Plant Cell Biotechnology and Molecular Biology 22(41&42):184-196; 2021 ISSN: 0972-2025

THE EFFECT OF SOLVENTS AND EXTRACTION METHODS ON PHARMACOLOGICAL ACTIVITIES OF LEAVES OF DANDANG GENDIS *(Clinacanthus nutans):* A REVIEW STUDY

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Article Information

Editor(s):
(1) Dr. Sylwia Okoń, University of Life Sciences, Poland.
Reviewers:
(1) Nehia Neama Hussein, University of Technology, Iraq.
(2) Aman Shah Abdul Majid, Quest International University, Malaysia.

Received: 10 May 2021
Accepted: 16 July 2021
Published: 22 July 2021

Review Article

ABSTRACT

***Clinacanthus nutans* Lindau *(C. nutans)* is one of the most common plants found and primarily used in Southeast Asia. Some studies have proven that the content of *C. nutans* can act as an anti-inflammatory, anti-analgesic, antiviral antibacterial, antivenom, and immune response activity, antioxidant and antidiabetic. Several extraction methods that have been widely done and the results prove the pharmacological effect of *C.nutans* and have widely applied are the extraction of meta methanol; also, the extraction method used for the determination of phenolic flavonoid content and antioxidant activity is by maceration of methanol solvents. This review aims to explain the chemical content, extraction methods used to identify the chemical compounds in *C. nutans* leaves and their relation to pharmacological activity. The information about *C. nutans* plants has collected from several recent research journals that have been accessed from web of science, Scopus, and Web of Mendeley. The methods widely used in identifying were through the isolation and extraction by using methanol, ethanol, and chloroform from various parts of *C. nutans* plants and the *in vitro* and *in vivo* methods used for biological activities test. Some research revealed that C. *nutans* leaves contained chlorophyll derivates. Many flavonoids found in C. *nutans* plants after the thin-layer chromatography (TLC) screening test. The chemical content of *C. nutans* is identified using various extraction methods and it has been proven that the content of *C. nutans* plants has pharmacological effects.**

Keywords: Chemical content; *Clinacanthus nutans*; dandang gendis; extraction method; pharmacological activity.

INTRODUCTION

C. nutans is a plant belongs to the family Acanthaceae. This plant can be found almost all over the country in Asia, such as Malaysia, Thailand, Singapore, Indonesia, also China. The plant is prevalent in Southeast Asia, which is used as a heat-lowering and diuretic drug [1]. The plant is gaining popularity in Malaysia and Singapore at various commercial products such as tea and beverages as anticancer substances [2].

The naming of the plant *C. nutans* is also different in each country in Asia. In Thailand, this plant is referred to as saled pangpon tua mea (female saliva) or phyo yo phaya plongtong [3]. In Malaysia, this plant is called Proboscis elephant or Sabah Snake Grass, a short bush with hairy branches and small oblong. In China, this plant is called a twist of flowers or crocodile flowers. In Singapore, this plant is called snake grass. In Indonesia, especially the Java region, this plant is referred to as dandang gendis.

Herbal products are much cheaper and low side effect than medical treatments [4,5]. About 85% to 90% of the world's population consume medicinal plants [6]. The use of herbs that affluent in the effectiveness of treatment has been beneficial in curing various health problems [7]. The community in the past has known this plant as an alternative treatment such as herpes virus infection treatment, anti-inflammatory, anticancer and antidiabetic. In addition, some scientific approaches to the use of this herbal remedy showed that the side effects are much less and have good effectiveness [8].

Taxonomy of this plant is classified into the kingdom (Plantae), phylum (Magnoliophyta), subclass (Astridae), family (Acantachae), genus (*Clinacantus landau*), species (*Clinacanthus nutans*) [9]. *C. nutans* is an herbal medicine that can grow up to 1 meter high with pubescent branches, cylindrical stems, and striated the leaves are simple, opposite, and narrow (2.5-long 13.0cm x0.5-width 1.5 cm) [9].

