ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 12, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

PARTIAL CHARACTERIZATION AND ANTIMICROBIAL PROPERTY OF SMALL PEPTIDES ISOLATED FROM THE FLOWERS OF *MILLINGTONIA HORTENSIS*

ANGAYARKANNI R*, SRIDEVI G

Department of Chemistry, Sriram Engineering College, Perumalpattu, Tiruvallur, India. Email: getinanitha@gmail.com

Received: 04 July 2018, Revised and Accepted: 09 August 2018

ABSTRACT

Objective: The flowers of *Millingtonia hortensis* were initially screened for the presence of Cu (II) ninhydrin-positive compounds. Purification and characterization of small alpha peptides from the flowers of *M. hortensis* have been done. Further elucidation of the antimicrobial properties of these small peptides is also taken as part of the work.

Methods: Using 80% aqueous ethanol the crude extract was prepared and screening was carried out by a circular paper chromatographic technique. Purification and characterization of small alpha peptides from the flowers of *M. hortensis* have been done. Further elucidation of the antimicrobial properties of these small peptides by disc diffusion method is also taken as part of the work.

Results: Based on the findings of UV–visible spectrophotometer, it is confirmed that the purified compound is a small peptide and might contain glycine, cysteine, and tyrosine or histidine. The result of antimicrobial studies proves the ability of small peptides to function as antimicrobial peptides.

Conclusion: It is concluded that the small peptides show an inhibitory effect against various Gram-negative and some Gram-positive bacteria.

Keywords: *Millingtonia hortensis*, Alpha peptides, UV–visible spectrophotometer, Fourier-transform infrared spectrophotometer, Antimicrobial activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i12.28297

INTRODUCTION

India is endowed with a rich wealth of medicinal plants. Plants, as extracts and in various other forms are being used for centuries in different traditional systems of medicine for the treatment of human ailments, particularly, those caused by pathogenic bacteria, fungi, as well as viruses. The effective plant constituents can combat the human and plant pathogenic bacteria, fungi, and viruses without toxic side effects and environmental hazards. It is because of these reasons that search for plant products having antimicrobial properties has intensified in recent years. The leaf sample of *Millingtonia hortensis* was characterized by the presence of flavonoids, and its antimicrobial properties were already discussed [1]. In this study, alpha peptides from the flowers of *M. hortensis* were isolated, purified, characterized, and screened for the antimicrobial property.

The majority of peptides occurring in nature come under the category of alpha peptides, wherein the alpha carboxyl group is engaged in the peptide bond formation. Small peptides with newly exploited biological activities such as vasoactive, hormone-like, and antimicrobial, and others have been recently given much attention [2]. The ability of small peptides to function as a powerful radiosensitizer has been characterized [3]. A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Bacterial drug resistance is emerging as one of the most significant challenges to human health [4]. Antimicrobial peptides (AMPs), a ubiquitous group of natural compounds, have attracted considerable attention due to their broad spectrum killing activity and novel modes of action [5-8]. These peptides are excellent candidates for development as novel therapeutic agents and a complement to conventional antibiotic therapy. Circular paper chromatography technique is mainly employed in this work. It provides a simple and inexpensive method for screening of smaller alpha peptides from the petals of white flowers.

METHODS

Plant materials

M. hortensis Linn. (Bignoniaceae), known as Indian cork tree, is a widely distributed perennial flowering tree grown in gardens throughout the plains in India. Fresh flowers were collected and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai-45.

Preparation of crude extract

1 g of white petals of *M. hortensis* were homogenized thoroughly, with a mortar and pestle for approximately 5 min, in 5 ml of warm (60°C) 80% aqueous ethanol. The ethanol extract was filtered through a Whatman No.1 filter paper. The filtered extract was centrifuged at 3000 rpm for 10 min. The clear supernatant thus obtained was used as the crude source of the small peptide [9].

Circular paper chromatography

The flower extract was spotted in the center of Whatman No.1 filter paper. The sample spotting may be repeating 15–20 times to ensure sufficient concentration of the component to be detected. The chromatography was carried in isopropanol: water (4:1, v/v) solvent system after the run which require approximately 20–40 min, the chromatogram was dried at ambient temperature for 30 min. The air-dried chromatogram was developed by spraying uniformly with Cu (II) ninhydrin reagent followed by drying at 60°C for 10 min [10].

