Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 4, No. 3, July 2019

Research Paper

Physical Interaction Of Chitosan-Alginate Entrapping Extract Of Papaya Leaf And Formation Of Submicron Particles Dosage Form

Budi Untari[1], Dina Permata Wijaya[1], Mardiyanto[1]*, Herlina[1], Via Angraeni[1], Ario Firana[1]

[1] *Department of Pharmacy Faculty of Science, Sriwijaya University (UNSRI)*
***Corresponding author**: mardiyantoUNSRI@gmail.com

Abstract
Research on physical interaction of chitosan-alginate entrapping extract of papaya leaf (*Carica papaya L*) into submicron particles formation has been performed. Preparation of papaya leaf extract into submicron particle dosage form of chitosan and sodium alginate polymer using ionic gelation method aimed to increase the solubility of extract. Submicron particles consisting of papaya leaf extract, chitosan, sodium alginate and $CaCl_2$ were combined using variation of stirrer speed of 500, 750, and 1000 RPM. The optimum formula obtained has a speed of 1000 RPM with the percent EE value of 71.90%. The results of submicron particles characterization such as diameter and particle size distribution (PDI) using particle size analyzer (PSA) tools were 189.2 nm and 0.330. The results of XRD revealed the changes of type of crystalinity form to amorphous on submicron particles. The results of FTIR revealed the physical interaction without shifting of wave number of carbonyl, amine, and hydroxyl group which indicated that there were no chemical interactions occurred. These data indicated that papaya extract can be formulated into submicron particles of chitosan-alginate polymer.

Keywords
Interaction, chitosan-alginate, papaya leaf, submicron-particles

Received: 20 June 2019, Accepted: 18 July 2019
https://doi.org/10.26554/sti.2019.4.3.64-69

1. INTRODUCTION

Solubility is one of the limitations of extract for production of the dosage form. Study regarding physical interaction is focussed to solve the problem of solubility (Akerholm and Salmen, 2001) Problem of solubility is relevant to bioavalability level of active ingredient in blood circulation of human. The classification system of bioavalabilty was classified by 4 types: biopharmaceutics classification system (BCS) class I to IV. BCS II is poor of solubility and good of permeation. Using of Hydrophylic polymer such as chitosan (Berger et al., 2004) which has interaction to active ingredients can increase the solubility (Kim et al., 2002).

DHF is a disease caused by the dengue virus that is transmitted through the bite of the *Aedes aegypti* mosquito (Moektiwardoyo et al., 2014; Natalia et al., 2013). Herbal medicines has already used (Lin et al., 2014; Jain et al., 2008) to encourage the pain of hemorrhagic fever (DHF) (Suwanbamrung et al., 2013; Zunjar et al., 2016). Papaya extract is also can be used as anti DHF (Ahmad et al., 2011). Vector control is one of the solutions to eradicate dengue diseases. One of them is by utilizing chemicals such as Abate® powder, but the use of these chemical larvacides produces a lot of residues that can inhibit the growth of other non-targeted organisms, influence human health and in repeated administration can lead to resistance cases.

An alternative strategies to reduce the negative effects of synthetic larvacides is by developing biolarvacides that are safe and environmentally friendly; one of them is by using herbal materials such as papaya leaves. Papaya leaves (*Carica papaya L*) are usually used as wound healing, anti parasite, and for dengue fever. Papaya leaf extract contains active ingredients such as the enzyme papain, carbaine alkaloid, and flavonoids. Flavonoid compounds can affect the respiratory system of adult mosquitoes, papain substances can inhibit larval growth, and carpaine alkaloids are toxic to the larvae of *Aedes aegypti* mosquitoes (Govindarajan and Karuppannan, 2011; Wahyuni, 2015).

The use of extracts still needs the development on phytopharmaca preparations because extracts are easily damaged by light, water vapor, metals, and microbes. Damage to the extract can be minimized by the presence of submicron particle technology, by creating material into submicron sized particles. The submicron form has the advantages of increasing solubility, increasing stability, reducing dosage administration, increasing delivery efficiency, and extending storage time (Singh and Rawat, 2017).

Preparation of submicron particles can utilize the biopolymeric materials such as chitosan, gelatin, albumin, and sodium alginate (Han et al., 2010). The use of biopolymeric materials in

this study was a combination of chitosan and sodium alginate. The combination of chitosan with sodium alginate will form a poly ion complex (as shown in Figure 1) that can entrap extract. Chitosan in the form of submicron particles has the advantage of mucoadhesive which can increase the bioavailability of active ingredient (Berger et al., 2004).

