Piper Crocatum Ruiz & Pav as A Commonly Used Typically Medicinal Plant From Indonesia: What Do We Actually Know About It? Scoping Review

Andina Setyawati[1,*], Mae Sri Hartati Wahyuningsih[2], Dwi Aris Agung Nugrahaningsih[2], Christantie Effendy[3], Sam Ibeneme[4]

[1]Department of Surgical and Medical Nursing, Faculty of Nursing, Hasanuddin University, Makassar, Indonesia.
[2]Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[3]Department of Medical and Surgical Nursing, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[4]Department of Medical Rehabilitation, Faculty of Health Sciences, University of Nigeria, Enugu Campus, Enugu, Nigeria.
[*]Corresponding Author: andina@unhas.ac.id

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Abstract

Aims: Piper crocatum Ruiz & Pav (P. crocatum) has been widely used medicinally among ethnic communities in Indonesia to treat a variety of diseases through various preparation-application techniques, duration, and frequency of use. Since research on the benefits of P. crocatum is still limited, our scoping review aimed to identify the key findings resulting from experimental studies regarding the medicinal uses of P. crocatum.

Methods: We conducted a scoping review using the framework developed by Arksey and O'Malley. Databases were searched using CAB Direct, ProQuest, ScienceDirect, SpringerLink, and Google Scholar. Data were extracted and tabulated using Microsoft Office Excel 2013.

Results: Sixteen articles fulfilled were included in the scoping review. Three key concepts were emerged from this scoping review: 1) Study characteristics indicated the most prevalent type of research was in vivo experiment (n=14) with the highest number of preparations using ethanol extracts (n=8) and P. crocatum sample were mostly sourced from Indonesia (n=9); 2) Seventeen screened metabolites were reported and the most frequently identified were steroids and flavonoids, followed by polyphenols and saponins; and 3) Eight potential activities of P. crocatum were reported as follows: anti-inflammatory, pro-wound healing, antibacterial, antioxidant, anti-necrotic, antitumoral, hepatoprotective, and anti-hyperglycaemic.

Conclusion: P. crocatum has various metabolites that might influence the reported potential therapeutic activities with different preparation-application techniques, uses, and duration, yet the scientific evidence conducted in clinical study is still limited. Future research needs to perform testing standardized preparations of P. crocatum to be developed as novel therapeutic agents in wounds, infective diseases, cancers, diabetes, and liver diseases.

Keywords: Piper crocatum, medicinal plant, potential activity, metabolite, scoping review

Introduction

Piper crocatum Ruiz & Pav (*P. crocatum)*, known as red betel leaf, has long been known by Indonesia communities as a medicinal plant with economic value that has benefits for treating various diseases i.e., hyperglycaemia, ulcer, cancer, gout, and hypertension (Permadi et al., 2014; Suri et al., 2021). This species originates from the island of South Sulawesi in Indonesia and is easily found in Asia-Pacific ountries. Due to its popularity as an effective remedy, its empirically used is also widely found in Vietnam, Malaysia, Thailand, and other Asian countries. Yet, the benefits of *P. crocatum* are still steped in traditional beliefs and required further evidence to be considered as a scientifically safe medicinal plant (Sharma et al., 2013). A recent study identifying the chemotaxonomic significance of *P. crocatum* confirmed that ethyl acetate fractions of methanol extract contained 15 phenolic compounds, two monoterpenes, four sesquiterpenes, a neolignane, and a flavonoid *C*-glycoside. These compounds might have different and contradictory potential activities (HongXu et al., 2019).

A study in the field of dentistry found methanol extracts of *P. crocatum* was shown to have pro-proliferative effects on baby hamster kidney cell line 21 (BHK-21) fibroblasts (Permadi et al., 2014). Conversely, another study in the field of palliative care found that methanol extracts of *P. crocatum* have antiproliferative activity in cancer cells (Emrizal et al., 2014). The findings of these studies show that *P. crocatum* has various active metabolites with different activities and some have contradictory activities. The inconsistent findings certainly need to be confirmed in further research before being applied in health clinical practice.

Regarding the complexity of experimental findings of *P. crocatum*, conducting a thorough review is needed to assess the collective evidence in a particular area of research interest (Snyder, 2019). Many traditional literature reviews have identified the benefits of *P. crocatum* including its antibiotic properties (Fadlilah, 2015), and pharmacological aspects (Parfati & Windono, 2016; Suri et al., 2021). However, all of these studies have been limited to general *Piperaceae* genus studies among which some included articles were recently published within 2005-2012 and most of them were unpublished scientific works (Parfati & Windono, 2016; Suri et al., 2021). Additionally, the traditional literature reviews often are lacking in thoroughness and are conducted ad hoc, rather than following a specific methodology (Snyder, 2019). There has been little to no attempt to date to synthesize the reliable evidence for therapeutic application in the administration of
P. crocatum according to recent original experimental studies. Therefore, this scoping review aimed to identify the key findings resulting from the recent experimental studies regarding the medicinal uses of the plant scientifically known as of *Piper crocatum Ruiz & Pav*.

