauer, A.W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. 45(4_ts) April 1966: 493–496, https://doi.org/10.1093/ajcp/45.4_ts.493

Bello, S. A. and Jamiu, A.T. 2017. Antibacterial activity of *Moringa oleifera* seed extracts on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Nigerian Journal of Microbiology*. 31(1) : 3873-3881

Blesson, J., Saji, C.V., Nivya, R.M. and Kumar, R. 2015. Synergistic antibacterial activity of natural plant extract and antibiotics againts methicillin resintant *S. auerus* (MRSA). *World Journal of Pharmacy and Phamarcceutical Sciences*. 4(3) : 741-763.

Burman, S., Bhattacharya, K., Mukherjee, D. and Chandra1, G. 2018. Antibacterial efficacy of leaf extracts of *Combretum album* Pers. against some pathogenic bacteria. *BMC Complementary and Alternative Medicine*. 18 : 213. https://doi.org/10.1186/s12906-018-2271-0

Davis, W. W. and Stout, T. R. 1971. Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. *Applied Microbiology*. 22(4): 659–665. PMID: 5002143; PMCID: PMC376382.

Fardiaz, S. 1992. *Mikrobiologi Pangan 1*. Jakarta: Gramedia Pustaka Utama. ISBN: 979-511-316-X. 308p.

Kheir, S.M., Kafi, S. K. and Elbir, H. 2015. Evoluation of the antifunggal activity of moring a oleifera seeds, leaves and flowers. *World Journal of Pharmaceautical Research*. 4 (2) : 18-25.

Kitula, R. A. 2007. Use of medicinal plants for human health in Udzungwa mountains forests: a case study of new Dabaga Ulongambi Forest Reserve, Tanzania. *Journal of Ethnobiology and Ethnomedicine*. 3 : 7. https://doi.org/10.1186/1746-4269-3-7

Krishnaiah, D., Sarbatly, R. and Bono, A. 2007. Phytochemical antioxidants for health and medicine – A move towards nature. *Biotechnol. Mol. Biol. Rev.* 1 : 97-104. https://doi.org/10.5897/BMBR2007.0009

Kurniawan, D. 2015. Uji aktivitas antijamur ekstrak etanol daun kelor (*Moringa oleifera* Lamk.) terhadap *Candida albicans* secara *in vitro*. *Jurnal Mahasiswa Fakultas Kedokteran Untan*. 3(1): 23p

Mashiar, M., Mominul, I., Sharma, A., Soriful, I., Atikur,

R., Mizanur, R. and Alam, M. 2009. Antibacterial activity of leaf juice extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. *Journal of Natural Science*. 8 : 219-227.

Sari, P., Sugita, P. and Santoso, A. 2019. Aktivitas antioksidan, antibakteri, dan toksisitas ekstrak kulit batang pohon kesambi (*Schleichera oleosa* (Lour) Oken). *Jurnal Jamu Indonesia*. 4(3) : 112-118.

Sari, R. K., Tina, L. and Fachlevy, A.F. 2017. Efektifitas biji kelor (*Moringa oleifera*) terhadap bakteri *Escherichia coli* dalam upaya pencegahan penyakit diare. *Jurnal Ilmiah Mahasiswa Kesehatan Masyarakat Unsyiah*. 2(6) : 1-8.

Thatavong, X. 2015. *Chemical consituents and biological activities from crude hexane extract of Schleichera oleosa* fruits. Chonburi: Burapha University.

Vandepitte, J., Engbaek, K., Rohner, P., Piot, P. and Heuck, C.C. 2003. *Basic laboratory procedures in clinical bacteriology* / J. Vandepitte ... [et al.], 2nd ed. World Health Organization. ISBN 9789790440005 (Indonesian) 167p. https://apps.who.int/iris/handle/10665/42696

Walter, A., Samuel, W., Peter, A. and Joseph, O. 2011. Antibacterial activity of *Moringa oleifera* and *Moringa stenopela* methanol and n-hhexane seed extracts on bacteria implicated in water bone diseases. *African Journal of Microbiology Research*. 5(2) : 153-157. https://doi.org/10.5897/AJMR10.457.