

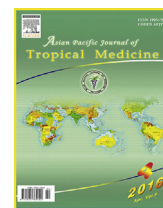
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journal homepage: <http://ees.elsevier.com/apjtm>Review <http://dx.doi.org/10.1016/j.apjtm.2016.03.011>***Clinacanthus nutans*: A review of the medicinal uses, pharmacology and phytochemistry**Ariful Alam¹, Sahena Ferdosh^{2*}, Kashif Ghafoor³, Abdul Hakim⁴, Abdul Shukor Juraimi⁵, Alfi Khatib¹, Zaidul I. Sarker^{1*}¹Faculty of Pharmacy, International Islamic University Malaysia (IIUM), Kuantan Campus, Kuantan 25200, Pahang, Malaysia²Faculty of Science, International Islamic University Malaysia (IIUM), Kuantan Campus, Kuantan 25200, Pahang, Malaysia³Department of Food Science and Nutrition, King Saud University, Riyadh 11451, Saudi Arabia⁴Institute of Tropical Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia⁵Department of Crop Science, University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

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ABSTRACT

Clinacanthus nutans Lindau is known as snake grass belonging to the Acanthaceae family. This plant has diverse and potential medicinal uses in traditional herbal medicine for treating skin rashes, insects and snake bites, lesions caused by herpes simplex virus, diabetes, and gout in Malaysia, Indonesia, Thailand and China. Phytochemical investigations documented the varied contents of bioactive compounds from this plant namely flavonoids, glycosides, glycolipids, cerebrosides and monoacylmonogalatosylglycerol. The pharmacological experiment proved that various types of extracts and pure compounds from this species exhibited a broad range of biological properties such as anti-inflammatory, antiviral, antioxidant, and anti-diabetic activities. The findings of toxicity study showed that extracts from this plant did not show any toxicity thus it can be used as strong therapeutic agents for specific diseased conditions. However, further experiments on chemical components and their mode of action showing biological activities are required to elucidate the complete phytochemical profile and assess to confirm their suitability for future drugs. This review summarizes the medicinal uses, phytochemistry and pharmacology of this plant in order to explore its therapeutic potential and gaps necessitating for prospected research work.

1. Introduction

Acanthaceae is one of the leading families of dicotyledonous flowering plant which included 250 genera and about 2500 species [1]. Most of them are tropical herbs, shrubs, or twining vines; some are epiphytes. A few of them are spread in temperate regions. Species from this family mainly distributed in Indonesia and Malaysia, Africa, Brazil, and Central America [2]. Plants of this family can be grown in most of the habitat, comprising dense or open forest, bushes, damp fields and valleys, sea shores and marine regions, swamps, and mangrove areas [3]. Acanthaceae

family is considered as one of the largest sources of medicinal plants providing effective traditional medicines against specific health impediments and those can be simply predictable morphologically by their simple, differing, decussate, whole leaves, zygomorph flowers and their superior ovary.

Clinacanthus nutans (*C. nutans*) Lindau is one of the important species from this family and has been used as vital herbal medicines in tropical Asia (Figure 1). At the moment this plant attracts much attention of many researchers for its medicinal potency [4,5]. Thai ministry of public health has been considered this plant as a main remedy for the treatment of skin inflammations and lesion caused by virus [6].

In the botanical viewpoint *C. nutans* (Burm. f.) Lindau and *Clinacanthus siamensis* Bremek are two different species and often been mistaken due to their alike morphology. These two species have different pharmacological characteristics, molecular aspect and anti-herpes simplex virus (HSV) type 1 and type 2 activities [7]. This plant is well recognized as anti-snake venom activity used by the traditional curers in Thailand. This plant is

