International Food Research Journal 25(4): 1698-1702 (August 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antimicrobial properties of the acetone leaves and stems extracts of *Clinacanthus nutans* from three different samples/areas against pathogenic microorganisms

Kong, H. S. and *Abdullah Sani, N.

Food Science Programme, School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi Selangor, Malaysia

Article history

Received: 23 April 2017
Received in revised form: 12 May 2017
Accepted: 13 May 2017

Keywords

Antifungal
Antimicrobial
Botany
Traditional medicine Asia and Oceania

Abstract

Clinacanthus nutans is the native medicinal herbs that grow in tropical climate, mainly can be found in Malaysia and Thailand. *Clinacanthus nutans* is used to boost immunity and for detoxification. It is claiming to promote bowel movement and other health promotions, such as skin care, diuresis and cancer (such as lung, ovary, uterine, prostate, nasopharyngeal and breast). This study focused on the effects of solvents towards antioxidant and antimicrobial properties of *C. nutans* (Belalai Gajah/ Sabah Snake Grass) leaves and stems. Freeze-dried leaves and stems were homogenized before testing its antimicrobial properties. The 70% acetone (Merck, Germany) was prepared by dissolving fine powder of *C. nutans* in 1:10 solvent ratio with 70% acetone using ultrasonic extraction for 1 h in an ultrasonic bath (Soniclean, Thebarton, Australia). Ten bacteria (*Bacillus cereus, B. subtilis, Escherichia coli, Klebsiella pneumonia, Listeria monocytogenes, Pseudomonas aureginosa, Salmonella typhimurium, Shigella boydii, Staphylococcus aureus* and *S. epidemidis*) and four fungi (*Aspergillus niger, Candida albicans, Rhizopus stolonifer* and *Saccharomyces cerevisiae*) were used in the antimicrobial study employing minimum inhibitory concentration (MIC) and minimal microcidal concentration (MMC) methods. The data obtained were statistically analysed using SPSS Inc. Version 22 (Chicago, USA). The MIC values of acetone extracts for the selected bacteria and fungi ranged between 6.25 – 100.00 mg/ mL while the MMC values of acetone extracts for the selected bacteria and fungi were between 25.00 - >100.00 mg/ mL. Recommendation on phytochemicals investigation should be conducted to determine the potential of compounds toward antioxidant and antimicrobial properties.

Introduction

Clinacanthus nutans is the native medicinal herbs that grow in tropical climate, mainly can be found in Malaysia and Thailand. The *C. nutans* has been utilised for its benefits and functions according to folklore, especially in the Southeast Asia region. *C. nutans* is used to boost immunity, detoxification, health promotion, promote bowel movement, skin care, diuresis, cancer such as lung, ovary, uterine, prostate, nasopharyngeal and breast. It is widely used for home decoration, tea and bath (Siew *et al*., 2014). The washed leaves of *C. nutans* can be freshly eaten or blended with apple and drink as fruit juice. *C. nutans* is also used to treat skin affections, insect and snakebites and swellings due to fall or boils (Chiwapreecha *et al*., 2014).

There are very few studies on the phytochemical, antioxidant, antimicrobial and anti-inflammatory properties of *C. nutans*. The phytochemical compounds that can be found in *C. nutans* plants are flavonoids, betulin, phytosterols such as stigmasterol, lupeol and β-sitosterol; sapopenin and diterpenes, which contribute to antimicrobial and anti-inflammatory properties (Sakdarat *et al*., 2009; Yang *et al*., 2013). The leaves of *C. nutans* contains C-glycosidic flavones such as orientin, vitexin, and isovitexin with concentration of 2.55-17.43, 0.00-0.86, 0.00-2.01 and 0.00-0.91 mmol/ g respectively (Chelyn *et al*., 2014). Petroleum ether extracts of *C. nutans* has the radical scavenging activity of 82.00 ± 0.02%, compared with ascorbic acid and α-tocopherol corresponding values of 88.7 ± 0.0 and 86.6 ± 0.0%, respectively (Arullappan *et al*., 2014). Chloroform leaves extract of *C. nutans* is a good antioxidant agent against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and galvinoxyl radicals, but less effective in negating nitric oxide and hydrogen peroxide radicals (Yang *et al*., 2013). Higher ferric reducing antioxidant power (FRAP) in six month old buds of *C. nutans* than one month old buds is recorded with the values of 488 μM of Fe(II)/ g and 453 μM of Fe(II)/ g respectively

*Corresponding author.
Email: *norrasani@ukm.edu.my*
Fax: 03-89213232

(Fong *et al*., 2014).

