Journal of Applied Pharmaceutical Science Vol. 12(08), pp 009-017, August, 2022
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2022.120802
ISSN 2231-3354

A comprehensive review on the phytoconstituents and biological activities of *Nyctanthes arbor-tristis* L.

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ARTICLE INFO

Received on: 23/02/2022
Accepted on: 26/06/2022
Available Online: 04/08/2022

Key words:
Herbal medicine, night-flowering jasmine, pharmacology, secondary metabolite, traditional use.

ABSTRACT

Nyctanthes arbor-tristis (NAT) is one of the Indonesian herbal medicines which belongs to the genus *Nyctanthes* and the Oleaceae family. Traditionally, NAT is used to reduce fever and pain and treat hemorrhoids, irregular menstruation, cough, and asthma. This review article aimed to examine the phytoconstituents and pharmacological activities of NAT from different plant parts. The literature search was conducted through PubMed and Google Scholar databases. Our literature study showed that NAT has various secondary metabolites, such as steroids, terpenes, flavonoids, phenol, iridoid glycosides, and alkaloids. These compounds are distributed in the leaves, seeds, flowers, and stem bark. Different chemical structures of secondary metabolites are present in each plant part, and each demonstrates distinct pharmacological activities. The most common activities found in the literature are antimicrobial, analgesic, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, antitussive, and antimalarial activities. Although this plant offers a promising source for drug discovery, the pharmacological and phytochemical data are still limited. Further studies are needed to explore the potency of NAT as natural products benefiting human health.

INTRODUCTION

Indonesia is a country with abundant biodiversity including medicinal plants. The abundance of medicinal plants encourages people to use traditional medicine based on natural ingredients. Traditional medicine is not based on scientific approaches. Traditional medicine is based on cultural knowledge, which means that the practice of traditional medicine is a part of their culture and habit. People prefer using traditional medicine with natural ingredients because they perceive natural ingredients to be safer than synthetic drugs. In addition, traditional medicine is affordable as medicinal plants are often grown in home yards (Elfahmi *et al*., 2014; Moreira *et al*., 2014).

Nyctanthes arbor-tristis (NAT) is native to South Asia. Its distribution ranges from Northern Pakistan and Nepal throughout North India to Southeast Thailand. Currently, this plant is widely cultivated in the tropics and subtropics. The genus *Nyctanthes* includes two types of species, namely NAT and *Nyctanthes aculeata*. NAT is a native plant of India and is used as a sacred plant in religious ceremonials, whereas *N. aculeata* is a native plant of Thailand. The scientific research and literature study on *N. aculeata* are still limited. In this narrative review, we provide comprehensive information on the pharmacological and phytochemical aspects of NAT and discuss its prospect as a potential source of bioactive molecules for drug discovery (Wallander and Albert, 2000).

NAT has fragrant flowers resembling jasmine. The flowers only grow in the afternoon until the evening and fall in the morning. Thus, this plant is called "night jasmine." In Ayurveda, each part of this plant can be used to treat various diseases, including digestive problems, as an antidote to reptile venoms, as a tonic, laxative, diaphoretic, and diuretic, and as a remedy to arthritis (Rangika *et al*., 2015). In Indonesia, NAT is usually used as a traditional medicine to treat fever, hemorrhoids, irregular menstruation, pain, cough, and asthma.

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The plant parts used comprise leaves, stem bark, and flowers (Medicinal Herb Index in Indonesia, 1986). The extracts of NAT have been pharmacologically investigated as antimicrobial, analgesic, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, cough suppressant, and antimalaria agents (Chaudhary *et al*., 2018; Ghosh *et al*., 2015; Godse *et al*., 2016; Kakoti *et al*., 2013; Michael *et al*., 2013; Mousum *et al*., 2018).

Although the research on NAT is still limited, this plant has the potency to be developed as a drug or source of bioactive molecules based on its traditional use and recent scientific findings. In this review, we describe the chemical structure diversity of NAT phytoconstituents and provide the pharmacological activities of its leaves, flowers, seeds, and bark. This comprehensive review presents scientific information that motivates researchers and scientific communities to find scientific data related to NAT.