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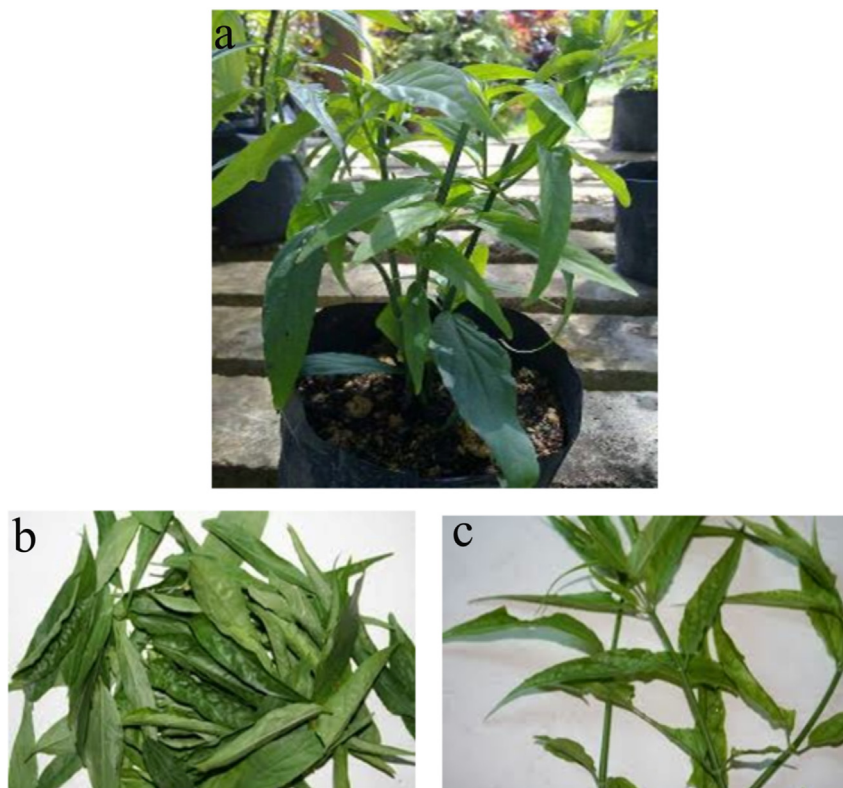


Figure 1. *C. nutans* (Burm. f.) Lindau. (a) whole plant; (b) leaves; (c) leaves with stem.

traditionally used as natural medicine in Malaysia, Indonesia and Thailand for treating certain diseases such as skin rashes, scorpion and insect bites, diabetes mellitus, fever and diuretics [8,9]. Pharmacological studies proved that this plant possessed a broad range of antimicrobial activity especially treating genital herpes and varicella-zoster virus (VZV) lesions diagnosed in immunocompromised people [10].

This plant is economically very important owing to the medicinal uses and herbal products. Different kinds of topical preparations such as cream, lotions, capsule, tablet, herbal tea, concentrated extract and secondary metabolites products are available in the market. Preparation of cream using alcoholic extract of dried leaves can relieve pain and heal herpes infection [11]. Most of the products have not much popularity among the consumers because of the lack of pharmacological information.

2. Botany

The vernacular name of this plant in English is snake grass; Belalai gajah, Sabah snake grass in Malaysia; Dandang Gendis, Ki tajam (Sunda) in Indonesia; Phaya yo, Phaya plongtong in Thailand; twist of flowers, alligator flower, e zuihua in China [12–14]. *Clinacanthus burmanni* Nees, *Clinacanthus burmanni* var. *robinsonii* Benoist are the synonym of *C. nutans* (Burm. f.) Lindau. Taxonomically this plant can be classified by kingdom: Plantae; phylum: Magnoliophyta; class: Magnoliopsida; subclass: Asteridae; order: Lamiales; family: Acanthaceae; genus: *Clinacanthus* Lindau; species: *C. nutans* (Burm. f.) Lindau [12].

C. nutans is a perennial herb which can grow up to 1 m tall with pubescent branches and cylindrical, striate, and glabrescent stems. The leaves are simple, opposite, narrowly elliptic-oblong or lanceolate (2.5–13.0 cm long × 0.5–1.5 cm wide) [15]. This

shrub is about 1 m tall, and stems cylindrical, striate and glabrescent. The petiole is 0.3–2.0 cm, sulcate, bifariously pubescent and leaf blade lanceolate-ovate, lanceolate or linear-lanceolate [16]. The leaves are apex acute or acuminate and exsculptate; dentate or subentine margins. Both surfaces of leaves are pubescent when young then glabrescent. The leaf base are cuncate, obtuse rounded or truncate; often oblique. Petiole is 3–15 mm long [17].

