IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Estimation of Antimicrobial Properties of Shrimp Extract on E. Coli

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Abstract: *The research aimed at the study of antimicrobial properties of shrimp extract on E. coli. We used the Agar well method and loaded the concentrated sample of shrimp in this experiment. After incubating for 24 hours, we observed a limited area of inhibition in E. coli. The sample was extracted by crushing the shrimp waste in a mortar and pestle. Methanol was used as a solvent to extract the chemical components of the shrimp paste. After filtering the waste, the methanol was evaporated and the remaining sample was added to sterile distilled water and loaded in the agar plate. To obtain a uniform inhibition zone the Agar well method is occasionally beneficial for the assay of viscous materials. The Agar well diffusion method is extensively used to evaluate the antimicrobial activity of shrimp extracts. Then, a hole with a diameter of 6 to 8 mm has punched aseptically with a sterile cork borer or a tip, and a certain amount of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending on the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.*

Keywords: Shrimps, antimicrobial, Escherichia coli, shrimp waste

I. INTRODUCTION

Shrimps are found near the seafloor along most coasts and estuaries, as well as in rivers and lakes. Most shrimp species are marine, with only about a quarter found in freshwater[1]. Marine species can be found from the tropics to the poles at depths of up to 5000 meters (16000 feet). Shrimps are eaten by a variety of animals, including fish and seabirds, they play important roles in the food chain and provide food for larger animals ranging from fish to whales. Shrimp tails with muscular muscles can be delicious to eat, and they are widely caught and farmed for human consumption[1]. Shrimp waste (dried head or shell) was discovered to have a high protein content as well as a high level of minerals, particularly Ca, P, Na, and Zn. The chemical makeup and yield of edible shrimp are as follows. Shrimp weighing 37.3 g and measuring 15.5-23.4 cm yielded 47.2 to 55.1% (mean 51.3%).

Shrimp had an average composition of 76.74% water, 0.91% fat, 1.71% ash, 0.49% total phosphorus, and 22.07% total protein, with the latter consisting of 76.5% pure protein[2]. A shrimp protein was high in arginine, histidine, and proline, but low in threonine, methionine, valine, lysine, and tryptophane. Amine nitrogen accounted for 39.5% of total nonprotein nitrogen. It was mostly made up of free amino acids like glycine, arginine, proline, and alanine. The amino acid profile of dried shrimp shells was found to be higher than that of shrimp heads. In both dried samples, glutamic acid was the most abundant amino acid. The saturated: unsaturated fatty acid ratio for dried shrimp heads was 1: 1.63 and 1: 1.51 for dried shrimp shells. Aflatoxins (B1, B2, G1 & G2) concentrations did not appear to be present in the dried shrimp shell or head. The two dried samples also demonstrated their bacteriological safety. Their protein digestibility in vitro (IVPD) ranged from 60.44 to 67.68%[2]. The total saturated fatty acids in the two suggested recipes were found to be lower than the total unsaturated fatty acids. The panelists found potato croquette recipes containing 5% dried shrimp shell or head to be of acceptable overall quality (aroma, taste, appearance, texture, and color). Shrimp waste production from shrimp processing industries has increased dramatically in recent years. The continued production of this biomaterial without corresponding technological development has resulted in waste collection, disposal, and pollution issues.

Shrimp waste contains a variety of bioactive compounds, including chitin, pigments, amino acids, and fatty acids. These bioactive compounds are used in a variety of applications, including medicine, therapies, cosmetics, paper, pulp,

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textile industries, biotechnology, and food[3]. This current review article discusses the utilization of shrimp waste as well as an alternative technology to replace hazardous chemical methods that address future trends in total shrimp waste utilization for bioactive compound recovery. Chitin is the second most abundant polymer in nature after cellulose. Chitin is primarily derived from crustacean shells such as shrimp and crab. Chitin is regarded as an important material due to its biodegradability, biocompatibility, non-toxicity, low immunogenicity, and thermal stability[3].

