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Pharmacological effects of *Clinacanthus nutans* Lindau and its potential cosmeceutical values: A comprehensive review

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Abstract

Clinacanthus nutans is largely cultivated in Asia tropical regions and predominantly native to Malaysia, Indonesia, Thailand and China. It is an important species from the Acanthaceae family and regarded as one of the primary contributor to the medicinal plants. This plant had been traditionally used as treatment of poisonous snake bites, skin rashes, dysentery, haematoma, rheumatism and injuries. Public Health Ministry of Thailand had used it as the primary therapy for lesion treatment due to virus. Phytochemicals recovered from this plant are chlorophyll derivatives, flavones, isoflavones and sulphur-containing glucosides. They exhibited various pharmacological effects such as anti-herpes simplex virus, anti-inflammatory and anti-cancer via various mechanisms. They also can potentially be exploited as the main ingredients in the cosmetic products including the lightening, anti-aging as well as anti-acne products since it exhibited the presence of compounds that had been proven to have anti-tyrosinase activity (melanin reduction), anti-oxidants (protection from UV radiation), anti-microbial towards *Propionibacterium acnes* and anti-inflammatory activities.

Keywords: Anti-cancer; anti-herpes simplex virus; anti-inflammatory; *Clinacanthus nutans*; cosmetic values.

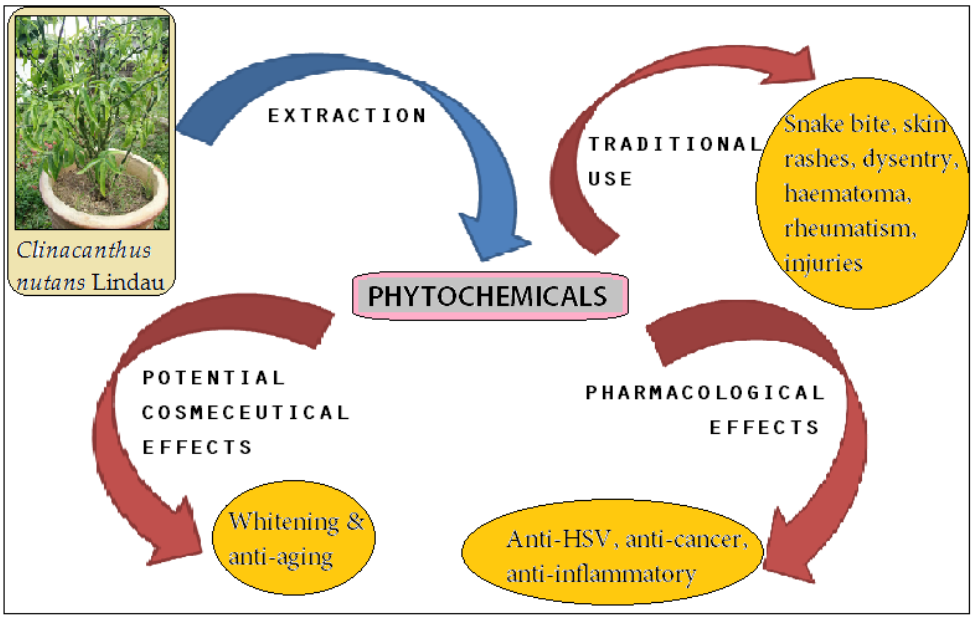

Figure 1: Graphical abstract

INTRODUCTION

Clinacanthus nutans (*C. nutans*) Lindau or Sabah Snake Grass (Figure 2) is a plant with the characteristics of tall, erect and usually form rambling bushes. *Clinacanthus nutans* plant is predominantly native to Malaysia, Indonesia, Thailand and China and it is largely cultivated in tropical regions in Asia including Southeast Asia [1]. This plant has several vernacular names. For example, in Malaysia, this plant is known with the name of Belalai Gajah while in Thailand, it is known as Saled Pangpon Tua Mea and Phaya Plongtong. Its vernacular name in Indonesia and China are Dandang Gendis and Ezuihua, respectively [2, 3]. *Clinacanthus nutans* (*C. nutans*) Lindau is an important species belonging to one of the primary families of flowering dicotyledonous plant which is Acanthaceae family [3]. Table 1 shows the details of *C. nutans*'s taxonomy [2, 4].

