A minireview on phytochemical and medicinal properties of *Clinacanthus nutans*

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**ABSTRACT**
*Clinacanthus nutans* (Burm.f) Lindau has attracted considerable attention in the past two decades, particularly in Southeast Asia, due to its medicinal properties. It has broadly been used as a folk medicine to treat various illnesses ranging from snake bites, viral infection, inflammation, diabetes, and cancers. Natural phytochemical constituents isolated from this plant possess medicinal properties that can help maintain general health and prevent or treat certain diseases. This paper aims to provide an updated overview of its ethnomedicinal uses, phytochemical constituents, pharmacological activities, and toxicity.

**INTRODUCTION**
Despite tremendous advancement in therapeutic intervention, plant-based traditional medicines remain popular worldwide for treating various ailments. Medicinal plants represent a vast reservoir of bioactive molecules that are invaluable for treating a broad spectrum of diseases and promising sources for discovering interesting compounds for therapeutic intervention. Nevertheless, only about 15% of plant species have been thoroughly studied for their potential medicinal values (Süntar, 2020).

*Clinacanthus nutans* (Burm. f) Lindau is a perennial plant belonging to the family of Acanthaceae. The synonyms of *C. nutans* are *Clinacanthus burmanni* Nees, *Justicia nutans* (Burm. f), and *C. nutans* var. robinsonii Benoist (Aslam et al., 2014). It is widely found in Asian nations including Indonesia, Malaysia, Thailand, Vietnam, and China (Arullappan et al., 2014). In Malaysia, it is also recognized as “Belalai Gajah” in Malay, Sabah Snake Grass, and “You Dun Cao” or “E zui hua” in Mandarin. It has various vernacular names such as “Ki Tajam (Sundal),” and “Saled Pangpon Tua Mea” (Saliva of the female mongoose) in Thailand, and “Gendis” and “Dandang Gendis” in Indonesia (Aslam et al., 2014; Zulkipli et al., 2017).

*Clinacanthus nutans* can grow up to 1 m in height with cylindrical, striate, and hairless stems (Fig. 1). The stems are small, soft, thin, and slightly curved, resembling an elephant trunk. The leaves are simple, opposite to its leaf blade narrowly elliptic and oblong or lanceolate shapes. Its leaf surface is pubescent when young and becomes glabrescent at maturity. The flowers are yellowish and are formed in clusters at the top branches and branchlets (Shim et al., 2013; Yahaya et al., 2015).

**Ethnobotanical uses**
*Clinacanthus nutans* is a well-known folk medicine, especially in Thailand and Malaysia. It has reportedly been used for various ailments such as snake bites, viral infection [herpes simplex viruses (HSV-1 and HSV-2), varicella-zoster virus (VZV), and human papillomavirus (HPV)], and cancer (Kamarudin et al., 2017; Kongkaew and Chaiyakunapruk, 2011; Kunsorn et al., 2013). Apart from that, *C. nutans* extracts are also useful in treating inflammation disorders such as swelling, bruises, strains, sprains of injuries, and rheumatism. It has also been used for gastrointestinal diseases, dysuria, diabetes, fever, and regulating the menstrual cycle (Shim et al., 2013).
Its fresh leaves are commonly served alone or together with herbs as tea because of their nutritional and antioxidant properties. Besides that, it is also used as a home remedy for cancer patients. In Thailand, the alcoholic extract was applied externally for skin irritations, snake/insect bites, and lesions caused by HSV (Sakdarat et al., 2009). The leaves could be consumed alone or mixed with other juices (Aslam et al., 2014) for general well-being. The combination of leaves and stem was also used to make an infusion or decoction to treat hepatitis infection (Teshima et al., 1998). The lotions and creams made solely from the plant had been reported by Sakdarat et al. (2006) to treat minor skin inflammation and insect bites.

