Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

Check for updates

Antibacterial activity of ethanol extract from *Morinda citrifolia* L. leaves and fruits collected in Tan Hung district, Long An province, Vietnam

Nguyen Thị Thanh Thao [1], Dang Thi Ngoc Thanh [2], Hoang Minh Tam [2,*] and Pham Van Ngot [1]

[1] *Faculty of Biology, HCMC University of Education, 280 An Duong Vuong Street, Ward 4, District 5, Ho Chi Minh City, Vietnam*

[2] *Faculty of Natural Science Pedagogy, Sai Gon University, 273 An Duong Vuong Street, Ward 3, District 5, Ho Chi Minh City, Vietnam.*

Magna Scientia Advanced Biology and Pharmacy, 2023, 10(01), 019–024

Publication history: Received on 30 August 2023; revised on 08 October 2023; accepted on 11 October 2023

Article DOI: https://doi.org/10.30574/msabp.2023.10.1.0062

Abstract

The study investigated antibacterial activity of *Morinda citrifolia* L. collected in Tan Hung district, Long An Province in Vietnam. Antimicrobial activity of *Morinda citrifolia* L. was evaluated based on size of antibacterial zones of ethanol leaf and fruit extracts by agar well diffusion method. The results showed that ethanol leaf and fruit extracts produced growth inhibition zones against tested bacteria with diameters of smaller than 9.0 mm (1.80 - 6.72 mm and 1.66 - 8.06 mm, respectively). The lowest ethanol concentration of extracts inhibited growth of tested bacteria was 200 mg mL^{-1} and their antibacterial activity increased with increase in tested concentrations. In general, antibacterial effects of fruit extracts were higher than those of leaf extracts on bacterial growth. Antibacterial activity of ethanol extracts of *Morinda citrifolia* L. leaves and fruits were evaluated at weak degrees, according to Manuanza's rating scale of antibacterial zones.

Keyword: Antibacterial activity; Antibacterial zone; *Morinda citrifolia* L.; Fruit extract; Leaf extract

1. Introduction

Genera Morinda belonging to Rubiaceae with 102 species were widely distributed in tropical, sub-tropical and temperate countries [1]. Traditionally, Morinda were applied to treat various diseases. They were used as a therapeutic remedy to various diseases such as bacterial, helminthic infection, malaria, diabetes, hepatitis, cancer and inflammation. The antimicrobial potential of Morinda was widely studied [1, 2]. Methanol extract from Tahitian *M. citrifolia* fruits was antagonistic to *Candida albican* and *Staphylococcus aureus* [3]. Indian *M. citrifolia* fruit extracts by methanol, ethyl acetate and hexane were against a wide range of bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Vibrio harveyi*, *Klebsiella pneumonia*, *Shigella flexneri*, *Salmonella paratyphi* A, *Aeromonas hydrophila*, *Vibrio cholera*, *Chromobacterium violaceum* and *Enterococcus faecalis*. Methanol extract showed the most effects on all the tested micro-organisms, ethyl acetate extract was effective on all the tested micro-organisms but *Pseudomonas aeruginosa* and *Klebsiella pneumonia* while hexane extract had no effects on all tested microorganisms. The extracts by methanol and ethyl acetate of *M. citrifolia* fruits also had positive effects on tested fungi. The percentage of inhibition against *Trichophyton mentagrophytes* of the methanol ethyl acetate extracts were of 79.3% and 62.06%, respectively. The methanol extract presented about 50% inhibition against *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp.. However, none of the extracts were against *Candida albicans* and *Aspergillus* species [4]. Different extracts of *M. tinctoria* fruits exhibited their activities against microbes. Ethanol and methanol extracts of mature fruits inhabited the growth of *S. typhii* and *K. pneumonia*. Antimicrobial activity of *M. tinctoria* extracts caused by various secondary metabolites [5]. Leaf extracts by hexane, chloroform, ethyl acetate and methanol of *Morinda tinctoria* Roxb were evaluated against nine human pathogens by agar well diffusion method.

* Corresponding author: Hoang Minh Tam

The results showed that they had positive effects on *Proteus vulgaris* (halo ring diameter of 24 mm), *Klebsiella pneumonia* (halo ring diameter of 21 mm) and *Enterococcus feacelis* (halo ring diameter of 21mm) [6]. Presently, antimicrobial activity of *M. elliptica* leaves was reported by using agar disk diffusion method. The research showed that the extracts by n-hexane, dichloromethane, ethyl acetate, and methanol were inhibited against to *E. coli* and *S. typhii and S. aureus* and inhibited the growth of fungi *A. brasiliences* and *A. flavus* [7].

