

## BIOACTIVITY OF CHEMICAL COMPOUNDS FROM MORINGA SEEDS (*Moringa oleifera*) AND ANTIBACTERIAL ACTIVITY TEST AGAINST *Streptococcus pyogenes* BACTERIA

H. Hidayat✉, M.M. Musawwa and F.I. Fajarwati

Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Islam Indonesia, Jl. Kaliurang Km 14,5 Yogyakarta 55584, Indonesia

✉Corresponding Author: [habibihidayat13@uii.ac.id](mailto:habibihidayat13@uii.ac.id)

### ABSTRACT

Moringa seeds are a class of edible oils that have activities such as antioxidants, antiaging, emollients, hair care, and skin lightening. This study aims to identify the potential of chemical compounds from moringa seed oil as an antibacterial against *Streptococcus pyogenes* bacteria. Analysis by GC-MS showed that Moringa seed oil contains 40% oleic acid which is the compound with the highest peak. The antibacterial activity of Moringa seed oil was categorized as strong with clear zones of 11 mm and 15 mm respectively by Moringa seed oil which was extracted using soxhletation and MAE methods. Moreover, the characterization using GC-MS identified the presence of high oleic acid in Moringa seed oil with an area of 40%. The extraction results identified their functional groups by Fourier Transform Infrared (FTIR). Phytochemical test results showed that the oil from Moringa seeds was proven to contain alkaloids, flavonoids, and polyphenols. FTIR analysis showed several functional groups consisting of C=O, CH<sub>3</sub>, CH<sub>2</sub> groups, C-O esters, and O-H acids.

**Keywords:** Moringa, Antibacterial, Streptococcus, Bacteria.

RASĀYAN J. Chem., Vol. 16, No. 3, 2023

### INTRODUCTION

The Moringa plant (*Moringa oleifera* Lamk) is a plant with high nutritional value, growing in tropical and subtropical areas. Moringa seeds are a class of edible oils that have activities such as antioxidants, antiaging, emollients, hair care, and skin lightening.<sup>1</sup> Moringa seeds (*Moringa oleifera* Lam.) are plants that contain unsaturated fatty acids with a high oleic acid content of 66.12% of the total fatty acids. Moringa plants are one of the natural ingredients that are widely used for consumption needs and alternative medicine in every part such as flowers, leaves, fruit, fruit, seeds, stems, bark, and roots. Moringa leaves can be efficacious as antimicrobial against the activity of *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* bacteria, and anti-inflammatory. Flowers can be efficacious as an anti-inflammatory, antihypertensive diuretic. Seeds can be efficacious as minerals/vitamins, antimicrobial, and anticancer. Moringa bark can be efficacious as an antimicrobial, and diuretic, and the roots can be efficacious as an antimicrobial, anti-inflammatory, and analgesic. Another extraction method besides using the soxhletation method is the Microwave Assisted Extraction (MAE) extraction method which utilizes microwave radiation to heat the solvent quickly and efficiently. The difference between conventional extraction and extraction using MAE is the length of time used and the results of the analysis. Then, Moringa seed oil to determine its compound composition and antibacterial activity against *Streptococcus pyogenes*. The results of this study are expected to provide an overview of the use of Moringa seed oil as a raw material for the development of the food, cosmetic, medicine, and alternative fuel industries in the future.

### EXPERIMENTAL

Moringa seeds, *Streptococcus pyogenes* bacteria, ethanol 96%, ethanol 70%, label paper, plastic wrap, filter paper, aqua dest, aquabidest, nutrient broth (Sigma Aldrich), nutrient agar (Sigma Aldrich), Mueller Hinton media (MERK).

#### Identification of Moringa Seeds

Extraction of moringa seeds with microwave and soxhletation methods, after that, the sample was filtered

to separate the moringa seed powder from the solution. Moringa seed extract solution was obtained and then evaporated for 4 h to separate the solvent from the Moringa seed oil extract. And then, characterization of Moringa seed oil using GC-MS.

### Enrichment of *Streptococcus pyogenes*

Prepare the bacterial growth medium using a nutrient broth medium. then put 10 mL of liquid nutrient broth that has been sterilized into a test tube add 1 ose of *Streptococcus pyogenes* bacteria and spread it evenly into the liquid medium. Cover the test tube using aluminum foil and plastic wrap then incubate for 24 h at 37 °C.

