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Abstract
Oxidative stress is a pathogenic factor in the complications of diabetic. Sambiloto herb (*Andrographis paniculata* (Burm f.)Ness.) and dewandaru leaves (*Eugenia uniflora* Linn.) are traditional medicines that have an antidiabetic and antioxidant activity, respectively. In this study, the extract of these plants were formulated into an effervescent tablet to improve taste, physical properties and patient acceptability. Effervescent tablet that showed the best physical properties and taste responses was then tested for the antidiabetic and antioxidant activities.

The sambiloto herbs and dewandaru leaves were extracted by maceration method with petroleum ether and 70% ethanol. Tablets were formulated using mannitol as filler and different concentration of polyvinylpyrrolidone (PVP) as binder. Tablets were prepared by using wet granulation method and evaluated for weight variation, hardness, disintegration time and friability. Antihyperglycaemic was tested using oral glucose tolerance test. The antioxidant property was observed by using DPPH method in vitro.

All the formulation showed hardness and weight variation within limit, but not the friability. Increasing PVP concentration improved physical properties of tablets i.e. decreased the friability and increased the granular flow and hardness, however generated a longer disintegration time. Tablet F1 containing 2% of PVP and 24% of mannitol was the most promising formulation with the disintegration time of less than 2 minutes and high acceptability of the taste and appearance by respondents. The study indicated that effervescent tablet F1 in the dose of 18.35 mg/kg BW lowered the blood glucose level of diabetic rats (P<0.05) and showed antioxidant activity with the IC50 value of 40.71 µg/ml.

Keywords: effervescent tablet, *Andrographis paniculata* (Burm f.) Ness., *Eugenia uniflora* Linn., antidiabetic, antioxidant

Introduction
Oxidative stress is an important pathogenic factor in the developments of diabetic mellitus complications. Diabetic causes abnormalities in the metabolic that produces excessed mitochondrial superoxide 1. High concentration of reactive oxygen species (ROS) in a biological system contributes for the cellular damage of lipids, membranes, proteins, and DNA. This will lead to microvascular and cardiovascular diabetic complications such as neuropathy, retinopathy, nephropathy and multi-organ atherosclerosis 2. In recent years, antioxidants such as vitamin C, vitamin E, bioflavonoids, minerals, etc., have been studied widely for its important role in the protection against ROS 3-4.

Sambiloto herb (*Andrographis paniculata* (Burm f.)Ness.) and dewandaru leaves (*Eugenia uniflora* Linn.) are traditional medicines that have an antidiabetic and antioxidant activity, respectively. Previous studies showed that *A. paniculata* herb can reduce blood glucose level on normal, diabetic rats, or non-diabetic rabbit 5-9. The extract of *A. paniculata* was also reported to decrease oxidative stress in diabetic rats 10,11. *E. uniflora* is a widely distributed plant over the world. This plant contains polyphenolic compounds such as flavonoid that has an antioxidant activity 12-14.

One challenge in the formulation of the extract of herbal medicines is the solubility that usually very low, thus limited the absorption of the active compound. The other problem is the taste of the extract that sometimes unacceptable by consumers, in this case the very bitter taste of *A. paniculata* extract 15. In this study, the extract of these plants were formulated into an effervescent tablet to improve solubility, taste, physical properties and patient...
acceptability. Effervescent tablet that showed the best physical properties and taste responses was then tested for the antidiabetic and antioxidant activities.

Material and Methods

Materials

Plant materials and Chemicals

The herb of *A. paniculata* and leaves of *E. uniflora* were collected from Tawangmangu, Surakarta, Indonesia. The plants were identified in the *Balai Besar Penelitian dan Pengembangan Obat da Obat Tradisional* (BBPPTOOT) Tawangmangu. Polyvinylpyrrolidone, citric acid, tartaric acid, sodium bicarbonate, aspartame, magnesium stearate, silica gel GF254, cellulose plate, DPH (1,1-diphenyl-2-picrylhidrazyl), vitamin E were purchased from Sigma. GOD-PAP reagent and glucose was from DiaSys. Other chemicals and solvents were purchased from Brataco.