In Malaysia, cancer patients have claimed their recovery from cancer illnesses after drinking a decoction made from C. nutans leaves over a certain period. Currently, various testimonies asserted the effectiveness of this herb for different cancer treatments and prevention. Due to the lack of scientific study and evidence, tremendous efforts have been invested in understanding and deciphering the molecular interactions of the phytochemical compounds and their molecular targets in the last few decades.

### Chemical constituents

Extraction, isolation, and characterization of phytochemicals from various parts of C. nutans revealed a wide range of bioactive compounds. It consists of lupeol, stigmasterol, β-sitosterol, betulin, and myricyl alcohol (Arullappan et al., 2014; Charuwichitratana et al., 1996; Chavalittumrong et al., 2013; Dampawan et al., 1977; Teoh et al., 2017). Six C-glycosyl flavones such as orientin, isoorientin, isomollupentin 7-O-b-gluco-pyranoside, schaftoside, isovitexin, and vitexin had been isolated from the butanol and water extracts of this plant. The butanol fraction was found to have five sulfur-containing glucosides: clinacoside A, clinacoside B, clinacoside C, and cyclocclinacoside A1 (Teshima et al., 1998). Moreover, its hexane and chloroform leaf extracts possessed chemical constituents such as 13-hydroxy-(13-S)-phaeophytin b, papurin-18-phytly ester, and pheophorbide (Ayudhya et al., 2001). A few phaeophytins, chlorophyll derivatives, namely, 132-hydroxy-(132-R)-phaeophytin b, 132-hydroxy-(132-S)-phaeophytin a, and 132-hydroxy-(132-R)-phaeophytin a, were also found in the chloroform extracts (Sakdarat et al., 2009). These compounds have shown potent antiviral activities, particularly against HSV by affecting viral entry into host cells.

Satakhum et al. (2001) had reported two glycosyl cerolipid compounds (1-O-palmitoyl-2-O-linolenoyl-3-O-[α-D-galactopyranosyl-(1″6′)-O-β-D-galactopyranosyl]-glycerol and 1,2-O-dilinolenoyl-3-O-β-D-galactopyranosyl-glycerol) from the leaves. Nine cerebrosides and a monoacylmonogalactosylglycerol were isolated in the leaf ethyl acetate fraction (Tuntiwachwuttikul et al., 2004). An antimicrobial compound, known as 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, was isolated from the chloroform leaf extract (Yong et al., 2013). Besides having phenolic compounds such as gallic and caffeic acids, the methanol leaf extracts also have flavonoids constituents like catechin and kaempferol (Ghasemzadeh et al., 2014).

Chemical constituents with the established biological functions or potential therapeutic benefits are summarized in Table 1. Phytochemicals such as catechin, betulin, lupeol, kaempferol, squalene, and vitamin E are known to possess antioxidant, anticancer, and anti-inflammatory activities. Molecular docking analysis has predicted several bioactive compounds that could potentially be used in preventing disorders linked to hyperuricemia, wound healing, and hyperlipidemia. Among them were clinacoside A-C, cyclocclinacoside A1, shahtoside, vitexin, orientin, isovitexin, and isoorientin (Narayanaswamy et al., 2016).
Pharmacological activities

Traditionally, C. nutans is a folk medicine for alleviating various health complications and ailments. In Thailand, numerous studies have shown that C. nutans leaf extracts exhibited excellent biological activities against viruses, inflammation, and cancer. In clinical trials, creams blended with the plant extract showing positive effects in alleviating patients with HSV and VZV infections (Charuwichtratanata et al., 1996). Other studies also discovered that the medicinal products from this plant had replaced acetylovit topically to treat herpes simplex and herpes zoster in hospitals (Kongkaew and Chaiyakunapruk, 2011; Vachirayonstien et al., 2010).

Sriwanthana et al. (1996) stated that C. nutans was traditionally used in cancer patients as primary complementary or alternative herbal remedies. In Thailand, the aerial parts of the plant prepared in decoction form showed an anticancer effect on KB cells (human nasopharyngeal carcinoma cell line) and mammary cancer-bearing rats by promoting the activity of natural killer cells (Na-Bangchang et al., 2012). People in Malaysia also have the same practice where they mixed C. nutans leaf in various formulations such as herbal or fruit juices. Many of them claimed to have recovered after using it for a certain period (P’ng et al., 2013). Thus, attempts to understand its modes of action are carried out mainly using C. nutans leaf extracts.

