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FORMULATION SPAN 40 NIOSOME OF AQUEOUS FRACTION MENIRAN (*PHYLLANTHUS NIRURI* L.)

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ABSTRACT
Meniran (*Phyllanthus niruri* L.) is used for many conditions such as diabetes mellitus, hypertension, obesity and hyperlipidemia. The active constituens to which the antidiabetic activity of *P. niruri* L. has been attributed include flavonoids. Flavonoid glycosides have been the physical problem of delivering a drug across the membrane. The aim of the present study were total flavonoids content and to develop a niosomal formulation for aqueous fraction of *P. niruri* L. Methode in this study are analysis the total flavonoids content and characterization of the niosomes. The total flavonoids content from extract and aqueous fraction with rutin standard were 12.32±0.53 and 20.07±1.23 (RE/g) respectively. The niosomes preparation used a thin layer film hydration technique with solven hydration 0.5; 1; 2; 3 and 4% aqueous fraction of *P. niruri* L. Niosomes span 40 can be hydration in temperature 80^{o}C for 20 minutes to concentration 0,5% and 1-3% for 50 minutes while 4% can't released. The particle size of the optimal delivery system prepared with Span 40 100 µmol and cholesterol 20 µmol was about 5 µm.

KEYWORDS: Aqueous fraction of *P. niruri* L., total flavonoids and niosome span 40.

INTRODUCTION
Phyllanthus niruri L. (Phyllanthaceae) is a small herb well known its medicinal properties and widely used worldwide. It has a long history in herbal medicine systems such as Indian Ayurveda, Traditional Chinese Medicine (TCM), and Indonesian Jamu. Different parts of herb including roots, leaves, and seeds are used medicinal purposes. It is often used in the traditional system of medicine for a variety of disease including diabetes, diuretic, problems of stomach, astringent, liver, kidney and spleen, asthma and bronchial infections (Petel et al., 2011). Extracts of the plant had been reported to have pharmacological effects such as antioxidant and hepatoprotective (Lim & Murtijaya, 2007; Sabir & Rocha, 2008, Pramyothin, Ngamtin, Poungshompoo, & Chaichantipyuth, 2007; Faremi, Suru, Fafunso, & Obioha, 2008; Sarkar & Sil, 2007), antimutagenic and anticarcinogenic (Sripanidkulchai, et al., 2002), antiplasmodial (Ajala, et al., 2011), antibacteria (Eldeen, et al., 2011), antiinflammatory and allodynic (Kassuya et al. 2006), hipoglicemic dan hipocholesterol (Adeneye, et al., 2006).

The active constituens to which the antidiabetic activity of *P. niruri* L. has been attributed include flavonoids. Natural product such as rutin, quercetin, silymarin, curcumin, emetine, quinine, etc., have found aplications in health care system to their wide biological activities. Among different groups of natural products, flavonoids play an important role in the treatment of antidiabetic (Sharma et al. 2013). Niosomic surfactant based vesicles are formed from the self-assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structures (Bayindir & Yuksel 2010). The aim in the present study were total flavonoids content and to develop a niosomal formulation for aqueous fraction of *P. niruri* L. Total flavonoids content extract and aqueous fraction of *P. niruri* L determinate such as marker and characterization of the niosomes Span 40.

MATERIAL AND METHODS
Aerial parts of *Phyllanthus niruri* L., were supplied by the Herbal Anugerah Alam Yogjakarta Indonesia Distribution Center of Herbal/materal Jamu. Span 40, cholesterol, rutin from sigma Aldrich. Rotary evaporator (BUCHI R-100), Spectrophotometer Shimadzu type 2450 UV-Vis. All reagents were pro analysis grade.

1. Extraction and Purification
The air-dried areal part of *P. niruri* L. 1.1285 kg were powdered and than extracted with 70% ethanol at room temperature for eight times. Each extraction was continued for 2 days. Filtrates were combined and concentrated using a rotary evaporator at a temperature 50^{o}C to give dark brown syrup about 284.14 g (2.52% based on the dry weight). A part of the crude extract

168,94 g was suspended in destilled water (240 ml) and then partitioned with n-hexan and EtOAc successively. The fractions were concentrated under reduced pressure at 50°C to remove solvents. The n-heksan, EtOAc and water fraction were 5.63, 10.47 and 51.40 g respectely.

