Optimisation and Development of *Aegle marmelos* Incorporated *Prunus amaygdalus var dulcis* Gum Capsule Film

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Abstract

*Prunus amaygdalus var dulcis* gum commonly known as almond gum is a polymer. Capsules made up of almond gum was used as a stand-in to gelatin capsule and the fusion of *Aegle marmelos* was found to help prevent peptic ulcer. Anti-bacterial property of almond gum helps increasing the shelf life of capsules. To standardize and formulate *Aegle marmelos* incorporated *Prunus amaygdalus var dulcis* gum capsule and to assess the microbial and solubility properties of *Aegle marmelos* incorporated *Prunus amaygdalus var dulcis* gum capsule. The samples were formulated and standardized using five grams of almond gum and one gram of *Aegle marmelos*. Glycerol and sorbitol were used as plasticizers. Hydroxy Propyl Methyl Cellulose and carrageenan gum were used as a binding agent for the formulation of *Aegle marmelos* incorporated almond gum capsule and it entails antioxidant, anti-ulcerative and anti-bacterial property. Predominantly, the anti-ulcerative property was due to the tannin content. The microbial content and solubility properties were also determined and compared with gelatin capsules. The swelling property and moisture content of the sample decreased as the concentration increased. The total bacterial count of *Aegle marmelos* incorporated almond gum capsule of AMV1 was 5.0± 0.042 CFU/g and for AMV2 was 7.0±0.023 CFU/g when compared with standard (98.0 ± 0.05 CFU/g) and is significant at 5% level. The water solubility content of standard was 25% and for AMV1 and AMV2 was 80% and 97% respectively and found to be significant at 5% level. The swelling index of AMV1 was higher at 40 % when compared to standard (20%) and AMV2 (30%) and is due to presence of high volume of water using method I. Using method II, the swelling index of AMV1 was higher at 9.3 compared to other two samples of standard (-6.3) and AMV2 (-3.8) and is because of the medium density of the material. The moisture content of AMV1 was higher at 23% as determined by two methods when compared with standard and AMV2. The tannin content in *Aegle marmelos* helps to prevent peptic ulcer in individuals and almond gum, a natural polymer is a best vegetarian capsule alternative to gelatin capsule. The present study proved that anti-bacterial and solubility properties of *Aegle marmelos* incorporated almond gum and is far better in health perspective as compared to normal commercial capsule.

**Keywords:** Almond gum, *Aegle marmelos*, *Prunus amaygdalus*, vilvam, capsule, peptic ulcer, solubility, swelling index, moisture content, total bacterial count.

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1. Introduction

Almond gum is an exudate gum of sweet almond tree *Prunus amaygdalus*. Almond gum looks like an ice stone or little rock. The components present in almond gum can be used in the capsule shell formulation and it also has medicinal properties. Almond gum has medicinal purposes like to treat diarrhoea, dysentery, stomach ulcer, helps to gain weight and acts as a natural cooling agent. It is also used in pharmaceutical industries as an emulsifier, thickener, binder and stabilizer (Intiyyaz and Jahan, 2017). Almond gum consists 2.45, 0.85 and 92.36% of proteins, fats and carbohydrates respectively. Carbohydrates particularly comprise of 46.83% arabinose, 35.49% galactose and 5.97% uranic acid with traces of rhamnose, mannose and glucose. Rich sources of minerals namely sodium, potassium, magnesium, calcium and iron is present in gum exudate. Natural polysaccharides possess anti-cancerous, antioxidant and anti-inflammatory properties. Therefore, it is of great concern among the food scientists to find out alternate and new sources of biopolymers for use in food industry (Bashir and Haripriya, 2016).