Despite controversial findings, some research findings revealed the chemoprevention and chemotherapy potential of C. nutans. For instance, the aqueous extract was found to possess significant antiproliferative activity against cervical cancer (HeLa) and erythroleukemia (K-562), while the chloroform extract showed profound inhibitory effects against K-562 and Raji cells (Yong et al., 2013). However, only Teoh et al. (2017) demonstrated its root extracts to exhibit antiproliferative and proapoptotic activities on breast cancer cell line, MCF-7.

Apart from that, its ethanol leaf extracts were also shown to have protective effects of DNA integrity by retaining the supercoiled structure of plasmid DNA under riboflavin photoreaction treatment (Yuann et al., 2012). Besides that, the leaf extract also elicited antioxidant activity and prevented the rupture of red blood cells induced by free radicals (Pannangpetch et al., 2007).

Male mice repeatedly administrated with the leaf methanol extracts at different concentrations of 250, 500, and 1,000 mg/kg for 14 days continuously showed a significant increase of acetylcholinesterase (AChE) activity in several organs (e.g., heart, liver, and kidney) but not in the brain. This indicated that the extract did not cause neurological dysfunctions in the treated mice (Lau et al., 2014). However, the bioactive compounds that contribute to the activation of AChE remain unknown.

The only polysaccharide–peptide complex reported so far was isolated from the leaves. It was made of 87.25% carbohydrate and 9.37% protein. This complex inhibited the growth of gastric cancer cells (SGC-7901) in a concentration-dependent manner. It also increased the nitric oxide production in RAW264.7 cells, suggesting an immunomodulating effect (Huang et al., 2016).

The metabolomic findings on C. nutans aqueous extracts at the concentrations of 500 and 1,000 mg/kg ameliorated the physiological sickness behavior in lipopolysaccharide-induced rats after 2 weeks. This improvement was correlated with anti-neuroinflammatory metabolite biomarkers’ expression from nuclear magnetic resonance spectra compared to the sera obtained from the control group (Azam et al., 2019). Additional and updated studies supporting the pharmacological actions of C. nutans are deliberated and depicted in Table 2.

Toxicity

Several toxicological assessments of C. nutans extracts have been carried out on animals, but no lethal toxicity was reported so far. P’ng et al. (2013) observed no lethality and abnormal behavior in mice administrated with the methanolic leaf extracts (300, 600 and 900 mg/kg) for up to 14 days. Both treated and control groups showed no significant differences in terms of serum biochemical tests. In another study, the subchronic toxicity of the ethanol leaf extract was assessed in male Sprague Dawley rats for 90 days (Asyura et al., 2016). The results demonstrated hepatotoxicity and renal toxicity with no mortality in rats treated with 125–250 mg/kg of plant extracts. Conversely, Roslan et al. (2017) documented subacute toxicities in the female BALB/c

