Life Science Journal

Websites:
http://www.lifesciencesite.com
http://www.sciencepub.net

Emails:
editor@sciencepub.net
sciencepub@gmail.com

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Antibacterial and antioxidant properties of alkaloids extracted from *Morinda citrifolia* (Rubiaceae)

Abdul Mushin M. Shami [1]*, Koshy Philip [1] and Sekaran Muniandy [2]

[1.] Institute of Biological Science, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[2.] Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
aashbio@yahoo.com

Abstract: *Morinda citrifolia* have been used as a treatment for many diseases such as dysentery, heart diseases, AIDS, cancers and others. The purpose of the study was to determine the antibacterial and antioxidant activities from alkaloids extracted from of *M. citrifolia* fruits. **Methods**: Well diffusion assays were used to determine minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). These parameters were used to evaluate antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa*, MRSA and *Helicobacter pylori*. Assays for radical scavenging activity and superoxide dismutase-like activity were used to evaluate antioxidant activity. LC-MS analysis was used to identify alkaloids while studies with the scanning electron microscope (SEM) revealed mode of action. **Results:** Alkaloids extracts from the fruits of *M. citrifolia* exhibited significant inhibition against all strains of test bacteria except *P. aeruginosa*. Alkaloids extracted from the fruits showed antioxidant activities. LC-MS analysis of alkaloids of the *M. citrifolia* fruits identified specific compounds in these extracts. SEM analysis of the interaction of these substances with the bacteria showed significant morphological changes of cell wall, membrane and destruction of the targeted bacterial cells. **Conclusions:** It could be concluded that the alkaloids extracts of the fruits of this plant had good antibacterial and antioxidant effects. The results suggest that these substances can be a new source of antimicrobials and antioxidants.
[Shami A, Philip K, Muniandy S. **Antibacterial and antioxidant properties of alkaloids extracted from *Morinda citrifolia* (Rubiaceae).** *Life Sci J* 2021;18(2):6-11]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 2. doi:10.7537/marslsj180221.02.

Keywords: *Morinda citrifolia;* alkaloids; antibacterial; antioxidant; mode of action

1. Introduction

M. citrifolia L. is one important plant used as a medicine in many countries of the world. It belongs to the Rubiaceae family and comprises 80 species. This plant is found in South East Asia, Caribbean countries, Australia and Central-South America. The common names of this plant are *Noni*, Indian mulberry, *nuna*, and *mengkudu* (Nelson, 2006; Potterat & Hamburger, 2007). Noni is rich in phytochemical compounds which can be extracted from different parts of the plant (Singh, 2012). The compounds found in this plant have many bioactive properties such as antibacterial and antioxidant activities (Potterat & Hamburger, 2007). Past studies on the extracts from the fruit, leaves and roots of *M. citrifolia* have documented antibacterial activity against a wide spectrum of microorganisms (Natheer *et al.*, 2012; Selvam *et al.*, 2009) and antioxidant activity by using different assays such as the DPPH and SOD assays (Pongnaravane *et al.*, 2006; Zin *et al.*, 2002).

One of the bioactive compounds present in this plant is alkaloids (chemical compounds containing nitrogen atoms). Ralph Heinicke documented that the *M. citrifolia* fruit contains a natural precursor called proxeronine. This compound merges with an enzyme in the human intestine called proxeroninase to result in xeronine (Heinicke, 1985; Lim, 2013). Xeronine plays the part of directing the protein in the human body to fold into an appropriate conformation to perform properly. In this way, xeronine ensures the performance of protein in overcoming a variety of health problems (Wang *et al.*, 2002).

The aim of this study is to determine the antibacterial and antioxidant activities from the alkaloids extracts of the fruits of *M. citrifolia*. LC-MS analysis and mode of action of alkaloids extracts were also investigated.

2. Material and Methods

Plant collection

The fresh ripe fruits of *M. citrifolia* were collected from Sendayan Valley, Seremban, Malaysia in November, 2010. This plant was identified at the

University of Malaya Herbarium and deposited as plant vouchers under the registration number KLU 22480. All samples were washed under tap water and dried in an oven at 40ºC for 3 days. The plant materials were then put through a grinder with a mesh size of 2 mm.

