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CONTENT

PREFACEi
EDITORSii
CONFERENCE COMMITTEEiii
CONTENTiv
PHARMACY1
COMMUNITY PHARMACISTS' COUNSELLING SKILLS ON OVER-THE-COUNTER (OTC) MEDICATIONS
FORMULATION AND OPTIMIZATION OF CAFFEINE NANOEMULSION USING FACTORIAL DESIGN STUDY
EFFECT OF COMBINATION SODIUM ALGINATE-GELATIN 1%: 2% CONTENT IN CHARACTERISTIC AND ANTIMICROBIAL ACTIVITY OF PROBIOTIC MICROSPHERES Lactobacillus acidophilus
ANTIDIABETIC ACTIVITY OF POWDER AND ETHANOLIC EXTRACT OF ANTLION (Myrmeleon sp.) ON WISTAR STRAIN WHITE MALE RATS WITH GLUCOSE PRELOAD
ANTIBACTERIAL AND ANTIBIOFILM POTENTIAL OF ETHANOLIC EXTRACT FROM BINTARO FLOWER (Cerbera odollam) AGAINST Staphylococcus aureus ATCC 6538
STRUCTURE MODIFICATION AND MOLECULAR MODELING OF 1-(BENZOYLOXY)UREA DERIVATIVES AS ANTICANCER DRUG CANDIDATES
CHARACTERIZATION AND THE RELEASE TEST OF ANTI-AGING TRETINOIN IN NANOEMULSION USING OLIVE OIL
OF OVALBUMIN-ALGINATE MICROSPHERES
ANTIHYPERCHOLESTEROLEMIC EFFECT OF Arcangelisia flava STEM EXTRACT IN HYPERLIPIDEMIC RATS
GREEN TEA EXTRACT EFFECT ON BLOOD GLUCOSE LEVEL AND LIVER HISTOPATHOLOGY IN DIABETIC MICE
THREE-WAVELENGTH SPECTROPHOTOMETRIC METHOD VALIDATION FOR DETERMINATION OF PREDNISONE TABLET CONTAINING COLORING DYES
INFLUENCE OF OLEIC ACID ON THE IN VITRO PENETRATION OF DICLOFENAC SODIUM GEL 43
ANTIOXIDANT ACTIVITY OF METHANOL EXTRACTS FROM THE STEM BARK OF MANGROVE PLANT Rhizophora mucronata
PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY of MANGROVE PLANT Soneratia sp 51
EFFECT OF SOLID LIPID NANOPARTICLE (SLN) AND NANO STRUCTURE LIPID CARRIER (NLC) SYSTEM ON ANTIOXIDANT STABILITY OF TOMATO EXTRACT (LIPID: CETYL ALCOHOL AND ISOPROPYL MYRISTATE)
EFFECTIVENESS OF BINTARO (Cerberra odollam Gaertn.) LEAF ETHANOLIC EXTRACT AGAINST Staphylococcus aureus IN-VITRO BIOFILM FORMATION