### Table 1. Phytochemical compounds found in C. nutans with known biological functions.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Chemical constituent</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem and root</td>
<td>Lupeol</td>
<td>Dampawan et al. (1977), Teoh et al. (2017)</td>
</tr>
<tr>
<td>Leaf and root</td>
<td>Stigmasterol</td>
<td>Charuwichiratanata et al. (1996), Teoh et al. (2017); Haron et al. (2019)</td>
</tr>
<tr>
<td>Leaf, stem and root</td>
<td>β-sitosterol</td>
<td>Aruallappan et al. (2014); Teoh et al. (2017)</td>
</tr>
<tr>
<td>Root</td>
<td>Betulin, squalene</td>
<td>Teoh et al. (2017)</td>
</tr>
<tr>
<td>Leaf and stem</td>
<td>Orientin, isoorientin, isomollupentin 7-O-b-glucopyranoside, schaftoside, isovitexin, vitexin, clinacoside A, clinacoside B, clinacoside C, cycloclinacoside A1</td>
<td>Teshima et al. (1998)</td>
</tr>
<tr>
<td>Leaf</td>
<td>Myricyl alcohol</td>
<td>Chavalittumrong et al. (2013)</td>
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<tr>
<td></td>
<td>1,2-O-dilinolenoyl-3-O-β-D-galactopyranosyl-glycerol</td>
<td>Satakun et al. (2001)</td>
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<tr>
<td></td>
<td>1,2-benzemedicarboxylic acid, mono(2-ethylhexyl) ester</td>
<td>Yong et al. (2013)</td>
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<tr>
<td></td>
<td>Gallic acid, caffeic acid</td>
<td>Ghasemzadeh et al. (2014), Rahim et al. (2016)</td>
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<tr>
<td></td>
<td>Catechin, kaempferol</td>
<td>Ghasemzadeh et al. (2014)</td>
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<tr>
<td></td>
<td>Protocatechuic acid, cinnamic acid, chlorogenic acid, ferulic acid</td>
<td>Sarega et al. (2016a)</td>
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<tr>
<td></td>
<td>Vitamin E, phytol</td>
<td>Haron et al. (2019)</td>
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<tr>
<td></td>
<td>Myricetin, orientin, isoorientin, vitexin, isovitexin, isookanin, apigenin, ferulic acid</td>
<td>Aliyu et al. (2020)</td>
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</tbody>
</table>
The pharmacological activities of *C. nutans*.