Table 1: Taxonomic classification and nomenclature of *C. nutans*.

Taxonomic Classification	Nomenclature
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales Bromhead
Family	Acanthaceae Juss
Genus	*Clinacanthus* Nees
Species	*nutans*- Lindau

Figure 2: *Clinacanthus nutans* or also known as *Belalai Gajah*

Properties of C. nutans

Other than *C. nutans*, there are about 2500 species and 250 genera included in the family of Acanthaceae and most of them are twinning vines, bushes and tropical plants [4]. Regarding Acanthaceae, it comprises of several subfamilies which are Acanthoideae, Avicennioideae, Nelsonioideae and Thunbergioideae [5]. Typically, the plants under this family are originated from pantropical and subtropical species which may be obtained in Madagascar, Indo-Malaysia, Brazil, Africa as well as Central America while few species can be found in Mediterranean, China, Australia and the United States [5, 6] .

Since they are originated from same families, the plants under the umbrella of Acanthaceae have a number of shared features for instance the leaves are organised in opposite sets (decussate), estipulate and normally entire-margined, with round to quadrangular stems, zygomorph flowers and superior ovary [3, 6]. Some of the shared features of Acanthaceae family as mentioned previously can be observed in the *Clinacanthus nutans* and its morphologies can be viewed in Table 2 [6-8].

Table 2: Morphologies of *C. nutans* (Deng et al. 2012; Ying 2013).

Morphology	Description
General	Perennial herbs and the maximum height is 1 metre with pubescent branches
Stems	Torete, striate and glabrescent
Leaves	Simple, opposite, narrowly elliptic oblong or lanceolate; apex – acute or acuminate, base – cuneate, obtuse, rounded or truncate; leaf blade – lanceolate-ovate, lanceolate or linear-lanceolate; surfaces – pubescent when young then glabrescent; petioles – sulcate, bifariously pubescent and 0.3-2 cm long.
Flowers	located in dense cymes (5-8 flowers) at the top of branches and branchlets; calyx – densely glandular-pubescent; stamens – two, inserted in the throat, more or less appressed against the upper lip; ovary is compressed, 2-celled, 2 ovules in each cell
Capsule	Oblong, basally contracted into a short, solid stalk, 4-seeded

Ethnobotanical use of Clinacanthus nutans as traditional medicine

Ethnobotany is the study on the way ethnics of a particular region and culture exploiting and making use of particular local plants [9]. Acanthaceae family is popular for its vast variation of its tropical, subtropical environments and some of them in temperate regions. The major areas of its distribution are Malaysia, Indonesia, Africa, Brazil and Central America [10]. Acanthaceae family is regarded as one of the primary contributor to the medicinal plants. They usually serve as the effective traditional remedies to counter particular health disorders [3]. As for *Clinacanthus nutans*, one of Acanthaceae members, also possesses medicinal values in which this plant had been initially used to treat poisonous snake bites, skin rashes and lesions [2, 11]. Thus, one of its major ethnobotanical use is as the traditional medicine in several countries as mentioned previously [3]. In Indonesia, *Clinacanthus nutans* is usually used in the form of decoction (fresh leaves boiled with hot water) for the treatment of diabetes mellitus, difficulty urinating and bowel inflammation (dysentery) [1].