### Antibacterial Activity Test Against *Streptococcus pyogenes*

The antibacterial activity test included a positive control test using the antibiotics ampicillin and erythromycin. The method used in this test is the agar diffusion method. A sterile 8 mm paper disk was placed on the media so that the sample was then dripped with the sample, the petri dish was wrapped in plastic wrap and stored in an incubator at 37 °C for 24 hours then the diameter of the clear zone was measured.

### Phytochemical Test

Phytochemical testing aims to determine the secondary metabolites contained in Moringa seed oil. The secondary metabolites that were tested qualitatively include alkaloids, flavonoids, and polyphenols. And then, characterization of Moringa seed oil using GC-MS and FTIR.

## RESULTS AND DISCUSSION

Macroscopic identification of bacteria, namely by gram staining aims to see the shape of the micro-organisms of the bacteria and classify the bacteria from the chemical reaction that is caused by the gram staining solution.



Fig.-1: *Streptococcus Pyogenes* from a Microscope

The morphology of the bacteria as a result of gram staining in the form of coccus (Fig.-1) from a microscope with a magnification of 40X belongs to the group of gram-positive bacteria. Gram-positive bacteria will be purple in color because, in the gram staining process, the bacterial cells tested will interact and bind to the crystal violet-Lugol complex so that it becomes purple.<sup>2,3</sup> The antibacterial activity test was conducted to determine whether Moringa seed oil has activity against the growth of *Streptococcus pyogenes* bacteria. This test is carried out by observing the inhibition zone, which is marked by a clear area formed around the paper disc.<sup>4</sup> The test method is carried out using the disc diffusion method, where in the process the test sample is placed in agar media which has been planted with a number of test bacteria which will diffuse into the agar medium and then incubated for a certain time. The clear/clear area indicates the presence of inhibition of the growth of microorganisms on the surface of the agar medium. This method has advantages and disadvantages. The advantages are that it is easy to do, does not require special equipment, and is relatively inexpensive, while the disadvantage is that the size of the clear zone formed depends on the conditions of incubation, inoculum, diffusion, and preincubation.<sup>5</sup> The *Streptococcus pyogenes* bacteria that will be used before are rejuvenated first to regenerate the bacteria so that young and uncontaminated bacteria are obtained. There are two kinds of media used in the antibacterial activity test. The first medium, namely nutrient agar is a solid medium used for testing antibacterial activity. Nutrient agar media was used as a source of nutrition for the growth of both gram-positive and gram-negative bacteria. The second medium,

nutrient broth is a liquid medium used for the maintenance of bacterial isolates and for bacterial rejuvenation. Bacterial rejuvenation was carried out by taking one needle of *Streptococcus pyogenes* bacteria then put in nutrient broth media, incubating for 24 h. Parameters of whether bacterial growth occurs or not are seen from the turbidity, if the solution is cloudy then bacteria grow and if the solution is clear then there is no bacterial growth. The results of bacterial rejuvenation showed that the nutrient broth medium was turbid which indicated that the medium was dominated by *Streptococcus pyogenes* bacteria, thus indicating that the bacteria could grow well.

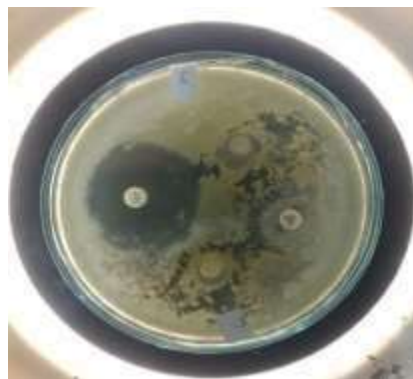


Fig.-2: Moringa Seed Oil Antibacterial Activity

Table-1: Data of Antibacterial Activity

| Sample                          | Resistance Diameter (mm) |
|---------------------------------|--------------------------|
| Positive control (Erythromycin) | 28                       |
| Positive control (Ampicillin)   | 10                       |
| Moringa seed oil (Soxhletation) | 11                       |
| Moringa seed oil (MAE)          | 15                       |

