



Extraction and Antioxidant Potential of Metabolites from Sand Lobster (*Thenus unimaculatus*)

Balu Arasukumar^a, Gopal Prabakaran^a and B. Gunalan^{a,b*}

^aCentre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India.

^a PG and Research Department of Zoology, Thirukilanchiappar Government College of Arts College, Virudhachalam - 606001, Cuddalore (Dist), Tamil Nadu, India.

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Corresponding Author Email: aquagunal@gmail.com

Abstract

The present study was aimed to explore the antioxidant potential of *Thenus unimaculatus* extract by methanolic and chloroform. The yield was calculated, and the maximum was found in chloroform 49% followed by methanol 45%. The DPPH radical scavenging effect has showed concentration depended and the results have been recorded 13, 21, 33, 41 and 54% in methanol and 4, 6, 11, 19 and 24% in chloroform at the concentration of 1-3mg/ml. The ferrous ion chelating ability of *Thenus unimaculatus* methanolic and chloroform extracts has showed dose depended and the result has shown in Fig.2. The methanolic extract has showed 14, 28, 37, 53 and 69% and the chloroform extract has showed 6, 13, 21, 30 and 42%. The hydroxyl radicals scavenging effect of methanolic extract has showed 3, 7, 11, 16 and 29 % and the chloroform extract has showed 14, 26, 34, 48 and 61%. Methanolic extract has showed potential antioxidant radicals scavenging metabolites and it's an alternate source for development of drug in pharmaceutical industry.

Keywords

Sand lobster, DPPH, Ferrous ion chelation, Hydroxyl radical scavenging.

INTRODUCTION

The lobster yield was estimated globally about 165,367 and the production cost was estimated for \$3.32 billion in 2004 (Holmyard and Franz 2006). Recently the production and cost were gradually increased 304,000 tons (captures and aquaculture) in 2012 (Sabatini 2015). Lobster production can be found across the world; however, the majority of production is concentrated in only three countries: Canada (34%), America (29%), and Australia (11%) (Annie and McCarron 2006). The *Thenus*

unimaculatus are locally called as "sand lobster" is renowned according to their mound through in mangrove zone. The mound is made by burrowing the muddy soil by using their appendages to capture the food and their behavior in destroying the apical plant shoots has classify this mud lobster as pest (Hassan et al., 2015). The crustaceans of mud lobster are comprised in marine organisms that have several potentials in natural antibacterial and antioxidant sources (Kiran et al. 2014; Oliveira et al., 2016). Lobsters are commercially processed into different

value-added products namely fresh lobster meat, picked lobster meat, canned lobster, lobster medallion, whole cooked lobsters, and frozen lobsters (Holmyard and Franz 2006; Nguyen et al., 2017). While processing, the inedible parts are traditionally discarded comprising heads, shells, roe, and livers. The types and proportion of lobster processing by-products generated differed depending on the processing process but on average accounts for around 75% (w/w) of the starting material. As a result of this, the annual estimate of LPBs produced from the major lobster processing countries namely Canada, America, and Australia) is about 50,000 tons. Hence, the present study was aimed to explore the antioxidant potential of *Thenus unimaculatus* extract by methanolic and chloroform.

MATERIALS AND METHODS

Sample collection

The *Thenus unimaculatus* lobster was collected from the Mudasalodai fish landing center (Cuddalore Dist, Tamil Nadu; Lat. 11° 29' N; Long. 79° 46' E) and identified through the morphological key characters. The tissue part was dissected out and washed with distilled water and cut into clean pieces air dried in room temperature.

Extraction of metabolites

The extraction of secondary metabolites from dried tissues of *Thenus unimaculatus* was done by following the method of Moovendhan et al. (2015). Briefly, 100g of the dried tissue sample was soaked in Methanol and Chloroform individually at the ratio of 3:1 and kept for 3 days, after that the solvent was filtered through the Whatman No.1 filter paper and the solvent was completely concentrated by rotary evaporator with reduced pressure. The resultant residues were stored at 4°C for bioassay and spectral analysis.

