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ANALGETIC ACTIVITY DETERMINATION OF PAPAYA LEAVES (*Carica papaya* L.) AND BASIL LEAVES (*Ocimum sanctum* L.) EXTRACTED USING NATURAL DEEP EUTECTIC SOLVENT

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ABSTRACT

Natural deep eutectic solvents (NaDES) are obtained from an adequate mixture of hydrogen bond acceptors (HBA) and donors (HBD), which can bond through the interaction of hydrogen bonds to form a eutectic with a low melting point. NADES has been implemented in several studies as an effective extraction method, but it is still not widely used. *C. papaya* leaves and *O. sanctum* leaves have been widely known to have various pharmacological activities. The aim of this study was to determine the best analgesic ability of the NADES extract of *C. papaya* and *O. sanctum* leaves. The extraction process used was Microwave-Assisted Extraction (MAE). The liquid extract was tested for antioxidant activity and analgesic activity using doses of 600 mg/kgBW, 800 mg/kgBW, and 1000 mg/kgBW. The results show that The IC_{50} values of the NADES extract of *C. papaya* and *O. sanctum* leaves were 920 ppm and 882 ppm, respectively. Both analgetic percentages are equivalent to the positive control starting from a concentration of 800 mg/kgBW, where the analgetic percentage of the NADES extract of papaya leaves and basil leaves is 59.80% and 54.57% respectively that included in weak category. It can be concluded that both NADES extracts of papaya and basil leaves have analgetic capabilities.

Keywords: Natural Deep Eutectic Solvent (NADES), *Carica papaya*, *Ocimum sanctum*, analgetic activity.

INTRODUCTION

Herbal medicine in developing countries is still used as the main treatment, namely around 75-80% of the total population. Herbal medicines are more culturally accepted because they are more affordable, more suitable for the body, and have relatively mild side effects (Nur, 2018). Herbal medicines that are often sought after are pain relievers. The large number of cases of herbal analgesic medicines containing chemicals has prompted the need for further research to test the natural ingredients that can be used as pain relievers.

Research shows that n-hexane and ethyl acetate extracts of *C. papaya* leaves can protect against pain, while ethanol extracts of *C. papaya* leaves have comparable abilities to aspirin (Hasimun et al., 2014). The ethanol extract of *C. papaya* leaves has analgesic activity at concentrations ranging from 80 mg/kgBW (Sasongko et al., 2016), 600 mg/kgBW (Hasimun et al., 2014), 300 mg/kgBW and 600 mg/kgBW (Afrianti et al., 2015). *C. papaya* leaves contain various enzymes, including papain, which have analgetic and anti-inflammatory activities (Mikaili et al., 2012). Papaya leaves also contain flavonoids and have high antioxidant activity (Nisa et al., 2019).

Apart from *C. papaya*, *O. sanctum* also has potential as an analgetic. Based on quantitative tests, *O. sanctum* contains phenols, alkaloids, and flavonoids (Borah and Biswah. 2018). Several studies have shown that *O. sanctum* ethanol extract at doses of 250

mg/kgBW, 500 mg/kgBW (Hannan et al., 2011), and 800 mg/kgBW can provide an analgetic effect (Rustam and Arifin, 2020). From the background above, researchers are interested in determining the greatest analgesic potential between *C. papaya* and *O. Sanctum* leaves using environmentally friendly solvents.

RESEARCH METHODS

Equipment and Materials

Equipment: Oral syringe (Terumo), stopwatch, analytical balance (Radwag AS160C12), Ohaus digital scale CR2200, sentrifuge (80-1 *Table top low speed centrifuge*), oven microwave (*rewez multifunctional* EM820ABT), Oven (*Drying Oven*), spectrophotometer (Shimadzu UV mini-1240), glassware commonly used in laboratories.
Materials: *Carica papaya* L leaves, *Ocimum sanctum L.*leaves, mefenamic acid (Opistan®) as positive control, aquadest as negative control, aqua pro injection, citric acid (Weifang Ensign Industry), Glucose (Gulshan Polyols Limited), DPPH (2, 2-Diphenyl picrylhydrazyl) (Merck), methanol (Pratama Sains Global), acetic acid (Merck), Na-CMC (Pratama Sains Global), , Vitamin C, Mayer and Wagner reagent, alcohol 96%, magnesium, HCl, ferric chloride.
Animal model: Male mice (balb/c) 2-3 month weight 20-25 gram.

