

Clinacanthus nutans, Yesterday's Practice, and Future's Drug: A Comprehensive Review

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ABSTRACT

Background: *Clinacanthus nutans* (CN) is individual from *Acanthaceae*, native to Southeast Asian countries. An herb with prominent therapeutic uses in treating cancer, inflammation, diabetes, renal dysfunction, digestive disorder, and snakebite. Base on the latest literature, this review aimed to provide a comprehensive review of ethnopharmacology, pharmacology, toxicology, phytochemistry, clinical applications of CN and finally the critical analysis of research findings.

Methods: Information about CN collected from an online database (Web of Science, Science direct, Scopus, EBSCO, Pub med, and Google scholar).

Results: The numbers of chemical compounds identified or isolated from leaves, and stem of CN are nearthirty-five. Crude preparations and isolated phytochemicals through *in-vitro* and *in-vivo* testing model exhibited significant antioxidant, anticancer, anti-inflammatory, anti-analgesic, antibacterial antiviral, antivenom, immune response activity, with no toxicological effects. Further, CN in the form drinks, tea, or cosmetics products entered local and international markets.

Conclusions: The scientific research publications justify some of the traditional uses of this herb. Both positive and negative results recorded in the same testing model. The inconsistency in biological reports may be attributed to the source of the test article. More systematic research is warranted to construct finger print of CN and standardized test article to establish consistent preclinical and clinical data. Therefore, this review provides a valuable reference for future investigation on CN as herbal medicine.

Keywords: *Clinacanthus nutans*, Herbal medicine, Ethnopharmacology, Preclinical, clinical, Phytochemistry, Southeast Asia.

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INTRODUCTION

Clinacanthus nutans (CN) is indigenous to Southeast Asian countries, namely South China, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam^{1,2}. It is an annual plant grows in a tropical environment, as an erect herb or rambling shrub with green and cylindrical stem and lanceolate leaf blade.^{1,2} Despite the accessibility of modern medicine, CN has been used as a remedial herb. In some cases, it is the first step in health care and in others, it provides a real alternative to primary health care approaches in treating conditions such as kidney dysfunction, gastrointestinal diseases, inflammation, viral infection lesions, and snake or scorpion venom. Use of herb with rich medicinal history, has been beneficial in dealing with disease condition³. The scientific approach toward the application of these drug candidate reduces side effect, increases their efficacy⁴. This review presents the regional medicinal uses, pharmacological activities, toxicology, clinical application, and phytochemicals content, as well as marketed products of CN.

Kingdom	Plantae
Family	Acanthaceae
Genus	<i>Clinacanthus</i>
Species	<i>nutans</i>
Native	Southeast Asia
English name	Sabah snake grass
Local name	
Indonesia	Kitajan (Sunda); Dandang Gendis (Java); Gendis (Central Java)
Malaysia	Belalai Gajah
Thailand	Phaya yo, Phaya plongtong
South China	E zui hua (鳄嘴花)

METHODS

Relevant information on the modern and traditional application of CN as therapeutic or food agent sourced from leading databases. Web of Science, Science direct, Scopus, EBSCO, Pub med, and Google scholar, website ministry of agriculture; Malaysia, and databases of University Sains Malaysia are the sources. The search was performed with, *Clinacanthus nutans* as key word.

RESULTS AND DISCUSSION

Medicinal uses

In China, recipes involving the different parts of CN, individually or in combination, play a key role in traditional Chinese medicine to treat various inflammatory conditions such as atheroma, contusion, strains, sprains, rheumatism, high uric acid, and gout. Chinese healers administered this herb to regulate mental function, relieve pain, anemia, and jaundice^{1, 2, & 5}. The combination of CN and other herbs also has been utilized to treat various medical conditions. Topical use of CN combined with other traditional herbal medicines was reported to be 96.7% effective in treating cervical ectopic columnar epithelium, qi stagnation, and blood stasis; nourishing the spleen; removing dampness; clearing away heat, detoxification; and removing blood stasis pain. There are no records of severe side effects⁵. Consumption of CN also was reported to prevent fatty liver when the herb was prepared in a very specific manner⁶. In addition, distiller's yeast made from CN and 18 healing herbs was reported to have the effects such as: strengthening the body; promoting urination; discharging kidney stones; promoting blood circulation; invigorating the kidney; and treating

diseases such as rheumatoid arthritis, gout, irregular menstruation and dysmenorrhea, easy convulsion, insomnia, chest distress, palpitations, cerebral thrombosis, hyperosteo-geny, and traumatic injury⁷.

