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EXTRACTION, PRELIMINARY CHEMICAL SCREENING AND CHARECTERISATION OF *PORTUNUS SANGUINOLENTUS* EXOSKELETON

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ABSTRACT

Crustaceans make up a large group of arthropods which include crabs, lobsters, shrimp, krill etc. The aim of this work was to carry out the preliminary chemical screening of various extracts of *Portunus sanguinolentus* (Three spotted swimming crab) exoskeleton for identification of chemical constituents and to carry out the physicochemical studies so as to determine the level of minerals, moisture content, extractive value and to analyze the presence of active constituents in extract due to which they possess different pharmacological activities and also with a goal of increasing awareness of the beneficial effects of crab exoskeleton. Extracts of exoskeleton were prepared using chloroform, ethyl acetate and ethanol by simple maceration. Then the chemical analysis of these extracts were carried out and found the presence of beta carotenoids, non reducing sugar, proteins, minerals etc. Further chromatographic and spectrometric studies of these extracts were performed and confirmed the presence of these chemical constituents.

KEYWORDS: Portunus sanguinolentus, Marine sources, physicochemical studies, exoskeleton.

INTROUCTION

Marine environment is considered to be more biologically diverse than terrestrial environment. In some marine ecosystems such as coral reefs or deep sea floor, experts estimate that the biological diversity is higher than tropical rain forest. Even though, marine organisms are considered as vast resource of medications to treat various ailments, its 29% is only explored.

In recent years, marine natural products bioprospecting yielded a considerable number of drug candidates. Although there are only a few marine derived products currently in market, several new compounds from marine origin are now under clinical trial for drug development. Many different marine organisms have been explored for bioactive compounds. Examples for marine invertebrates are sponges, coelenterates, crustaceans, tunicates, echinoderms, corals, algae, bryozoans and some microbes which include bacteria, fungi and cyanobacteria.[1]

Crustaceans make up a large group of arthropods which include crabs, lobsters, cray fish, shrimp etc. Of these, crabs are found to be an excellent source of readily digested high quality protein and essential amino acids. Another important feature of crustaceans is its hard exoskeleton (carapace), which contain various nutritional components like proteins, minerals, fatty acids etc. Major components of crustacean shell are beta carotenoids, calcium carbonate, minerals, fatty acids and beta-1, 3- linked glucan. Also these compounds are found to be relatively more in marine crabs, belongs to the family Portunidae. Besides nutritive value these compounds are claimed to possess, anti-inflammatory, anti-coagulant, anti-arthritic, hepatoprotective, wound healing and cardioprotective activities.[2]

METHADOLOGY

SAMPLE COLLECTION AND EXTRACT PREPARATION

Fresh crabs were collected from southern coastal area of kerala in month of October 2015 and are authenticated by Dr Biju Kumar, Dept of Aquatic biology, University Kerala, Karyavattom, Thiruvananthapuram.

The shells were separated, cleaned and washed with distilled water and dried under sunlight for two days. Then it is finely powdered and kept for simple maceration using chloroform, ethyl acetate and ethanol, for 5 days each with occasional stirring. Then the slurry was filtered and the liquid extracts were concentrated to yield dry extracts.

1. Preliminary chemical screening[3]

For the identification of various active principles in *Portunus sanguinolentus* exoskeleton extracts, various chemical tests were carried out.

a) ***Test for carbohydrates***

- Molisch's test - A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's reagent and formation of brick red colour confirmed the presence of carbohydrate.
- Test for non-reducing sugar - To a small quantity of extract, 1-2ml of hydrochloride and then 0.1g of sodium bicarbonate. After the fizzing sound has been ceased equal volume of benedict's reagent was added, mixed and heated in boiling water bath for 5 minute.

