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Review Article

Medicinal Properties of *Clinacanthus nutans*: A review

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Abstract

To date, medicinal plants are the most important resources in the discovery of new drugs. Clinacanthus nutans has been used traditionally in Thailand folk medicine to promote overall well-being. A few biological constituents of C. nutans and their physiological functions have been evaluated in previous studies. However, the mechanisms of action, potency and efficacy of the plant are still not well understood. In this review, the pharmacological properties of C. nutans such as anti-inflammatory effects, anti-proliferation, anti-venom and anti-bacterial activities, and their underlying mechanisms of action are presented and discussed.

Keywords: *Clinacanthus nutans: Anti-inflammatory, Anti-proliferation, Anti-venom, Anti-bacterial properties*

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INTRODUCTION

Complementary and alternative medicine (CAM) is becoming popular nowadays. It encompasses a wide range of practices, knowledge and treatments which are not related to modern medical profession. These include massage therapy, acupuncture, chiropractic and herbal foods [1-6]. Countries in which CAM is widely used include Southeast Asia, United States, United Kingdom and Australia [7]. Studies indicate that CAM is mostly used in treatment and management of chronic diseases but less used in depressive disorders [3]. It has some advantages over conventional medicines. For example, herbal products are cheaper than conventional treatments [8,9]. Although the use of CAM is popular amongst patients, there is lack of scientific evidence on its clinical effectiveness. In addition, safety issues related to CAM remain of concern to scientists and health care providers [9,10].

Clinacanthus nutans is a medicinal plant and a member of the family Acanthaceae. The plant is popular in many tropical countries such as Thailand, Malaysia and Indonesia due to its ready availability and medicinal properties. The plant has different names based on the native languages of the countries. In Thailand, *C. nutans* is recognized as *Phaya Yo* or *Phaya Plong Thong* whereas it is named *Belalai Gajah* or *Sabah Snake Grass* in Malaysia. It is a short shrub with hairy branches and small oblong

leaves. Six to seven pairs of side veins are found under the leaves while white internodes and vertical strips are distributed throughout the stem. The branches are topped with dull red-coloured flowers with green base and yellow streaks on the lower lips [11-15].

In Thailand, *C. nutans* leaves are traditionally used for treating skin diseases and bites from snakes and insects [13,16]. A topical cream containing ethanolic extract of *C. nutans* leaves has been used in the treatment of viral infections such as herpes genitalis, varicella-zoster and herpes simplex [16-19]. Moreover, *C. nutans* is used as an anti-inflammatory agent to relieve swelling [12,20,21]. A cross-sectional study in Malaysia has discovered that *C. nutans* is able to act as an anti-diabetic agent [22]. Due to the pharmaceutical properties of *C. nutans*, the Thai Ministry of Public Health has shortlisted it as one of the medicinal plants for public healthcare [23]. Moreover, a survey of ethnobotanical applications of medicinal plants showed that *C. nutans* ranks amongst the top 5 commonly used plants in Singapore [24]. Previous studies have shown the presence of stigmasterol, β-sitosterol, lupeol, myricyl alcohol, botulin, sulphur-containing glycosides and glycoglycero-lipids in *C. nutans* [16,25-29]. In addition, six C-glycosyl flavones have been isolated and characterised from the leaves and stem of *C. nutans,* while chlorophyll derivatives (phaeophytins) were isolated from the leaves [13,15]. Some cerebrosides and glycerol derivatives have also been isolated from the leaves of *C. nutans* [16]. Reports from previous studies suggest that *C. nutans* is safe for consumption. Ames test revealed no evidence of mutagenic or carcinogenic effects after exposure to aqueous extract of *C. nutans* leaves [30]. No mortality or morbidity was found in animals following single and repeated dose administrations of the plant extract [30]. Furthermore, studies on the toxic effect of *C. nutans* on human gingival fibroblasts showed that the cells survived well after exposure to the plant extract [31]. In this review, the medicinal properties of *C. nutans* and its mechanism of action are discussed.