In Indonesia, an aqueous decoction from fresh CN leaves is the typical prescription to treat patients suffering from gastrointestinal problems, dysentery, dysuria, and a hyperglycemia⁶. A mixture comprised 17 plants plus CN in the form of tablets, dispersible tablets, capsules, soft capsules, ointment or extract patented as a treatment for abnormal amenorrhea⁸.

Another patent in the form of herbal powder containing CN uses to treat edema after breast cancer surgery, promoting diuresis and nerve damage, and stopping blood with a success rate of 93.33%⁹.

In Malaysia, CN is one of the most common herbs used as complement or alternative to current medicine after disease diagnose¹⁰. It is used to lower high levels of blood cholesterol, glucose, uric acid, and blood pressure. In addition, a drink made from CN is used to detoxify the body and is beneficial for dialysis and treating kidney dysfunction¹¹. Siew *et al* (2014) conducted a cross-sectional study of 240 diabetic volunteers from Malaysia; 7.9% of the patients reported using CN as an alternative medicine to complement their biological treatment. Other medicinal plants used by the patients were *Momordica charantia* (30.4%), *Orthosiphon stamineus* (24.2%), and *Allium sativum* (13.3%)¹². Both Malaysian and Thai, administer CN in hepatitis and herpes simplex¹³.

In Thailand, CN is celebrated as an anti-snake venom agent and as a treatment for scorpion bites and nettle rash. Thai traditional healers also use the plant to treat fever and inflammatory conditions¹⁴. An independent claim reported that herbal pills consisting of *Tinospora cordifolia* (1–20 g), *Andrographis paniculata* (1–20 g),

Phyllanthus amarus (1–20 g), CN (1–20 g), and *Cinnamomum camphora* (1–20 g) are useful for treating premenstrual pain, migraine, and sleeplessness and as an anti-AIDS virus treatment¹⁵. These Thai herbal tablets also are supposed to be useful for treating diabetes and body pain, increasing appetite, nourishing the pancreas, reducing blurriness or reddening of the eyes, treating stomachache, indigestion (dyspepsia), Parkinson's, aphthous ulcers of the tongue, melasma, blisters, and all kinds of cancer, acting as a laxative, and treating colds, reducing allergies, and nourishing the body. These effects are all claimed to be true, but no biological data are provided¹⁵. Furthermore, CN is used to treat herpes simplex virus (HSV) infection, varicella zoster virus (VZV) lesions, skin rashes, and insect and snake bites¹⁶.

A survey on administration of fresh medicinal herbs in Singapore revealed that among the 414 plants surveyed, CN was among the five most popular used plants, and the leaves were the most used part¹⁷.

Pharmacological activity

Antioxidant activity

The antioxidant activity of CN has been evaluated in a number of *in vitro*, *ex vivo*, and *in vivo*, studies. The hydro alcoholic preparation of CN leaves has been subjected to antioxidant screening such as the DPPH radical scavenging assay, the ferric reducing antioxidant power assay, and phorbol myristate acetate (PMA)-induced reactive oxygen species production in rat macrophages the tested sample illustrated the antioxidant activity and protective effect against free radical¹⁸. Another study considered the age of the plant as a factor for bioactivity. The report said that radical scavenging activities were significantly higher in buds and leaves of 12 months old with fifty percent inhibition of DPPH radical

at 64.6. The activity of the extracts was compared to those of hydroxytoluene (68.0%), caffeic acid (70.4%), and α -tocopherol (71.2%) at a concentration of 100 $\mu\text{g/ml}$ ¹⁹.