b) ***Test for Proteins***

- Millions test: 3ml extracts were dissolved in 10ml of millions reagent. White precipitate is formed which on warming the white precipitate dissolves giving red coloured solution.
- Biurette test: 3ml of the extract was mixed with 4% sodium hydroxide and few drops of 1% copper sulphate solution were added, violet or pink colour not appeared. To 3ml of extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

c) ***Test for Steroids***

- Salkowski reaction - 1ml of concentrated sulphuric acid was added to 1ml of extract via sides of test tube and kept for one minute red colour is produced in one layer.
- Libermann-Buchnard test - Small portion of extract was dissolved in 2ml chloroform.To this 1ml of acetic anhydride and 2ml of concentrated sulphuric acid was added through sides of test tube.

d) ***Test for terpenoids***

- Extract was treated with vanillin- sulphuric acid reagent orange red colour is produced.

e) ***Test for diterpenes***

- Copper acetate test – Small amount of extract was dissolved in water and treated with 3-4 drops of copper acetate solution, emerald green colour is produced.

f) ***Test for carotenoids***

- 1.025g of sample is dissolved in 1ml of hexane. To this 2-3ml of sodium nitroprusside and 1N sulphuric acid is added, disappearance of colour indicate presence of carotenoids.

2. Physico-chemical studies[3]

- ***Determination of ash value***
- Determination of total ash: 2-4g of the sample was weighed in a crucible and was spread evenly and ignited slightly increasing the temperature to 500-600 degree Celsius until it turns white, it was cooled in a dessicator and weighed.
- Determination of acid insoluble ash: 25 ml of dilute hydrochloric acid was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.
- Determination of water soluble ash: 25 ml of water was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

$$\text{Percentage ash value} = \frac{\text{Initial weight taken} \quad \text{x } 100}{\text{Weight of ash}}$$

- ***Determination of extractive value***
- Water soluble extractive value: 5 grams of the powdered drug was macerated with 100ml of water for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight .The percentage of water soluble extractive value was calculated with reference to the air dried drug.
- Alcohol soluble extractive value: 5 grams of the powdered drug was macerated with 100ml of alcohol for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight and the percentage of water soluble extractive value was calculated with reference to the air dried drug.
- Ethyl acetate soluble extractive value: 5 grams of the powdered drug was macerated with 100ml of ethyl acetate for 24 hours with occasional shaking for the first 6 hours and then

left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat con

- tainer at 105 degrees until constant weight and the percentage of water soluble extractive value was calculated with reference to the air dried drug.
- Chloroform soluble extractive value: 5 grams of the powdered drug was macerated with 100ml of chloroform for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight and the percentage of water soluble extractive value was calculated with reference to the air dried drug.

3. Chromatographic analysis[4]

- ***Thin layer chromatography***

100 gram of silica gel G was dissolved in sufficient amount of water and was coated on the glass plate. Solvent system chosen was acetone: hexane [5:5]. Extracts were dissolved in sufficient vehicle to make up a concentration of 1mg/ml .The spots were made 1 cm from the bottom of the glass slide. The glass plate was kept in to the chamber after chamber saturation and allowed to run 2/3rd of the glass plate. R_f value was calculated.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute.}}{\text{Distance travelled by the solvent front}}$$

- ***Paper chromatography***

Extracts were dissolved in sufficient vehicle to make up a concentration of 1mg/ml. The spots were made 1 cm from the bottom of chromatographic paper. Solvent system chosen was acetone: hexane [5:5]. Then the spotted paper was kept in to the chamber after chamber saturation and allowed to run 2/3rd of the paper. The paper was air dried and kept for two dimensional developments in same solvent system. Finally R_f value of all spots were calculated.

4. Spectrometric analysis[4]

- ***Ultra violet spectroscopy***

0.1g of extracts was dissolved in 100ml of corresponding solvents and uv analysis was done in UV spectrophotometer schimadzu 1800.

- ***Fourier transform infrared spectroscopy***

0.1g of extracts was underwent FTIR analysis in FTIR spectrophotometer sepctrum400.