Methods

This scoping review was performed according to the six steps of framework developed by (Arksey & O'Malley, 2005) as follows: 1) identifying the research question; 2) identifying relevant studies; 3) study selection; 4) charting the data; 5) collating, summarizing, and reporting results; and 6) consultation. Steps one and two were set based on the acronym of PCC (population, concept, and context). The next four phases also followed a standard flow diagram of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Review (PRISMA ScR) to complete step three (Higgins et al., 2019; Tricco et al., 2018).

Identifying the research questions

The research questions were set based on the acronym of PIO (population,

Intervention, and Outcome). The guiding question was as follows: What are the identified potential activities of *P. crocatum* in experimental research findings?

Identifying relevant studies

The literature was searched by two independent reviewers (A.S and N.J) until June 2021. The following databases were screened: CAB Direct, ProQuest, ScienceDirect, and SpringerLink. Moreover, a hand search was performed on Google Scholar. The selection of keywords was in accordance with Medical Subject Headings (MeSH) and the researchers consulted with a librarian (Table 1). During the search process, we used Boolean operators "AND" and "OR", wildcards, and truncations to extent the search for different forms of a word.

Table 1. List of keywords used in search of the literature

Population	Intervention	Outcome
patient OR woman OR m* OR cell OR rat	"piper croc* OR red betel*	act* OR potential OR benefit OR effect

m* (mice, mouse, men, male), piper croc* (*Piper crocatum, Piper crocatum Ruiz & Pav, Piper crocetin*), red betel* (red betel Lynn, red betel leave, red betel root, red betel stem), act* (action, activity)

Study Selection

Study selection was the next important step in this scoping review. We used the inclusion and exclusion criteria to provide appropriate studies that matched with the purpose of study (Prajapati et al., 2014). This scoping review also performed a review framework to describe the results of studies selection. The studies included in this scoping review were experimental studies on the application of *P. crocatum* as a medicinal plant. The selected articles were original articles with quantitative studies, published within a ten years timeframe (2011-2021) and written in English and Indonesian. Articles written for the purpose of the veterinary health field were excluded. To reduce duplication of data, searching was conducted by two persons, and then, results were checked by three reviewers. The consensus was achieved between all authors with the final decision on the article selection following the quality check using SYRCLE's Risk of Bias Tools for Animal Studies (Hooijmans et al., 2014), Checklist for Reporting In Vitro Studies (CRIS) Guideline (Krithikadatta et al., 2014), and the JBI checklist for quasi-experimental study (Joanna Briggs Institute, 2020)

Data collection

Study characteristics, test material of *P. crocatum*, targeted subject, study design, location where *P. crocatum* was obtained, detailed intervention, screened metabolites, investigated potential activity and the parameters, and the effective dosage were extracted and tabulated using Microsoft Office Excel 2013. The reviewers assessed the full text articles and in duplicate assessment. Disagreements on the eligibility to include articles were resolved in discussion in order to reach consensus. Studies that met the inclusion and exclusion criteria were selected for full analysis.

Charting the data

We did data extraction using the Excel template that was prepared based on the purpose of the study and the outcomes were explored. The excel template we used for charting the data in this scoping review included author and year, origin pf *P. crocatum,* subject, material, detailed intervention, screened metabolite, potential activities, parameter, and effective dosage.

Collating, Summarizing, and Reporting Results

Step five of this scoping review describes the results of the reviews that have been

done by applying several steps, i.e., collating, summarizing, and reporting the results of data extraction that have been reviewed.

Results

Initially, the search retrieved 508 scientific articles. After removing the duplicates, 501 articles were selected. After reading the titles and abstracts, 477 articles were excluded. From the 31 selected articles, 15 articles were excluded for not meeting the eligibility criteria. After carefully analyzing the complete manuscripts, 16 studies were included in this scoping review (Figure 1).

