Characterization, Phytochemical Screening Of *Phyllanthus Emblica* L. Fruit Nanoherbal And Determination Of The Estrus Cycle Of Female Rats

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Abstract.
One of a plant that is thought to have quite high antioxidant activity is kemloko (Phyllanthus emblica L.). This plant is a material that is often used by the community as a traditional medicine. This plant in India has been used to treat cancer, diabetes, liver (liver), heart problems and anemia. This biological activity is thought to be caused by the presence of bioactive compounds from secondary metabolites contained therein, especially compounds from the phenolic and flavonoid groups. The short length of the estrus cycle of rats makes them ideal for investigation of changes occurring during the reproductive cycle. The estrus cycle lasts four days and is characterized as: proestrus, estrus, metestrus and diestrus, which may be determined according to the cell types observed in the vaginal smear. Since the collection of vaginal secretion and the use of stained material generally takes some time, the aim of the present work was to provide researchers with some helpful considerations about the determination of the rat estrus cycle phases in a fast and practical way. This study began with macroscopic examination, examination of water content, examination of water-soluble extract levels, examination of ethanol-soluble extracts, examination of total ash content, and also an examination of acid-insoluble ash levels, phytochemical screening of Phyllanthus emblica L. fruit nanoherbal and determination of the estrus cycle of female rats. The results of phytochemical screening showed that the compound of Phyllanthus emblica L. Fruit nanoherbal contained a class of secondary metabolites of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. And the estrus cycle of female rats can be determined by observing changes in vaginal epithelial cells.

Keywords: *Characterization, Phytochemical Screening, Phyllanthus emblica L. Fruit Nanoherbal and Estrus Cycle.*

I. INTRODUCTION

To determine the content of chemical compounds in plants, a phytochemical analysis is carried out. Phytochemical screening is a qualitative examination of chemical content to determine the class of compounds contained in a plant. The examination was carried out on secondary metabolites that have health benefits such as alkaloids, glycosides, flavonoids, terpenoids, tannins, and saponins (Harborne, 2006).According to research by Liu et al., (2007), P. emblica fruit contains phenolic compounds, such as geraniin, quercetin 3-β-D glucopyranoside, kaempferol 3-β-D glucospiranoside, isochorylagin, quercetin, and kaempferol. In addition, this plant also contains gallic acid, ellagic acid, 1-O-galloylbeta-D-glucose, 3-ethylgalic acid and corilagin (Zhang et al., 2003). Ascorbic acid is also found in P. emblica plants (ElDesouky et al., 2008). The acidic hydroxyl groups in these phenolic compounds are thought to play an important role in oxidation-reduction reactions in the body (Huang et al., 2005).Research related to the analysis of the content of phenolic compounds, flavonoids and antioxidant activity of P. emblica fruit originating from various regions in China with different geographical environments has been carried out (Liu et al., 2007).The reproductive cycle of female rats is called estrus cycle and is characterized as proestrus, estrus, metestrus (or diestrus I) and diestrus (or diestrus II) (Long & Evans, 1922; Freeman, 1988).

The ovulation occurs from the beginning of proestrus to the end of estrus (Young et al., 1941; Schwartz, 1964). From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 days (Long & Evans, 1922; Freeman, 1988; Mandl, 1951), and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle (Spornitz et al., 1999; Marcondes et al., 2001).During the estrus cycle, prolactin, LH and FSH remain low and increase in the afternoon of the proestrus phase. Estradiol levels begin to increase at metestrus, reaching peak levels during

proestrus and returning to baseline at estrus. Progesterone secretion also increases during metestrus and diestrus with a decrease afterwards. Then the progesterone value rises to reach its second peak towards the end of proestrus (Sportnitz et al., 1999; Smith et al., 1975).

II. METHODS

2.1 Characterization of P. emblica Fruit Nanoherbal

Examination of the features of P. emblica Fruit Nanoherbal involves determining the water content, the water-soluble extract content, the ethanol-soluble essence content, the total ash content, and the acid-insoluble ash content (Yuniarti, 2022).

2.2 Phytochemical Sreening of P. emblica Fruit Nanoherbal

The flavonoid compounds, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids in P. emblica Fruit Nanoherbal are tested as part of the phytochemical screening (Martiningsih and Suryanti, 2017).