EXTRACT AND ROSELLE FLOWER PETAL WATER EXTRACT62
INHIBITORY EFFECT OF NON-POLAR AND SEMI-POLAR FRACTIONS OF ETHANOLIC EXTRACTOR OF Guazuma ulmifolia Lamk. LEAVES ON RAT PREADIPOCYTES PROLIFERATION AND DIFFERENTIATION
THE INFLUENCE OF PHARMACEUTICAL CARE SERVICES MODEL IN PRESCRIPTION DRUGS ON PHARMACIST'S BEHAVIOR IN PHARMACEUTICAL CARE
COCRYSTAL OF ATORVASTATIN CALCIUM – MALONIC ACID
IN SILICO STUDY OF ACRYLAMIDE TOXICITIES USING TOXTREE METHOD AND ITS ANALYSIS IN POTATO CHIPS USING HPLC METHOD79
IMPACT OF CISPLATIN BASE CHEMOTERAPY ON QUALITY OF LIFE IN INDONESIAN PATIENTS WITH CERVICAL CANCER81
Arcangelisia flava LEAVES ETHANOLIC EXTRACT SUPPRESSES CANCER CELL LINES VIA NON APOPTOTIC PATHWAY83
BANANA AND PLANTAIN AS MEDICINAL FOOD87
FORMULATION AND OPTIMIZATION OF CARBOPOL AND ETHYL CELLULOSE AS FLOATING-MUCOADHESIVE SYSTEM OF DILTIAZEM HYDROCHLORIDE TABLET BY FACTORIAL DESIGN 92
DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACT OF ROBUSTA AND ARABICA COFFEE LEAVES96
MICROBIAL ASSAY OF CYPROFLOXACIN IN A BONE IMPLANT (CHITOSAN —BOVINE HYDROXYAPATITE WITH CROSS-LINKER GLUTARALDEHYDE) TOWARDS Staphilococcus aureus ATCC25923100
IN VITRO α -GLUCOSIDASE INHIBITORY ACTIVITY OF VARIOUS TEA (Camellia sinensis L.) EXTRACTS
B-CELL EPITOPE PREDICTION of Mycobacterium tuberculosis Ag85A ANTIGEN108
EFFECT OF ETHANOLIC EXTRACT OF Annona muricata L SEEDS POWDER TO DECREASE BLOOD GLUCOSE LEVEL OF WISTAR MALE RATS WITH GLUCOSE PRELOAD112
PROTEIN DENSITY AND QUALITY OF KORO KRATOK (Phaseolus lunatus L. Sweet) AND KACANG TUNGGAK (Vigna unguiculata (L.) Walp)116
HYPERTENSION MANAGEMENT APPROACH THROUGH MODIFIABLE RISK FACTORS IN JEMBER REGION COMMUNITY HEALTH CENTER121
ASSOCIATION BETWEEN AEROALLERGEN SENSITIZATION AND THE SEVERITY OF ASTHMA IN PEDIATRIC PATIENTS126
ANALYSIS OF FACTORS THAT RELATED MATERNAL SEVERE PREECLAMPSIA TO THE ASPHYXIA OF NEW BORN BABY IN SOEBANDI HOSPITAL JEMBER REGENCY130
AN ANALYSIS OF THE ASPECTS OF HEALTHCARE SERVICE QUALITY IN RELATION TO PATIENT LOYALTY133
SYNERGISTIC COMBINATION OF Curcuma xanthorrhiza, Ficus septica AND DOXORUBICIN INHIBITS METASTASIS OF BREAST CANCER THROUGH INHIBITION MMP-9 ACTIVITY137
ASSOCIATION BETWEEN IGE SERUM LEVEL AND SEVERITY OF ASTHMA IN CHILDREN 142