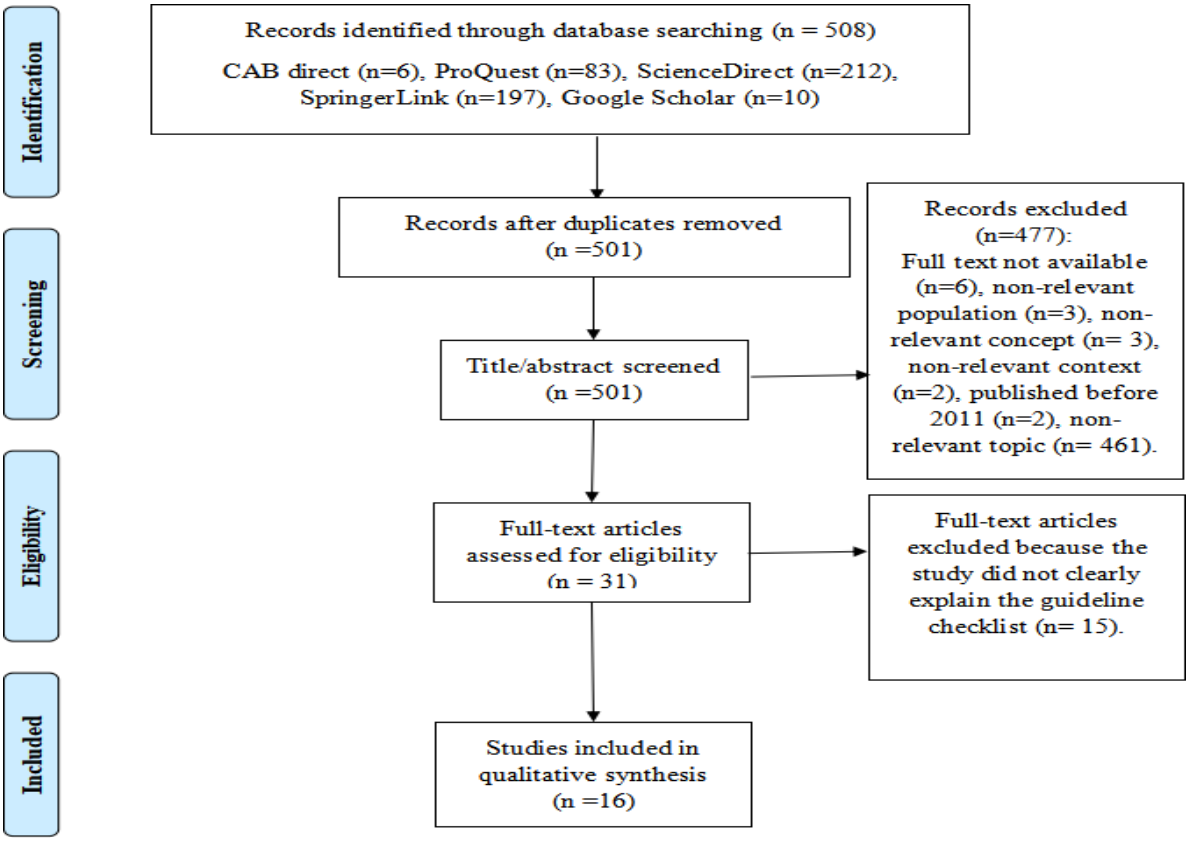

Figure 1. Flowchart of the selection process of articles according to the PRISMA statement

Study Characteristics

The study characteristic was shown in table 1. Most included studies were conducted in vitro. We reported five in vitro studies using subject samples of lipopolysaccharide-induced murine macrophage cell line (RAW 264.7) (Laksmitawati et al., 2017), cells with liver injury model (Ginting et al., 2021; Lister et al., 2020), pathogenic bacterial strains (Astuti et al., 2014), and Hela cells (Anugrahwati et al., 2016). Nine studies were in vivo studies using subjects of rats (Dewi et al., 2014; Fitriyani et al., 2011; Kendran et al., 2013; Nasi et al., 2015; Wurlina et al., 2019), mice (Fatmawaty et al., 2019; Hartini et al., 2018; Saputra et al., 2018), and *Nauplii* (Emrizal et al., 2014). Two studies were clinical studies using subjects of postpartum mothers with perineal wound (Siagian et al., 2021) and fingers of ten volunteers (Kusuma et al., 2017).

Eight included studies used *P. crocatum* test material of ethanol extract (Anugrahwati et al., 2016; Dewi et al., 2014; Ginting et al., 2021; Kendran et al., 2013; Kusuma et al., 2017; Laksmitawati et al., 2017; Lister et al., 2020; Wurlina et al., 2019) , fractions of methanol extract (Emrizal et al., 2014; Fitriyani et al., 2011; Hartini et al., 2018), isolation of antioxidant fractions (Fatmawaty et al., 2019), ethyl acetate extracts (Astuti et al., 2014), water extracts (Saputra et al., 2018), and decoctions (Nasi et al., 2015; Siagian et al., 2021).

We also identified that all *P. crocatum* used in the studies were obtained from Indonesia including from Bogor-West Java (Fatmawaty et al., 2019; Ginting et al., 2021; Kusuma et al., 2017; Laksmitawati et al., 2017; Lister et al., 2020), Yogyakarta (Anugrahwati et al., 2016; Astuti et al., 2014), Tawangmangu-Central Java (Hartini et al., 2018), and Tanah Datar-West Sumatra (Emrizal et al., 2014). Six studies did not report the place where they obtained *P. crocatum* (Dewi et al., 2014; Fitriyani et al., 2011; Kendran et al., 2013; Nasi et al., 2015; Saputra et al., 2018; Siagian et al., 2021; Wurlina et al., 2019), Detailed interventions in the included studies were reported in table 1.

The secondary metabolite contained in P. crocatum

Six studies were found performing metabolite screening of *P. crocatum*, but only four studies reported the screening method. The screening methods used in these studies were photochemical analyses (Fatmawaty et al., 2019), nuclear magnetic spectra (NMR) data observation (Emrizal et al., 2014), thin layer chromatography (TLC) bioautography (Astuti et al., 2014), and gas chromatography mass spectrometry (GCMS) (Anugrahwati et al., 2016). Seventeen metabolites contained in *P. crocatum* were identified in this scoping review, as shown below:

1. Saponins were observed in ethanol extracts (Kusuma et al., 2017), methanol extracts (Fitriyani et al., 2011) and antioxidant isolation (Fatmawaty et al., 2019).
2. Polyphenols were observed in ethanol extracts (Kusuma et al., 2017), methanol extracts (Fitriyani et al., 2011), and fractions of methanol extract (Emrizal et al., 2014).
3. Quinones, monoterpenoid, and sesquiterpenoids were observed in ethanol extract (Kusuma et al., 2017).
4. Tannins were observed in ethanol extracts (Kusuma et al., 2017) and methanol extracts (Fitriyani et al., 2011).
5. Flavonoids were observed in ethanol extracts (Kusuma et al., 2017), antioxidant isolation (Fatmawaty et al., 2019), and fractions of methanol extracts (Emrizal et al., 2014).
6. Steroids were observed in ethanol extracts (Kusuma et al., 2017 and methanol extracts (Fitriyani et al., 2011).
7. Glycosides, triterpenoids, and essential oils were observed in antioxidant isolation (Fatmawaty et al., 2019).
8. Alkaloids were observed in methanol extracts (Fitriyani et al., 2011). and antioxidant isolation (Fatmawaty et al., 2019)
9. Terpenoids were observed in fractions of methanol extract (Emrizal et al., 2014) and ethyl acetate extracts (Astuti et al., 2014).
10. Neophytadiene, elemicin, and propionic acid were observed in ethanol extracts (Anugrahwati et al., 2016).
11. Neolignanes were observed in fractions of methanol extracts (Hartini et al., 2018).

Regarding the extracts screened by the included studies, we resumed that the metabolites most commonly found in all preparations were flavonoids followed by polyphenols that were found in nearly all preparations except for fractions of antioxidant. Metabolites contained in ethanol extracts were similar with methanol extracts since they have the same five metabolites i.e., polyphenols, flavonoids, saponins, tannins, and steroids. In the form of separated compounds, fractions of methanol extracts only showed three of the same three compounds as methanol extracts i.e., polyphenols, flavonoids, and neolignanes. Not mentioning the details of the preparation technique, the metabolites contained in the antioxidant fractions were difficult to be involved in further review discussions.

Potential activities observed in the included studies.

Seven issues of potential activities with different indicators were identified from the

twelve articles in this scoping review. These potential activities were retrieved from the results regarding the purpose of each study. Extracts of *P crocatum* were reported to have the following potential activities (table 3):

Anti-inflammatory activities
Three studies reported anti-inflammatory activities of *P. crocatum* ethanol extracts. These activities were examined using enzyme-linked immunosorbent assay (ELISA) methods. Regarding the results, a decrease of tumor necrosis factor-alpha (TNF-α) was observed in cells with liver injury model at an effective dose of 25 µg/mL (Ginting et al., 2021; Lister et al., 2020) and of 100 µg/mL (Ginting et al., 2021) and RAW 264.7 cells at an effective dose of 50 µg/mL (Laksmitawati et al., 2017). Among other indicators, a decrease of nitrite oxide (NO) and interleukin-1β (IL-1β) level were also observed in 50 µg/mL ethanol extract administration on RAW 264.7 cells (Lister et al., 2020). The 50 µg/mL ethanol extracts were also reported to show decreased inflammatory percentages in carrageenan-induced rats (Fitriyani et al., 2011).

Pro-wound healing activities
Two studies reported pro-wound healing activities of *P. crocatum*. Shorter length of days of healing was found in a clinical study of postpartum mothers with perineal wounds by orally administering decoction of 4-5 leaves in 500 ml of water (Siagian et al., 2021). Another study observed the wound improvement in skin histology of male Wistar rats after 12.5% methanol extract administration (Wurlina et al., 2019).
Anti-necrotic activities.
Two studies observed anti-necrotic activities of *P. crocatum*. Decreased in the percentage of death cells and increased in the percentage of live cells were found in cells with liver injury model after ethanol extract applications at a doses of 25 and 100 µg/mL (Ginting et al., 2021; Lister et al., 2020). The percentage of death cells were identified using apoptotic activity assay.

Antibacterial activities
Two studies reported antibacterial activities of *P. crocatum.* The growth of bacteria was examined using the diffusion technique. A maximum zone of inhibition of *E. coli, P. aeruginosa, S. aureus, and C. albicans* were showed after administering 10% ethanol extracts (F3) lotion formula in volunteers' finger (Kusuma et al., 2017). In accordance with this study, decreased in the growth of *Bacillus subtilis, E. coli,* and *S.aureus* were shown in in vitro studies administering 31.25, 125 and 250 µg/mL ethyl acetate extracts (Astuti et al., 2014).

Antioxidant activities
Three studies reported antioxidant activities of *P. crocatum* through a decrease of reactive oxygen species (ROS) level. The ROS levels were identified using flow cytometry analysis and ROS assay. As much as 8 mg/200 g antioxidant fractions decreased ROS levels in mice (Fatmawaty et al., 2019). In similar findings, 25 and

100 µg/mL of ethanol extracts also decreased ROS levels in cells with liver injury model via increasing glutathione peroxidase (GPX) (Ginting et al., 2021; Lister et al., 2020). In a study of another indicator, there was also increases in cytochrome P450 2E1 (CYP2E1) gene expressions, a membrane-bound protein that catalyzes the oxidation process (Ginting et al., 2021). GPX and CYP2E1 were examined using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) (Ginting et al., 2021; Lister et al., 2020).