Purification of alpha peptides

Five or more circular paper chromatograms of the flower extract were run simultaneously using Whatman No.1 filter paper discs (12 cm). One of them was developed with the Cu (II) ninhydrin reagent and eluted by soaking in 80% ethanol [10].

UV–visible spectrophotometry

The purified flower extract of *M. hortensis* was studied for its absorption spectrum in an ultra-violet visible spectrophotometer. The UV region

scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm [11].

Fourier-transform infrared (FT-IR) spectrophotometer studies

The purified flower extracts were studied for its absorption spectrum using SHIMADZU FT-IR spectrophotometer. IR spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in a spectrum is a direction of the amount of materials present [12].

Antimicrobial activity

The bacterial cultures, namely *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aureus,* and *Streptococcus,Shiegella* were used. Disc diffusion method was followed to inoculate the culture [13]. Nutrient broth was prepare and taken in sterile test tubes. A loopful of test organisms were taken from the respective agar slants and inoculated into the broth. The inoculum was allowed to develop certain turbidity (say 0.5–1 h). Mueller-Hinton agar was prepared and poured into sterile Petri dishes as the media for test microbes. The surface of the agar plate was streaked with the test organism by means of a sterile swab dipped into the inoculum. The sterile discs were placed in the Petri dish and about 50 µl of the purified solution of the flower extracts was loaded into the discs. The sample solution was loaded in a consecutive volume of 10 µl with intermittent air drying. The loaded discs were placed on the surface of the agar plates with the help of a sterile forceps. Antibiotic discs such as tetracycline were used as standards. The plates were incubated at 18-24 h at 37°C. The results were recorded as a measurement of the diameter of the zone of inhibition and compared with the standard charts [14].

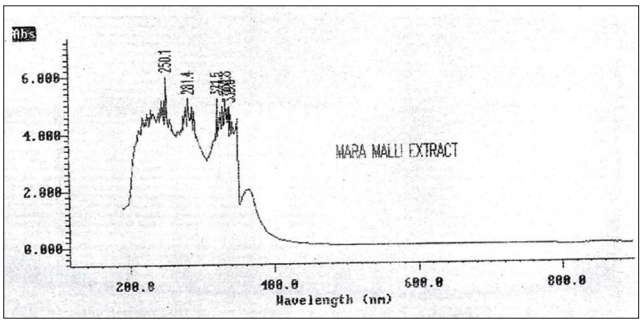

Fig. 1: Absorption spectrum of purified compound from *Millingtonia hortensis* (A peak at around 210 nm is indicating the presence of a peptide bond in the compound)

RESULTS AND DISCUSSION

In our preliminary studies as a part of the screening process for Cu (II) Ninhydrin-positive compounds in flowers of medicinal value, the following result was obtained.

The Cu (II) – ninhydrin-positive compounds which may be a small peptide, an amino acid amide or an amino acid ester were purified from the flowers *M. hortensis* [10].

The reaction between peptide and Cu (II) ninhydrin is due to a complex formation rather than due to an oxidation reaction of the ninhydrin. Evidence was provided by Ganapathy *et al*., for a reaction sequence in which peptides first react with Cu (II) ion to form a complex which then reacts with ninhydrin to give yellow chromophore [10].

The peptide nature of the purified compound was first established by scanning the compound in the UV–Visible spectrophotometer (SHIMADZU) from 200 nm to 900 nm. A single sharp peak at around 210nm was obtained indicating the presence of peptide bond in the compound [15] (Fig. 1).

The purified compounds from these flowers were also studied by FT-IR spectrophotometer spectrophotometry. The sharp peaks at 1758.96 cm^{-1} and 1662.52 cm^{-1} indicate the presence of C = O and N – H groups, respectively, in the compound [16,17] (Fig. 2).

Amino acids produce a red color, and their esters produce a yellow color with Cu (II) ninhydrin reagent (Fig. 3). It is also a general procedure to distinguish all amino acids and their carboxyl group derivatives such as amino acid esters, amino acid amides, and even oligopeptides containing up to five amino acid residues [10] except for the derivatives of L-Proline and L-Hydroxyproline, which do not produce any colored products.