RESULTS

Constituents	Test	Chloroform extract Absence/Presence	Ethyl acetate extract Absence/Presence	Ethanol extract Absence/Presence
Carbohydrates	*Molisch test *Test for non reducing sugar	+ +	+ +	+ +
Proteins	*Million's test *Biurette test	– –	– –	+ +
Steroids	*Salkowski test *Libermann buchnard reaction	– –	+ +	+ +
Terpenoids	*Vanillin sulphuric acid test	+	+	+
Diterpenes	*Copper acetate test	+	+	+
Carotenoids	*Nitroprusside test	+	+	+

- **PRELIMINARY CHEMICAL SCREENING**

Total ash value	1.5 g
Water soluble ash value	5.97%
Acid insoluble ash value	17.6%
Water soluble extractive value	9.31%
Alcohol soluble extractive value	51.59%
Chloroform soluble extractive value	22.23%
Ethyl acetate soluble extractive value	19.21%

- **PHYSICOCHEMICAL EVALUATION**

- **CHROMATOGRAPHIC ANALYSIS**

- ***Thin layer chromatography***

Extracts	Spots	R_f value
Chloroform extract	1 2 3	0.60 0.75 0.83
Ethyl acetate extract	1 2 3	0.47 0.65 0.85
Ethanol extract	1 2 3 4	0.25 0.603 0.85 0.99

Photographs of TLC

Chloroform extract

Ethyl acetate extract

Ethanol extract

- ***Paper chromatography***

Extracts	Spots	R_f value
Chloroform extract	1 2 3	0.98 0.9 0.96
Ethyl acetate extract	1 2 3	0.98 0.64 0.90
Ethanol extract	1 2 3	0.984 0.85 0.87

