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Antibacterial potential of methanolic and hexanic extracts of mud lobster (*Thalassina anomala*) from Bintulu, Sarawak, Malaysia

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ABSTRACT

Present study demonstrated the antibacterial potential of three different body parts, abdomen, carapace and cheliped of mud lobster (*Thalassina anomala*) which were extracted with methanol and hexane. Three test bacteria were used in this study namely *Bacillus cereus*, *Escherichia coli*, and *Salmonella enterica*. Present study found that in general, methanolic and hexane extracts of different body parts had variable inhibitory responses on different test bacteria with methanolic extract had greater inhibitory response than hexane extract. In methanol extraction, *B. cereus* significantly had greatest negative impact ($p<0.05$) on growth after treated with cheliped (21 mm zone of inhibition) extract of mud lobster as compared to carapace (9.2 mm) and abdominal (8.8 mm) extracts of mud lobster. Overall, the growth of *S. enterica* was much affected with the presence of all methanolic body-part extracts of mud lobster. In hexane extraction, *B. cereus* growth was not affected with all body-part extracts of mud lobster. However, *E. coli* and *S. enterica* were affected with no significantly different ($p>0.05$) among body-part extracts and between them. Present study concludes that the extracts of mud lobster of all body-part in methanol possess greater inhibitory effect on bacterial growth than extracts in hexane thus the methanolic extract of mud lobster has the potential to be exploited as a natural source of antibacterial agent.

KEYWORDS: Antibacterial; hexane; methanol; mud lobster; *Thalassina anomala*

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INTRODUCTION

Mud lobster, *Thalassina anomala* or locally known in Malaysia as "*ketam busut*" or "*udang ketak*" is recognized according to their mound made in mangrove area. The mound is built by burrowing the muddy soil using their appendages to seek food and their behaviour in destroying the apical plant shoots has classify this mud lobster as pest [1]. The crustaceans of mud lobster are included in marine organisms that have great potential in natural antibacterial sources [2, 3]. Astaxanthin in crustacean is widely investigated and used in several applications such as in pharmaceuticals, chemicals, food and animal feed [4]. It has strong antioxidant activity presence in eggs and body tissue of crustaceans [5]. Several communities in Southeast Asia believed that the boiled *T. anomala* could potentially reduce asthmatic problem [1]. The asthmatic potential might be associated with the antioxidant properties that can be extracted from the crustacean *T. anomala*. Several recent studies demonstrated the plausible link between antioxidant and anti-asthmatic potential [6,7,8]. Extracts from several crustacean species have shown to exhibit antibacterial properties [9,10,11] also Karimzadeh and Pormehr [11] reported that the prawn shell extracts could inhibit the growth of pathogenic *Bacillus subtilis*, *Staphylococcus aureus*, and *Vibrio cholera* and relate the activity to carotenoids content in prawn shell. Antibacterial potential from different body-part of crustacean may be important for investigation and may express different responses as demonstrated in mud crabs [12]. To the best of our information, no literature is available on the antibacterial potential of *Thalassina* species. Therefore, present study was conducted to assess the three body-part of mud lobster (*Thalassina anomala*) namely carapace, cheliped and abdomen and extracted each with methanol and hexane and then exposed to test bacteria for antibacterial potential.

MATERIALS AND METHODS

Species of mud lobster, *Thalassina anomala* (Figure 1) was collected at Kuala Tatau, Bintulu, Sarawak, Malaysia (3° 4' 24.46" N; 112° 48' 30.99" E). The collection was rinsed by running tap water to remove any dirt materials. The samples were kept in the freezer -4°C to preserve the mud lobster until further use. At sample processing, the sample was thawed in room temperature for 4 hours then washed with tap water.

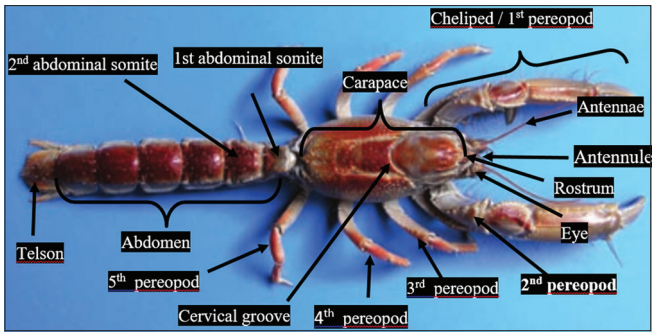

Figure 1: *Thalassina anomala* in Kuala Tatau, Bintulu, Sarawak, Malaysia.