In Thailand, the leaves of *C. nutans* have been freshly benefited for relieving of insect bites and skin rashes and the leaves extracts have been traditionally utilised for the herpes simplex virus (HSV) infection treatment in primary health care in Thailand [4, 12]. Ministry of public health of Thailand even adopted this plant as the primary therapy for the skin inflammations and lesion treatments due to virus. *C. nutans* also is a popular anti-snake venom and remedy for scorpion bites among the traditional practitioner of Thailand which is believed to be attributed to the cell lysis effect [3].

Other than Thailand and Indonesia, this plant also has been widely used in China where it is applied for the treatment of inflammation and/or tenderness due to bruises on eye, haematoma, anxieties, rheumatism and injuries [1]. In Malaysia, the fresh leaves are prepared by infusion by boiling the fresh leaves with water and drank as herbal tea [13]. It is also used in the treatment of skin rashes, snake bites, lesions caused by herpes simplex virus, diabetic myelitis, fever and diuretics [2].

Phytochemistry review of Clinacanthus nutans

Several phytochemistry studies from *Clinacanthus nutans* had been screened and it showed that this plant, specifically the leaves part contains a vast variety of bioactive compounds that exert beneficial effects as mentioned previously [2, 3, 14]. The example of the bioactive compounds that have been isolated from *C. nutans* include the chlorophyll derivatives (pheophytin) which originated from the leaves of *C. nutans* by using chloroform as solvent of extraction [3, 15, 16]. The pheophytins from chloroform extract such as 13^2-hydroxy-(13^2-*R*)-phaeophytin b, 13^2-hydroxy-(13^2-*S*)-phaeophytin a, and 13^2-hydroxy-(13^2-*R*)-phaeophytin a, had been demonstrated to have anti-herpes simplex activity [16, 17].. Other than that, the other compounds related to chlorophyll (derivatives) and had been found in chloroform extract of *C. nutans* leaves are 13^2-hydroxy-

(13^2-S)-chlorophyll b, 13^2-hydroxy-(13^2-R)-chlorophyll b, 13^2-hydroxy-(13^2-S)-phaeophytin b [3, 16].

Other than chlorophyll derivatives, the other major phytochemical discovered from this plant is C-glycosyl flavones (subclass of flavonoid group) which consist of shaftoside, orientin, isovitexin, and vitexin [4, 15, 18]. These compounds hold crucial bioactive activities for instance; antimicrobial activity exerted by isoorientin, vitexin; hepatoprotective activity of isoorientin, and antioxidant activity showed by isovitexin [15, 19, 20]. Then, the other phytochemical that may be extracted from *C. nutans* is sulfur-containing glucosides. According to previous studies, these compounds had been isolated from the leaves and stem of this plant by butanol soluble fraction of the methanol extract. The compounds are clinacoside A, clinacoside B, clinacoside C, cycloclinacoside A1 and cycloclinacoside A2 [4, 21, 22].

In one study conducted by Yong et al. discovered that the chloroform extract of *C. nutans* exhibit potent anti-oxidant activity and the gas chromatography-mass spectrometry (GC-MS) analysis had discovered fourteen phytochemical constituents with the major compound namely 1,2-benzenedicarboxylic acid,mono (2-ethylhexyl) ester (28.6%) [23]. Other than that, a study conducted by Che Sulaiman et al. had discovered 28 phenolic and fatty acids with some major compound such as alpha tocopherol, stearic acid, palmitic acid and linoleic acid from ethyl acetate extract of *C. nutans* leaves [14]. Cinnamic acid, protocatechuic acid, caffeic acid, ferulic acid, and chlorogenic acid had been screened in the phenolic composition analysis of *C. nutans* extracts in which these compounds exhibit potent anti-oxidant activities [24]. The summary of phytoconstituents present in *C. nutans* had been presented in Table 3.

Table 3: Phytochemicals screened from *C. nutans* plant.