METHODS

The literature search was carried out through the PubMed and Google Scholar databases. To obtain the most recent information, we only used articles ranging from 2010 to 2021 in this study. The keywords for the database search were "*Nyctanthes arbor-tristis* L.; phytochemistry," "*Nyctanthes arbor-tristis* L. isolation," "*Nyctanthes arbor-tristis* L. study *in vitro*," "*Nyctanthes arbor-tristis* study *in vivo*," and "*Nyctanthes arbor-tristis* L. pharmacological study." The data included in this work were original research articles only. Unpublished data, including theses and conference articles, were excluded from this study.

Botanical aspect of *Nyctanthes arbor-tristis*

NAT is a vascular plant and a member of Tracheophyta. The plant belongs to Magnoliopsida or Dicotyledoneae (which includes flowering plants with two pieces of seeds). The plant is a member of the Oleaceae family and genus *Nyctanthes* (Sharma *et al*., 2021). The term "*Nyctanthes*" comes from the words nykhta (night) and anthos (flower), thus referring to a night flower. NAT is also called "the sad tree" or "tree of sorrow." NAT is often called "night jasmine." Its flower has a fragrant aromatic odor. This woody plant reaches approximately 10 m in height and has a grey bark surrounded by shells. It usually grows and lives for up to 20 years. The leaves are ovate and tapered and sit in the opposite position, whereas the mature fruits are brown with a diameter of approximately 2 cm. The flowers grow in the armpits or terminals consisting of 2–7 chalices with rectangular stems. The flower crown is orange with a white lobe 5–15 mm long, and two stamens can be found at its top. The petals are ovoid with an orange to red color combined with white. This plant usually blossoms in the afternoon until evening (Hiremath *et al*., 2016; Mishra *et al*., 2016a; Mishra *et al*., 2016b; Sharma *et al*., 2021).

Phytoconstituents of *Nyctanthes arbor-tristis*

Table 1 indicates that NAT has a high diversity of phytochemicals, and it has the potential to be further explored as a bioactive molecule or a drug candidate. NAT contains various phytochemical constituents. Table 1 presents the reported phytochemical constituents from different parts of the plant (flowers, leaves, stems, and seeds). Phytochemical screening showed that NAT contains steroids, terpenes, flavonoids, iridoid glycosides, carbohydrates, and alkaloids. The ethyl acetate extract of the NAT flower contains stigmasterol, rengyolone, 2-phenylethyl β-D-glucopyranoside, and n-tetradecyl-β-D-glucopyranoside; the methanol and ethanol extracts contain crocin and crocetin (Pawar *et al*., 2019). The leaf contains β-sitosterol, nyctanthic acid, 1-(8-hydroxy-7-((4-nitrophenyl)(phenylamino)methyl)quinoline-3-yl) propane-2-one, 2-(8-hydroxy-7-((4-nitrophenyl)(phenylamino) methyl)quinoline-3-yl) acetic acid, arbortristoside C, calceolarioside A, arborside A, arborside B, lupeol, and betulinic acid (Ashwini and Rekha, 2019; Bhadouria *et al*., 2012; Chaudhary *et al*., 2018; Karan *et al*., 2019; Mishra *et al*., 2016b). From the seeds of NAT, an iridoid-derived compound, namely arbortristoside C, was identified from the methanolic extract (Vajravijayan *et al*., 2020). The stem contains 21α-hydroxyfriedel-4-(23)-en-3one, β-sitosterol, 1-triacontanol, friedel-1-ene-3-one, pelargonic acid, and lignoceric acid (Kumari *et al*., 2017).

Biological activities of *Nyctanthes arbor-tristis*

Table 2 presents the recent studies (last 10 years) on the pharmacological effects of NAT. The extracts from the leaves, flowers, seeds, and stems of NAT demonstrate various biological activities. The leaf is well studied compared with the flowers, seeds, and stem bark. Although the comprehensive information regarding the bioprospective of NAT is limited, the pharmacological activities from its different parts are discussed in this review. These pharmacological activities include antioxidant, anti-*Malassezia*, antihyperglycemic, antihyperlipidemia, antimalaria, hepatoprotective**,** antitussive, analgesic, anti-inflammatory, anticancer, antiproliferation, and antigenotoxic activities.