Inhibition tests conducted using Moringa oil with disc method against *Streptococcus pyogenes* bacteria showed varying results. These results have an influence on the development of *Streptococcus pyogenes* bacteria and also these results indicate that Moringa seed oil can act as an antibacterial. The diameter of the widest inhibition is the yield of Moringa seed oil extracted using the MAE method with a power of 15 mm. The yield of Moringa seed oil extracted using the soxhletation method with an inhibitory diameter of 11 mm. In the process of testing the antibacterial activity, two standard antibiotics were used, namely erythromycin and ampicillin. Based on the zone of inhibition, the highest ability of the antibiotic to inhibit *Streptococcus pyogenes* was the antibiotic erythromycin, which was 28 mm while ampicillin was 10 mm. Moringa seed oil samples were in the category of being able to inhibit bacteria strongly compared to ampicillin-positive controls which were included in the moderate growth inhibition response category (Table-2), while erythromycin was an isolate that had a very strong inhibitory response compared to Moringa seed oil and ampicillin because based on the David Stout method of measuring the strength of antibacterial power, it is divided into several categories.

The results of the gas chromatography analysis for Moringa seed oil using the MAE method produced a chromatogram with 15 fatty acid peaks as shown in Fig.-3.

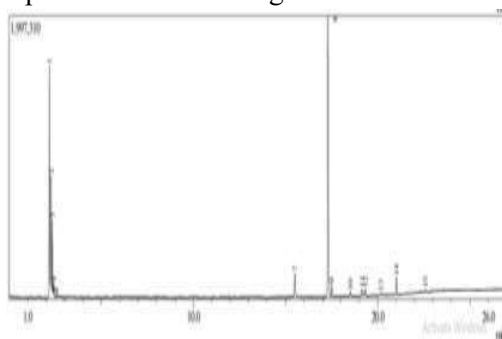


Fig.-3: Results of GC-MS Analysis

Table-2: Classification of Inhibitory Zone Response

| Clear zone diameter (mm) | Growth barrier response |
|--------------------------|-------------------------|
| ≤5                       | weak                    |
| 5-10                     | medium                  |
| 10-20                    | strong                  |
| ≥20                      | very strong             |

The fatty acids in the first Moringa oil were shown at the seventh peak with a retention time of 15,505 having an area of 3.69% which was thought to be palmitic acid because it had mass spectra that matched the mass spectra of methyl hexadecanoate from WILEY7.LIB. The eighth peak with a retention time of 17,305 having an area of 40% is oleic acid because it has mass spectra that match the mass spectra of 9-octadecanoic Acid from WILEY7.LIB is the compound with the highest peak. The mass spectra of the main component of the extracted fatty acid methyl ester (tR= 17.305) produced fragment ion peaks at m/z:41, 55, 69, 74, 97, 110, 123, 137, 166, 180, 194, 207, 222, 235, 246, 264, 266, and 296. Fragments with m/z 296 are oleic acid molecular ions having the molecular formula  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$ . The ninth peak with a retention time of 17,505 has an area of 1.72% which is thought to be stearic acid because it has mass spectra that are in accordance with Octadecanoic Acid/Stearic acid from WILEY7.LIB. The tenth peak with a retention time of 18,524 has an area of 0.76% which is suspected to be Olealdehyde. The eleventh peak with a retention time of 19,141 has an area of 0.97% which is suspected to be linoleic acid because it has mass spectra that correspond to the mass spectra of 9-Octadecanoic Acid from WILEY7.LIB. The twelfth peak with a retention time of 19,334 having an area of 0.96% is suspected to be arachidic acid because it has mass spectra that correspond to the mass spectra of eicosanoic acid (arachidic acid) from WILEY7.LIB. The fourteenth peak with a retention time of 21,017 having an area of 2.61% is suspected to be behenic acid because it has mass spectra that correspond to the mass spectra of docosanoic acid (behenic acid) from WILEY7.LIB. The fifteenth peak with a retention time of 22,595 having an area of 0.27% is suspected to be lignoceric acid because it has a mass spectrum that corresponds to the mass spectra of tetracosanoic acid (lignoceric acid) from WILEY7.LIB.