The evaluation of submicron particle characters was observed by analyzing at the percent encapsulation efficiency (% EE), zeta potential value, and stability test. Stability parameters can be seen from the zeta potential and polydispersity index (PDI) produced. High zeta potential relates to the stability of particles. The benefits of this study are to provide information about; the physical interactions between papaya leaf extract to chitosan-alginate on the submicron formulation of particles; for the development of science especially in the pharmaceutical field for an effort to decrease the incidence of dengue hemorrhagic fever in Indonesia.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials are utilized in this research were: extract of papaya leaves (*Carica Papaya L.*) from Indralaya region, some solvents with analytical grades were obtained from Merck® such as ethanol, ethyl acetate, acetic acid, and HCl. Pure materials were obtained from Sigma-Aldrich such as quercetin, chitosan, sodium alginate, calcium chloride, NaCMC, sodium hydroxide and aquabidest from Otsuka®.

2.2 Methods

2.2.1 Identification of Flavonoids

Simplicia were weighed of 0.5 g that has been crushed in a mortar and placed in a test tube, Addition of 5 mL ethanol and heat were needed for 5 minutes. The extraction was filtered and the filtrate was obtained. Then, a few drops of concentrated HCl were added and followed by enter ± 0.2 mg of magnesium powder. Results that indicate the formation of red colour was used to present the flavonoids.

2.2.2 Determination of The Amount of Total Flavonoids in Extracts

As much as 1 g of extract was dissolved in a 25 mL volumetric flask with 15% ethanol as much as 15 mL and stirred until homogeneous. 96% ethanol was added to the boundary mark. Measurement of the absorbance of the sample was conducted at a wavelength of 374.8 nm using quercetin as marker.

2.2.3 Preparation of Polymers

- **Preparation of Chitosan**
  Chitosan preparation as much as 36 mg of chitosan powder was dissolved in 60 ml of 1% acetic acid solution in the cup glass then homogenized with a magnetic stirrer at a speed of 750 rpm for 30 minutes at room temperature until the dissolved chitosan solution was obtained 20 mL chitosan solution containing 12 mg for each formula.
- **Preparation of Sodium Alginate**
  Preparation of 9.6 mg of sodium alginate was dissolved with 60 mL API in a Beaker glass then homogenized with a magnetic stirrer at a speed of 750 rpm for 30 minutes at room temperature until the sodium alginate solution dissolved completely. The stock solution was pipetted of 20 mL (sodium alginate solution contains 3.2 mg) for each formula.
- **Preparation of Calcium Chloride**
  The 0.018 M calcium chloride concentration used in 40 μL for each formulas then was homogenized with a magnetic stirrer at a speed of 750 rpm for 30 minutes at room temperature until the solution of calcium chloride was obtained completely.

2.2.4 Formula of Submicron Particles

Variation in stirring speed was used in each formula. Preparation of submicron formula of chitosan and sodium alginate particles entrapping extract of papaya leaves was used the variation the concentration of calcium chloride as in Table 1. The amount of extract of formula (F1, F2, F3) in this study was based on the results of the preliminary study which has the most potential to inactivated *Aedes aegypti* mosquito larvae which used; chitosan as much as 12 mg; sodium alginate 3.2 mg and calcium chloride 18 mM of 40 μL (Moradhaseli et al., 2013).

Table 1. Formula of Submicron Particles Entrapping Extract of Papaya Leaf

Parameters	Amount and Condition		
	F1	F2	F3
Papaya extract (ppm)	1059	1059	1059
Chitosan (mg)	12	12	12
Natrium alginate (mg)	3,2	3,2	3,2
CaCl2 0,018 M (mL)	40	40	40
Stiring (rpm)	500	750	1000

2.2.5 Formation of Submicron Particles Entrapping Extract of Papaya

The formation of submicron particles entrapping extract of papaya leaves was conducted by using ionic gelation method as shown in Figure 1. The difference in these three formulas was found in variations in stirring speed. The way to make a formula was as follows: Ethanol extract of papaya leaves added to a 40 ml chitosan solution into a Beaker glass using a magnetic stirrer with a speed of 500 RPM for each formula and as mass 1 in making submicron particles (Pal et al., 2012). Prepare sodium alginate solution above the magnetic stirrer with variations in the speed of 500 RPM taken 20 mL for each formula and as mass 2. Perform mass mixing 1 with mass 2 by dropping it using a 50 μL micro pipette on top of the magnetic stirrer and continue to the mixing process for 1 hour with the speed of 500 RPM. The solution of calcium chloride was added with a volume of 40 μL

