

## A Review of Therapeutic Potentials of *Clinacanthus nutans* as Source for Alternative Medicines (Ulasan Potensi Terapeutik *Clinacanthus nutans* sebagai Punca Perubatan Alternatif)

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### ABSTRACT

*Clinacanthus nutans*, also known as Sabah Snake Grass, is an ethno-medicinal plant species belonging to the family Acanthaceae and has been widely used as a traditional remedy in Malaysia and Thailand to treat various diseases. The species contains massive amount of phytochemical compounds useful to human health. Various studies have reported that among the compounds found in the species are those with anti-venom properties reported to be rarely present in other medicinal plants. A number of pharmacological research studies have also documented that extracts of *C. nutans* contain high antioxidants, as well as compounds with anti-cancer, anti-inflammatory and anti-viral properties. Isolation and identification of these phytochemical compounds have gained global attention due to their well-known beneficial values. The present review article is a compilation of previous pharmacological studies on *C. nutans* with a view of gaining further interest in exploring alternative sources of medicine.

**Keywords:** *Clinacanthus nutans*; medicinal plant; pharmacological constituents

### ABSTRAK

*Clinacanthus nutans* atau dikenali sebagai Belalai gajah, ialah tumbuhan ubatan daripada famili Acanthaceae. *C. nutans* digunakan secara meluas di Malaysia dan Thailand dalam perubatan tradisi untuk merawat pelbagai jenis penyakit. *C. nutans* mempunyai kandungan fitokimia yang tinggi. Justeru, dapat memberi manfaat kepada manusia. Antara semua potensi terapeutik yang terdapat dalam *C. nutans*, anti bisa merupakan kelebihan pokok ini. Anti-bisa merupakan potensi terapeutik yang jarang ada dalam tumbuhan ubatan. Selain itu, berdasarkan kajian farmakologi yang telah dijalankan, ekstrak *C. nutans* juga tinggi antioksidan dan mempunyai anti kanser, anti keradangan dan anti-virus. Pengasingan dan mengenal pasti kandungan fitokimia dalam *C. nutans* telah menjadi perhatian di seluruh dunia kerana kebaikannya kepada kesihatan. Tujuan ulasan kertas ini adalah untuk mengumpulkan kajian farmakologi yang telah dijalankan terhadap pokok *C. nutans*.

**Kata kunci:** *Clinacanthus nutans*; juzuk farmakologi; tumbuhan ubatan

### INTRODUCTION

Utilization of natural products such as medicinal plants as sources of medicines has been practiced through much of human history. Medicinal plants consist of wide range of bioactive compounds that have been used in the maintenance of health including prevention, diagnosis, improvement or treatment of a large number of human physical illnesses. Medicinal plants have been utilized by approximately 70-80% of world population as complementary and alternative medication as their primary medicines (Ekor 2013). In modern medicine manufacturers, 25% of drugs derived from natural products including medicinal plants (Dias et al. 2012). It has been reported that 350000 of higher plant species in nature have been identified and documented and about 80000 species have been discovered to have medicinal properties. However, only 5000 of the plants have been comprehensively studied of their bioactive compounds and their biological activities useful to human health (Dar et al.

2017; Yuan et al. 2016). Due to cheaper price and lower risks of side effects, medicinal plants derived products have been accepted globally (Marušek et al. 2010).

The Acanthaceae family is classified as one of the biggest dicotyledonous flowering plants families in the plant kingdom, comprising of around 346 genera and 4300 species (Khan et al. 2017). The family is distributed mainly in the subtropical regions including Malaysia, Indonesia, Thailand, Central America, Africa and Brazil (Khan et al. 2017).

One of the species belonging to the Acanthaceae family is *Clinacanthus nutans*, is a very popular herb in Thailand and Malaysia. There are several common names of *C. nutans* such as 'Pokok Stawa Ular' or 'Belalai Gajah' among community in Malaysia and Brunei, 'Dandang Gendis' and 'Kijatan' among the Indonesian, 'Phaya Yo' and 'Phaya Plongtong' among the Thais, and 'E Zui Hua' among Chinese community in China (Arullappan et al. 2014; P'ng et al. 2013). The taxonomy classification of *C. nutans* is as follows (Mat Ali et al. 2010):