CORRELATION OF CD4 WITH TOTAL LYMPHOCYTE COUNTS IN HIV PATIENTS145
DENTISTRY
Determinants of HIV/AIDS Awareness and Knowledge in Tanah Papua, Indonesia 149
THE ABILITY OF ANTI-INFLAMMATORY JATROPHA CURCAS LEAF EXTRACT AT COX-2 EXPRESSION ON MONOCYTES WERE EXPOSED LPS
NOVEL METHOD THYROID HORMONE MEASUREMENT
ROBUSTA COFFEE BEANS INCREASE LEVELS OF TNF- α AS A RESPONSE TO Streptococcus mutans
THE LEVELS OF TNF-A IN GINGIVAL CREVICULAR FLUID (GCF) OF OSING TRIBE WOMEN WITH OCCLUSAL DISHARMONY165
Effects of Robusta Coffee Bean Extract (Coffea robusta) on the Viability of Neutrophils Exposed by Porphyromonas gingivalis
ROBUSTA COFFEE BEANS DECREASE OF INFLAMMATION IN DENTAL CARIES
The Progressive Low Chronic Inflammation on Oral Tissues In Elderly
DENTAL CARIES IN PREGNANT WOMEN WHO VISITED POSYANDU OF SEVERAL PUBLIC HEALTH CENTERS IN JEMBER
Role of Chemoattractant Chemokine (SDF-1/CXCR4) In Bone Marrow Niche 185
Establishment of a Rat Model of Temporomandibular Joint Osteoarthritis using Intraarticular Injection of Complete Freund's Adjuvant190
PUBLIC HEALTH
RECIPROCAL DETERMINISM "DAKOCAN" CHALLENGE EFFORTS TO REDUCE HIV AND AIDS CASES IN JEMBER DISTRICT
IRON TABLETS DISTRIBUTION OF PREGNANT WOMAN IN THE DISTRICT AND CITY OF EAST JAVA PROVINCE
RISK MANAGEMENT OF DUE TO EXPOSURE TO PESTICIDE POISONING FOR TOBACCO FARMERS IN THE JEMBER DISTRICT
AN OVERVIEW OF MOTHER KNOWLEDGE AFTER GIVING BIRTH ABOUT EXCLUSIVE BREASTFEEDING
DISASTER PREPAREDNESS AT PUBLIC HEALTH CENTER (PHC) BY SCORING ANALYSIS OF GENERAL ASPECTS, HEALTH CARE, SURVEILLANCE, ENVIRONMENTAL SANITATION AND LOGISTICS
INDEPENDENT FAMILY PLANNING IN RURAL AND URBAN AREAS GRESIK DISTRICT 215
UNMET NEED FOR FAMILY PLANNING ON ELIGIBLE COUPLE IN INDONESIA: 2007 IDHS DATA ANALYSIS
Shells That Have been Polluted by lead around Youtefa Bay in Jayapura City That Have Potential Risk Of Non Carcinogenik
DESIGN AND IMPLEMENTATION DIARRHEAL SURVEILANCE REPORT INFORMATION SYSTEM WITH WATERFALL METHOD IN HEALTH DEVELOPMENT OF JEMBER

URINE THIOCYANATE LEVELS229
UNDERWEIGHT AND MORBIDITY STATUS AMONG UNDER FIVE YEARS CHILDREN IN SURABAYA
CONDOM USE AMONG EXIT CLIENTS OF FEMALE SEXUAL WORKERS FOR PREVENTION HIV/AIDS IN MAKASSAR
THE SOCIAL SUPPORT AND PREVALENCE EMESIS GRAVIDARIUM ON PREGNANT MOTHER IN TRIMESTER I AT PUSKESMAS KEMBARAN I BANYUMAS REGENCY241
NURSING245
We need a bigger bomb: a community attempt on fighting dengue fever in a suburban Surabaya, Indonesia
APPLICATION OF STANDART NURSING LANGUAGE (NANDA, NOC. NIC) USING SOCIAL MEDIA: INSTAGRAM® TO INCREASE INFORMATION SEEKING BEHAVIOUR AND MOTIVATION OF NURSING STUDENT
THE EFFECT OF ONION (Allium ascalonicum L.) COMPRES TOWARD BODY TEMPERATURE OF CHILDREN WITH HIPERTERMIA IN BOUGENVILLE ROOM DR. HARYOTO LUMAJANG HOSPITAL253
ACHIEVEMENT OF BLOOD PRESSURE TARGET WITH MEDICATION ADHERENCE AND SODIUM CONSUMPTION IN SAIFUL ANWAR GENERAL HOSPITAL OUTPATIENT CLINIC257
EFFECT OF INSTRUCTIONAL VIDEO OF SPLINTING PROCEDURE TO NURSING STUDENTS SPLINTING SKILL (PREHOSPITAL SETTING)261
THE CORRELATION BETWEEN NURSE PERFORMANCE & THE LEVEL OF JAMKESMAS PATIENT SATISFACTION IN DAHLIA II WARD, NGUDI WALUYO WLINGI HOSPITAL266
How To Maintain High Quality Cardiopulmonary Resuscitation In Adults : Literature Review270
SMOKING BEHAVIOUR AMONG MIDDLE AND LATE ADOLESCENTS IN A SUB DISTRICT OF MALANG DISTRICT, EAST JAVA, INDONESIA275
THE DIFFERENCES DECLINE BREAST ENGORGEMENT CONDUCTED CONVENTIONAL METHODS (BREAST MASSAGE) WITH HERB YEAST-KATU282