Antioxidant effect

Antioxidants are compounds that can prevent oxidative stress due to excessive free radicals in the body. This oxidative stress can induce lipid, lipoprotein, and DNA damage, thus triggering various diseases. The methanol extract from NAT leaves has an antioxidant activity, and these have been tested *in vitro* using different methods. The half-maximal inhibitory concentration (IC_{50}) values of 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging, hydroxyl radical scavenging, nitric oxide scavenging, and superoxide radical scavenging assays are 57.93, 98.61, 91.74, and 196.07 µg/ml, respectively. These antioxidant activities may be correlated with the phenolic content of the extract, which reaches 78.48 ± 4.26 mg tannic acid equivalence per gram. Phenolic compounds exhibit antioxidant activity by counteracting free radicals, thus causing their free radical reduction (Michael *et al*., 2013).

The ethanol and aqueous extracts from the seeds and leaves of NAT showed concentration-dependent activity in a DPPH radical scavenging assay. The ethanol and water extracts of NAT leaves demonstrated stronger antioxidant activity than those of the seeds. Interestingly, betulinic acid was identified as an active antioxidant compound with an IC_{50} of 18.03 µg/ml with the DPPH assay. This compound might interfere with the oxidation process by donating an electron to neutralize free radicals (Karan *et al*., 2019; Patel and Gokhale, 2016; Sousa *et al*., 2021). Other studies evaluated the antioxidant activities of different NAT flower extracts (ethanol, ethyl acetate, and water extracts) using DPPH radical scavenging, superoxide radical scavenging, lipid peroxidation, and reducing power assays. The study revealed

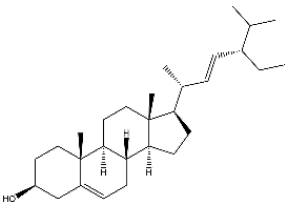
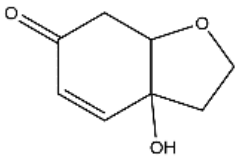
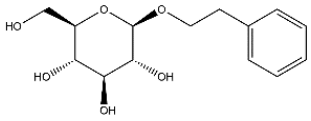
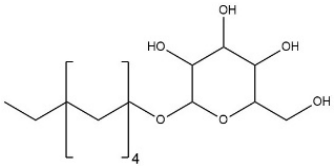
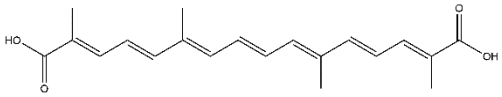
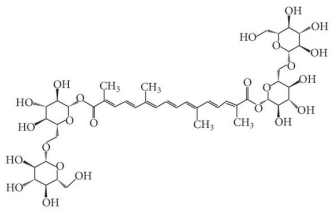
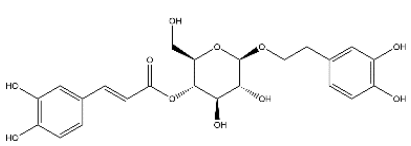
that the ethanol extract of NAT flower demonstrated the highest antioxidant activity with IC_{50} values of 406.37 ± 2.45, 269.66 ± 18.48, and 2004.14 ± 8.31 in DPPH, lipid peroxidation, and superoxide radical scavenging assays, respectively. This finding indicated that although the extract showed a medium degree of antioxidant activity, its reducing power is weak. The difference in the antioxidant potency may be due to the solubility of antioxidant compounds in various solvents used in the extraction process (Mishra *et al*., 2016a; Patel and Gokhale, 2016).

