Design of Polymeric Nanoparticles of \textit{Emblica officinalis} extracts and study of \textit{in vitro} therapeutic effects

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Abstract

The therapeutic values of commonly available plants are of more interest due to its beneficial pharmacological activities. \textit{Emblica officinalis} (EO) belongs to Euphorbiaceae family, a most commonly available fruit plant in the tropical and subtropical regions of India. This plant is observed to show several medicinal properties and especially, its fresh fruit juice is used for ophthalmic, carminative, digestive, stomachic, laxative, aphrodisiac, rejuvenative, diuretic, antipyretic purposes and to enhance the memory. The objective of present work is to extract the active ingredients present in the dried fruits of \textit{Emblica officinalis} (EO) by cold maceration using ethanol and hot decoction process using water. The crude extracts are developed into polymer-herbal nanoparticle by solvent evaporation process using polyvinyl pyrrolidine (PVP) as the polymer and characterized for its size and stability. The entrapment efficiency and release study is performed for the nanoparticle formulations. The crude herbal extracts and their nanoparticle formulations are evaluated for anti-oxidant and anti-inflammatory activity by \textit{in vitro} methods. Also the stability and skin irritation test are performed to understand the effect of the formulation.

Keywords: \textit{Emblica officinalis}, Maceration, Decoction, Herbal Nanoparticles, Anti-oxidant, Anti-inflammatory

Introduction

\textit{Emblica officinalis} (EO), commonly called as the Indian gooseberry has a very reputed place in the field of Ayurveda as is believed that the plant EO was the first plant on earth. This is a native Indian plant commonly seen in tropical and subtropical regions. There are several therapeutic properties believed and proved for the parts of the plant. The fruit exhibits several activities like antioxidant, immune modulatory, antipyretic, analgesic, cytoprotective, anti-tussive and gastro protective. They are also found to be beneficial in the treatment against cancer, diabetes, liver diseases, heart problems etc. From past many decades \textit{Emblica Officinalis} was used in home as a memory enhancer and to lower cholesterol levels. It is also helpful in neutralizing snake venom and as an antimicrobial agent (1, 2).

There are several compounds primarily identified in EO like tannins, carbohydrates, amino acids and alkaloids. It is also observed that the fruits juice contained a high amount of vitamin-C about 478.56 mg/100 mL. Also when the EO fruit juice is blended with other fruit juices, there is boost up effect on the nutritional content and quality, in terms of vitamin-C.

Low molecular weight (<1000) of hydrolysable gallotannins (EOT) isolated from the fresh juice of EO proved to contain the emblicanin A, emblicanin B, punigluconin and pedunculagin, specifically showed a significant antioxidant effect...
by the *in vitro* tests performed (3, 4). Even the *in-vivo* test showed to exert a significant antioxidant effect against iron-overload hepatotoxicity and it elevated the rat frontal cortical and striatal concentrations of superoxide dismutase, catalase and glutathione peroxide and reduce lipid peroxidation, especially in the brain region of rats. EO usually protects the cells against free radical damage by giving an antioxidant protection. There were several studies that proved the enhancement of the activity of immune system as there was a constant increase in the white blood count and the strengthening of the immune system. This fruit showed a reduced UV-induced erythema and was observed to be an excellent free-radical quencher. Even the chelating ability of the fruit to compounds like iron and copper, helped in the inhibitory activity of MMP-1 and MMP-3 was proved (5, 6, 7).

With all these efficient properties of EO, the present work concentrate on designing herbal extract nanoparticle formulation which can improve the potential activities. The entrapment efficiency and release studies of the formulations are tested and compared to the crude extracts. The formulation is exposed to test its improvement of Anti-oxidant and Anti-inflammatory properties.

**Materials and Methods**

**Plant material collection:** The fruits of *Emblica officinalis* (Euphorbiaceae family) was collected, identified and authenticated by Dr. Ravichandran, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, India.

**Extraction by cold maceration:** The fruits of EO was first cut into small pieces, seed removed and dried completely without any amount of moisture present in it. This dried fruit was then pulverised and made into fine powder. About 75g of the powder was taken in a beaker and a required amount of petroleum ether was poured to the content and allowed to macerate overnight. The next day the content of primary extract filtrate was discarded and the residue was air dried for 30 min. This dry sample was again treated with 100mL of ethanol and kept overnight for extraction. The next day the filtrate was collected and evaporated in a water bath for 24 hours at 70°C. The dry extract was obtained after the evaporation process was complete (8).

