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Analgesic and Antipyretic Activity of Sweet Orange Peel Methanol Extract

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Abstract

An analgesic-antipyretic drug widely used is paracetamol, which has various health benefits and several adverse effects. Therefore, various natural products have been extensively studied as alternative analgesic-antipyretics, one of which is sweet orange peel. This study aimed to investigate sweet orange peel's analgesic and antipyretic activity by in vivo methods. This experimental study evaluated the analgesic and antipyretic effects of sweet orange peel extract extracted by the maceration method. The analgesic effect was evaluated by tail immersion (Maximum Possible Analgesia) and acetic acid-induced writhing method (total abdominal writhing). Meanwhile, the antipyretic effect was evaluated by the brewer yeast-induced hyperpyrexia (body temperature) method. This study showed that sweet orange peel methanol extract significantly increased the maximum possible analgesia value (132.79%) and reduced the number of abdominal writhing (44.05%) at the highest dose of 750 mg/kg BW. It indicated analgesic activity from sweet orange peels. Meanwhile, the antipyretic effect of sweet orange peel methanol extract was observed from 1-4 hours after administration, and the highest percentage inhibition of body temperature 4 hours after administration was found in a moderate dose, that was 5.98% (P value: 0.042). Therefore, it can be concluded that sweet orange peel methanol extract has analgesic and antipyretic effects with an optimal dose range of 500-750 mg/kg BW.

Keywords: Analgesic; Antipyretic; Sweet Orange; Peel; Methanol.

INTRODUCTION

Estimated 50 million adults in the United States suffer from chronic pain daily, with 19.6 million adults experiencing high-impact chronic pain that interferes with daily life or work activities (Raja et al., 2020; Treede, 2018; Wang & Meng, 2021). Various injuries and illnesses are commonly occupied by pain and fever. Nonsteroidal anti-inflammatory drugs (NSAIDs) are usually prescribed for the relief of pain and fever; however, some patients may express some adverse drug effects in the gastrointestinal tract, such as perforation, hemorrhage, ulceration, and obstructions (Subedi et al., 2016). Paracetamol or acetaminophen has been widely used since 1955 and that commonly used equally with other NSAIDs. These drugs are over-the-counter drugs with analgesic antipyretic activity. Due to this reason, an irrational application of these drugs has widely occurred, including the recreational use of sports, to relieve pain from athletic activity or as prophylaxis before exercise (Esh et al., 2017).

The paracetamol utility was high in some countries, which may be due to the public having an opinion about paracetamol as a medicine for all diseases (pill for every illness). Dorji et al. (2018) reported that 72.1% of 441 outpatients in Phuentsholing General Hospital, Bhutan (India) was prescribed paracetamol in the last year (Dorji et al., 2018). The paracetamol utility was also high in Indonesia. Surya et al. (2018) reported that 34 parents (69%) of 50 parents in Laksana Kumara Kindergarten preferred paracetamol as an antipyretic for their children (Surya et al., 2018).

Other than the benefits, paracetamol also has some adverse drug effects due to the metabolite of paracetamol, including acetanilide and phenacetin. Paracetamol must be used judiciously; when prescribed with a higher dose (7.5-15 grams/24 hours), it may become hepatotoxic or ineffective when it is too low. More than 28 billion combination forms of paracetamol were distributed worldwide in 2005, and around 401 deaths were reported associated with paracetamol or paracetamol combination drugs in 2009 by the Association of Poison Control Centers. Furthermore, paracetamol-induced liver failure is recently reported as the second leading cause of liver transplantation in the United States (Khosravi et al., 2011). (Bebenista dan Nowak, 2014).

Due to the information above, looking for a natural product with similar efficacy with minimal adverse effects is essential. Herbs have been widely used as an

alternative treatment. However, direct used herbs were not effective and unadjusted. Thus, it required a procedure to form the form of a pharmaceutical. The direct application of herbs was high in some undeveloped countries because of the cheaper cost than synthetic drugs (Salmerón-Manzano et al., 2020).