Morinda were widely dispersed in Vietnam with approximately ten species, namely, *Morinda officinalis*, *Morinda cochinchinesis*, *Morinda parvifolia*, *Morinda umbellate*, *Morinda citrifolia, Morinda persicaefolia*...and traditionally applied as a therapeutic remedy to various diseases [8]. However, potential antibacterial activity of Morinda have been limitedly studied. Therefore, to understand more about their importance in field of medicine, study was conducted. The aims of this study were to assess antibacterial activity of ethanol extract from *Morinda citrifolia* L. leaves and fruits collected in Tan Hung district, Long An province, Vietnam.

2. Material and methods

2.1. Preparation of *M. citrifolia* L. leaves and fruits

Leaves and fruits of *M. citrifolia* L. grown at Tan Hung district, Long An Province, Vietnam were collected. The sample were washed with water to remove sand and dust, left to dry at room condition for one - two days. *M. citrifolia* L. leaves and fruits were oven-dried at 50 °C to constant mass. Finally, the dried leaves and fruits were separately grounded into powder stored for further uses [9].

2.2. Preparation of ethanol extracts of *M. citrifolia* L. leaves and fruits

Each fifty grams of the leaf and fruit powder of *M. citrifolia* L. were separately immersed in 500 mL of ethanol 96° (1:10 (w/v) for 48 hours. The mixtures were then filtered through Whatman filter paper No. 4. The residues were re-exacted two times in ethanol with the same ratio of the powder and solvent (w/v) [10]. Extracts of leaves and fruits were evaporated at room condition, with a rotary evaporator (Rotary Evaporator RE301, Yamato Scientific) and then stored in screw cap bottles at 4°C for further uses.

2.3. Preparation of bacterial suspension

Tested bacteria were included two groups: Gram (+) and Gram (-) bacteria. The first groups included *Bacillus subtilis, Bacillus cereus*. The second group contained *Pseudomonas aeruginosa*. The bacteria were cultured in liquid LB media and shook at 37 °C, 120 rpm. After 48 hours of culture, the suspension of each bacterium was determined cell density with a spectrophotometer at 600 nm and adjusted to the McFarland standard 0.5 corresponding to 1.5 x 10^8 CFU mL^{-1} and called standard bacteria suspension used for later experiments [11].

2.4. Antibacterial activity screening

2.4.1. Antibacterial activity of ethanol leaf and fruit extracts

Agar well diffusion method was applied to determine antibacterial activity of extracts of *M. citrifolia* L. leaves and fruits [12]. 10 mL of standard bacteria suspension of each strain was added to a 190 mL conical flask consisting of warm sterilized LB medium (40 – 50 °C) and mixed well. Bacterial cell density was reduced to 5 x 10^6 CFU mL^{-1}. The media were then poured into petri dishes. Using a sterile round glass pipe (Φ = 6 mm) created four wells on each medium plate. 20 µL of ethanol leaf and fruit extracts with each concentration of 200 mg mL^{-1}, 400 mg mL^{-1}, 600 mg mL^{-1}, 800 mg mL^{-1} and 1000 mg mL^{-1} were applied in well of each medium plate, respectively. Medium plates' wells containing 20 µL of ethanol 70° were used as negative controls and medium plates' wells containing 10 µL of aqueous 1.0 mg mL^{-1} solution of tetracycline or gentamicin were used as positive controls. Experimental dishes were kept at 5 °C for three hours and inoculated at 37 °C for one – two days.

The antibacterial activity of extracts produced zones of growth inhibition against the test bacteria. The antibacterial strength of extracts was proportional to diameter magnitude of antibacterial zones. The rating scale of Manuanza's antibacterial zones was used to evaluate degrees of bacterial growth inhibition of the extracts [13].

2.5. Experiment design and data analysis

Experiments were set up in RCRD type and repeated three times. Data for quantitative experiments was analyzed by using One-way ANOVA. Comparisons of means were carried out following Duncan's test at 5% level of confidence with the support of IBM SPSS Statistics software version 20.0.