Research Procedure

1. **Extraction**
   - ***Carica papaya* L leaves and *Ocimum sanctum L.* leaf simplicia preparation.**
     C. papaya and *O. sanctum* leaves were cleaned, chopped, dried in an oven at 50°C for 24 hours, and ground using a blender (Variation et al., 2018).
   - **NADES preparation.**
     NADES consisted of citric acid and glucose in a weight ratio of 3:1. From each weight ratio, 375 g citric acid and 125 g glucose were weighed. Next, 500 mL distilled water was added. The mixture was heated at 50°C and stirred slowly until a homogeneous and clear liquid formed. The NADES solvent was then stored in a closed container (Meiliyani, et al., 2019).
   - ***Carica papaya* L leaves and *Ocimum sanctum L.* leaves extraction**
     Simplicia leaves (100 g) of *C. papaya* and *O. sanctum* were extracted with NADES (citric acid and glucose) at a simplicia and solvent ratio of 1:5. Place in a microwave at medium power for 5 minutes. It remains at room temperature (Meiliyani et al., 2019).
2. **Extract Standardization**
   Testing of density, water-soluble extract, ethanol-soluble extract, and loss of drying level was carried out based on procedures from the Ministry of Health of the Republic of Indonesia (2000).
3. **Antioxidant Activity Test using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay**
   A DPPH solution (100 ppm) was prepared in methanol as a free radical. Vitamin C was prepared at concentrations of 2 ppm, 4 ppm, 8 ppm, and 10 ppm in methanol. The NADES extracts of *C. papaya* and *O. sanctum* leaves were diluted to concentrations of 100, 200, 300, 400, and 500 ppm. Absorbance measurements were performed on the sample mixture and DPPH after 30 minutes of incubation at a wavelength of 517 nm. Measurements were carried out on a blank solution (DPPH:methanol solution) (1:1); (Vitamin C: DPPH) (1:1) ; (DPPH solution: test sample) (1:1). The percentage inhibition and IC_{50} values were calculated using a linear regression formula.
4. **Analgetic Activity Test**
   The analgesic activity test received a statement of ethical suitability with number: 065/kepk-bth/XI/2021. 40 healthy male mice (*Mus musculus*) adapted for one week were divided into 8 groups, consisting of a negative control group (aquadest 0.2 ml/20 gBW), positive control group (mefenamic acid 1.3 mg/20gBW), *and C. papaya* and *O. sanctum*

leaf extract groups (600 mg/kgBW, 800 mg/kgBW, and 1,000 mg/kgBW). Before treatment, mice were fasted for approximately 18 hours without food intake but were still given water to ensure that absorption was not disturbed by the influence of food (Sentat et al., 2018). The mice were treated orally according to their group. After 5 minutes, mice were intraperitoneally injected with acetic acid. The frequencies of writhing were recorded every 10 minutes for 60 minutes and the analgetic percentage was calculated.

Data Analysis

The research data were collected and processed using the SPSS statistical data analysis method to compare the NADES treatment groups of *C. papaya* and *O. sanctum* leaf extracts at doses of 600 mg/kgBW, 800 mg/kgBW, and 1000 mg/kgBW with positive and negative controls.

RESULTS AND DISCUSSION

1. Extraction results of *C. papaya* and *O. sanctum*

In this study, the use of NADES as a green solvent, combined with a non-conventional microwave-assisted extraction method (MAE), is expected to obtain efficient, easy, fast, and environmentally friendly extraction conditions. The MAE method has several advantages: the extraction process requires a very short time, reduces heat gradients, produces high levels of target compounds, and minimizes solvent use (Ahmad et al., 2018). The NADES solvent used is food grade, so it is safe to use even if evaporation is not performed.