CHEMICAL CONSTITUENTS OF *C. NUTANS*

Recently, Chelyn *et al* conducted a study on the flavone C-glycoside content of the leaves of *C. nutans* from different geographical locations in Malaysia [13]. Preliminary thin layer chromatography screening of the samples demonstrated two distinguishable green and yellow fluorescent bands. Flavone C-glycosides with apigenin backbone were found in the green color band, comprising shaftoside, isovitexin and vitexin. On the other hand, the yellow color band was identified as flavone C-glycosides containing luteolin backbone, consisting of isoorientin and orientin. Among all the compounds identified, only shaftoside was present in all the samples tested, regardless of geographical location from where the samples were collected. The other compounds were present in samples from specific geographical regions. This might be due to differences in climate and soil, and differences in stages of maturity of the plants harvested. Sample handling processes such as drying temperature and methods, as well as storage conditions might also affect the composition of the flavonoids. Since shaftoside is the only stable flavonoid in all samples, it has been suggested for use as a chemical marker for *C. nutans* raw material [13]. In another study, Sakdarat *et al* reported the presence of chlorophyll derivatives in chloroform extract of leaves of *C. nutans* [15]. In that study, compound 1, a dark green amorphous solid was identified as 13^2-hydroxy-(13^2-S)-phaeophytin-b through proton (^{1}H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR). Compound 2, which appeared as a green powder, displayed signals corresponding to amine, ester and hydroxyl functional groups in the infrared (IR) spectrum, indicating that it is a 13^2-hydroxy-(13^2-S)-phaeophytin-a. Compound 3, identified as 13^2-hydroxy-(13^2-R)-phaeophytin a by IR, ^{1}H NMR and ^{13}C NMR, was closely equivalent to compound 2. Results from antiviral tests showed that these compounds inhibited HSV activity [15].

Chromatographic purification of ethanol extract of *C. nutans* showed that cerebrosides appeared as a colorless solids while monoacylmonogalactosylglycerol was in pale yellow color. The cerebrosides had a glycosphingolipid structure, with sugar moieties, amide function and long chain aliphatic and olefinic groups. Methanolysis of the sample yielded methyl glucoside, trihydroxy long-chain base and fatty acid methyl esters, which further confirmed the presence of cerebrosides. Sugar and glycerol moieties were present in the monoacylmonogalactosylglycerol. The sugar was a β-D-galactopyranose while the long chain fatty acid was linolenic acid. These compounds did not exhibit anti-HSV and anti-inflammatory activities [16]. The chemical constituents of ethanolic extract of the aerial parts of *C. nutans* and their bioactivities have been reported [32]. Four new clinamides and 2-*cis*-entadamine-A showed antiviral activities against dengue virus, as well as anti-inflammatory and immune-protective activities.

Based on the structure of the active constituents of *C. nutans*, Janwitayanuchit *et al* synthesized

some 1,2-O-diacyl-3-O-β-glycopyranosyl-*rac*-glycerols in order to investigate the stereochemical influence of C-2 in the glycerol backbone on anti-HSV property [33]. It was shown that the presence of olefinic fatty acyl moieties produced higher inhibitory effects against HSV-1 and HSV-2. However, the sugar moiety (glucose or galactose) and stereochemistry at C-2 had no significant effect on anti-viral activity.

A novel polysaccharide-peptide compound has been isolated from *C. nutans* extract. The complex comprised about 87.25 % carbohydrate and 9.37 % protein. Further analysis revealed that the complex was composed of D-glucose, L-arabinose, D-mannose, D-galactose and L-rhamnose [34]. The phytochemical composition of the plant changes as a function of age. The levels of total flavonoids and total phenolic compounds were highest in 6-month-old buds [35]. The flavonoids isolated in that study included catechin, quercetin, kaempferol, luteolin, as well as phenolic acid, caffeic acid and gallic acid [35].

MEDICINAL PROPERTIES OF *C. NUTANS*

Anti-viral properties

Herpes simplex virus (HSV) is classified into two antigenic types: HSV-1 and HSV-2. Both viruses contain linear and double-stranded DNA molecules with approximately 150 kbp. Infection with HSV-1 causes cold sores, encephalitis, corneal damage, blindness and herpetic whitlow; whereas genital herpes which is a common sexually transmitted disease is mainly caused by HSV-2 [36]. Currently, there are various types of antiviral drugs available in the market for the management of HSV infections, the most commonly used of which is Acyclovir™ (ACV). However, studies have shown that the presence of mutated or resistant strains of HSV affects the efficacy of ACV [37]. Hence, CAM can be explored as a potential treatment for HSV infections.