3. Result and discussion

3.1. Antibacterial activity of ethanol leaf extract

Results of antibacterial activity of ethanol extract of *Morinda citrifolia* L. leaves on three tested bacteria were presented in Table 1 and Figure 1. Ethanol extracts showed inhibition of tested bacteria's growth at the lowest concentration of 200 mg mL^{-1} (E5). When applied higher concentration, antibacterial activity increased. For Gram (+) bacteria, the lowest concentration of the extract affected growth of *B. cereus* with diameter of bacterial inhibition zones about 5.06 mm. Although at higher concentrations, the extract produced larger rings of bacterial inhibition up to 6.69 mm in diameter at concentration of 1000 mg mL^{-1} (E1), but the results were not statistically different. However, to *B. subtilis*, greater concentration of the extract caused increases in sizes of antibacterial zones and the effects had statistical differences among three tested extract concentration groups, 800 mg mL^{-1} (E2) - 1000 mg mL^{-1} (E1); 400 mg mL^{-1} (E4) - 600 mg mL^{-1} (E3) and 200 mg mL^{-1} (E5). Antibacterial effects of the extract on *B. subtilis* (diameter of halo rings arranged from 1.87 to 4.99 mm) were lower than those on *B. cereus* (diameter of halo rings arranged from 5.06 to 6.69 mm). For Gram (-) bacteria, *P. aeruginosa*, extract concentration of 200 mg mL^{-1} created an antibacterial ring with diameter of 2.63 mm. When higher extract concentrations (E4 – E1) were applied, larger diameter of antibacterial rings arranged from 2.63 to 6.72 mm were produced and were statistically different among tested extract concentrations. Extract effects on *P. aeruginosa*'s growth inhibition was lower than those on *B. cereus* 's but higher than *B. subtilis's*. Extract's antibacterial activity was much lower than those of positive controls (Gentamicin and Tetracycline). The ethanol leaf extract showed inhibition against bacterial growth at all tested concentrations. In summary, antibacterial activity of the ethanol leaf extract was just at weak level.

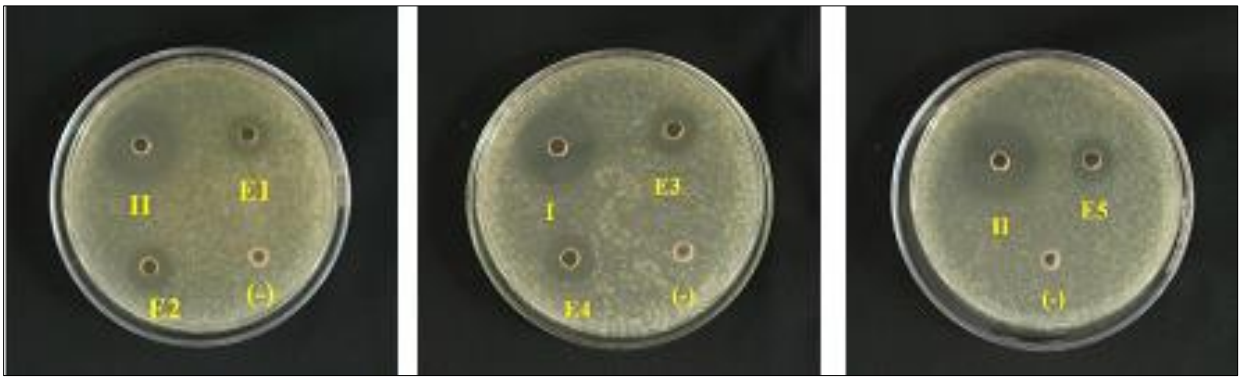
Table 1 Antibacterial effects of ethanol leaf extract on tested bacteria

Ethanol extract/Antibiotics	**Diameter of antibacterial zones (mm)**		
	Gram (+) bacterium		**Gram (-) bacterium**
	B. cereus	***B. subtilis***	***P. aeruginosa***
E1	$6.69^{a} \pm 0.63$	$4.99^{c} \pm 0.43$	$6.72^{d} \pm 0.65$
E2	$6.39^{a} \pm 0.48$	$4.58^{c} \pm 0.27$	$5.02^{c} \pm 0.37$
E3	$6.08^{a} \pm 0.36$	$3.17^{b} \pm 0.21$	$4.16^{bc} \pm 0.33$
E4	$5.92^{a} \pm 0.34$	$3.13^{b} \pm 0.18$	$3.86^{b} \pm 0.41$
E5	$5.06^{a} \pm 0.40$	$1.87^{a} \pm 0.14$	$2.63^{a} \pm 0.25$
E0	-	-	-
Gentamicin (I)	$17.18^{b} \pm 1.70$	$23.18^{e} \pm 0.34$	$16.28^{f} \pm 1.14$
Tetracycline (II)	$15.72^{b} \pm 1.30$	$17.61^{d} \pm 1.29$	$15.17^{e} \pm 0.57$

