Evaluation of Gastroprotective Activity of Cemot Leaves (Passiflora foetida L.) Extracted by Ultrasonic Assisted Extraction (UAE) Against Ethanol-Induced Gastric Lesions in Rats

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ABSTRACT

Peptic ulcers is one of the global health problems in the world. However, most of these drugs have unwanted side effects and drug interactions that this research focus on the use of plant products as anti gastric ulcer. The phytochemical of Passiflora foetida L. leaves or better known by the name of the Central Borneo with 'Cemot' such as flavonoid, alkaloids and tannin have potential as a gastroprotective. In this study used the method of extraction of non-conventional, namely the UAE, because it has advantages compared with other conventional methods. This study aims to investigate the gastroprotective activities of ethanol extract of Passiflora foetida leaves-UAE. Cemot extract was obtained by UAE method using a concentration of ethanol (70%), the ratio of sample/liquid (10g/mL) for 3 minutes. Three groups of rats treated with a respective dose of the extract (50, 100 and 200 mg/kg BW), one group of rats treated with omeprazole 36 mg/kg BW for seven days before peptic ulcer induction using ethanol. The activity gastroprotective with measurement of lesion of gastric ulcer index and the percentage of inhibition ulcer. Group of rats treated with 200 mg/kg BW extract showed a decreased the ulcer index and ulcer inhibition than the other extract dosage groups, respectively 2.83 and 35.34%. Conclusion: Based on the results of this study concluded that the cemot extract has potential as a gastroprotective.

Keywords: Passiflora foetida L.; UAE; gastroprotective; gastric lesions; ethanol

INTRODUCTION

Gastric ulcer is one of the global public health problems and affects a large number of people around the world (Chan & Leung, 2002). Gastric ulcer disease affects 4 million people worldwide each year (Zelickson et al., 2011). The oral administration of ethanol will penetrate the mucous layer of the stomach (epithelium) resulting in increased mucosal permeability and epithelial barrier and the diffusion of hydrogen ions that can damage the gastric mucosa. Ethanol can lead to increased free radical production, disruption to the microcirculation of mucous membranes, and a decrease in the production of mucilage or mucus (Srikanta et al., 2007). In clinical practice, some classes of drugs such as histamine receptor antagonists (H2), proton pump inhibitors (PPI) and antacids used as a treatment of peptic ulcers (Root et al., 2014). However, based on the clinical evaluation of the use of this conventional treatment in the long term can cause side effects such as hypersensitivity, arrhythmia, impotence, gynecomastia and hematopoietic disorder (Sheen & Triadafilopoulous, 2011).

However, most of these drugs have unwanted side effects and drug interactions that

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this research focus on the use of plant products as anti gastric ulcer. The use of herbal medicine is growing widely with a recent study where traditional medicine used to treat diseases in several generations (Hernani, 2011). One of the natural ingredients as an alternative therapy from plants as gastroprotective is Passiflora foetida L. Community in Central Kalimantan to know this plant with its region name is "Cemot". The alternative approach with the research of phytochemical compounds from natural ingredients as a new treatment therapy against gastric ulcer is relatively cheaper and safer, especially use in higher doses (Root et al., 2014). The cemot plants (Passiflora foetida L.) grows in the tropical state of Indonesia found in the forests of Central Kalimantan. The cemot plants (Passiflora foetida L.) is a type of plant that is publicly exposed to propagate in other crops. This plant found in aqueous areas such as swamp and rivers (Lim, 2012). Figure 1 below shows the plants of the cemot with some parts of the plant.

The cemot plants have activities as anti-inflammatory, antitumor, anticancer, antihypertotoxicity and antimicrobial. In general, the cemot leaves are still limited in use as soups and as fresh vegetables. The main phytochemical compounds from cemot leaves include alkaloids, phenols, glycosides, flavonoids and the cyanogenic
that can serve as antioxidants (Lim, 2012; Duke, 2009). The efficacious of cemot leaves are relieve to heat, insomnia, colds, headaches, and asthma (Quattrocchi, 2012).

In previous studies showed that the ethanol extract of cemot leaves had activity as an anti-peptic ulcer and antioxidant. Results showed significant in lowering the ulcer index at doses of 100 and 200 mg/kg BW by 64.30% and 71.66% compared to the control group. Also, results showed significantly (p < 0.01) decreased pH in the rat stomach and increased the activity of glutathione in treatment groups. It has an antioxidant activity which is one of the most critical defence factors involved in gastric anti-ulcer effect (Sathish et al., 2011).