**Extraction by hot decoction:** Another 75g of EO powder was taken in a beaker with 100mL of distilled water. This was boiled to 100°C for 15 min and stirred continuously. Then the solution was filtered and the filtrate was taken for solvent evaporation process. The process was done for 24 hours at 70°C till a dry powder extract of the fruits was obtained (9, 10).

**Preparation of Polymeric Nano-particles:** The nanoparticles of herbal extract with polymer were formulated by solvent evaporation process. In this process an aqueous solution of the extract was prepared by dissolving 0.02 g of dry extract with 10ml of water and 0.01g of Pluronic F68 was added as a surfactant. The organic phase containing the polymer was prepared with 0.05g, 0.10g and 0.15g of PVP with 2ml of dichloro methane (DCM) separately. The aqueous solution was sonicated for 20 min at 100 Kv using a probe sonicator (P250 Vibronics, India), into which the organic solvent was poured in drops. Then the mixture was placed for constant stirring in a magnetic stirrer for 20 min, until the organic solvent is completely evaporated (11, 12). This process was performed for the extracts obtained by cold maceration and hot decoction process to formulate the nanoparticles in different composition of polymer. A blank nanoparticle containing the polymer and surfactant (without extract) was also formulated for comparative studies.

**Calibration of the EO extract by UV-Visible Spectrophotometer:** The aqueous extract was dissolved in water, whereas the ethanol extract was dissolved in small amount of ethanol, followed by addition of water, and then suitably diluted. The solutions were scanned for its
specific UV-Visible range of absorbance maxima. Then the absorbance of the different serial diluted samples was measured at the $\lambda_{\text{max}}$, and a standard calibration curve was plotted with concentration against absorbance.

**Entrapment efficiency of the polymeric herbal nanoparticles:** The nanoparticle suspension formulated with the extract and polymer was ultra centrifuged at 18,000 rpm for 30 minutes in a cooling centrifuge apparatus (Sigma 3K30, Germany) at -10°C and then the supernatant solution was diluted suitably to measure the absorbance, from which the concentration of drug in supernatant was calculated using the standard calibration data. The entrapment efficiency of the extract in the polymeric nanoparticles was calculated using the formula (13),

\[
\% \text{ Entrapment Efficiency} = \frac{\text{Total drug content} - \text{Drug content in supernatant}}{\text{Total drug content}} \times 100
\]

**Particle size and surface charge analysis of the nanoparticles:** The nanoparticle formulations of both the extracts were analysed by a zeta analyser (Malverm Nano Series ZS, UK) to verify for the particle size based on dynamic light scattering technique and the zeta potential based on charge conductivity principle, to ensure the uniformity of size distribution and the stability of the formulation, respectively (14).

**In-vitro anti-Inflammatory bioassay:** The *in-vitro* anti-inflammatory bioassay was performed based on the protein denaturation principle method. A mixture containing a total of 5ml was prepared that consisted of 0.2ml of egg albumin with 2ml of varying concentrations of the sample (aqueous crude extract prepared by cold and hot maceration and their respective nanoparticles in separate test tubes) so that final concentrations become 30, 60, 120, 240, 500 µg/ml with 2.8ml of phosphate buffered saline (PBS, pH 6.4) in each solution. Distilled water of similar volume was used as control. Then these samples were incubated at 37 ± 2°C for 15 minutes and then heated for 5 minutes at 70°C. After cooling, the absorbance of the solutions was measured at 660 nm by using the respective blank. Diclofenac sodium was used as a reference drug, which was treated similarly with egg albumin and the final concentration (78.125, 156.25, 312.5, 625, 1250 µg/mL) were measured for its absorbance (15). The percentage inhibition of protein denaturation was calculated for all the formulations and crude extract using the formula,

\[
\% \text{ Inhibition} = \frac{V_t}{V_c - 1} \times 100
\]

Where, $V_t$ = absorbance of test sample, $V_c$ = absorbance of control.