Some studies have investigated the various benefits of natural products in Indonesia. Sweet orange is one of the natural products widely utilized for various products such as beverages, pharmaceuticals, or cosmeceuticals. However, the peel becomes a wasted product for these industries. Due to this reason, some studies have been performed to investigate the benefits of sweet orange peels. Malleshappa et al. (2018) reported that some types of orange (*Citrus aurantifolia, Citrus reticulata, Citrus aurantium, Citrus grandis*, and *Citrus medica*) had a significant anti-inflammatory effect by decreasing paw edema volume in carrageenan-induced rats. On the other hand, Malleshappa also demonstrated the analgesic effect of these Citruses by increasing reaction time in tail immersion assay and hot plate assay (120 and 150 minutes). Another study by Ahmed et al. (2019) reported a similar result whether hydroethanolic Citrus extract, naringin, and naringenin had hepatoprotective effects in APAP (N-Acetyl-p-aminophenol)-Induced liver injury. This protective effect was indicated by increased antioxidant defense systems and inflammation and apoptosis suppression. Furthermore, Schneider et al. (2020) also reported that *Citrus reticula ethanol extract* had an analgesic effect by the formalin-induced hyperalgesia assay (control: 501.5 ± 40.0 seconds; *C. reticulata* 300 mg/kg: 161.8 ± 41.1 seconds), carrageenan model (control at hour 4th: 82.5 ± 9.6 %; *C. reticulata* 300 mg/kg at 4th hour: 47.5 ± 6.5%) and Complete Freund's Adjuvant model (control: 501.5 ± 40.0 s; *C. reticulata* 300 mg/kg: 161.8 ± 41.1 sec). However, none of these studies investigated analgesic and antipyretic activity from sweet orange peel. Thus, this study aimed to investigate analgesic and antipyretic activity from sweet orange peel methanol extract by in vivo models.

MATERIALS AND METHODS

Study Design

This experimental study was performed between September 2022 to December 2022 in Pharmacology Laboratory, Universitas Prima Indonesia. This study evaluated analgesic and antipyretic activity from Sweet orange peel methanol extract by in vivo model. Acetic Acid-Induced Writhing and Tail Immersion methods evaluated the analgesic activity of sweet orange peel. Meanwhile, the antipyretic activity was evaluated by Brewer Yeast- Induced Hyperpyrexia method. This study protocol has been approved by Health Research Ethics Committee Universitas Prima Indonesia with letter no. 018/KEPK/UNPRI/XII/2022.

Materials

This study used some materials, including sweet orange peels, distilled water, magnesium powder, amyl alcohol, Mayer, Bouchard, and dragendorff reagent, iron (III) chloride, hydrogen sulfate, 95% ethanol, lead (II) acetate, isopropanol, chloroform, Molisch reagent, Lieberman-Burchard reagent, acetic acid, blood tube, paracetamol, Sodium Carboxyl Methyl Cellulose (SCMC), acetic acid, brewer's yeast, and ketamine.

Extraction Process

Sweet orange peel was cleaned, cut, and dried without sunlight exposure to form a dry simplicial. After that, dry simplicia meshed into simplicial powder. This simplicial powder is then extracted by the maceration method. It was soaked into 95% methanol as the solvent in a ratio of 1:3 for three days and regularly stirred. After three days, it was filtered, and the residue was macerated again in the same way two times. Meanwhile, all filtrate from each maceration was collected to evaporate by rotary evaporator, forming a concentrated sweet orange peel methanol extract. Finally, the extract yield was determined by dividing the extract mass by fresh simplicial mass and multiplying it by 100% (Chiuman et al., 2023; Gulo et al., 2021; Suhartomi et al., 2020).

Phytochemicals Screening

The obtained extract underwent phytochemical screening to identify the presence of some phytochemicals, including flavonoid, tannin, phenol, saponin, alkaloid, steroid/ triterpenoid, and glycoside (Chiuman et al., 2023; Girsang et al., 2019; Widowati et al., 2018).

Oral Suspension Formulation

A gram of concentrated extract and 150 milligrams of paracetamol were suspended into 0.5% SCMC to form sweet orange peel extract and paracetamol suspensions. Meanwhile, 0.5% SCMC was formulated by dissolving 0.5% grams of SCMC into ten millilitres of distilled water (Chiuman et al., 2021; Mutia et al., 2021).