Table I. Yield and Results of Extract Standardization

Sample	Liquid extract Yield (%)	Density (g/ml)	Water-Soluble extract %	Ethanol-Soluble Extract %	Loss on Drying (%)
***C. papaya* leaves extract**	386.5	1.24	3.11	2.98	55.47±1.22
***O.sanctum* leaves extract**	315.2	1.25	2.71	5.56	54.00±0.82

A **B**

Figure 1. The appearance of *C. papaya* leaves NADES extract (A), *O. sanctum* leaves NADES extract (B)

The *C. papaya* and *O. sanctum* leaf extracts used in this research did not undergo any evaporation process at all, resulting in a very high yield of *C. papaya* and *O. sanctum* extracts as shown in **Figure 1**. The standard parameters of the extract can be seen in **Table I.**

2. Antioxidant Activity Test Result

Previously it was known that the total phenolic, total flavonoid content and IC_{50} of *C. papaya* water extract were respectively 57.6 µg gallic acid equivalence (GAE)/g dry weight, 0.34 µg quercetine equivalence (QE)/g dry weight and 247 µg/mL (Mandal et al., 2015). Phenolic compounds, especially(-OH), are responsible for the antioxidant activity of the phenolic aromatic ring. Flavonoids are phytoconstituents with antimicrobial, anti-inflammatory, antimutagenic, and anticarcinogenic activities (Chagas et al., 2022).

Methanolic extract from *O. sanctum* leaves has been reported to contain a total phenolic content of 87.13 mg GAE/g and a flavonoid content of 221.97 mg quercetin equivalence (QE)/g. In the same study, it was also revealed that the EC_{50} value of methanolic extract for radical scavenging was 7.32 µg/mL (Chaudhary et al., 2020). The antioxidant compounds present in *O. sanctum* include essential oils, terpenes, phenolics, and flavonoids (Agarwal et al., 2017). Supported by Chaundar et al. (2020), there is a correlation between the antioxidant activity and the levels of polyphenols and flavonoids. **Table II** shows that the antioxidant capacities of papaya and basil extracts were low.

Table II. IC_{50} Value of *C. papaya* and *O. sanctum* leaves NADES extract compared to vitamin C (vitamin C daily intake is 100 mg)

Sample	IC_{50} (ppm)	Equivalence to vitamin C	Equivalent daily intake (mg)
Vitamin C	50	-	100
***C. papaya* leaves**	920	18.4	1,840
***O. sanctum* leaves**	882	17.64	1,764

3. Analgetic Activity Test Result

The presence of the alkaloid nicotine and choline in *C. papaya* leaves is responsible for their anti-inflammatory activity (Zhou et al., 2012). Other compounds, such as phenolics, carotenoids, and glucosinolates have been shown to modulate the levels of cytokines, transcription factors, and antioxidant enzymes (Pandey et al., 2016).

The aqueous extract of *O. sanctum* (100 mg/kgBW) was tested for anti-inflammatory and analgesic activity in rats, and the percent inhibition of *O. sanctum* was 23.85%. It is suspected that the compound in *O. sanctum* that acts as an analgesic is eugenol, which is abundant in *O. sanctum* leaves, is suspected to act as an analgesic (Umamageswari & Kudagi, 2015). The analgesic mechanism of *O. sanctum* may involve antagonizing prostanoid receptors or inhibiting the synthesis of prostaglandins (Kumar et al., 2011).

Table III summarizes the comparison of the number of writhes in each group per 10 minutes compared with the positive and negative controls at each time point. It was observed that the NADES extract of *C. papaya* leaves had a real effect in the 20th minute at a dose of 800 mg/kgBW, while the NADES extract of *O. sanctum* leaves had a real effect at a dose of 1000 mg/kgBW. **Figure 2** shows the overall analgesic percentage of both the NADES extracts of *C. papaya* and *O. sanctum* leaves provide analgesic effects at a dose of 800mg/kgBW, which is comparable to that of the positive control.

Table III. Comparison of various extract concentration on writhing responses in mice during 60 min