Domain : Eukaryota; Kingdom : Plantae; Phylum : Tracheophyta; Class : Magnoliopsida; Order : Lamiales; Family : Acanthaceae; Genus : *Clinacanthus*; Species : *nutans*; Botanical name : *Clinacanthus nutans*

The species is an annual shrub that can grow up to 1 - 3 meters tall. It has green cylindrical, smooth and striated stems. The leaves shapes are simple with lanceolate-ovate and the arrangement of leaves are opposite. The leaf margin is sub-entire to sinuate-crenate with an acuminate apex and cuneate to rounded base. The leaf grows approximately 1 - 4 cm in width and 7 - 12 cm in length. The flowers of *C. nutans* are dark red in color with approximately 4 - 6 cm across.

*C. nutans* has long been used as a traditional medicine among local communities in Asian countries such as Malaysia and Thailand. Among the parts of the plant most commonly used are the leaves, taken orally either in dried or fresh form (Shim et al. 2013). Leaf extract is well-known to possess anti-venom properties against snake bites, insect and scorpion stings (Tejasen & Thongthaap 1978; Thongharb & Tejasen 1977). In addition, the extract has been reported to be effective for the treatment of herpes simplex, and varicella zoster viruses and skin rashes (Kongkaew & Chaikyunpruk 2011; Shim et al. 2013; Wirotasangthong & Rattanakiat 2006). Other uses of the extract include to cure fever, diarrhea, diabetes and hepatitis infections (Teshima et al. 1998, 1997; Tiew et al. 2014; Sriwanthana et al. 1996).

#### PHYTOCHEMICAL CONSTITUENTS OF *C. NUTANS*

In the last few decades, studies on phytochemical contents in plants have widely gained attention of numerous researchers. Bioactive compounds isolated from medicinal plants have become the biggest contributors in pharmaceutical and nutraceutical industries in the world (Tiwari et al. 2011). Numerous phytochemical screening tests have been conducted to identify the functional groups present in plants. Phytochemical screenings of *C. nutans* extracts by using different extraction solvents and plant parts have been reported and are shown in Table 1.

However, phytochemical screening tests are limited to functional groups present in plants. In order to determine the specific bioactive compounds, further analyses are required. The more common techniques in isolation and identification of phytochemical compounds in plant extracts include thin layer chromatography (TLC), liquid chromatography mass spectrometry (LCMS), high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GCMS) (Fong et al. 2014). The phytochemical compounds of *C. nutans* that have been isolated and identified are shown in Table 2.

#### THERAPEUTIC ACTIVITY OF *C. nutans*

**Anti venom** Extracts from *C. nutans* have been traditionally used by local people especially in southern Thailand and North-Western Malaysia as remedy for envenomation of

venomous animals such as bees, scorpions and snakes. A number of *in vitro* and *in vivo* studies have been conducted to examine the anti-venom properties of *C. nutans*. Extracts of the plant were prepared by using water, ethanol and aqueous ethanol as extraction solvent. The first attempt was made on *in vivo* tests of *Laticauda colubrine* snake venom extract on mice and Mongrel dogs by Levey (1969). The tests failed to exhibit anti-venom properties after the extracts were administered to the test subjects. Similar results were also obtained by Cherdchu et al. (1977) on their *in vivo* studies of *Naja naja siamensis* snake venom. The test subjects were given *C. nutans* extracts, however, no anti-venom effect was observed. On other tests on the snake venom, *C. nutans* was extracted using water and 95% ethanol. The results showed that water extracts of *C. nutans* were able to reduce mortality of test subjects from 100% to 63%. Meanwhile, ethanolic extracts of *C. nutans* failed to exhibit anti-venom properties (Thongharb & Tejasen 1977). On *in vitro* studies of effectiveness of *C. nutans* extract found that water extract of *C. nutans* exhibited 46.51% efficiency on *Heterometrus laoticus* scorpion venom. Aqueous ethanol extract of *C. nutans* was reported to be ineffective against *Apis mellifera* Linn. bees' venom (Uawonggul et al. 2011, 2006).