Tail-Immersion Method

Analgesic activity was evaluated by tail immersion and abdominal writhing methods. Firstly, the tail immersion method was performed among twenty-five male Wistars rats, which were grouped into five groups: Control, Standard, Sweet Orange Peel Extract-1, 2, and 3. The control group received a milliliter of 0.5% SCMC; the Standard group received 10 ml/ kg BW of paracetamol suspension; Sweet Orange Peel Extract-1, 2, and 3 received 2.5 ml/ kg BW, 5.0 ml/ kg BW, and 7.5 ml/ kg BW of sweet orange peel extract suspension, respectively. An hour before and after the treatment, all rats were placed into a restrainer to fix the rat's body, and the tail was pulled out from the restrainer. Then, the rat tail was dipped 3 cm from the distal tail in heated distilled water at 55ºC, which was heated by a hotplate.

The maximum time for a dipped rat tail was 15 seconds. Analgesic activity was described by Maximum Possible Analgesia (MPA), which was obtained by the following formula (Salim et al., 2021):

$$MPA: \frac{Reaction\ time\ of\ sample\ group - Reaction\ time\ of\ Control\ Group}{15\ detik - Reaction\ time\ of\ Control\ Group}\ x\ 100\%$$

Acetic Acid-Induced Abdominal Writhing Method

Besides the tail immersion method, the acetic acid-induced abdominal writhing method is also used to evaluate analgesic activity. It required a 0.7% acetic acid solution formulated by dissolving 0.7 ml of 100% glacial acetic acid into a hundred milliliters of distilled water in a hundred milliliters volumetric flask. After that, all rats were grouped into five groups, including Control, Standard, Sweet Orange Peel Extract-1, 2, and 3, that received some treatment as described in the tail immersion method. Fifteen minutes after treatment, all rats were injected intraperitoneally with 0.7% acetic acid solution. Five minutes after acetic acid injection, the number of abdominal writhing was counted for twenty minutes (Saini & Singha, 2012; Salim et al., 2021).

Brewer Yeast-Induced Hyperpyrexia

This study evaluated the analgesic activity and antipyretic activity of sweet orange peel extract. This study used Brewer yeast-induced hyperpyrexia model to investigate the antipyretic activity of sweet orange peel extract. This study used a 15% brewer's yeast suspension formulated by dissolving 15 grams of brewer's yeast in 100 ml of normal saline. Then, 20 grams of the last suspension is dissolved with 100 ml of distilled water to form a 20% brewer's yeast solution. This solution was subcutaneously injected at 10 ml/kg BW. However, the body temperature was initially measured by a rectal thermometer before the injection, and the body temperature was also measured 24 hours after the injection. After that, all rats were grouped into five groups Control, Standard, Sweet Orange Peel Extract-1, 2, and 3, which received some treatment as described in the analgesic activity assay. The antipyretic activity was analyzed from body temperature an hour to five hours after the treatment. Finally, all rats were sacrificed for blood sampling intracardiac using three milliliters syringe with a 23 G needle. The blood sample obtained was then filled into the EDTA tube. Before taking the rat blood, it was anesthetized using chloroform. The EDTA blood sample was investigated for routine blood count at the Health Laboratory, North Sumatra Provincial Health Office (Saini & Singha, 2012; Salim et al., 2021; Sivamurugan et al., 2016; Veronica et al., 2017).

Data Analysis

Phytochemicals screening of sweet orange peel extract, initial body weight, number of writhing, reaction time, and body temperature were analyzed by descriptive statistics. Then, the analysis was continued based on data distribution by Shapiro-Wilk. If the data distribution is normal, then one-way ANOVA analyzed it. If data distribution is not normal, and then Kruskal-Wallis analyzed it.