Experimental Animals

A total number of 20 Wistar male rats weighing about 150-200 gram and age of 3 months were purchased from Department of Pharmacology, Faculty of Pharmacy Muhammadiyah University of Surakarta. The animals were maintained under standard condition and allowed free access to pellet diet and water ad libitum.

Experimental design

Extraction procedure

The ethanolic extract of *A. paniculata* and *E. uniflora* was collected by maceration method. Dry powder of *A. paniculata* herbs (4 kg) and *E. uniflora* leaves (3 kg) was macerated using petroleum ether (3 x 7L). The dried powder was then macerated again using ethanol (2 x 7L). The filtrate was evaporated using vacuum rotary evaporator to remove the solvent. The product was washed with warm water and freeze-dried for 2 days to obtain dry powder.

Identification of extracts

The extracts were examined for the viscosity, color, odor and taste according to the standard. TLC was carried out to determine the flavonoid and the andrographolide qualitatively of the *E. uniflora* and *A. paniculata* extract, respectively.

Formulation of effervescent tablet containing extract of *A. paniculata* and *E. uniflora*.

The effervescent tablet of 3000 mg was prepared as follows: the *A. paniculata* herb extract (114 mg), *E. uniflora* leaves extract (32 mg), polyvinylpyrrolidone (PVP) as binder (60, 90, 120, 150 mg for F1, F2, F3 and F4, respectively), magnesium stearate (15 mg), citric acid (281 mg), tartaric acid (562 mg), and sodium bicarbonate (956 mg) and mannitol (32 mg), polyvinylpyrrolidone (PVP) as binder (60, 90, 120, 150 mg for F1, F2, F3 and F4, respectively), magnesium stearate (15 mg), citric acid (281 mg), tartaric acid (562 mg), and sodium bicarbonate (956 mg) and mannitol (32 mg). The extracts were diluted with ethanol 96%, and mixed with the binder. Mannitol and aspartame were then added and formed into a paste and granulated using mesh 14. The granules were oven dried at 50°C for one day. Citric acid, tartaric acid, sodium bicarbonate and magnesium stearate were added and the granules were resized using mesh 14. The granules were then transferred to the hopper of a single punch tableting machine (Korsch-Germany) after tested for the flow properties. All of the processes were carried out in a room with a RH of 40-60% and a temperature of 21°C.

The physical test of the effervescent tablet i.e. hardness, weight variation, disintegration time/effervescent time, and friability test were carried out to confirm their conformity with monographs of Indonesian Pharmacope and other literatures.

Hardness test were carried out using Monsanto hardness tester on 10 tablets of each formula. The average weight was determined by randomly picking 20 tablets and recording the weight of each tablet using a sensitive balance. Friability of the tablet was determined using Erweka TA-20 friabilator. The disintegration time was determined by randomly taking three tablets. One tablet was individually placed in a beaker containing about 200 ml of water at 10, 25 and 60°C and the time of complete dissolution indicated by the cease of gas bubble for each tablet was recorded using a stop watch.

Oral Glucose Tolerance Test

Rats were fasted overnight for 12-14 h and assigned randomly into 4 groups.

Group I: Negative control, received sodium CMC 0.5%

Group II: Positive control, received Tolbutamide 100 mg/kg BW

Group III: Placebo, received solution of effervescent tablet without extract (18.35 mg/kg BW)

Group IV: Received solution of effervescent tablet F1 (18.35 mg/kg BW)

Baseline blood glucose level (BGL) was determined. Thirty minutes before extract treatment, all the rats were loaded with 4.5 g/kg BW glucose solution, and then orally treated according to their perspective grouping. Blood samples were then collected to determine BGL prior to treatment and after 30, 60, 90, 120, 180, 240 and 300 min of treatment. BGL was determined using enzymatic method, GOD-PAP using spectrophotometer (StarDust FC 15). Blood glucose level was calculated as the area under curve (AUC0-300) with an equation as follows:

\[
AUC_{0-300} = \frac{t_1 - t_0}{2} x(C_0 + C_1) + \frac{t_2 - t_1}{2} x(C_2 + C_1) + \cdots + \frac{t_n - t_{n-1}}{2} x(C_n + C_{n-1})
\]