The flowers are sordidly yellow or greenish yellow and dense cymes at the top of branches and branchlets; always covered with 5- α cymules [18]. The calyx of flower about 1 cm long with granular-pubescent. Corolla is dull red with green base, about 3.0–4.2 cm. The stamen is exerted from the throat of corolla. The ovary is compressed into two cells and each cell has two ovules. The styles are filiform with shortly bidentate. Capsule is oblong basally wrapped into 4-seeded short stalk [18].

3. Ethnomedicinal uses

C. nutans has been traditionally used for a long time in different regions of Asia due to their different pharmacological effects. Usually the fresh leaves are boiled with water and consumed as herbal tea in Malaysia. In Thailand, an alcoholic extract of fresh leaves is used externally for treatment of skin rashes, snake and insect bite, HSV, and VZV lesions [19]. Sometimes the leaves are consumed as raw material or mixed with apple juice, sugarcane or green tea and provide as fresh drink. It is also prominent in Thailand as anti-snake venom amongst the traditional healers. The mode of action of this plant is attributed to be its anti-cell lysis property rather than an anti-neuromuscular transmission blocker. It has been also used to treat scorpion bites and nettle rash. In China the whole plant is

used in various manners to treat inflammatory conditions like hematoma, contusion, strains and sprains of injuries and rheumatism [16,20,21]. This plant is widely used for treating gastrointestinal complications. Indonesian and Thai traditional curers prepare herbal medicines from *C. nutans* to treat dysentery. In Indonesia they boil a handful of the fresh leaves and boil them in five glasses of water till the water level recedes to about three glasses and the bouillon is given in a dose of 1 glass each time [13]. It is also used to treat diabetes by boiling 7–21 fresh leaves in two glasses of water until the water level comes to one glass and serve twice daily. The decoction is made by boiling of 15 gm of fresh leaves for 15 min and consumed daily for the treatment of dysuria. Likewise this plant is also used in the treatment of fever. Whereas Chinese healers deal with the plant as useful in controlling menstrual function, relieving pain, anemia, jaundice and setting of fractured bones [13,21].

4. Phytochemistry

Phytochemical investigation of this plant shows that *C. nutans* contains a wide range of bioactive compounds (Figure 2). The vital phytochemical are stigmasterol (1) [22], lupeol (2), *b*-sitosterol (3) [23], belutin [24] and myricyl alcohol [25,26] are found. There are six known *C*-glycosyl flavones isolated from the *n*-BuOH and water soluble fractions of the methanolic extract of this plant in Thailand; they are vitexin (4), isovitexin (5), schaftoside (6), isomollupentin 7-*O*-*b*-glucopyranoside (7), orientin (8) and isorientin (9) [27]. The *n*-BuOH soluble fractions from methanolic extract of stems and leaves of this plant contain five glucosides [27].

A mixture of cerebrosides (10) and a monoacylmonogalactosyl glycerol (2*S*)-1-*O*-linolenoyl-3-*O*-*b*-dgalactopyranosylglycerol (11) were isolated from the EtOAc-soluble fraction of the ethanolic extract of the fresh leaves of *C. nutans* [28]. The hexane and chloroform extract of this plant were used for isolation of 13-hydroxy-(13-*S*)-phaeophytin *b*, pupurin-18-phytyl ester and phaeophorbide-*a* derived from the extracts [29]. Anti-HSV effective trigalactosyl and digalactosyl diglycerides (12) were isolated from leaves extract from this plant [30].

There were eight compounds related to chlorophyll *a* and chlorophyll *b* isolated from the chloroform extract of leaves 13²-hydroxy-(13²-*S*)-chlorophyll *b*, 13²-hydroxy-(13²-*R*)-chlorophyll *b*, 13²-hydroxy-(13²-*S*)-phaeophytin *b* [9], 13²-hydroxy-(13²-*R*)-phaeophytin *b* (13), 13²-hydroxy-(13²-*S*)-phaeophytin *a* (14), 13²-hydroxy-(13²-*R*)-phaeophytin *a* (15) [10], purpurin 18 phytyl ester and phaeophorbide-*a* [9]. Four new sulfur-containing compounds, clinamides A-C (16–18) and 2-cis-entadamide A (19) and three known compounds, entadamide A (20) [31], entadamide C [32], and trans-3-methylsulfinyl-2-propenol [20] were isolated from the ethanolic extract of the aerial parts of *C. nutans* [33].