Table 2. Percent EE and Equivalent Amount of Quercetine in Submicron Particles

Formula	Amount of Extract	%EE	Amount of Extract in submicron particles	Amount of Quercetine in submicron particles
1	1059 mg	68.12±2.01	721.39±26.01 mg	0.3910±0.08 mg
2	1059 mg	69.07±2.68	731.45±36.41 mg	0.3964±0.15 mg
3	1059 mg	71.90±3.02	761.42±29.16 mg	0.4127±0.24 mg

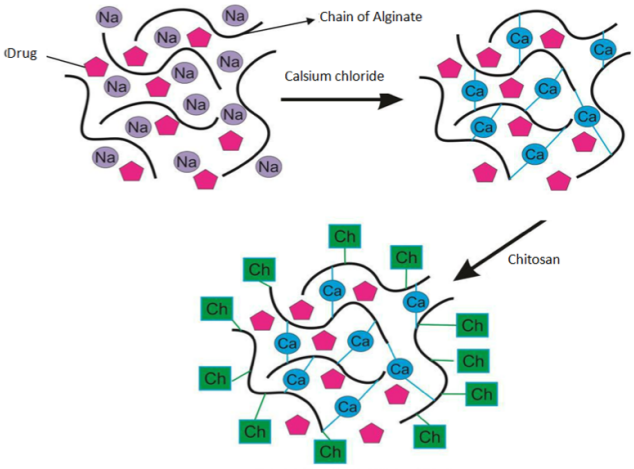

Figure 1. Ilustration of polyionic interaction between drug, alginate, and chitosan

for each formula (Moradhaseli et al., 2013). After mass 1 and mass 2 were mixed, then add distilled water for each formula to 100 mL.

2.2.6 Purification of Particles and Determination of Percent EE

Submicronn particles purification of sodium alginate chitosan particles encapsulated papaya leaf extract was conducted by 30 mL of the solution inserted into Vivaspin® 300 kDa to separate submicron particles entrapping papaya leaf extract and soluble impurities in distilled water. The centrifugation process was carried out for 15 minutes until there were 2 phases; the phase that was absorbed and the phase that was not absorbed by Vivaspin® 300 kDa. The un-absorbed phase was separated after added as much as 30 mL of aquadest into an absorbed phases again and centrifuged again. This treatment was done three times so that the pure papaya leaf extract particles were obtained.

Determination of the percent EE submicron extract of papaya leaf particles was carried out using a UV-Vis spectrophotometer. The calibration curve was made in the series of concentration 0.01; 0.02; 0.03; 0.04 and 0.05 mg /mL from the quersetine stock solution with a concentration of 1 mg/mL.

$$\%EE = \frac{\Sigma ACF - \Sigma ACS}{\Sigma ACF} x100\% \quad (1)$$

noted: ACF : active compound of formula ; ACS : active compound of supernatant

2.2.7 Characterization of Particles

Determination of particle characterization includes diameter and particle distribution was used the PSA tool through the DLS (Dynamic Light Scattering). After that 50 μL of submicron solution of chitosan sodium alginate particles entrapping extract of papaya leaf was pipetted then of 50 μL and put it into cuvette. Diameter and PDI measurements were carried out with a scattering angle of 90° detector.

2.2.8 Fourier Transform Infrared (FTIR) Measurement

The dispersed particles of the best formula and the equal amount of polymer mixture were carried out for FTIR test. By measuring it was needed 5 mg mixture of sodium alginate, chitosan, with extract. As a comparison spectra, 5 mg of sodium alginate, chitosan and extract were also needed. The sample zone was carefully cleaned because this zone would be passed through an IR beam. Spectra of environments were documented to minimize errors after measurements were taken. Percentage (%) of intensity arranged in such a way as to rotate the stressing grid. Only spectra with an intensity of 90% were documented.

2.2.9 X-Ray Diffraction (XRD) Measurement

An optimum composition of three formulas was characterized by XRD (X-Ray Diffraction). This measurement was needed 5 mg mixture of sodium alginate, chitosan and also extract. For comparison, 5 mg of sodium alginate, chitosan and extract were used. The sample was compacted and flattened on the aluminum holder for further measurement using the XRD tool. The scanning mode was a continuous scan on the 2θ axis.