Antitumoral activities

Three studies reported antitumoral activities of *P. crocatum.* The half maximal inhibitory concentration (IC50) values of 2.04; 1.34 and 2.08 µg/mL were reported respectively from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay of n-hexane, ethyl acetate and butanol fractions originated from methanol extract (Emrizal et al., 2014). Lethal concentration 50% (LC50) was also reported in the evidence of antitumor activity. The cytotoxicity test showed that LC50 value of concentrated ethanolic extract was 0.81 + 0.26 mg/ml (Anugrahwati et al., 2016). In vitro studies using cancer cell lines as target therapy also showed antitumor activity. Decreased in the growth of cell lines derived from human colon carcinoma (WiDr) and human breast cancer cell lines (T47D) were shown after administering 31.25, 125 and 250 µg/mL ethyl acetate extracts (Astuti et al., 2014).

Hepatoprotective activities

Two studies reported hepatoprotective activities of *P. crocatum*. Decreased levels in aspartate aminotransferase (AST) were reported after administering orally 450 mg/kg fraction of methanolic extracts in eight weeks old male Balb/c mice (Hartini et al., 2018). These results had non-toxic effects on the liver, and 2% ethanol extract did not have effect on alanine transferase ALT and AST activity on alloxan- induced *Rattus novergicus* (Kendran et al., 2013).

Anti-hyperglycaemia activity

One study reported anti-hyperglycaemia effects of *P. crocatum*. Decreased in blood glucose levels were observed on day 21 after administering 2% ethanol extracts at 50 and 100 mg/ WB in *Rattus novergicus* (Dewi et al., 2014), on fifth month after administering 1.2-2.4 decoction in Wistar rats (Nasi et al., 2015), and on the fifth days after applying 2.8 g/kg BW of water fraction in *Mus musculus L* (Saputra et al., 2018).

Reporting results in six of eight potential activities, ethanol extracts were supposed to have the most therapeutic activities (i.e., anti-inflammatory, anti-necrotic, antibacterial, antioxidant, antitumor and anti-hyperglycaemia), followed by methanol extracts and its fractions (i.e., pro-wound healing, anti-tumor, and hepatoprotective). In addition, ethyl acetate extracts commonly had anti-bacterial and anti-tumor activities, while water extracts commonly had pro-wound healing and anti-hyperglycaemia activities.

Table 2. Characteristics of the included studies

Article No.	Author, year	Sample	Test material	Study design	Origin of *P. crocatum*	Intervention
1	(Laksmitawati et al., 2017)	Lipopolysaccharide-induced murine macrophage cell line (RAW 264.7)	Ethanol extract of *P. crocatum*	In vitro experiment	Traditional Medicine Research Center, Bogor, West Java, Indonesia	*P. crocatum* at concentration of 50 µg/mL was applied in cell line (detailed intervention was not reported in methods section)
2	(Siagian et al., 2021)	Postpartum mothers with perineal wounds, >20-> 35 years old (18 people as intervention group and 18 people as control group)	4-5 *P.crocatum* leaves boiled in 500 ml of water (decoction)	Quasi-experiment (clinical study)	Not reported	200 ml of *P. crocatum* decoction is given orally, twice a day (morning and evening), for 7 days
3	(Kendran et al., 2013)	20 alloxan-induced *Rattus novergicus* (*Sprague Dawley*), 190-210 grams	2% ethanol extract of *P.crocatum*	In vivo experiment	Not reported	Alloxan-induced rats were given *P. crocatum* extract 50 mg/kg (P3) and 100 mg/kg (P4) on day 3 to day 30
4	(Kusuma et al., 2017)	Finger of 10 volunteers	Antimicrobial lotions (F1–F3) of *P. crocatum* ethanol extracts formulated in concentrations 5, 7.5 and 10% (w/w)	Experiment (clinical study)	Bogor, Indonesia	A finger lubricated with 0.5 g of *P.crocatum* lotion was made on the media in a petri dish after 1 minute and were incubated at 25°C for 2–3 days
5	(Fatmawaty et al., 2019)	Mice	Isolation of antioxidant fractions conducted using organic solvent extraction techniques	In vivo experiment	Aromatic Research Center (Balitro) in Bogor, Indonesia	The extract was administered to the mice daily for 10 days with the dose of 4 mg/200 g (Group 1), 8 mg/200 g (group 2), and 16 mg/200g (group 3), on the tenth day, mice were given maximum physical activity (swimming for 20 minutes)