**Antioxidants** Antioxidants activities of *C. nutans* have been quantified by using different types of *in vitro* assays. Different types of solvents and extraction methods have been used to determine inhibition potential of the extract. The more commonly used method to determine antioxidant content is 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The highest inhibition was obtained by 4 mg/mL of leaves petroleum ether extract with 82% DPPH inhibition (Arullappan et al. 2014). Comparison between 1, 6 and 12 months old leaves and bud found that methanolic extract of 12 months old buds exhibited the highest DPPH inhibition with the IC<sub>50</sub> of 64.6 µg/mL followed by 12 months old leaves with IC<sub>50</sub> of 112.1 µg/mL (Ghasemzadeh et al. 2014). Other investigation has been carried out by using fermented and unfermented hot water leaves extract of *C. nutans*. The results showed that unfermented *C. nutans* leaves extract possessed higher DPPH inhibition compared with fermented *C. nutans* leaves extract (Barek et al. 2015). According to the experiment conducted by Yong et al. (2013), DPPH inhibition was also affected by extraction solvents used. In their studies, the leaves of *C. nutans* were extracted by using water, methanol and chloroform. Based on the results obtained, the highest DPPH inhibition was recorded from leaves extracted by using chloroform followed by methanol and water extraction procedures (chloroform > methanol > water). Another factor that may affect DPPH inhibition is extract storage duration. According to Raya et al. (2015), DPPH inhibition of aqueous ethanolic extracts of *C. nutans* was drastically reduced by 69.97% from first day to four days of storage duration. Several other studies on DPPH inhibition have also been conducted. Different types of extraction solvents, extraction methods, postharvest methods and plant parts

TABLE 1. Phytochemical screening results of *C. nutans* extract

Functional group	Part of plant	Extraction solvent	Result
Alkaloid	Leaf	Water	Present (Nurulita et al. 2008)
	Leaf	100% methanol	Absent (Rahim et al. 2016)
	Leaf	100% chloroform	Present (Ho et al. 2013)
	Leaf	70% methanol	Absent (Sekar & Rashid 2016)
Carbohydrates	Leaf	70% methanol	Present (Sekar & Rashid 2016)
Diterpenes	Leaf	Water	Present (Ho et al. 2013)
Flavonoids	Leaf	100% methanol	Present (Ho et al. 2013; Rahim et al. 2016)
	Leaf	100% chloroform	Present (Sekar & Rashid 2016)
	Leaf	70% methanol	Present (Nurulita et al. 2008)
Glycosides	Leaf	100% chloroform	Present (Nurulita et al. 2008)
	Leaf	70% methanol	Present (Ho et al. 2013)
Phenolic compound	Leaf	100% methanol	Present (Ho et al. 2013)
Phytosterol	Leaf	100% methanol	Present (Nurulita et al. 2008)
	Leaf	70% methanol	Present (Ho et al. 2013)
	Leaf	Water	Present (Nurulita et al. 2008)
Protein and amino acid	Leaf	70% methanol	Present (Sekar & Rashid 2016)
Quinone	Leaf	Water	Absent (Nurulita et al. 2008)
Saponin	Leaf	100% methanol	Present (Ho et al. 2013; Rahim et al. 2016)
	Leaf	70% methanol	Absent (Sekar & Rashid 2016)
	Leaf	Water	Present (Nurulita et al. 2008)
Steroids	Leaf	70% methanol	Present (Ho et al. 2013)
	Leaf	100% methanol	Present (Ho et al. 2013)
	Stem	Light petroleum	Present (Dampawan et al. 1977)
Tannin	Leaf	100% methanol	Absent (Ho et al. 2013; Rahim et al. 2016)
	Leaf	70% methanol	Present (Sekar & Rashid 2016)
Triterpenoids	Leaf	Water	Present (Nurulita et al. 2008)
	Leaf	100% methanol	Present (Ho et al. 2013)

have been examined (Chomnawang et al. 2007; Hamid et al. 2016; Khoo et al. 2015; Lee et al. 2014; Pannangpetch et al. 2007; Tiew et al. 2014; Wanikiat et al. 2008).

The second most commonly used method in determination of antioxidant is ferric reducing antioxidant power (FRAP) assay. Generally, FRAP assay is conducted to measure the ability of crude extract to reduce ferric iron [Fe (III)] to ferrous ion [Fe (II)]. Based on the results obtained by Berek et al. (2015), unfermented herbal tea derived from leaves of *C. nutans* exhibited higher FRAP inhibition of 438.80 mg/L compared to fermented herbal tea derived from leaves of *C. nutans* with 344.80 mg/L. The study conducted by Ghasemzadeh et al. (2014) showed that six-month old flower buds of *C. nutans* exhibited higher FRAP activity with 488  $\mu$ M of Fe (II)/g compared to one month old leaves which exhibited 209  $\mu$ M of Fe (II)/g of FRAP activity. The comparison of FRAP activity was difficult due to different standard was used such as gallic acid, caffeic acid, vitamin C and ascorbic acid (Hamid et al. 2016; Pannangpetch et al. 2007).