**Anti-oxidant activity by ferric reducing assay:** Different concentrations of the crude extracts obtained by cold and hot maceration at their respective nanoparticles at various concentration (100, 200, 300, 400 and 500µg/ml) was dissolved in 1 ml of methanol separately. To each test tube 2.5 ml of phosphate buffer of pH 6.6 and 2.5 ml of 1% potassium ferri cyanide was added. These tubes were kept in water bath at 50°C for 20 minutes and then cooled rapidly. The cooled samples were mixed with 2.5 ml and 0.5 ml of 10% trichloroacetic acid and 0.1% ferric chloride respectively and this mixture was incubated for 10 min. A Perl's Prussian blue colour was formed due to the presence of iron (II)-ferricyanide complex formation. This was determined by measuring the absorbance at 700 nm (16, 17, 18).

**In vitro release studies:** The extracts obtained by cold maceration and hot decoction, and their nanoparticles prepared with different ratios of polymers was evaluated for the percentage of release of the extract constituents, for 6 hours with first one hour samples estimated for every 15 min followed by sampling at every one hour to estimate the immediate or sustained release effect of extract from the polymeric nanoencapsulation complex (19). 2ml of the nanoparticle formulation was placed in a plastic tube with one side sealed with dialysis membrane and this tube is placed into the basket of USP type I apparatus (DS 8000 Labindia, India) and
dipped into the dissolution chamber containing 100 mL of distilled water as media with the bath temperature of 37 ± 0.5ºC. The study was carried at 100 rpm and the samples of 10 mL were withdrawn using a syringe at the end specified predetermined time intervals. The collected samples were analyzed using UV-Vis Spectrophotometer at the λmax.

**In-vitro drug release kinetics:** Kinetic modeling was performed to understand the kinetics and mechanism of extract release from the nanoparticles. In these analysis results of *in vitro* dissolution studies were fitted with various kinetic equations like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi’s model (cumulative % drug release vs. square root of time), Hixon model (Cube root of drug % remaining in matrix vs. Time) and Korsmeyer-Peppas (Log cumulative % drug release vs. log time). The R² and n values obtained from the linear plots were used to analyze the mode of extract realease from the nanoparticulate system (20).

**Results and Discussion**

**Calibration of the crude extract:** The crude ethanolic and aqueous extract of EO dissolved in suitable solvent, diluted in distilled water and scanned in a UV-spectrophotometer have shown the maximum absorbance wavelength at 273.7 nm. The serially diluted samples of both the extracts exhibited the absorbance values for the calibration as shown in Table 1 and their regression was found to be 0.999 and 0.993 for ethanolic and aqueous extract respectively (Fig. 1).

**Entrapment efficiency:** Amount of extract entrapped in the polymeric nanoparticle determined using UV-VIS spectrophotometer showed that both the extracts of different nanoparticle formulation had higher entrapment efficiency in the range of 58 – 70%. There was no significant difference observed in the formulations prepared with different concentrations of the polymer, since the surfactant level, amount of extract used and all other process variables were kept constant during the nanoparticles development.

**Particle size and zeta potential of nanoparticles:** The average size of the synthesised nanoparticles of ethanolic and aqueous extract was found to lie within the range of 550-825 nm. Size range of polymeric nanoparticles as 100-1000 nm could provide stable colloidal dispersion (14). Surface charge measured in terms of zeta potential plays a major role for nanoparticles stability as the standard criteria. The zeta potential of the synthesized herbal nanoparticles was found to be -7.49 mV to -13.0 mV. Zeta potential greater than +25mV and less than -25mV correlate to higher stability of the nanoparticles(12). Thus the below

![Fig. 1. Calibration curve of *Emblica officinalis* extract (a) Ethanolic (b) Aqueous](image-url)
tabulated values illustrated that the synthesised nanoparticles exhibited average stability.

The results of entrapment, size and charge of the nanoparticles are shown in table 2, where E1, E2, E3 – Nanoparticles of EO ethanolic extract with 50 mg, 100 mg and 150 mg PVP, respectively, A1, A2, A3 – Nanoparticles of EO aqueous extract with 50 mg, 100mg, 150 mg PVP, respectively.