Table-3: GC-MS Data Analysis

| Peak | (Minutes) | Area(%) | Weight | Compound Name                      |
|------|-----------|---------|--------|------------------------------------|
| 7    | 15,505    | 3.69    | 270    | Hexadecanoic acid/ palmitic acid   |
| 8    | 17,305    | 40      | 296    | 9-Octadecanoic acid/oleic acid     |
| 9    | 17,505    | 1.72    | 298    | Octadecanoic acid/ stearic acid    |
| 10   | 18,524    | 0.76    | 312    | Olealdehyde                        |
| 11   | 19,141    | 0.97    | 282    | 9-Octadecanoic acid/linoleic acid  |
| 12   | 19,334    | 0.96    | 326    | Eicosanoic acid/ arachidic acid    |
| 14   | 21,017    | 2.61    | 354    | Docosanoic acid/behenic acid       |
| 15   | 22,595    | 0.27    | 382    | Tetracosanoic acid/lignoceric acid |

The following is the structure of the fatty acid compounds found in Moringa seed oil which were analyzed by GC (Fig.-4). The phytochemical test was carried out to determine the content of active compounds contained in Moringaseed oil. This test is carried out by the tube method by taking a small sample of oil, and then adding reagents according to the compound to be identified. The results of phytochemical tests on Moringa seed oil extracted using the soxhletation and MAE methods qualitatively are shown in Table-4. The positive Moringa seed oil contains secondary metabolites of alkaloids, flavonoids, and polyphenols which are indicated by the extract changing color after being given the reagent. This indicates that Moringa seed oil has potential as an antibacterial (Table-3).

Table-4: Phytochemical Analysis Data

| Test type  | Reactor                                                        | Reaction Results                     | ket        |
|------------|----------------------------------------------------------------|--------------------------------------|------------|
| Alkaloids  | Dissolved in a solvent fraction<br>+ Dragendorff reagent       | Yellow solution<br>Orange solution   | (+)<br>(+) |
| Polyphenol | Dissolved in a solvent fraction<br>+ 3 drops FeCl <sub>3</sub> | Greenish solution<br>Purplish green  | (+)<br>(+) |
| Flavonoids | + Mg powder                                                    | There are bubbles and deposits of Mg | (+)        |

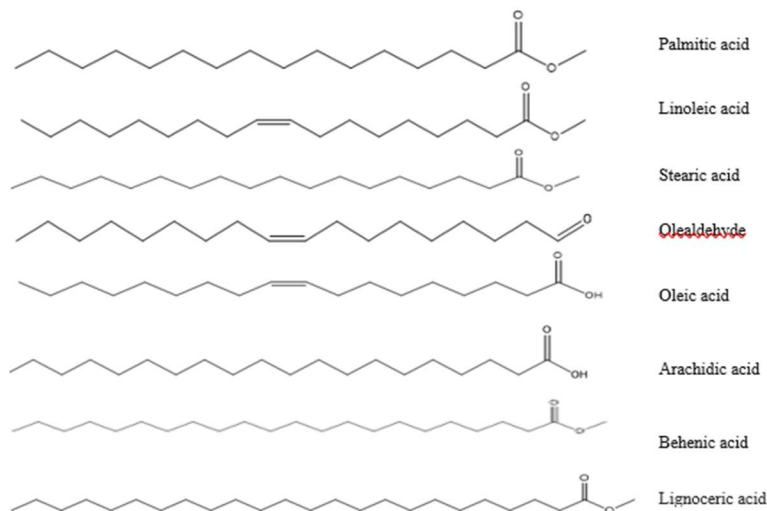


Fig.-4: Fatty Acid Compounds By GC

Active substances which are antibacterial compounds derived from Moringa seed oil are alkaloids, polyphenols, saponins, and terpenoids.<sup>13</sup> The results of the identification of the IR spectrophotometer in the form of peaks provide information on the functional groups contained in the fraction. The results of identification with FTIR can be seen in Fig.-5. Each fraction obtained spectral data in the IR region in the wave number range of 3000-400  $\text{cm}^{-1}$ .