(E0): negative control, (E1): 1000 mg mL^{-1}; (E2): 800 mg mL^{-1}; (E3): 600 mg mL^{-1}; (E4): 400 mg mL^{-1}; (E5): 200 mg mL^{-1}, Gentamicin and Tetracycline: positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

A study of evaluating antibacterial activity of *Morinda citrifolia* of Natheer et al., 2012 showed that ethanol extract of *Morinda citrifolia* inhibited *Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas diminuta* and *S. aureus* ATCC 6538 with antibacterial halo zone diameters of 6 mm, 7 mm, 8 mm, 6 mm, 10 mm and 13 mm, respectively [14]. According to Sunder et al., 2012, when conveyed antimicrobial activity of *Morinda citrifolia* solvent extracts, average bacteria inhibition zones of ethanol extract was about 8.0 mm against *B. cereus* were not found. The best activity against *S. aureus* ATCC6538 was obtained [15].

Manuanza's rating scale of antibacterial zones used to evaluate the degree of bacterial inhibition of the extract. Antibacterial activity of an extract was assessed as being strongly active if diameter of antibacterial zones was equal or greater than 15 mm, moderately active if diameter of antibacterial zones was between 10 mm and 14 mm; and weak activity if diameter of antibacterial zones was equal or smaller than 9 mm [13]. Thus, the ethanol leaf extract showed inhibition against bacterial growth at all tested concentrations. However, reported antibacterial activity of the ethanol leaf extract was at weak to moderate levels because diameters of bacterial inhibition zones at all tested extract concentrations were smaller than 14 mm.



(E1): 1000 mg/mL; (E2): 800 mg/mL; (E3): 600 mg/mL; (E5): 200 mg/mL; (-): negative control (I)-Gentamicin and (II)-Tetracycline.

Figure 1 Some results on the halo zones of ethanol extract of leaves

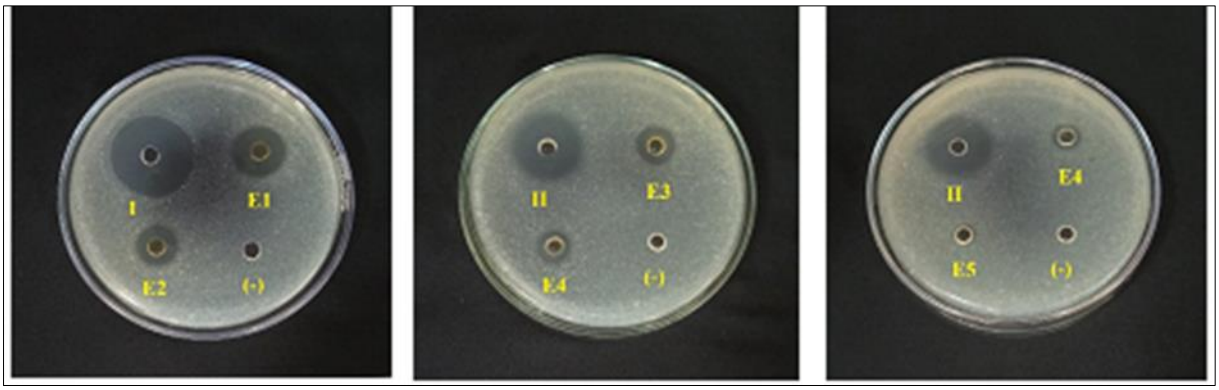
3.2. Antibacterial activity of ethanol fruit extract