3. RESULTS AND DISCUSSION

3.1 Identification of Extract and Quantification of Flavonoids

Flavonoids of extract was tested by adding Mg and HCl powder to produce a reddish-orange solution that occurs due to the formation of flavillium salts (Agung et al., 2017). The sample was also shown the positive saponins in the presence of stable foam which shows the presence of glycosides which are capable of forming foam in the water. The combination of structures constituent such as saponin (hydrophilic), hydrophobic groups can act as active surfaces in foam formation. Positive results were obtained in tannin testing with the color change of the sample being blackish blue. The simplicia positively contains steroids in

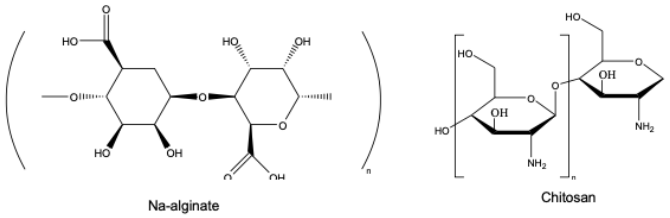

Figure 2. The chemical structures of biopolymer Na-alginate and chitosan

the testing of steroid/triterpenoid compounds after reacted with anhydrous acetic acid and concentrated H_2SO_4 which forms a blue acetyl steroid complex.

3.2 Preparation of Extract and Dispersed Polymers

Preparation of submicron particles component consists of papaya leaf extract, chitosan, sodium alginate, and $CaCl_2$, which was dissolved in the appropriate solvent. Papaya leaf extract preparation was carried out by dissolving papaya leaf extract into distilled water then filtered. Chitosan powder requires 1% acetic acid for its dissolution process. 1% acetic acid solvent was chosen because chitosan dissolves in acid and acetic acid was an organic acid solvent. Chitosan is also soluble in mineral acids because chitosan has an amine in acidic pH and chitosan protonated. Chitosan powder is insoluble in water, concentrated alkali, alcohol and acetone. Preparation of sodium alginate was carried out by means of sodium alginate powder dissolved in aquadest and $CaCl_2$ also using aquadest because it could dissolve well in water due to the presence of carboxyl groups in alginates and Cl-ions on $CaCl_2$ and then interact in aquadest and then obtain clear solutions for manufacture of submicro particles of papaya leaf extract. The chemical structures of chitosan dan Na-algiante was presented in Figure 2.

3.3 Formation of Submicron Particles Entrapping Extract of Papaya

The first stape of formation submicron particles was dissolving papaya leaf extract into aquadest then filtered. This aims to facilitate well-mix solution. Chitosan solution as mass 1 and sodium alginate as mass 2. Mass 1 was added into mass 2 drop by drop to produce mass 3. Drop by drop while stirring technique was used so that there was no fast-aggregation of particles and produce spheric particles. The next stape, mass 3 was sonicated using a sonicator cleaner. The sub-micro particles of the papaya leaves was obtained by adding aquabidest ad 100 mL then divided into three formulas with a volume of 10 mL. The difference between formulas 1, 2 and 3 was in variations in the speed of stirring using a magnetic stirrer. Stirring speed in formula 1 was 500 rpm, formula 2 750 rpm, and formula 3 1000 rpm. The image of products F1, F2 and F3 was shown in Figure 3.

3.4 Determination of Percent EE

Determination of percent EE (%EE) using the supernatant phase of the particle entrapping extract of papaya leaf extract was

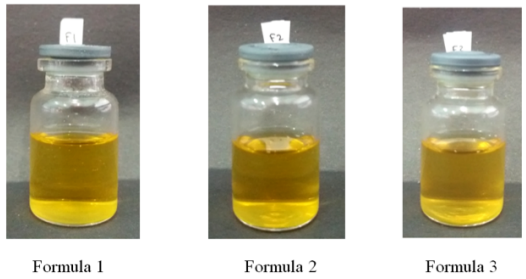

Figure 3. The submicron particles of the formula (F) F1;F2;F3

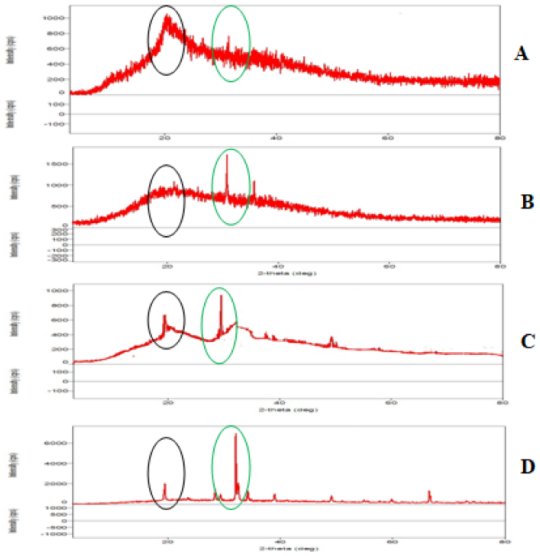

Figure 4. XRD spectra of A= complex(extract-biopolymer) compared to B= extract, C= chitosan, and D= Na-alginate.