A study conducted by Yong et al. (2013) on hydrogen peroxide scavenging activity of *C. nutans* leaves extract found that methanol, chloroform and water extracts showed low hydrogen peroxide scavenging activity. Among the

solvents used, methanolic extract of *C. nutans* exhibited the highest with 34% of scavenging activity at 100  $\mu$ g/mL. However, in the study on nitric oxide scavenging activity, water extract of *C. nutans* leaves significantly produced the highest nitric oxide scavenging activity with 32.33% at 100  $\mu$ g/mL. In yet another study on nitric oxide scavenging activity, 10 mg/mL aqueous extract of *C. nutans* was able to inhibit 90% of nitric oxide (Wong et al. 2014).

Other studies on antioxidant assay such as galvonoxyl scavenging activity found that chloroform extracts of *C. nutans* possessed highest (12.25 mg trolox equivalent/g extract) followed by methanol and water extracts (Yong et al. 2013). On metal chelating activity, *C. nutans* demonstrated strong chelating activity with 10 mg/mL water extract of *C. nutans* exhibited 90% of metal chelating activity (Wong et al. 2014). A study on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or commonly known as ABTS cation radical scavenging activity found that unfermented extract of *C. nutans* leaves exhibited higher ABTS cation radical scavenging activity compared to fermented extract (Berek et al. 2015). Furthermore, 80% methanol extract of *C. nutans* possessed high scavenging activity with 65 mg trolox equivalents/g extract (Sarega et al. 2016). *C. nutans* extracts were also proved to inhibit

TABLE 2. Phytochemical compounds of *C. nutans*

Phytochemical class	Phytochemical compound	References
Terpens-Tripenoids	Lupeol [1,2]	Dampawan et al. 1977; Le et al. 2017;
Terpenes-Phytosterols	$\beta$ -sitosterol [1] Stigmasterol [5]	Dampawan et al. 1977; Tuntiwachwuttikul et al. 2004
Phenolic compounds	Shaftoside Vitexin Isovitexin Isomollupentin 7-O- $\beta$ -glucopyranoside Orientin Isoorientin Gallic acid Apigenin 6,8-di-C- $\alpha$ -L-arabinopyranoside Quercetin Kaemferol	Dampawan et al. 1977; Huang et al. 2015; Teshima et al. 1998
Sulfur-containing glycosides compounds	Clinacoside A Clinacoside B Clinacoside C Cycloclinacoside A1 Cycloclinacoside A2	Teshima et al. 1998
Sulfur containing compounds	Clinamides A Clinamides B Clinamides C Clinamides D Clinamides E 2-cis-entadamide A Entadamide A Entadamide C	Hamid et al. 2016; Tu et al. 2014

superoxide radical scavenging activity by using methanol and 70% ethanol as extraction solvents (Wanikiat et al. 2008).

The antioxidant capacity of *C. nutans* was analyzed by using phorbol 12-myristate 13-acetate (PMA) induced peroxide production in rat macrophages and protective effect against peroxy radicals initiator (AAPH)-induced oxidative hemolysis. Both of these methods are rarely used in determination of antioxidant capacity of plant. Based on the results obtained, both of these methods were able to protect the cells from oxidative damage caused by free radicals (Pannangpetch et al. 2007).

**Anti-cancer** It has been documented that *C. nutans* was traditionally being used in cancer treatment as it was believed to have phytochemicals that can inhibit cancer cells. However, the efficiency, inhibition percentages and dosages needed have not been well-defined. Hence, *in vitro* and *in vivo* studies have been conducted by using different types of extraction solvents to find the efficiency of the *C. nutans* extracts against different types of cancer cells. The extraction solvents used included water, methanol, ethanol, aqueous ethanol, aqueous methanol, ethyl acetate, dichloromethane, chloroform, hexane, and petroleum ether. Most of the studies used leaf parts. Other than leaf, the stems, roots and buds of the plant have also

been tested. Anti-cancer agents from crude of plant extract were considered as strong when the  $IC_{50}$  exhibited was less than 20  $\mu$ g/mL.