Medicinal plants contain chemical compounds (bioactive secondary metabolites such as terpenes, phenolics and alkaloids, from plant secondary metabolism) exhibiting different biological and pharmacological activities such as antimicrobial and antioxidant (Stefanović *et al.*, 2015). Therefore, *C. nutans* was investigated for determining its antimicrobial properties to prove its potential to become new sources for natural antimicrobial agents.

Methods and Materials

Plant material preparation

Fresh and healthy leaves and stems of Sabah snake grass *(C. nutans)* were obtained from Yik Poh Ling (YPL) and You Dun Chao (YDC) Company, Malaysia. The leaves and stems were washed with water to remove sand and dust particles. The leaves were then freeze-dried using ALPHA freeze dryer (Hampshire SO50 4NU, UK), homogenized into 0.5 mm size using Universal cutting mill (FRITSCH, Idar-Oberstein, Germany) before the experiment. All the experiments were conducted in triplicates.

Extraction of plant material

The preparation of plant material and extract was done adopting the method of (Hassan *et al.*, 2015) with minor modifications. The 70% acetone (Merck, Germany) extracts were prepared by dissolving fine powder of *C. nutans* in 1:10 solvent and aqueous ratio with 70% acetone using ultrasonic extraction for 1 hour in an ultrasonic bath (Soniclean, Thebarton, Australia). Then the extracts were filtered with the aid of a Bucker funnel and Whatman filter paper #1. The extracts were preserved in airtight bottles at -40°C for further use.

Microorganisms

The microorganisms used in this study consisted of ten bacteria (five Gram positive and five Gram negative bacteria) and four fungi (two yeasts and two moulds). The Gram positive bacteria were *Bacillus cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *Listeria monocytogenes* (ATCC 51774), *Staphylococcus aureus* (ATCC 25923) and *S. epidemidis* (ATCC 12228). The Gram negative bacteria were *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Pseudomonas aureginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028) and *Shigella boydii* (ATCC 9207). The yeasts were *Candida albicans* (ATCC 90028) and *Saccharomyces cerevisiae* (ATCC 4098), while the mould were *Aspergillus niger* (ATCC 16404) and *Rhizopus stolonifer* (ATCC 6227). The control microorganisms which were stored in a -40°C refrigerator were thawed at room temperature and after cultivation on Tryptone Soya Agar (CM0131, Oxoid, England) and Sabouraud´s Dextrose Agar (CM0041, Oxoid, England) incubated for 24 hours at 37°C (for bacteria) or 25°C (for fungi) except for A. niger that was incubated for 72 hours. A single colony was picked with a sterile loop and transferred into a nutrient broth (CM0001, Oxoid, England)). The broth was then incubated with shaking for 24 hours except for A. niger that was incubated for 72 hours. The density of the microbial suspensions were adjusted to equal to the 0.5 McFarland standard (0.5 mL of 0.048 M $BaCl_2$ (1.17% m/v $BaCl2.2H_2O$) was added to 99.5 mL of 0.18M H_2SO_4 (1% v/v)) by adding sterile nutrient broth. To aid comparison, test and standard were compared against a white background with a contrasting black line. Freshly prepared microbial suspensions were containing 10^8 CFU/mL of bacteria, 10^6 CFU/mL of yeast and mould.

Stock solutions preparation

The sample stock solution was used to prepare eight different concentrations, i.e. 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/ mL by serial two-fold dilutions.