In another study, the antioxidant activities of the chloroform, methanol, and aqueous extracts of CN were assessed using DPPH, galvinoxyl, nitric oxide, and hydrogen peroxide based radical scavenging model. The chloroform extract had the strongest antioxidant activity against the DPPH free radical and galvinoxyl radicals, but it was less effective at negating nitric oxide and hydrogen peroxide radicals²⁰. Arullappan *et al* (2014) also found that the petroleum ether extract exhibited higher DPPH radical scavenging (82.00 %) than ethyl acetate and methanol crude preparation²¹.

The protective activity of a CN extract against oxidative stress on plasmid DNA integrity in *Escherichiacoli* was tested and compared to that of superoxide dismutase activity and total phenolic contents of green tea. The reference activities were higher than that of the CN extract. However, the addition of riboflavin increase protective effect of CN²².

Anticancer activity

Numerous studies have assessed the anticancer activities of CN. Evaluation of cytotoxicity against different cancer cell line has been the most common approach. In one study, the antineoplastic activity of CN was tested on HepG2, IMR-32, NCL-H23, SNU-1, Hela, LS-174T, K-562, Raji, and IMR-32 cancer cells using the MTT assay. The strongest antiproliferative activity was observed with the chloroform extract on the K-562 (91.28 \pm 0.03%) and Raji (88.97 \pm 1.07%) cell lines at 100 $\mu\text{g/ml}$ of the extract, and CN exhibited mild activity on other cell lines and no activity on IMR-32 cells²⁰. Another study tested the effects of extracts

of CN on human cervical adenocarcinoma (HeLa). The results revealed 50% growth inhibition at 56.8 $\mu\text{g/ml}$. The effect was considered significant compared to 76% inhibition induced by the positive control (tamoxifen)¹⁹.

Bangchang *et al.* (2012) evaluated the cytotoxic effect of aqueous extract from a mixture of herbs that included CN. The extracts showed inhibition of growth of KB cells, with an IC₅₀ value of greater than 100 $\mu\text{g/ml}$ ²³.

In another study, human Saos-2 osteosarcoma cells were exposed to CN methanolic extract under normoxia or hypoxia (0.5% oxygen). The MTT assay was performed after 24h of exposure, and the hypoxia-driven firefly luciferase reporter kit showed that the extract was not toxic to cells²⁴. Mixture of CN and entadamides was patented for antitoxicity in the malign cell, anti-inflammatory and immune system boosting²⁵. Aqueous extract of CN leaves extract also has chemo-preventive potential against colon cancer²⁶.

Anti-inflammatory and analgesic activities

Traditional healers agree that a typical application of CN is to alleviate inflammation, and results of *in vitro* and *in vivo* pharmacological investigations support this claim. The reports showed the notable dose-dependent activity of the CN in suppressing the ethylphenylpropionate (EPP) and carrageenan-induced inflammation²³. The authors related the effect to neutrophil function and migration, which was confirmed by the reduction of myeloperoxidase activity in the EPP-induced rat ear edema model. CN had further significant inhibitory effects on neutrophil functional responsiveness without cytotoxic and apoptotic effect on neutrophil²⁷.

In another, the 95% ethanolic preparation of CN leaves was used in the

carrageenan(1%)-induced inflammatory model; the result was less than 35% anti-inflammatory and low anti-analgesic activity²⁸. The ethanolic extract from a mixture of Thai traditional herbs that included CN exhibited mild but significant inhibition of IFN-gamma/TNF-alpha-induced apoptosis in HaCaT keratinocyte cells²⁹.

Immune response activity

The CN extract also was tested for its effect on the immune system using the lymphocyte proliferation response and the natural killer (NK) cell activity assay²⁶. The report says CN strengthen the immune system, by increasing lymphocyte proliferation and reducing the activity of NK cells. CN extract also suppresses the release of IL-2 and increases in IL-4. This result partially explains the mechanism of cell-mediated immune response activity of the CN extract³⁰.