No	Phytochemical group	Phytochemical	Reference
1	Chlorophyll derivatives (pheophytin)	i. 13^2-hydroxy-(13^2-*R*)-phaeophytin A ii. 13^2-hydroxy-(13^2-*R*)-phaeophytin B iii. 13^2-hydroxy-(13^2-*S*)-phaeophytin iv. 13^2-hydroxy-(13^2-S)-phaeophytin B v. 13^2-hydroxy-(13^2-S)-chlorophyll B vi. 13^2-hydroxy-(13^2-R)-chlorophyll B	Sakdarat et al. 2006; Sakdarat et al. 2009; Chelyn et al. 2014; Alam et al. 2016
2	C-glycosyl flavones	i. Shaftoside ii. Orientin iii. Isovitexin iv. Vitexin	Teshima et al. 1997; Chelyn et al. 2014; Kumar et al. 2015
3	Sulfur-containing glucosides	i. Clinacoside A ii. Clinacoside B iii. Clinacoside C iv. Cycloclinacoside A1 v. Cycloclinacoside A2	Teshima et al. 1998; Kumar et al. 2015; Mustapa et al. 2015
4	Phenolic acid	i. Alpha tocopherol ii. Stearic acid iii. Palmitic acid iv. Linoleic acid v. Cinnamic acid vi. Protocatechuic acid vii. Caffeic acid viii. Ferulic acid ix. Chlorogenic acid	Che Sulaiman et al. 2015; Sarega et al. 2016

Pharmacological effects of *clinacanthus nutans*

Anti-herpes simplex virus activity of Clinacanthus nutans

Recently, acyclovir and other nucleoside derivatives are drugs of choice for HSV infections treatment [12]. Acyclovir is a guanosine analogue that will be mistakenly phosphorylated by HSV-encoded thymidine kinase, the second and third phosphate groups. The triphosphorylated nucleotide will specifically block the DNA polymerase of the HSV in growing viral DNA chain which lead to chain termination and inhibition of replication [25]. Other than conventional medicines, many of chemical compounds can be found in plants (medicinal plants) they are well-known to be used traditionally and in healthcare in many countries. An enormous number of plants, have been confirmed to demonstrate anti-viral activity [26, 27]. It was discovered that medicinal plant are good sources for natural products that possess promising anti-HSV drug including *C. nutans* [12].

Extracts from fresh leaves of *C. nutans* have been traditionally used for treatment of viral infection specifically herpes simplex virus. The extracts even is used for the treatment of the infection in primary care in Thailand [2 -4]. Herpes simplex virus can be described into two distinctive serotypes with 40% sequence homology, which are type 1 (HSV-1) and type 2 (HSV-2) infections [28]. The reported assay of the anti-HSV activity by *C. nutans* is done by infecting the vero cells (lineage of cells used in cell cultures) with both HSV-1 and HSV-2 which then incubated to let the plaque of HSV is formed. The plaque reduction assay was performed by comparing the plaque count on the vero cell with the treatment of *C. nutans*'s extracts, fractions or purified compounds and without the treatment to discover the plaque formation inhibition potency [12]. In a study, at non-toxic concentration, glycoglycerolipids which are monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) purified from chloroform extract from *C. nutans* exhibited 100% replication inhibition of HSV-1 and HSV-2 at post-infection stage. MGDG and DGDG from *C.nutans* exerted similar action as acyclovir in the treatment of HSV infection, which is by inhibiting the virus replication. However, the specific mechanism of how the glycoglycerolipids MGDG and DGDG inhibit the replication of HSV is till now not very clear [29]. However, it was suggested monoglyceride had been reported to exhibit anti-viral effects against viruses by damaging its envelopes which suggested that MGDG and DGDG are the possible recent drug candidates for anti-HSV [30, 31]. The HSV envelope disruption can result to the permanent inhibition of infectivity and also its virulence by avoiding the formation of fusion pore and thus the entry of genome-containing capsid into the cytoplasm [32. 33]. Other than chloroform extract, hexane, dichloromethane and methanol extract also had been studied on both HSV serotypes and all the extracts showed anti-HSV-1 and anti-HSV-2 with more than 50% plaque formation inhibition [3].