2. Total Flavonoid content as Marker Compound

a. Calibration curve of rutin

Total flavonoid was measured by alluminium chloride colorimetric method. In this method rutin was used as solution standard. 5 mg of rutin was dissolved in ethanol: destilled water (1:1) and then diluted to 70, 80, 100, 120, 140 and 160 µg/ml. The standard solution was added to 5 ml volumetric flask. To the flask was added 0.15 ml 5% $NaNO_2$, after six minutes 0.15 ml 10% $AlCl_3$ was added. After six minutes, 2 ml 4% NaOH was added and the volume was made up to 5 ml with ehtanol : destilled water (1:1). After 15 minutes a calibration curve was made by measuring the absorbance of the dilutions at 509,5 nm (λmax of rutin) with a Shimadzu type 2450 UV-Vis spectrophotometer. The total flavonoid content was expressed as mg rutin equvalens (RE) (John et al. 2014).

b. Stock Solution of extract and aqueous fraction

Stock solution of sample was prepared by dissolving 25 mg of the ethanolic extract and fraction of *P. niruri* L. in 25 ml ethanol : destilled water (1:1).

c. Preparation of Test Solution

2 ml of each extract stock solution was added to 5 ml volumetric flask. To the flask was added 0.15 ml 5% $NaNO_2$, after six minutes 0.15 ml 10% $AlCl_3$ was added. After six minutes, 2 ml 4% NaOH was added and the volume was made up to 5 ml with ehtanol : destilled water (1:1). After 15 minutes a calibration curve was made by measuring the absorbance of the dilutions at 509,5 nm (Linn 2013).

3. Solubility study

The solubility of rutin and aqueous fraction were determined in mixture ethanol/water different ratio. An excess quantity of the sample was added in 5 ml of each mixture ethanol/water in different ratio at room temperature.

4. Preparation of aqueous fraction *P. niruri* L. (AFP) niosome

Niosomes were prepared by film hydration method was used surfactant span 40 in level 100 µmol and 20% cholesterol of span 40 levels used in the formula. Before encapsulating AFP in niosomes a formulation study was done without active agent to observe the vesicle formation and to choose appropriate parameters to use in formulation preparation. Span 40 and cholesterol dissolved in chloroform and then organic solvent was removed under vacuum by rotary evaporator (BUCHI R 100) at themperature 45±2°C and the flask rotated at 150 rpm until a film was obtained. This film was stored in a desiccator to remove residual organic solvents (Bayindir & Yuksel 2010; Jin et al. 2013).

5. Preparation of AFP-Loaded in Niosome

In this study, the solution sample containing AFP 0.5, 1, 2, 3 and 4%. The film was hydrated with 10 ml of a mixture of ethanol/water (1:4) containing AFP. Rotary epavoration was performed again at 80°C until hydration was achieved. The size of the noisome particle is minimized using a probe type sonicator (Singh 2011).

6. Photo microscopy of AFP niosomes

Vesicle dispertions were characterized by photo microscopy for vesicle for vesicle formation and morphology. Samples of niosome formulations were examined under optical microscope and photographed at magnification of 40 to 100x (Singh 2011).

RESULTS AND DISCUSSION

Total Flavonoid Content

Flavonoid are plant secondary metabolites widely distributed in the plant kingdom. To perform the calculations of total flavonoid content in the studied plant using G.C. Bag el al., method, a standar curve is needed which is obtained from a series of different rutin concentrations. Concentration values of extract and aqueous fraction were obtained from Rutin standard curve, by interpolating to the X-axis. TFC was calculated by using the following formula (Bag & Devi 2015; John et al. 2014).

$$TFC = \frac{RxD.FxVx100}{W}$$

Where, R-Result obtained from the standard curve, D.F-Dilution factor, V-Volume of stock solution, 100-for 100 g dried plant and W-weight of plant in the experiment.

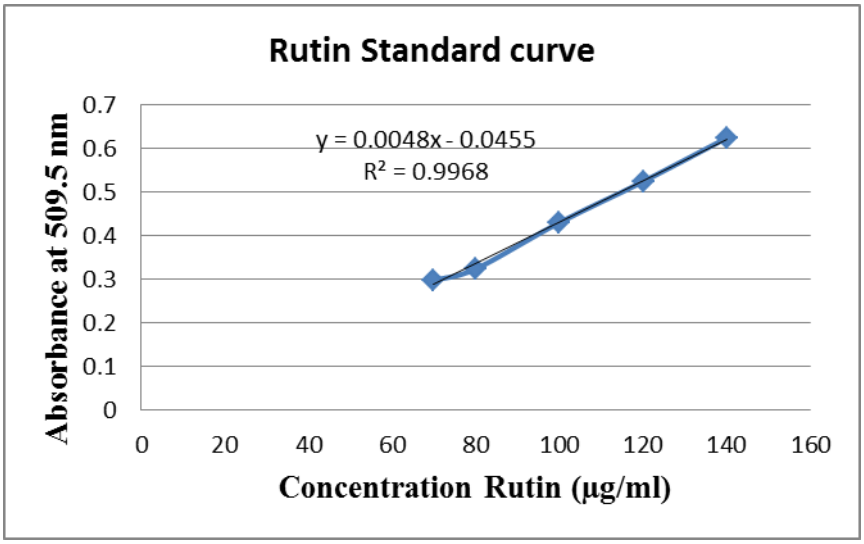

Figure 1: Calibration curve of rutin.