STRUCTURE LIPID CARRIER (NLC) SYSTEM ON ANTIOXIDANT STABILITY OF TOMATO EXTRACT (LIPID: CETYL ALCOHOL AND ISOPROPYL MYRISTATE)

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INTRODUCTION

Tomato extract was known contains antioxidants (Chauhan et al., 2010), such as lycopene, that are easily degraded by UV-B rays. The mechanism lycopene as an antiaging is to reduce skin regeneration and increase the thickness of the epidermis (Sahasrabuddhe, 2011). antioxidant, lycopene easily degraded when exposed to light, heat, and oxygen (Chauhan et al., 2011). Drug delivery system based on nanolipid carrier system, such as: Solid Lipid Nanoparticles (SLN) was appropriate to stabilize lycopene, as lipophilic substance (Wissing and Muller, 2003; Helgason et al., 2009; and Okonogi dan Riangjanapatee, 2014). One of the limitations of SLN is the drug molecule that has been trapped expulsed easily during storage. It is caused of the orderdness of lattice lipid crystal. Replacement part of solid lipid using liquid lipid (called as nanostructure lipid carriers or NLC)

In this research have been tried to be compared the ability of SLN and NLC to improve the stability of antioxidant tomato extract. Using 20% total amount of lipid, extract tomato-SLN made from cetyl alcohol as solid lipid and extract tomato-NLC made from difference ratio between cetyl alcohol and isopropyl myristate (IPM) such as: 9:1 and 7:3. Tween 80 and Kollipor were used as surfactant and co-surfactant. IPM was choose because of its enhancer and emollient effect so easily to apply to the skin (Vadgama et al, 2015).

can reduce the orderdness of lattice lipid crystall and

it will repaired it (Kaur et al, 2015).

MATERIALS AND METHODS

Material:

Tomato extract, contain of 20% lykopene (CN Lab Nutrition, Asian Group),, DPPH (Sigma Aldrich), cetyl Alcohol (PT.Bratachem), Isopropyl Myristate (Sigma Aldrich), Kolliphor® (Sigma Aldrich), Tween 80 (Sigma Aldrich), Metanol (Sigma Aldrich), Aquadest, Instrument:

Ultra Turax IKA T-25, Hot-plate stirer , pH meter SCHOTT CG-842, cone and plate viscometer Brookefield, Spectrophotometer Shimadzu UV-1800, X-ray Diffractometry (XRD), , Delsa™ Nano Submicron Particle Size.

METHOD:

Determination of Tomato Extracts IC₅₀ Value by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Spectrophotometry Methode (Muller, 2011). IC50 is the concentration of sample that can inhibite 50% free radical activity, further more call as antioxidant power. Various concentration of workong standard tomato extract solution were reacted with 0.004% solution free radical inhibition DPPH The concentarion determined from the absorbance spectrophotometer, absorbance measurements were taken at three wavelengths, namely 505 nm, 515 nm, 525 nm. Next percent inhibition be calculated by:

% peredaman =
$$\left\{\frac{A_{kontrol} - A_{sampel}}{A_{kontrol}}\right\} \times 100\%$$

IC₅₀ was obtainded from correlation regression curves between percent free radical inhibition and concentration of extract solution, that indicate 50% inhibition of free radical activity.

Preparation of SLN and NLC tomato extract. SLN tomato extract was made of 0,25% tomato extraxt that contain of 20% lycopene (CN Lab Nutrition, Asian Group), 20% cetyl alcohol as solid lipid, 5% tween 80, and 5% kollipor and aquadest ad 100% as aqueous phase. NLC tomato extract had same compotition with SLN, but there were cetyl alcohol replacement with IPM, with two different ratio, such as: 9:1 and 7:3. SLN and NLC tomato extract were made by High Shear Homogenization (HPH) method with a speed of 24,000 rpm conducted for 8 minutes at 4 cycle with Ultra Turax T-25.