*Aegle marmelos* is a medicinal plant and commonly known as vilvam in Tamil and other common names of *Aegle marmelos* are Aluvigam, Kuvilam, Mavilangai, Vilvm. *Aegle marmelos* is mainly known for its anti-diabetic and anti-ulcerative property. The tannin content in *Aegle marmelos* is responsible for curing / healing peptic ulcer. Tannin present in leaves is known to be skimmianin, also named as 4, 7, 8-trimethoxy furo-quinoine (Nilesh et al., 2012). *Aegle marmelos* leaves consists of more than 30 identified medicinal components and the major constituents of the leaf were identified to be tannins, skimmianin, essential oil (mainly caryophyllene, cineole, citral, citronellal, d-limonene andeugenol), sterols and/or triterpenoids, including lupeol, β and γ-sitosterol, α- and β-amyrin, flavonoids (mainly rutin) and coumarins, including aegeline, marmesin and umbellifero (Arul et al.,2005). Thus, the almond gum consists of natural polymer that can be used in the formulation of capsule along with other compounds and *Aegle marmelos* helps in preventing peptic ulcer. To our knowledge there is no such reports available on gum capsule shell and fusing nutrient into the capsule shell. Therefore, the aim of the study was to standardize and formulate *Aegle marmelos* incorporated *Prunus amaygdalus* var *dulcis* capsule and to assess the microbial and solubility properties of *Aegle marmelos* incorporated *Prunus amaygdalus* var *dulcis* capsule.

2. Materials and methods

2.1 Materials

Almond gum was purchased from local market in Coimbatore, Tamil Nadu. Vilvm leaf was preferred and procured from local households in Coimbatore, Tamil Nadu. Hydroxy propyl methyl cellulose, carrageenan gum, glycerol and sorbitol of food grade was purchased.

2.2 Formulation and standardisation of almond gum capsule (film)

2.2.1 Purification of almond gum

Twenty grams of almond gum weighed, washed using distilled water to remove impurities. It was dried for 6 hours at 70°C in cabinet dryer. The dried sample was ground into a fine powder using mortar and pestle, sieved using sieve no.25 to get fine uniform powder without any bigger particles or lumps. The almond gum capsule film was tried with two variations namely variation 1 (AMV1) and variation 2 (AMV2). For AMV1, three grams of almond gum was taken and treated with alkali. Alkali treatment was done by soaking almond gum (3 g) in 50 ml of 2% sodium hydroxide for 15 minutes. The alkali treatment was carried out to remove unwanted minute particles and debris present in the almond gum. The almond gum due to its swelling property after soaking for 15 minutes weighed to 60 grams. It was then rinsed using distilled water 4 to 5 times to remove the sodium hydroxide present in the almond gum. The almond gum was then filtered using muslin cloth to remove the minute floating particles and the weight of almond gum after alkali treatment was found to be 60 grams. For AMV2, 5.0 grams of almond gum and 100 ml of 2% sodium hydroxide were taken and the same procedure was carried out as mentioned for AMV1.

2.2.2 Standardization of almond gum capsule (film)

The film forming solution was formulated in a beaker by mixing 3 grams of 2% sodium hydroxide treated almond gum (AMV1) and added 50 ml of distilled water into the beaker for 30 minutes at 80°C. Hydroxy Propyl Methyl Cellulose (HPMC) was added in the beaker. HPMC is a polymer cellulose that gives physical appearance of gelatin in the formulation of gum capsule. To this, added one gram of carrageenan...
gum by mixing into the distilled water which acts as a binding agent. One ml of each sorbitol and glycerol were added to the solution. After the mixture melts completely, the resultant solution was further incubated for 15 mins at 60°C to remove and break the air bubbles formed if any. After gently stirring the solution, 15 ml of the solution poured into petri dishes of 5 mm diameter. The petri dish containing the film forming solution could dry at room temperature. It took ten hours for drying. After 10 hours, the film was formed.

2.3. Incorporation of Aegle marmelos into the standardised almond gum capsule (Film)

The fresh Aegle marmelos leaves collected were cleaned by removing stalks and stems and washed thoroughly. Then dried in room temperature (37°C) for 3 to 4 days. Drying at room temperature (shade drying) helps to prevent the loss of nutrients. Dried leaves were collected and finely powdered using mixer and sieved to get fine powder. To the standardised almond gum capsule solution i.e., for AMV1 and AMV2, one gram of Aegle marmelos was added into the film forming solution, stirred well using glass rod to ensure uniform distribution of Aegle marmelos powder and allowed to dry at room temperature.