6	(Wurlina et al., 2019)	20 male wistar rat	Methanol extract of *P.crocatum*	In vivo experiment	Not reported	The extract *P. crocatum* 50% (P2), 25% (P3), 12.5% (P4) was given two drops using Pasteur pipette. On the tenth day, the skin histology of each rat was taken
7	(Lister et al., 2020)	Hydrogen peroxide - induced HepG2 cells (liver injury model)	Ethanol extract of *P.crocatum*	In vitro experiment	Pabuaran Cilendek Timur, Bogor, West Java, Indonesia	H2O2-induced HepG2 + RBLE 25 mg/mL (Group IV) and H2O2-induced HepG2 + RBLE 100 mg/mL (group V)
8	(Ginting et al., 2021)	Acetaminophen-induced HepG2 cells	Ethanol extract of *P.crocatum*	In vitro experiment	Pabuaran Cilendek Timur, Bogor, West Java, Indonesia	RBLE 25 mg/mL (Group IV); and RBLE 100 mg/mL (group V)
9	(Emrizal et al., 2014)	Nauplii	Green gum of n-hexane 24.22 g (9.56 %), a green gum of ethyl acetate 53.17 g (20.99 %) and a brown gum of butanol 61.23 g (24.18 %) fractions originated from methanol extract	In vitro experiment	Batu Sangkar village, Kabupaten Tanah Datar, West Sumatra, Indonesia	The samples (triplicate) to be assayed were dissolved in DMSO (dimethyl sulfoxide) (2 mg/400 µl or 2 mg/1000 µl) and diluted serially (10, 20, 30 and 50 µl/5 ml) in seawater. About 10–20 nauplii were added to each set of tubes containing the samples.
10	(Astuti et al., 2014)	Five pathogenic bacterial strains [*Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhi (S. typhi)*]	Ethyl acetate extracts of *P. crocatum* obtained by liquid-liquid partition of fermentation broth of endophytes followed by evaporation	In vitro experiment	Medicinal Plant Garden, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia	Graded doses of *P. crocatum* extracts (31.25-500 µg/disc) were prepared with each consecutive disc containing half amount of the consecutive dose, and were deposited on the surface of agar plates and then were incubated for 24 h at 37 °C
11	(Anugrahwati et al., 2016)	Hela cells	Ethanol extract of *P. crocatum*	In vitro experiment	Turi, Sleman Yogyakarta, Indonesia	Concentrated extract of *P. crocatum* leaves was diluted to specific concentrations then was added into certain wells together with the cells in cultured media and incubated for 24 h

12	(Hartini et al., 2018)	Eight weeks old male Balb/c mice with 25–30 g bodyweight	*P. crocatum* fraction of methanolic extract	In vivo experiment	Tawangmangu, Central of Java, Indonesia	The neolignanes Pc-1 and Pc-2 at the dose of 5 mg/kg (Groups A and B), treated the extract with the dose of 150, 300, and 450 mg/kg (Groups C, D, and E), given orally once in a day for 14 days. At 15th day (day 0) and 25th day 0.2 ml L. monocytogenes (5×103 cfu/ ml) injected intraperitoneally to all the mice. the macrophage phagocytic activity of given 450 mg/kg Pc-extract was equal to 5 mg/kg of Pc-1 and Pc-2.
13	(Dewi et al., 2014),	20 white male rats *(Rattus novergicus)* induced aloxan	Ethanol extract of *P. crocatum*	In vivo experiment	Not reported	Suspension of red betel leaf extract 2% (dose of 50 mg/kg BW) (group III) and suspension of red betel leaf extract 2% (100 mg/kg BW) (group IV)
14	(Fitriyani et al., 2011)	Carrageenan-induced rat	Methanol extract of *P. crocatum*	In vivo experiment	Not reported	25, 50 and 100 mg/WB were applied within 180 hours (group III, IV and V)
15	(Nasi et al., 2015)	12 wistar rats	Decoction of *P. crocatum*	In vivo experiment	Not reported	1.2 and 2.4 ml of *P. crocatum* decoction (Group D and B) within 5 months.
16	(Saputra et al., 2018)	24 sucrose-induced male mice (*Mus musculus L*)	Water extract of *P. crocatum*	In vivo experiment	Not reported	0.7, 1.4, 2.1, and 2.8 g/kg BW of *P. crocatum* water extracts were applied 5 days.

Table 3. Screened P. crocatum metabolites of included studies

No	Screened Metabolites	Article No.
1	Saponins	[4], [5], [14]
2	Polyphenols	[4], [9], [14]
3	Quinones	[4]
4	Tannins	[4], [14]
5	Steroids	[4], [5], [9], [14]
6	Monoterpenoid	[4]
7	Sesquiterpenoids	[4]
8	Flavonoids	[4], [5], [9]
9	Glycoside	[5]
10	Alkaloids	[5], [14]
11	Triterpenoid	[5], [14]
12	Essential oils	[5]
13	Terpenoids	[9], [10]
14	Neophytadiene	[11]
15	Elemicin	[11]
16	propionic acid	[11]
17	Neolignanes	[12]

Table 4. Potential activities observed and parameters in included studies

No	Potential activity	Parameters [Article No.]	Dosage [Article No.]
1	Anti-inflammatory	Decreased in TNF-α [1] [7] [8]	25 µg/mL [7] [8], 50 µg/mL [1], 100 µg/mL [8] of ethanol extract
		Decreased in NO [1]	50 µg/mL ethanol extract [1]
		Decreased in IL-1β level [1]	50 µg/mL ethanol extract [1]
		Decreased in inflammatory percentage [14]	50 µg/mL methanol extract [14]
2	Pro-wound healing	Faster in the length of days of healing [2] Improved in the skin histopathology [6]	4-5 leaves boiled in 500 ml of water [2] 12.5% methanol extract [6]
3	Antibacterial	Showed maximum zone of inhibition of *E. coli, P. aeruginosa, S. aureus, and C. albicans* [4]	10% ethanol extracts (F3) lotion formula [4]
		Decreased in the growth of *Bacillus subtilis, E. coli, S.aureus* [10]	31.25, 125 and 250 µg/mL ethyl acetate extracts [10]
4	Antioxidant	Decreased in ROS level [5][7][8]	8 mg/200 g antioxidant fractions [5]
		Increased in GPX gene expression [7][8]	25 and 100 µg/mL of ethanol extracts [7][8]
		Increased in CYP2E1 gene expression [8]	
5	Anti-necrotic	Decreased in the percentage of death cells [7][8]	25 and 100 µg/mL of ethanol extracts [7][8]
		Increased in live cells percentage [8]	
6	Anti-tumoral	IC50 values of 2.04; 1.34 and 2.08 µg/mL [9]	Green gum of n-hexane 24.22 g (9.56 %), a green gum of ethyl acetate 53.17 g (20.99 %) and a brown gum of butanol 61.23 g (24.18 %) fractions originated from methanol extract [9]
		LC50 value of 0.81 + 0.26 mg/ml [11]	Concentrated ethanolic extract [11]
		Showed maximum zone of inhibition of WiDr and T47D cell lines [10]	31.25, 125 and 250 µg/mL ethyl acetate extracts [10]
7	Hepatoprotective activity	Decreased in AST [12]	450 mg/kg fraction of methanolic extracts [12]
		Had no effect on ALT and AST activity [3]	2% ethanol extract [3]
8	Anti-hyperglycaemia	Decreased in blood glucose rate [13] [15] [16]	2% ethanol extracts at 50 mg/kg BW and 100 mg/kg BW [13]
		Increased number of Langerhans islet [15]	1.2 and 2.4 ml of decoction [15] 2.8 g/kg BW of water extracts [16]