Anti-*Malassezia*

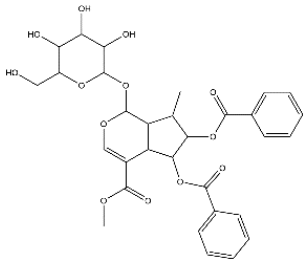
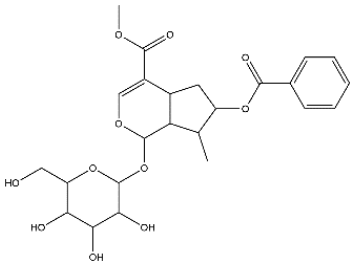
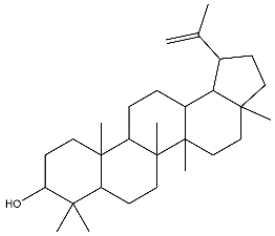
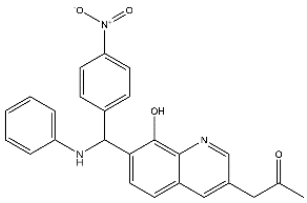
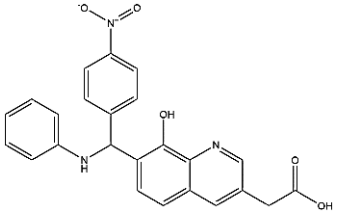
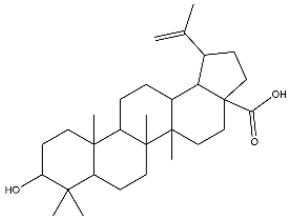
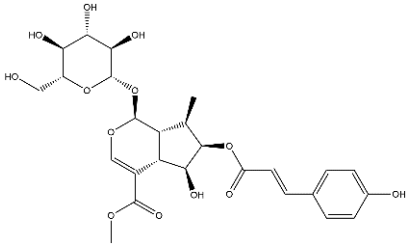
Malassezia is a type of eukaryotic fungus and normal microbiological flora of human and animal skin. However, *Malassezia* spp. can cause several diseases, including pityriasis versicolor, seborrheic dermatitis, and folliculitis (Han *et al*., 2017). In addition, the interaction of this fungus with the host can stimulate hypersensitivity and activate immunoglobulin E (IgE) production and T cell reactivity. It produces Mala s1, a major allergen that triggers the production of IgE and causes skin diseases (Saunders *et al*., 2012).

The ethanol extract of NAT leaves prevented *Malassezia* infection, as assessed by a microdilution method. The ethanol extract inhibited the growth of two *Malassezia* strains with minimum inhibitory concentrations of 1.05 and 1.47 µg/ml against *M. globosa* and *M. restricta*, respectively. The cytometric analysis demonstrated that the active compounds were β-sitosterol

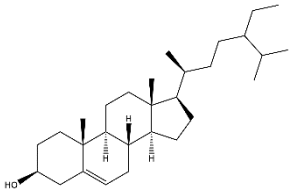
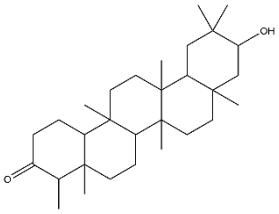
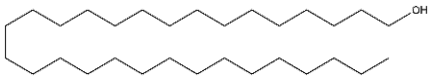
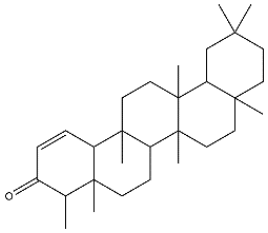
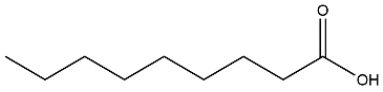
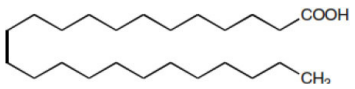
Table 1. Reported data of compounds present in *Nyctanthes arbor-tristis*.

Name of compound	Structure	Part of plant	Reference
Stigmasterol		Flower	
Rengyolone		Flower	(Haque *et al*., 2019)
2-Phenylethyl-β-D-glucopyranoside		Flower	
n-Tetradecyl-β-D-glucopyranoside		Flower	
Crocetin (Ag-NY1)		Flower	(Gadgoli and Shelke, 2010; Khanapur *et al*., 2014)
Crocin		Flower	(Khanapur *et al*., 2014; Pawar *et al*., 2019)
Calceolarioside A		Leaf	(Mishra *et al*., 2016b)

Continued

Name of compound	Structure	Part of plant	Reference
Arborside A		Leaf	(Chaudhary *et al*., 2018)
Arborside B		Leaf	
Lupeol		Leaf	(Ashwini and Rekha, 2019)
1-(8-Hydroxy-7-((4-nitrophenyl)(phenyl amino)methyl) quinoline-3-yl) propane-2-one		Leaf	(Bhadouria *et al*., 2012)
2-(8-Hydroxy-7-((4-nitrophenyl) (phenylamino)methyl) quinoline-3-yl) acetic acid		Leaf	
Betulinic acid		Leaf	(Karan *et al*., 2019)
Arbortristoside C		Leaf and seed	(Chaudhary *et al*., 2018; Vajravijayan *et al*., 2020)