The selection of the method of extraction must be considered to obtain the levels of compound optimum. The use of conventional methods has many shortcomings, such as the magnitude of the volume of the solvent, requires the extraction time is long and raises the risk of degradation of the components of the compound thermolabile (Ballard et al., 2010). The use of UAE as a method of extraction of a non-conventional range because of the time efficiency and reduce the amount of solvent for the extraction, so that it can save expenditure costs (Chemat et al., 2016). The rendemen high indicates the number of bioactive components contained in it (Nurhayati et al., 2009). This study used UAE may increase the yield of the extract.

This study aims to investigate the gastroprotective activities of ethanol extract of *Passiflora foetida* leaves-UAE. Further tests on one of the gastroprotective parameters are the measurement of gastric lesions of the animal gastric organ that has induced ethanol absolute.

**METHODOLOGY**

**Materials**

Rotary vacuum evaporator (Buchi, Germany), centrifuge (Heraeus-Christ GmbH, Germany), digital ultrasonic cleaner (Krisbow), Nikon 1 J5 Mirrorless Digital Camera of lens10-30 mm and shoot 20.8 megapixels. Sodium chloride solution (0.9%) obtained from PT. Otsuka Indonesia, Lawang, East Java, Indonesia. Ethanol with concentration 70 % and absolute ethanol (Merck, Darmstadt, Germany). Ketamine hydrochloride and xylazine purchased from Alfasan Co.(Woerden, The Netherlands).The samples of cemot leaves as much as 2 kg obtained from Tumbang Samba village, Katingan Regency, Central Kalimantan. The test animal used in the research was rats of Wistar strain rats aged 2-3 months with a weight between 200-250 grams obtained from the Animal Clinic of Agriculture and Food Service of Yogyakarta.
The treatment of the test animal group conducted for seven days per oral (while still given drinking only). After seven days of treatment, the test animals were fasted for 24 hours without feeding and given only drinking. The induction of peptic ulcers by the administration of absolute ethanol on the 8th day of each animal group K-2, K-3, K-4, K-5, and K-6. After 3 hours of induced absolute ethanol (1 mL/200g BW), then euthanasia with combination between the ketamine and xylazine intraperitoneal (i.p) (Trinovita et al., 2018).

Measuring Gastric Lesions
The euthanasia stage was performed in rats using a combination of ketamine and xylazine (i.p) and carried out the surgery and the removal of the rat gastric organ. The gastric organs opened along the minor curvature, then washed with 0.9% NaCl and then stretched and observed on the inner surface of the stomach. The ulcer index calculation is performed by following the inner surface (mucosa) of the stomach using a magnifying glass or loup. After that, the measurement of the gastric lesion diameter formed by raising the length and width of gastric lesions using long-term assistance. Furthermore, the calculation of lesions of the average diameter of the individual organ and score gave according to Table II (Suzuki et al., 1976).

The ulcer index value (UI) is calculated based on the comparison between the total number of scores with the number of animals in each group. The percentage inhibition of ulceration calculated using the following formula below (Raji et al., 2004):

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\text{Inhibition of ulceration} = \left(\frac{\text{UI of control} - \text{UI of treated}}{\text{UI of control}}\right) \times 100\%
\]

Data Analysis
The data obtained is the number of lesions formed. The statistical data is analyzed using SPSS 22 software (IBM Corporation, Armonk, NY, United States of America). The statistical analysis used in this study is a test of one-way analysis of variance.
RESULTS AND DISCUSSION

The Analysis of Compounds in Extract

The rendemen of the extract cemot leaves-UAE by 42.3815 %. According to previous research results rendemen of the extract cemot leaves by using the method of maceration of the show amounted to 10.85 %. Obtaining the results of the rendemen with the method of the UAE is high compared to other conventional methods such as maceration (Wijaya et al., 2018). The selection of extraction UAE methods based on time efficiency, amount of solvent used, cost-effectiveness, and prevents degradation of various compounds (Guaman et al., 2016). Extraction by UAE method influenced by several factors, such as extraction time, solvent ratio, power, temperature extraction, and sample rate against solvents (Joeng et al., 2015). The study conducted testing, including the concentration of ethanol solvent, the ratio of the sample to the solvent, and the extraction time.