2.2.1 Alkaloid Examination

Added 1 mL of HCl 2N to 0.5 g of extract, followed by 9 mL of distilled water. two minutes of heating in a water bath Cooled and filtered, the filtrate was then divided among three tubes. To the first tube, two drops of Mayer's reagent are added. To the second tube, two drops of Bouchard's reagent were introduced. In the third tube, two drops of Dragendoff's reagent were added. Two of the three test tubes were positive for alkaloids, indicating a positive outcome (Deshmukh et al., 2012; Alabri et al., 2014).

2.2.2 Flavonoid Examination

Weighed 10 grams of extract, added 100 milliliters of distilled water, and then heated for five minutes. Filtered while hot, then Mg powder, 1 mL HCl (p), and 2 mL amyl alcohol were added (Syahputra et al., 2021).

2.2.3 Saponin Examination

The tube was filled with as much as 0.5 g of extract and 10 ml of hot distilled water before being violently shaken for 10 minutes. With one drop of HCl 2N, foam production was stimulated (Amir et al., 2011).

2.2.4 Tannin Examnination

One g of extract was added to 10 mL of distilled water, which was then filtered and diluted until colorless. To 2 ml of diluted filtrate solution, 2 drops of 1% FeCl3 reagent were added (Amir et al., 2011).

2.2.5 Glikosida Examination

Overall, 7:3 To a combination of 96% ethanol and distilled water, 1 g of extract was added, followed by 10 mL of HCl 2N and 10 minutes of refluxing. Before filtering, 20 mL of the filtrate was combined with 25 mL of distilled water and 25 mL of Pb(CH3COO)2 0.4 M, shaken, and left to stand for 5 minutes. Using a mixture of 20 mL chloroform-isopropanol (3:2) and adequate anhydrous sodium, the filtered findings were extracted. filtration and evaporation afterward, dissolve in 2 mL of ethanol. The residue was treated with 2 ml of water and 5 drops of Molisch reagent, followed by the addition of H2SO4 (p) (Syahputra et al., 2021).

2.2.6 Steroid / Triterpenoid Examination

One gram of extract was weighed in total. Two hours of maceration with 20 mL of n-hexane, followed by filtration. The filter was evaporated, and the remaining substance was dripped with Libermann-Bouchart reagent (Nasution et al., 2022).

2.3 Determination of the Estrus Cycle of Female Rats

a. Female rats were acclimatized for 14 days, given sufficient standard food and drink and lighting was regulated for 12 hours during the day and 12 hours at night.

b. The stages of the estrus cycle were determined in adult female rats to determine fertile animals, by:

   i. A small amount of 0.9% NaCl was taken with a pipette, then inserted into the rat's vagina

   ii. 0.9% NaCl was aspirated and reinserted, done several times, then the liquid was placed on top of the object glass

   iii. Allowed to dry, then dripped with 0.1% methylene blue solution

iv. After drying, they were observed under a microscope with a ratio of 10x10 to see if the rats were in the pre-estrus, estrus, meth-estrus and estrus phases.

c. Fertile female rats are characterized by the presence of stem cells in vaginal smears, namely during the estrus phase (Long and Evans, 1922; Mandl, 1951).

III. RESULT AND DISCUSSION

3.1 Result of The *P. emblica* Fruit Nanoherbal Characterization

The results of macroscopic examination of the *P. emblica* fruit were that the fruit was yellowish green in color, had a sour taste with a characteristic aroma, was round in shape and had a diameter of approximately 1.3-2.5 cm.The results of *P. emblica* fruit nanoherbal characterization include determination of water content, water soluble extract content, ethanol soluble extract content, total ash content and acid insoluble ash content which can be seen in Table 1.

Table 1 Results of *P. emblica* Fruit Nanoherbal Characterization

No	Parameter	Result (%)
1	Determination of water content	7,63
2	Determination of water-soluble extract content	28,86
3	Determination of ethanol-soluble extract content	47,17
4	Determination of total ash content	4,30
5	Determination of acid-insoluble ash content	0,29

The results of determining the water content of the *P. emblica* fruit nanoherbal obtained 7.63%, this is in accordance with the standardization of the water content of simplicia in general with the condition that it is not more than 10% (Ministry of Health RI, 1995). The results of the determination of the *P. emblica* fruit nanoherbal showed that the water-soluble extract was 28.86%; while the content of the extract dissolved in ethanol was 47.17%.Determination of the ash content of the *P. emblica* fruit nanoherbal showed a total ash content of 4.30% and an acid insoluble ash content of 0.29%. Determination of ash content aims to determine the mineral content of the sample. The minerals present in the sample can come from organic oxides. High total ash content indicates the presence of metal inorganic substances (Ca, Mg, Fe, Cd, and Pb), some of which may come from impurities. High levels of heavy metals can be harmful to health, therefore it is necessary to determine the acid-insoluble ash content to ensure that the sample does not contain heavy metals exceeding the established limit. Determination of the ash content in the acid is intended to determine the amount of silicates, especially sand, contained in simplicia by dissolving the total ash using hydrochloric acid (WHO, 2011).