Continued

Name of compound	Structure	Part of plant	Reference
β-sitosterol		Leaf and stem	(Kumari *et al*., 2017; Mishra *et al*., 2016b; Nirmal *et al*., 2012)
21α-Hydroxyfriedel-4-(23)-en-3-one		Stem	
1-Triacontanol		Stem	
Friedel-1-ene-3-one		Stem	(Kumari *et al*., 2017)
Pelargonic acid		Stem	
Lignoceric acid		Stem	

and calceolarioside A. Both compounds effectively bind to the specific targeting site of Mala s1 of the fungus in the molecular docking study and inhibit the production of tumor necrosis factor-α (TNF-α) in the stimulated splenocytes. NAT is considered a potential natural product for preventing *Malassezia* infection (Mishra *et al*., 2016).

Antihyperglycemia and antihyperlipidemic

Diabetes is a disease caused by disorders of the endocrine system. This damage causes an increase in glucose levels because of the disturbance in insulin secretion. Diabetes in the long term will affect an increase in low-density lipoprotein (LDL), a decrease in high-density lipoprotein (HDL), which causes lipid dysregulation, reactive oxygen species production, and reduction of antioxidant status (Luo *et al*., 2019). Lipid disorders also trigger the occurrence of coronary heart disease. The ethanol extract of NAT leaves demonstrated antihyperglycemic and antihyperlipidemic activities in albino rats. It decreased blood sugar levels at the dose of 400 mg/kg given for 28 days in high-fat-diet (HFD) and streptozotocin (STZ) induced rats. The extract significantly reduced the blood sugar level from 437.4 ± 21.18 mg/dl to 191.3 ± 21.51 mg/dl ($p < 0.001$). Interestingly, the lipid profile was also significantly improved. The total cholesterol, triglyceride, vLDL, and LDL decreased by 36.3%, 21.6%, 21.6%, and 60.3%, respectively, whereas HDL increased compared with the control group (HFD–STZ-induced diabetic rats). The ethanol extract of NAT also decreased the malondialdehyde (MDA) level and increased the superoxide dismutase (SOD), catalase, and glutathione reductase enzyme levels. MDA is a marker of lipid peroxidase and can cause cross-linking of the polymerization of proteins, nucleic acids, and other macromolecules. Meanwhile, SOD, catalase, and glutathione reductase are enzymatic antioxidants that have an important role in the defense of the immune system. Although the active compound responsible for the antihyperglycemic and antihyperlipidemic activities remains unknown, previous studies showed that the ethanol extract of NAT leaves was able to suppress hyperglycemia-mediated oxidative stress and to inhibit inflammatory pathways upon nuclear factor kappa B activation that are crucial in diabetes and hyperlipidemia (Luo *et al*., 2019; Mousum *et al*., 2018). The inhibition of these biomarkers suggested that NAT has the potential to be a natural source for the development of immunomodulatory and chemoprevention agents.

Apart from the ethanol extract of NAT leaves, the aqueous extract of NAT flowers showed hypoglycemic and hypolipidemic activities in healthy adult imprinting control region male mice.

Table 2. Summarized data of pharmacology activities from *Nyctanthes arbor-tristis*.

Part of plant	Sample	Pharmacological activity	Study design	Reference
Leaf	Methanol extract	Antioxidant	*In vitro*	(Michael *et al*., 2013)
	Aqueous extract	Antioxidant	*In vitro*	(Patel and Gokhale, 2016)
	Ethanol extract	Antioxidant	*In vitro* and *in vivo*	(Chaudhary *et al*., 2018; Patel and Gokhale, 2016)
	Betulinic acid isolate	Antioxidant	*In vitro*	(Karan *et al*., 2019)
	Ethanol extract	Anti-*Malassezia*	*In vitro*	(Han *et al*., 2014; Mishra *et al*., 2016b)
	Ethanol extract	A decrease in hyperglycemia and hyperlipid	*In vivo*	(Mousum *et al*., 2018)
	Aqueous extract	Antimalaria	Clinical study	(Godse *et al*., 2016)
	Ethanol extract	Antimalaria	*In vitro* and *In vivo*	(Agrawal and Pal, 2013)
	Ethanol extract	Hepatoprotective	*In vivo*	(Chaudhary *et al*., 2018)
	Carbohydrate polymer (CP) isolate	Antitussive	*In vivo*	(Ghosh *et al*., 2015; Nosáľová *et al*., 2012)
	Betulinic acid isolate	Anti-inflammatory and anticancer	*In vitro*	(Karan *et al*., 2019)
	Ethanol extract and Lupeol isolate	Inhibition matrix metalloproteinase, inhibition angiogenesis, inhibition proliferation of glioma cells	*In vitro*	(Ashwini and Rekha, 2019)
Flower/tubular calyx	Ethanol extract	Antioxidant	*In vitro*	(Mishra *et al*., 2016)
	Ethanol extract and crocin isolate	Antigenotoxic	*In vitro*	(Pawar *et al*., 2019)
	Aqueous extract	Hypoglycemic activity and hypolipidemia	*In vitro* and *in vivo*	(Rangika *et al*., 2015)
	Ethanol extract	Antiproliferation	*In vitro*	(Khanapur *et al*., 2014)
	Ethyl acetate fraction			
Stem	Methanol extract	Analgesic and anti-inflammatory	*In vivo*	(Kakoti *et al*., 2013)
Seed	Arbortristoside C isolate	Hypoglycemic activity	*In vitro*	(Vajravijayan *et al*., 2020)