The purified fraction of *M. hortensis* showed maximum antibacterial activity against *Streptococcus* (Fig. 4c), moderate antibacterial activity against *Escherichia coli* (Fig. 4a), *Shiegella* (Fig. 5a) and less activity against *Pseudomonas aureus* (Fig. 5b), *Proteus* (Fig. 4b) and shows no activity against *Klebsiella* (Fig. 5c).

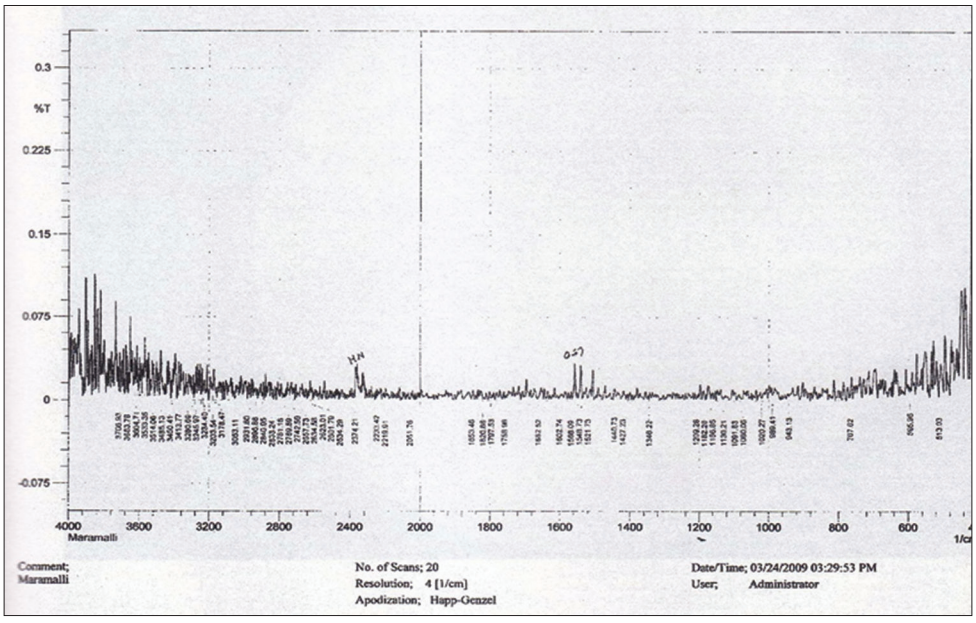

Fig. 2: Fourier-transform infrared spectrum of the purified compound from *Millingtonia hortensis*

The zone of inhibition for antibacterial activity was given in Table 1. Chloramphenicol was used as standard (S) about concentration 20 µg/µl for all the bacterial strains. Two different concentrations were taken, 10 µg/µl (C) and 20 µg/µl (M) to study the antibacterial activity. The results showed that ethanolic extract of the flower of *M. hortensis* has maximum effect against *Streptococcus, Shigella, and E. coli* than other strains. This observation provides strong circumstantial evidence that small peptides play an important role in plants antimicrobial defense system [17].

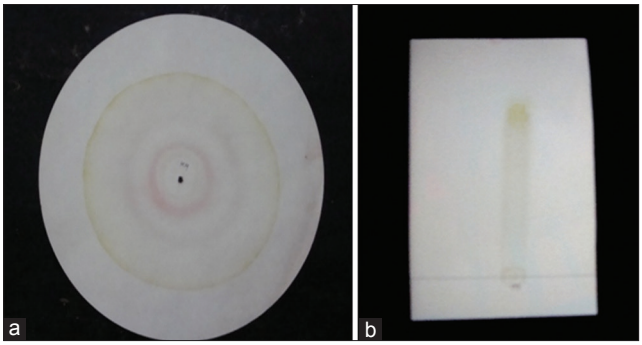

Fig. 3: (a) Circular chromatogram, (b) Thin-layer chromatogram of flower extracts of *Millingtonia hortensis* sprayed with Cu (II) – ninhydrin reagent

AMPs provide a rich resource for the development of novel antibiotic compounds [18,19]. However, peptide drugs are rather larger complex molecules and thus pose challenges in terms of stability and delivery. The ability to capture the essential properties of AMPs in simple easy to prepare molecules that are abiotic in origin and proteolytic offers many advantages [20].