5. Pharmacological effects

5.1. Anti-inflammatory activity

C. nutans has been used as anti-inflammatory agents for the treatment of insect bites and allergic responses and as remedies for herpes simplex and VZV lesions. Different pharmacological effects of plant extracts and isolated compounds are presented in Table 1. The 80% ethanol extract of aerial part of this plant

showed a significant inhibition on the generation of superoxide anion and the elastase release by activated neutrophils. The inhibition was produced by 10 µg/mL ethanolic extract of *C. nutans* at 68.33% [33]. In this study MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide was used as the elastase substrate for observing elastase release and superoxide anion formation examined by detecting the superoxide dismutase-inhibitory reduction of ferricytochrome *c*. The petroleum extract of leaves produced highest cytotoxic effect as compared to the ethyl acetate and methanol extract. The increasing concentrations of the extracts showed variability on the percentage of cell viability of HeLa cells. On the other hand optimum cytotoxic activity of petroleum extract was found on HeLa cells at the incubation period 72 h. The inhibition of K-562 cells proliferation was observed at concentration of 20.0 µg/mL with an IC₅₀ of 20.0 µg/mL after 72 h incubation [34].

Methanol extract of the leaves were tested by topical application at the dosage of 3, 6, 9 mg/20 µL acetone on edema experimentally ethyl phenylpropionate-induced rat. The extract at all concentrations showed the most potent inhibition at 15 min underlying prevention of the release and/or the effect of histamine and serotonin considered as initial inflammatory mediators of this pathway [6]. Oral administration of methanolic extracts at the dosages of 50, 100 and 200 mg/kg on carrageenan-induced edema in the hind paw of male Sprague–Dawley rats was observed. An equivalent volume of 0.5 mL/kg was administered 1 h before the animals were induced edema on the plantar side of the right hind paw by intradermal injection of carrageenan and results showed significant reduction of foot volume by volume displacement technique [6]. Methanolic extracts of *C. nutans* were tested in four cancer cell lines NCI-23, HeLa, K-562, and Raji and no anti-proliferative activity was found. Whereas mild anti-proliferative activity found in IMR32, SNU-1, and LS-174T cell lines. A dosage of 100 µg/mL exhibited (41.88 ± 2.81)% inhibitions in HepG2 cell lines at 100 µg/mL. The dosage at a concentration of 100 µg/mL was tested on umbilical vein endothelial cells and lower percentage of inhibition found compared to the other cancer cells [5].

Treatment of HepA tumor-bearing mouse models with 30% ethanolic extract of aerial part (3 and 10 mg/kg) exhibited antitumor activity compared to the fluorouracil-treated mice as positive control. The same study reported that upsurge of thymus indices, IL-2 and IFN-γ levels in serum which indicate the potential antitumor and immunomodulatory properties of *C. nutans* [35].

Phytosterols are soluble in polar and dipolar solvents while phytostanols, a derivative of phytosterols that are present in non-polar solvent extraction like petroleum ether. In this plant, some of the phytosterols present are stigmasterol, lupeol and β-sitosterol. Phytosterols in combination with other bioactive compounds are able to exert cytotoxic effect against cancer cell lines [36].

5.2. Antioxidant activity

Petroleum ether (82.0%) extract of whole plant strongly scavenged 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) with a concentration of 4.0 mg/mL while methanolic extract of stem produced 70.0% radical scavenging activity at 10.0 mg/mL concentration. The crude ethanol extract within 1–300 µg/mL concentration demonstrated highest scavenging activity of (67.65 ± 6.59)% and with IC₅₀ of (110.4 ± 6.59) µg/mL [37]. The