Photographs of paper chromatography

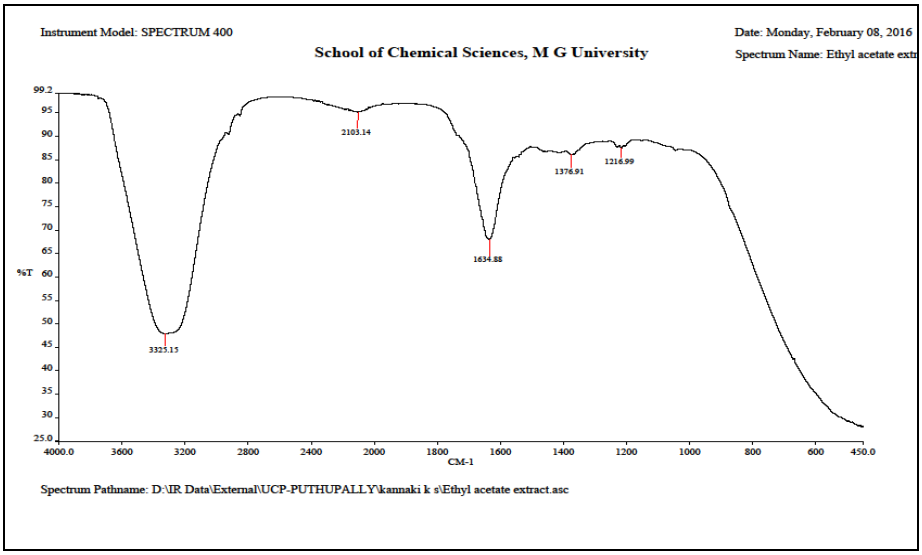
Chloroform extract

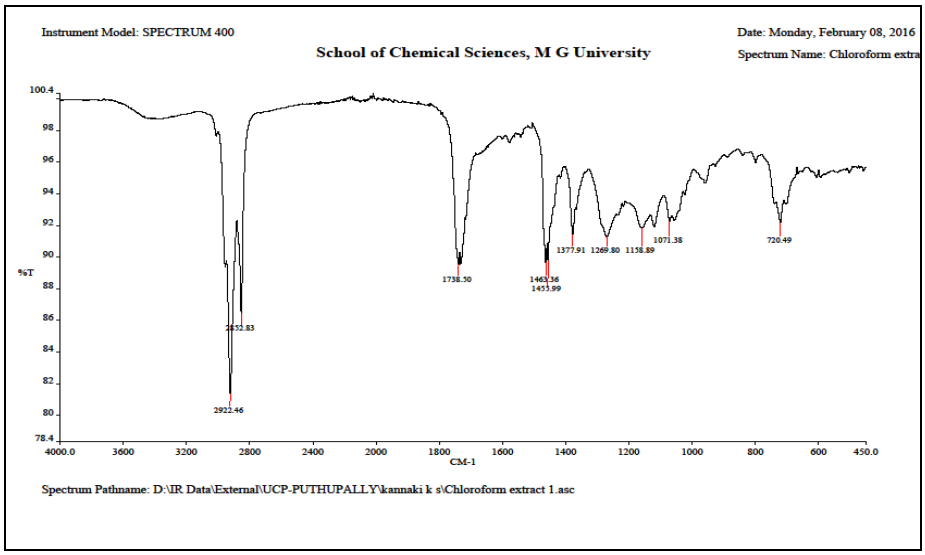
Ethyl acetate extract

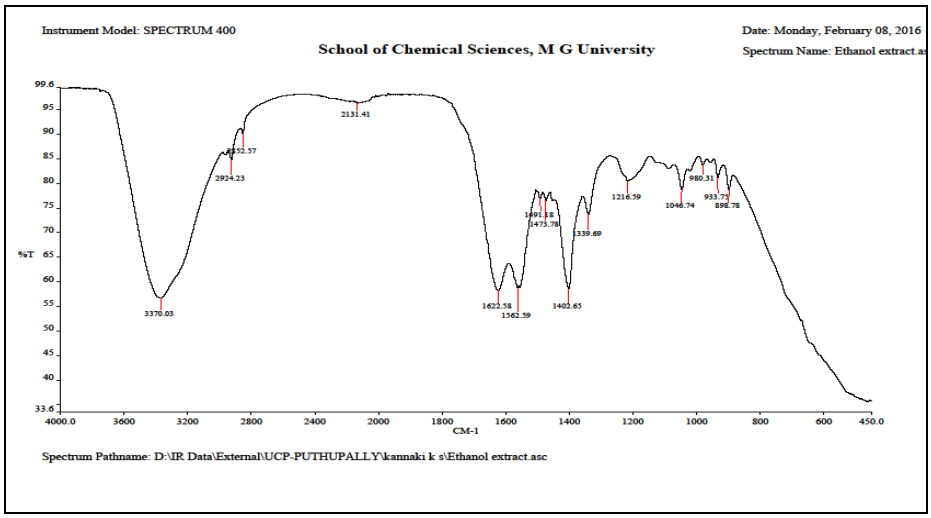
Ethanol extract

- **SPECTROMETRIC ANALYSIS**

✓ **FTIR**

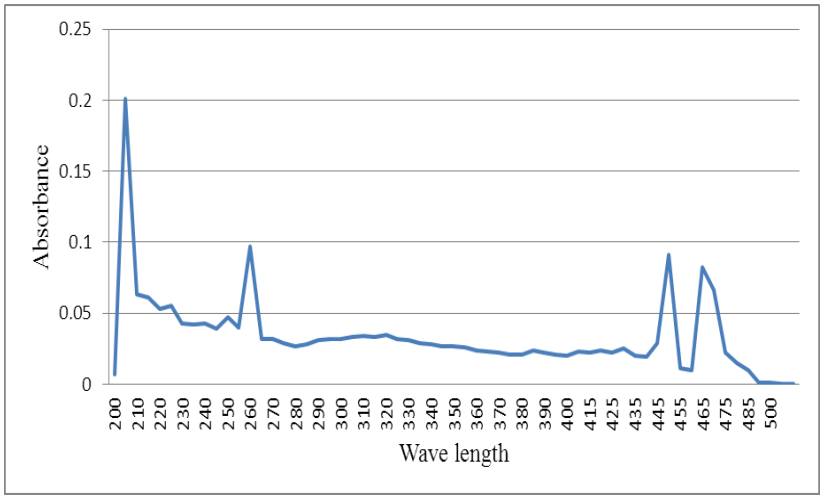




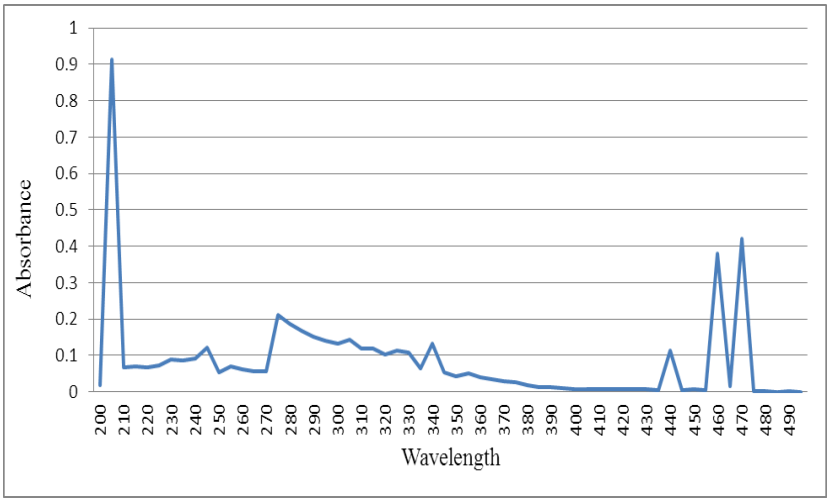


✓ **UV Analysis**

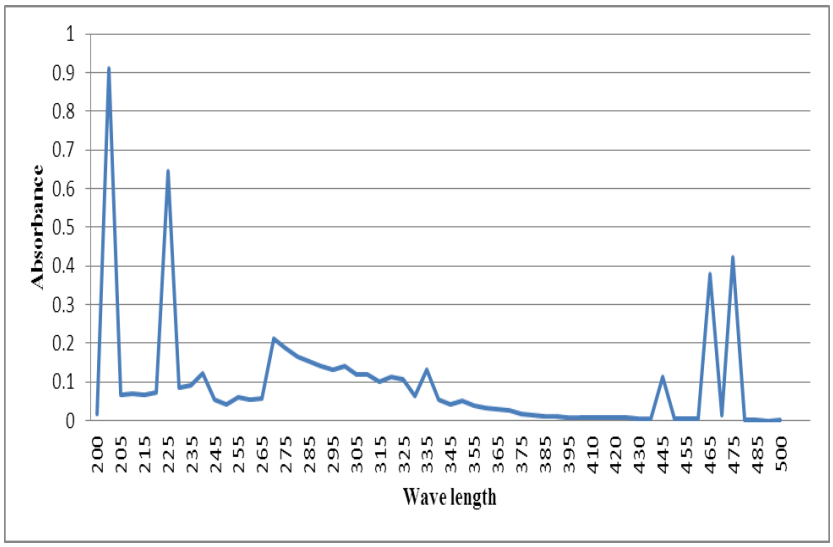
- ***Chloroform extract***



- ***Ethyl acetate extract***



- ***Ethanol extract***



DISCUSSION

- The preliminary chemical tests of the three extracts showed the presence of carbohydrates, carotenoids, diterpenes, terpenoids and steroids. In addition the ethanol extract showed the presence of proteins.
- The ash value and extractive value helped to determine the amount of minerals and the limit of exhaustion of drugs.
- The presence of active constituents is been confirmed by chromatography and spectral analysis. The R_f values obtained are in close proximity with standard R_f values of marine carotenoids [Astaxanthin monoester, Astaxanthin diester, Canthaxanthin, Leutin and Beta carotene] and the lambda max of active constituents are also coinciding with standard values.
- FTIR spectroscopy confirmed the presence of various active constituents by showing the peaks corresponding to the functional groups.

CONCLUSION

In present study the extraction, preliminary chemical screening and characterization of *Portunus sanguinolentus* exoskeleton was done. The chemical screening revealed the presence of active constituents responsible for cardio protective activity. Physicochemical studies like ash value, extractive value suggests that it has better stability, no adulteration, least exhaustion. Further extractive value suggests that the active constituents are more soluble in ethanol. Chromatographic and spectral analysis of the extracts confirmed the presence of active constituents, the results are comparable with standard values.

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