Characterisation of sample. Furthermore, sample were characterized such as: pH, viscosity, particle size and its distribution were measured by pH meter, cone and plate viscosimeter and DelsananoTM particle size analyzer respectively. Characterization be held before and after UV-B exposure.

Measurement antioxidant stability. Each sample were exposed with 32.400 Joule/hour UV-B radiation for 2, 5, 9, 15, and 21 hours. After reacting with DPPH solution, the aborbance was observed by spectrophotometer and were calculated the percent inhibition of free radical activity using equation 1. Furthermore the stability of antioxidant were interpreted based on constanta of percent scavenging activity decreasing in antioxidant power (k value) on the appropriate reaction order.

RESULT AND DISCUSSION

IC50 of tomato extract. The regression equation obtained was y = 637,23x + 7.7944 with a correlation coefficient was r = 0.9973. IC50 values can be obtained from the regression equation by entering a value of y = 50% to obtain the value of x, namely the concentration of tomato extract, which can inhibite 50% free radical activity of DPPH. IC50 value of tomato extract obtained is equal to 0.0662%.

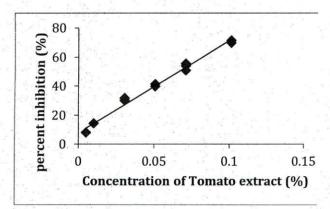


Figure 1. Calculation curve of percent inhibition of free radical activity

Further more, preparation SLN-tomato extract and NLC-tomato extract using extract tomato concentration at least twice to its IC₅₀.

Character of SLN-tomato extract and NLC-tomato extract.

All character of sample presented in table1. All sample had pH in between skin pH range. It was mean all sample did not have potential iritation for skin. There were no significan change of pH relatively after 21 hour UV-B exposure.

Table 1. Character of tomato extract-SLN and tomato extract-NLC

		Mean	Mean	mean	Mean
Sampl	e .	pH ± SD	diameter	PI ± SD	Viskositas
		(%KV)	(nm) ±	(%KV)	(cPs) ± SD
		1 1 4 4 5	SD (%KV)		(%KV)
	SLN	5,73 ±	274.17 ±	0,47 ±	7733,33
		0,20	8,38	0,02	±
Without		(3,49)	(3,06)	(3,34)	1419,72
UV-B					(18,36)
exposure	NLC	5,82 ±	203,54 ±	0,29 ±	1916,33
	9:1	0.07	5,64	0,01	± 152,67
		(1,21)	(2,77)	(4,14)	(7,97)
	NLC	5,87 ±	187.8 ±	0,20 ±	1231,00
	7:3	0,14	31.15	0,10	± 331,39
		(2,46)	(1,66)	(4,78)	(26,92)
	SLN	5,68 ±	411,27 ±	0,33 ±	6845,33
		0,28	11,24	0,10	±
After		(4,93)	(2,73)	(29,59)	1003,23
UV-B					(14,66)
exposure	NLC	5,64 ±	229,72 ±	0,30 ±	1232,56
for 21	9:1	0.09	13,75	0,01	± 300,,24
hours		(1,60)	(5.98)	(2,76)	(24,36)
	NLC	5,83 ±	235,92 ±	0,24 ±	906,12 ±
	7:3	012	8,78	0,01	171,14
		(2,06)	(3,72)	(5,31)	(18,89)

NLC had viscosity lower than SLN. Viscosity NLC with ratio cetyl alcohol: IPM 7:3 lower than 9:1. It was consequence of solid lipid replacement by liquid lipid. This phenomena were the same with the particle size.

UV-B exposured for 21 hours decreased the viscosity but increased particle size. Based on Stokes law, viscosity invers relate with floculation rate of droplet emulsion. Therefore the particle size of NLC with ratio cetyl alcohol: IPM 7:3 increased significant compare with others after UV-B radiation exposure, but indicated more homogen in size.