The decrease of blood glucose level was calculated as follows:

\[
\text{% decreasing BGL} = \frac{AUC_{0-300} \text{ of Negative control} - AUC_{0-300} \text{ of sample}}{AUC_{0-300} \text{ of Negative control}} \times 100\%
\]
DPPH radical scavenging activity
DPPH radical scavenging activity was performed by the method of Utami, et al. (2005). Samples were prepared by dissolving one effervescent tablet F1 into 250.0 ml of water. Series concentration of ethanolic extract of *E. uniflora*, etanolic extract of *A. paniculata* and solution of vitamin E in methanol were used as references. Ethanolic DPPH (400 \( \mu \text{M} \)) was used in the reaction mixture. Serial dilutions of the test sample were combined with the DPPH solution. The reaction mixtures were incubated for 30 min at 37°C and the change in absorbance at 517 was measured. Mean values were obtained from triplicate experiments. Radicals scavenging percent was calculated using the equation,

\[
\text{Percent radicals scavenging} = \left( \frac{\text{absorbance of the control} - \text{absorbance of the sample}}{\text{absorbance of the control}} \right) \times 100\%.
\]

Percent radicals scavenging were then plotted against concentration, and the equation for the line was used to obtain the IC\(_{50}\) value.

Taste responses
The taste and appearance of the effervescent tablet was tested on 20 respondents. Respondents were given questionnaire whether they could accept the taste and the appearance of the effervescent tablet. The marketed effervescent tablet containing thyme extract, bitter orange peel and chicory root was used as reference.

Statistical analysis
All experiments were run in triplicate. The data were analyzed by SPSS software. The differences between treatments were determined by using one-way analysis of variance (ANOVA) and the LSD test, values of \( p \leq 0.05 \) being considered as significantly different.

Results and Discussion
The maceration process of *A. paniculata* herbs resulted in brown, sticky powder, specific odor and very bitter taste of extract with the yield of 3.3%. The *E. uniflora* extract showed light brown, dry, odorless and bitter powder with the yield of 3.35%. The TLC profile of the extract identified flavonoid content from *E. uniflora* leaves and andrographolide from *A. paniculata* (Figure 1). Those compounds were still remained after tableting process, as can be seen in spot from the tablet solution (2), compared to the spot from the extract (1). The TLC analysis showed a Rf of 0.81 and 0.75 for *E. uniflora* and *A. paniculata*, respectively, and the similar spot of the tablet solution informed that there is no degradation during tableting process.

Effect of PVP as binder on effervescent tablet properties
In this research PVP was used as binder agent with different concentration. The binder concentration affects the granular flow hence influences the weight uniformity of tablets. Figure 2A showed the decreasing of granular flow time as increasing PVP concentration. The granular flow time fulfilled the requirement for a good tablet which is less than 10 sec for 100 g granules. Increasing PVP solution concentration rise the compatibility of the granules, hence enhance the flow rate of the granules. As shown in Figure 2A, the flow rate of the granules did not effect the weight variation of the tablet. All the formulations showed mean weight of around 3000 mg, and weight variation within limit, which is less than 5%. Weight uniformity will ensure the uniformity of the dose, to achieve a similar therapeutic effect.
uniformity (A) and the relation of the hardness and the friability (B) as a function of increasing PVP concentration.

The results showed that formulation of effervescent tablet containing *A. paniculata* and *E. uniflora* with 2-8% PVP produced tablet within acceptable limits, but not the friability (Figure 2B). Increasing concentration of PVP as binder resulted in significant difference of hardness and friability (p≤0.05). F1-F4 formulation resulted in tablet hardness of 5.16, 5.78, 5.76 and 6.56 kg and friability of 16.88, 16.08, 12.01 and 8.87 for F1, F2, F3 and F4, respectively. Binder concentration influenced the inter-particles binding, hence affected the hardness and friability. Friability above 1% was an indication of poor mechanical resistance of the tablets.