2.4. Evaluation of Aegle marmelos incorporated almond gum capsule (Film)

The Aegle marmelos incorporated capsule film was subjected to further analysis. This includes analysis of microbial content and solubility properties.

2.4.1 Analysis of microbial content

Microorganisms is one of the risk factors to health and can spoil food. It includes bacteria, viruses, yeasts and molds. Microbial count of standard, AMV1 and AMV2 was done using standard procedures (FSSAI 2016).

2.4.2 Analysis of Solubility Properties

2.4.2.1 Water solubility test

About one gram of standard, AMV1 and AMV2 were taken and cut it into small pieces in three different test tubes. To the test tubes, added 10 ml of distilled or deionized water. Stirred gently with a glass stirring rod. This was done for 20 minutes. The solubility of the sample i.e., soluble or insoluble was recorded.

2.4.2.2 Swelling Index

Two methods were used to find the swelling index and the best method was identified.

Method 1 - One gram of sample namely standard, AMV1 and AMV2 were reduced to fine pieces and introduced into 25 ml measuring cylinder stoppered with glass. Added 25ml of water and shook vigorously for every 10 minutes for one hour and kept at room temperature. The volume occupied by sample, including sticky material was measured (Sarojini et al., 2010).

Method 2 - The swelling power of samples was determined. In a centrifuge tube added one gram of sample (standard, AMV1 and AMV2) and mixed 10 ml of distilled water, heated in a heating mantle at 80°C for 30 mins. After centrifugation at 1000 rpm for 15 minutes, the supernatant was discarded and paste weight was measured. The procedure was repeated three times to get concordant values. The swelling power of the sample was calculated using the formula. \( \text{Swelling power(g/g)} = \frac{\text{Weight of the paste}}{\text{Weight of the sample}} \)

2.4.2.3 Moisture content

Method 1 - Moisture content plays a major role in capsule formation. Moisture content was measured automatically using moisture balance.

Method 2 - Moisture content was expressed as percentage weight loss on drying. Two grams of ground sample i.e., standard, AMV1 and AMV2 was weighed and oven dried. Water was removed on drying at 105°C for 5 hours.

\[ \% \text{ Loss on Drying} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \]

2.4.2.4 Process of extrusion of Aegle marmelos incorporated almond gum capsule (film)

The film forming solution was formulated. The temperature (80°C) was maintained using heating mantle. Hydroxy Propyl Methyl Cellulose (HPMC) and carrageenan gum was also added. One ml of each sorbitol and glycerol were added to the solution by maintaining the constant temperature. Added one gram of...
Aegle marmelos powder into the solution. After the mixture melts completely, the resultant solution was further incubated for 15 mins at 60˚C. 15 ml of the solution was poured into petri dishes of 5 mm diameter after gentle stirring. The petri dish containing the film forming solution could dry at room temperature. It took ten hours for drying. After 10 hours, the film was formed. Further the film can be used for making soft capsule.

Other type of fabrication for making hard capsule shell was made by dipping glass rod into the warm solution and let it out drying for 10 hours. The dried cap was removed from the rod and stored.

3. Results and Discussion

3.1 Microbial content

**Total Bacterial count**-The analysis of microbial content includes total plate count which helps to denote the shelf life of the material. These tests were carried out for standard, AMV1 and AMV2 of Aegle marmelos incorporated almond gum capsule (film). Total bacterial count values were recorded for AMV1 and AMV2 in comparison with standard using the FSSAI procedure. The optimum total bacterial count of hard gelatin capsule should be less than 1000 CFU/g. The total bacterial count of the standard was 98.0 ± 0.05 CFU/g. The total bacterial count of Aegle marmelos incorporated almond gum capsule of AMV1 was 5.0± 0.042 CFU/g and for AMV2 was 7.0±0.023 CFU/g. The total bacterial count difference between AMV1 and AMV2 was +2.0 CFU/g. The bacterial count is extremely low than the standard due to the antibacterial effect of almond gum. This property of bacterial count indicates the long shelf life of the capsules. The Aegle marmelos incorporated almond gum capsule can be stored for long time with proper storage and handling (Table I). The disc’s diffusion method as given by Bouaziz et al., (2016) is used for antibacterial activities of SEAG.