Antibacterial and antiviral activities

Petroleum ether, ethyl acetate, and methanol crude extracts of CN significantly inhibited the growth of *Bacillus cereus* (ATCC11778), *Escherichia coli* (ATCC25922), *Salmonella enteric Typhimurium* (ATCC14028), and *Candida albicans* (ATCC10231)²¹. Jayavasud *et al* (2013) reported anti-HSV-2 activity of CN extracts via inhibition of plaque formation in the baby hamster kidney cell line³¹. In another study, the effects of a methanol extract of CN on a common cause of genital herpes (HSV-2 (G)) and five clinical HSV-2 isolates were assessed; the results showed no antiviral activity from CN in the plaque inhibition assay¹³.

CN has strong virucidal and bactericidal activity against aquatic animals' pathogens. Notably, in controlling viral disease in the black prawn (*Penaeus monodon*)³², and inhibiting yellow-head

Rhabdoviridae infection in black tiger shrimp both in *in vivo* and *in vitro* model³³. CN also showed mild anti-*Aeromonas hydrophila* activity in fish and shrimp³⁴. Anti HSV-2 observed with either isolated compound from CN.

CN showed virocidal activity against VZV³⁵, HSV-1, HSV-2³⁶ HSV-2 (strain G) and HSV-1F³⁷. Negative result relating to the anti-HSV-2 activity was also reported³¹.

Antivenom activity

To test the Thai folk claim that CN has an anti-snake bite and scorpion venom effects, two different models were used³³. Water Extract of CN leaves suppressed up to 46.51% of *Heterometrus laoticus* scorpion venom-induced cell lysis³⁸. While, no protective effect was observed against lethal doses of *Naja naja siamensis* crude cobra venom when CN extract was given orally or intraperitoneally to mice. Pretreatment with a mixture of CN extract and alpha-amylase or beta-amylase also did not stop the lethal activity of the venom. The authors concluded that the extract could not antagonize the action of cobra venom³⁹.

Cosmetic uses

A herbal extract mixture composed of CN extract and 35 other medicinal herbs up-regulates dynein in dermal fibroblasts or keratinocytes. The ability of the agent to modulate cytoplasmic dynein was determined by measuring the expression level of cytoplasmic dynein in a cell that has been contacted with the active agent⁴⁰. Another patent reported that an extract from the air-growing part of CN along with *Siegesbeckia orientalis* L., *Laggera pterodonta* Benth., *Momordica cochinchinensis*, *Azadirachta indica* Juss., *Acanthus ilicifolia* L., or *Cassytha filiformis* L. possesses hair growing activity⁴¹.

Toxicological studies

In vitro toxicity

Cytotoxicity of the aqueous extract of CN was tested against Vero cells (African green monkey, kidney epithelial cells) for 5 days, and no toxic activity of the extract on the tested cells was observed at a median convulsant dose of more than 2000 $\mu\text{g/ml}$ ⁴². However, another study reported that the petroleum extract obtained from sequential extraction (petroleum ether, ethyl acetate, and methanol) exhibited strong cytotoxicity when tested against HeLa cells and K-562 cells, with respective IC_{50} values of 18.0 and 20.0 $\mu\text{g/ml}$ ²¹.

In vivo toxicity

Oral toxicity (14 days duration) of the methanol extract of CN leaves was evaluated in two different studies, one with repeated dosing of male rats at 0.3, 0.6, and 0.9 g/kg ⁴³ and the other with single administration of 2 g/kg of test sample to female rats⁴⁴. No signs of toxicity, morbidity, or mortality were observed in male and female rats. The extract caused no adverse effects, and no alteration was observed in liver and kidney parameters. Furthermore, no organ damage and changes in gross histology were observed in male and female rats^{43,44}. Further, the No-observed-adverse-effect level of water extract of CN was reported to be up to 2 g/kg in both male and female rats in sub-chronic study⁴⁵.

Clinical Trials

A placebo-controlled trial was performed to evaluate the anti-VZV properties of a topical formulation of CN extract in 51 volunteers suffering from VZV infection. The patients received treatment five times per day for 7–14 days until they recovered from the lesions. The treatment showed significant ($p < 0.01$) efficacy in

crusting lesions within 3, 7, and 10 days. Compared with placebo-treated patients, pain alleviation was faster in the drug treated group compared⁴⁶. A double-blind clinical study found that cream made from CN was more effective at treating HSV patients compared to placebo-treated patients⁴⁷.