Chitin and its derivatives are therefore widely used in cosmetics, food, agriculture [8,] tissue engineering, wastewater treatment, and packaging material applications. According to reports, shrimp accounts for approximately 45% of processed seafood, and the shrimp processing industry generates massive amounts of by-products such as shrimp shells. These shrimp by-products are typically used in the production of low-value animal feeds and biological fertilizers. Chitin, proteins, and minerals are the main components of shrimp shells[4]. Chitosan, a partially deacetylated chitin, can stimulate the synthesis of many plant defense proteins. Phytoalexins, phenols from cell walls, and callose It can also prevent the growth of a wide variety of fungi and bacteria. Drug resistance in microorganisms has been steadily increasing. As a result, novel bioactive compounds with anti-bacterial and anti-fungal properties against common clinical pathogens were required.

The inhibitory activity of shrimp chitosan (98% deacetylated) against Escherichia coli was studied on cell age, reaction temperature, pH value, and salts. The age of a bacterial culture affected its susceptibility to chitosan, with late exponential phase cells being the most sensitive. Chitosan's bactericidal effects were enhanced by higher temperatures and acidic pH. Sodium ions may form complexes with chitosan, reducing its activity against E. coli. Chitosan also caused E. coli cells to leak glucose and lactate dehydrogenase.

Chitosan, a deacetylated chitin derivative that reduces the fraction of acetylated amine groups, has been proposed as a potential antimicrobial agent. Chitin is a fibrous polysaccharide with a polysaccharide structure. It is common in the outer skeletons of insects and crustaceans like shrimp, crabs, and lobsters. Chitosan film has been used as a bio-functional material in recent years because it is biocompatible and well tolerated by living tissues. Chitosan's antifungal and antibacterial activity appears to be mediated by electrostatic forces between the protonated NH2 group in chitosan and the negative residues on cell surfaces[4]. The number of protonated NH2 groups in chitosan increases as the degree of deacetylation increases (DD). As a result, the DD of chitosan influences its antimicrobial activity. Chitosan is typically produced from crustacean shells (crabs, shrimp, and crayfish) through chemical or microbiological processes.

Recent research on shrimp/crab by-products as natural astaxanthin sources. The goal is to provide an overview of scientific knowledge on astaxanthin's chemical and biochemical properties, as well as an overview of its content in crustacean by-products. To identify the limitations of successful astaxanthin exploitation, we reviewed papers on extraction protocols in search of environmentally friendly processes or green chemistry techniques for the recovery of this metabolite. The article describes astaxanthin's bioactive properties and health benefits.

Carotenoids are a type of lipid-soluble pigment that is found in many photosynthetic organisms as well as some non-photosynthetic bacteria and fungi and is responsible for their red, orange, and yellow colours. These colour characteristics are the result of their chemical structure, which is primarily a long polyynic carbon chain, while derivatization of their base structure results in different colour hues and tones, among other properties. Plants, algae, yeasts, fungi, archaea, and eubacteria primarily biosynthesize them, but they can also be found in animals and humans, who absorb and deposit them through diet and various metabolic reactions.

Carotenoids are essential pigments, structural and functional components in a wide range of aquatic organisms (primary carotenoids), or are produced as a result of environmental exposure (secondary carotenoids). Photoautotrophic organisms are the primary sources of carotenoids; they can be found in their primary consumers or secondary consumers, where they are absorbed directly or modified through metabolic reactions.

Astaxanthin (crabs, shrimps, lobsters, and so on); fucoxanthin, -carotene; lutein; siphonaxanthin; mytiloxanthin; zeaxanthin (in microalgae, seaweeds, corals, shellfish, and so on); saproxanthin and myxo (in bacteria from the Flavobacteriaceae family); halocynthiaxanthin.