Discussion

Research on herbal medicine in the past century has focused on many aspects, including pharmacognosy, quality control, experimental basis, and clinical tests for efficacy (Benzie & Wachtel-Galor, 2011). Most studies included in this scoping review were conducted on a pre-clinical experimental. Although clinical trials of this herbal medicine should be encouraged to further widen their forum of acceptance, pre-clinical studies need to be performed carefully to estimate the potential benefits and adverse reactions of *P. crocatum.* Considering the limited studies of *P. crocatum* as a medicinal plant, preclinical studies in vitro and/ or in vivo have to be done to gain a clear basis for study in humans.

We found that most common herbal preparations used by the studies were ethanol extracts, followed by methanol extracts. According to the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ethanol and methanol are recommended solvents to be used in extraction since they have limited toxic potentials to humans (World Health Organization, 2018). Metabolites contained in ethanol extract are similar with methanol extract and they have the same five metabolites i.e., polyphenols, flavonoids, saponins, tannins, and steroids. In the form of separated compounds, fractions of methanol extracts only shows three of the same three compounds as methanol extracts i.e., polyphenols, flavonoids, and neolignanes. This result is in accordance with the theory that fractionation is a separation process in which a mixture is divided into a number of smaller quantities (fractions) with higher content of target substances (Prayitno et al., 2018). In reporting polyphenols and flavonoids as major compounds in these extracts, factually flavonoids are a class of polyphenols that play a role in the characteristics including flavor, color, and pharmacological activities (Nabavi et al., 2020).

Methanol has been known to be able to attract more polyphenols. Similarly, a recent study confirmed that methanol was the best solvent for the extraction of total polyphenol content. Another study identified that ethanol solvents are suitable for flavonoid extraction (El Houda Lezoul et al., 2020). However, a recent study of *Salvia hispanical L* seeds showed the same amounts of polyphenols and flavonoids were found in both methanol and ethanol extracts (Gema et al., 2020). These atypical results reflect how the favour temperature and the different species of plants would result in different yields of extracts. In the conventional method, *n*-butanol and methanol are the suitable solvents in producing saponins and tannins contained in leaves. This method is recommended for an efficient extraction of bioactive compounds (Le et al., 2018). One study of Binahong leaves showed that methanol extracts had the highest saponins, followed by ethanol extracts (Wardatun et al., 2019). Regarding with these discussions, methanol extract is suitable for yielding the greater amounts of polyphenols, saponins and tannins while ethanol extract is suitable for yielding the greater amounts of flavonoids. The selection of secondary metabolites is based on the purpose of targeted potential activities.

Our scoping review noticed that ethanol extracts of *P. crocatum* were supposed to have the most therapeutic activities (i.e., anti-inflammatory, anti-necrotic, antibacterial, antioxidant, antitumor and anti-hyperglycaemia), followed by methanol extract and its fractions (i.e., pro-wound healing, antitumor, and hepatoprotective). In addition, ethylacetate extracts commonly had anti-bacterial and anti-tumour activities, while water extracts commonly had pro-wound healing and anti-hyperglycaemia activities.

Identifying most compounds in only ethanol extracts, methanol extracts of *P. crocatum* need to be further studied in an extended scope of activities, because

scientists discovered that methanol extracts as highly polar solvents have a high effectiveness of antioxidant, anti-inflammatory, antibacterial, and anti-hyperglycaemia (Altemimi et al., 2017). Antioxidant activities were determined by total flavonoids and total phenols (Prayitno et al., 2018), because of the existence of their hydroxyl group as scavengers of various free radicals in several diseases (El Guiche et al., 2015). We identified that antioxidant activities of *P. crocatum* are indicated by decreased in ROS levels and increased in GPX and CYP2EI gene expressions. In accordance with these findings, GPX gene expressions along with superoxide dismutase (SOD) and catalase (CAT) have been shown to avoid the cell membrane damage induced by oxidation in lipid peroxidation cases. Additionally, the CYP2EI of cells with a liver injury model was increased by *P. crocatum* administration in one included study, and it was explained that CYP2E1 may regulate the development of liver diseases trhough mediated mitogen-activated protein kinase c-Jun N-terminal Kinase (JNK) signaling (Schattenberg & Czaja, 2014).