Fruit extract showed positive effects on targeted bacteria at any tested concentrations (Table 2, Figure 2) but were smaller than 50% compared with positive controls (Gentamicin and Tetracycline). The antibacterial activity of the extract increased with increase in concentration. The lowest concentration of the extract causing antibacterial activity was 200 mg mL^{-1} (E5). Positive Gram *B. cereus* was the most sensitive to the extract. E1 and E2 corresponding concentrations of 800 – 100 mg mL^{-1} caused the most inhibition of bacterial growth with halo zone diameters of 7.04 mm and 8.06 mm, respectively. However, there was not statistical difference between them. Positive Gram *B. subtilis* was the least sensitive to the extract. The highest concentration inhibiting tested bacteria's growth was 1000 mg mL^{-1} (E1) causing halo zones with average diameter of 4.86 mm and followed by 800 mg mL^{-1} (E2) producing halo zones with average diameter of 3.5 mm. *P. aeruginosa,* a negative Gram bacteria was more sensitive than *B. subtilis* but less sensitive than *B. cereus* to the extract. Among tested concentrations, E1 (1000 mg mL^{-1}) and E2 (800 mg mL^{-1}) produced the biggest antibacterial zones with diameters of 5.76 mm and 6.11 mm, respectively. However, there was not statistical difference between E1 and E2's antibacterial effects on *P. aeruginosa*. In summary, antibacterial activity of the ethanol fruit extract was just at weak level.

Table 2 Antibacterial effects of ethanol fruit extract on tested bacteria

Ethanol extract/Antibiotics	**Diameter of antibacterial zones (mm)**		
	Gram (+) bacterium		**Gram (-) bacterium**
	B. cereus	***B. subtilis***	***P. aeruginosa***
E1	$8.06^{b} \pm 0.32$	$4.86^{c} \pm 0.45$	$6.11^{c} \pm 0.58$
E2	$7.04^{b} \pm 0.28$	$3.50^{b} \pm 0.38$	$5.76^{c} \pm 0.56$
E3	$3.20^{a} \pm 0.18$	$2.34^{ab} \pm 0.18$	$4.27^{b} \pm 0.12$
E4	$2.89^{a} \pm 0.16$	$1.95^{a} \pm 0.19$	$3.82^{b} \pm 0.19$
E5	$1.86^{a} \pm 0.15$	$1.66^{a} \pm 0.10$	$1.70^{a} \pm 0.17$
E0	-	-	-
(I) Gentamicin	$21.14^{d} \pm 1.59$	$22.04^{e} \pm 0.91$	$15.23^{d} \pm 0.50$
(II)Tetracycline	$16.97^{c} \pm 1.08$	$17.78^{d} \pm 1.54$	$15.06^{d} \pm 0.24$

(E0): negative control, (E1): 1000 mg mL^{-1}; (E2): 800 mg mL^{-1}; (E3): 600 mg mL^{-1}; (E4): 400 mg mL^{-1}; (E5): 200 mg mL^{-1}, Gentamicin and Tetracycline: positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.



(E1): 1000 mg/mL; (E2): 800 mg/mL; (E3): 600 mg/mL; (E5): 200 mg/mL. ; (-): negative control (I)-Gentamicin and (II)-Tetracycline.

Figure 2 Some results on the halo zones of ethanol extract of fruits

To ethanol fruit extract, antibacterial activity occurred to most tested bacteria and was higher than methanol and ethyl acetate. Maximum average zone of bacterial growth inhibition of ethanol extract was about 7.5 mm [14]. Natheer et al., 2012 when investigating evaluation of antibacterial activity of *Morinda citrifolia by* disc diffusion method, also found that the ethanol extract of *Morinda citrifolia* L. fruits demonstrated antimicrobial activity inhibiting the growth of all studied bacteria: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus sp., Klebsiella pneumoniae, Pseudomonas diminuta, Pseudomonas fluorescens and Enterobacter cloacae S. aureus* ATCC 6538 and *E. coli* ATCC 25922...[15]. Extract concentration of 1000 μg/disc produced antibacterial rings with average diameters arranged from 6 mm to 9 mm. Another study of antimicrobial activity of ethanol extract of *Morinda citrifolia* fruits reportet that it inhibited the growth of both Gram positive and Gram negative bacterial strains [16]. The highest antibacterial activity with 12mm zone of inhibition against *Klebsiella pneumonia* and moderate activity with 11mm zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains were found. The least activity with antibacterial zone of 10 mm against *Bacillus subtilis* was recorded. Thus, the ethanol fruit extract showed inhibition against bacterial growth at all tested concentrations. However, reported antibacterial activity of the ethanol fruit extract was evaluated at weak to moderate level, according to Manuanza's rating scale of antibacterial zones.