The results showed that total flavonoid content in the extract ethanolic (12.32±0.53 RE/g) lower than the aqueous fraction (20.07±1.23 RE/g). Rutin also known as quercetin-3-rutinoside is a flavonol glycoside was isolated from *P. niruri* L (Bagalkotkar et al. 2006; Prabodh Shukla 2012). Base on the result of total flavonoid content, the aqueous fraction of *P. niruri* L. (AFP) then made the form drug delivery system that is niosomes.

Solubility profile

The observation solubility of rutin and AFP in in mixture ethanol/water different ratio are shown in table 1.

Table 1: Solubility profile.

Solvent	Solubility	
	Rutin	AFP
Ethanol	Very soluble	Very soluble
Water	Very slightly soluble	Very slightly soluble
Ethanol/water (1:4)	Slightly soluble	Slightly soluble
Ethanol/water (1:1)	Soluble	Soluble

Preparation of aqueous fraction *P. niruri* L. (AFP) niosome

Niosomes will form when hydrated with aqueous phase. Niosomes can trap lipophilic material in the lipid double layer, and ensnare the hydrophilic material in the intra niosome portion (Sankhyan, A., 2012). The process was made niosomes begins by hydrated thin layers of cholesterol and surfactants. Span 40 is a nonionic surfactants were preferred because of low toxicities. Span 40 is an amphiphilic compound having a hydrophilic group and a lipophilic group. Span 40 has hydrophilic groups that are hydrophilic and lipophilic alkyl chains (Rowe, R. C., Sheskey, P.J., Quinn 2009). Span 40 also showed percent entrapment efficiency better then span 20 and span 60 in the niosomes of paclitaxel. Cholesterol is added to the formulations as membrance stabilizing agent. Interaction between cholesterol and the surfactant bilayer depend on both the structure of the lipid chain and the hydrophilic head group, incorporation of cholesterol at increasing amounts causes to broaded and eventually disappear the gel to liquid phase transition of lipid bilayers (Bayindir & Yuksel 2010). In this study of rotavapor speed used four variations of that is 60, 90, 150 and 210 rpm. The process was removed organic solvent at a temperature of 45±2^{o}C for 10 minutes. The results of the optimization showed that at 60 rpm and 90 rpm, the thin layer obtained was uneven in which tends to thicken at the bottom flask. The thin layer formed at a speed of 150 rpm shows more evenly on the surface of the round bottom flask, while at 210 rpm the thin layer formed is unevenly visible where there is a thin layer of hollow due to air bubbles or foam due to the high rotation speed. The results of this optimization was obtained that the process of made thin layer niosomes coating done at rotavapor speed 150 rpm.

Table 2: Result optimization rotation speed at procces removed organic solvent.

Formula	F1	F2	F3	F4
Span 40 (µmol)	100	100	100	100
Chloroform (ml)	10	10	10	10
Speed (rpm)	60	90	150	210
Result	Thin layer was unevenly thickened at bottom flask	Thin layer was unevenly thickened at bottom flask	Thin layer evently	Thin layer was unevenly and there were hollow part

Preparation of AFP-Loaded in Niosome

The niosomes thin layer hydration process with various concentration 0.5, 1, 2, 3 and 4% of AFP. In the hydration process in which themperature optimazation was used to hydration was achieved is 80^{o}C and the time required to obtain the niosomal suspension at each concentration is different. Solution containing 0.5% AFP was hydration obtained for 20 minutes while solution containing AFP 1-3% were hydration obtained takes 50 minutes and then concentration 4% was not hydration achieved because passes saturated. The suspension niosomal of AFP showed fig.2.

Figure 2: Suspension nisomal span 40 of AFP.

Photo microscopy

The vesicle forming ability of Span 40 was investigated after sonication application. In the present study the size of particles were studied using an optic microscope. The studies with the microscope gave a qualitative idea about the size and shape of the vesicle (Bayindir & Yuksel 2010). The size particles niosomal AFP The results obtained niosome shaped speris and have particles size of 5µm.

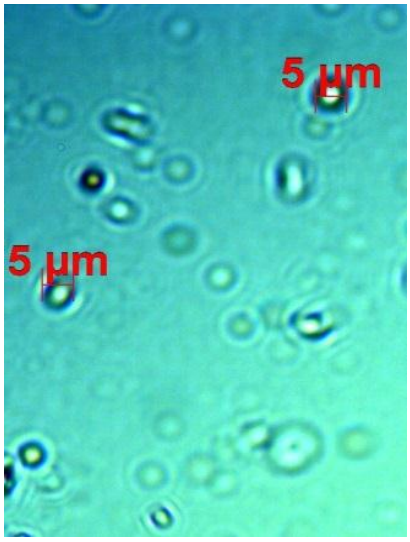

Figure 3: Photomicrograph nisomal of AFP.