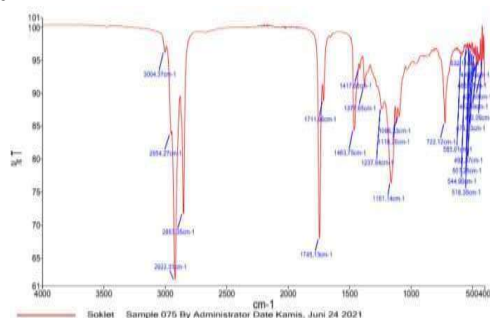


Fig.-5: Moringa Seed Oil FTIR Spectrum by Soxhletation

The IR spectrum of Moringa seed oil extracted using the soxhletation method showed absorption that appeared in the area of 1745.13  $\text{cm}^{-1}$  which is a carbonyl group ( $\text{C}=\text{O}$ ) with a sharp characteristic that is thought to be a functional group of flavonoid and polyphenolic compounds. Then the  $\text{CH sp}^3$  group was obtained at a wave number of 1377.65  $\text{cm}^{-1}$ . Furthermore, the  $\text{C}-\text{O}$  group appears at wave number 1118.2  $\text{cm}^{-1}$  which is a group found in the three flavonoid compounds, polyphenols, and alkaloids. In the area of 1463.75  $\text{cm}^{-1}$ , it shows the presence of the  $\text{C}=\text{C}$  Aromatic functional group. The aromatic ring shows a peak in the 1650-1450  $\text{cm}^{-1}$  region, which has a low degree of substitution and has  $-\text{CH}_2-$  bonds in the 1417.66  $\text{cm}^{-1}$  region and  $\text{CH}$  at 2954.27  $\text{cm}^{-1}$  absorption. the presence of the  $\text{OH}$  group. The acid that appears with an absorption peak of 3004.37  $\text{cm}^{-1}$  is a functional group found in flavonoid, polyphenolic, and alkaloid compounds. The absorption in the area of 1096.33  $\text{cm}^{-1}$  is the absorption area of the  $\text{C}-\text{N}$  group which is a functional group found in alkaloid compounds. Based on these data, it was concluded that the fatty acids of Moringa seed oil were oleic acid, methyl palmitic, linoleic acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid. The highest fatty acid content in Moringa seed oil is 9-octadecanoic acid or oleic acid by 40%. The largest fatty acid in Moringaoil is oleic acid.<sup>7,8</sup>

Oleic acid is a straight-chain carboxylic acid (containing a  $\text{COOH}$  group) in lipids, which is formed by a biocatalyst (enzyme) through a linear combination of acetic ( $\text{C}_2$ ) units involving the desaturation of saturated fatty acids so as to allow the formation of unsaturated fatty acids such as linoleic acid, linolenic acid, arachidonic, etc (Fig.-6).<sup>9</sup> Stearic acid has antibacterial activity against *Streptococcus aureus* concentration of 1.00  $\text{mg/mL}$  in a test medium.<sup>10</sup> Antioxidants prevent skin pigment generation by reducing

photodamage. In addition, some antioxidants were shown to increase skin hydration to revitalize the skin.<sup>11</sup> Fatty acids have no antibacterial activity against gram-negative bacteria, except those containing low-carbon chain fatty acids (< C8), especially in the form of monoacylglycerol. This is closely related to the inability of triacylglycerols (long chain fatty acids) to interact or penetrate the cell wall/membrane system to disrupt the bacterial cell wall/membrane permeability system because the triacylglycerol structure is large and long.<sup>1</sup>

