RESEARCH NOTE

The antimicrobial activity of plants in the vicinity of a geothermal area in Perak, Malaysia [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

We wish to report the study of the antimicrobial activity of plants collected in the vicinity of a geothermal area in Perak, Malaysia. This is the first report of these species in the vicinity of the geothermal area. The plants are *Cleome icosandra*, locally known as Maman Pasir of the family Cleomaceae, and *Stachytarpheta* species of the family Lamiaceae. Both are subshrubs and are believed to have specific biological activities as a result of living in such extreme areas. Methanol extracts of both plants revealed no antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Keywords

geothermal, Cleomaceae, Lamiaceae, antimicrobial

Open Peer Review

Approval Status ? ✓

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2. Anang W M Diah , Tadulako University, Palu, Indonesia		

Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: No competing interests were disclosed.

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Introduction

Extreme environments provide a new scaffold for the study of natural products for drug discovery. These environments are a challenge to live in, therefore the plant species that are found there must have specific survival mechanisms. This increases the possibility of discovering unique biologically active compounds. The polar region is an example of an extreme environment that has been explored. In McMurdo Sound, Antarctica, benthic invertebrates are exposed to significant predation by sea stars and potentially infectious water-column microorganisms, which suggests that chemically-mediated defense strategies would be an advantage. For example, discorhabdin C (Figure 1), found in the sponge *Lantrunculia apicalis*, is alkaloid and possesses a unique structure with anti-tumor and antimicrobial activity, and plays a role in the sea star tube-foot retraction assay¹⁻³.

Malaysia, although the climate is tropical, is rich in various geography, including geothermal areas in the form of hot springs. The biodiversity of geothermal areas in Malaysia has yet to be fully studied, therefore, an opportunity to investigate the bioactivities of plants that grow near geothermal areas, has risen. Plants that survive here are unique because of their ability to adapt to extreme temperature. The most heat-tolerant plants are mosses and lichens, which can survive ground temperatures of 70°C. Prostrate kanuka

Figure 1. Discorhabdin C. Alkaloid discorhabdin C found in the sponge *Lantrunculia apicalis*.

(*Kunzea ericoides* var. *microflorum*) is a low, spreading variety of the kanuka shrub that only grows in geothermal areas. It can tolerate ground temperatures of up to 55°C⁴. Additionally, grasses such as *Dichanthelium lanuginosum* and *Paspalum laeve* Michx were also found in geothermally heated environments, namely in the Yellowstone National Park⁵ and Hot Springs National Park, Arkansas⁶, respectively. Locally, *Hyptis suaveolens*, located within the hot spring enclosure in the Tambun area of Perak is used to combat fever, and soothe headaches and skin rashes⁷.

Among the data presented in the 2nd edition of the National Antibiotic Guideline in conjunction with the Annual Scientific Meeting on Antimicrobial Resistance in 2015 in Putrajaya, Malaysia, was resistance of *Escherichia coli* to Ampicillin, which had risen to more than 50 percent. *Streptococcus pneumoniae* resistance towards Erythromycin had increased from 18.2 percent in 2013 to 28.1 percent in 2014, and *Acinetobacter baumannii* resistance against Meropenem had increased from 47.7 percent to 57.3 percent within seven years^{8,9}. Additionally, *Paeruginosa* is generally resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment¹⁰. Hence, research for the discovery of new antibiotics and drugs against these bacteria is very much needed.

Methods

Plant material

Plants were collected by hand on the 26th of April 2016 in the vicinity of Ulu Slim, Perak, Malaysia (Figure 2). It should be noted that these plants do not have flowers or any significant morphology that allows their identification, so identifying them was a challenge.

Extraction

C.icosandra and *Stachytarpheta* sp. whole plant samples were washed with distilled water, dried then extracted with methanol for five days at room temperature. 0.51g of *C.icosandra* and 0.45g of *Stachytarpheta* sp. were used for the extraction, from which the

Figure 2. Geothermal area in Ulu Slim, Perak, Malaysia, where the plants were collected.

crude extract was obtained. A total of 56.8 mg crude extract of *C.icosandra* and 65.6 mg crude extract of *Stachytarpheta sp.* was obtained, after solvent removal using a rotary evaporator.

