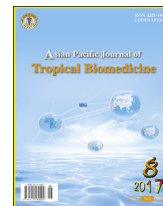


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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2017.07.003>Comparison study of anti-microbial activity between crude extract of *Kappaphycus alvarezii* and *Andrographis paniculata*

Xin Qi Chuah, Wenlynn Mun, Swee Sen Teo*

Department of Biotechnology, UCSI University, Kuala Lumpur, Malaysia

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ABSTRACT

Objective: To evaluate the antimicrobial properties of *Kappaphycus alvarezii* (*K. alvarezii*) and *Andrographis paniculata* (*A. paniculata*) and to compare the microbial inhibition activities between these two crude extracts.

Methods: Both *K. alvarezii* and *A. paniculata* were extracted with methanol before the commencement of antimicrobial properties studies. There were a total of eight species of bacteria, including Gram-positive bacteria *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enterica*. The antimicrobial activity was tested by disk diffusion method.

Results: Crude extract of *K. alvarezii* was found not effective against both Gram-positive and Gram-negative bacteria. In contrast, *A. paniculata* showed higher inhibition towards the growth of Gram-positive bacteria compared to Gram-negative bacteria. Results revealed that *Bacillus subtilis* was susceptible at lower concentration of *A. paniculata* crude extract however *Staphylococcus epidermidis* was the most susceptible towards *A. paniculata* at higher concentration. Although the inhibition zones produced by the crude extract were smaller than that of the positive control, streptomycin disc, *A. paniculata* crude extract still can be considered as potential antimicrobial agents either because it is a natural product or the active compound which is yet identified from its crude extract.

Conclusions: Crude extract of *K. alvarezii* has zero inhibition in bacteria growth whereas *A. paniculata* exerted higher inhibition towards Gram-positive bacteria. The bioactive compounds contained by *A. paniculata* can be evaluated in order to yield a better vision towards the mode of action.

1. Introduction

Infectious diseases are one of the major causes of morbidity and mortality worldwide. The highly dependent usage of broad-spectrum antibiotics and immunosuppressive agents has been highly related to the occurrence of multi-drug resistant microbial strains [1]. The antimicrobial agents which were established in the market often accompanied with side effects such as gastrointestinal upset, drug sensitivity and disturbances of guts microflora. These synthetic drugs are not only expensive and inadequate for disease treatment but build up the resistant type of pathogenic microorganisms [2]. Thus, attention has been

paid to natural products which contain bioactive compounds that compatible with synthetic drugs without exerting toxic effects on users.

Kappaphycus alvarezii (*K. alvarezii*) is a type of red algae which is abundant in tropical Asia such as Malaysia and Philippines. This marine algae is edible and its cultivation cycle is every 45 days. The fast growing properties and its relatively large amounts of antioxidants, phytochemicals, polyphenols, vitamins and minerals provide the impetus to study the medical uses such as antimicrobial [3]. Besides, *Andrographis paniculata* (*A. paniculata*) that originates from the family of Acanthaceae is a bitter herb, namely as ‘Hempedu Bumi’ in Malaysia [4]. Functional properties of *A. paniculata* can be illustrated as treating liver disorder, bowel complaints of children, common cold, colic pain and upper respiratory tract infection [5]. From over 28 species of small annual herbs classified under the genus of *Andrographis*, *A. paniculata* is the most popular and proven as medicinal herb for centuries over the world [6].

*Corresponding author: Swee Sen Teo, UCSI University, No.1, Jalan Menara Gading, Taman Connaught, Cheras 56000, Kuala Lumpur, Malaysia.

E-mail: teoss@ucsiuniversity.edu.my (S.S. Teo).

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Based on studies, the constituents of bioactive compounds originate from various parts of *A. paniculata*, mainly in leaves. The derivatives of this plant exhibit pharmacological activities such as antibacterial, antiviral and immunostimulatory [7].

Antimicrobial drugs have caused a dramatic change not only in the treatment of infectious disease but also fate to mankind. Effective results may not be achieved and may lead to a wrong prognosis if an unsuitable antimicrobial agent is preferred over the treatment of infection with drug-resistant microorganisms. Hence, both of these natural products fulfil the interest in discovering potential antimicrobial treatment by comparing the susceptibility of bacteria towards various concentration of crude extracts.