CONCLUSION

From the results obtained in our experiments, we have concluded the following: Presence of Cu (II) – ninhydrin-positive compounds in the flowers medicinal plants. They may small peptides, amino acid amides, or amino acid esters. Based on the findings of UV–visible and FT-IR experiment, it is confirmed that the purified compound is a small peptide and might contain glycine, cysteine, and tyrosine or histidine. The result of antimicrobial studies proves the ability of small peptides to function as AMPs (Table 1). It is also shown that these compounds exert a wide range of activity against bacterial pathogens.

AUTHOR'S CONTRIBUTION

The author's had done all the work regarding sample collection, peptide isolation, purification, characterization and antimicrobial activity.

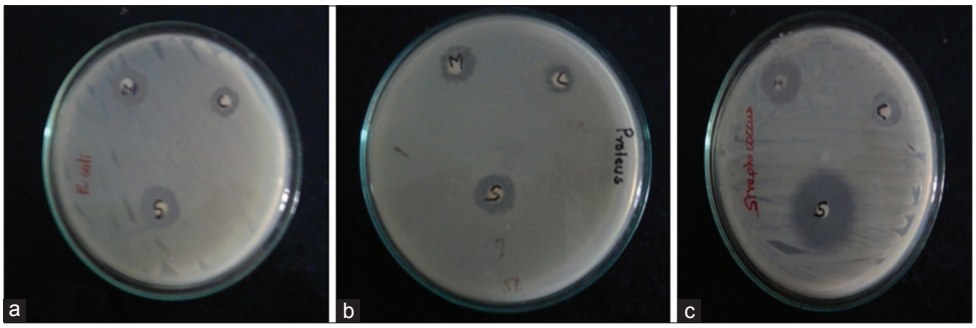

Fig. 4: (a) Antibacterial activity of *Escherichia coli.* (b) Antibacterial activity of Proteus (c) Antibacterial activity of *Streptococcus.* Antimicrobial activity of flower extracts of *Millingtonia hortensis* extract by Disc diffusion method, C-10 µg/µl, M-20 µg/µl, S-20 µg/µl

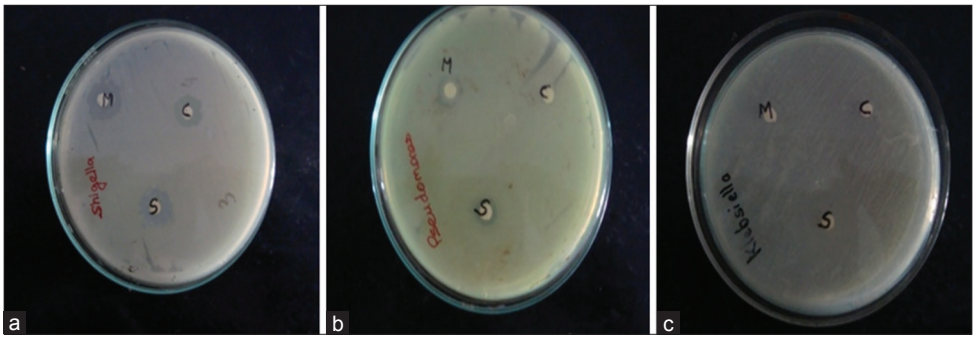

Fig. 5: (a) Antibacterial activity of *Shigella* (b) Antibacterial activity of *Pseudomonas* (c) Antibacterial activity of *Klebsiella.* Antimicrobial activity of flower extracts of *Millingtonia hortensis* extract by Disc diffusion method, C-10 µg/µl, M-20 µg/µl, and S-20 µg/µl

Table 1: Antibacterial activity of *M. hortensis* ethanolic extract

Zone of inhibition mm							
S. No.	**Concentration of sample µg/µl**	***E. coli***	***Proteus***	***Streptococcus***	***Shigella***	***Klebsiella***	***Pseudomonas***
1	10 (C)	7.2	6.1	6.5	7.3	-	-
2	20(M)	8.1	6.4	9.8	9.4	-	4.2
3	20 (S)	12	10	18	14	-	5

M. hortensis: Millingtonia hortensis, E. coli: Escherichia coli

CONFLICTS OF INTEREST

The author has no conflicts of interest.