3.2 Result of *P. emblica* Fruit Nanoherbal Phytochemical Screening

The results of phytochemical screening of *P. emblica* fruit nanoherbal were carried out to obtain information on the class of secondary metabolites contained therein. The results of the phytochemical screening can be seen in Table 2.

Table 2 Result of *P. emblica* Fruit Nanoherbal Phytochemical Screening

No	Secondary Metabolites	P. emblica Fruit Nanoherbal
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Tannins	+
6	Steroids/triterpenoids	+

Description: (+) = contains the substance being examined

In Table 2 it shows that the *P. emblica* fruit nanoherbal contain groups of chemical compounds, namely alkaloids, flavonoids, glycosides, saponins, tannins, steroids and triterpenoids.

3.3 Result of the Estrus Cycle of Female Rats Determination

The estrus cycle of female rats is influenced by hormone levels. The estrus cycle of female rats lasts 4-5 days. The rat estrus cycle consists of 4 stages. These stages are proestrus, estrus, metestrus, and diestrus

(Goldman et al, 2007).In their reproductive activity, rats have polyestrus properties, which means they have an estrus cycle more than twice a year. The estrus cycle is influenced and regulated by reproductive hormones and lasts for 4-6 days. The first estrus cycle occurs after 1-2 days from the start of the opening of the vagina which occurs at the age of 28-29 days (Malole and Pramono 1989). The detection of the estrus cycle can be done using the pap smear technique (vaginal review), by looking at the vaginal epithelium using a microscope so that it can be distinguished into proestrus, estrus, metestrus and diestrus (Partodiharjo, 1992).Observation of the estrus cycle of female rats was carried out every morning by observing vaginal smears. This aims to increase the possibility of rat pregnancy because the heat period is known. Into the vaginal opening of the female rat, 0.9% NaCl was inserted through the blunt tip of the pipette and withdrawn slowly and carefully. The vaginal fluid that had been taken was dripped on a glass object, allowed to dry and then 0.1% methylene blue dye was added and then examined under a microscope with a magnification of 10x10. The results of observing the rat estrus cycle through vaginal smears can be seen in Figure 1.