Ethanol solvent concentrations show that the higher levels of ethanol used then the fewer extracts produced. It means that the more concentrated extracts obtained. The higher the ethanol fraction, the more it will evaporate when the rotary spare vacuum evaporator vaporizer so that the resulting extract is also fewer than the less solvent containing the composition of ethanol. The longer the extraction time causes the phytochemical compounds damaged due to the heat inflicted by the ultrasonic waves. The heat effect is one of the effects of ultrasonic waves, so the longer the extraction time, then the temperature in the mixture is increasing (Legay et al., 2011). Extraction using the UAE method with a short time can prevent the degradation of compounds (Dai & Mumper, 2010). The extraction time with the UAE method is shorter compared to conventional methods (Cvjetko et al., 2016; Guaman et al., 2016). The extraction time is selected based on the vibration efficiency of the Ultrasonicator tool, which can accelerate the displacement of compounds into the solvent. The effects of ultrasonic vibrations can expedite the extraction process. The optimum condition for the extraction of anthocyanins from grape skin is 9 minutes (Jeong et al., 2015). The tethering of the ultrasonic waves produces vibrations that can increase the displacement of active compounds into solvents. Such fluctuations provide an intensive stirring effect so that the compound displacement process and the extraction time is shorter (Chemat et al., 2016; Falleh et al., 2012). On the extraction method with the UAE, the larger volume of solvents will lower the yield value. The more significant the amount of the solvent causes the ultrasonic energy to absorbed higher to raise the temperature, so that sample only absorbs the rest of the power from the ultrasonic apparatus. It affects the extraction process because the compound not correctly extracted. The results of phytochemical screening showed that extract cemot leaves-UAE contained multiple compounds. It can see in Table III.

According to the results of previous studies on the extract cemot leaves with the method of maceration, which has done shows that the levels of flavonoids 256.500 ± 0.534 (mg/ml QE), steroid 30.547 ± 0.951(mg/mL), alkaloids 15.940 ± 0.269 (%), saponins 4.082 ± 0.008 (%) and tannins 4.250 ± 0.069 (mg/ml QE) (Wijaya et al., 2018). Based on
the results of phytochemical screening extract *P.foetida* leaves with the different extraction methods showed the acquisition levels of various compounds. The results of phytochemical extract *P.foetida* leaves-UAE show the levels of the compound higher than the method of maceration, and it is suspected can affect the mechanism of the compounds contained in the leaves *P.foetida* non as anti gastric ulcer.

**Evaluation of Gastric Lesions Measurement**

The evaluation of gastric lesions measurement parameters done with short term assistance. Figure 2 shows the macroscopic description of lesions in the gastric organs in each treatment group. The normal group does not indicate lesions of the stomach. The negative groups (K-2) indicate a large number of lesions in the stomach and the larger size of the lesions and tend to the perforation of the stomach organs.

In this study, measurements of gastric lesions and the calculation of ulcer index as well as percentage inhibition of ulcer as one parameter evaluation parameter as gastroprotective. It is seen in Table III below, which shows the result of the ulcer index and ulcer inhibition percentage in each treatment group.

Table I shows that groups of extract A (50 mg/kg BW), B (100 mg/kg BW) and C (200 mg/kg BW) were statistically significant (p <0.05) when compared with the negative group. Increasing doses of cemot leaves extract, followed by the decreased formation of gastric lesions formed. The normal groups do not form gastric lesions so that the percentage of ulcers inhibition of 100%. However, in negative groups, the inhibition of ulcers is 0 % due to the negative group as a control group in the calculation of percentage inhibition of ulcer to facilitate the calculation of percentage inhibition of ulcer for control group positive and group of Cemot leaf extract at various dosage variations.

One of the parameters in evaluating its effectiveness as a gastroprotective is the healing of lesions in the stomach. The ethanol-induced ulcer has used for the evaluation of new anti-ulcer drugs. The test animals were fasting for 24 hours before being induced with ethanol. The duration of fasting can affect the process of absorption, and rats showed a significantly emptying time of the stomach more quickly than larger animals (Vermeulen *et al.*, 1997). The empty stomach can stimulate the ulcer quickly and can reduce the prostaglandin cytoprotection to facilitate mucosal damage. Gastric acid secreted can cause the occurrence of autodigestion due to weak mucous defence factor. According to previous studies that the long interval of three-hour induction before surgery is the optimum time based on the results of the orientation that can show the highest index value (Fadlina *et al.*, 2008). It is associated with the absorption of ethanol in the stomach. Absorption of ethanol is faster in the lower intestine than in the stomach so that when the stomach is empty, it will speed up the absorption of ethanol in the stomach. It can also stimulate the formation of gastric lesions and can facilitate the observation of lesions in the stomach. The stomach condition is empty or