DPPH radical scavenging activity

The DPPH radical scavenging activity of crude methanolic and chloroform extract of *Thenus unimaculatus* was determined as described by Seedevi et al. (2017). Briefly, the DPPH (0.1mM) solution was mixed with 100% methanol and the solution matrix was added with different concentrations of methanol and chloroform extract such as 1, 1.5, 2, 2.5 and 3mg/ml. The aliquot was mixed fine and allowed to stand for 15 mins at the room temperature. The frequent reduction of DPPH free radicals was assessed through decrease of absorption at 517 nm wavelength. The BHT (Butylated Hydroxytoluene) was taken for standard radical scavenger and a result of the scavenging

ability of the crude tissue was determined by standard formula.

Ferrous ion chelating assay

The ferrous ion chelating activity of crude methanolic and chloroform extract of *Thenus unimaculatus* was estimated by the method of Decker and Welch (1990). The crude extract was taken for 1-5mg/ml and then it was individually mixed with 1ml of 2g/l acetic acid containing 3.7 ml of methanol and 0.1ml of 2 mmol FeCl₂ solution. The reaction was initiated by adding 0.2ml of 5mmol/l ferrozine solution, after 10 min incubation at temperature, the absorbance was taken at 562 nm wavelength against blank. The EDTA (Ethylenediamine tetra acetic acid) was used as standard for ferrous ion chelating agents.

Hydroxyl radical scavenging assay

The hydroxyl scavenging effect crude methanolic and chloroform extract of *Thenus unimaculatus* was determined through Fenton reaction as described by Smirnoff and Cumbes protocol. The hydroxyl radical was formed through Smirnoff and Cumbes procedure. Sodium phosphate buffer (150 mM, at pH of 7.4) containing 10mM Ferrous sulphate, 10mM ethylenediamine tetra acetic acid, 2mM Sodium salicylate, hydrogen peroxide 30%. The crude extract was prepared with different concentrations 0.5- 2.5 mg/ml. The solution was allowed to stand for 37°C for 1 h, then the sample was absorbance was read by spectrophotometer (Shimadzu, Japan) at the wavelength of 510nm. The Butylated hydroxyanisole (BHA) was used as standard scavenger. The hydroxyl scavenging effect was estimated by standard equation.

RESULTS AND DISCUSSION

The most common traditional utilization of Lobster Processing by Products (LPBs) is their use as a foundation of nutrients for soil amendment measured as an informal way of disposal (Cousins 1997), but it brings no economic benefits for lobster producers. More recently LPBs have been studied as an important bioresource in the recovery of marine functional ingredients, nutraceuticals, and pharmaceuticals for numerous applications (Giyose et al., 2010; Nguyen et al., 2017). In the present study, the secondary metabolites from *Thenus unimaculatus* tissues was extracted by using organic solvents such as Methanol and Chloroform based on the polarity polar and non-polar solvents. The yield of the *Thenus unimaculatus* tissue extracts were estimated 45 and 49% in Methanol and Chloroform respectively. The yield has showed superior in non-polar solvent chloroform than the polar solvents, due