The extract (at doses of 500 and 750 mg/kg) lowered fasting blood glucose by 49% and 39%, respectively. Additionally, the random blood glucose level was significantly reduced (32%) by the extract after 4 hours of treatment. In the absorption phase, the aqueous extract of the NAT flower also inhibited glucose absorption from the intestines and increased the diaphragmatic glucose uptake by 85% and 64%, respectively. The α-amylase enzyme activity was inhibited by 16.66%, whereas the levels of total cholesterol and triglycerides were inhibited. The level of HDL increased. Another study revealed that the antidiabetic activity of the aqueous extract of NAT was due to the capability of the extract to inhibit α-amylase glucose absorption and increase glucose transport to cell membranes. The extract mainly contained tannins and flavonoids that were able to inhibit α-amylase and prevented the hydrolysis of starch and oligosaccharides into maltose, maltotriose, and simple sugars. Thus, they might be responsible for the antihyperglycemic activity (Rangika *et al*., 2015; Siraj *et al*., 2013; Wickramaratne *et al*., 2016).

Antimalaria

Malaria is an endemic disease transmitted by mosquitos infected by a *Plasmodium* parasite. The current malaria drugs, namely quinine and artemisinin, are derived from medicinal plants. The main burden in malaria therapy is the progression of drug resistance. Therefore, researchers continually explore new sources, including medicinal plants, for the discovery of malaria drugs. NAT is one of the promising plants for combating malaria. A study on Swiss albino mice infected with *Plasmodium berghei* showed that the ethanol extract of NAT leaves reduced proinflammatory mediators (TNF-α, interleukin-6, and NO). The ethanol extract of NAT leaves contained active iridoid glycosides responsible for the antiplasmodial activity. Although the mechanism of action of the iridoid glycosides as antimalaria agents is unclear, previous studies demonstrated that these iridoid glycosides inhibited IL-6 and TNF-α production in mice induced with *P. berghei* (Agrawal *et al*., 2013; Agrawal and Pal, 2013). The ethanol extract of NAT leaves also showed a toxic effect against *Plasmodium falciparum* strain 3D7 (IC_{50}: 77 ± 7 µg/mL) (Kumari *et al*., 2012).

The most advanced experimental data regarding the antimalarial effect of the aqueous NAT leaf extract originated from a clinical study involving 20 participants strongly suspected of having malaria. The extract was given thrice a day for 7 days to treat malaria. The results showed that 10 out of 20 patients experienced heat and parasite clearances confirmed by polymerase chain reaction. Ten patients showed persistent but decreased parasitemia. Meanwhile, 4 out of 10 patients required chloroquine for further treatment. In addition, the increase in platelet number and normalization of plasma lactic acid were observed. The levels

of inflammatory cytokines, including TNF-α, were decreased (Godse *et al*., 2016).

Hepatoprotective

The liver is an organ that is responsible for the metabolism, secretion, storage, and detoxification of endogenous and exogenous compounds. The ethanolic extract of NAT leaves (500 mg/kg) showed a hepatoprotective effect by decreasing the aspartate aminotransferase, alanine aminotransferase, *alkaline phosphatase*, and total *bilirubin* levels in Wistar male rats induced by antituberculosis drugs (isoniazid, rifampicin, and pyrazinamide). The presence of nyctanthic acid, β-sitosterol, and arborsides A, B, and C in the extract may be crucial for the hepatoprotective effect of β-sitosterol (Kim *et al*., 2014); no data is available regarding the hepatoprotective mechanism of action of these compounds. Further study in this area of research is needed.