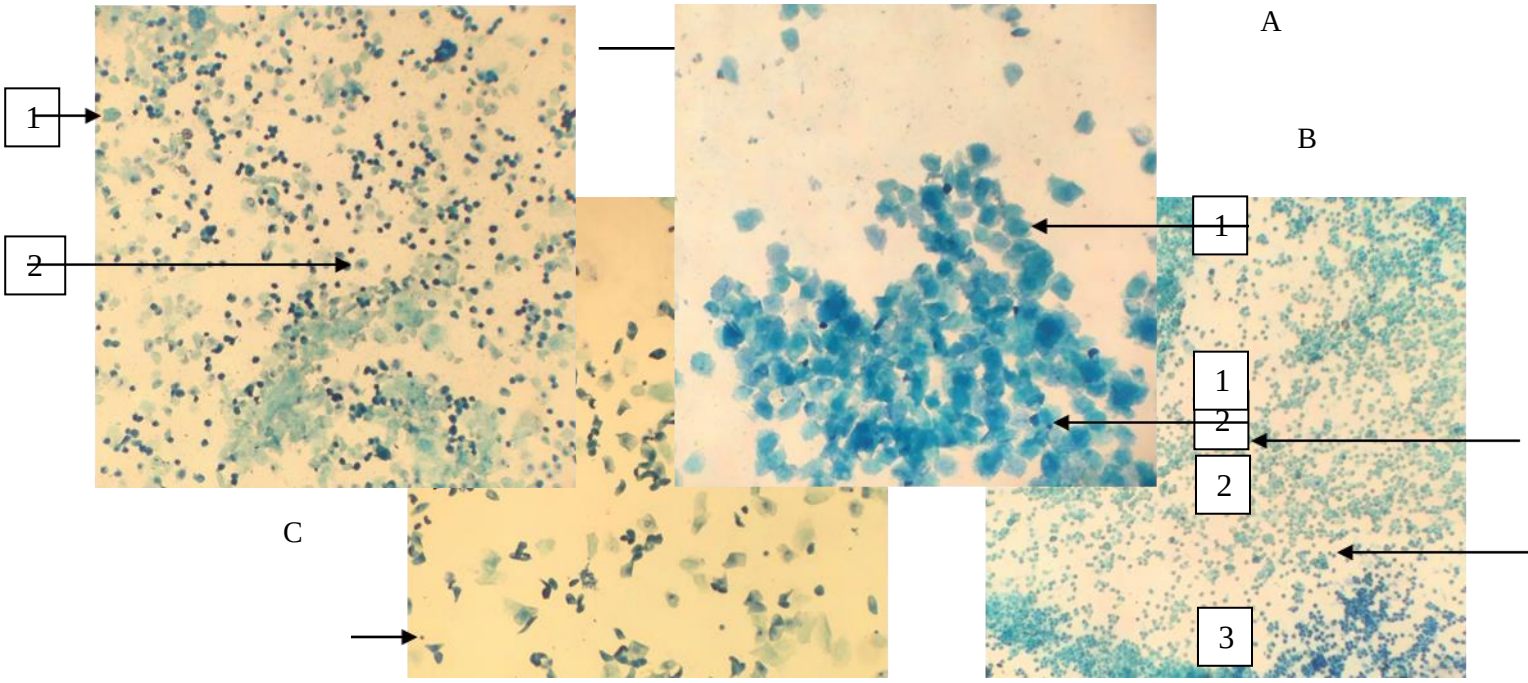

Fig 1. Vaginal smears in one estrus cycle with methylene blue stain

Description:
A = Proestrus phase
B = Estrus phase
C = Metestrus phase
D = Diestrus phase
1 = Horned epithelial cells
2 = Nucleated epithelial cells
3 = Leukocyte cells

Female rats that were in estrus were characterized by the presence of horned epithelial cells which were more dominant than nucleated epithelial cells. The estrus phase begins with the presence of cornification cells. Cornification cells, are horn cells that have a box shape, either rectangular or more (irregular) usually these cells do not overlap each other. Pavement (epithelial pavement) cells or horned epithelium have an irregular shape and cells stack on top of each other. The proestrus phase is indicated by the presence of nucleated epithelial cells. Nucleated epithelial cells have a nearly spherical oval shape with a clear nucleus in the center. (Lohmiller and Swing 2006). Vaginal biological cells during the metestrus phase consist of many leukocytes along with the presence of nucleated cells and pavement cells (Lohmiller and

Swing, 2006). Leukocyte cells that are found in abundance and accompanied by the presence of nucleated cells indicate the diestrus phase.The lust cycle is the time interval or distance between one lust and the next. The reproductive cycle is generally divided into 4 phases or periods, namely: proestrus, estrus, metestrus and diestrus. (Toelihere 1981; Guyton 1994;).

In the Estrus phase, copulation is possible. The estrus phase lasts 12 hours. The duration of this phase is the same as in white rats (Rattus sp) (Baker et al. 1979; Smith & Mangkoewidjojo 1988). A distinctive feature is the presence of activity very high running around under the influence of estrogen. Estrus is a period of high estrogen secretion. Estrogen from mature Graaf follicles causes various changes in the reproductive tract, the uterus is tense, the vaginal mucosa grows rapidly, and there is mucus secretion (Toelihere 1981).Many mitoses occur within the vaginal mucosa and new cells accumulate, while the surface layer becomes squamous and horny. These horned cells exfoliate into the vaginal lumen (Baker et al 1979).Metestrus is the phase immediately after estrus in which the corpus luteum begins to grow. The corpus luteum is a change in the shape of the Graafian follicle in the final stage which changes function after ovulation (McDonald 1980). Metestrus is largely under the influence of progesterone which the corpus produces luteum. The metestrus stage in white-tailed rats occurs between 15 - 21 hours and in white rats (Rattus sp) 21 hours after ovulation takes place.Diestrus, is the last period in the estrus cycle. In this period the corpus luteum matures and the effect of progesterone becomes more pronounced. The endometrium is thicker and the glands are enlarged (Toelihere, 1981). The distrusion stage of the white-tailed rat occurred between 45 and 54 hours earliercompared to white rats (Rattus sp) which ranged from 57 to 70 hours (Baker et al, 1979).

IV. CONCLUSION

P. emblica fruit nanoherbal contain groups of chemical compounds, namely alkaloids, flavonoids, glycosides, saponins, tannins, steroids and triterpenoids.The estrus cycle lasts 3 to 4 days consisting of 12 hours proestrus, 12 hours estrus, 15 to 21 hours metestrus and 45 to 54 hours diestrus.

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