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**ORIGINAL RESEARCH**

# Antimicrobial plant extract incorporated gelatin beads for potential application in pharmaceutical industry

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**ABSTRACT**
Gelatin is a gelling protein, which has widely been applied in the food and pharmaceutical industries. Most commercial gelatins are derived from mammalian sources, mainly pigskin and cowhide but for many socio-cultural reasons alternative sources are increasingly demanded. Fish skin forms a major portion of the fishery waste and this can be processed into gelatin which solve the problem of waste disposal and also create value added products. In this study, fish skin wastes of both marine and fresh water fishes were collected from various localities and gelatin extraction was done by using Grossman and Bergman method. The gelatin content obtained from both types of fishes was compared and marine fish waste found to have higher content of gelatin. The plant leaves of *Psidium gaujava* (Guava), *Portulaca olaraceae* (Common Purslane), *Annona muricata* (Soursop) were taken to analyze their antimicrobial activity by standard disc diffusion method against *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp. and from the result *P. gaujava* plant leaf extract is found to have highest antimicrobial activity. The plant leaf with highest antimicrobial activity is then incorporated to the marine fish gelatin for the preparation of gelatin beads by emulsion cross linking method. Gelatin beads thus obtained has potential application in pharmaceutical, medical and food industry.

**KEY WORDS:** *Fish gelatin, Plant leaf extract, Antimicrobial activity, Gelatin beads*

## Introduction

Gelatin is a mixture of purified protein which is practically odorless and tasteless. It swells and softens in water, solubilizes in hot water and upon cooling to 35–40°C, it forms a gel. Gelatin is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties such as texturization, stabilization and emulsification. Though the global demand for gelatin has been increasing over the years concerns still persist among consumers with regard to its usage and this is mainly due to religious sentiments. Gelatin from marine sources is a possible alternative to bovine gelatin (Kim and Mendis, 2006).One major advantage of marine gelatin sources is that they are not associated with the risk of outbreaks of Bovine Spongiform Encephalopathy. Fish gelatin is acceptable for Islam, and can be used with minimal restrictions in Judaism and Hinduism. Processing of fish lead to enormous amount of fish skin waste that could be processed into gelatin, thus solving the problem of fish waste disposal in addition to creating value added products (Karim *et al*., 2009 and Arnesen *et al*.,2002).

Biodegradable and biocompatible polymers can be used as carriers of pharmaceutical active ingredients and are a useful strategy for the enhancement of their stability characteristics (Rijo *et al*., 2014). Gelatin beads can be used for this

purpose, as these beads could provide a larger surface area and possess an easier estimation of diffusion and mass transfer behavior (Khan *et al*., 2014). The use of herbal extracts as therapeutic agents has aroused interest in both developing and developed countries. The environmentally benign materials like plant extract have numerous benefits of eco-friendliness, compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol.

Many research papers reported the antibacterial efficiency of plant extracts like *Psidium gaujava* (Guava), *Portulaca olaraceae* (Common Purslane), *Annona muricata* (Soursop) against variety of bacterial species. Therefore, medicinal plant extracts having well established therapeutic importance can be incorporated into the gelatin beads. The fish gelatin can be used in microencapsulation and to make soft-gel capsules. The gelatin beads with the antibacterial property of the plant extract can be further utilized to fill the gelatin capsules with one or more bead populations using a commercial capsule filling equipment (Princely *et al*., 2015). It provides prolonged release of pharmaceutical compounds and protection from atmospheric agents (moisture, light, heat, oxidation (Khan *et al.,* 2014)).

## Materials and Methods

### Extraction of Gelatin

#### Fish skin preparation

Marine and fresh water fish skin wastes were collected from various localities of Thrissur, Kerala. Marine water fishes include Bluefin trevally fish (*Caranx melampygus*), Garfish (*Belone belone*) and fresh water fishes include Opheocephalus (*Channa striata*), Pearl spot (*Etroplus suratensis)*. At the lab, the skin was cleaned from any attached muscle and scales using sharp knife. Only 10 g of each fish skin samples were acquired. After cleaning the skin, it was chopped into fine small pieces (0.5×0.5 cm) and placed into 100 ml flask.

#### Extraction

The extraction procedure was conducted according to Grossman and Bergman (1992), with slight modifications. The fish were thawed prior to the experiments. The accurately weighed fish were cleaned and washed with tap water followed by peeling the fish skin using a sharp scalpel. The fish skins were thoroughly rinsed in excess water to remove superfluous materials. The fish skins were soaked in 0.2% sodium hydroxide for 40 min. After washing out sodium hydroxide, two successive acid incubations were performed, each for 40 min, first in sulphuric acid (0.2%) and then in a citric acid solution (1.0%). The acid solutions were drained and the samples were washed with cold water once.

The final extraction of gelatin was performed in distilled water at 45°C for 18 h. Solubilized gelatin was separated from residual skin fragments by filtration through a Whatman No.4 filter paper, collected and kept at –80°C for at least 24 h. Residual water was removed by lyophilization. The gelatin content obtained from each sample was further evaluated.

#### Antimicrobial screening of plant extracts

The plant leaves of *Psidium gaujava* (Guava), *Portulaca olaraceae* (Common Purslane), *Annona muricata* (Soursop) were taken for screening on the basis of their antimicrobial activity. The solvent which was used for the extraction of these leaves was 75% ethanol.

#### Extraction

Each plant leaves were sliced in to small pieces, kept in petriplates and oven dried.1 gm of each dried plant leaves were crushed with 5 ml of 75% ethanol in mortar and pestle. The aqueous extract transferred to Eppendorf tubes, kept for overnight incubation. It was centrifuged at 10,000 rpm for 10 min and supernatant transferred to labeled Eppendorf tubes. The extract was stored at 4°C for further analysis.