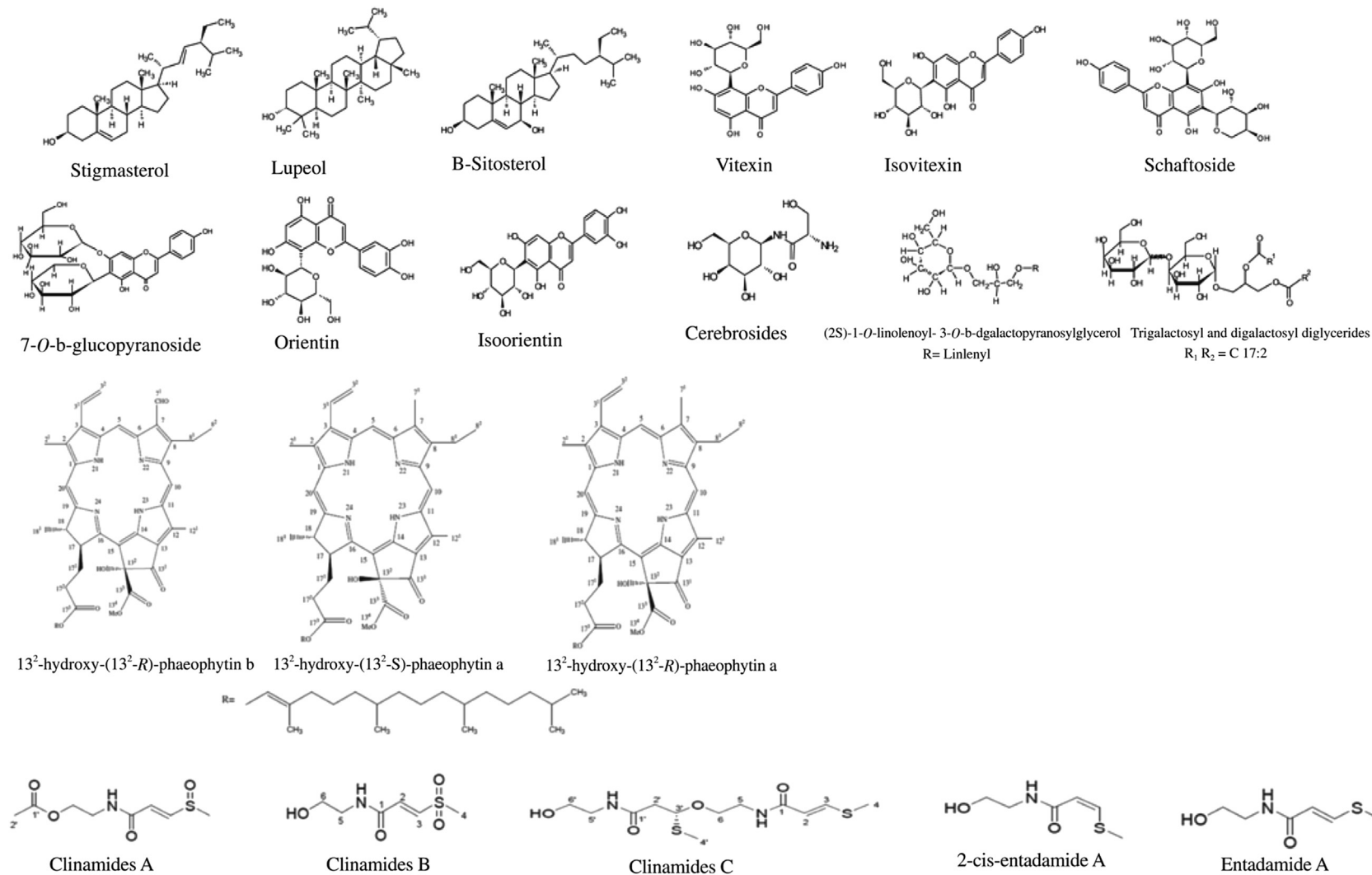


Figure 2. Structure of different bioactive compounds from *C. nutans*.

Table 1
Pharmacological effects of *C. nutans*.

