Research Article

Formulation and Characterization of Bioadhesive Vaginal Cream of Nanocapsule of Parang Romang (*Boehmeria virgata* (Forst) Guill) Leaf Extract

Latifah Rahman[1], Surya Ningsi[2], Lukman Muslimin[1] and Marianti A. Manggau*[1]
[1]Faculty of Pharmacy, Hasanuddin University, South Sulawesi, Makassar, Indonesia.
[2]Faculty of Science, Alauddin State Islamic University, South Sulawesi, Makassar, Indonesia.
***Corresponding author's E-mail:** winati04@yahoo.co.id

Accepted on: 10-12-2014; **Finalized on:** 31-01-2015.

ABSTRACT

This research aimed to create a nanocapsules formula of *B. virgate* leaf extract that has the best physical character and to found bioadhesive vaginal cream formula of nanocapsules of *B. virgata* leaf extract that meets pharmaceutical requirements. Nanocapsules was done by variation of the core and the coating concentrations using an ionic gelation method (N1, N2, N3, N4 and N5), and the best nanocapsules formula was formulated in to bioadhesive vaginal cream with variations base cream (F1, F2 and F3). The results showed that N3 has the best physical character: yield (76.80%), encapsulation efficiency (19.49%), LE (4.23%) and particle size (149.0 nm – 262.7 nm). After N3 was formulated in to bioadhesive vaginal cream, we found that F3 with HPMC K100M as cream base polymers showed the biggest bioadhesion (13 g), pH6.8 and biggest viscosity (53200cPs) and meets pharmaceutical requirements.

Keywords: Nanocapsules, *B. virgate* leaf extract, vaginal bioadhesive cream, formulation

INTRODUCTION

Consideration of alternative cancer drug use is emphasized primarily in plants and herbs. Herbal medicines become popular because of its use for treating various kinds of diseases with low toxic effects and better therapeutic[1-3]. However, some limitations in the use of herbal extracts/active substances of plants, such as instability at acidic solution, metabolism by the liver, low solubility in water, and other causes of drug levels in the blood below therapeutic concentrations resulting in low or no therapeutic effects[4-6].

The use of nanoparticles as drug delivery systems for anticancer therapy has a great potential for cancer therapy in the future[7]. Nanoparticles are an efficient delivery system for hydrophilic and hydrophobic substances[8,9]. Nanoparticle-encapsulated formulation could enhance cellular uptake and thereby improve bioactivity[10]. Several studies have been reported: nanoparticle-encapsulated of curcumin (*Curcuminlongma*)[11,12], *Gelsemiumsempervirens* ethanol extract[13], thymoquinone-loaded PLGA nanoparticles[14] and *Polygalasenega* ethanol extracts[15] were more active than non-encapsulated extract.

Plants remain an important source of new drugs, approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world[16,17]. One of the plants whose often used by Makassar People as anticancer is ParangRomang (*Boehmeriavirgata* (Forst) Guill), Family of Urticaceae[18]. *B. virgate* n-hexane, ethylacetate and n-Butanol extracts have antiproliferative activity against HeLa cancer cells: IC503.453; 12.096 and 168.66 ug/mL, on bladder 5637: 1.4; 3.96; and 2.18 ug/mL, respectively[19]. IC50 of *B. virgata* ethanol extract on HeLa is 9.40 ug/mL, on macrophage cell is 29.10 ug/mL and selective on HeLa cell cancer compared with macrophage[20]. *B. virgate* methanol extract on WiDr, T47D and Vero cell are 18.925, 12.732 and 16.022, respectively[21].

Vaginal delivery system is an important route of drug delivery for local and systemic route. Creams, foams, gels, irrigations, tablets and other traditional dosage forms used through in vaginal cavity have short act due to self-cleaning action ofvagina[22] but bio adhesive drug delivery systems have been developed to decrease the self-cleaning action of vagina[23]. This research aimed to create a bioadhesive vaginal cream from nanocapsule of *B. virgate* leaf extract with good pharmaceutical character.

MATERIALS AND METHODS

Materials

Methanol, n-hexane, chitosan, sodium tripolyphosphate, acetic acid and acetone were obtained from Merck-Indonesia. Hydroxypropyl methylcellulose K15M and hydroxypropyl methyl cellulose K100M were obtained from Shin-Etsu-China. Sorbitanmonooleate, polysorbate80, propylene glycol, stearylalcohol, isopropylmyristate, methylparaben, propylparaben, bulylatedhydroxyanisole, cellophanemembranes, aquadest and citratebufferpH4 (pharmaceutical grade).