In Thai folk medicine, *C. nutans* is commonly applied onto viral lesions or rashes on the skin. It has been demonstrated that the size of virus plaque became smaller after incubation with *C. nutans* extract, suggesting that *C. nutans* extract could affect the intracellular activity of HSV-2 [20]. Some bioactive constituents of *C. nutans* such as polyphenolics, glycosides and terpenes have been identified as promising anti-HSV agents [12, 16]. The anti-HSV activity was further evident after it was shown that *C. nutans* leaf extracts produced anti-HSV-1 and HSV-2 properties [12]. The plaque reduction assay revealed that *n*-hexane extract of *C. nutans* had a stronger anti-HSV-1 activity than dichloromethane extract or methanol extract. On the other hand, the three extracts produced only slight anti-HSV-2 activities. These findings demonstrate the potential of *C. nutans* as an antiviral agent.

In another study, Sakdarat *et al* tested the anti-viral activities of three active compounds isolated from *C. nutans* [15]. Inhibition of HSV was exhibited with 132-OH-(132-S)-phaeophytin, 132-OH-(132-R)-phaeophytin-a and 132-OH-(132-S)-phaeophytin-a at concentrations of 1.96, 3.11 and 3.11 µM, respectively. In the pre-viral entry stage, 100% inhibition of viral activity was produced by all the tested compounds. However, at the post-viral entry stage, viral activity was inhibited by 30 %. Thus, the three compounds inhibited HSV-1 infection before the virus entered the host cell. These findings indicate that the compounds might be acting through interference with the viral envelope structure that is required for adsorption and entry of the virus into the host cells. Extracts of *C. nutans* might also have a direct inactivation action on the virus, thereby inhibiting its activity [20].

Clinical trials have been carried out to evaluate the antiviral efficacy of *C. nutans* extracts. A meta-analysis revealed that the use of *C. nutans* cream against herpes genitalis caused by HSV-2 demonstrated full recovery and 100 % crusting relative to placebo [19]. In addition, combination of *C. nutans* extract and ACV produced synergistic antiviral results [15,19]. In another study, a randomized, placebo-controlled trial demonstrated that when used topically, *C. nutans* extract improved healing of VZV lesions and reduced pain scores more rapidly in the treated group [38]. A cream formulated with *C. nutans* was also found to be effective in the treatment of herpes zoster, in another study [39]. Although *C. nutans* extract was effective in the treatment of VZV infection, its mode of action still remains unclear. Hence, further studies should be conducted to elucidate its mechanism of action. *C. nutans* extract has also been shown to be protective against human papillomavirus infection through prevention of viral particle binding to the cell receptor [40,41]. In addition, ethanolic extract of the aerial parts of *C. nutans* has been shown to be moderately effective against the dengue virus at a concentration of 31.04µg/mL [32,42].

Anti-inflammatory activity

Inflammation is an immune response which

eliminates pathogens such as microbes from the body. It is a protective mechanism which helps to get rid of infections and injuries via migration of leukocytes and proteins from circulation to the infected or damage sites. Consequently, this defence involves polymorphonuclear neutrophils, especially with respect to acute and chronic inflammations. Thus, dysregulation of neutrophil functions leads to the production of pro-inflammatory mediators, toxic reactive oxygen species and release of myeloperoxidase (MPO) and elastase, resulting in inflammation-induced tissue lesions [43,44].