A mixture of 17 plants of *C. nutans* in the form of tablets, capsules, or extract patents is used to overcome abnormal menstruation [10]. Another patent in an herbal powder containing *C. nutans* is used to cure oedema after breast cancer surgery [11]. Previous studies has been proven that *C. nutans* has an effectiveness in inhibiting alpha-glucosidase and strong antioxidant activity, thus it can be used as an alternative treatment for diabetes [12].

The *Clinacanthus* plant has traditionally been widely used before. In Malaysia, the use of this plant after diagnosing the disease is the most widely used alternative treatment in Malaysia [13]. In China, this traditional herbs is used to treat a variety of inflammatory conditions such as, rheumatism, high uric acid and gout [14-16]. In Malaysia, fresh leaves are used by boiling it with hot water, and consumed as an alternative medicine for antioxidants and treatment of cancer patients at home. While in Thailand, fresh leaves of *C. nutans* are used as a treatment for skin rashes, snake bites, treatment for wounds on the virus simplex herpes, diabetes mellitus, fever, etc. [17]. In addition, some of its commonly known properties are effective in the treatment for skin rash drugs, recovery of snakebites and insects, against herpes simplex virus, restoring varicella-zoster virus lesions and other viral infections [18, 19]. Furthermore, the survey on the application of ethnobotanical medicinal plants showed that *C. nutans* Previous research has also demonstrated the presence of stigmasterol, ß-Sitosterol, Lupeol, Myricyl alcohol, botulin, sulphur-containing glucosides (Clinacoside A-C cycloaclosida A1 and A2) and glycoglycerol-lipids in *C. nutans* [20]. In addition, six C-Glycolysyl flavones (vitexin, isovitexin, shaftoside, isomollupentin, 7-0-ß-glucoronoside, orientin and isoorientin) have been isolated and characterized from the leaves and stems of *C. nutans*. At the same time, chlorophyll derivates were isolated from the leaves of *C. nutans*. Some studies have also proven that this plant is safe for consumption [21].

Ames trials methods showed no evidence of mutagenic or carcinogen effects after exposure to

liquid extracts of *C. nutans* leaves. No mortality or morbidity was found in animals on repeated administration of *C. nutans* leaf extract [22]. Furthermore, studies on the toxic effects of *C. nutans* on human gingival fibroblasts showed that cells became much better after administering *C. nutans* plant extract [23].

Based on the discovery of Zhang et al., during the early stages of growth, the leaves have a low phenolic and flavonoid content, while the total content of alkaloids gradually increases during the growth and developmental stage [24]. The metabolite contents of plants from the same species is estimated to differ in terms of environmental factors. In some cases, the accumulation of secondary metabolites is influenced by geographical altitude and local ecological conditions such as rainfall, humidity, temperature, water variability, exposure to soil microorganisms and variations in soil pH and nutrition. In addition, environmental factors also interact with plant genetics resulting in variable gene expression that ultimately leads to changes in the concentration of secondary metabolites to affect the quality of medicinal plants [25].

Ethanol and ethyl acetate extract in *C. nutans* are also reported to have a high antioxidant effect and function to defend the body from the damage of free radicals. These results were correlated to the research of Males et al. [26] and Ghasemzadeh et al. [27], which confirmed that the phenolic content of *C. nutans* is very high. The activity of DPPH young leaves and young stems of *C. nutans* was found much higher than in adult leaves and stems [26]. Phenolic is a common compound that contributes to antioxidant properties and pro-oxidant properties that have effectiveness as anticancer substances [28].

Previous research has reported that the anticancer effects of *C. nutans* leaves may differ depending on the extraction method used, and the extracts selection of selected plant parts. Chloroform extract from *C. nutans* leaves has the greatest antiproliferative effect against the erythroleuchemical K-562 cell series (IC_{50} 47.70 µg/mL) and human Raji Burkitt lymphoma line (IC_{50} 47.70 µg/mL), but not against human neuroblastoma IMR-32 cellular channels. In addition, methanol extract showed cytotoxic activity against Hep Cells G2 and MDA-MB-231 (IC_{50} 13.3 and 187 µg/mL respectively) but not againts A549, HT-29, MCF-7 and CRL 1739. Ether petroleum extract from *C. nutans* leaves also has a cytotoxicity effect on human cervical cancer cells (HeLa). Ethyl acetate extract's using *C. nutans* stems showed a cytotoxicity effect on cancer cells, whereas the leaves extract could not resist against the same cancer cells [29].