Groups	Writhing responses observed at-(minute)						Analgesic activity (%)
	10	20	30	40	50	60	
Negative control (*aqua pro injection*)	12.20±1.32*	17.00±2.09*	23.00±2.28*	26.40±2.87*	23.00±1.09*	14.80±2.85*	-
Positive control (mefenamic acid)	6.00±1.78$^{\#}$	7.20±1.60$^{\#}$	6.60±1.35$^{\#}$	6.60±1.01$^{\#}$	4.40±1.01$^{\#}$	2.40±1.35$^{\#}$	**70.70**
C. papaya extract (600 mg/kgBW)	9.80±0.74$^{*\#}$	12.40±1.01$^{*\#}$	16.00±0.89$^{*\#}$	18.20±1.46*	15.80±1.60*	10.20±1.60*	**29.10**
C. papaya extract (800 mg/kgBW)	6.00±0.89$^{\#}$	7.80±1.16$^{\#}$	9.20±0.97*$^{\#}$	8.40±2.65$^{\#}$	9.00±1.41*$^{\#}$	7.00±0.89*$^{\#}$	**59.80**
C. papaya extract (1,000 mg/kgBW)	6.40±1.62$^{\#}$	7.40±1.01$^{\#}$	7.40±1.01$^{\#}$	7.60±2.05$^{\#}$	5.80±1.32$^{\#}$	2.80±1.72$^{\#}$	**66.98**
O. sanctum extract (600 mg/kgBW)	9.20±1.30$^{*\#}$	14.80±1.30*	16.80±1.92$^{*\#}$	18.20±5.54*	12.40±2.07$^{*\#}$	9.80±6.26*	**30.39**
O. sanctum extract (800 mg/kgBW)	5.00±1.87$^{\#}$	13.4±1.67$^{*\#}$	12.40±4.16$^{*\#}$	8.00±3.47$^{\#}$	8.00±1.87$^{\#}$	6.00±3.74$^{\#}$	**54.57**
O. sanctum extract (1,000 mg/kgBW)	2.60±1,82$^{*\#}$	5.40±1,14$^{\#}$	7.40±2.97$^{\#}$	6.20±1.30$^{\#}$	5.00±0.71$^{\#}$	3.60±2.41$^{\#}$	**74.09**

(*) $p<0.05$ compared to the positive control, ($^{\#}$) $p<0.05$ compared to the negative control

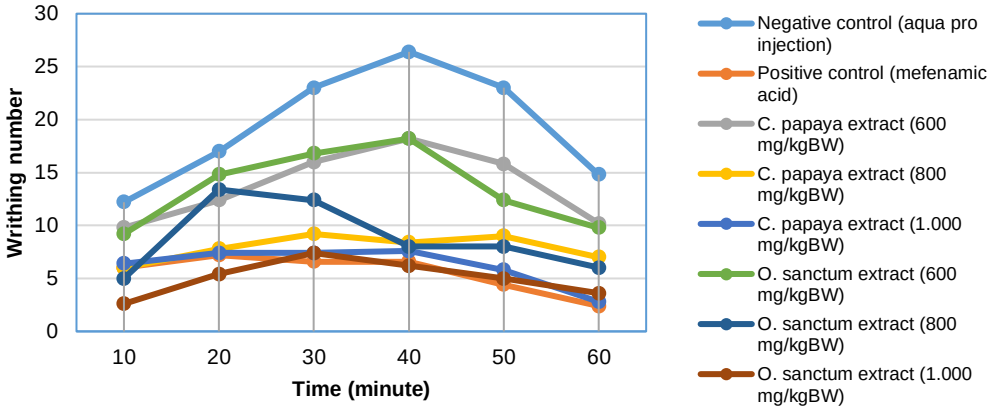

Figure 2. Comparison of various extract concentration on writhing responses in mice during 60 minutes

The percentage of analgesic protection is defined as the ability of a test substance to reduce the writhing response induced by acetic acid in mice. The percentage of analgesic protection was determined by comparing the average number of wriggles in the test group with that in the negative control group. For the analgesic activity test, acetic acid was used as an inducer. Acetic acid is an established procedure in analgesic testing, in which an abdominal constriction response can occur. Acetic acid can induce pain by releasing endogenous mediators, such as prostacyclines, which involve the activation of sensory C-fibers through the activity of the enzyme COX (Satyanarayana et al., 2004). Possibilities that may apply to the analgesic mechanism of *C. papaya* and *O. sanctum* extracts compared to NSAID Where COX will be inhibited and furthermore there will be inhibition of the release of endogenous pain mediators.

CONCLUSION

It can be concluded that both *C. papaya* and *O. sanctum* leaf NADES extracts provide analgesic activity at a dose of 800 mg/kgBW. The antioxidant mechanism is not considered to have a significant influence on its analgesic ability.

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