Alkaloid extracts from *M. citrifolia* fruit

100 gram of the dried fruit powder was added to the mixture of ethanol-chloroform 1:3 with 2% of strong ammonia solution and refluxed for 6 hr. Extraction was conducted with 2N HCl and the extract was made alkaline with strong ammonia. The solution was extracted with chloroform and washed with distilled water. Chloroform was then evaporated until the solvent was removed at 40ºC using a rotary evaporator (Heidolph WB2000, Germany). The product yield was 0.1% of original material. This method is based on Smita & Sushma (2010). These extracts were weighed to 0.1g and dissolved in 1 ml of dimethyl sulfoxide 5% (DMSO). They were diluted to 100, 50 and 25 mg/ml.

Determination of Antimicrobial Activities

For this study, four species of bacteria were used. *S. aureus* (RF 122), *E. coli* (UT181), *B. cereus* (ATCC 14579), and *P. aeruginosa* (PA7) were procured from cultures maintained at the Fermentation Technology Laboratory in the Microbiology Division, Institute of Biological Sciences, University of Malaya, Malaysia. Other strains used in this study included methicillin-resistant *S. aureus* (MRSA) (ATCC BA-43) and *H. pylori* ATCC 43504. Antibacterial activities were measured using well diffusion assay. The positive control used was 10 mg/ml of tetracycline, while the negative control was 5% DMSO. All extracts were checked for their respective MIC values using a standard protocol (Andrews, 2001). MBC values were determined by sub-culturing the MIC assay tubes onto Muller-Hinton agar (Difco, Detroit, MI, USA), and represent the dilution at which growth was detected.

Determination of antioxidant activities of plants

DPPH radical scavenging assay

Free radical scavenging activities were determined using the method of Bozin *et al.* (2008) The reagent of the assay is 2, 2- diphenyl-1-picrylhydrazyl solution (Sigma Aldrich GmbdH, Germany). The percentage of DPPH radical scavenging activity of the resulting solutions was calculated using the following equation:

DPPH radical scavenging activity (%) = [($A_{control} - A_{sample}$) / $A_{control}$] x100

Ascorbic acid (10 mg/ml) was used as a positive control of the assay.

IC_{50} was calculated using linear regression plots. The IC_{50} values represent the concentrations of samples that are required to scavenge for 50% of DPPH free radicals.

Superoxide dismutase activity assay

Superoxide dismutase (SOD) activities were determined using a SOD Assay Kit-WST (Dojindo Molecular Technologies, Gaithersburg). The protocol used in this study was modified from Sakudo *et al.* (2005). The positive control was ascorbic acid (10 mg/ ml).

LC-MS analysis

Alkaloids extracted from *M. citrifolia* fruits were identified through the Agilent 6530 quadrupole time-of-flight liquid chromatography mass spectrometer (Agilent Technologies, USA) with binary pump and automatic sampler, while the data were analysed by Agilent MassHunter Workstation Software B.01.03.

Effect of alkaloids extracts from *M. citrifolia* by scanning electron microscope

Bacterial culture (*B. cereus*) was incubated into nutrient broth overnight at 37ºC. This culture (1 ml) was added to one milliliter of alkaloids extracts from *M. citrifolia* fruits. The bacterial cells were dehydrated in ascending concentrations of ethanol, and dried using liquid CO_2, and the cells mounted on a stub. These cells were coated with gold, and examined using an SEM (Model: JEOL JBM 7001F, UK).

Statistical analysis

Data is expressed as mean ± SD. Statistical analyses were carried out using SPSS version 17. One-way ANOVA followed by Duncan's multiple comparison were used to compare the values of samples with the control. A P value < 0.05 was deemed as indicating significant differences. Each treatment was duplicated thrice and each experiment was repeated at least twice.

3. Results

Antibacterial activity

The zones of inhibition for the alkaloid extract from the fruits of *M. citrifolia* as 10.16 mm for *S. aureus*, 10.33 mm for *E. coli*, 11.66 mm for *B. cereus*, 11.83 mm for MRSA and 16.66 mm for *H. pylori* at a high concentration of this extract (100 mg/ml). No result was obtained for *P. aeruginosa* (Figure 1).