Phytochemical compounds are an important content in plants, considered to be effective in its utilization as alternative medicine. *C. nutans* stems and leaves are usually used to identify phytochemical contents. Still, the growth stage affects the content of phytochemicals in it, as in the early stages of *C. nutans* has a low content of phenolics and flavonoids ,while the alkaloid content increases gradually. But other factors that determine the phytochemical content in it are the length of storage, light exposure, insect attacks and the length of temperature during storage [30].

This review will discuss *C. nutans* completely, in terms of chemical content, extraction methods and pharmacological activity of *C. nutans*, obtained from a number of recently published research journals, to be a source of research references in the future.

DISCUSSION

Chemical Content of *C. nutans*

Previous studies have shown that *C. nutans* contains stigmasterol, ß-Sitosterol, Lupeol, Myricyl alcohol [31], botulin sulphur-containing glucosides (Clinakosida A-C cycloaclosida A1 and A2) and glycoglycerol-lipids. Six Flavone C-Glycolysyl (vitexin, isovitexin, shaftoside isomollupenti,7-0-ß-glucopyranoside, orientin and isoorientin) as well as chlorophyll derivates that have been isolated and characterized from the leaves and stems of *C. nutans* [21].

Stigmasterol and Beta-sitosterol are two of the phytosterols that often appear in human food. In addition, purpurin-18 phytyl esters, phaeophorbide as well as chlorophyll a and related compounds which have been identified from chloroform

extracts [32]. Han et al. showed that Beta-sitosterol could be used as anti-polypheric substances in human mast cell line 1 cells [33], and 5-sulphur glucosides content isolated from n-BuOH-soluble parts on methanol extract and leaves on plant material. A mixture of cerebrosides and Monoasil-monogalactosyl glycerol have isolated from EtOAc soluble fractions using ethanol extract from fresh leaves of *C. nutans*. C-glycosyl flavones, containing sulphur glycosides, glycoiserolipids, a mixture of nine cerebrosides and amonaacylmonogalatosyl glycerol, a derivative of chlorophyll entadamides [34]. 13-hydroxy-(13-S)-phaeophytin b, purpurin-18-ohytyl ester and phaeophorbide are isolated from n-hexane and chloroform extract from *C. nutans* leaves. Trigalactosyl and digalactosyl diglycerides are isolated from leaves extract and have an antiviral effect on herpes simplex virus.

C. nutans n-hexane and chloroform leaves extract contain 132-hydroxy-(132-S)-chlorophyll-b,132 hydroxi- (132R)- chlorophyll- b,132-hydroxy-(132-s)-phaeophytin-b,132-hydrox (132-R)-Phaeophytin-b,132-hydroxy-(132-S)-phaeophytin-a,132-hydroxy-(132-R)-Phaeophytin a, purpurin-18-phytyl ester and phaeophorbide-a. Three chlorophyll derivates (phaeophytins) isolated in chloroform extract from *C. nutans* leaves.

Those three chlorophyll derivates are known to be composed of 3 structures related to chlorophyll a and chlorophyll b, named 132 hydroxy (132-R)-Phaeophytin-b,132 hydroxy(132-S) Phaeophytin a, and 132-Hydroxy-(132-R)-Phaeophytin [17]. The recent research revealed that different content in *C. nutans* plant samples obtained from different regions. By using thin layer chromatography, two differences were found in green and yellow fluorescence. C-glucosides with apigenin backbone were found in green colour bonds containing shaftosid, isovitexin and vitexin. While in yellow bonds, flavon-c glucosides consisted of luteolin containing isoorientin and orientin were identified. Of all samples tested, regardless of the difference in the sample area, flavonoids that found in almost of all the samples were schaftoside. Nevertheless, there are several factors that cause the content of other flavonoids could not be detected, such as working and technical procedures, temperature conditions and even storage method.