Antimicrobial assay

The antimicrobial assay consisted of the applied agar diffusion method, or Kirby-Bauer method^{11,12}. The assay consists of inoculating an agar plate with the chosen microorganisms, followed by placement of paper discs which have been impregnated with a known concentration of antibiotic. After incubation, the inhibition zone is measured.

In our assay, three bacterial isolates of *E.coli*, *S.aureus* and *Paeruginosa* were grown in nutrient broth at 37°C overnight, using a shaking incubator. The three bacterial isolates were subcultured from an existing culture, so we are unable to state the strain of bacterial isolates. Plates were swabbed with cotton wool impregnated with the microorganisms. Individual filter paper discs (Whatman, Cat No 1001 110; 6 mm diameter) were saturated with 10 µL solution containing approximately 1 mg/mL of crude extract and dried for 15 minutes in the laminar flow before they were placed on the top of cultured nutrient agar. The plates were then sealed with parafilm and inverted before being incubated at 37°C overnight.

After incubation, the inhibition halo was measured with a ruler ($\pm 0.5\text{mm}$); the measured distance was from the edge of the paper disc to the widest part of the inhibition halo. The average distance across the inhibition halos from the filter paper was taken as the level of inhibition of bacterial growth for each sample.

Results and discussion

Both plants were collected in the vicinity of the geothermal site at Ulu Slim, Perak, Malaysia. Water temperature was 90°C at the time of collection. Upon collection, plants were immediately identified by a botanist and then underwent methanol extraction. Both plants were identified to be *C.icosandra* (also called *C.viscosa*), locally known as Maman Pasir of the family Cleomaceae. In addition, *Stachytarpheta* species of the family Lamiaceae were also collected. Further species identification needs to be conducted for this particular plant; they are identified based on plant morphology and taxonomy. It should be noted that both plants are very small, subshrub-like and only consist of leaves that are absent of flowers. We believe that these plants may possess unique, biologically active compounds. We proceeded to evaluate their antimicrobial activity, but crude methanol extracts of both plants revealed no activity against the three tested bacteria *E.coli*, *S.aureus* and *Paeruginosa* (Table 1, Figure 3)

Table 1. Diameters of the zones of inhibition of methanol crude extracts of *C.icosandra* and *Stachytarpheta sp.* tested against *E.coli*, *S. aureus* and *P. aeruginosa*. (-) = no inhibition.

Sample	Concentration of crude extract	Diameter of the zone of inhibition (mm)		
		<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>
<i>C.icosandra</i>	1 mg/ml	-	-	-
<i>Stachytarpheta sp.</i>	1 mg/ml	-	-	-

Figure 3. Antimicrobial assay of crude methanol extracts of *C.icosandra* and *Stachytarpheta sp.* The figure displays petri dishes containing filter paper discs with saturated crude methanol extracts of *C.icosandra* (C) and *Stachytarpheta sp.* (D), in (i) *E.coli*, (ii) *S. aureus* and (iii) *P. aeruginosa*. No inhibition halo was observed.

Conclusions

This is the first record taken of plants in the vicinity of the geothermal area in Ulu Slim, Perak, Malaysia. Plants near these unexploited environments of extreme condition should be further studied for their potential in drug discovery.

