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Characterization of oil extracted from freshwater edible crab (*Barytelphusa cunicularis*)

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Abstract
The present study has been conducted in order to find out chemical, physical and biological properties of oil Extracted from Freshwater Edible Crab (*Barytelphusa Cunicularis*). The freshwater crab *Barytelphusa cunicularis* is a main species in Marathwada region. The crabs are collected from local market of Aurangabad (MS) India. We analyzed oil and estimated its different values such as saponification value, free fatty acid value, peroxide value. Furthermore oil is subjected to other tests such as: Cholesterol test, Acrolein Test, Qualitative test of Vitamin A, solubility test, protein test, Density test, Refractive index, and Antimicrobial activity test. Outcomes are discussed in details.

Keywords: *Barytelphusa cunicularis,* cholesterol test, antimicrobial activity test

1. Introduction
Literature survey revealed that fresh water crabs have been given relatively less attention than marine water crabs. But now this scenario has been getting better over the past few decades [1]. Researchers studied the systematics and biology of freshwater crabs of the different regions of the world. The volunteer contributions of some researchers should not be ignored [2].
Earlier we studied literature available on crabs and Supercritical Fluid Extraction. We extracted crab oil using Supercritical Fluid Extraction instrument at different temperature and pressures [3-6].
There is very little work related to freshwater crab such as structure and properties of the exoskeleton of the sheep crab [7], morphometry of freshwater crab [8], lesions in gills [9], and nutritional properties of freshwater crab [10], Fatty Acid Profile [11], amino acid composition in tissues [12], nutritive value of hard and soft shell crabs [13].

2. Material and Method
The crabs (*Barytelphusa Cunicularis*) are purchased from local market, at Aurangabad District (Maharashtra) India. The crab meat is dried in oven for 8 hours at 50 ^{o}C. After proper drying, the dried crab meat is subjected extraction process in order to obtain crab oil. Extracted oil from the freshwater crab *Barytelphusa Cunicularis* is used as a sample for all tests.

2.1 Saponification value
Solid oil sample is melted. It is filtered through filter paper to remove impurities. 1 gram sample is taken into 250 ml flask and connected to a condenser. 50 ml alcoholic KOH is added in to the flask. Blank sample is prepared with just 50 ml of alcoholic KOH in 250 ml flask. Few boiling heads is added to the flasks. Flask is connected to the condenser. Sample is boiled on water bath until it is clear and homogeneous, indicating complete saponification. Sample is allowed to cool somewhat. The inside of the condenser is washed down with little deionized distilled water. Flask is disconnected from condenser. It is then allowed to cool to room temperature. 1 ml phenolphthalein is added to sample and titrated with 0.5 N HCl (from burette) until the pink color just disappear. The volume of titrant is recrded. Same procedure is performed with sample blank.

2.2 Free fatty acid value
1 gram sample is taken into Erlenmeyer flask. 100 ml neutralized ethanol and 2 ml phenolphthalein indicator is added in to the flask. Flask is shaken to dissolve the mixture completely and titrated with standard base, shaking vigorously until the endpoint is reached. This is indicated by a slight pink color that persists for 30 seconds. The volume of titrant used is recorded.

2.3 Peroxide value
1 gram oil sample (to the nearest 0.001 g) is taken into 250-ml glass-stopper Erlenmeyer flasks.30 ml acetic acid-chloroform solution is added to the flask and swirled to dissolve.0.5 ml saturated KI solution is added. The flask is kept stand with occasional shaking for 1 min. After 1 minute 30 ml deionized distilled water is added. Sample is titrated with 0.1 N sodium thio-sulfate solution, with vigorous shaking until yellow color is almost gone. 0.5 ml 1% starch solution is added, and titration is continued, shaking vigorously to release all iodine from chloroform layer, until blue color just disappears. The volume of titrant used is record. Same procedure is performed with sample blank.

2.4 Cholesterol test
In a clean and dry test tube, 5ml chloroform and 1 ml sample (crab oil) is taken and mixed well, 2 ml concentrated H_2SO_4 is slowly added through the side of test tube and mixed well. A reddish color is seen in the upper chloroform layer and a yellow color with green fluorescence is seen in the lower acid layer.