Diffraction Pattern Determination of Lipid Cetyl alcohol and in the system SLN, NLC (9:1) and NLC (7:3). From diffractogram (fig. 2 and table 2) were known that declining intensity of the crystal lattice cetyl alcohol after made to be SLN. It was effect of adding surfactant in the lipid. Furthermore the intencity more decline once created NLC.



Figure 2. Difractogram cetyl alcohol, cetyl alcohol in SLN, NLC (9:1) and NLC (7:3)

The addition liquid lipid (IPM) reduce the orderdness of lattice lipid crystal. According to Jenning (2000) the orderdness of lattice crystal corresponds to the capability of lipid to entrap the drug. The drug was more difficult to be inserted in the particle lipid that have ordered lattice crystal and was easier to expelled from the carrier

Table 2. The main diffraction peak intensity of the diffraction pattern of the sample

			Angle 20	
	Sample _			
1		7,06°	21,76°	24,70°
	Cetyl Alcohol(CA)	1523,72	3459,99	1668,71
	CA in SLN	211,33	3415,49	1068,65
	CA in NLC (9:1)	237,02	3257,35	833,49
	CA in NLC (7:3)	190,32	2449,97	737,46

Antioxidan Stability Test. From the result of the reaction order determination of changes percent free radical activuty inhibition on each exposure time of each sample was known that the reaction according to first order. Furthermore, the constanta of percent scavenging activity decreasing in antioxidant power (k value) every sample was calculated by making the regretion curve plot time versus log percent inhibition (fig.3). Using the equation for the first order can be calculated the value of k, as presented in the table 3.

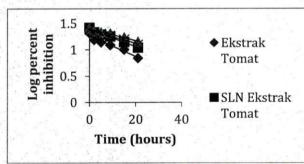


Figure 3. Change rate of log percent free radical activity inhibition of sample over time

First orde reaction means that the change was occurring exponentially with time (Martin et al., 2008). In this case that means decreasing antioxidant power depends only on the concentation of ingridient in tomato extract that has antioxidant power. The greater the value of k indicates that the rate of change of percent inhibition faster, or the sample was unstable. It was known that constanta of percent inhibition free radical activity decreasing of tomato extract was $4.58 \times 10^{-2} \pm 0.0045/$ hour faster than SLN and NLC. This was due to the lipophilic antioxidants ingredients in tomato extract, exposed to the conditions that could degrade the material. Meanwhile, when they were made in

nanolipid such as SLN and NLC, the materials were encapsulated in the system.

Table 3. Mean constanta of change rate of percent free radical activity inhibition of sample over time (based in orde 1 reaction)

Sample	Mean constanta (k) of change rate of percent		
	inhibition between time		
	(/hour) ± SD* (%KV)		
Tomato Extract (TE)	4,58 x 10 ⁻² ± 0,0045 (9,87)		
TE - SLN	$3,82 \times 10^{-2} \pm 0,0019 (4,93)$		
TE - NLC 9:1	$2,59 \times 10^{-2} \pm 0,0011 (4,19)$		
TE - NLC 7:3	$2,83 \times 10^{-2} \pm 0,0006 (2,05)$		

The antioxidant power tomato extract in the NLC was more stable than in the SLN form. It was seen from the smaller of k value. It was the impact of reducing of lattice lipid crystal orderdness (fig. 2). Therefore the active ingredient was not easily expulsed out. In this case, the more IPM addition in the system by changing the ratio of cetyl alcohol: IPM from 9: 1 to 7: 3 was not very meaningfull in stability effect. Decreasing orderdness of lipid crystal lattice was not necessarily improve the drug entrapment. There were need optimal conditions that produce optimal trapping anyway.

CONCLUSION

The conclussion of this research were:

- Antioxidant stability of tomato extract increased in nanolipid system carrier with total amount of lipid cetyl alcohol 20%
- Replacing part of solid lipid (cetyl alcohol) to liquid lipid (isopropyl myristate/IPM) to be NLC system in ratio 9:1 and 7:3 could increased antioxidan stability of tomato extract.
- Increasing isopropyl myristate ratio in NLC system from 9:1 to be 7:3 did not increase antioxidan stability of tomato extract meaningfuly

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