Antitussive

A cough is a body protection mechanism against foreign objects that enter the respiratory tract. A cough is also a sign of body abnormality or the presence of certain diseases. Codeine and dextromethorphan are the most common drugs for suppressing a cough. However, they have major side effects due to sedation, respiratory depression, and mucus viscosity disturbance influence. The carbohydrate polymer (CP) isolated from the aqueous extract of NAT leaves reduced coughs in guinea pigs induced by citric acid by up to 66.5% at a dose of CP 50 mg/kg. The activity was slightly lower than that caused by codeine phosphate (62.1%). Previous studies showed that CP decreased airway irritation by protecting cough receptors and suppressing pathological cough reflexes. In addition, *in vivo* test results showed that CP had a significant effect when administered. The use of NAT for the treatment of coughs is empirically based on the traditional knowledge of Indian tribes (Ghosh *et al*., 2015; Nosáľová *et al*., 2012).

Anti-inflammatory and analgesic

Inflammation is the body's response to injuries caused by foreign invaders, such as viruses, bacteria, unwanted proteins, chemicals, and other agents. Inflammation also includes pathological events underlying various disorders. Currently, anti-inflammatory drugs are divided into two groups: steroids and nonsteroidal anti-inflammatory drugs (NSAIDs). However, the use of these drugs is commonly accompanied by undesired side effects. The use of NSAIDs, such as aspirin, is associated with gastric bleeding, whereas the long-term application of steroid drugs may lead to low resistance to infection, weight gain, and moon face syndrome. Alternatively, alternative anti-inflammatory agents are needed to overcome these problems. The methanolic extract of NAT stem bark was reported to have anti-inflammatory and antianalgesic activities. In the hot-plate, tail-flick, and tail-immersion assays of mice, the methanol extract of NAT stem bark (500 mg/kg) demonstrated analgesic activity. However, the analgesic activity was still lower than that of morphine, a natural opiate with potent analgesic activity (Kakoti *et al*., 2013).

The ethyl acetate extract of NAT leaves contains betulinic acid. The betulinic acid inhibited proinflammatory enzymes (COX-1, COX-2, and 5-*lipoxygenase)* with IC_{50} values of 10.34, 12.92, and 15.53 µg/ml, respectively. The extract was also reported to contain β-sitosterol with anti-inflammatory and analgesic activities. The anti-inflammatory activity was tested on colony-bred adult Wistar rats (150–200 g), whereas the analgesic activity was tested in the hot-plate test using acetic acid-induced writhing in mice (given intraperitoneally at 50 mg/kg). β-Sitosterol inhibited the production of proinflammatory mediators (prostaglandins and bradykinin) and exerted *in vivo* antinociceptive activity. In addition, an alkaloid-derived compound, namely 2-(8-hydroxy-7-((4-nitrophenyl)(phenyl amino) methyl)quinoline-3-yl) acetic acid, was identified to have anti-inflammatory activity due to its structural similarity with indomethacin, a clinically proven NSAID targeting COX enzymes (Bhadouria *et al*., 2012; Karan *et al*., 2019; Nirmal *et al*., 2012). From these data, NAT demonstrated anti-inflammatory and analgesic activities in several experimental models.