RESULTS AND DISCUSSION

Physical Characteristics and Phytochemicals Screening

This study used sweet oranges obtained from a traditional market in Medan. This study used 836 grams of fresh sweet orange peel that was dried into 490 grams of dried sweet orange peel powder, and this dried powder was extracted into 75 grams of concentrated sweet orange peel extract. After that, the obtained sweet orange peel extract underwent phytochemicals screening, and it showed that sweet orange peel methanol extract has some phytochemicals, including alkaloid, saponin, flavonoid, and tannin.

Analgesic Activity of Extract

Tail Immersion Method

The obtained sweet orange peel methanol extract underwent both analgesic and antipyretic assay. Analgesic activity assay by tail immersion method in all groups was described in Table 1.

Table 1. Reaction Time of All Groups in the Tail Immersion Method.

Group	Reaction Time, *Seconds*		P-Value
	Mean	SD	
Control	4.33	0.76	
Standard	12.97	2.19	
Sweet Orange Peel Extract-1	7.80	1.26	< 0.05
Sweet Orange Peel Extract -2	8.50	1.99	
Sweet Orange Peel Extract -3	10.08	3.06	

Based on Table 1, all groups showed a significant difference in reaction time, which can be seen from P-Value < 0.05. The Standard group expressed the highest reaction time, and the lowest reaction time was expressed by the Control group. Further analysis was performed to determine Maximum Possible Analgesic (MPA), that was indicated the analgesic potency from the sample compared to a control group, and Maximum Possible Analgesia in all groups was described in Table 2.

Table 2. Maximum Possible Analgesia of All Groups in Tail Immersion Method.

Group	**Maximum Possible Analgesia (%)**
Control	Ref
Standard	199.54
Sweet Orange Peel Extract-1	80.14
Sweet Orange Peel Extract -2	96.30
Sweet Orange Peel Extract -3	132.79

Table 2 above showed that the highest MPA value was found in the standard group, that was 199.54%, followed by Sweet Orange Peel Extract-3 (132.79%), 2 (96.30%), and the lowest one was found in Sweet Orange Peel-1, which was 80.14%.

Acetic Acid-Induced Abdominal Writhing Method

On the other hand, this study also used the acetic acid-induced abdominal writhing method to investigate the analgesic activity from sweet orange peels, and the result of the Acetic acid-induced abdominal writhing method was described in Table 3.

Table 3. Number of Abdominal Writhing of All Groups in Acetic Acid-Induced Abdominal Writhing Method.

Group	**No. Writhing**		**P-Value**
	Mean	**SD**	
Control	16.80	1.92	
Standard	8.80	1.92	
Sweet Orange Peel Extract-1	11.00	2.34	< 0.05
Sweet Orange Peel Extract -2	10.00	1.87	
Sweet Orange Peel Extract -3	9.40	1.14	

Table 3 showed that all groups showed a significant number of abdominal writhing, which was shown by P-Value < 0.05. The lowest abdominal writhing was found in the standard group, and the highest in the control group. However, this study evaluated the analgesic potency in acetic acid-induced abdominal writhing by percent inhibition of abdominal writhing, described in Table 4.

Table 4. Percent Inhibition of Abdominal Writhing of All Groups in Acetic Acid-Induced Abdominal Writhing Method.

Group	**Percent Inhibition of Abdominal Writhing (%)**
Control	Ref
Standard	47.62
Sweet Orange Peel Extract-1	34.52
Sweet Orange Peel Extract -2	40.48
Sweet Orange Peel Extract -3	44.05

Table 4 showed that the highest percent inhibition of abdominal writhing was found in the standard group, that was 47.62%, followed by the Sweet Orange Peel Extract-3 (44.05%), 2 (40.48%), and the lowest one was found in Sweet Orange Peel Extract-1, that was 34.52%.

Antipyretic Activity

Other than analgesic activity, this study also investigated the antipyretic activity of sweet orange peel, which was used as evaluated outcome and body temperature in all groups described in Table 5.

Table 5. Body Temperature of All Groups in Brewer's Yeast-Induced Hyperpyrexia Method.