Additionally, this study discovered content of chlorophyll derivates in *C. nutans* leaves extract. The first content was a dark green amorphous, identified as 132-hydroxy-(132-S) phaeophytin-b through protons-1 (1H) and carbon -13 (13C) NMR. The second content appears like green powder that showed interconnected signals to the amine, ester and functional hydroxyl group in the infrared spectrum IR, indicated that it was 132-hydroxy-(132 -S)-Phaeophytin-a. The third content identified as 132-hydroxy-(132 R)-phaeophytin a by IR, 1H NMR and 13C NMR, which was almost the same as the second content. The results of antiviral tests showed that its content inhibits the activity of herpes simplex virus.

The polysaccharides peptide content in *C. nutans* extract has been isolated, and the result of its complex content consists of 87.5% carbohydrates and 9.37 % protein. Another analysis showed that the complex is compose of D-glucoside, L-Arabinosa, D-mannosa, D-galactose and L-Rhamnosa [35]. Phytochemical content in plants changes functions according to the age of plants. The level of the overall number of flavonoids and the overall content of phenol are very high at the age of 6 months growth [27]. Flavonoids isolated in the study consisted of catechin, quercetin, kaempferol, luteolin, phenol acid, caffeic acid and gallic acid [21]. Methanol extract consists of lupeol, botulin, stigmasterol, sitosterol, amirin, vitamin E, and campesterol [36].

The antioxidant and anti-inflammatory tests using polyherbal *C. nutans* and *Elephantos scaber* extraction method, succesfully determined the active compounds through electrospray ionization mass spectrometry (ESI-MS). The active compounds were shown in spectrophotometry detection (LC-MS) provided by fragmentation patterns. Detected content was flavonoids that may be identified in all bioactive fractions (ethyl acetate, n-butanol fraction) of new polyherbal formulations [37].

The study of *C. nutans* content in different parts of the plant, with a comparison of long storage,

found higher content of phenols and flavonoids on the young leaves and stems of young plants *C. nutans*. At the same time, the content of phenolic and flavonoids was relatively low on the part of matured plants leaves and stems, as well as the total phenol content which affected by the storage long duration of *C. nutans* [37]. The potency of high phytochemical content in *C. nutans* makes it an alternative drug that can treat skin rashes, snakebite recovery, fight herpes simplex virus, restore varicella-zoster virus lesions, as well as anti-herpes, anti-hepatitis, anti-inflammatory, and antidiabetic [37,38].

Extraction Method

Pelik lin Tech has investigated the chemical composition and cytotoxic properties of *C. nutans* using root extract. According to their research, the roots became the target of solvent extraction using methanol and ethyl acetate. Antiproliferative effects of root extract tested at concentrations of 10 to 50 µg/mL on MCF-7, and HeLa using a 3-(4,5dimethylazhol-2-yl) 2.5 MTT test, and 72 hours of morphological changes were observed under a light microscope. The effect of pro-apoptosis on root extracts was examined using cytometry analysis of RT-PCR flow, while chemical compound of root extract was detected using GC-MS [36].

The study on antioxidants and wound healing activities of polyherbal fractions of *C. nutans* and *Elephantopus scaber* was conducted through a combination of two leaves of the *C. nutans* plant and *Elephantopus scaber* with a ratio of 1:1, extracted with ethanol for 16 hours, then the crude extract was then glued with rotary evaporator vacuum. Ethanol extract was diffractometer with different solvents n-hexane, chloroform, ethyl acetate, n-butanol and water [17].

Raya et al., conducted research to determine the content of different phytochemicals in the plant part and the length of storage time by selecting samples (*C. nutans*) that had previously been planted for three months. There were two stages of sample treatment, the first stage was the collection of young leaves and stems, and the second stage was careful parting. The extraction method used ethanol 80% and shaken for 2 hours at a temperature of 50°C, then filtered with Whatman paper No. 1, then analysed for its total phenol and flavonoid content [37].