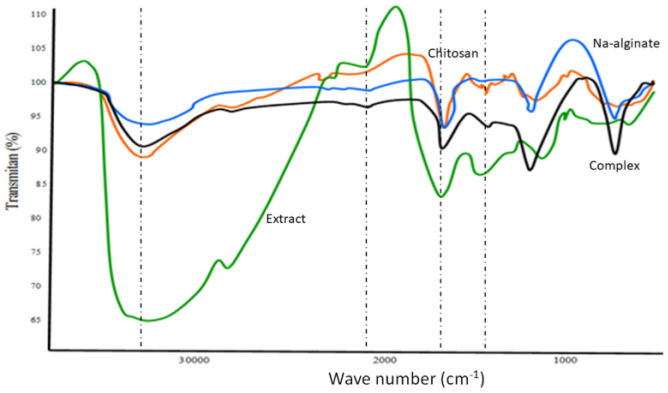

Figure 5. FTIR spectra of complex, extract, Na-alginate and chitosan

analyzed by UV-Vis spectrophotometer instrument. The %EE value obtained in formulas (F) 1, 2 and 3 were 68.12; 69.07; and 71.90 mg with no-significant different (p>0.5). The results of %EE was obtained in F3 have the highest %EE value so that the particles of papaya leaf extract are well protected by the sodium alginate chitosan polymer. The higher %EE value indicates that more extracts are absorbed or encapsulated by the polymer used, so that the extract content obtained is also increasing. Based on the %EE obtained, the concentration of extract in each F 1, 2, and 3 was 721.39; 731.45; and 761.42 mg. Quercetin content encapsulated in F 1, 2, and 3 were 0.3910; 0.3964; and 0.4127 mg quercetin. The results of the percent EE submicron particles extract of papaya leaf can be seen in Table 2.

3.5 Characterization of Particles

Determination of diameter and size distribution was done using a PSA tool. The samples analyzed for this test used the optimal formula, namely the F3 to determine the diameter value and particle size distribution. Determination of the diameter was carried out to determine the particle size formed from the preparation of submicron particles. Measurement of diameter using the PSA instrument produced data of 189.2 nm. The results of diameter measurements showed that the optimal formula analyzed entered the submicron range of particles so that it was expected to increase the availability of active substances. Particles size distribution parameters or PDI values were determined to detect the level of uniformity of size. Particle size distribution will affect the stability of the particles. PDI measurements in F 3 showed a value of 0.330.

3.6 Physical Interaction by XRD and FTIR

The results of XRD measurement was displayed in Figure 4 and the FTIR measurement was in Figure 5. The interaction study aims to determine the possibility of physical interactions between papaya extract and various polymers (chitosan and sodium alginate) without chemical shifting. Determination of the presence or absence of interactions can be determined by identifying the characteristics of peaks at wave numbers in FTIR and 2θ in XRD.

FTIR spectra showed that there was a widening peak of the OH group overlapping with NH, and the presence of CO groups in the spectrum which showed COOH groups. The fingerprint area (1500 - 500 cm^{-1}) which was the identity of a compound indicates the presence of CO groups from COOH.

According to XRD measurement, chitosan and Na-alginate polymers (both of these polymers) were contacted with X-rays, as was the case with chitosan na-alginate particles entrapping papaya leaf extract as seen in Figure 4. Both polymers exhibit a crystal structure as in dark circles. When the polymer and papaya leaf extract become particles, there was no peak at 2θ in a green circle. The results of XRD revealed the changes of type of crystalinity form to amorphous on submicron particles.

4. CONCLUSIONS

Based on the results which has been, the summary of this research could be stated as follows: The optimum formula obtained has a speed of 1000 RPM with the %EE value of 71.90%. The results of submicron particles characterization such as diameter and particle size distribution (PDI) using particle size analyzer (PSA) tools were 189.2 nm and 0.330. The results of XRD revealed the changes of type of crystalinity form to amorphous on submicron particles. The results of FTIR revealed the physical interaction without chemical shifting.