Data availability

All data required to re-analyse the study has been provided in the main body of text.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

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References

1. Amsler CD, McClintock JB, Baker BJ: **Secondary metabolites as mediators of trophic interactions among Antarctic marine organisms.** *American Zoologist*. 2001; **41**(1): 17–26.
[Publisher Full Text](#)
2. Wada Y, Fujioka H, Kita Y: **Synthesis of the marine pyrrolotriquinone alkaloids, discorhabdins.** *Mar Drugs*. 2010; **8**(4): 1394–1416.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Perry NB, Blunt JW, McCombs JD, *et al.*: **Discorhabdin C, a highly cytotoxic pigment from a sponge of the genus *Latrunculia*.** *J Org Chem*. 1986; **51**(26): 5476–5478.
[Publisher Full Text](#)
4. <http://www.teara.govt.nz/en/hot-springs-mud-pools-and-geysers>.
5. Stout RG, Al-Niemi TS: **Heat-tolerant flowering plants of active geothermal areas in Yellowstone National Park.** *Ann Bot*. 2002; **90**(2): 259–267.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Scully FJ: **Grasses of Hot Springs National Park and Vicinity.** *Rhodora Journal*. 1992; **44**.
7. Wiart C: **Medicinal plants of the Asia-Pacific: drugs for the future?** *World Scientific*. 2006; **527**.
[Reference Source](#)
8. Bernama: **Malaysian hospitals at forefront in war against antimicrobial resistance: Subramaniam.** *The Sun Daily*. 2015.
[Reference Source](#)
9. Ministry of Health, Malaysia: **Garis panduan antibiotik kebangsaan – edisi kedua bersempena Annual Scientific Meeting on Antimicrobial Resistance 2015.** Rintangan antibiotik (antibiotic resistance) ancaman kepada kesihatan global. [Press release]. 2015.
[Reference Source](#)
10. Lister PD, Wolter DJ, Hanson ND: **Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms.** *Clin Microbiol Rev*. 2009; **22**(4): 582–610.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Bauer AW, Kirby WM, Sherris JC, *et al.*: **Antibiotic susceptibility testing by a standardized single disk method.** *Am J Clin Pathol*. 1966; **45**(4): 493–496.
[PubMed Abstract](#)
12. Sati SC, Khulbe K, Joshi S: **Antibacterial Evaluation of the Himalayan medicinal plant *Valeriana wallichii* DC. (Valerianaceae).** *Res J Microbiol*. 2011; **6**(3): 289–296.
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Anang W M Diah

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This paper report is very interesting as it discusses on the antimicrobial activity of the plants from a specific area in Perak, Malaysia. Although the authors tried a simple experiment as the first study to these plants extracts, further investigations need to be done to evaluate this. The extraction method used is appropriate but the authors should explain why choosing only the methanol solvent. Other researchers have different solvents to extract these families' plants such as ethanol[ref1]⁻². Different solvents should have different level of metabolite compounds. In addition, the discussion in the paper is too short to explain the results. The authors should explain Figure 3 with the notation of B and N(-) in those dishes. What are these? If B and N(-) as the controls, these should be mentioned in the experiment part and explained in discussion. These can be used to compare the inhibition zone between the methanol extracts and why they perform similar result.

References

1. Sudhakar M, Rao ChV, Rao PM, Raju DB, et al.: Antimicrobial activity of *Caesalpinia pulcherrima*, *Euphorbia hirta* and *Asystasia gangeticum*. *Fitoterapia*. 2006; **77** (5): 378-80 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Kozłowska M, Laudy AE, Przybył J, Ziarno M, et al.: CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS FROM LAMIACEAE FAMILY. *Acta Pol Pharm*. **72** (4): 757-67 [PubMed Abstract](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 10 August 2017

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Mohamed A. Ghandourah

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The manuscript by Bakri *et al.* describes the evaluation of antimicrobial activity of two plant extracts collected from geothermal area in Malaysia. However, several points should be corrected/clarified before acceptance.

- 1- The criteria for collection of those two plants; the existence in an extreme area is not a guarantee for antimicrobial activity.
- 2- The introduction section should survey previous work on the plants under investigation.
- 3- The name of the botanist with complete affiliation in addition to herbarium number should be mentioned.
- 4- The exp. section is not complete.
- 5- Please report the diameter of inhibition zones of each test.
- 6- No need to mention the structure of cpd isolated from marine organism.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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