The study of Haron et al*.,* regarding *in vitro* cytotoxic activity in *C. nutans* extract in Hela cells using methanol extract n-hexane, dichloromethane, diffractionated water as extraction media. The study determined the antiproliferative activity of the extract against HeLa cells. Most of the cytotoxic extracts were further analysed with apoptosis and cell cycle tests, and phytochemical constituents were filtered with gas chromatography-mass spectrophotometry (GC-MS) [28]. The extraction method used was the maceration of methanol solvent. This extraction process was repeated three times [39].

Commonly, previous studies used methanol for phytochemical screening of medicinal plants, and is considered as a volatile organic compound suitable for GC-MS analysis. In addition, the use of methanol 80% was reported as an efficient extraction solvent for detecting bio phenol compounds [40]. Diamonds et al., have conducted in vitro antioxidant, cytotoxic and phytochemical tests on *C. nutans* leaf extract. The tests used the dried *C. nutans* leaves which were mashed and soaked for 3 days. Extraction process was carried out using solvents n-hexane, dichloromethane, ethyl acetate and ethanol extracts collected separately and then filtered with Whatman paper No.1, then glued using a rotary evaporator at a temperature of about 40°C, and stored at a temperature of -20°C before further analysis of TPC (Total phenolic content), TTC (Total tannin content), and TFC (Total flavonoid content) [28].

Methanol extract of *C. nutans* leaf contains polyhydroxy compounds and glycerine. While hexane fraction contain fatty acids and terpenoids. According to the journal Antidiabetic, the extracts were reported to have antioxidant activity [41].

The journal comprehensively reviewed and discussed one of the extraction methods in determining the phytochemical content of *C. nutan*s leaves, using microwave-assisted extract and pressure microwave-assisted extract, supercritical carbon and Soxhlet. It concluded that microwave-assisted extract is the best method

because it only uses a short extraction time and posseses a good selectivity level [42].

The study of *in vitro* test was conducted using antiproliferative tests and sulforhodamine B test against HeLa cells to observe the cytotoxic activity of *C. nutans* leaf extract against HeLa cells. In addition, cytotoxicity was determined using the formula described [43].

Pharmacological Activities

C. nutans has many pharmacological effects such as antibacterial, antiviral, antioxidant, antiproliferative, anti-inflammatory and antivirus against varicella-zoster virus and herpes simplex virus 2 [44]. In addition, consuming *C. nutans* was reported to prevent fatty liver when this herb is prepared appropriately [45]. Besides, 18 potent herbs were reported to have such effects in maintaining the body's immune system, destroying kidney stones, improving blood circulation, and treating many diseases [46].

Anticancer activities

The second leading cause of death in the world is cancer, and it is estimated that cancer will account for 9.6 million deaths in 2025 [47]. Cervical cancer is the fourth most common cancer among women worldwide; about 570,000 new cases reported in 2018 represented 6.6% of all cancers in women. Currently, the recommended treatment program for these cancers involves surgery, chemotherapy, radiation or other methods [48]. In the development of cancer, many things or factors behind it, such as genetics, health, nutrition, personal habits and the environment.

C. nutans is a plant belongs to the family Acanthaceae. This plant is one of the natural products that are traditionally potential in treating cancer, including cervical cancer in humans and genital herpes. A cell study found that *C. nutans* methanol extract shows cytotoxic activity against SelG2 and MDA-MB-231 but is not contrary to A549, HT-29, MCF-7 and CRL 1739 Cells**.** Additionally, extracts from different parts of the plant may have different effects on cancer cells [49]. In Malaysia, Singapore, and Thailand, the herbs made from *C. nutans* are traditional herbal remedies used to treat cancer patients. Some patients claimed to have recovered due to a consistent consumption of juice of the *C. nutans* [50]. *C. nutans* has become a potential medicinal plant for treating cancer. By using GC-MS analysis on *C. nutans*, it leads to bioactive compounds that can treat cancers such as 2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one, phytol, phenol 2,4 bis (1,1 dimethyl ethyl), 10, 13-octadecadienoic acid, methyl ester, oleic acid, octadekaanoic acid and vitamin E [51].