5. ACKNOWLEDGEMENT

The acknowledgements are addressed to Sriwijaya University (UNSRI) for Kompetitif Research Grant of Academic Year 2019 using funding of PNBP. The acknowledgements are also expressed to UII and UGM Jogjakarta for helping of samples measurements.

REFERENCES

Agung, N., H. Heryani, S. Jae, and H. Park (2017). Identification and quantification of flavonoids in Carica papaya leaf and peroxynitrite-scavenging activity. *Asian Pacific Journal of Tropical Biomedicine*, **7**(3); 208–213

Ahmad, N., H. Fazal, M. Bilal, H. Abbasi, and I. Fazal (2011). Dengue fever treatment with Carica papaya leaves extracts. *Asian Pacific Journal of Tropical Biomedicine,*, **1**(4); 330–333

Akerholm, M. and L. Salmen (2001). Interactions between wood polymers studied by dynamic FT-IR spectroscopy. *polymer*, **42**(3); 963–969

Berger, J., M. Reist, J. Mayer, O. Felt, and R. Gurny (2004). Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics*, **57**(1); 35–52

Govindarajan, M. and P. Karuppannan (2011). Mosquito larvicidal and ovicidal properties of Eclipta alba (L.) Hassk (Asteraceae) against chikungunya vector, Aedes aegypti (Linn.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, **4**(1); 24–28

Han, J., Z. Zhou, R. Yin, D. Yang, and J. Nie (2010). Alginate–chitosan/hydroxyapatite polyelectrolyte complex porous scaffolds: Preparation and characterization. *International Journal of Biological Macromolecules*, **46**(2); 199–205

Jain, M., L. Ganju, A. Katiyal, Y. Padwad, K. Mishra, S. Chanda, D. Karan, K. Yogendra, and R. Sawhney (2008). Effect of Hippophae rhamnoides leaf extract against Dengue virus infection in human blood-derived macrophages. *Phytomedicine*, **15**(10); 793–799

Kim, K., K. Adachi, and Y. Chujo (2002). Polymer hybrids of functionalized silsesquioxanes and organic polymers utilizing the sol–gel reaction of tetramethoxysilane. *Polymer*, **43**(4); 1171–1175

Lin, L., W. Hsu, and C. Lin (2014). Antiviral natural products and herbal medicines. *Journal of Traditional and Complementary Medicine*, **4**(1); 24–35

Moektiwardoyo, W., T. Ami, Y. Susilawati, Y. Iskandar, O. Halimah, and Z. Eli (2014). The Potential of Dewa Leaves (Gynura Pseudochina (L) D.C) and Temu Ireng Rhizomes (Curcuma aeruginosa Roxb.) as Medicinal Herbs for Dengue Fever Treatment. *Procedia Chemistry*, **13**; 134–141

Moradhaseli, S., Z. Abbas, S. Ali, M. Nasser, S. Saman, and R. Mehrasa (2013). Preparation and characterization of sodium alginate nanoparticle containing ICD-85 (venom derived peptides). *International journal of innovation and applied studies*, **4**; 534 – 542

Natalia, N., N. Lucimeire, S. Santana, M. Sampaio, F. Maria, and L. Oliva (2013). The component of Carica papaya seed toxic to A. aegypti and the identification of tegupain, the enzyme that generates it. *Chemosphere*, **92**(4); 413–420

Singh, P. K. and P. Rawat (2017). Evolving herbal formulations in management of dengue fever. *Journal of Ayurveda and Integrative Medicine*, **8**(3); 207–210

Suwanbamrung, C., S. Promsupa, T. Doungsin, and S. Tongjan (2013). Risk factors related to dengue infections in primary school students: Exploring students' basic knowledge of dengue and examining the larval indices in southern Thailand. *Journal of Infection and Public Health*, **6**(5); 347–357

Wahyuni, D. (2015). New Bioinsecticide Granules Toxin from Ectract of Papaya (Carica Papaya) Seed and Leaf Modified Against Aedes Aegypti Larvae. *Procedia Environmental Sciences*, **23**; 323–328

Zunjar, V., R. P. Dash, M. Jivrajani, B. Trivedi, and M. Nivsarkar (2016). Antithrombocytopenic activity of carpaine and alkaloidal extract of Carica papaya Linn. leaves in busulfan induced thrombocytopenic Wistar rats. *Journal of Ethnopharmacology*, **181**; 20–25