CONCLUTION

The total flavonoids content from extract ethanolic (12.32±0.53 RE/g) lower than the aqueous fraction (20.07±1.23 RE/g). The size particles niosomal AFP The results obtained niosome shaped speris and have particles size of 5µm.

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REFERENCES

1. Adeneye, a a, Amole, O.O. & Adeneye, a K., 2006. Hypoglycemic and hypocholesterolemic activities of the aqueous leaf and seed extract of Phyllanthus amarus in mice. *Fitoterapia*, 77(7-8): 511–4.
2. Ajala, T.O. et al., 2011. The antiplasmodial effect of the extracts and formulated capsules of Phyllanthus amarus on Plasmodium yoelii infection in mice. *Asian Pacific journal of tropical medicine*, 4(4): 283–7.
3. Bag, G.C. & Devi, P.G., 2015. Research Article Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley 1. International Journal of Pharmaceutical sciences Review and Research, 30(28): 154–159.
4. Bagalkotkar, G. et al., 2006. Phytochemicals from Phyllanthus niruri Linn. and their pharmacological properties: a review. *The Journal of pharmacy and pharmacology*, 58: 1559–1570.

5. Bayindir, Z.S. & Yuksel, N., 2010. Characterization of Niosomes Prepared With Various Nonionic Surfactants for Paclitaxel Oral Delivery. Journal of Pharmaceutical Science, 99(4): 2049–2060.
6. Eldeen, I.M.S. et al., 2011. In vitro antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven Phyllanthus sp. *South African Journal of Botany*, 77(1): 75–79.
7. Faremi, T.Y. et al., 2008. Hepatoprotective potentials of Phyllanthusamarus against ethanol-induced oxidative stress in rats. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 46(8): 2658–64.
8. Jin, Y. et al., 2013. Development of a novel niosomal system for oral delivery of Ginkgo biloba extract. International Journal of Nanomedicine, 421–430.
9. John, B. et al., 2014. Total Phenolics and Flavonoids in Selected Medicinal Plants From Kerala., International journal Pharmacy and Pharmaceutical Science, 6(1): 0–2.
10. Kassuya, C.A.L. et al., 2006. Antiinflammatory and antiallodynic actions of the lignan niranthin isolated from Phyllanthus amarus. Evidence for interaction with platelet activating factor receptor. *European Journal of Pharmacology*.
11. Lim, Y.Y. & Murtijaya, J., 2007. Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods. *LWT - Food Science and Technology*, 40(9): 1664–1669.
12. Linn, S., 2013. RESEARCH ARTICLE ESTIMATION OF TOTAL FLAVONOIDS CONTENT (TFC) AND ANTI OXIDANT ACTIVITIES OF METHANOLIC WHOLE PLANT EXTRACT OF BIOPHYTUM., 3(4): 33–37.
13. Prabodh Shukla, B.G. and P.S., 2012. ISOLATION OF RUTIN FROM PHYLLANTHUS AMARUS. *International Journal of pharmaceutical sciences and research*, 3(04): 1198–1201.
14. Pramyothin, P. et al., 2007. Hepatoprotective activity of Phyllanthus amarus Schum. et. Thonn. extract in ethanol treated rats: in vitro and in vivo studies. *Journal of ethnopharmacology*, 114(2): 169–73.
15. Rowe, R. C., Sheskey, P.J., Quinn, M.., 2009. *Handbook of Pharmaceutical Exipients* 6th Editio., London: Pharmaceutical Press and American Pharmacists Association.
16. Sabir, S.M. & Rocha, J.B.T., 2008. Water-extractable phytochemicals from Phyllanthus niruri exhibit distinct in vitro antioxidant and in vivo hepatoprotective activity against paracetamol-induced liver damage in mice. *Food Chemistry*, 111(4): 845–851.
17. Sankhyan, A Pawar, P., 2012. Recent Trends in Niosome as Vesicular Drug Delivery System. *Journal of Applied Pharmaceutical Science*, India, 2012: 20–32.
18. Sarkar, M.K. & Sil, P.C., 2007. Hepatocytes are protected by herb Phyllanthus niruri protein isolate against thioacetamide toxicity. *Pathophysiology*.
19. Sharma, S. et al., 2013. Rutin: therapeutic potential and recent advances in drug delivery. *Expert Opinion on Investigational Drugs*, 22(8): 1063–1079.
20. Singh, C.H., 2011. Formulation, Characterization, stability and invitro evaluation of nimesulide niosomes. *Pharmacophore*, 2(3): 131–148.
21. Sripanidkulchai, B. et al., 2002. Antimutagenic and anticarcinogenic effects of Phyllanthus amarus. *Phytomedicine*, 9: 26–32.