The part of carapace, cheliped and abdomen were divided and wrapped in aluminium foil. Then, the samples were put in the oven and dried at 50°C for two weeks. The weight of sample was taken every day and stop until constant weight achieved. The dried samples were ground and homogenized using an industrial grinder (650W, 28000 rpm) to fine powder form. Each part of *T. anomala* were extracted with methanol and hexane as a solvent by using 2:5 ratio and vortexed for 30 seconds. Then, samples were placed in 50°C water bath for 10 minutes before centrifuged (Bench Top Centrifuge ROTINA-38) for 3000 rpm for 10 minutes. Aqueous and organic layers were separated with new Falcon tube and fresh solvent was added until the aqueous layer become colourless. The concentrated extracts were transferred into sterile Eppendorf or 15 mL Falcon tube. Then, the sample was stored in -4°C to reduce any contamination and degradation of bioactive compound [13].

The extracts were tested against pure culture of potential pathogenic bacteria obtained from the Culture Collection of Laboratory of Microbiology and Plant Pathology, Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus. Three species of bacteria were used as test organism including two Gram-negative bacteria: *Escherichia coli* and *Salmonella enterica*; and a Gram-positive bacterium, *Bacillus cereus*, all cultures were maintained in nutrient agar medium. Mueller-Hinton Agar (MHA) was used in disc diffusion method to determine antibacterial activity by measuring the diameter of inhibition zone. Under aseptic condition, 100 μL of bacteria from broth culture was pipetted onto MHA plates. The bacteria were spread evenly on surface of the agar using sterile hockey stick. The 25 μL from each crude extraction was pipette onto Whatman No. 1 filter sterile paper disc with 6 mm diameter and let it dried for a while. Then, the paper disc was impregnated on the spread plate of bacteria with sterile forceps. The agar plates were kept at room temperature for 15 minutes to allow the extract on disc to absorb into agar before incubation at 37°C for 18 to 24 hours. Then, the tetracycline (30 μg per disc) and 10% of acetic acid were used as positive controls. Treatments were assigned in completely randomized design under laboratory condition. The antibacterial activity determined after incubation by the diameter of the inhibition zone formed around the disc. The inhibition of *T. anomala* extracted with methanol and hexane were compared with positive controls. The examination was observed as for resistant (< 7mm inhibition zone) and inhibition (intermediate inhibition: 7-10 mm; moderate inhibition: 11-15 mm; strong inhibition: 16-20 mm) [13]. All data were analysed for variance and compare means by Duncan New Multiple Range Test (DNMRT) using Statistical Analysis Software (SAS) version 9.4. The results were expressed as mean milimeter diameter ± standard error (mm±SE).

RESULTS AND DISCUSSION

There were significance differences ($p<0.05$) among three different body-part extracts of mud lobster (*T. anomala*) with the greatest inhibition was observed on cheliped extract (21.0 mm) against *B. cereus* and the lowest inhibition was found on carapace (9.2 mm) and abdominal (8.8 mm) extracts (Table 1). The antibacterial potential of cheliped extract against *B. cereus* was comparable ($p>0.05$) with acetic acid (18.0 mm) and significantly ($p<0.05$) higher than tetracycline (13.2 mm). This phenomenon was found differently on *E. coli* where abdominal extract (21.2 mm) of mud lobster was at greatest as compared to cheliped (13.2 mm) and carapace (10.0 mm) extracts. This indicates that each part of the body has different chemical composition as reported by Boßelmann and co-workers [14] that American lobster *Homarus americanus* and the edible crab *Cancer pagurus* have different chemical composition in different parts of skeleton and also between two species. The difference affects its interactions with other organisms such as microbial communities [15].

The effect of abdominal extract against *E. coli* was no different significantly ($p>0.05$) between acetic acid (22.8 mm) and tetracycline (15.8 mm). Among body-part extracts of mud lobster on *S. enterica*, there were no significance difference ($p>0.05$). However, there were significance difference ($p<0.05$) when compare with positive controls, tetracycline and acetic acid. The extracts from abdomen and cheliped were no significant different with acetic acid with considerably higher inhibition level while carapace extract and tetracycline were similar and considerably lower inhibition level. In the antibacterial potential among organisms, abdominal extract showed strongest inhibition on *E. coli* and *S. enterica* and the weakest when against *B. cereus*. Carapace extract against *S. enterica* was at greatest inhibition (significant different, $p<0.05$) as compared to *B. cereus* and *E. coli*. Cheliped extract had significantly higher inhibitory effect against *B. cereus* and *S. enterica* as compared to *E. coli*. Among organisms, the inhibitory effects were comparable with tetracycline and acetic acid.