Anti-inflammatory activity of C. nutans

Inflammation is a self-limiting and defensive mean involving immune response against damaging stimuli such as tissue injury, microbial infection and any other deleterious conditions [34, 35]. However, chronic inflammation and other pathogenic event may be resulted from out-of-control inflammation for instance the chronic retention of pro-inflammatory cytokines at the site of inflammation [36]. *Clinacanthus nutans* had been used traditionally for the treatment of inflammation and many of the articles discussed about this plant mentioned about the popular use of this plant's leaves as anti-inflammatory [17, 20, 23, 37].

The extract of *C. nutans* by 80% ethanol exhibited anti-inflammatory as well as immune-modulating activity. The phytochemical investigation of this extract in this study revealed that the compounds that may contribute to the mentioned activities are clinamides A, B, C and 2-*cis*-entadamide A [38]. In a study that utilise two *in vivo* models of neutrophil-dependent acute inflammation (carrageenan-induced paw oedema and ethyl phenylpropiolate-induced ear oedema in rats) to investigate the effects of extracts on human neutrophil responsiveness reported that the methanol extract of *C. nutans* whole plant showed potent dose-dependent inhibitory activities in both edema models. There was a significant reduced in the myeloperoxidase activity signifying the anti-inflammatory activity of the extract was observed [39, 40].

The mechanism of action of anti-inflammatory activity of *C. nutans* extracts from leaf and stem parts by using polar and non-polar solvents was also studied. The ability of the extracts to inhibit the activation of toll-like receptor-4 (TLR-4) thus blocks the production of nitric oxide (NO) and induction of nuclear factor-κB (NF-κB) was determined by Mai et al., 2016. The inhibition of those events reduced the pro-inflammatory cytokines and cascades thus inflammation per se. In this study, all the extracts which contained phenolic and flavonoid compounds exhibited the inhibition of those mentioned events and reduce the production of cytokines. Thus the mechanism of anti-inflammatory effect of this plant extract are likely due to the inhibition of TLR-4, reduction of NO production and retardation of NF-κB induction [40].

In another study that also aimed in elucidating the mechanism of action of anti-inflammatory effects of *C. nutans* extracts reported that *C. nutans* ethanolic leaf extract which might contained clinamides A-C, 2-cis-entadamide A, entadamide A, entadamide C, and trans-3-methylsulfinyl-2-propenol inhibit arachidonic acid inflammation pathway (figure 3) by modulating the induction of cytosolic phospholipase A_2 (PLA_2) expression by histone deacetylase [38, 41]. From phospholipids, PLA_2 stimulates the generation of arachidonic acid and formation of pro-inflammatory eicosanoids [41]. According to figure 3, the production of arachidonic acid is the initial and main step of the arachidonic acid pathway that leads to the production of pro-inflammatory mediators such as leukotrienes, prostaglandins and thromboxanes by the metabolism of lipooxygenase (LOX) and cyclooxygenase (COX) enzymes. Since COX can induce a wide range of inflammatory stimuli, it becomes the potential target in the development of anti-inflammatory drugs (NSAIDs) [42]. Therefore, as *C. nutans* inhibits the level of PLA_2 expression, there will be no activation of arachidonic pathway thus reduces the inflammatory biochemical mediators [41. 42]. As mentioned before, C-glycosyl flavones (subclass of flavonoid group) which consist of shaftoside, orientin, isovitexin, and vitexin had been identified in *C. nutans* [4, 15, 22]. Flavones (apigenin, chrysin and luteolin) had been proven to be the inducer of inflammatory gene modulator which is peroxisome proliferator activated receptor gamma (PPARγ) and inhibits the pro-inflammatory enzymes activities such as the metabolism of cyclooxygenase-2 (COX-2) in the arachidonic acid pathway as well as nitric oxide synthase [43].