Table 1 shows the values of MIC and MBC for alkaloid extracted from the fruit of *M. citrifolia* had MIC/MBC at 50 mg/ml for *S. aureus*, *E. coli*, MRSA and *H. pylori* while *B. cereus* was inhibited at 25 mg/ml. No result was detected for *P. aeruginosa.*

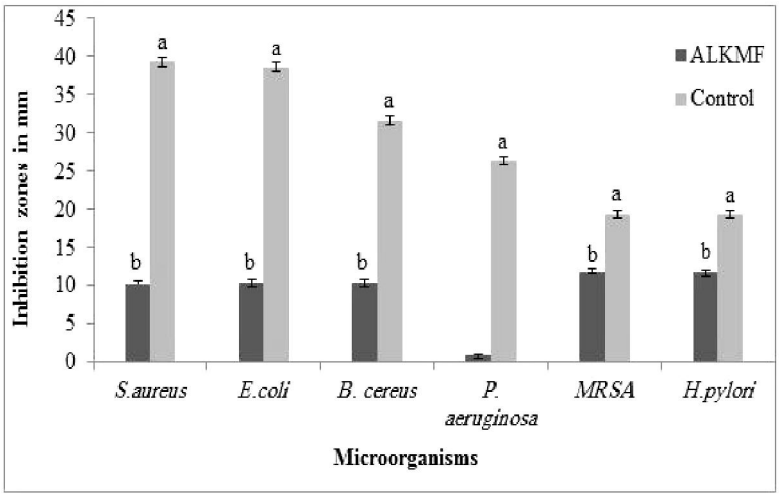


Figure 1. The inhibition zones of alkaloids extracts from the fruits of *M. citrifolia* fruit (ALKMF) on selected test microorganisms.

Table 1. MIC and MBC of alkaloids extracted from *M. citrifolia* fruit (ALKMF) on selected microorganisms.

Bacteria	**Plant extracts (mg/ml)**	
	MIC	**MBC**
S. aureus	50.00	>50.00
E. coli	50.00	>50.00
B. cereus	25. 00	>25.00
P. aeruginosa	Na	Na
MRSA	50.00	50.00
H. pylori	50.00	50.00
		0.00

Na–non active at high concentration

Antioxidant activity

The results to determine the DPPH radical scavenging activity of the alkaloid extract from *M. citrifolia* fruit showed 27.71% (IC_{50} 18.05 mg/ml) compared to ascorbic acid as a positive control at 96.59% (IC_{50} 5.18 mg/ml) (Figure 2). While the inhibition rate of SOD-like activity of the alkaloid extract from *M. citrifolia* fruit was at 27.01% it was at 96.97% for ascorbic acid (Figure 3).

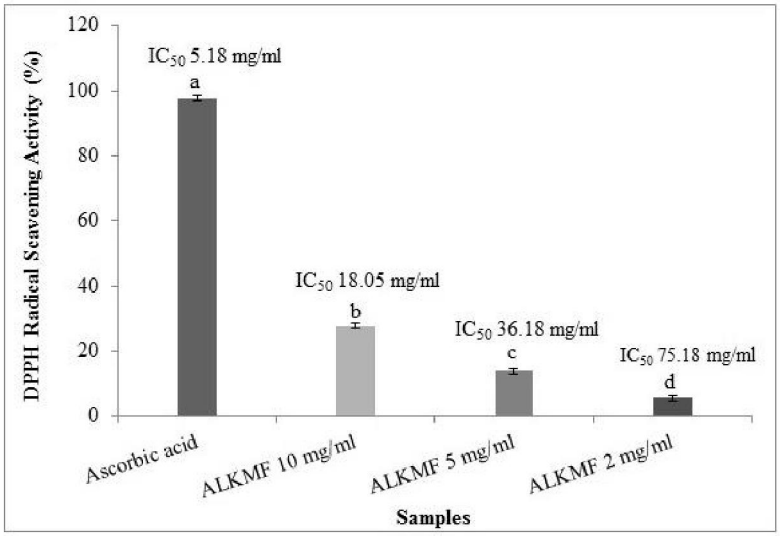
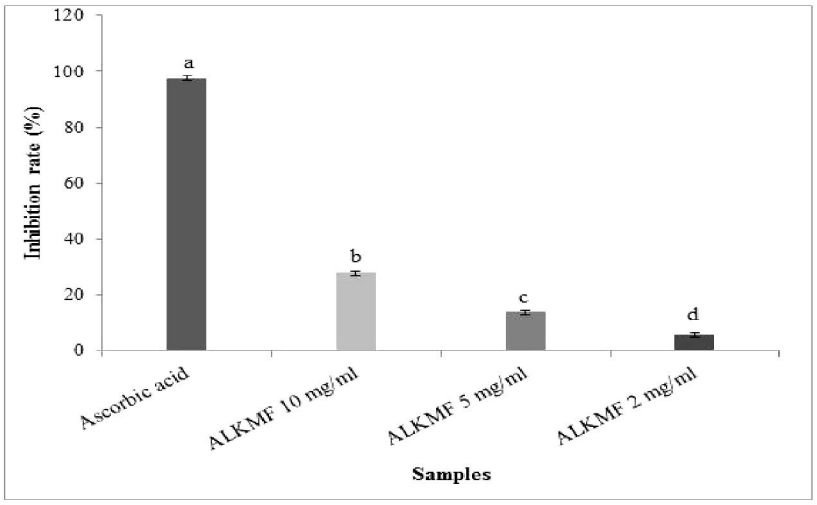