![Disintegration time](image1)

**Figure 3. Disintegration time of effervescent tablets containing *A. paniculata* and *E. uniflora* (A) and the acceptability of the taste and appearance of the tablets by respondents (B).**

Disintegration time studies indicated the promising formulation of F1, as the tablet could disintegrate in less than 2 minutes at 25°C (Figure 3A). Increasing PVP concentration inhibited penetration of water into the tablet, resulted in increasing disintegration time. This data was supported by acceptability of the F1 by respondents that showed acceptability of 65% and 85% for the taste and appearance, respectively. Taste is an important factor in the development of effervescent tablet, especially the one containing herbal extract. The addition of mannitol and aspartame as filler and sweetener agent could mask the bitter taste of the *A. paniculata* and *E. uniflora* extracts, and produced tablet with good appearance.

![Disintegration time](image2)

**Figure 4. Antihyperglycaemic effect of the effervescent tablet containing *A. paniculata* and *E. uniflora***

**Determination of antidiabetic activity**

The effect of effervescent tablet solution containing *A. paniculata* and *E. uniflora* and tolbutamide is shown in Figure 4. The blood glucose level of glucose-loaded rats with a treatment of effervescent solution and tolbutamide suspension showed significant differences (p≤0.05), compared to the negative control or placebo group. The solution of effervescent tablet decreased the blood glucose level by 13.1% than the negative control (solution of sodium CMC 0.5%). Decreasing blood glucose level of the test solution compared to the placebo by 16.57% indicated that the antihyperglycaemic effect was resulted from the extract ingredients, and that the excipients such as PVP, mannitol or aspartame did not take any role. Mannitol is a sugar alcohol with sweetness half as sucrose, whereas aspartame is an artificial sweetener with sweetness more than 200 times than sucrose. Both Mannitol and aspartame are safe for diabetic patients because they do not increase the blood glucose level. However, antihyperglycaemic activity of the positive control (tolbutamide) was still superior than the effervescent tablet, with the % decreasing blood glucose level of 24.61% and 27.66% to the negative control and placebo, respectively.
Determination of antioxidant activity

_E. uniflora_ contains flavonoids that have been proven for their antioxidant activities. The flavonoids were observed for their antioxidant activity through their radical scavenging effects. In this research, radical scavenging activity of the effervescent tablet containing _A. paniculata_ and _E. uniflora_ was determined using DPPH assays. The result of antioxidant activity of the samples was shown in Figure 5. Among the samples, the effervescent tablet showed relatively high scavenging activity with an IC50 of 40.71±0.38 μg/ml, although lesser than vitamin E and _E. uniflora_ extract (p≤0.05). The antioxidant activity of the effervescent tablet was due to the presence of _E. uniflora_ extract that showed an excellent radical scavenging activity with an IC50 of 3.44±0.38 μg/ml, which is better than vitamin E with an IC50 of 7.59±0.03 μg/ml. The _A. paniculata_ extract showed the lowest scavenging activity, with an IC50 of 2222.49±4.29 μg/ml.

Conclusion

As conclusions, formulation of the extract of _A. paniculata_ and _E. uniflora_ using PVP as binder and mannitol as filler resulted in an effervescent tablet that fulfilled all the requirements, except for the friability and disintegration time. Increasing concentration of PVP produced tablet with more hardness and less friability, but lack in disintegration time. Tablet F1 containing 2% of PVP and 24% of mannitol was the most promising formula with the disintegration time of less than 2 minutes and high acceptability of the taste and appearance by respondents. The study indicated that the solution of the effervescent tablet containing _A. paniculata_ and _E. uniflora_ lowered the blood glucose level of diabetic rats and showed high antioxidant activity in vitro.

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AUTHORS’ CONTRIBUTIONS
Authors contributed equally to all aspects of the study.

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CONFLICTS OF INTEREST
The authors declare that they have no competing interests.