Earlier studies by Liew et al. (2012) found that methanol extracts of leaf of *C. nutans* were ineffective against osteosarcoma cell lines (cultured Saos-2) for bone tumor. A study by Yong et al. (2013) found that chloroform extracts of *C. nutans* leaves were most effective against human erythroleukemia cell lines (K562) for acute myeloid leukemia. In addition, Yong et al. (2013) also reported that *C. nutans* extracts were able to inhibit other cancers cell lines including human liver hepatocellular carcinoma cell line (HepG2) for liver cancer, human neuroblastoma cell line (IMR-32) for nerve tissue cancer, human lung adenocarcinoma (NCI-H23) for lung cancer, human gastric cancer cell line (SNU-1) for gastric cancer, human colon adenocarcinoma (LS-174T) for colon cancer, human cervical cancer cell line (HeLa) for cervical cancer and human Burkitt's lymphoma cell line (Raji) for lymphatic disorder. *C. nutans* extracts were also proven to be effective against skin cancer by inhibiting human melanoma cell lines (D24 and MM418C1) and breast cancer by inhibiting human breast carcinoma (BT474) and human breast adenocarcinoma (MCF7) cancer cell lines (Fong et al. 2014). Several reports have been published to support that *C. nutans* extracts were able to inhibit HeLa, HepG2,

MCF-7 and K-562 cancer cell lines (Arullappan et al. 2014; Ghasemzadeh et al. 2014; Hamid & Yahya 2016; Teoh et al. 2017).

**Anti-inflammatory** Anti-inflammatory inhibition can be determined by carrying out *in vitro* and *in vivo* tests on the targeted cells. One of the well established assays to test anti-inflammatory is lipopolysaccharide (LPS)-induced inflammation macrophage model. Based on the results obtained in several studies, polar extracts (methanol and dichloromethane) of *C. nutans* leaves possessed higher anti-inflammatory activity with  $IC_{50}$  value < 22  $\mu\text{g/mL}$  compared to non-polar extracts (hexane and diethyl ether) in inhibition of LPS- induced TLR-4, LPS- stimulated nitric oxide and LPS- stimulated cytokines production (Mai et al. 2016). A study by Tu et al. (2014) found that 80% methanol of *C. nutans* extracts was able to reduce superoxide anion with an inhibition of 28.52%. By using other types of anti-inflammatory assay which was *N*-formyl-methionyl leucyl-phenylalanine (fMLP), 80% ethanol and methanol extracts were able to inhibit neutrophil responsiveness. The results showed that 30% superoxide formation was inhibited at 10  $\mu\text{g/mL}$  of both extract. Meanwhile, 68.33% elastase release was inhibited at 10  $\mu\text{g/mL}$  of 80% methanol extract (Tu et al. 2014; Wanikiat et al. 2008).

For *in vivo* study, the types of experiment models used to study anti-inflammatory properties of *C. nutans* were ethyl phenylpropionate (EPP) induced rat ear oedema, acetic acid induced vascular permeability, carrageenan induced paw oedema and granuloma pouch model (Satayavivad et al. 1996; Wanikiat et al. 2008). By using experiment model EPP induced rat ear oedema, application of 9 mg EPP/ear of methanol extract on the rat resulted in 44.4% myeloperoxidase (MPO) reduction after 120 min and 79% inhibition of oedema at 15 min (Wanikiat et al. 2008). Meanwhile, acetic acid induced vascular permeability model showed that butanol extract at 540 mg/kg exhibited higher anti-inflammatory properties compared to water, methanol and chloroform extracts. Butanol extract at 270 mg/kg also showed high anti-inflammatory properties in carrageenan induced paw oedema model (Satayavivad et al. 1996).

**Anti-bacterial activity** Extracts of *C. nutans* had also been tested on several gram-positive bacteria to study their antibacterial properties. A total of 16 bacteria strains had been examined including *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Micrococcus luteus*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Streptococcus* sp., *Aeromonas hydrophila*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Salmonella enteric*, *Vibrio harveyi* and *Vibrio parahaemolyticus*. Of the extraction solvents used from the studies, only polar such as water, methanol and ethanol and semi-polar such as chloroform and ethyl acetate exhibited low antibacterial inhibitions (Arullappan et al.