Pharmacological activity	Part use	Extract/fraction/dose tested	Dose tested/route of administration	Animals/cell line culture	Experimental model (<i>in vivo/in vitro</i>)/ clinical trial	Result	Reference
Cytotoxic study	Leaves	Petroleum ether	18.0 and 20.0 µg/mL	HeLa and K-562 cells	<i>In vitro</i>	Strongest cytotoxic activity	[34]
Cholinergic modulation	Leaves	Methanol	250 mg/kg, 500 mg/kg, 1 000 mg/kg bw	Male mice	<i>In vivo</i>	Induce AChE activity	[46]
Anti-papillomavirus infectivity	Leaves	Stock solution in DMSO	Different concentrations in different compounds	293FT cells	<i>In vitro</i>	Can inhibit infection cause by HPV	[19]
Anti-viral activity on VZV	Aerial part	Topical formulation	5 times 7–14 d	Human	Clinical trial	VZV lesion healed and reduce pain scores	[45]
Anti-inflammatory activity	Aerial part	80% ethanol extract	10 µg/mL ethanolic extract	Human neutrophils	<i>In vitro</i>	Strongest elastase release inhibitory effect at 68.33%	[33]
Anti-HSV type 1 activity	Aerial part	Compounds 13,14,15 in DMSO	5.89, 6.21 and 6.21 µM	<i>In vitro</i>	Plaque reduction assay using Vero cell line	100% inhibition activity, IC ₅₀ of each compounds 1.96, 3.11, 3.11 nM	[10]
Dengue virus	Aerial part	80% ethanol extract	31.04 µg/mL	Naïve Huh-7 cells	<i>In vitro</i>	Moderate anti-dengue virus activity	[33]
Acute toxicity study	Leaves	Methanol	300 mg/kg, 600 mg/kg and 900 mg/kg (a) 250 mg/kg, 500 mg/kg	Rats	Rats <i>in vivo</i>	No toxicological effects in liver and kidney (a) Induce AChE activity (b)	[42,43]
Anti-oxidant, protective effect against oxidative induced hemolysis	Leaves, stem	Petroleum ether, methanol	4 mL extract in 1 mL DPPH	–	DPPH assay	Radical scavenging Activity 82% at concentration of 4.0 mg/mL (Leaves) 70.0% of radical scavenging activity of 10.0 mg/mL	[34]

radical scavenging activity of chloroform, methanol and water extracts from leaves were examined using Trolox as a standard and highest antioxidant activity found for chloroform extract (7852.63 ± 449.90) μg Trolox eq/g extract and the antioxidant activity declined in case of methanol and water extract [5]. Moreover nitric oxide scavenging activity observed only in water extract to the extent (32.33 ± 0.97)% using 100 $\mu\text{g}/\text{mL}$ dosage and a relatively mild hydrogen peroxide scavenging activities recorded for chloroform, methanol and water extract [5]. The presence of alkaloids, flavonoids and flavones may be also a possible reason for higher radical scavenging activity [38].

5.3. Immune response activity

The effect of ethanolic extracts of leaves on modulating *in vitro* cell-mediated immune response was studied by observing human competent cells obtained from healthy human with no previous history of immune related complications and none were taking immunosuppressive drugs. Concentrations at a level of 0.5, 2.5, and 5.0 $\mu\text{g}/\text{mL}$ could induce remarkable proliferation of lymphocytes but drastically decrease at 2.5 and 5.0 mg/mL of extracts. The activity of natural killer cells considerably decreased at the concentrations of 1 and 5 mg/mL but the level of interleukin-2 formation extract treated mononuclear cells was unobservable. In case of interleukin-4 it was induced by the extract to the concentration of 2.5 and 5.0 mg/mL [39].

5.4. Anti-herpes simplex viral activity assay

The activity of hexane, dichloromethane, and methanol extracts of leaves were examined on HSV type 1 (KOS), type 2 (Baylor 186) and Vero cells by plaque reduction assay. Every extracts showed HSV-1 and HSV-2 activities with more than 50% inhibition of plaque formation (30 PFU/25 μL) at a concentration of 100 $\mu\text{g}/\text{mL}$. Hexane extract showed moderate inhibition with lowest IC_{50} values of HSV-1 with selectivity index >50.36 and (65.13 ± 2.22) $\mu\text{g}/\text{mL}$ of methanol extract that inhibited HSV-2 with selectivity index >24.59 .

Three different bioactive compounds isolated from *C. nutans* (compounds 13, 14, 15) and their anti-herpes simplex viral activity was examined. For doing anti-HSV-1F activity, the cytotoxicity of all the compounds were investigated and found that 5.89, 6.21 and 6.21 μM of compound 1, 2, 3, respectively were the maximal concentration which were not toxic to Vero cells. The sub-toxic concentrations of each compound were used in anti-HSV-1F study and the concentration of 1, 2 3 compounds exhibited 100% inhibition activity and IC_{50} of those compounds were 1.96, 3.11 and 3.11 nM, respectively. The experiment also assumed that the compounds 1–3 may interfere with the virion envelope structures or mask viral glycoproteins, which are necessary for adsorption and entry into host cell [10].