The anti-inflammatory effect of *C. nutans* leaf extract on neutrophils has been demonstrated using ear and paw edema rat models [21]. In addition, inhibition of neutrophil marker enzyme, MPO activity was found to be associated with reduced neutrophil migration. Furthermore, *C. nutans* exerted concentration-dependent inhibitory effects on chemotaxis and chemokinesis of neutrophils. This in turn attenuated superoxide anion generation as well as the release of MPO and elastase. The anti-inflammatory effect of *C. nutans* was further tested on recurrent aphthous stomatitis [45]. In a clinical evaluation, patients were instructed to apply the *C. nutans* in orabase to the lesion 4 times daily. Results obtained showed that *C. nutans* treatment shortened the healing time when compared to placebo, although the duration of pain was not affected [45]. In a docking study, some active phytochemical constituents of *C. nutans* were shown to bind to human neutrophil elastase enzymes involved in inflammation [46]. These compounds included β-sitosterol, clinacoside A-C, cycloclinacoside A1, lupeol, shaftoside, vitexin, isovitexin, as well as orientin and isoorientin. Isovitexin and isoorientin showed preference for HNE, nitric oxide synthase, squalene synthase, xanthine oxidase, HNE, and matrix metalloproteinases II and III. On the other hand, clinacoside B produced the lowest binding energy for all the candidate enzymes except xanthine oxidase and squalene synthase, while orientin and vitexin docked and bound to nitric oxide synthase and HNE only. All the compounds were predicted to have inhibitory potential against cytochrome P4502D6, with the exception of isoorietin and orientin.

Mai *et al* elucidated the mechanism involved in the anti-inflammatory property of *C. nutans* through the application of lipopolysaccharide (LPS)-treated RAW264.7 macrophages and human embryonic kidney cells transfected with Toll-like receptor-4 (TLR-4) [47]. Extracts of *C. nutans* reduced the expression of nitric oxide (NO) and cytokines, and also inhibited the expression of LPS-triggered TLR-4 inflammatory proteins like ERK, p65, p38, c-Jun N-terminal kinases and interferon regulatory factor 3.

Antioxidant properties

Chemotherapy drugs and radiotherapy may induce oxidative stress resulting in cell damage. These are unwanted side effects of cancer therapy which may be reduced by compounds with antioxidant properties. *C. nutans* extracts are potential cytoprotective antioxidant agents. In a comparative study of antioxidant properties of various solvent extracts of *C. nutans*, higher DPPH and galvinoxyl radical scavenging activities were obtained with $CHCl_3$ when compared with methanol and aqueous extracts, while the aqueous extract exhibited the highest NO radical scavenging activity [14]. A comparative study of DPPH radical scavenging activity between young and old buds of *C. nutans* revealed that buds aged 12 months were more active than 6 month-old, with IC_{50} values of 64.6 and 73.5 µg/mL, respectively [35]. However, the younger buds had higher activity in FRAP assay. In another comparative *in vitro* study, ethyl acetate and ethanol extracts of *C. nutans* were shown to have higher DPPH radical scavenging, oxygen radical absorbing and ß-carotene bleaching potential than extracts from dichlomethane and hexane [48].

Anti-cancer properties

Chloroform extract of *C. nutans* has been shown to be capable of inhibiting the proliferation of some human cancer cells, when compared to the aqueous and methanol extracts which exerted relatively weak inhibitory effects [14]. Interestingly, the cytotoxic effect and percentage of inhibition were significantly lower in endothelial cells exposed to the three extracts [14]. The bioactive component of *C. nutans*, CNP-1-2 inhibited the growth of human gastric cancer cells SGC-7901 [34]. Similarly, it has been demonstrated that extracts of 6-month-old *C. nutans* buds exhibited significant anticancer activity against HeLa cancer cells [35]. In addition, *C. nutans* extracts have produced anti-carcinogenic effects against MCF-7 cells [48]. These results show that the extracts of *C. nutans* exert cancer-inhibitory properties, thereby supporting their use in cancer treatment.

Huang *et al* showed that *C. nutants* ethanolic extract exhibited potent tumoricidal effect in tumour-bearing mice [49]. ICR mice injected with HepA hepatocarcinoma tumour cells received *C. nutans* treatment (3 and 10 mg/kg) for 10 days, resulting in significant reduction in tumour size,

when compared to the untreated group. The hepatoma cells were in apoptotic state after the treatment. This was confirmed by increased Bax and Caspase-3 protein expressions in the cells. Furthermore, the hepatoma cells showed reduced proliferation with de-activation of Akt protein. Although *C. nutans* extracts inhibit various types of cancer, studies have shown that they are not toxic to hypoxic human Saos-2 osteosarcoma cells, which are known to be resistant to radiotherapy and chemotherapy [50].