A number of studies have assessed the anticancer activity in *C. nutans*. Evaluation of cytotoxicity against different cancer cells has become a common approach. A study of antineoplastic activity of *C. nutans* has been investigated on cancer cells Hep G2, IMR-32, NCL-H23, SNU-1, HeLa, LS-174T, K-562, and IMR32 using MTT testing. The strongest antiproliferation activity that has been examined using chloroform extract on K-562 (91.28;0.03%) cell lines at 100 µg/mL against extracts of *C. nutans,* showed mild activity on other cell lines and no activity in IMR-32 cells. Evaluation of cytotoxicity effect on diluted extracts of herbal mixtures consisting of *C. nutans*, showed inhibition to kb cell growth, with IC_{50} values greater than 100 µg/mL [21]. One of the cytotoxic properties test results showed that the proliferation of MCF-7 cells was inhibited by methanol and ethyl acetate root extract of *C. nutans* leaves, with IC_{50} values of 35 and 30 µg/mL, respectively. GC-Ms analysis revealed that root extracts are rich in terpenoids and phytosterol [36].

In addition, ethanol extract of *C. nutans* leaves showed tumor effect on experiments in mice, rat ICR was circumscribed with hepatocarcinoma hepA tumor cells and administered *C. nutans* 3 and 10 mg/kg for ten days, resulting in a decrease in tumor cell size compared to the group without certain treatment [52].

Anti-inflammatory activities

C. nutans plant contains chemical compounds that can act as anti-inflammatory. Physicians agree that the type of *C. nutans* application may reduce inflammation. The pharmacological traceability results in *in vitro* and *in vivo* experiments support

this assumption. The report provided the number of doses that must be considered in reducing Ethylphenylpropiolate (EPP) and carrageenan that cause the inflammation.

Isovitexin and isoorientin isolated from *C. nutans* showed potentials in the docking and binding with all of the six targeted enzymes (xanthine oxidase [XO], nitric oxide synthase [NOS], human neutrophil elastase [HNE], matrix metalloproteinase [MMP 2 and 9], and squalene synthase [SQS]). Moreover, clinacoside B (*C. nutans* constituent) exhibited the least binding energy for the target enzymes, except for XO and SQS. Interestingly, all of the selected ligands from *C. nutans* showed the potential to dock and bind with HNE [53].

Researchers also attributed the effect on the function and migration of neutrophils, which has been confirmed through a reduction of myeloperoxidase activity in EPP, causing a form of rat ear edema (rat). *C. nutans* expresses anti-inflammatory effects by inhibiting toll-like receptor 4 (TLR-4) [54]. On the other hand, 95% of ethanol processing on *C. nutans* leaves that have been used in carrageenan (1%), causes an inflammatory model. The result was less than 35% of anti-inflammatory and low analgesic activity. An ethanol extract comes from a traditional Thai herbal blend containing *C. nutans*, which is very mild but could act as a significant inhibitor against IFN-gamma/TNF-alpha-reduction of apoptosis in HaCaT keratinocytes cells [21]. Other research revealed that optimum cytotoxic activity in petroleum extracts was found in Hela cells which incubated for 72 hours. Inhibition of proliferation cells K-562 has been found at a concentration of 20.0 µg/mLwith IC_{50} at 20.0 µg/mL after incubation for 72 hours [55].

Antioxidant activity

Radiotherapy and chemotherapy drugs can induce oxidative stress and produce cell damage. This is an undesirable side effect in cancer therapy that can reduce antioxidant compounds. *C. nutans* extract has the potential to be an antioxidant agent for cell protection. Research comparing an antioxidant against various solvent extracts in *C. nutans*, discovered that DPPH and galvinoxyl radical arrests were indicated higher by chloroform compared to methanol and water extracts.