Determination of minimal inhibitory concentration (MIC)

Broth Macro-dilution

The minimal inhibitory concentration (MIC) were determined by a broth macro-dilution method (Petrus *et al.*, 2011) with a minor modification using nutrient broth. Sterile centrifuge tubes were arranged in sets of rows for each bacterial inoculum to cover the different concentration of *C. nutans* extracts in triplicate. Each concentration of *C. nutans* extracts were transferred to the tubes accordingly, containing of the selected bacteria and fungi. The negative control acetone had no influence on the growth. The contents in the tubes were mixed throughly and incubated at 37°C (for bacteria) or 25°C (for fungi) for 24 h except for A. niger that was incubated for 72 hours. The evaluation for the inhibition of microbial growth was based on the color change of 2% para iodonitrotetrazolium chloride (INT) to pink, while absence of growth was indicated by absence of color change. The MIC endpoint is the lowest concentration of *C. nutans* extracts at which there is no visible growth in the tubes.

Table 1. MIC and MMC values of acetone extracts of *C. nutans* leaves

Test microorganism	YPL non-shaded area (mg/mL)		YDC non-shaded area (mg/mL)		YDC shaded area (mg/mL)	
	MIC	MMC	MIC	MMC	MIC	MMC
Gram positive bacteria						
B. cereus	12.50	>100.00	12.50	>100.00	12.50	>100.00
B. subtilis	12.50	25.00	12.50	25.00	12.50	25.00
L. monocytogenes	12.50	50.00	12.50	50.00	12.50	50.00
S. aureus	6.25	25.00	12.50	25.00	12.50	25.00
S. epidemidis	12.50	25.00	12.50	25.00	12.50	25.00
Gram negative bacteria						
E. coli	12.50	25.00	12.50	25.00	12.50	25.00
K. pneumonia	12.50	25.00	12.50	25.00	12.50	25.00
P. aureginosa	12.50	25.00	12.50	25.00	12.50	25.00
S. typhimurium	12.50	25.00	12.50	25.00	12.50	25.00
S. boydii	12.50	25.00	12.50	25.00	12.50	25.00
Fungi						
A. niger	12.50	100.00	12.50	100.00	12.50	100.00
C. albicans	6.25	25.00	6.25	25.00	6.25	25.00
R. stolonifer	12.50	>100.00	6.25	>100.00	6.25	>100.00
S. cerevisiae	6.25	50.00	12.50	50.00	12.50	50.00

Determination of minimal microcidal concentration (MMC)

The method used and described below is an amended version of the procedure described in the BSAC Guide to Sensitivity Testing and was adopted for determining the minimal microcidal concentration (MMC) of *C. nutans* extracts. After MIC determination of *C. nutans* extracts, an aliquot of 10 µL from all tubes in which no visible growth was observed were seeded in Tryptone Soya Agar or Sabouraud´s Dextrose Agar. The plates were then incubated for overnight at 37°C (for bacteria) or 25°C (for fungi) except for A. niger that was incubated for 72 hours. The MMC endpoint is defined as the lowest concentration of antimicrobial agent that kills >99.9% of the initial bacterial population where no visible growth of the bacteria was observed on the nutrient agar plates.

Results and Discussion

Determination of antimicrobial activity of acetone extracts of C. nutans *on MIC and MMC evaluation*

A test material's potency in terms of the concentration at which it will inhibit growth of or completely kill 1 x 10^6 (one million) challenge microorganisms during a 18 to 20 hours period of incubated exposure is called MIC or MMC (antibiotic's mechanism is based on inhibiting cell wall formation) respectively. The MIC values of acetone extracts for the selected bacteria and fungi ranged between 6.25 – 100.00 mg/ mL while the MMC values of acetone extracts for the selected bacteria and fungi were between 25.00 - >100.00 mg/ mL.

The lowest MIC in all acetone leaves extracts (6.25 mg/ mL) were found against *C. albicans.* The MIC values for *B.cereus* (food contaminant) were 12.50 mg/ mL for all leaves and stems extracts. However, the MMC value was higher (>100.00 mg/ mL). It means the acetone leaves and stems extracts can inhibit the growth of *B. cereus* but it cannot kill the *B. cereus.* Low MIC value (6.25 mg/ mL) of acetone leaves extract of YPL non-shaded area was shown against *S. aureus* which are food-borne pathogens causing nosocomial and community acquired infections (Akindolire *et al*., 2015) and against *S. cerevisiae* which commonly used for brewing and baking. Low MIC value (6.25 mg/ mL) of acetone leaves extract of YDC non-shaded and shaded areas were shown against *R. stolonifer* where commonly found on bread surface. The MIC value with 12.50 mg/ mL of acetone leaves and stems extracts in all samples were shown against *B. subtilis* (causes food spoilage), *L. monocytogenes*, *S. epidemidis* (causes endocarditis, most often in patients with defective heart valves), *E. coli* (produce Shiga-like toxins cause diarrheal disease in humans) (Paton *et al.*, 1996), *K. pneumonia* which causes nosocomial infections, community-acquired pneumonia, liver abscess, and urinary tract infections (Melot *et al*., 2015), *P. aureginosa* (nosocomial infections), *S. typhimurium* (the most popular causes of bacterial food-borne disease in humans and deaths globally) (Paião *et al.*, 2013; Srikanth *et al.*, 2011) and *S. boydii* (causes dysentery in humans through faecal-oral contamination).