to the presence of the lipids. Similarly, Zohir et al. (2018) have been reported the minimal extract yield in mud lobster (*Thalassina anomala*) from Bintulu, Sarawak, Malaysia by extract methanol and hexane. Additionally, Varadharajan and Soundarapandian (2013), have been recorded similar level of metabolites (Chitin) extract from *Scylla serrata* shell. The results revealed that the secondary metabolites extraction yields differed by body parts and solvent systems and polar and non-polar are also showed better yield. The antioxidant activities namely, DPPH radical scavenging effect, ferrous ion chelating effect and hydroxyl radical scavenging effect of *Thenus unimaculatus* methanol and chloroform extracts were screened by *in vitro* methods and the results were shown in figures (Fig.1, Fig.2, Fig.3) respectively. The DPPH radical scavenging ability of *Thenus unimaculatus* methanolic and chloroform extracts has showed concentration depended and the results have been recorded 13, 21, 33, 41 and 54% in methanol and 4, 6, 11, 19 and 24% in chloroform at the concentration of 1-3mg/ml respectively (Fig.1). The standard scavenger BHT has showed promising scavenging effect between 18 and 90% at the concentration of 1-3mg/ml respectively. From the results the polar (Methanol) has showed more scavenging effect than non-polar extract (Chloroform). The ferrous ion chelating ability of *Thenus unimaculatus* methanolic and chloroform extracts has showed dose depended and the result has shown in Fig.2. The methanolic extract has

showed 14, 28, 37, 53 and 69% and the chloroform extract has showed 6, 13, 21, 30 and 42% at the same time the EDTA (Commercial chelator) has showed promising effect 21-97% at the concentration of 1-5mg/ml respectively (Fig.2). The maximum effect was recorded in methanol 69% followed by chloroform 42%, when compared to the non-polar the polar solvent extract methanol has showed maximum Fe ion chelating effect. The hydroxyl radical scavenging activity of *Thenus unimaculatus* methanolic and chloroform extracts has showed concentration depended and the result has shown in Fig.3. The methanolic extract has showed 3, 7, 11, 16 and 29 % and the chloroform extract has showed 14, 26, 34, 48 and 61% at the same time the EDTA (Commercial chelator) has showed promising effect 35-98% at the concentration of 0.5-2.5mg/ml respectively (Fig.3), in this scavenging effect the chloroform extract has showed highest effect followed by methanol. In the present study the methanolic (Polar) extract has showed better radical scavenging effect than the non-polar solvent extract. The same biological activity was noticed by Moovendhan et al. (2014), who has been evaluated the *Pinna nobilis* methanolic extract against the clinical pathogens. Correspondingly, Vijayalakshmi et al. (2018) extracted the carotenoids a by products from lobster collected from Visakapattinam coast and explored the structural features and biological activities.