Group	**Body Temperature (oC)**						
	Before Induction	**After Induction**	**1st Hour**	**2nd Hour**	**3rd Hour**	**4th Hour**	**5th hour**
Control	36.84 ± 0.30	38.60 ± 0.68	36.58 ± 0.48	38.04 ± 0.78	37.12 ± 0.47	37.06 ± 0.30	36.88 ± 0.16
Standard	36.70 ± 0.40	38.34 ± 0.44	37.20 ± 0.74	36.46 ± 0.44	36.68 ± 0.54	36.46 ± 0.24	36.64 ± 0.42
Sweet Orange Peel Extract-1	36.96 ± 0.43	38.50 ± 0.55	38.44 ± 0.71	37.78 ± 0.63	37.34 ± 0.46	36.94 ± 0.45	36.66 ± 0.26
Sweet Orange Peel Extract -2	36.98 ± 0.41	39.10 ± 0.23	38.42 ± 0.68	37.34 ± 0.30	36.98 ± 0.25	36.76 ± 0.30	36.66 ± 0.30
Sweet Orange Peel Extract -3	36.60 ± 0.39	38.16 ± 0.65	37.66 ± 0.87	36.68 ± 0.43	36.84 ± 0.57	36.46 ± 0.42	36.38 ± 0.40
P-Value	**0.601**	**0.084**	**0.025**	**0.001**	**0.252**	**0.042**	**0.230**

Table 5 above showed no significant difference in body temperature in all groups either before or after brewer yeast injection. It can be seen from the P-Value of either before (P value: 0.601) or after (P value: 0.084) induction that was lower than 0.05. The mean body temperature before and after induction ranged between 36.60^{o}C-36.98^{o}C and 38.16^{o}C-39.10^{o}C, respectively. It indicated that the brewer yeast injection increased the body temperature. After the induction, all groups received some treatment based on their group. All groups reported significantly decreasing body temperature an hour to four hours after receiving the treatment. It can be seen from the P-Value in 1st (P-value: 0.025), 2nd, and 4th (P-value: 0.043) hour after the treatment, which was

lower than 0.05. However, the body temperature decrease was insignificant 5 hours after the treatment and can be seen from P-value > 0.05 (P value: 0.230). Furthermore, the percent inhibition of body temperature is described in Table 6.

Table 6. Percent Inhibition of Body Temperature of All Groups in Brewer's Yeast-Induced Hyperpyrexia Method.

Group	Percent Inhibition of Body Temperature (%)				
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th hour
Control	5.23	1.45	3.83	3.99	4.46
Standard	2.97	4.90	4.33	4.90	4.43
Sweet Orange Peel Extract-1	0.16	1.87	3.01	4.05	4.78
Sweet Orange Peel Extract -2	1.74	4.50	5.42	5.98	6.24
Sweet Orange Peel Extract -3	1.31	3.88	3.46	4.45	4.66

Table 6 showed that the highest percent inhibition of body temperature 4 hours after treatment was found in the Sweet Orange Peel Extract-2, which was 5.98%, followed by the standard group (4.90%), 3 (4.45%), 1 (4.05), and the lowest one was the control group, that was 3.99%. After evaluating the rats' body temperature, all rats in this study were sacrificed for intracardiac blood sampling. This blood sample is then used for routine blood counts, including RBC or Red Blood Cell and WBC or White Blood Cell. The number of RBC and WBC from rat blood samples in all groups was described in Table 7.

Table 7. RBC and WBC Counts in All Groups.

Group	RBC	WBC
Control	8.20 ± 0.35	7.86 ± 0.97
Standard	4.27 ± 1.63	7.66 ± 0.36
Sweet Orange Peel Extract-1	7.68 ± 4.08	8.00 ± 0.29
Sweet Orange Peel Extract -2	5.89 ± 2.23	8.70 ± 0.34
Sweet Orange Peel Extract -3	3.97 ± 1.83	8.04 ± 0.85
P-Value	**0.030**	**0.149**

Table 7 showed no significant difference in the number of white blood cells or White Blood Cells in all groups and can be seen from P value > 0.05 (P value: 0.149). However, all groups showed significant differences in the number of Red Blood Cells (RBC), which can be seen from P value <0.05. The highest RBC level was found in the control group, which was 8.20 ± 0.35 x 10^6/mm^3, followed by the Sweet Orange Peel Extract-1 (7.68 ± 4.08 x 10^6/mm^3), 2 (5.89 ± 2.23 x 10^6/mm^3), Standard (4.27 ± 1.63 x 10^6/mm^3), and the lowest one was the Sweet Orange Peel Extract-3, that was 3.97 ± 1.83 x 10^6/mm^3.