**TABLE I**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Bacterial Count (CFU/g) (7th day)</th>
<th>Paired ‘t’ test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>98.0 ± 0.05</td>
<td>S Vs AMV1 607.5**</td>
</tr>
<tr>
<td>AMV1</td>
<td>5.0 ± 0.042</td>
<td>S Vs AMV2 275.2**</td>
</tr>
<tr>
<td>AMV2</td>
<td>7.0 ± 0.023</td>
<td>AMV1 Vs AMV2 11.13**</td>
</tr>
</tbody>
</table>

**- Significant at 5% level (p<0.05), AMV1 – AMV1, AMV2 – AMV2, S-Standard

Statistical analysis of bacterial count present in Aegle marmelos incorporated almond gum capsule revealed that there was significance at 5% level when the samples were compared. It was observed that a statistical significance of 5 % was observed when standard and AMV1; standard and AMV2; and AMV1 and AMV2 were compared. It shows that there existed less bacterial count in Aegle marmelos incorporated gum capsule when standard and AMV1 and AMV2 were compared. This was due to the incorporation of Aegle marmelos in AMV1 and AMV2, respectively. Whereas low statistical significance was noted when AMV1 and AMV2 were compared. This is due to the low percentage concentration of Aegle marmelos in AMV1 and AMV2. Hence, this shows that the total bacterial count in standard, AMV1 and AMV2 is different and very slight variation was noted comparing AMV1 and AMV2. It can be observed that Aegle marmelos incorporated almond gum capsule has long shelf life when compared to the standard.
3.2. Solubility properties of *Aegle marmelos* incorporated almond gum capsule (Film)

The solubility properties of *Aegle marmelos* incorporated almond gum capsule (Film) includes water solubility test, swelling index, moisture content and. It was done for standard, AMV1 and AMV2 using standard procedures and is shown in Table II. Figures 2, 3 and 4 depict the solubility properties of *Aegle marmelos* incorporated almond gum capsule.

**TABLE II**

**SOLUBILITY PROPERTIES OF *AEGLE MARMELOS* INCORPORATED ALMOND GUM CAPSULE (FILM)**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Water Solubility Test (%)</th>
<th>Swelling Index</th>
<th>Moisture Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method 1</td>
<td>Method 2</td>
<td>Method 1</td>
</tr>
<tr>
<td>Standard</td>
<td>25</td>
<td>20%</td>
<td>3.0 ±0.03</td>
</tr>
<tr>
<td>AMV1</td>
<td>80</td>
<td>40%</td>
<td>9.3 ±0.21</td>
</tr>
<tr>
<td>AMV2</td>
<td>97</td>
<td>30%</td>
<td>5.5 ±0.13</td>
</tr>
<tr>
<td>Pairs</td>
<td>Paired ‘t’ test</td>
<td>7.366NS</td>
<td>4.305NS</td>
</tr>
<tr>
<td>S Vs AMV1</td>
<td>254.6**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S Vs AMV2</td>
<td>196.7**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMV1 Vs AMV2</td>
<td>38.17**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**- Significant at 5% level (p<0.05), NS - Not Significant; AMV1 – AMV1, AMV2 – AMV2, S-Standard**

3.2.1. Water solubility test

The water solubility test was done for standard, AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) using standard procedure. The water solubility content of standard was found to be 25% and the solubility level for AMV1 and AMV2 was 80% and 97% respectively. According to (Laohakunjit and Noomhorm, 2004) hydroxyl group increases the solubility. Therefore, hydroxyl propyl methyl cellulose belonging to hydroxyl group present in AMV2 is higher i.e., 5% than in AMV1 (2 %). Thus, AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) showed higher solubility compared with other samples of standard and AMV1.