<table>
<thead>
<tr>
<th>Plant parts and extracts</th>
<th>Pharmacological activities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plant (methanol extract)</td>
<td>Suppressed ethyl phenylpropionate-induced ear edema and carrageenan-induced paw edema in dose-dependent manner.</td>
<td>Wanikiat et al. (2008)</td>
</tr>
<tr>
<td>Leaf (ethanol)</td>
<td>Exhibited antioxidant activity by protecting the plasmid DNA from riboflavin photoreaction.</td>
<td>Yuann et al. (2012)</td>
</tr>
<tr>
<td>Leaf (hexane, dichloromethane and methanol extracts)</td>
<td>Possessed antiviral activities against HSV-1 and HSV-2.</td>
<td>Kansorn et al. (2013)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Showed antibacterial activity against <em>Staphylococcus aureus</em> and <em>Escherichia coli</em>.</td>
<td>Ho et al. (2013)</td>
</tr>
<tr>
<td>Leaf (chloroform extract)</td>
<td>Exhibited antiproliferative effects on various human cancer cells such as erythroleukemia (K-562), liver hepatocellular carcinoma (HepG2), lung (NCI-H22), gastric (SNU-1), colon adenocarcinoma (LS-174T), cervical (HeLa) and Burkitt’s lymphoma (Raji); but no effect on neuroblastoma (IMR-32) and normal human umbilical vein endothelial cells (HUVECs).</td>
<td>Yong et al. (2013)</td>
</tr>
<tr>
<td>Leaf (ethanol extract)</td>
<td>In <em>vitro</em> studies showed that keratinocyte cells (HaCat) were protected from IFN-γ/TNF-α induced apoptosis.</td>
<td>Thongrakard and Tencomnann (2013)</td>
</tr>
<tr>
<td>Aerial part (ethanol extract)</td>
<td>Four new compounds (clianamides A-C and 2-cis-entadamide A) were isolated from ethanol extract but none of them showed anti-inflammatory, immunomodulating and anti-dengue virus activities.</td>
<td>Tu et al. (2014)</td>
</tr>
<tr>
<td>Leaf and stem (petroleum ether and ethyl acetate)</td>
<td>Both petroleum ether leaf and stem extracts and ethyl acetate stem extract inhibited the growth of HeLa cells.</td>
<td>Arullappan et al. (2014)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Increased AChE activity in liver, kidney and heart but not in the brain of male mice. No neurological disorder was observed.</td>
<td>Lai et al. (2014)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Inhibited the growth of triple negative breast cancer cell line, MDA-MB-231.</td>
<td>Nasir and Bohari (2015)</td>
</tr>
<tr>
<td>Leaf (ethanol and ethyl acetate extracts)</td>
<td>Both extracts showed growth inhibitory effects on breast cancer cell line, MCF-7.</td>
<td>Salihamin et al. (2015)</td>
</tr>
<tr>
<td>Aerial part (ethanol extract)</td>
<td>Inhibited hepatoma xenograft in mice by increasing the expression of proapoptotic protein while downregulating proliferating cell nuclear antigen (PCNA) and phosphoprotein kinase B (p-AKT). Immunomodulating activity was evidenced through the increase of IFN-γ and IL-2 in serum.</td>
<td>Huang et al. (2015)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Induced apoptosis in human melanoma cells (D24).</td>
<td>Fong et al. (2016)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Exerted central and peripheral antinociceptive activity through the activation of opioid receptor and nitric oxide mediated pathway. However, it is cyclic guanosine monophosphate (cGMP)-independent.</td>
<td>Rahim et al. (2016)</td>
</tr>
<tr>
<td>Leaf (ethanol extract)</td>
<td>Exhibited neuroprotective effects on astrocytes and endothelial cells under hypoxic condition by downregulating histone deacetylases (HDAC1/6).</td>
<td>Tsai et al. (2016)</td>
</tr>
<tr>
<td>Leaf (water and methanol extracts)</td>
<td>Showed termination of hyperlipidemia-associated oxidative stress in rats by increasing the antioxidant enzymes activity and expression of antioxidant genes in serum and liver. Prevented insulin resistance by upregulating of insulin receptor, phosphotidyl inositol-3-phosphate, adiponectin receptor and leptin receptor genes in rats fed with high fat and high cholesterol diet.</td>
<td>Sarega et al. (2016a)Sarega et al. (2016b)</td>
</tr>
<tr>
<td>Leaf and stem (polar extract)</td>
<td>Showed anti-inflammatory effect by inactivating the cytokine production and Toll-Like receptor-4 in lipopolysaccharides-induced RAW264.7 cells.</td>
<td>Mai et al. (2016)</td>
</tr>
<tr>
<td>Leaf (aqueous extract)</td>
<td>Exhibited anti-cancer effects on human cervical cancer cells (HeLa) while normal kidney cell line (Vero) was not inhibited.</td>
<td>Zakaria et al. (2017)</td>
</tr>
<tr>
<td>Root (methanol and ethyl acetate extracts)</td>
<td>Promoted apoptosis by suppressing BCL-2 in MCF-7 cells via mitochondria-dependent or independent pathway.</td>
<td>Teoh et al. (2017)</td>
</tr>
<tr>
<td>Leaf and stem (chloroform and hexane extracts)</td>
<td>Exhibited antiproliferative effects on non-small cell lung cancer (A549), nasopharyngeal cancer (CNE1) and liver cancer (HepG2) cell lines. Hexane extract induced sub-G1 phase arrest and apoptosis via oxidative stress. At high concentrations (&gt;100 µg/ml), the upregulation of caspases 8, 9 and 3/7 was observed.</td>
<td>Ng et al. (2017)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Cytotoxic effects were found in various cancer cell lines such as HepG2, A549, HT-29, MDA-MB-231, MCF-7, and CRL 1739. Cytochrome P450 inhibitory (CYP3A4 &amp; CYP2E1) activities were also reported in human liver microsomes.</td>
<td>Quah et al. (2017)</td>
</tr>
<tr>
<td>Leaf (water extract)</td>
<td>Exhibited anti-angiogenic activities in endothelial cells.</td>
<td>Ng et al. (2018)</td>
</tr>
<tr>
<td>Leaf and stem (acetone extracts)</td>
<td>Showed antimicrobial properties against pathogenic microorganisms.</td>
<td>Kong and Abdullah Sani (2018)</td>
</tr>
<tr>
<td>Leaf (acetone extracts)</td>
<td>Inhibited lymphoma SUP-T1 cells by inducing apoptosis through the loss of mitochondrial membrane potential. Besides increases of reactive oxygen species (ROS) and calcium ion, the upregulation of CHOP and IRE-1α proteins (ER stress) was also observed.</td>
<td>Lu et al. (2018)</td>
</tr>
<tr>
<td>Leaf (water extract)</td>
<td>Sonicated water extract had the highest nitric oxide inhibitory effect in lipopolysaccharide-interferon-gamma-induced RAW264.7 macrophages. <em>H-NMR</em> metabolomic analysis suggested C-glycosyl flavones abundantly found in this extract plays an important role in anti-inflammatory effect.</td>
<td>Khoo et al. (2019)</td>
</tr>
<tr>
<td>Leaf (methanol extract)</td>
<td>Decreased nitric oxide and malondialdehyde levels in blood in mice treated with 200 mg/kg and 1,000 mg/kg of leaf extract. Mitotic cells, tumor weight, and tumor volume were reduced. No inflammatory and adverse reactions related to splenocytes activities were found in all treated mice.</td>
<td>Haron et al. (2019)</td>
</tr>
<tr>
<td>Leaf extracts (after liquid portioning of crude methanol extract)</td>
<td>Dichloromethane extract inhibited the proliferation of cervical cancer by inducing apoptosis and cell cycle arrest. Hexane and dichloromethane extracts showed better cytotoxicity effects on MCF-7 but the dichloromethane extract exhibited less inhibitory effect on normal breast cells. Through molecular docking, palmitic acid and linolenyl alcohol in dichloromethane extract were found to have the highest binding affinity with p53-binding protein Mdm-2.</td>
<td>Nik Abd Rahman et al. (2019)Ismail et al. (2020)</td>
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mice organs after administering 300, 2,000 and 5,000 mg/kg of the ethanol leaf extract for 28 days.