5.5. Antimicrobial activity

Ethyl acetate fractions from leaves of this plant were tested against *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica* Typhimurium and *Candida albicans* using minimum inhibitory concentration and minimum bactericidal or fungicidal assays. The fractions and crude extracts demonstrated inhibition against

all tested microorganisms within the range between 1.39 mg/mL and 6.31 mg/mL against *B. cereus* and *C. albicans*. Flavonoids and phenolic compounds that are synthesized universally in medicinal plants can induce antibacterial response due to the presence of carbonyl group [40].

5.6. Antivenom activity

This plant has been traditionally used for a long time as a remedy for envenomation snakes or venomous insects like scorpions and bees especially in the southern Thailand and North-Western Malaysia. There was no antivenin activity found by analyzing the ability of the extract to defuse the inhibitory effects of neurotoxins of *Naja naja siamensis* on neuromuscular transmission [41]. A report stated by Watson in botanical medicine that there is other components in the venom of the snake which can be neutralized by the plant extract [20].

5.7. Anti-dengue activity

The ethanolic extract from aerial part of the *C. nutans* showed moderate anti-dengue virus activity in the IC_{50} 31.04 $\mu\text{g}/\text{mL}$. Naïve Huh-7 cells and cultured in Dulbecco's modified Eagle's medium were used in that study [33].

6. Toxicity

The Sprague Dawley female rats were subjected to 14 d oral administration of methanol leaves extract of *C. nutans* at a dose of 300 mg/kg, 600 mg/kg and 900 mg/kg and the result showed no toxicological effect on the liver and kidney that causes injury [42]. The effect of methanol extract of this plant on acetylcholinesterase enzyme activity which is responsible for terminating the cholinergic nerve transmission by hydrolyzing the acetylcholine into choline and acetate was elucidated [43]. The experiment discovered that administration of 250 mg/kg, 500 mg/kg and 1000 mg/kg dose of methanol leaves extract of *C. nutans* on the activity of AChE in Balb/C male was able to modulate cholinergic neurotransmission by activating AChE activity in mice kidney, liver and heart [43]. It was reported that ethanolic extract of *C. nutans* leaves at the highest dose of 1.3 g/kg given orally, subcutaneously or intraperitoneally did not produce any signs of acute toxicity in mice [44].

7. Clinical trial

7.1. Treatment of recurrent aphthous ulcer

A double blind controlled trial was conducted to assess the efficacy of *C. nutans* in orabase for the treatment of recurrent aphthous stomatitis. Total forty-three subjects with aphthous stomatitis complain were hired for the trial and the effect of extract tested against triamcinolone acetonide in orabase and placebo. The result showed that *C. nutans* in orabase provide better healing of the ulcer as compared to placebo but was less so when compared to triamcinolone acetonide in orabase [45].

7.2. Anti-VZV infection

A topical formulation of *C. nutans* extract was prepared and its effect on 51 patients with VZV infection examined through

randomized, placebo-controlled trial. The result was promising crusting on lesion formed within 3 d and healing process occurred within 7 d. The patients were treated 5 times with the preparations per day for 7–14 d until lesion healed and the pain scores also reduced significantly. There were no side effects detected during the progress of treatment [45].

8. Conclusion

C. nutans has been broadly used as traditional medicine in several countries in Asia. All parts of this plant have been used in the treatment and prevention of several complications especially for viral infection, cancer and skin inflammation caused by insect bites. Flavonoids are the main bioactive compounds in this plant and different extracts have been found to possess biological activity. Less toxicity of this plant represent the possible uses as therapeutic remedy for several ailments.

This review has presented a comprehensive view about the phytochemistry and pharmacology of *C. nutans*. However the research is very limited in some areas and further study on phytochemicals and their mode of actions revealing pharmacological effects are required to fully understand in concern with the traditional uses. In addition majority of medicinal studies were conducted using crude and poorly other solvent extracts. In such case more bioactive compounds should be identified through bioassay guided isolation. More clinical studies on the toxicity of extracts from different parts and the isolated compounds from this plant need to be assessed for ensuring the safe application as modern medicines.

Conflict of interest statement

We declare that we have no conflict of interest.

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