Discussion

This study demonstrated the analgesic and antipyretic activity of sweet orange peel methanol extract. Both analgesic and antipyretic assays showed that the sweet orange peel had analgesic and antipyretic activity. This analgesic and antipyretic activity from sweet orange peel was not better than the standard group that received paracetamol. However, this analgesic and antipyretic activity from sweet orange peel was better than the control group that received only SCMC, a vehicle of extract, and another active compound.

The International Association for the Study of Pain (IASP) 2020 defined pain as an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage. Various organizations, including World Health Organization, have widely accepted and adopted this definition. This definition has also been revised many times; however, the meaning of this definition has remained the same from time to time. Several pain-related terms include nociception, nociceptive system, and noxious stimulus. Nociception is an activity that can be observed in response to an adequate stimulus to the nervous system, in contrast to pain, a subjective experience that can be said to only exist in people who feel it. The nociceptive system is a warning system for an adequate stimulus, while a noxious stimulus is a stimulus that can damage or threaten normal tissue (Raja et al., 2020; Treede, 2018).

According to the pain definition, this study evaluated the analgesic effect of sweet orange peel methanol extract using acetic acid-induced abdominal writhing and tail immersion. The abdominal writhing method evaluated analgesic activity at either central or peripheral levels. Meanwhile, tail immersion was used to evaluate

analgesic activity at the peripheral level via thermal stimulation of nociceptors (Fan et al., 2014; Moniruzzaman & Imam, 2014; Saha et al., 2013).

Acetic acid-induced abdominal writhing was performed by intraperitoneal injection of acetic acid, which induced an inflammatory response in the peritoneum. This injection induced local inflammation by activating arachidonic acid metabolism, initiating cyclooxygenase (PGE2 and PGE2α) or lipoxygenase pathways in the peritoneum tissue. Both cyclooxygenase products such as PGE2 or PGE2α and lipoxygenase products accumulate in the peritoneal fluid and cause various inflammation cascades, including increased capillaries permeability, swelling, and the release of various endogenous mediators which stimulate pain in the nerve ends nociceptor (Afsar et al., 2015).

This study demonstrated analgesic activity by acetic acid-induced abdominal writhing and tail immersion methods from sweet orange peel. It indicated the analgesic activity from sweet orange peels at central and peripheral levels. This analgesic activity was reported from Maximum Possible Analgesia and percent inhibition of abdominal writhing. An increase of sweet orange peel extract dose followed to increase Maximum Possible Analgesia and percent inhibition of abdominal writhing. The highest Maximum Possible Analgesia and percent inhibition of abdominal writhing at all tested sweet orange peel extract doses were found in the highest dose (750 mg/ kg BW), 132.79% and 44.05%, respectively.

This study investigated analgesic activity from sweet orange peel and antipyretic activity. Typically, human body temperature is ranged between 36.7°C to 37°C (98°F–98.6°F) for oral thermometer, and it can be 0.6°C (1°F) higher than oral thermometer for rectal thermometer or 0.6°C (1°F) lower than oral thermometer for axillar thermometer. When the human body can not keep this normal body temperature, it is defined as a fever and requires antipyretic drugs (Estella et al., 2022).

Some methods can be used to evaluate the antipyretic activity of herbs. One of these methods is brewer's yeast-induced hyperpyrexia models. Brewer's yeast is a lipopolysaccharide (exogenous pyrogen) which is a component of the cell wall of gram-negative bacteria. When pyrogens such as lipopolysaccharide (LPS) or brewer's yeast enter the body by escaping the natural barrier, this brewer's yeast then binds to an immunological protein called Lipopolysaccharide Binding Protein (LBP). It promotes the synthesis and release of various endogenous cytokines, such as IL-1, IL-6, and TNF-α, which easily cross the blood-brain barrier and act on the preoptic/anterior hypothalamus. Thereby, it activates the arachidonic acid pathway for the synthesis and release of prostaglandin E2, which was synthase by the Cyclooxygenase-2 pathway, leading to increased body temperature (Eldahshan & Abdel-Daim, 2015; Santra et al., 2014).