**Anti-oxidant activity of the nanoparticles:** The results of anti-oxidant assay performed for 5 different concentrations (100, 200, 300, 400, 500 µg/mL) for the ethanolic extract nanoparticles (E1, E2, E3) and aqueous extract nanoparticles (A1, A2, A3) was compared with the standard values taken from Ascorbic acid as shown in Table 3. As the data of nanoparticles were compared with standard Ascorbic acid the Fig. 3 showed the higher reducing power of the EO nanoparticles converting ferrous to ferric which depict the scavenging of the free radicals. The increase in absorbance of the reaction mixtures indicated increased reducing power. The ethanolic extract nanoparticles showed that E1 formulation with 50mg of PVP showed a higher activity than the formulations E2 and E3, due to the faster release of the EO extract from the polymeric coating with low amount of polymer (50 mg), than the formulations E2 and E3 with higher polymer concentration (100 and 150 mg respectively). In case of the aqueous extracts A1, A2 and A3, even with increasing amount of polymeric coating in all the three formulations, they showed an increased consumption of the free radicals than the standard Ascorbic acid or E2 and E3 formulation confirming the highest potential activity of the aqueous extract of EO. The antioxidant activity found in all the EO formulations showed increased activity and this could be due to the presence of high Flavonoids in the extracts that was confirmed from the phytochemical analysis done previously (18).

![Fig. 2. Size and Zeta potential of Emblica Officinalis Nanoparticles (a) Ethanolic extract with 50mg PVP (b) Ethanolic extract with 100mg PVP (c) Ethanolic extract 150 mg PVP (d) Aqueous extract with 50 mg PVP (e) Aqueous extract with 100 mg PVP (f) Aqueous extract 150 mg PVP](image)
**Anti-inflammatory activity:** EO showed a very high protein denaturation effect *in vitro* when egg albumin was used as the protein (15, 21). EO extract polymeric nanoparticles of different concentrations showed a higher anti-inflammatory activity when compared to Diclofenac sodium as a standard. The comparative evaluation shown in Fig. 4 depicted a cumulative evaluation of the standard and the nanoparticles formulated from both extracts, wherein the higher activity in the nanoparticle formulations was observed, especially with decreasing concentration of the polymeric coat in ethanolic extract formulation as E1 showed higher activity than E2 and E3. But all the aqueous extracts A1, A2 and A3 showed higher activity than the ethanolic and the standard Diclofenac sodium, inspite of the changes in concentration of polymer used. This proved that the percentage inhibition effect was higher in the nanoparticle formulation of aqueous extract prepared when compared to the ethanolic extract samples, due to the presence of active anti inflammatory constituents in the aqueous extract of *Emblica Officinalis*.

**Release studies from nanoparticles:** The release of the extract from the nanoparticles prepared with different concentrations of polymer was compared for both the extracts (Fig. 5). Nanoparticle formulation A1 containing aqueous extract with 50 mg of polymer, have shown the highest release of 56% at the end of 6 hours. Whereas, when the polymer composition was increased to 100 mg and 150 mg in A2 and A3 formulation respectively, the drug release was found to decrease to 30% and 26% due to the higher polymer coating or encapsulation. A similar profile was observed in ethanolic extract nanoparticles as 33%, 30% and 23% release of the extract at the end of 6 hours from E1, E2 and E3 respectively. Also in case of the ethanolic extract nanoparticles, there was a lag time observed in the release profile, as the extract was released form the nanoencapsulated system only after 1.5 hours. Whereas in case of aqueous extract sample, the extract release was observed...
from initial 15 min, providing linear release profile. When the aqueous and ethanolic extract nanoparticles were compared, we could observe that the percentage release was higher in aqueous extract due to its exposure to aqueous dissolution media. The significant reason for the faster dissolution of the aqueous extract may be due to the presence of aqueous soluble active constituents in the sample exposed to the aqueous dissolution media. Also the nanosize of the sample and its solubilization played a special role in the dissolution rate of the formulations.