2. Methods and methods

2.1. Sample preparation

K. alvarezii was purchased from the cultivation site in Sabah, Malaysia. They were drained and soaked in water to remove excessive salts and contaminants. Next, they were dried in 60 °C oven and weights were measured daily until they achieved constant. Besides, fresh *A. paniculata* was self-cultivated and sampled for extraction. Both leaves and branches of *A. paniculata* were cleaned and dried at 40 °C until constant weight is achieved.

2.2. Methanolic extraction of *K. alvarezii* and *A. paniculata* crude extracts

Approximately 10 g of each *K. alvarezii* and *A. paniculata* were grounded into powder form by using mortar and pestle with the aid of liquid nitrogen. To perform the extraction of active components from the samples, both of the species were macerated with 100 mL of 70% methanol in a conical flask to obtain a ratio of 1:10. It was then continued with the incubation of 2 h on the orbital shaker at 180 rpm. After 2 h, the extracts were decanted and filtered into a round evaporation flask. The filtrates of both *K. alvarezii* and *A. paniculata* were concentrated through evaporation by using vacuum rotary evaporator at 45–50 °C. The crude extracts were kept in –20 °C for further usage.

2.3. Preparation of sample extract concentrations

A total of 20 mg of *K. alvarezii* extract was dissolved in 0.2 mL 5% (v/v) dimethyl sulfoxide (DMSO) to achieve a final concentration of 100 mg/mL. The crude extract was then diluted with respective volume of 5% DMSO to reach the final concentration of 25, 50, 75 and 100 mg/mL in final volume of

0.01 mL. The steps were repeated and applied on *A. paniculata* crude extract.

2.4. Antimicrobial susceptibility test

A total of eight bacteria strains were obtained from Microbiology Laboratory of UCSI University Kuala Lumpur. Bacteria strains included *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Salmonella enterica*. All of the bacteria were transferred to nutrient broth for activation before the day of susceptibility test. The bacteria strains were spread evenly on Mueller–Hinton agar (MHA) by using sterile cotton swab. Next, sterile paper discs with approximately 6 mm in diameter were placed on the MHA and impregnated with 10 µL of desired concentration of *K. alvarezii* and *A. paniculata* crude extracts. Streptomycin susceptibility disc (10 µg) was used as positive control and 5% DMSO solution was served as negative control. All of the tests were conducted in triplicates and they were all incubated at 37 °C overnight. Inhibition zones (mm) were measured using Vernier caliper.

3. Results

3.1. Methanolic extraction yield

Successful prognosis of bioactive compounds from natural products are largely based on the type of solvent used in extraction [8]. It has been showed by researchers that those samples extracted from organic solvent showed distinct antibacterial activity [1]. Methanol has a polarity index of 5.1 and it is mostly used for extracting various polar compounds such as phenolic components [9]. The extraction yields of *K. alvarezii* and *A. paniculata* were 15.42% and 12.60%, respectively. Although the yield of *K. alvarezii* was slightly higher than *A. paniculata*, this did not affect the downstream experiments.

3.2. Antimicrobial susceptibility test

Crude extract of *K. alvarezii* did not pose any inhibitory effect on all the eight types of microorganisms. Gram-positive bacteria encountered an inhibition after the treatment of *A. paniculata* crude extract as low as 50 mg/mL. However, Gram-negative bacteria appeared to be resistant towards the treatment of *A. paniculata* (Table 1).

Table 1

Inhibition zone diameter (mm) of *A. paniculata* extract using 5% DMSO (v/v) against different test microorganisms.