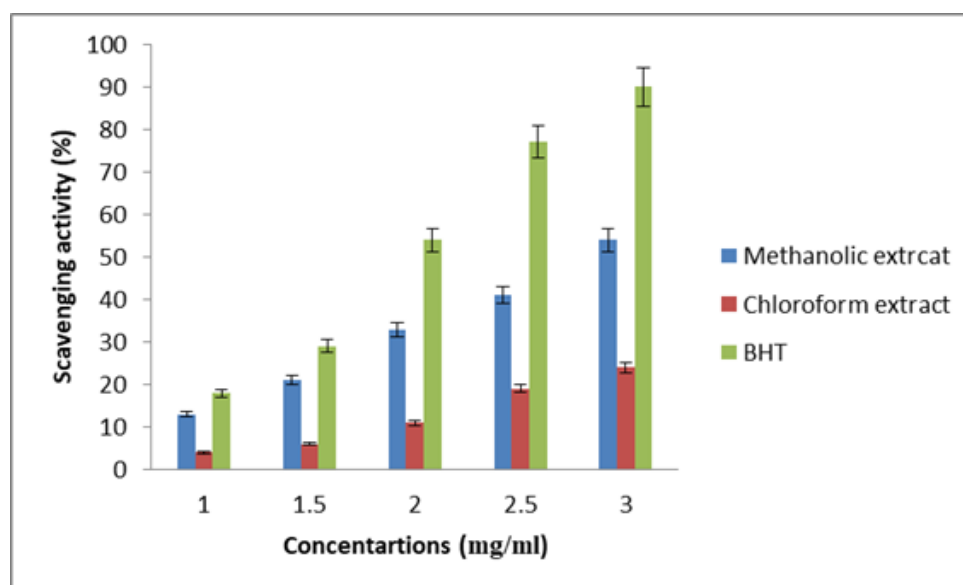


Fig.1. DPPH radical scavenging effect

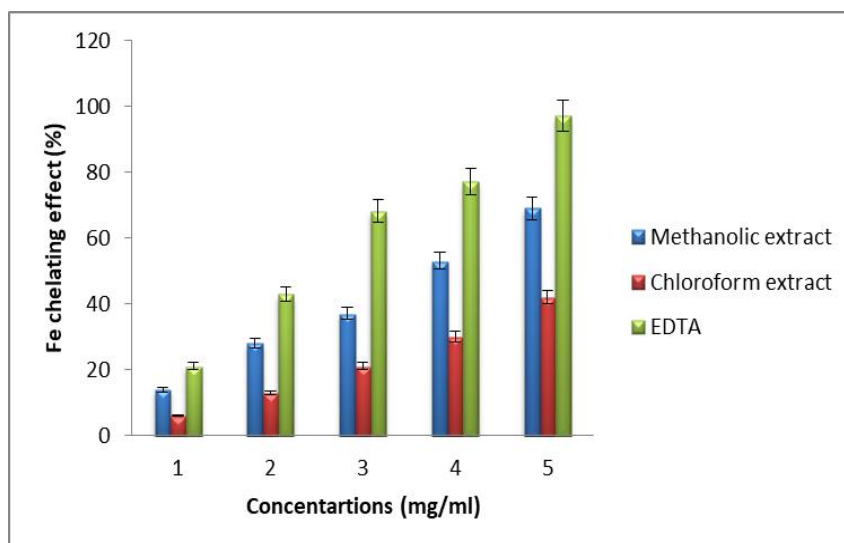


Fig.2. Ferrous ion chelating effect

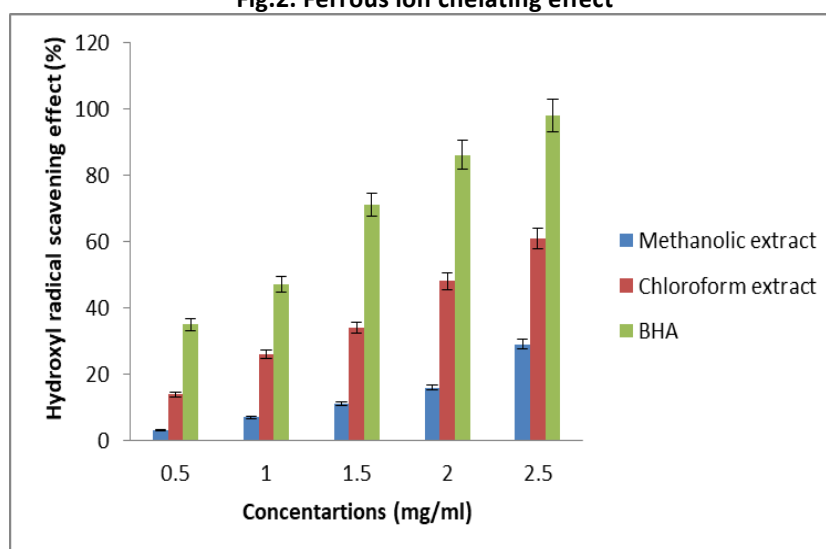


Fig.3. Hydroxyl radical scavenging effect

CONCLUSION

In the present study, the secondary metabolite was extracted by methanol and chloroform solvents system from the tissue of lobster *Thenus unimaculatus* and the yield has showed high level. The antioxidant effect of the crude extract was evaluated, and activities of the methanol has showed promising scavenging effect than the chloroform extract. In conclusion, the yield was differed from the body parts and depends on the polarity and the polar solvent is more suitable metabolite extraction and having potential antioxidant radicals scavenging metabolites, even though some high purification and secondary evaluation and mechanism related study to development of drug in pharmaceutical industry.

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