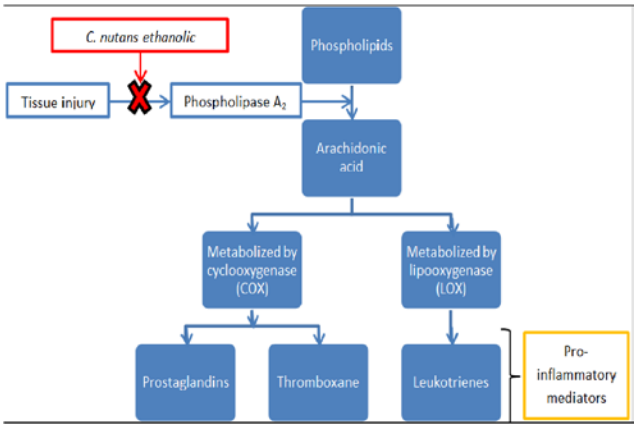

Figure 3: Arachidonic acid pathway of inflammation

Anti-cancer activity of C. nutans

Figure 4 shows that the escalated level of free radicals that eventually caused the cancer pathology. Several studies had shown that elevation of free radicals level in cells such as reactive oxygen species (ROS) or reactive oxygen nitrogen (RONS) is closely related to the increase process of tumour generation (tumorigenesis) [23, 44, 45]. Normally functioned cells maintain ROS level inside a non-toxic range by regulating and maintaining the its generation as well as its scavenging systems [44]. The excessive and chronic increase of ROS endogenous level and other free radicals will lead to adaptive alterations that contribute to the tumorigenesis, metastasis, and drug resistance in various categories of cancer cells [44, 45].

Apart from the evidence of free radicals increases the cancer growth and expansion, uncontrolled free radicals generation also can result in the adverse injury to DNA, lipid and cellular protein. This will result in the instability of genome and eventually promotes cancer formation [44, 46]. In addition, the activation of oncogenes that occurs in cancer cells can cause the significant increase in RONS level. The surge of RONS will worsen the condition of the cancer and promotes its expansion [46]. Since extract of *C. nutans* has high potential of scavenging free radicals, it

can be utilised to prevent all the adverse impact of chronic high level of free radicals as mentioned previously (tumorigenesis, metastasis and drug resistance) [23, 47].

The study had been done to evaluate *C. nutans*' potential to be utilised for the prophylaxis as well as treatment of cancer. This study had been done after this plant gained popularity and said to be useful for anti-tumour effects as been widely reported in Malaysian newspaper claiming that this plant had saved many various cancer types [23]. In this study, it had been reported that the chloroform extract of *C. nutans* (with the major constituent of 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester) showed high anti-oxidant and anti-proliferative activities against liver hepatocellular carcinoma, human lung cancer, human gastric cancer, human colon adenocarcinoma, human erythroleukemia, human cervical cancer and human Burkitt's lymphoma cell lines. The possible mechanism of *C. nutans* extract exerted anti-cancer activity is by utilizing its anti-oxidant ability to scavenge the free radicals (ROS, RONS) and shield normal healthy cells from DNA damage and restrict the advancement of cancer cells [23].