Test microorganisms	Inhibition zone diameter (mm) in different concentrations (mg/mL)								
	50	75	100	200	400	600	800	1000	+ve
<i>B. cereus</i>	2.40 ± 2.77	2.13 ± 2.47	4.73 ± 1.98	7.80 ± 5.65	10.97 ± 1.11	13.47 ± 1.72	14.27 ± 1.80	15.73 ± 1.22	21.53 ± 0.69
<i>B. subtilis</i>	4.47 ± 1.84	4.57 ± 1.89	5.10 ± 2.17	8.77 ± 0.34	10.27 ± 0.66	11.40 ± 0.75	12.27 ± 0.48	13.50 ± 1.31	17.80 ± 0.50
<i>E. coli</i>	–	–	–	–	–	–	–	–	12.80 ± 0.41
<i>K. pneumoniae</i>	–	–	–	–	–	–	–	–	14.47 ± 0.45
<i>P. aeruginosa</i>	–	–	–	–	–	–	–	–	10.87 ± 0.31
<i>S. enterica</i>	–	–	–	–	–	–	–	–	13.30 ± 0.71
<i>S. aureus</i>	–	–	–	2.77 ± 3.91	2.80 ± 3.96	2.87 ± 4.05	9.83 ± 0.82	11.12 ± 0.95	14.37 ± 0.37
<i>S. epidermidis</i>	–	–	–	12.60 ± 0.93	13.53 ± 0.75	15.27 ± 0.33	16.83 ± 0.42	19.57 ± 1.65	20.67 ± 0.38

Data are expressed as mean ± standard deviation; n = 3; – = no inhibition zone.

4. Discussion

According to literature, different types of algae family contained variant of properties; for example: brown seaweed tends to possess higher activity against antimicrobial than red and green seaweed extracts [9,10]. The efficacy of algae species towards the inhibition of bacteria growth may depend on the algae species, extraction method and concentration of the extract. For instances, *E. coli* and *P. aeruginosa* were resistant to both *K. alvarezii* and *A. paniculata* crude extracts.

These may be due to both *E. coli* and *P. aeruginosa* are only affected by crude extracts extracted by using ethanol as mentioned by Silva et al. [11], which suggested that different natural components could have favour in various polarity of organic solvent during extraction. Moreover, the bioactive compounds which act actively in antimicrobial activity are strictly influenced by natural factors including temperature, light intensity and salinity [12]. This piece of information can be used to explain the factor that leads to the distinct results [13] compared to the results obtained whereby Rhodophyta that showed the highest antimicrobial activity were obtained from India.

Apart from that, *A. paniculata* crude extracts did perform minor inhibition effect towards Gram positive bacteria such as *B. cereus*, *B. subtilis*, *S. aureus* and *S. epidermidis*. At the concentration of 50 mg/mL until 1 000 mg/mL, the inhibition zone increased gradually towards both *B. cereus* and *B. subtilis*. Besides, *S. aureus* and *S. epidermidis* were only susceptible when they were treated with 200 mg/mL crude extract of *A. paniculata* onwards. In contrary, all Gram negative bacteria tested in this experiment remained resistant towards the treatment of *A. paniculata* and *K. alvarezii*. Briefly, Gram positive bacteria can be distinguished by its thick peptidoglycan whereby Gram negative bacteria contains thin peptidoglycan which sandwiched between an outer membrane and inner cytoplasmic cell membrane. On top of that, Gram negative bacteria contained its major component present on the outer cell membrane, known as Lipopolysaccharides (LPS) [14]. It is believed that Gram negative bacteria tested in this experiment were more resistance than Gram positive bacteria may be due to the presence of LPS on its outer membrane, serving as physical barrier from its surrounding [15,16]. Despite of that, there should be other possible reasons resulting this output, which required further investigation on effect of bioactive compounds that present in both *K. alvarezii* and *A. paniculata*.

Natural bioactive compounds are eventually being utilised to target different bacteria strains instead of using synthetic drugs. Based on the results obtained in this experiment, *K. alvarezii* exerted no effect on the eight strains of bacteria whereas *A. paniculata* particularly inhibited the growth of *B. cereus*, *B. subtilis*, *S. aureus* and *S. epidermidis*. Hence, *A. paniculata* has the potential to become an anti-bacterial drugs towards Gram positive bacteria with further investigation on its bioactive compounds.

Conflict of interest statement

All authors declare no conflicts of interest.

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