Figure 2. DPPH scavenging activity with IC_{50} values of alkaloids extracts of *M. citrifolia* fruits (ALKMF).



L(+
ıs
al :4
in ıs
1. ıe
(N :4
pe
H

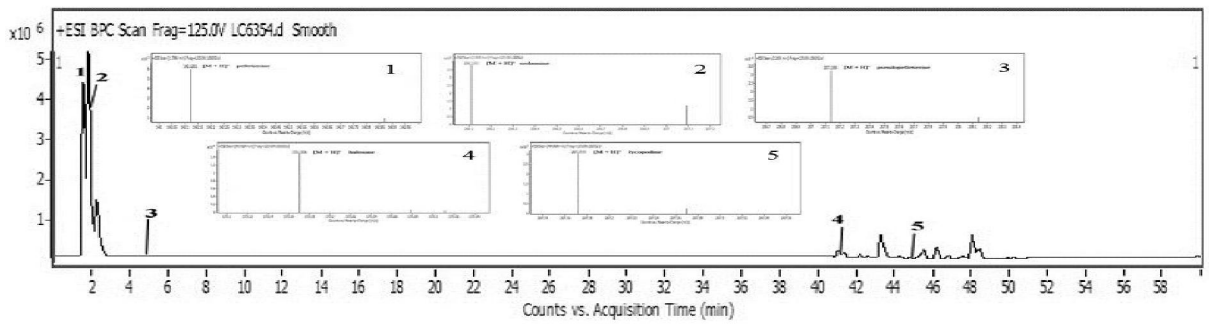


Figure 4. LC chromatograms and MS/MS data of the major of alkaloids extracted from the fruit of *M. citrifolia* (1) pelletierine, (2) sedamine, (3) pseudopelletierine, (4) halosine and (5) lycopodine.

Effect of alkaloids extracts from the fruit of *M. citrifolia* fruits by scanning electron microscope

The effects on bacterial cells treated with alkaloid extract from from the fruit of *M. citrifolia* were observed with the utilization of a scanning electron microscope (Figure 5). The morphological changes included swelling, rupture in cell walls and cell lysis which eventually culminates in the death of the cell. Untreated cells exhibited an unchanged shape.

4. Discussions

The alkaloids extract of the fruits of *M. citrifolia* have antibacterial activity against all test bacterial strains including MRSA and *H. pylori*. This plant is known to produce alkaloids as secondary metabolites and these have antibacterial properties (Koyama *et al.*, 2008; Lim, 2013; Pandey & Barve, 2011). Costa *et al.* (2010) reported that alkaloids *O*-methylmoschatoline, lysicamine and liriodenine from the bark of *Guatteria hispida* had antibacterial activity against *S. epidermidis*. Yang *et al.* (2012) reported that the new alkaloids from the twigs of *Kopsia hainanensis exhibited antibacterial properties against S. aureus*. MIC and MBC values of alkaloids extracted from in this study showed significant antibacterial effects against all test bacterial strains with *B. cereus* being most sensitive. The current study is the first study on alkaloids isolated from these plants and its antibacterial activity against pathogenic bacteria

including clinically important antibiotic resistant bacteria such as MRSA and *H. pylori*.