Forty-three volunteers with a minor type of recurrent aphthous stomatitis volunteered in a double-blind control trial. Patients applied CN or triamcinolone in orabase or the placebo four times a day and were monitored three times during treatment to record pain and ulcer healing duration. A significant ($p < 0.01$) difference in duration of the ulcer healing period but not in pain recovery was detected. Thus, CN in orabase form could shorten ulcer recovery time, but it was less effective than triamcinolone acetonide in orabase⁴⁸.

Phytochemicals

Different extracts from CN have been investigated phytochemically and chemically. Twenty-five known and unknown compounds and nine mixtures of compounds have been isolated from CN extracts. Six known C-glycosyl flavones⁴⁹, two phytosterols, one triterpenoid⁵⁰, two glycolipids⁵¹, belutin⁵², five sulfur-containing glycosides⁵³, and eight compounds related to chlorophyll a and chlorophyll b¹⁴ were found. A mixture of nine cerebrosides and a monoacylmonogalatosylglycerol separated from the ethanolic leaf extract of CN (Table 1)⁵⁴.

Mustapa *et al* (2015) counted the method of extraction as another determinant of phytochemicals yield from CN leaves. They employed microwave-assisted extraction (MAE), pressurized microwave-assisted extraction (PMAE), supercritical carbon dioxide extraction (SFE) and Soxhlet to compare the level of recovery for bioactive compounds: phenols, flavonoids,

phytosterols, and β -sitosterol. The analysis showed that the best method for acquiring polyphenol and flavonoids content was MAE and phytosterols and β -Sitosterol yields were highest in SFE. P-MAE had amild effect on extraction of polyphenol and flavonoids. Authors conclude the MAE as the best method since in shorter period extraction, higher and yield better selectivity⁵⁵. He further describes the presence of diterpene, triterpene, fatty acids and other bioactive compounds.

Product in market

The flamboyance of therapeutic claims and support of research reports about CN garnered local and international market attention. Different preparation and formula such as tea, drinks, cosmetic products, supplement capsule, and pure dried powder of plant were seen in shelves of groceries. Some of these products and their claimed properties listed in table 2 and presented in figure 2.

CONCLUSIONS

CN is herb with a rich history of medicinal uses in south China and some Asian countries. It is credited as a remedy to treat varieties medical conditions. Some of the traditional uses proved through *in-vitro* and *in-vivo* biological testing models and clinical trials while some not. The analysis of the reports also highlights inconsistency in pharmacological activity of CN. The Further the studies showed the content of phytochemical vary in the sample with different growth condition, age, method preparation. This may explains the wide range traditional usage, inconsistency in pharmacological activity, and the difference between the level efficacy claimed by folk and the pharmacological activity observed in a laboratory environment.

Base on theses report we suggest the highly monitored process for preparation of

test sample from farm to laboratory. Inclusive of quality control of raw material with anexact record of plant origins, growth conditions, climate, altitude harvest seasons, processing, and /or any other causes that may affect chemical constituents. As illustrate in literature any difference in above factors can lead to the variant content of phytochemical and biological activity. Standardization of test article identification and quantification of the chemical marker in reference to pharmacological activity are the steps to be considered in achieving evidence base herbal medicine. We hope that this review emphasizes on the importance of CN and can provide new directions for researchers in the future.

Competing interests

There is no competing interest.

Authors' contributions

All the authors participated in collecting, evaluating, and analyzing the information. All authors contributed in drafting the article and approved the final draft for publications.

ACKNOWLEDGEMENTS

The authors would like to thank the Malaysian Ministry of Agriculture for providing financial support for this project (grant no.: 304/PFARMASI/650622/K123), Universiti Sains Malaysia (USM) through the USM- Postgraduate Research Grant Scheme (USM- PRGS) (grant no.: 1001/PFARMASI/846069), Nature Ceuticals Sdn Bhd and the School of Pharmaceutical Sciences, University Sains Malaysia for facilitating the work and Universiti Sains Malaysia fellowship scheme.