Plant material

B. virgata leaves obtained from Malino-Gowa, South Sulawesi-Indonesia. The plant was identified by Herbarium Bogoriense (Bogor, West Java, Indonesia). The leaves was washed, dried (38 °C) and ground to fine powder. The dried ground leaves were extracted three times with ethanol at room temperature using

maceration method. Extract was filtered (Whatman), evaporated (Buchi) and freeze-dried (Scanvac). The ethanol extract was partitioned by liquid-solid method with n-hexane. The n-hexane extract was evaporated (Buchi) and freeze-dried (Scanvac).

Nanocapsule Formulation

Nanocapsules were prepared using chitosan biodegradable polymer carried out by mechanical stirring ionic gelation method. Nanocapsules made with various concentration of chitosan polymer (Table 1).

Table 1: Nanocapsule Formula of *B. virgata* leaf extract

Formula	*B. virgata* leaf extract (mg)	Chitosan (mg)	Extract : Chitosan
N1	75	75	1:1
N2	75	150	1:2
N3	75	225	1:3
N4	75	300	1:4
N5	75	325	1:5

Chitosan solution in acetic acid 1% and *B. virgata* leaf extract in acetone were mixed. Tween 80 was added while stirred at 1.000 rpm for 150 minutes. Sodium tripolyphosphate was added gradually during stirring, centrifuged at 5.000 rpm for 20 minutes. The precipitate was re-suspended in aqua dest to remove un-trapped drug and nanocapsules were freeze-dried. To determine entrapment of *B. virgata* leaf extract using ultraviolet (UV) visible spectrophotometer (Agilent) at λ_{max} value of 408 nm.

The percent Encapsulation Efficiency (EE) was calculated as:

$$EE\ (\%) = \frac{\text{Actual extract loading}}{\text{theoretical drug loading}} \times 100$$

Loading Efficiency (LE) of extract was calculated as:

$$LE\ (\%) = \frac{\text{Encapsulated extract (gram)}}{\text{Nanocapsule (gram)}} \times 100$$

Bioadhesive Vaginal Cream Formulation

Sorbitanmonooleate, stearylalcohol, isopropylmyristate and propylparabenwere melted (oil phase). Hydroxypropyl methylcellulose, polysorbate80, propylene glycol, aqua dest and methylparaben were heated (water phase). The oil phase was added to the water phase while adding butylatedhydroxyanisole and nanocapsules (0.00816%) at 40 ^{o}C temperature. A homogenous cream was obtained. There are 3 formula base cream based on the concentration ratio of HPMC K15M and HPMC K100M namely F1 (1:0), F2 (1:1) and F3 (0:1).

Vaginal Adhesion Measurements

Vaginal Adhesion was measured on tensile strength to break ties of cellophane membrane (1 x 2 cm) and cream (1 gram). Cellophane membrane was soaked in citrate buffer pH 4 for 1 hour, cream was placed on cellophane membrane for 15 minutes. Tensile strength is weight needed to break cream and cellophane membrane[24,25].

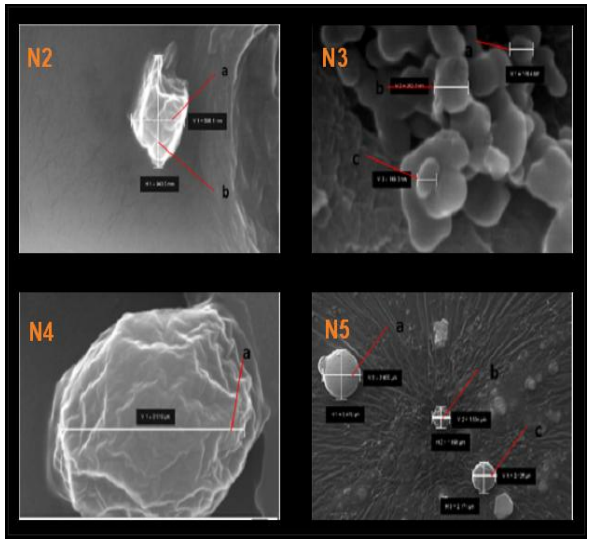
Stability Testing of Bioadhesive Vaginal Cream

Stability testing of bioadhesive vaginal cream used accelerated (5 ^{o}C and 35 ^{o}C) for 10 cycles. Stability of cream was determined include creaming volume, pH, viscosity, emulsion type and homogeneity[26].