REFERENCES

1. Kumar MV, Rajagopal VS. Antioxidant, antimicrobial, antiproliferative activities and *in silico* drug likeness prediction of purified MHH compound, isolated from *Millingtonia hortensis* Linn. Int J Pharm Pharm Sci 2015;2015:251-7.
2. Pimenta AM, De Lima ME. Small peptides, big world: Biotechnological potential in neglected bioactive peptides from arthropod venoms. J Pept Sci 2005;11:670-6.
3. Xu Bo. Interfering with DNA damage signals: Radio Sensitizing Prostate Cancer using Small Peptides. New Orleans: Louisiana State Univ; 2006. p. 561-3.
4. Marshall SH, Arenas G. Antimicrobial peptides; natural alternatives to chemical antibiotics and a potential for applied biotechnology. Electron J Biotechnol 2003;6:271-5.
5. Tamayo R, Portillo AC, Gunn JS. Mechanisms of Bacterial Antimicrobial Resistance to Antimicrobial Peptides in Mammalian Host Defense Peptides. Cambridge: Cambridge University Press; 2004. p. 323-48.
6. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389-95.
7. Pillai KS. Medicinal plants and their applications. Nat Sym Med Plants 2001;90:24-6.
8. Sadasivam S, Manickam A. Biochemical Methods. 2nd ed. New Delhi: New Age International (P) Limited Publishers; 2004. p. 80.
9. Van Eldik LJ, Grossman AR, Inversion DB, Watterson DM. Isolation and characterization of calmodulin from spinach leaves and *in vitro* translation mixtures. Proc Natl Acad Sci USA 1980;77:1912-6.
10. Ganapathy V, Ramachandramurthy B, Radhakrishnan AN. Distinctive test with Cu^{2+} - ninhydrin reagent for small alpha peptides separated by paper chromatography. J.Chromatogr 1981;213:307-16.
11. Wilson K, Walker J. Principles and Techniques of Biochemistry and Molecular Biology. 7th ed. Cambridge: Cambridge University Press; 2010. p. 499-510.
12. Wilson K, Walker J. Principles and Techniques of Biochemistry and Molecular Biology. 7th ed. Cambridge: Cambridge University Press; 2010. p. 540-3.
13. Arunkumar K, Chandrashekar KR. Phytochemical evaluation and *in vitro* antimicrobial and antioxidant studies of leaf and stem bark extracts of *Polyalthia fragrans* (dalz.) Bedd.–an endemic species of Western Ghats. Int J Pharm Pharm Sci 2017;2:20-4.
14. Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibily tests: Dilution and disc diffusion methods. Man Clin Microbiol 1999;51:1526-43.
15. Goldfarb AR, Saidel LJ, Mosovich E. The ultraviolet absorption spectra of proteins. J Biol Chem 1951;193:397-404.
16. Porter EA, Wang X, Lee HS, Weisblum B, Gellman SH. Non-haemolytic beta-amino-acid oligomers. Nature 2000;404:565.
17. Tawfeeq AA, Mahdi MF, Abaas IS, Alwan AH. Isolation, quantification, and identification of rosmarinic acid, gas chromatography-mass spectrometry analysis of essential oil, cytotoxic effect, and antimicrobial investigation of *Rosmarinus officinalis* leaves. Asian J Pharm Clin Res 2018;6:126-32.
18. Kohli RM, Walsh CT, Burkart MD. Biomimetic synthesis and optimization of cyclic peptide antibiotics. Nature 2002;418:658-61.
19. Yount NY, Yeaman MR. Multidimensional signatures in antimicrobial peptides. Proc Natl Acad Sci U S A 2004;101:7363-8.
20. Nüsslein K, Arnt L, Rennie J, Owens C, Tew GN. Broad-spectrum antibacterial activity by a novel abiogenic peptide mimic. Microbiology 2006;152:1913-8.