2014; Cheeptham & Towers 2002; Chomnawang et al. 2009, 2005; Direkbusarakom et al. 1998; Ho et al. 2013; Wong et al. 2014). Among all the solvents, ethyl acetate fraction was the most effective with high anti-bacterial properties against *B. cereus* and *E. coli* growth at 1.39 mg/mL (Arullappan et al. 2014).

**Anti-fungal activity** To date, studies on anti-fungal properties of *C. nutans* extract have been very limited. Only two reports are available as references. In the report by Cheeptham and Towers (2002), 5 mg/mL of 95% ethanol leaves extract of *C. nutans* was tested on *Candida albicans* and *Aspergillus fumigatus*. The results showed that, the extracts were ineffective to exhibit fungicidal effect on both species of fungus. In contrast to study by Arullappan et al. (2014), ethyl acetate extract of *C. nutans* was able to exhibit anti-fungal effect on *C. albicans* at minimal concentration of 1.39 mg/mL.

**Anti-viral activity** The anti-viral properties, *C. nutans* extracts have been tested on several types of viruses including varicella zoster virus (VZV), herpes simplex virus (HSV), fish pathogenic virus, crustaceans (shrimp and prawn) infectious virus and mosquito borne virus. The mode of action of anti-viral properties of *C. nutans* was examined through three different stages of treatment which included pre-infection, post-infection and direct inactivation pathways (Thongchai et al. 2008).

The anti-varicella zoster virus (VZV) was evaluated by conducting *in vitro* study and clinical trials. Based on the *in vitro* study, organic extract of *C. nutans* showed a potential to inhibit VZV Kawaguchi strain by direct inactivation stage (Thawaranantha et al. 2017). Meanwhile, in clinical trials, *C. nutans* cream formulated with 5% *C. nutans* extracts were tested for their ability to encounter VZV infections. The results obtained showed that the application of *C. nutans* cream by patients experienced lesion healing within seven days and produced higher percentage of curing with lower side-effects and pain scores (Charuwichitratana et al. 1996; Sangkitporn et al. 1995).

For herpes simplex virus (HSV), the potential of *C. nutans* extracts to act as anti-HSV were tested using various extraction solvents including ethyl acetate, chloroform, methanol, dichloromethane and hexane (Kunsorn et al. 2013; Thongchai et al. 2008; Yoosook et al. 1999). Kunsorn et al. (2013) reported that *C. nutans*, extracted with hexane, exhibited higher inhibitory effect ( $IC_{50}$ : 32.05  $\mu\text{g/mL}$ ) on HSV-1-KOS strain compared to *C. nutans* extracted with methanol ( $IC_{50}$ : 64.93  $\mu\text{g/mL}$ ). However, in order to inhibit HSV-2-Baylor 186 strain, methanol extract of *C. nutans* exhibited higher inhibitory effect ( $IC_{50}$ : 65.13  $\mu\text{g/mL}$ ) as compared to hexane extract ( $IC_{50}$ : 72.62  $\mu\text{g/mL}$ ). Among the results obtained, ethyl acetate extract of *C. nutans* displayed the best anti-HSV inhibition with the lowest  $IC_{50}$  value recorded (7.6  $\mu\text{g/mL}$ ) (Thongchai et al. 2008). Based on clinical trials, topical application of 5% cream of *C. nutans* extract resulted in crusting of lesions in 98.6% of patients within three days of application. The

injury healed entirely in 88.9% of patients after seven days of cream application compared to patients who received placebo and acyclovir as treatments of herpes simplex virus infections (Yoosook et al. 1999).

Ethanol extracts of *C. nutans* have also been tested on three strains of fish pathogenic viruses: Infectious hematopoietic necrosis virus (IHNV), *Oncorhynchus masou* virus (OMV) and infectious pancreatic necrosis virus (IPNV). The studies were to examine the ability of *C. nutans* as anti-viral agent. In a direct inactivation pathway, 100% inhibition on plaque formation of IHNV and OMV strains were achieved. However, 0% inhibition was recorded by IPNV strains. In pre-infection treatments, all the strains showed inhibitory effects with the highest being recorded by IPNV strain (74%) followed by OMV (54%) and IHNV (31%). In post-infection treatments, plaque formations were reduced by 48% (OMV), 25% (IHNV) and 3% (IPNV), respectively (Direkbusarakom et al. 1996).