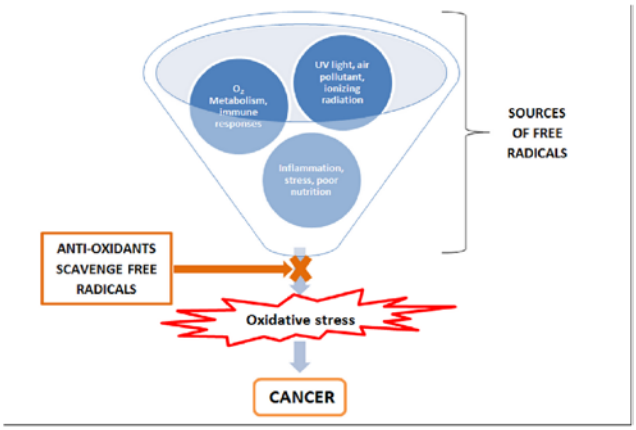

Figure 4: Cascade of cancer pathology due to the sources of free radicals

Other than the current mechanism of anti-cancer drugs, another potential mechanism of anti-tumour agents is by exerting the effect of replacing the immunosuppressive activities caused by tumour cells by stimulating immune effectors [48, 49]. *C. nutans* also can exert other mechanism of anti-tumour effects which is by up-regulation of immune response. Huang et al. (2015) investigated the immunomodulatory activity and anti-tumor of *C. nutans* 30% ethanol extract containing shaftoside, orientin, vitexin, isoorientin, isovitexin and 6,8-apigenin-C-α-l-pyranarabinoside towards Hepatitis A tumour-bearing mouse [19]. The result of this study showed that 30% ethanol *C. nutans* extract exhibited anti-tumour activity by indirectly enhancing the immunologic functions since in this study, the extract considerably increases the immune effectors such as the thymus indices, interferon gamma and interleukin-2 levels in the serum of the mouse [19]. Other than cytotoxic effect, anti-tumour agents also stimulate host immune system and this effect cooperates with the cytotoxic effect of the agents for successful tumour elimination [49, 50]. The pharmacological effects of different extracts and compounds from *C. nutans* is summarized in Table 4.

Table 4: Pharmacological activities of extracts and compounds from *C. nutans*.

Phamacological activity	Extraction method / bioactive compound	Mechanism of action / receptor involved	Reference
Anti-herpes simplex virus	i. Monogalactosyl diglyceride ii. Digalactosyl diglyceride	Exhibited 100% replication inhibition of HSV-1 and HSV-2 at post-infection stage. The specific mechanism and receptor involved are not clear.	Pongmuangmul et al. 2016
Anti-inflammatory	Ethanol extract (Clinamides A-C, entadamide A, entadamide C, trans-3-methylsulfinyl-2-propenol)	Arachidonic acid inflammation pathway by modulating the induction of cytosolic PLA_2 expression	Tu et al. 2014; Tan et al. 2016
	i. Phenolics ii. Flavonoids	Inhibit the activation of TLR-4 thus blocks the production of nitric oxide (NO) and induction of NF-κB (reduce pro-inflammatory reaction).	Mai et al. 2016
	i. Apigenin ii. Chrysin iii. Luteolin	Inhibits the pro-inflammatory enzymes activities such as COX-2 and nitric oxide synthase by modulating PPARγ	Shim et al. 2013
Anti-cancer	Chloroform extract (major constituent: 1,2-Benzenedi carboxylic acid, mono(2-ethylhexyl) ester)	Scavenging of the free radicals (ROS, RONS) and shield normal healthy cells from DNA damage and restrict the advancement of cancer cells.	Yong et al. 2013
	30% chloroform extract (shaftoside, orientin, vitexin, isoorientin, isovitexin and 6,8-apigenin-C-α-L-pyranarabinoside)	Exerts anti-tumor activity by increasing the immune effectors such as the thymus indices, interferon gamma and interleukin-2 levels	Huang et al. 2015

Potential cosmeceutical effects of *c. Nutans*

Potential whitening effect of C. nutans extracts

The common and well-known whitening agents that widely being used in market are hydroquinone, kojic acid, arbutin, magnesium ascorbyl phosphate, licorice extract, aloesin, azelaic acid, soybean extract and niacinamide [51, 52]. The typical mechanism of whitening agents is by inhibiting tyrosinase activities, which is the key enzyme in the melanin biosynthesis (Figure 5). To be more specific, the most common target for the down-regulation of melanogenesis is direct inhibition of tyrosinase enzyme activity [51].