4. Conclusion

Ethanol leaf and fruit extracts of *Morinda citrifolia* L. collected in Tan Hung district, Long An province, Vietnam showed antimicrobial activity against tested bacteria. The leaf and fruit extracts produced growth inhibition zones with diameters of 1.80 - 6.72 mm and 1.66 - 8.06 mm, respectively. Both extracts revealed antibacterial potential at the lowest concentration of 200 mg mL^{-1} and activity of inhibiting bacterial growth increased with increase in tested concentrations. Fruit extract's antibacterial activity was higher than that of leaf one. However, both were evaluated at weak levels according to Manuanza's rating scale of antibacterial zones.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the support of Sai Gon University and Ho Chi Minh City University of Education.

Disclosure of conflict of interest

There is no conflict of interest to be disclosed.

References

[1] Ninh KB, Vu HG, Tran ML, Le QL, Ninh TN, Do TT, Nguyen HN, Nguyen XC, Phan VK, Chau VM. Two new 11-noriridoids from the aerial parts of *Morinda umbellate*. Phytochem. Lett. 2013; 6(2), 267–269.

[2] Ali M, Kenganora M, Manjula SN. Health Benefits of *Morinda citrifolia* (Noni): A Review. Pharmacognosy Journal. 2016; 8(4):321-334.

[3] West BJ, Palmer SK., Deng S, Palu AK. Antimicrobial activity of an iridoid rich extract from *Morinda citrifolia* fruit. Curr. Res. J. Biol. Sci. 2012; 4 (1), 52–54.

[4] Jayaraman SK, Manoharan MS, Illanchezian S. Antibacterial, antifungal and tumor cell supression potential of *Morinda citrifolia* fruit extracts. International Journal of Integrative Biology. 2008; 3 (1), 44.

[5] Ramesh S, Muthubalaji R and Elangomathavan R. Phytochemical and *in vitro* Antimicrobial assay of Fruit extracts of *Morinda tinctoria* Roxb. International Journal of PharmTech Research. 2014; 6 (2), 834-841.

[6] Deepti K, Umadevi P, Vijayalakshmi G, Vinod polarao B. Antimicrobial activity and phytochemical analysis of *Morinda tinctoria* Roxb. leaf extracts. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(3): S1440-S1442.

[7] Wakawa HY, Ahmad FB, Pukuma SM, Bulama H, Malgwi JM. Antibacterial and antifungal activities of leaf extract of *Morinda elliptica*. Borneo Journal of Resource Science and Technology. 2022; 12(2):96-100.

[8] Pham HH. An illustrated flora of Vietnam (vol. III). Vietnam: Tre Publishing House; 1998.

[9] Nguyen KPP. Methods of organic compound isolation. HCMC: Publishing House of VNUHCM; 2007.

[10] Gupta VN, Roy. A. Comparative study of antimicrobial activities of some mangrove plants from Sundarban Estuarine. J. Med. Plants Res. 2012; 6(42):5480-5488.

[11] Ha BS, Dang TTNT, Huynh NVA, Pham TTL, Hoang MT. Study on organic matter decomposition and siderophore production of rhizobacteria isolated from black pepper grown in Loc Ninh, Binh Phuoc province, Vietnam. World Journal of Advanced Research and Reviews. 2023; 17(03):009–017.

[12] Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6(2):71-79.

[13] Muanza DN, Kim BW, Euler KL, Williams L. Antibacterial and antifungal activities of nine medicinal plants from zaire. Int. J. Pharmacog. 1994; 32(4): 337-345.

[14] Natheer SS, Sekar C, Amutharaj P, M. Syed Abdul Rahman MSA, Khan KF. Evaluation of antibacterial activity of *Morinda citrifolia, Vitex trifolia* and *Chromolaena odorata*. African Journal of Pharmacy and Pharmacology. 2012; 6(11): 783-788.

[15] Sunder J, Singh DR, Jeyakumar S, Kundu A, Kumar De A. Antibacterial activity in solvent extract of different parts of *Morinda citrifolia* plant. J. Pharm. Sci. & Res. 2011; 3(8):1404-1407.

[16] Srinivasahan V, Durairaj B. Antimicrobial activities of hydroethanolic extract of *Morinda citrifolia* fruit. Int.J.Curr.Microbiol.App.Sci. 2014; 3(9): 26-33.