Our scoping review found that anti-inflammatory activities were reported in studies that administered ethanol and methanol extracts of *P. crocatum*, indicated by decreased levels in TNF-α, NO, IL-1β level, and inflammatory percentages. Some metabolites contained in *P. crocatum* that may have contribution on these activities were phenolic compounds, alkaloids, saponins, steroids, terpenoids and essential oils (Mohammed et al., 2014). Different phenolic compounds including flavonoids, condensed tannins and gallotannins are attested to inhibit molecular targets of pro-inflammatory mediators in inflammatory responses (Fawole et al., 2009). Treatment with flavonoids has been shown to decrease lipopolysaccharide (LPS)-induced TNF-α production and inhibit endotoxin shock in infected mice through activation of nuclear factor (NF)-κB and extracellular signal-regulated kinase (ERK) pathways. Other inflammatory cytokines, such as IL-1β along with TNF-α were decreased by attenuation of LPS-induced by polyphenols in TNF-α-related diseases including obesity, type 2 diabetes mellitus (T2DM), rheumatoid arthritis (RA), and inflammatory bowel diseases (IBS) (Kawaguchi et al., 2011). Since the level of NO is high during the presence of inflammation, therapy targeting NO depletion is needed in infection diseases. In line with our scoping review finding methanol extract decreased NO, an in vitro study found that methanol extract of *Curcuma zedoaria* inhibited the synthesis of NO by 78% through reducing endothelial nitrite oxide synthase (eNOS) and inflammation nitrite oxide synthase (iNOS) levels in association with reduced oxidative DNA and protein damage in diabetes-induced rats (Borchard et al., 2012).

Similar with its antioxidant and antiinflammation activity, secondary metabolites contained in *P. crocatum* that may have contribution to antitumor activities were alkaloids, terpenoids, polyphenols, and flavonoids compounds (Agarwal et al., 2014). Alkaloid compound bind tubules and inhibit microtubule component formation in the mitotic phase resulting in metaphase stops (Rosyadi et al., 2020). Terpenoids act as anticancer agents through blocking the cell cycles in the G2/M phase via stabilizing spindle threads in the mitotic phase resulting the inhibition of mitosis process and proliferation (Rosyadi et al., 2020).. Possessing antitumor activities, methanol and ethanol extracts of *P. crocatum* should be retreated in a specific manner to neutralize the toxicity prior to use as wound healing agent. They must be properly processed to remove the unwanted substances. Herbal materials derived from the same species but different in processing methods may show different therapeutic activities (World Health Organization, 2018).

P. crocatum is one of the wound healing product used by cultures and ethnic groups in Asian countries. The secondary metabolite contained in *P. crocatum* may have a pivot role on pro-wound healing activities reported in this scoping review. Saponin is

known to ameliorate wound healing trough anti-bacterial and anti-microbial activities (Shedoeva et al., 2019). Polyphenols are also believed to act by disintegrating of bacterial cell walls through the hydrophobic components of phenolic compounds, altering the intercellular function by hydrogen binding to enzymes (Guimarães et al., 2021). A type of polyphenols, glycoside polyphenol is reported to regulate cell adhesion of dermal fibroblast during wound healing by increasing E-cadherin through calcium binding sites (Pomari et al., 2013).

Our scoping review identified that *P.* crocatum has various active substance and some are evidenced to have therapeutic activities in experimental basis. Future research of
P. crocatum needs to confirm the secondary metabolites in either methanol or ethanol extracts (and the fractions) through a standardized method to gain consistent potential activities against certain diseases since *P. crocatum* shows various characteristics which are beneficial to human health.

Limitations
The present research had some limitations. In order to compile a comprehensive and broad scoping review, we retrieved both the quantitative and qualitative studies based upon their methodological quality. As a result, a number of small-scale, uncontrolled studies were included whose results should be interpreted cautiously. Whilst every attempt was taken to develop a search strategy that utilised a number of global databases, extra time could have been taken to improve upon this with more iterative steps.

Contribution to global nursing practice
P. crocatum is found to have many therapeutic effects, but it is dose dependent with certain processing techniques. However, *P. crocatum* is widely used by the community to treat various diseases. Thus, literacy related to the effects of P. crocatum and how exactly it is processed can be integrated into education as an integration of holistic nursing care. In the end, it is expected to increase the competence of nurses in providing quality education to patients, among nurses and prospective nurses. As study of this in human is still limited, the challenge of implementing holistic nursing care in patients using *P. crocatum* as herbal therapy is that more detailed discussion and information is needed to clarify the therapeutic effects, side effects and safety aspects related to duration of therapy, frequency of therapy, and preparation-application techniques in human.

Conclusion
P. crocatum had various metabolites that might play a role in the reported potential therapeutic activities at different preparation techniques, yet the scientific evidence conducted in clinical study is still limited. Future research needs to be performed with standardized preparations of *P. crocatum* in order to be developed as novel therapeutic agents in wounds, infective diseases, cancers, diabetes, and liver diseases.

Author Contribution
All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest
Authors state no conflict of interest.

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