RESULTS AND DISCUSSION

Surface Morphology and Particle size of Nanocapsules

Chitosan nanocapsules using ionic gelation process was occurred by interaction between the positive charge on the amino group of chitosan with sodium tripolyphosphate as negative charge (NTPP) to produce ionic cross-link[27,28]. The size of nanocapsules depends on the concentration of chitosan in solution and STPP[29].



N2 a = 586.1 nm a = 2,519 nm b = 940.5 nm b = 1,634 nm
N3 N5 c = 149 nm
a = 178.4 nm a = 3,455 nm b = 262.7 nm c = 2,125 nm
N4

Figure 1: SME photos include: surface morphology and particle size of nanocapsules. The surface morphology and particle size showed generally not spheres and have non-uniform particle size.

Nanocapsules can be identified visually through the clarity changes of solution from clear to opaque when STPP was added to a solution of chitosan. It indicates changes of chitosan from dissolved particle to the nanometers in size, micro-scale particle and aggregate[30]. The visual observation showed: N1 is clear; N2, N3, N4 and N5 are opaque. The surface morphology and particle size showed generally not spheres and have non-uniform particle size. The N2 and N3 showed particle size which are less than 1000 nm (nanocapsules diameter range) but N4 and N5 are in the micrometer scale (microcapsules diameter range).

Particle size is affected by coating concentration, greater concentration of coating make more bonds formed between chitosan cross and NTPP. Chitosan matrix will be

increased the hardness and strength of chitosan particles on nanocapsule surface[31].

Encapsulated Efficiency and Loading Efficiency

Table 2: The result of Yield, EE and LE calculations

Formula	Yield (%)	EE (%)	LE (%)
N1	-	-	-
N2	49.89	1.57	0.72
N3	76.80*	19.49	4.23
N4	77.01*	9.49	1.61
N5	84.47*	10.53	1.34

*good yield (> 75%)

Encapsulation Efficiency (EE) was calculated by the reduction of total extract added and non-encapsulated extract in the supernatant, non-adsorbed extract was measured using UV-Vis spectrophotometer. The EE of N2, N3, N4 and N5 are 1.57%, 19.49%, 9.49% and 10.53%, respectively. N3 has the greatest encapsulation efficiency because has smaller particle size and greater surface area.

Loading efficiency of (LE) is to determine the percentage of the loaded extract in nanocapsule formed. The LE of N2, N3, N4 and N5 are 0.72%, 4.23%, 1.61% and 1.34%, respectively. N3 has the biggest LE (in every 1 gram of nanocapsule contains 42.3 mg of extract).

These data indicates that N3 has the best physical character and to use in preparation of bioadhesive vaginal cream.

Stability Test of Cream

Creaming Volume, pH and Viscosity

Table 3: The result of creaming, pH and viscosity measurements

Formula	Creaming Volume (%)		pH		Viscosity (cPs)	
	B	A	B	A	B	A
F1	0	0	6,5	6,5	38400	38800
F2	0	0	6,7	6,7	42000	56000
F3	0	0	6,8	6,8	53200	54800

(cPs= centipoises, B= before freeze-thaw cycle, A= after freeze-thaw cycle, o/w= oil in water and H= homogeny)

There are 3 kinds formula of bioadhesive vaginal basecream: F1 (HPMC K15M), F2 (HPMCK15M and HPMCK100M) and F3 (HPMC K100M). Polymer differentiation of bioadhesive vaginal base cream aims to get the best composition of cream with the greatest adhesion. Nonionic emulsifiers base cream no-irritate in vaginal lining and avoid the interaction between emulsifiers and the unknown compound of extract[32,33].

According to pharmaceutical requirements for cream, F1, F2, and F3 can be categorized as good stability cream. Adhesion test showed that F1, F2, and F3 have bioadhesion 10, 11 and 13 g, respectively. F3 showed the greatest bioadhesion (13g); pH 6.8 and biggest viscosity (53200cPs).

Viscosity indicates that the greater viscosity has greater bioadhesive power on vaginal surface[34], pH 6.8 - 7.8 indicates no-irritate on vaginal surface[35].