Antioxidant activity of alkaloids extracted from the fruit of *M. citrifolia* exhibited DPPH radical scavenging activity. In addition, alkaloids extracted from the fruit of *M. citrifolia* have inhibition of SOD-like activity. Past studies show the fruit of this plant are rich in alkaloids such as xeronine (Lim, 2013). These compounds have antioxidant activity and show inhibition of microsomal lipid peroxidation induced by $Fe2^{+}$/ ascorbate, CCl4/NADPH or $Fe3^{+}$ ADP/NADPH. Further, these alkaloids increased deoxyribose degradation by the hydroxyl radical and increased antioxidant capacity (Ubeda *et al.*, 1993).

LC-MS analysis of alkaloids extracted from *M. citrifolia* fruit identified five major compounds namely pelletierine, sedamine, pseudopelletierine, halosine and lycopodine. Heinicke (1985) documented that *M. citrifolia* fruit contains a natural precursor named proxeronine. This compound merges with an enzyme in the human intestine called proxeroninase to result in xeronine (Heinicke, 1985; Lim, 2013). The current study is the first report documenting that these alkaloids have antibacterial and antioxidant activities.

The effects of alkaloids extracted from the fruit of *M. citrifolia* of showed changes in morphology of bacterial cells such as swelling of entire cell, rupture in cell wall, cell lysis–ultimately result in cell death with the spreading of cell debris. Past studies reported that the alkaloids, berberine and piperine intercalate in cell wall and or DNA (Atta-ur-Rahman & Choudhary, 1995). Obiang-Obounou *et al.* (2011) found sanguinarine extracted from the root of *Singuinarina canadensis* cause distorted septa in MRSA with rare discerned separation and cell lysis of treated cells by using TEM.

5. Conclusions

In conclusion, this is the first report that studied antibacterial activity antioxidant capacity and mode of action with LC-MS analysis in alkaloids extracts from *M. citrifolia* fruit. Alkaloids extracts of *M. citrifolia* fruit had antibacterial activity against all strains of test bacteria including MRSA and *H. pylori*. However, *P. aeruginosa* has resistance against alkaloid extracts of *M. citrifolia* fruits. Alkaloids extract from *M. citrifolia* fruit have antioxidant activity. SEM observation of the mode of action of alkaloids extracted from the *M. citrifolia* fruit on bacterial cells showed changes in cell morphology such as swelling of cells, rupture in cell wall and cell lysis. LC-MS analysis of alkaloids extracted from this plant identified important compounds which may be used to develop biopharmaceuticals against infectious diseases and antioxidants source in future.

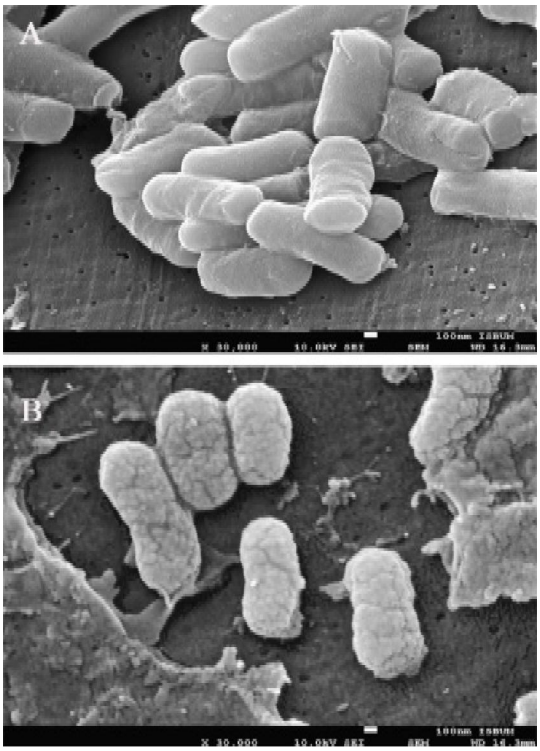


Figure 5 Effect of alkaloid extracted from the fruit of *M. citrifolia* by scanning electron microscope. (A) Control: *B. cereus*. (B) Shows changes in morphology with swelling rupture in cell walls with cell debris and cell death.