Solubility is defined as the ability to be dissolved and it also denotes the digestible property of the food particle. Drug absorption and physiological availability depends on the drug substance being in the dissolved state at the site of drug absorption (Mahato and Narang, 2018). Statistical analysis of water solubility of *Aegle marmelos* incorporated almond gum capsule revealed that there existed a 5 % significant difference when all the three samples namely standard and AMV1; standard and AMV2; and AMV1 and AMV2 were compared.

**Figure 1**

**Total Bacterial Count of *Aegle marmelos* Incorporated Almond Gum Capsule (film)**

The solubility properties of *Aegle marmelos* incorporated almond gum capsule (Film) includes water solubility test, swelling index, moisture content and. It was done for standard, AMV1 and AMV2 using standard procedures and is shown in Table II. Figures 2, 3 and 4 depict the solubility properties of *Aegle marmelos* incorporated almond gum capsule.
3.2.2. Swelling index

**Method 1** - The mean values of swelling index (using method 1) of standard, AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) revealed that standard had a swelling index of 20%, AMV1 had a swelling index of 40% and AMV2 had a swelling index of 30% as shown in Table II. The swelling index of AMV1 was higher compared to standard and AMV2. This is due to high volume of water.

**Method 2** - The mean values of swelling index (method 2) was analysed for standard, AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film). The mean swelling index (using method 2) of standard, AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) formulations were 3.0 ± 0.03, 9.3 ± 0.21, 5.5 ± 0.13, respectively. The swelling index of AMV1 is higher compared to other two samples of standard (-6.3) and AMV2 (-3.8) and this is because of the medium density of the material. The swelling property is also due to carrageenan gum in the almond gum capsule film. Figure 3 shows the swelling property of the sample. The value of AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) was found to be similar when compared with the results of (Sarojini et al., 2010). The results can be compared with edible active packaging enriched films as given by Srisuruthi and Balasasirekha (2020).

Statistical analysis of swelling index of *Aegle marmelos* incorporated almond gum capsule revealed that there existed no significant difference when all the three samples were compared.

3.2.3. Moisture content

**Method 1** - The moisture content (method 1) of standard, AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) as determined using digital moisture balance revealed 17%, 23%, and 20% respectively. The moisture content of AMV1 was slightly higher compared to other samples.

**Method 2** - The moisture content (method 2) of standard capsule was found to be 15%. The moisture content of AMV1 and AMV2 of *Aegle marmelos* incorporated almond gum capsule (Film) was recorded as 23% and 20% respectively and is shown in the Figure 4. The moisture content of the AMV1 is 8% and that of AMV2 is 5% higher when compared with the standard.

Statistical analysis of moisture content of *Aegle marmelos* incorporated almond gum capsule revealed that there existed no significant difference when all the three samples namely standard and AMV1; standard and AMV2; and AMV1 and AMV2 were compared. Overall, the moisture content should be between 15% and 30%.

![Figure 2](image-url)

**Figure 2**

Water Solubility of *Aegle marmelos* Incorporated Almond Gum Capsule (Film)
Swelling Index and Moisture content of Aegle marmelos Incorporated Almond Gum Capsule (Film)

4. Conclusion

The present study proved that the Aegle marmelos incorporated almond gum capsule has health benefits like anti-ulcerative, antioxidant and anti-bacterial effect than the normal commercial capsule. Commercial capsules are tasteless and nutrient less. Addition of Aegle marmelos incorporated almond gum capsule helps to prevent various diseases particularly peptic ulcer as they are rich in tannin. It can be stated that the addition of medicinal plants and herbs as part of capsule formation may improve the antioxidant level apart from the normal therapeutic treatment. Introducing plant-based gums and mucilage in the pharma field can help to reduce chemical materials in the drug formulation. Thus, the Aegle marmelos incorporated almond gum capsule (film) is cost-effective, eco-friendly and non-toxic compared with their analogues. Thus, the present study was an effective alternative for a synthetic and chemical-based capsule.

References