Antioxidant activity in *C. nutans* has been evaluated in a number of studies using in vitro, ex vivo and in vivo experiments. Alcoholic hydro preparations on *C. nutans* leaves have been targeted for antioxidant detection, such as DPPH radical catcher measurement, antioxidant power reduction of phorbol myristate acetate (PMA)-induced oxygen, production reaction species in rat macrophages. Samples tested described the antioxidant activity and protective effects on free radicals. Other studies explained that plant age could be a factor in bioactivity. The study revealed that radical capture activity was significantly higher in plants and leaves at 12 months old, with 50% inhibiting radical DPPH at 64.6% extract activity compared to a 100 µg /ml concentration of hydroxytoluene (68.0%), caffeine acid (70.4%) and alpha-tocopherol (71.2%).

Extracts from *C. nutans* are shown to have a high free radical and non-free radical cleansing activity. Such scavenging activity may be caused by the presence of alkaloids, flavonoids and factory flavones. The significance of reduction cannot be in the form of superoxide anions and inhibition of elastase, released in activated neutrophils [55]. However, younger plants had activity on higher FRAP measurements. Compared to other in vitro studies, ethyl acetate and ethanol extracts in *C. nutans* showed higher DPPH radical capture, as well as radical oxygen absorption and beta carotene purification potential than extracts from dichloromethane and hexane [56].

Other studies assessed antioxidant activity with chloroform, methanol and dilute extracts on *C. nutans* using DPPH, Galvinoxyl, and hydrogen peroxide nitric oxide. Based on the radical capture model, chloroform extract has the strongest antioxidant activity against DPPH free radicals and galvinoxyl radicals, but less effective in nitrogen monoxide and radical hydrogen peroxide [57]. In addition, some discoveries regarding higher antioxidant activity in *C. nutans* are followed by total phenolic content and total flavonoid content. This is because many antioxidant properties such as terpenes, sterols,

and saponins have contributed to this activity, not only phenolics [58,59].

Immune response activity

C. nutans extract was also tested for its effect on the immune system using proliferation lymphocyte response, and measurement of natural killer (NK) cell activity. The report explained that *C. nutans* can strengthen the immune system by increasing lymphocyte proliferation, and reducing the activity of natural killer cells [60]. *C. nutans* extract also suppresses the release of IL-2 and increases of IL-4. These results partly explain the mechanism in cells as an intermediary of immune response activity to *C. nutans* extracts [57].

Antivenom activity

Examining the Thailand society's assumption that *C. nutans* could heal snakebites and scorpions, the water extract on *C. nutans* leaves squeezed up to 46.51%, could cause cell lysis in scorpion *Heterometrus laoticus* venom. Meanwhile, there was no protective effect against lethal doses on the venom of cobra *Naja naja siamensis*, when *C. nutans* extract has been administered orally. The author concluded that the extract could not cope with cobra's venom [57].

Antioxidant and antidiabetic activity

The burden of type II diabetes mellitus increases more in developing and developed countries [61]. Leaf extract using *in vitro* bioassay Crude extract methanol 80% of this plant was further partitioned using different polarity solvents namely hexane, ethyl acetate-hexane (1:1,v/v), ethyl acetate, ethyl acetate, methanol (1:1,v/v) and methanol. All subfractions were analysed for antioxidants effects through 2,2-diphenyl-2 picrihidrazyl (DPPH) scavenging activity, iron-reducing power (FRAP) and xanthine oxidase test, followed by evaluation of antidiabetic through alpha glucose oxidase inhibitory test, peptidase–IV (DPP-IV) and glucose retrieval trials. Ethyl acetate fraction showed good antioxidant potential, while n-hexane fraction showed inhibition of alpha glucose oxidase enzyme and high DPP-IV. n-Hexane fraction also increased glucose uptake by relying on dosage. This data was an information on the use of *C. nutans* in preventing diabetes [62].

Type II diabetes currently reaches pandemic proportions. Although there have been advances in the treatment of the disease, its incidence and prevalence continue to increase. The effectiveness of type II diabetes management relies heavily on the ability of therapeutic agents to control complications, due to chronic hyperglycemia associated with long-term damage and failure of various organs such as eyes, nerves, kidneys and heart [63]. Antioxidant-rich supplementation in T2D patients can help in weakening diabetes complications by increasing total antioxidant levels and reducing oxidative stress [64].