Acknowledgements:

The authors would like to thank University of Malaya for the financial and lab facilities support for this study from IPPP grant (FP038-2010B).

Corresponding Author:
*Corresponding author:
AbdulMushin M. Shami
Institute of Biological Science
Faculty of Science, University of Malaya
50603 Kuala Lumpur, Malaysia
E-mail: aashbio@yahoo.com

References

1. Nelson C. *Morinda citrifolia* (noni). Species profiles for Pacific Island forestry. 2006;4:1-13.
2. Potterat O, Hamburger M. *Morinda citrifolia* (Noni) fruit-phytochemistry, pharmacology, safety. Planta Medica. 2007;73(3):191-199.
3. Singh DR. *Morinda citrifolia* L. (Noni): A review of the scientific validation for its nutritional and therapeutic properties. Journal of Diabetes and Endocrinology. 2012;3(6):77-91.
4. Selvam P, Raj K, Vimisha V, et al. Antimicrobial activity of fruit extracts of

Morinda citrifolia. J. Appl. Chem. Res. 2009;10:61-63.
5. Natheer ES, Sekar C, Amutharaj P, et al. Evaluation of antibacterial activity of *Morinda citrifolia, Vitex trifolia* and *Chromolaena odorata*. African Journal of Pharmacy and Pharmacology. 2012;6(11):783-788.
6. Zin Z, Abdul-Hamid A, Osman A. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. Food Chemistry. 2002;78(2):227-231.
7. Pongnaravane B, Goto M, Sasaki M, et al. Extraction of anthraquinones from roots of *Morinda citrifolia* by pressurized hot water: Antioxidant activity of extracts. The Journal of Supercritical Fluids. 2006;37(3):390-396.
8. Heinicke RM. The pharmacologically active ingredient of noni. The Bulletin Pacific Tropical Botanical Garden. 1985;15:10-14.
9. Lim TK. *Morinda citrifolia*. Edible Medicinal And Non-Medicinal Plants: Springer; 2013:715-753.
10. Wang MY, West BJ, Jensen CJ, et al. *Morinda citrifolia* (Noni): A literature review and recent advances in Noni research. Acta Pharmacologica Sinica. 2002;23(12):1127-1141.
11. Smita N, Sushma M. Preliminary physicochemical and phytochemical evaluation of *Morinda citrifolia* fruit extractives. International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2(1):150-154.
12. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48(suppl 1):5-16.
13. Bozin B, Mimica-Dukic N, Samojlik I, et al. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chemistry. 2008;111(4):925-929.
14. Sakudo A, Lee D, Li S, et al. PrP cooperates with STI1 to regulate SOD activity in PrP-deficient neuronal cell line. Biochemical and Biophysical Research Communications. 2005;328(1):14-19.
15. Pandey N, Barve D. Phytochemical and Pharmacological Review on *Annona squamosa* Linn. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011;2(4):1404-1412.
16. Koyama K, Hirasawa Y, Zaima K, et al. Alstilobanines A–E, new indole alkaloids from *Alstonia angustiloba*. Bioorganic & Medicinal Chemistry. 2008;16(13):6483-6488.
17. Costa E, Pinheiro M, Barison A, et al. Alkaloids from the bark of *Guatteria hispida* and their evaluation as antioxidant and antimicrobial agents. Journal of Natural Products. 2010;73(6):1180-1183.
18. Yang Y, Zuo W, Zhao Y, et al. Indole alkaloids from *Kopsia hainanensis* and evaluation of their antimicrobial activity. Planta Medica. 2012;78(7):1881-1884.
19. Ubeda A, Montesinos C, Payá M, et al. Antioxidant action of benzylisoquinoline alkaloids. Free Radical Research. 1993;18(3):167-175.
20. Atta-ur-Rahman M, Choudhary M. Diterpnoid and steroidal alkaloids. Natural Product Research. 1995;12:361-379.
21. Obiang-Obounou B, Kang O, Choi J, et al. The mechanism of action of sanguinarine against methicillin-resistant *Staphylococcus aureus*. The Journal of Toxicological Sciences. 2011;36(3):277-283.

1/17/2021