#### Antimicrobial Activity Test

The antimicrobial activity of plant leaf extracts were studied by standard well diffusion method on Muller Hinton Agar (MHA) plates. The media was autoclaved, cooled and poured in to the petriplates and kept for 30 min to solidify. The bacterial cultures (*Escherichia coli, Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp.) were swabbed on the MHA plates. Wells were prepared on the plates using sterile well puncture (5 mm diameter) and 100 µl of each plant leaf extracts were transferred to the wells made on the agar. The cultured plates were incubated at 37°C for 24 h without disturbing. The zone of inhibition was investigated.

**Selection of plant and synthesis of plant extract incorporated gelatin beads**

**Selection of plant**

Plant extract showing highest antimicrobial activity against the cultured bacteria (*Escherichia coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp.), based on the zone of inhibition was selected.

**Emulsion cross linking method**

In this method, plant leaf extract *Psidium gaujava* (Guava) was dissolved in aqueous gelatin solution, which was previously heated for 1 h at 40°C. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10 min at 35°C. Stirring was done for 10 min at 15°C. The produced beads were washed three times with acetone and isopropyl alcohol. It is then air-dried and dispersed in 5 ml of aqueous glutaraldehyde saturated toluene solution at room temperature for 3 hrs for cross linking. Afterward, beads were treated with 100 ml of 10 mm glycine solution containing 0.1% w/v of tween 80 at 37°C for 10 min (Khan *et al.,* 2014 and Reis *et al*., 2006).

## Results and Discussion

Gelatin was successfully obtained from the fish skin wastes of both marine and fresh water. The amount of gelatin obtained from each is given in Table 1. In this present study, 10 g of fish skin was used to extract gelatin and maximum gelatin content of 3.75 g was obtained from Marine water fish Bluefin trevally (*Caranx melampygus*).

**Table 1:** Gelatin Extraction

| Types of Fishes | Fish skin waste (g) | Gelatin extracted (g) |
|---|---|---|
| **Marine water Fishes** | | |
| **Bluefin trevally (*Caranx melampygus*)** | 10 | 3.75 |
| **Garfish (*Belone belone*)** | 10 | 2.85 |
| **Fresh water Fishes** | | |
| **Opheocephalus (*Channa striata*)** | 10 | 1.75 |
| **Pearl spot *(Etroplus suratensis*)** | 10 | 1.00 |

**Table 2:** Antimicrobial activity of plant leaf extracts

| Microorganisms | Zone of inhibition of *Psidium gaujava* (mm) | Zone of inhibition of *Portulaca olaraceae* (mm) | Zone of inhibition of *Annona muricata* (mm) | Zone of inhibition of control (75% ethanol) (mm) |
|---|---|---|---|---|
| ***Escherichia coli*** | 20 | 9 | 10 | 5 |
| ***Klebsiella* sp.** | 12 | 9 | 9 | 6 |
| ***Staphylococcus* sp.** | 23 | 11 | 12 | 5 |
| ***Salmonella* sp.** | 19 | 11 | 14 | 5 |
| ***Pseudomonas* sp.** | 22 | 11 | 11 | 6 |

Antimicrobial screening of therapeutic plant leaf extracts were done by standard well diffusion assay. From the result of antimicrobial study, *Psidium gaujava* plant leaf extract showed highest antimicrobial activity against *Escherichia coli, Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *and Salmonella* sp. than *Portulaca olaraceae*, *Annona muricata* and the control kept (Table 2, Figure 1).

*Psidium guajava* is commonly used as a medicine against gastroenteritis and child diarrhea and studies have shown that guava leaf extracts and essential oil are very active against *Staphylococcus* sp., *Salmonella* sp. etc. thus making up important potential sources of new antimicrobial

compounds (Goncalves *et al*., 2008). The *P. guajava* ethanol extract prepared were incorporated into the fish gelatin obtained, by emulsion cross linking method to generate the gelatin beads with the therapeutic property of the herbal plant. The successfully generated gelatin beads can be employed in various medical, food and pharmaceutical industry. This method is simple and can be easily adapted to the industrial scale.

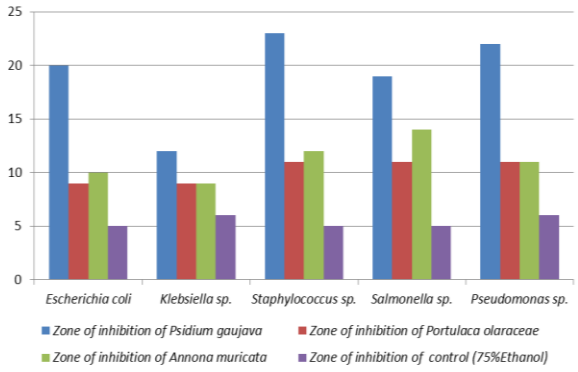


**Figure 1:** Antimicrobial activity of plant leaf extracts

This study may pave the way for further research and exploration of fish gelatin, as it fulfills the majority of consumer needs and meets the increasing demand for gelatin. Scaling up the extraction and production process of fish gelatin and securing control of the extraction conditions during the process pose a problem for processors. Therefore, more technological development research is required. The present investigation is successful in identifying the antimicrobial activity of selected medicinal plants against selected group of microbes and this property is exploited for the generation of therapeutic gelatin beads. The use of gelatin in pharmaceutical and biomedical applications had already gained importance because of its biocompatibility, biodegradability, absence of toxicity and allergic problems. Studies are now on-going and these benefits will carry the future developments which include the incorporation of this system into drugs for its sustained release, this system may even replace current standard of systemic antibiotic treatment.

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