2.5 Acrolein Test
A small amount of sulphuric acid or potassium bisulphate is taken in a dry test tube and 4-5 drops of oil is added. Test tube is heated cautiously and slowly. Irritatingly pungent smell of acrolein comes out of test tube.

2.6 Qualitative test of Vitamin A
2 drops of oil in I ml of chloroform is taken in a dry test tube, 2 drops of concentrated sulfuric acid is added. A blue coloration, gradually turning to brown red is observed. The test is based upon the dehydration of retinol by conc. sulfuric acid to yield colored products.

2.7 Solubility test
5 ml Hexane, Petroleum Either, Chloroform, Diethyl Ether, Carbon tetrachloride, Ethanol, Methanol, Ethyl Acetate, Diluted Acetic Acid is taken in 9 test tubes. Few drops of sample (oil) are added in each test tube. All test tubes are shaken well and allowed to stand for few minutes and observed that the mixture becomes homogenous.

2.8 Protein test
2-3 ml of test sample is taken in a clean test tube; an equal volume of sodium hydroxide is added and mixed thoroughly. Dilute cupric sulfate solution is Added drop by drop, mixing after each addition. A purple violet or pink violet color indicates the presence of proteins. It is important to note that excess addition of cupric sulfate solution is not desirable as it causes precipitation of copper hydroxide posing a difficulty in making out the purple-violet color change. The shade of the color produced depends upon the nature of the test protein, being pink for peptones, blue for gelatin and purple-violet to pink violet for other proteins. Since salts like ammonium sulfate and magnesium sulfate interfere with this test, the test is best done in their absence.

2.9 Density test
The mass of empty density bottle is measured using electronic balance. 10 ml of crab oil is measured by graduated cylinder and transferred it into the empty density bottle. Mass of the density bottle with the liquid (Crab Oil) is measured using electronic balance. The mass of oil is determined.
Dividing the mass of oil by 10 gives the density of oil.

2.10 Refractive index
A drop of sample is placed on the measuring surface beneath the View Point Illuminator. Looked through eyepiece and pressed the View Point Illuminator.
Reading is taken at the point where the contrast line (difference between light and dark areas) crosses the scale

2.11 Antimicrobial activity test
Antimicrobial activity is performed by pour plate method. Streptomycin is used as a standard antibiotic. Concentration of antibiotic is 100 µg/ml. Active cultures of two gram negative and two gram positive bacteria are used viz (E.coli & S. typhi) and (B. subtiles and S. aureus as gram positive bacteria. The size of well is 2mm. organism is spread on the agar plate [14].

The results are given in table 1

Table 1: The physical parameters of crab oil

Sr. No.	Test	Result
1	Saponification value	126.225 mg KOH/g
2	Free fatty acid value	34.782 mg KOH/g
3	Peroxide value	4.3 mEq/ Kg
4	Cholesterol test	Positive
5	Acrolein Test	Positive
6	Qualitative test of Vitamin A	Positive
7	Solubility test (at 25 ^{0}C)	Soluble: Hexane, Petroleum Either, Chloroform, Diethyl Ether, Carbon tetrachloride Insoluble: Ethanol, Methanol Partially soluble: Ethyl Acetate, Diluted Acetic Acid
8	Protein test	Negative
9	Density test	0.9410
10	Refractive index (at 30 ^{0}C)	1.423
11	Antimicrobial activity test	Negative

3. Conclusion
The qualitative tests carried out on oil indicate that, it contains cholesterol, glycerol and vitamin A. so the crab oil is a good source of vitamin A, but its excessive use has to be avoided since it also contains cholesterol to some extent.
The saponification value of oil is important from industrial point of view. The crab oil possesses good a sponification value i.e. 126 mg KOH/g. the free fatty acid content of crab oil obtained from study area was found to be 34.78 mg KOH/g. the peroxide value of crab oil is less i.e. 4.3mEq/Kg.
We tested the solubility of crab oil in various solvents, it was observed that the crab oil is insoluble in polar solvents like methanol and ethanol; hence hydrogen bonding or polar interactions are ruled out. It is soluble in non-polar solvents like hexane, carbon tetrachloride etc, and indicating maximum covalent nature of compound.
Though crab oil is a source of fat, it does not contain protein. We try to find out its antimicrobial activity. The tests are negative, indicating the crab oil cannot be used as antimicrobial agent.

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