Brewer yeast-induced hyperpyrexia in this study indicated that the administration of brewer yeast suspension significantly increased rats' body temperature in the range of 38.16^{o}C-39.10^{o}C. All doses of sweet orange peels revealed antipyretic activity within an hour after extract administration. It can be seen from the decrease in the rat's body temperature in the first 1 hour and continued to show a decrease in body temperature for the next 4 hours. Thus, the antipyretic activity reached the peak effect 4 hours after extract administration, and the highest antipyretic activity 4 hours after extract administration was found in the moderate dose of sweet orange peel extract, which was indicated by the highest percent inhibition of body temperature, 5.98%, among all tested extract doses. On the other hand, the line chart also clearly described the moderate dose line in line chart continued to increase and positioned above the other lines. In addition, increasing the sweet orange peel extract dose did not significantly increase the percent inhibition of body temperature. This study showed that the highest sweet orange peel extract did not reveal better antipyretic activity than the lower dose, which various factors can cause. Mintarto and Fattahillah (2019) reported an increased in body temperature affected by dehydration, blood flow velocity, and sweat secretion. This study did not control these factors, such as the beverages and food was not limited but was given freely (ad libitum), potentially affecting the rat body temperature. Furthermore, both foods and beverages also potentially affect the rat's body's metabolic rate and hydration level (Mintarto & Fattahilah, 2019).

The analgesic and antipyretic effects of sweet orange peel methanol extract are associated with the phytochemicals and the yield of the extract. Phytochemical screening reported that sweet orange peels contained alkaloids, saponins, flavonoids, and tannins. Limited previous studies investigated the antipyretic and analgesic effects of sweet orange peel methanol extract. However, several studies reported that alkaloids and flavonoids had analgesic activity. Flavonoids inhibit prostaglandins' biosynthesis, which is involved in the immunological response and product of cyclooxygenase and lipoxygenase pathways. In addition, flavonoids also affect protein kinase, one of the regulatory enzymes that can inhibit the inflammatory process (Eldahshan & Abdel-Daim, 2015). Meanwhile, alkaloids are also reported to inhibit the synthesis of prostaglandins, a product of the cyclooxygenase pathway (Gaichu et al., 2017). Hence, both flavonoids and alkaloids in sweet orange peel extract can inhibit prostaglandins' biosynthesis, thereby preventing the cascade of inflammation and resulting in analgesic and antipyretic effects.

Analgesic and antipyretic activity from sweet orange peel methanol extract can be affected by either phytochemicals or yield from sweet orange peel

methanol extract. Some previous studies have reported the yield value of orange peel extract, which can be a reference value. The yield values of ethanol and ethyl acetate extracts were 19.49% and 5.45%, respectively. This study reported that the yield value of sweet orange peel methanol extract was 14.90%, which was not much different from that of sweet orange peel ethanol extract. Nevertheless, the yield value of sweet orange peel methanol extract was higher than that of sweet orange peel ethyl acetate extract. This lower yield value indicates better extract quality, but this value is equal to the yield value of sweet orange peel ethyl acetate extract. The difference in yield values could be due to differences in the solvents and the duration of maceration in this study and these previous studies. Pandey and Tripathu (2014) reported that several factors affecting the extract's quality included the plant part, the solvent used for extraction, and the extraction procedure (Gulo et al., 2021; Pandey & Tripathi, 2014).

CONCLUSIONS

Overall, it can be concluded that sweet orange peel methanol extract has an antipyretic and analgesic activity with a range of dose 500-750 mg/ kg BW. The effective dose of analgesic activity of sweet orange peel methanol extract is 750 mg/ kg BW with central and peripheral acting. Meanwhile, the effective dose of antipyretic activity is 500 mg/ kg BW, which has revealed antipyretic activity within one hour of administration and is persistent until 4 hours after extract administration.

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