Anticancer

Cancer is one of the deadliest diseases in human life. Cancer progression can be initiated by angiogenesis (new formation of blood vessels to support cancer cell growth). The ethanol extract of NAT leaves at a dose of 320 µg/ml inhibited capillary formation (98.07%) in a chorioallantoic membrane experimental model. This inhibition was due to the capability of the extract to suppress secondary and tertiary vascular proliferation and inhibit angiogenesis through vascular endothelial growth factor and fibroblast growth factor pathways. These growth factors are involved in the progression of angiogenesis. Another key factor in angiogenesis, MMP (Matrix Metalloproteinase), was also inhibited by lupeol, a compound isolated from NAT leaves. Lupeol also exerted a cytotoxic activity on gliomas (brain tumors) with an IC_{50} of 10.75 µg/m. The mechanism of action of lupeol as an anticancer agent is mediated by its ability to suppress MMPs activity and to inhibit proliferation and angiogenesis induced by VEGF (Vascular Endothelial Growth Factor) and FGF (Fibroblast Growth Factor) (Ashwini and Rekha, 2019). Betulinic acid isolated from NAT leaves was proven effective in anticancer *in vitro* experiments on various types of human cancer cells. It demonstrated IC_{50} values of 6.53, 9.34, 14.92, 16.90, 17.07, 13.27, and 12.55 µM in HepG2, A549, HL-60, MCF-7, HCT-116, PC-3, and HeLa cells, respectively (Karan *et al*., 2019). The mechanism of action of betulinic acid as an anticancer agent is mediated by its activity to induce apoptosis by targeting the cell cycle at the S or G2/M phases in certain myeloma, gastric, and lung cancer cell lines. Interestingly, it was recently reported that betulinic acid also interfered with the NF-κB signaling pathway of cell survival by directly promoting ROS (Reactive Oxygen Species) overproduction leading to cell death (Park *et al*., 2021; Shen *et al*., 2019).

The ethanol extract of NAT flowers demonstrated antiproliferative activities in five types of cancer cell lines (Colo 205, Y79, K562, MCF7, and MDAMB231). Among these cell lines, Colo 205 showed the highest sensitivity against the extract (IC_{50}: 24 ± 6.63 µg/ml), which was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Khanapur *et al*., 2014). The ethyl acetate fraction of the NAT flower inhibited the proliferation of primary peripheral blood mononuclear cells from chronic lymphocytic leukemia (PBMC-CLL) and PMBCs from acute myeloid leukemia (PBMC-AML) with IC_{50} of 60.30 and 2.99 µg/ml, respectively. Compared with normal cells, the ethyl acetate fraction of the NAT flower

had selectivity index values of 2.06 and 102.50 against PBMC-AML and PBMC-CLL, respectively (Heendeniya *et al*., 2020). This finding indicated that the fraction has a higher selectivity against PBMC-CLL in cancer therapy. Further investigation of the bioactive compounds responsible for the anticancer activity of NAT is a promising direction.

Antigenotoxic

Genotoxicity refers to the damage of genetic information in cells caused by chemicals. This condition can lead to cell mutations and subsequent related diseases. The intensive use of NAT in traditional practices led to the need for genotoxic activity evaluation. The genotoxic activity of the ethanolic extract of the NAT flower and its constituent, crocin, was evaluated using the Ames test with the standard *Salmonella* assay. The experiment was carried out with and without S9 activation using *Salmonella typhimurium* strains TA 98, TA 100, and TA 102. The extract and crocin were nongenotoxic at doses of 125–2,000 µg with and without metabolic activation. The lack of genotoxicity data of other NAT extracts and their phytoconstituent encourages further studies to confirm the safety of complementary medicine. In addition, the genotoxicity effect of the direct and indirect interactions between extracts and phytoconstituent must be investigated (Pawar *et al*., 2019).

CONCLUSION

The data presented in this narrative review showed that NAT is a potential plant with a wide range of pharmacological activities. All parts of this plant, except the roots, have been investigated for their pharmacological activity and phytochemical constituents. NAT demonstrates antioxidant, anti-inflammatory, analgesic, antidiabetic, antihyperlipidemic, anticancer, antimicrobial, antigenotoxic, antimalarial, antitussive, hepatoprotective, and anti-*Malassezia* activities. In addition, NAT contains a variety of secondary metabolites, including steroids, terpenes, flavonoids, iridoid glycosides, and alkaloids. Further research focusing on the mechanism of the actions underlying the pharmacological effect of NAT is a promising direction. The lack of study on NAT toxicity is a major challenge for researchers to ensure the safety aspect before additional scientific evidence is provided.

ACKNOWLEDGMENTS

The authors thank the Faculty of Pharmacy, Universitas Gadjah Mada, for supporting this study (Grant No. UGM No.1.19.11/UN1/FFA.2/S1/KU PKKM/2021).

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Dewi NKSM, Fakhrudin N, Wahyuono S. A comprehensive review on the phytoconstituents and biological activities of *Nyctanthes arbor-tristis* L. J Appl Pharm Sci, 2022; 12(08):009–017.