The lowest value of MMC (25.00 mg/ mL) of acetone leaves and stems extracts in all samples areas were found against B. *subtilis, S. aureus, S.*

Table 2. MIC and MMC values of acetone extracts of *C. nutans* stems

Test microorganism	YPL non-shaded area (mg/mL)		YDC non-shaded area (mg/mL)		YDC shaded area (mg/mL)	
	MIC	MMC	MIC	MMC	MIC	MMC
Gram positive bacteria						
B. cereus	12.50	>100.00	12.50	>100.00	12.50	>100.00
B. subtilis	12.50	25.00	12.50	25.00	12.50	25.00
L. monocytogenes	12.50	50.00	12.50	50.00	12.50	50.00
S. aureus	12.50	25.00	12.50	25.00	12.50	25.00
S. epidemidis	12.50	25.00	12.50	25.00	12.50	25.00
Gram negative bacteria						
E. coli	12.50	25.00	12.50	25.00	12.50	25.00
K. pneumonia	12.50	25.00	12.50	25.00	12.50	25.00
P. aureginosa	12.50	25.00	12.50	25.00	12.50	25.00
S. typhimurium	12.50	25.00	12.50	25.00	12.50	25.00
S. boydii	12.50	25.00	12.50	25.00	12.50	25.00
Fungi						
A. niger	100.00	>100.00	100.00	>100.00	100.00	>100.00
C. albicans	12.50	50.00	12.50	50.00	12.50	50.00
R. stolonifer	12.50	>100.00	12.50	>100.00	12.50	>100.00
S. cerevisiae	12.50	50.00	12.50	50.00	12.50	50.00

epidemidis, E. coli, K. pneumonia, P. aureginosa, S. typhimurium and *S. boydii*. The result of acetone leaves extract of the current study was near to the previous study which shown that *C. nutans* methanolic leaves extract exhibited antibacterial effect against *S. aureus* (MIC > 12.5 mg/ mL), *S. epidermidis* (MIC > 12.5 mg/ mL), *B. cereus* (MIC > 12.5 mg/ mL) and *E. coli* (MIC 12.5 mg/ mL) (Yang *et al.* 2013). Higher amount of stem extract (MIC = 100.00 mg/ mL; MMC > 100.00 mg/ mL) was needed to against *A. niger* (produce organic acids especially citric acid) (Luna *et al*., 2015) compared to leaves extract (MIC = 12.50 mg/ mL; MMC = 100.00 mg/ mL).

Conclusions

Current study shown the antimicrobial properties of acetone extract of *C. nutans* leaves and stems were revealed toward pathogenic microorganism such as bacteria and fungi. Recommendation on the phytochemicals investigation should be conducted to determine the potential of compounds toward antimicrobial properties.

Acknowledgements

The authors would like to thank Universiti Kebangsaan Malaysia for providing the postgraduate scholarship (Zamalah) and the following project funds: FRGS/1/2014/STWN03/UKM/02/1, GSP-2013-019, STGL-007-2008 and DIP-2014-007.

References

Akindolire, M. A., Kumar, A. and Ateba, C. N. 2015. Genetic characterization of antibiotic-resistant Staphylococcus aureus from milk in the North-West Province, South Africa. Saudi Journal of Biological Sciences. *http://dx.doi.org/10.1016/j.sjbs.2015.10.011*

Arullappan, S., Rajamanickam, P., Thevar, N. and Kodimani, C.C. 2014. In vitro screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (acanthaceae) leaf extracts. Tropical Journal of Pharmaceutical Research 13(9): 1455-1461.