Emulsion Type and Homogeneity

Table 4: Emulsion Type and Homogeneity

Formula	Emulsion Type		Homogeneity	
	B	A	B	A
F1	o/w	o/w	H	H
F2	o/w	o/w	H	H
F3	o/w	o/w	H	H

(cPs= centipoises, B= before freeze-thaw cycle, A= after freeze-thaw cycle, o/w= oil in water and H= homogeny)

Table 4 reveals that conformation of emulsion type and homogeneity of all creams formula no changes.

CONCLUSION

B. virgata leaf extract can be formulated into bioadhesive vaginal cream and F3 has good pharmaceutical properties.

REFERENCES

1. Atmakuri LRand Dathi S, Current Trends in Herbal Medicines. Journal of Pharmacy Research, 3(1), 2009, 109-113.
2. Kelly JP, Kaufman DW, Kelley K, Rosenberg L, Anderson TE, Mitchell AA, Recent Trends in Use of Herbal and Other Natural Products. Archives of Internal Medicine, 165(3), 2005, 281-286.
3. Wachtel-Galor S and Benzie IFF, Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs, in Herbal Medicine: Biomolecular and Clinical Aspects, I.F.F. Benzie and S. Wachtel-Galor, Editors. CRC Press Llc.: Boca Raton (FL). 2011.
4. Firenzuoli F and Gori L, Herbal Medicine Today: Clinical and Research Issues. Evidence-Based Complementary and Alternative Medicine, 4(S1), 2007, 37-40.
5. Cardini F, Wade C, Regalia AL, Gui SLi W, Raschetti R, and Kronenberg F, Clinical Research in Traditional Medicine: Priorities and Methods. Complement Ther Med, 14(4), 2006, 282-287.
6. Rai MK, Herbal Medicines in India: Retrospect and Prospect. Fitoterapia, 65(6), 1994. 483-491.
7. Díaz M and Vivas-Mejia P, Nanoparticles as Drug Delivery Systems in Cancer Medicine: Emphasis on RNAI-Containing Nanoliposomes. Pharmaceuticals, 6(11), 2013, 1361-1380.
8. Lohcharoenkal W, Wang L, Chen YC and Rojanasakul Y, Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy. BioMed Research International, 2014, 2014, 12.
9. Upta S, Bansal R, Gupta S, Jindal N, and Jindal A, Nanocarriers and Nanoparticles For Skin Care and Dermatological Treatments. Indian Dermatol, 4(4), 2013, 267-272.