Anti-bacterial activities

Acne develops when bacteria such as *Propionibacterium acnes* and *Staphylococcus epidermis* multiply. *Propionibacterium acnes* is a common skin anaerobe behind or in the inspissated sebum. Scientists believe that *P. acnes* could produce certain organic acids that trigger the inflammatory response in acne [43]. Chomnawang *et al* incubated the two microbial strains with *C. nutans* extract and found that the growth of the bacteria was not significantly inhibited [51]. Similar results were obtained by Yang *et al.*, who however reported that the extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* with MIC of 12.5 mg/mL [52].

Anti-venom activity

C. nutans is a popular anti-snake venom in Thailand and Malaysia. However, it has been reported that aqueous extract of *C. nutans* had no inhibitory effect on *Naja siamensis* bite-induced neuromuscular transmission failure [53]. In another study, it was shown that extracts of *C. nutans* might prevent fibroblast cell lysis caused by *Heterometrus laoticus* scorpion venom [54], thus supporting the use of *C. nutans* as an antidote against scorpion venom.

Immuno-modulatory properties

C. nutans extract was found to enhance lymphocyte proliferation at the concentration range of 0.5 - 5 µg/mL [55]. However, it reduced the proliferation of lymphocyte at the concentration range of 1 - 5 mg/mL. At the higher concentration range of *C. nutans* extract, the activity of natural killer cells was significantly decreased while the level of IL-4 was enhanced. These results suggest the modulation of nonspecific cell-mediated immune responses, which might be useful in treating some viral infections.

Modulation of neurotransmission

Methanol extract of *C. nutans* leaves fed to Balb/c mice for 14 days activated acetylcholinesterase (AChE) and modulated cholinergic neurotransmission in mice kidney, liver, and heart [56].

Anti-nociceptive activity

Abdul Rahim *et al* showed that oral administration of methanol extract of *C. nutans* exerted both central and peripheral antinociceptive activities via activation of opioid receptors and modulation of L-arginine/NO-mediated pathway [57].

Neuro-protective effect

Studies have shown that *C. nutans* extract selectively inhibited histone deacetylase (HDAC)-1 and HDAC-6 expressions in neuronal cells, and also protected endothelial cells and astrocytes from hypoxic-induced cell death [58]. In addition, the *C. nutans* extract prevented neuronal cell death caused by oxygen/glucose deprivation [58].

Anti-hyperlipidemic effects

It has been demonstrated that water and methanolic extracts of *C. nutan* leaf lowered insulin, serum retinol binding protein-4 and fasting blood glucose in high fat and high cholesterol (HFHC)-fed rats [59]. Results obtained from studies of insulin resistance using homeostatic model showed that both extracts significantly improved insulin sensitivity in the HFHC-fed rats. The anti-hyperlipidemic effect of *C. nutans* was mediated through up-regulation of genes coding for phosphatidylinositol-3-phosphate, insulin receptor substrate, adiponectin receptor and leptin receptor [59]. The efficacies of aqueous and methanolic leaf extracts of *C. nutan* in attenuating oxidative stress were further tested in hyperlipidemia-induced rats [60]. The results indicated that both extracts increased the activities of serum antioxidant enzymes and upregulated hepatic antioxidant gene expressions.

FUTURE DIRECTION

Clinacanthus nutans is used as famous medicine among folklore healers in many countries. However, there is lack of scientific evidence on the effectiveness of this medical plant. Hence, a better understanding regarding the mechanisms of action of *C. nutans* is required. This will ultimately unveil the potential of *C. nutans* in the

treatment various diseases, and help in improving quality of life of patients. Although the role of *C. nutans* and its biological constituents have been widely documented in both *in vivo* and *in vitro* studies, the number of clinical trials carried out is limited. For example, the effect of *C. nutans* on skin rashes could be tested to further support the therapeutic efficiency of the plant. Furthermore, the possibility of *C. nutans* extracts being made into commercially available drugs remains to be explored.

CONCLUSION

Studies on the anti-viral, anti-inflammation, antioxidant, anti-cancer and anti-venom properties of *C. nutans* were reviewed in this paper. The progress of work *in vitro* and animal studies provides evidence that *C. nutans* could be explored further for its therapeutic potential.

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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