Imam et al., demonstrated that administering *C. nutans* and quercetin leaf extract orally to diabetic mice could lower the post-intervention blood glucose levels compared to untreated mice. Total cholesterol ($p<0.05$) was significantly lower in *C. nutans* compared to the diabetes control group. Levels of F2-isoprostane, an oxidative stress marker, are weakened in the presence of extracts. The activity of the enzyme aldose reductase increased by 64.99 and 0 % and total antioxidant activity by 22.29 and 126%, respectively. Sorbitol levels in kidneys, lenses, and nerves decreased in diabetic mice given *C. nutans* [65]**.** In addition, Musthapa Umar's research revealed that all chemicals, including quercetin, and flavonoid are well known to protect mice from oxidative stress [66].

Wound healing agent

Gene and skin therapy are common steps in wound healing. Wound care which is use non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, and topical corticosteroids has an effect on healing [67].

Muhammad Sahzad Aslam in his research on antioxidants and wound healing activities of the polymerbal fractions *C. nutans* and *Elephantopus scaber,* found the presence of several flavonoid-based compounds that work synergistically with lactone sesquiterpenes and other bioactive compounds. It concluded that flavonoids increase antioxidant activity that increases the rate of

wound contraction, and works synergistically with other bioactive compounds.

Antivirus activity

Herpes simplex virus infection is spread almost all over the world with different degrees of severity, which can be life-threatening, especially in patients with immune disorders. HSV-1 is often associated with orofacial mucosal lesions. HSV-2 is more commonly associated with genital herpes. This virus can cause primary and recurrent infections in viral reactivation through stimuli such as sunlight, stress and weakened immunity [68,69].

Currently, the drug choice of prophylaxis and treatment of HSV infection is acyclovir, a nucleoside analog that selectively inhibits replication of HSV with low host cell toxicity [70]. As a result, a new and more effective antiviral agent is needed that can replace or supplement the antiviral drugs used today [71].

Traditional treatments by using medicinal plants could be an alternative treatment due to its abundant content of chemical compounds. Some plants extract or pure compounds showed antiviral capabilities. Sirada Pongmuangmul studied the activity of the anti-herpes simplex virus from Monogalaactosyl diglyceride and digalactosyl diglyceride from the plant *C. nutans* collected and dried leaves, extracted with chloroform using a Soxhlet extraction tool at a temperature of 60^{o}C for 8 hours. Whole extracts were collected and solvents evaporated to dry with rotavapor. Raw extract stored at -20^{o}C prior to use in testing.

In addition, *C. nutans* traditional Thai herbal medicine showed profile as standard MGDG and DGDG using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The result showed that *C. nutans* demonstrated inhibition of 100% replication of HSV-1 in the post-infection stage at noncytotoxic concentrations. In addition, MGDG and DGDG from *C. nutans* were shown to have simplex anti-herpes activity at the same level as standard synthesis compounds. In the investigation of phytochemicals, flavonoids, steroids, triterpenoids, cerebrosides, two glycerolipids, and sulphur-containing glycerides were isolated from this plant *C. nutans* [72].

Modulation of nerve transmission effects

Methanol extract on the leaves of *C. nutans* is administered on mice for 14 days of the activation of acetylcholinesterase (AChE) and modulation of nerve transmission in the kidneys, liver, and liver mice [73].

Neuroprotective effects

A study has shown that *C. nutans* extract selectively inhibits Histone deacetylase (HDAC)-1 and (HDAC)-6 expressed in nerve cells, and protected endothelial and astrocytic cells from hipolic-induced cell death [76].

Anti-hyperlipidemic effect

It has been shown that water extract and methanol on the leaves of *C. nutans* may lower insulin, serum retinol binds to protein-4, and fasting blood glucose in fats and cholesterol is high in rats [74].

CONCLUSION

The chemical content of *C. nutans* can be identified using various extraction methods with different solvents. It has been proven that the content of all parts of the *C. nutans* can overcome various health problems, such as anti-inflammatory, anticancer, antiviral, antioxidant, antidiabetic and reduce the risk of side effects. This review also provides a reference on the specific phytochemical content of *C. nutans* along with the resulting pharmacological effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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