10. Khuda-Bukhsh AR, Bhattacharyya SS, Paul S and Boujedaini N, Polymeric Nanoparticle Encapsulation of a Naturally Occurring Plant Scopoletin and its Effects on Human Melanoma Cell A375. J. Chin Integr Med, 8(9), 2010, 853-862.
11. Bisht S, Feldmann G, Soni S, Ravi R, Karikar C and Maitra A, Polymeric Nanoparticle-encapsulated Curcumin ("nanocurcumin"): a Novel Strategy for Human Cancer Therapy. Journal of Nanobiotechnology, 5(1), 2007, 3.
12. Anand P, Nair HB, Sung B, Kunnumakkara AB, Yadav VR, Tekmal RR and Aggarwal BB, Design of Curcumin-loaded PLGA Nanoparticles Formulation With Enhanced Cellular Uptake, and Increased Bioactivity *in vitro* and Superior Bioavailability *in vivo*. Biochemical Pharmacology, 79(3), 2010, 330-338.
13. Bhattacharyya SS, Paul Sand Khuda-Bukhsh AR, Encapsulated Plant Extract (*Gelsemium sempervirens*) poly (lactide-co-glycolide) Nanoparticles Enhance Cellular Uptake and Increase Bioactivity *in vitro*. Experimental Biology and Medicine, 235(6), 2010, 678-688.
14. Ilaiyaraja N, Ambica P and Farhath K, Thymoquinone-loaded PLGA Nanoparticles: Antioxidant and Anti-microbial Properties. Intern Current Pharmaceu J., 2(12), 2013, 202-207.
15. Paul S, Bhattacharyya SS, Boujedaini N and Khuda-Bukhsh AR, Anticancer Potentials of Root Extract of *Polygala senega* and Its PLGA Nanoparticles-Encapsulated Form. Evidence-Based Complementary and Alternative Medicine, 2011.
16. Farnsworth NR and Soejarto DD, Potential Consequences of Plant Extinction in the United States on the Current and Future Availability of Prescription Drug. Econ. Bot., 39(3), 1985, 231-240.
17. Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, Sun JN, Han YF and Ko KM, New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evidence-Based Complementary and Alternative Medicine, 2013, 2013, 25.
18. Manggau MA and Lukman M, Efek Farmakologi Tanaman Antikanker Yang Digunakan Oleh Masyarakat Suku Makassar Sulawesi Selatan. Makassar: LepHas, 2012.
19. Manggau MA, Mufidah and Lindequist U, Antiproliferation Against Human Bladder Cancer 5637 Cell Line and Antioxidant Activity of Various Plant Extracts. The Indonesia J Nat Prod, 6, 2009, 247-250.
20. Lukman M, Wahyudin E, Subehan and Manggau MA, Cytotoxic Effect of Four Makassarese Medicinal Plants on Human Cervical Cell Lines and its Selectivity. Journal of Chemical and Pharmaceutical Research, 6(10), 2014, 851-855.
21. Wardihan, Muhammad R, Gemini A, Lukman M and Manggau MA, Selective Cytotoxicity Evaluation in Anticancer Drug Screening of *Boehmeria virgata* (Forst) Guill Leaves to Several Human Cell Lines: HeLa, WiDr, T47D and Vero Dhaka Univ. J Pharm Sci, 12(2), 2013, 123-126.
22. Acarturk F, Muco adhesive Vaginal Drug Delivery Systems. Recent Pat Drug Deliv Formul, 3(3), 2009, 193-205.
23. de Araujo Pereira RR and Bruschi ML, Vaginal Mucoadhesive Drug Delivery Systems. Drug Dev Ind Pharm, 38(6), 2012, 643-652.
24. Vermani K, Garg S and Zaneveld LJ, Assemblies for *in vitro* Measurement of Bioadhesive Strength and Retention Characteristics in Simulated Vaginal Environment. Drug Dev Ind Pharm, 28(9), 2002, 1133-1146.
25. Garg S, Vermani K, Garg A, Anderson RA, Rencher WB and Zaneveld LJD, Development and Characterization of Bioadhesive Vaginal Films of Sodium Polystyrene Sulfonate (PSS), a Novel Contraceptive Antimicrobial Agent. Pharmaceutical Research, 22(4), 2005, 584-595.
26. Sanjay B, Dinesh S and Neha S, Stability Testing of Pharmaceutical Products. J Applied Pharm Scien, 2(3), 2012, 129-138.
27. De Campos AM, Sánchez A and Alonso MaJ, Chitosan Nanoparticles: a New Vehicle for the Improvement of the Delivery of Drugs to the Ocular Surface. Application to cyclosporin A. International Journal of Pharmaceutics, 224(1–2), 2001, 159-168.
28. Gan Q, Wang T, Cochrane C and McCarron P, Modulation of Surface Charge, Particle Size and Morphological Properties of Chitosan–TPP Nanoparticles Intended for Gene Delivery. Colloids and Surfaces B: Biointerfaces, 44(2–3), 2005, 65-73.
29. Munin A and Edwards-Lévy F, Encapsulation of Natural Polyphenolic Compounds; a Review. Pharmaceutics, 3(4), 2011, 793-829.
30. Katas H and Alpar HO, Development and Characterisation of Chitosan Nanoparticles for siRNA Delivery. J Control Release, 115(2), 2006, 216-225.
31. Heurtault B, Saulnier P, Pech B, Venier-Julienne MC, Proust JE, Phan-Tan-Luu R and Benoît JP, The influence of Lipid Nanocapsule Composition on Their Size Distribution. European Journal of Pharmaceutical Sciences, 18(1), 2003, 55-61.
32. Kumar GP and Rajeshwarrao P, Nonionic Surfactant Vesicular Systems for Effective Drug Delivery: an Overview. Acta Pharmaceutica Sinica B, 1(4), 2011, 208-219.
33. Knowlton J and Pearce S, Surface Chemistry: Principles of the Colloidal State, in Handbook of Cosmetic Science & Technology, J. Knowlton and S. Pearce, Editors. Elsevier, 1993, 67-94.
34. Zhu Z, Zhai Y, Zhang N, Leng D and Ding P, The development of Polycarbophil as a Bioadhesive Material in Pharmacy. Asian Journal of Pharmaceutical Sciences, 8(4), 2013, 218-227.
35. Shankar NB, Kumar RP, Kumar NU and Bratac BB, Development and Characterization of Bioadhesive Gel of Microencapsulated Metronidazole for Vaginal Use. Irian J Pharm Res, 9(3), 2010, 209-219.

Source of Support: Nil, **Conflict of Interest:** None.