Astaxanthin accumulation in crustaceans has been proposed to play an important role as an immune-stimulating and antioxidant agent. Crustaceans have both endogenous enzymes and exogenous, nonenzymatic compounds that can scavenge free radicals. High irradiation, oxygenic photoautotrophy, and the presence of xenobiotics could all cause the production of reactive oxygen species (ROS). Superoxide dismutase, catalase, and peroxidase are the major antioxidant

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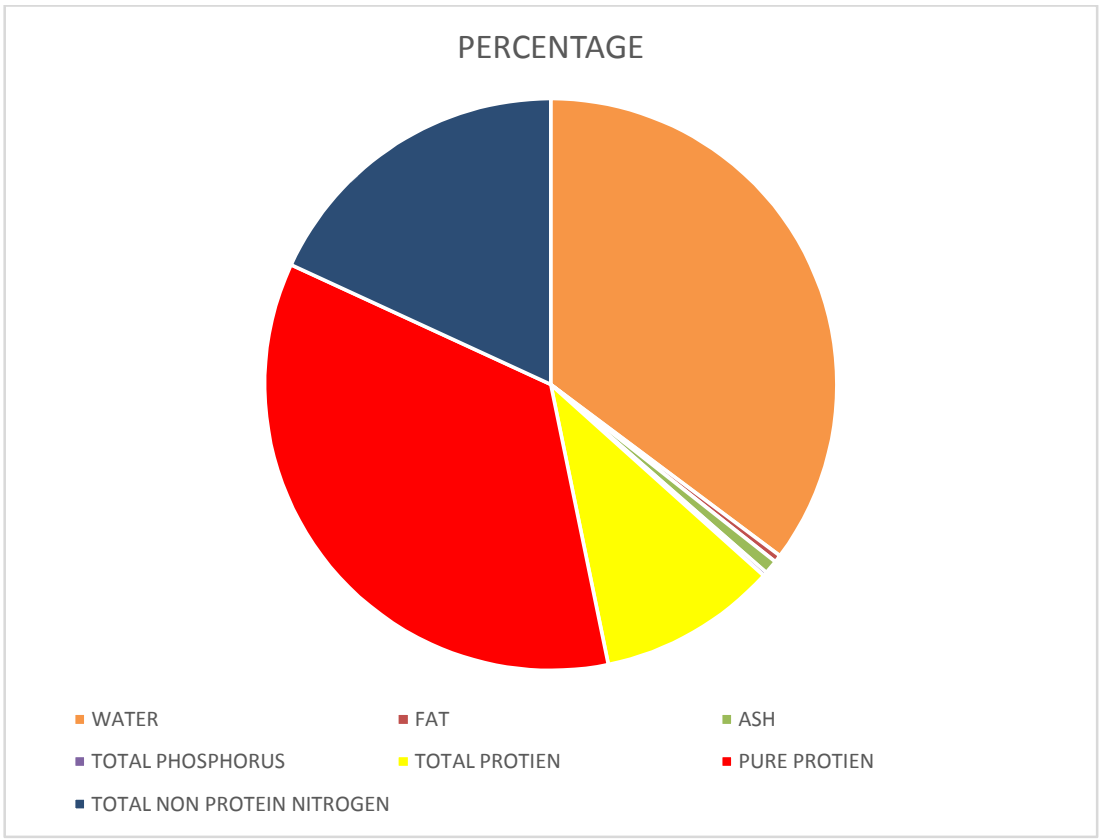
enzymes capable of detoxifying ROS. Crustaceans have peroxinectins, which are proteins involved in immune defenses and have peroxidase activity. Dietary intake provides nonenzymatic, exogenous compounds with antioxidant properties. Nonenzymatic antioxidants are classified as hydrophilic compounds found in aqueous cellular compartments such as vitamin C and glutathione, and lipophilic compounds found in cell membranes and associated with lipoproteins. As a result, carotenoids can regulate immunopathology and play an important role in immune defense modulation and evolution.

It has numerous health benefits and important pharmacological properties for the treatment of diseases such as diabetes, hypertension, cancer, heart disease, ischemia, and neurological disorders, and a potential role in the liver enzyme gamma-glutamyl transpeptidase, which is used as a diagnostic marker in medicine. Shrimps are the primary source of astaxanthin among crustaceans, and their presence protects them from the oxidation of polyunsaturated fatty acids and cholesterol. Finally, astaxanthin derived from shrimps is very effective against oxidative stress, which can cause a variety of diseases.