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Figure 2. The macroscopic lesions on the gastric surface

Description: K-1 = Normal Group (pre-treated); K-2 = Negative Group; K-3 = Positive Group; K-4 = Group Extract A dosage 50 mg/kgBW; K-5 = Group Extract B dosage 100 mg/kgBW; K-6 = Group Extract C dosage 200 mg/kgBW

(*yellow circle indicates lesions of gastric organs)
contains. It is crucial in the absorption of alcohol settings. At the stomach of a new state, perfect absorption occurs within 1 or 2 hours, but in the stomach, the condition contains full intake of food absorptive occurs up to 6 hours (Katzung et al., 2012). Based on previous optimization results showed that 80% ethanol (0.5 ml/kg BW) showed the most gastric lesion gains compared to concentration and other administration volumes. It can be seen in the visible image of lesions of the stomach organs (Fig. 2) indicating the size of ethanol-induced gastric lesions tends to be more significant to facilitate observation and measurement. Ethanol induces pepsin secretion that causes digestion of endogenous protein in the mucous layer of a gastric wall that precedes the inflammation. Ethanol also influences the secretion of hydrochloric acid by parietal cells and increases the severity of inflammation (Dias et al., 2000).

Table III shows that group of extract (50, 100 and 200 mg/kg BW) were statistically significant (p<0.05) when compared with the negative control group. Increasing doses of extract cemot leaves resulted in decreased formation of gastric lesions. A dose of 50 mg/kg BW was less effective in protecting the gastric wall than the doses of 100 and 200 mg/kg BW. The cemot leaves extract (200 mg/kg BW) group produced the highest decrease in gastric lesion formation compared to the other dose groups of cemot leaves extract, and showed the highest percentage inhibition of gastric lesion by 35.34%, but with a lower percentage obtained less than the positive control group (omeprazole) which received a percentage of 40.98%. According to the results of previous research showed an ethanolic extract of *P. foetida* at both the dose (100 mg dan 200 mg/kg p.o) levels significantly (p < 0.01) reduced the lipid peroxidase and alkaline phosphatase levels of ulcer animals. Antioxidant enzymes such as glutathione are present in oxygen handling cells, which are the first line of cellular defence against oxidative injury. They decrease the gastric mucosal damaging effect of absolute alcohol. There was a significant (p < 0.01) increase of glutathione activity in the ethanolic extract of *P. foetida* pretreated rats. Hence, the antioxidant activity of ethanolic extract of *P. foetida* may be one of the important defensive factors involved in its anti-ulcer effect. The present study reveals that *P. foetida* exhibits both anti-ulcer and antioxidant properties (Sathish et al., 2011). The group of dosage extract cemot leaf-UAE at various dosage variations shows the difference in results obtained. The increasing treatment of ulcer index and ulcer inhibition percentage obtained higher because of the thicker extracts obtained also increasingly concentrated.

Table III. Ulcer Index Results and Ulcer Inhibition Percentage

| Treatment group (n = 6) | Dosage | The diameter of ulcer (mm) | Ulcer Index | Inhibition of ulceration (%) |
|------------------------|--------|---------------------------|-------------|-----------------------------|
| Normal (pre-treated)   | -      | -                         | 0           | 100                         |
| Negative (0.5% CMC)    | 5 ml/kg BW | 2.40 ± 0.65               | 2.83        | -                           |
| Positive (omeprazole)  | 36 mg/kg BW | 1.17± 0.39 a             | 1.67        | 40.98                       |
| Extract A              | 50 mg/kg BW | 1.78± 0.40 a             | 2.67        | 5.65                        |
| Extract B              | 100 mg/kg BW | 1.47 ± 0.35 a            | 2           | 29.33                       |
| Extract C              | 200 mg/kg BW | 1.42± 0.29 a            | 1.83        | 35.34                       |

Results as the mean ± SD for three rats/group. Statistical comparison was performed using ANOVA followed by Tukey test

*a significantly different* (p<0.05) compared with the negative group

Description: K-1 = K-1 = Normal Group (pre-treated); K-2 = Negative Group; K-3 = Positive Group; K-4 = Group Extract A dosage 50 mg/kgBW; K-5 = Group Extract B dosage 100 mg/kgBW; K-6 = Group Extract C dosage 200 mg/kgBW.
CONCLUSION
The phytochemical compounds in the cemot leaves have a mechanism of action as gastroprotective with protecting the gastric from ethanol-induced gastric lesions with a decrease in the formation of gastric lesions.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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