On the other hand, ethanol extracts of *C. nutans* was recorded to have good anti-viral effects against yellow-head rhabdo-like virus (YRV-RNA) on black tiger shrimp (Direkbusarakom et al. 1996). In addition, 80% ethanol extracts of *C. nutans* had excellent anti-viral properties against dengue virus (DENV-2 Strain 16681) (Tu et al. 2014).

**Analgesic activity** Analgesic capabilities of *C. nutans* extracts have been investigated by conducting *in vivo* assays such as acetic acid writhing tests, formalin-induced paw licking tests and hot plate tests (Pongphasuk et al. 2005; Rahim et al. 2016; Satatyavivad et al. 1996). By using acetic acid writhing tests on mice, butanol extract at dose 90 mg/kg was more effective than water, methanol and chloroform extracts (Satatyavivad et al. 1996). On the other hand, by conducting formalin induced paw licking tests, application of methanol extracts of *C. nutans* leaves at late phase showed high inhibitory effects ( $EC_{50}$ : 227.7 mg/kg) in reducing pain as compared to early phase ( $EC_{50}$ : >500 mg/kg) (Rahim et al. 2016). By conducting hot plate tests using ethanol, methanol and butanol as extraction solvents of *C. nutans* leaves, only methanol extract at concentration of 500 mg/kg significantly delayed pain responses, while ethanol and butanol extracts did not show any analgesic effect on test samples (Pongphasuk et al. 2005; Rahim et al. 2016; Satatyavivad et al. 1996).

**Immunomodulating effects** The immunomodulatory effects of *C. nutans* were carried out by using methanol and ethanol as extraction solvents and tested on several types of lymphocytic cells. Experiments conducted by Sriwanthana et al. (1996) recorded that ethanol extracts of *C. nutans* leaves was able to increase lymphocyte proliferations at lower concentrations. In contrast, at higher concentrations of *C. nutans* extracts, lymphocytic cells production significantly decreased. In addition, 1 and 5 mg/mL of ethanol extracts of *C. nutans* were able to suppress natural killer (NK) activities and production of interleukin-4 (IL-4) increased at the concentration of 2.5 mg/mL of extracts

(Sriwanthana et al. 1996). Another study by Wanikiat et al. (2008) reported that methanolic whole-plant extracts of *C. nutans* exhibited fMLP dose dependent suppression inducing chemotaxis and chemokinesis of neutrophils. In the study, no cells apoptosis was observed.

**Toxicity** Toxicity tests have been carried out by using *in vivo* techniques to study toxicological effects of *C. nutans* extracts on animals. Based on the study by P'ng et al. (2012), acute oral toxicity of methanolic extracts of *C. nutans* were conducted on male Swiss albino mice at doses 900 and 1800 mg/kg. The results showed that no mortality and or side effects on liver, kidney, spleen, lung and heart were observed. In addition, Sprague Dawley male rats administered orally with methanolic leaf extracts of *C. nutans* at doses of 300, 600 and 900 mg/kg also showed no abnormality or toxicological effects on liver and kidney functions (P'ng et al. 2013). In another study by Lau et al. (2014), methanolic extract of *C. nutans* at doses of 250, 500 and 1000 mg/kg were able to activate acetylcholinesterase (AChE) activities in Balb/C mice kidneys, livers and hearts.

In a number of acute and sub-chronic toxicity studies, no toxicity or mortality were observed on male and female mice by oral administration of methanolic extracts of *C. nutans* at doses 5000 mg/kg for acute study and 50, 500 and 2500 mg/kg for sub-acute studies (Zakaria et al. 2016). Based on these recent findings, there has been no scientific evidence to prove that consumption of *C. nutans* leaves extract causes toxicity.

## CONCLUSION

Recent studies suggest *C. nutans* is a medicinal plant having huge potentials to be explored. The phytochemical compounds that have been isolated and identified proved that *C. nutans* can be utilized in the production of various health-healing products. Therapeutic constituents that have been tested showed that *C. nutans* is a suitable source for alternative medicine for human. The beneficial values of *C. nutans* can be maximized in pharmaceutical and nutraceutical applications in order to produce more modern medicines with low toxicity and side effects to human.

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