Antibiotic use in veterinary medicine and aquaculture has increased antimicrobial resistance in food-borne pathogens that can be transmitted to humans. The global concern is reflected in regulations from various agencies that have set the maximum allowed residue limits on antibiotics in various food matrices of animal origin. Monitoring residue levels in aquaculture species for routine regulatory analysis requires sensitive and selective methods. Because the most important step is sample preparation, several extraction methods have been developed. The goal of this review is to summarise the trends in the extraction of various antibiotic classes from shrimps and to compare the performance characteristics of the various approaches. Antibiotics like quinolone, Tetracyclines, Amphenicols, etc are extracted from shrimp by methods like LLE, SLE, SPE, etc.

II. CHEMICAL COMPOSITION OF SHRIMP



AIM: To study the effect of shrimp extract on E. coli.

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III. MATERIAL AND METHOD

3.1 Methodology

The devices used are stainless steel or ceramic cylinder. To obtain a uniform inhibition zone the Agar well method is applicable. In our laboratory, the agar-well method is occasionally beneficial for the assay of viscous materials. The Agar well diffusion method is extensively used to evaluate the antimicrobial activity of plants or microbial extracts. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a certain amount of theantibacterial agent or extract solution is injected into the well at the desired concentration. The agar plates are then incubated at the appropriate conditions for the test microorganism. The antimicrobial ingredient diffuses across the agar medium and stops the bacteria strain from proliferating. The spread plate technique is useful for separating microorganisms from a small sample volume dispersed over the surface of an agar plate. When the proper concentration of bacteria is used, distinct colonies are formed that are equally dispersed across the agar surface. In addition to using this technique for viable plate counts, in which the total number of colony-forming units on a single plate is enumerated and used to calculate the cell concentration in the tube where the sample was plated. Spread-plating is beneficial in enrichment, selection, and screening experiments. In most cases, the desired outcome for these three tests is the same as for plate counts, in which the distribution of discrete colonies forms across the surface of the agar. The goal isn't to ensure that every viable cell forms a colony.

Instead, only those cells in a population with a specific genotype should be supposed to thrive. The spread plate procedure may be preferred over the pour plate technique for an enumeration experiment if the end goal is to isolate colonies for further analysis because colonies grow accessibly on the agar surface, it becomes embedded in the agar with the pour plate procedure.

- Shrimp sample crushed in mortal and pestle
- 1 Petri dish
- Mac Conkey's agar
- Cork borer
- Pipette of 1 and 10 ml
- Saline suspension of ***E. coli***
- Spreader
- Distilled water
- Nichrome loop
- Methanol

Day 1-

Prepare the shrimp extract from the shrimp waste by crushing it in a mortar and pestle and adding methanol.

After extraction, evaporate the methanol and store it in sterile buffered distilled water.

Prepare one Mac Conkey agar Petri dish with the mentioned microorganisms by using the spread plate method and letting them rest for a few minutes.

Make 1 well in the centre of the plate using a sterile cork borer.

Transfer 0.05 to 0.2 ml of shrimp sample in the well of a plate aseptically using the pipette.

Incubate the plate in an incubator for 24 hours.

Day 2-

Measure the diameter of the zone of inhibition given by the sample of shrimp extract.

From this, we found the effect of the shrimp extract on the microorganism.

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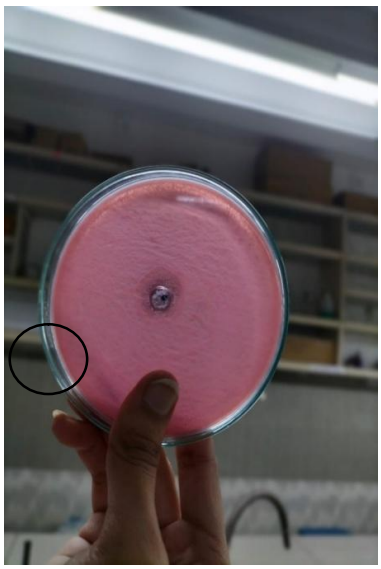
3.2 Sample Used

3.3 Observation



IV. RESULT

After incubation, a zone of inhibition was observed around the well of the sample. 19mm zone of inhibition was observed.

V. CONCLUSION

After experimenting, we were able to see a zone of inhibition of 19mm. Shrimp extract affects *E. coli.*

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