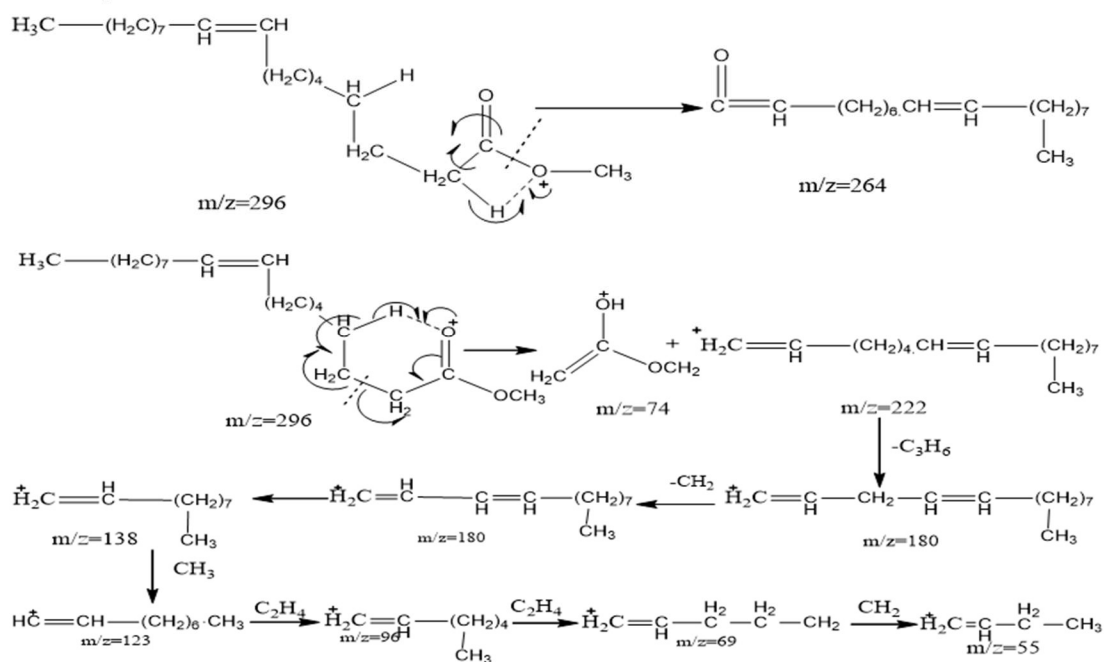


Fig.-6: Fragmentation of Oleic Acid

## CONCLUSION

Moringa seed oil extracted using the MAE method was the most active in inhibiting the growth of *Streptococcus pyogenes* bacteria. with the diameter of the inhibition zone is 15 mm, while the Moringa seed oil extracted using the soxhletation method is 11 mm. Moreover, the characterization using GC-MS identified the presence of high oleic acid in Moringa seed oil with an area of 40%.

## ACKNOWLEDGEMENTS

This study was financially supported by the Directorate Research and Community Service or Direktorat Penelitian dan Pengabdian Masyarakat (DPPM) Universitas Islam Indonesia, Yogyakarta, Indonesia.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

H. Hidayat <http://orcid.org/0000-0002-2191-0455>

N. Madina <http://orcid.org/0009-0002-3951-3383>

M.M. Musawwa <http://orcid.org/0000-0001-8929-8875>

F.I. Fajarwati <http://orcid.org/0000-0002-6224-1630>

**Open Access:** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## REFERENCES

1. Yulyuswarni, *Jurnal Kesehatan*, **12(1)**, 93(2021), <https://ejurnal.poltekkes-tjk.ac.id/index.php/JK>
2. H. Hidayat, *Indonesian Journal of Chemical Research*, **3(1-2)**, 6(2017), <https://doi.org/10.20885/ijcr.vol2.iss1.art2>
3. H. Hidayat, *Eksakta: Journal of Sciences and Data Analysis*, **15(1-2)**, 75(2015), <https://doi.org/10.20885/eksakta.vol14.iss1-2.art8>
4. H. Hidayat, E. Fadilah, Nurmadina and M. M. Musawwa, *IOP Conference Series: Earth and Environmental Science*, **463**, 012100 (2020), <https://doi.org/10.1088/1755-1315/463/1/012100>
5. G. Bonang, *Microbiology for the Health Profession, Issue 16*, Jakarta (1992)
6. Mardiana, *Antibacterial Activity Test of Amethyst Leaf and Seed Extract (Datura metel L) Against Escherichia coli and Bacillus cereus Bacteria*, Universitas Bengkulu, Bengkulu (2007)
7. N.A. Widyastuti, B. Susilo, *Extraction Study of Moringa (Moringa oleifera) Seed Oil Hydraulic Press with Variation of Heat Treatment*, Universitas Brawijaya, Malang (2013)
8. C. Omonhinmin, E. Olomukoro, A. Ayoola, E. Egwim, *AIMS Energy*, **8(1)**, 102(2020), <https://doi.org/10.3934/energy.2020.1.102>
9. R. B. Herbert, 1988. *Biosynthesis of Secondary Metabolites*, Chapman & hall. London (1988)
10. J.J. Kabara, A. L. In Branen and P. M. Davidson, *Antimicrobials in Foods*, Marcel Dekker, Inc. New York (1983)
11. S. Athikomkulchai, P. Tunit, S. Tadtong, P. Jantrawut, S.R. Sommano and C. Chittasupho, *Cosmetics*, **8(1)**, 2(2021), <https://doi.org/10.3390/cosmetics8010002>
12. Murhadi, *Jurnal Teknologi Industri dan Hasil Pertanian*, **14(1)**, 97 (2009), <https://jurnal.fp.unila.ac.id/index.php/JTHP/issue/view/17>
13. D. Djarwis, 2004, *Natural Material Organic Chemistry Research Techniques*, Univesitas Andalas, Padang.

[RJC- 8264/2023]