The C. nutans methanolic leave extract showed adverse effects on the CYP3A4 enzyme activity of human liver microsomes. If the plant extract is consumed together with a drug metabolized by CYP3A4, it can slow down xenobiotics’ metabolism in the body. The xenobiotic accumulation for extended periods may become potentially toxic in the body system (Quah et al., 2017). It was also found that the male and female mice showed no alteration of their body weight after administrated with the methanolic leaf extracts at 1,000 and 2,000 mg/kg. Besides, there were no hematological, biochemical, and histology signs of toxicity after 28 days (Abdulwahid et al., 2018).

The metabolomic analysis carried out using high-resolution proton nuclear magnetic resonance (1H-NMR) on the acute toxicity of rats administrated with a single dose of leaf water extract at 5,000 mg/kg for 15 days did not show significant metabolic abnormalities. These findings were in accordance with the results derived from physical, hematological, biochemical, and histopathological observation (Khoong et al., 2018).

On the contrary, Aliyu et al. (2020) suggested that precautions should be taken if they intend to use the plant extract as a health supplement after observing hepatic and kidney toxicities in mice continuously consumed with C. nutans ethanolic leave extract (1,000 mg/kg) for up to 28 days. Besides, a single dose at 2,000 mg/kg could also cause mild histological changes in the liver and renal with no hematological toxicity.

CONCLUSION

Even though extensive research has managed to shed light on the potential therapeutic values of C. nutans, scientific evidence is still scarce. For instance, many studies, including clinical trials, have shown a promising effect on antiviral activities, but its underlying molecular mechanisms are still unclear. More research focusing on multiple signaling pathways instead of targeting specific biological action will provide a better understanding of how these bioactive compounds exert their biological functions. Further investigations on the chronic toxicity effects of C. nutans plant extract also need to be done to ensure its safe consumption. These comprehensive data are essential in understanding of how these bioactive compounds exert their effects of cytotoxicity between extracts of Clinacanthus nutans and the induction of apoptosis by the crude methanol leaf extract in D24 human melanoma cells. BMC Complement Altern Med, 2016; 16:368.


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.

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