### Table 1. Calibration data of *Emblica officinalis* extract at 273 nm

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Absorbance of ethanolic extract</th>
<th>Absorbance of Aqueous extract</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>0.082</td>
<td>0.218</td>
</tr>
<tr>
<td>4</td>
<td>0.164</td>
<td>0.434</td>
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<tr>
<td>6</td>
<td>0.247</td>
<td>0.651</td>
</tr>
<tr>
<td>8</td>
<td>0.34</td>
<td>0.886</td>
</tr>
<tr>
<td>10</td>
<td>0.41</td>
<td>1.114</td>
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</table>

### Table 2. Physico chemical characterization of *Emblica officinalis* nanoparticles

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
</tr>
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<tbody>
<tr>
<td>Entrapment efficiency (%)</td>
<td>68.5</td>
<td>66</td>
<td>67.5</td>
<td>66.05</td>
<td>70.65</td>
<td>58.94</td>
</tr>
<tr>
<td>Size (nm)</td>
<td>548.1</td>
<td>793.3</td>
<td>629.7</td>
<td>693.9</td>
<td>825.8</td>
<td>770.9</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>-9.91</td>
<td>-13.0</td>
<td>-12.3</td>
<td>-11.4</td>
<td>-11.3</td>
<td>-7.49</td>
</tr>
</tbody>
</table>

### Table 3. Anti-oxidant activity of *Emblica officinalis* Nanoparticles by FRAP method

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Ascorbic acid</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.318±0.003</td>
<td>0.226±0.002</td>
<td>0.146±0.005</td>
<td>0.307±0.012</td>
<td>0.276±0.034</td>
<td>0.317±0.002</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.34±0.004</td>
<td>0.255±0.026</td>
<td>0.161±0.006</td>
<td>0.32±0.007</td>
<td>0.32±0.004</td>
<td>0.325±0.004</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.413±0.009</td>
<td>0.319±0.018</td>
<td>0.285±0.089</td>
<td>0.346±0.001</td>
<td>0.354±0.009</td>
<td>0.358±0.009</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.426±0.004</td>
<td>0.38±0.013</td>
<td>0.306±0.072</td>
<td>0.417±0.012</td>
<td>0.421±0.023</td>
<td>0.418±0.041</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.472±0.006</td>
<td>0.424±0.006</td>
<td>0.343±0.039</td>
<td>0.488±0.005</td>
<td>0.468±0.003</td>
<td>0.452±0.039</td>
<td></td>
</tr>
</tbody>
</table>

**Drug release kinetics**: The release study data fitted to various kinetic models revealed the mechanism of extract release from the polymeric nanoparticle formulations. As shown by the results in table 4, the $R^2$ value observed for all the nanoparticles of both aqueous extract and ethanolic extract showed the mechanism of release of the active constituents from the nanosystem could be attributed to Korsmeyer-Peppas model, which explain the diffusion of the extract from the polymeric matrix. Also the mode of diffusion was found to be super case II transport phenomenon based on the n-value > 0.89.

**Conclusion**

The development of herbal nanoparticles using polymeric substance was found to be very successful in providing linear release of the encapsulated extract containing active ingredients in it. The entrapment efficiency and required size could be achieved by optimizing the composition of the variables used and the
process parameters. Also the in vitro therapeutic effects of the formulated nanoparticles showed significant improvement in the anti oxidant and anti inflammatory activity of both the aqueous and alcohol extracted contents.

Acknowledgement
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References

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Table 4. Release kinetics of the Emblica officinalis Nanoparticles

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi R² values</th>
<th>Korsemeyer-Peppas</th>
<th>Hixon - Crowell</th>
<th>n-value</th>
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<tbody>
<tr>
<td>A1</td>
<td>0.9718</td>
<td>0.9406</td>
<td>0.7192</td>
<td>0.9761</td>
<td>0.9538</td>
<td>1.090</td>
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<tr>
<td>A2</td>
<td>0.9017</td>
<td>0.8743</td>
<td>0.5803</td>
<td>0.9606</td>
<td>0.8838</td>
<td>1.421</td>
</tr>
<tr>
<td>A3</td>
<td>0.8974</td>
<td>0.8913</td>
<td>0.6514</td>
<td>0.9005</td>
<td>0.8942</td>
<td>1.074</td>
</tr>
<tr>
<td>E1</td>
<td>0.9391</td>
<td>0.9041</td>
<td>0.6439</td>
<td>0.9787</td>
<td>0.9163</td>
<td>1.317</td>
</tr>
<tr>
<td>E2</td>
<td>0.9800</td>
<td>0.9562</td>
<td>0.7055</td>
<td>0.9931</td>
<td>0.9650</td>
<td>1.158</td>
</tr>
<tr>
<td>E3</td>
<td>0.9155</td>
<td>0.8959</td>
<td>0.6564</td>
<td>0.9310</td>
<td>0.9028</td>
<td>1.184</td>
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</table>


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