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Table 1: Phytochemicals identified or isolated from *Clinacanthus nutans*

S. No.	Major phytochemical	Group	Source	Reference
1	Stigmasterol	Phytosterols (Steroid)	50
2	Lupeol	Triterpenoid	Stem petroleum soxhlet extract	50
3	Beta-sitosterol	Phytosterols (Steroid)	Stem petroleum soxhlet extract	50
4	Belutin	Triterpene	52
5	Vitexin	C-glycosidic flavones	Leaf ethanolic extract	49
6	Isovitexin	C-glycosidic flavones	Leaf ethanolic extract	49
7	Shaftoside	C-glycosidic flavones	Leaf ethanolic extract	49
8	Orientin	C-glycosidic flavones	Leaf ethanolic extract	49
9	Isoorientin	flavones	Leaf ethanolic extract	49
10	Isomollupentin 7-O- β -glucopyranoside	C-glycosidic flavones	Leaf ethanolic extract	49
11	13 ² - hydroxy-(13 ² -S)-chlorophyll b	Chlorophyll a Chlorophyll b	Leaf chloroform fraction	14
12	13 ² -hydroxy-(13 ² -S)-phaeophytin b	Chlorophyll b	Leaf chloroform fraction	14
13	13 ² - hydroxy-(13 ² -R)-phaeophytin b	Chlorophyll b	Leaf chloroform fraction	14
14	13 ² -hydroxy-(13 ² -S)-phaeophytin a	Chlorophyll a	Leaf chloroform fraction	14
15	13 ² -hydroxy-(13 ² -R)-phaeophytin a	Chlorophyll a	Leaf chloroform fraction	14
16	Purpurin 18 phytol ester	Pheophytin	Leaf chloroform & hexan fraction	14
17	Phaeophorbide a	Chlorophyll a	Leaf chloroform fraction	14
18	Clinacoside A	Sulfur-containing glycosides	Leaves and stems extracted with MeOH	53
19	Clinacoside B	Sulfur-containing glycosides	Leaves and stems extracted with MeOH	53
20	Clinacoside C	Sulfur-containing	Leaves and stems extracted	53

		glycosides	with MeOH	
21	Cycloclinacoside A 1	Sulfur-containing glycosides	Leaves and stems extracted with MeOH	53
22	Triacetylcycloclinacoside A1	Sulfur-containing glycosides	Leaves and stems extracted with MeOH	53
23	Glycoglycerolipids	Ethanolicleaves extract	54
24	Nine Cerebrosides	Cerebrosides	Ethanollic leaves extract	54

Table 2: Some of Marketed products of *Clinacanthus nutans* and their claimed medicinal usage

S. No.	Trade name	Country	Claims
1	Clinacanthus burmani Nees (Dandang Gula) Powder	Indonesia	Diabetes
2	UTURN SSG plus drink (Mixed drink of CN*)	Malaysia	Protect kidney, detoxification, keep swelling caused by body fluid down, eliminate tumor, prevent various cancers and regulate periods.
3	Beveite sabah snake grass CN	Malaysia	Anti cancer
4	Teh daun belalai gajah	Malaysia	Cancer, High blood pressure, High uric acid, Diabetes, kidney dysfunction
5	Nutans Tea	Malaysia	Anti-cancer
6	Cher-aim Clinacanthus Nutan Balm	Thailand	Relieve muscle aches
7	CN Balm	Thailand	Relief of ache, sprain, muscular pain
8	Centella & Clinacanthus Herbal Bar Soap	Thailand	Cleans and nourishes the skin
9	Wangphrom Barleria Lupulina Green Herbal Balm (Herb mixture)	Thailand	Relieve itching and irritation,
10	Twin lotus herbal toothpaste	Thailand	whitening teeth and freshening breath
11	Herbal powder deodorant (Herb mixture)	Thailand	Anti sweating and odor for foot
12	Sabah Snake Grass / CN Capsule	Singapore	Healing grade plant, as traditional practices



Figure 1. Marketed products from *Clinacanthus nutans*.