Chelyn, J. L., Omar, M.H. Mohd Yousof, N.S.A. , Ranggasamy, R. , Wasiman, M.I. and Ismail, Z. 2014. Analysis of flavone c -glycosides in the leaves of *Clinacanthus nutans* (Burm. F.) Lindau by hptlc and hplc-uv/dad. Scientific World Journal 2014: Article number 724267.

Chiwapreecha, B., Janprasert, K. and Kongpakdee, C. 2014. Comparative anatomy of three medicinal plants in acanthaceae. Acta Horticulturae 1023: 229-232.

Fong, S.Y., Piva, T., Urban, S. and Huynh, T. 2014. Genetic homogeneity of vegetatively propagated *Clinacnathus nutans* (Acanthaceae). Journal of Medicinal Plant Research 8(25): 903-914.

Hassan, K. Z., Noor, H. M. and Kader, J. 2015. Antibacterial efficacy of three different extracts of Polygonum minus (Huds.). International Conference on Waste Management, Ecology and Biological Sciencess. Kuala Lumpur.

Luna, M. A. C., Vieira, E. R., Okada, K., Campos-Takaki, G. M. and Nascimento, A. E. D. 2015. Copper-induced adaptation, oxidative stress and its tolerance in *Aspergillus niger* UCP1261. Electronic Journal of Biotechnology 18(6): 418–427.

Melot, B., Colot, J. and Guerrier, G. 2015. Bacteremic

community-acquired infections due to *Klebsiella pneumoniae:* clinical and microbiological presentation in New Caledonia, 2008–2013. International Journal of Infectious Diseases 41: 29-31.

Paião, F. G., Arisitides, L. G. A., Murate, L. S., Vilas-Bôas, G. T., Vilas-Boas, L. A. and Shimokomaki, M. 2013. Detection of *Salmonella* spp, *Salmonella enteritidis* and *typhimurium* in naturally infected broiler chickens by a multiplex pcr-based assay. Brazilian Journal of Microbiology 44(1): 37-41.

Paton, A. W., Ratcliff, R. M., Doyle, R. M., Seymour-Murray, J., Davos, D., Lanser, J. A. and Paton, J. C. 1996. Molecular microbiological investigation of an outbreak of hemolytic–uremic syndrome caused by dry fermented sausage contaminated with shiga-like toxin-producing Escherichia coli. Journal of Clinical Microbiology 34: 1622-1627.

Petrus, E. M., Tinakumari, S., Chai, L. C., Ubong, A., Tunung, R., Elexson, N., Chai, L. F. and Son, R. 2011. A study on the minimum inhibitory concentration and minimum bactericidal concentration of nano colloidal silver on food-borne pathogen. International Food Research Journal 18: 55-66.

Sakdarat, S., Shuyprom, A., Pientong, C., Ekalaksananan, T. and Thongchai, S. 2009. Bioactive constituents from the leaves of *Clinacanthus nutans* Lindau. Bioorganic and Medicinal Chemistry 17: 1857 - 1860.

Siew, Y. Y., Zareisedehizadeh, S., Seetoh, W. G., Neo, S. Y., Tan, C. H. and Koh, H. L. 2014. Ethnobotanical survey of usage of fresh medicinal plants in Singapore. Journal of Ethnopharmacology 155(3): 1450 - 1466.

Srikanth, C. V., Mercado-Lubo, R., Hallstrom, K. and Mccormick, B. A. 2011. *Salmonella* effector proteins and host-cell responses. Cellular and Molecular Life Sciences 68(22): 3687–3697.

Stefanović, O. D., Tešić, J. D. and Čomić, L. R. 2015. Melilotus albus and Dorycnium herbaceum extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. Journal of Food and Drug Analysis 23(3): 417-424.

Yang, H. S., Peng, T. W., Madhavan, P., Shukkoor, M. S. A. and Akowuah, G. A. 2013. Phytochemical analysis and antibacterial activity of methanolic extract of *Clinacanthus nutans* leaf. International Journal of Drug Development and Research 5(3): 349-355.