Table 2 shows the different body-part of mud lobster extracted by hexane on antibacterial activity. Present study found that the growth of *B. cereus* was not affected by any mud lobster body-part hexane extracts. However, the growth of *E. coli* and *S. enterica* were mildly inhibited by the all body-part of hexane extracts as compared to higher inhibition zone as recorded in tetracycline and acetic acid.

When compared among test organisms, *S. enterica* was significantly ($p<0.05$) differed when exposed with hexanic

Table 1: Antibacterial potential of methanolic extracts of different body-part of *Thalassina anomala*

Test organisms	Zone of inhibition (mm±SE)				
	Abdomen	Carapace	Cheliped	Tetracycline	Acetic acid
Bacillus cereus	$8.8\pm0.6^{g,t}$	$9.2\pm0.4^{fg,tu}$	$21.0\pm0.5^{abc,xyz}$	$13.2\pm2.0^{defg,tuvw}$	$18.0\pm0.5^{bcd,wxy}$
Escherichia coli	$21.2\pm2.0^{abc,xyz}$	$10.0\pm1.6^{efg,tuv}$	$13.2\pm2.8^{defg,tuvw}$	$15.8\pm1.6^{cde,vwx}$	$22.8\pm2.9^{ab,yz}$
Salmonella enterica	$19.0\pm2.4^{abcd,wxyz}$	$16.8\pm2.6^{cd,wx}$	$21.4\pm2.1^{abc,xyz}$	$14.8\pm1.4^{def,uvw}$	$24.0\pm1.1^{a,z}$

Means with different superscript letters (a-g) within the same row differ significantly ($p<0.05$). Means with different superscript letters (t-z) within the same column differ significantly ($p<0.05$). Tetracycline=30μg/disc and Acetic acid=10% served as positive controls. Standard of error=SE

Table 2: Antibacterial potential of hexanic extracts of different body-part of *Thalassina anomala*

Test organisms	Zone of inhibition (mm±SE)				
	Abdomen	Carapace	Cheliped	Tetracycline	Acetic acid
Bacillus cereus	n.d.	n.d.	n.d.	$13.2^{c,x}\pm1.96$	$18.0^{b,y}\pm0.46$
Escherichia coli	$8.0^{de,vw}\pm0.32$	$7.2^{e,v}\pm0.2$	$7.4^{e,v}\pm0.24$	$15.8^{bc,xy}\pm1.56$	$22.8^{a,z}\pm2.89$
Salmonella enterica	$8.0^{de,vw}\pm0.45$	$8.0^{de,vw}\pm0.73$	$12.0^{cd,wx}\pm2.59$	$14.8^{bc,xy}\pm1.39$	$24.0^{a,z}\pm1.14$

Means with different superscript letters (a-f) within the same row differ significantly ($p<0.05$). Means with different superscript letters (u-z) within the same column differ significantly ($p<0.05$). Positive Controls: Tetracycline: 30μg/disc; Acetic acid: 10%; Not detected=n.d

extract of cheliped as compared to *E. coli* and *B. cereus*. A comparison between methanol and hexane extraction indicating that the methanol exhibited stronger inhibition than hexane. This is in agreement with Kiran *et al.* [1] which reported that the methanol extraction of several sea invertebrates were higher in antibacterial activity as compared to water extraction. Hexane extraction in most biological compounds exerted weakness in almost all biological activities such as antioxidant, antimicrobial, and anticancer [16,17]. This suggests that the polarity is an important aspect to improve the effectiveness of beneficial chemical composition found in the extract such as in this study, polar of methanol and non-polar of hexane where demonstrated greater and weaker activities, respectively. The antibacterial responses as shown in this study may have an association with the metabolite that can be found in the mud lobster extracts that affects or inhibits the growth of microorganisms as shown by Laith *et al.* [12] that relate the metabolite from whole mud crabs methanolic extract which possess antibacterial activity.

CONCLUSION

In conclusion, this study indicates the antibacterial potential in different body-part of mud lobster, *Thalassina anomala*. The highest potential antibacterial was found at methanolic cheliped extract. *Escherichia coli* and *Salmonella enterica* were considerably much affected with the extracts as in hexane or methanol with the latter much stronger. Thus, mud lobster (*Thalassina anomala*) extract has the potential as a natural antibacterial agent.

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