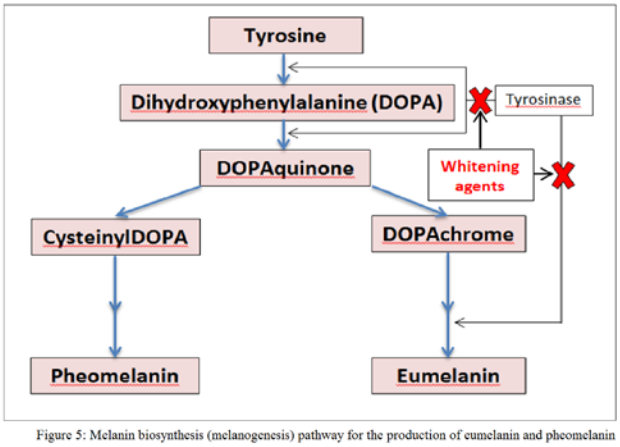

Figure 5: Melanin biosynthesis (melanogenesis) pathway for the production of eumelanin and pheomelanin

Other flavonoids had mostly contribute to the discovery of the newly discovered natural tyrosinase inhibitors for example biochanin A which directly attacks the tyrosinase

activity [51]. Several other product that had chemical structure and activity similar to biochanin A are calyosin (isoflavone), taxyfolin [53] and luteolin [54]. Other than directly inhibiting the tyrosinase activity, there are also whitening agents that exert different mechanism with same outcome which are by accelerating the tyrosinase degradation for instance linoleic acid [51].

For *C. nutans* plant, there is a phytochemical screening study that discovered the above mentioned whitening compounds that make it a potential natural source of lightening agents. There were several whitening compounds can be pointed out from the result of this screening which are quercetin, rutin, protocatechuic acid, cinnamic acid, caffeic acid, p-coumaric acid (p-CA), vanillic acid and linoleic acid [11]. These potential compounds had been proven to possess whitening effect via several studies that had been done for the screening of natural anti-tyrosinase activity from plants extracts for example the p-CA. It is reported to be a typical secondary metabolite of various plants and its structural similarity with tyrosine make it able to compete with tyrosine for the active site of tyrosinase and directly inhibit its activity. Other than p-CA, cinnamic acid and caffeic acid are also structurally similar to tyrosine and have the anti-tyrosinase activity yet their potencies are weaker than p-CA [51, 55]. Another compound that also can be found in *C. nutans* plant is quercetin which is a well-known natural flavonoid that had been demonstrated to be a strong tyrosinase inhibitor [56].

As mentioned above, the phytochemical screening study also discovered linoleic acid, vannilic acid and protocatechuic acid in *Clinacanthus nutans.* These compounds also had been proven to exert whitening effect. However, they applied different mechanism as compared to p-CA, cinnamic acid and caffeic acid (direct inhibition of tyrosinase activity). The other mechanism that produce the same whitening outcome is by indirectly diminishes the tyrosinase enzyme activity by accelerating the tyrosinase degradation as exerted by linoleic acid [51].

Linoleic acid had been proven to specifically increase the ubiquitin-dependent degradation which eventually lead to the acceleration of enzyme (tyrosinase) degradation [57]. The other anti-melanogesis mechanism reported is the inhibition of tyrosinase gene expression. Example of compounds that had been discovered to adapt the mechanism are vanillic acid and protocatechuic acid [51].

In a phenolic composition analysis of the leaves of this plant, it was reported that *C. nutans'* leaves aqueous-methanol and hot aqueous extracts possess a compound as being mentioned before which are protocatechuic acid [24].

Potential anti-aging effect of C. nutans

The aesthetic problem of aging skin can be prevented and solved by using anti-aging product. It can prevent and treat aging skin. Aging skin is characterised by diminished skin elasticity (wrinkles), increased skin roughness (skin dryness) and patchy skin tone (pigmentation disorder) [58, 59]. The main factors that cause this skin condition (skin aging) are escalation of reactive oxygen species (ROS) due to the ultraviolet radiation exposure [60], skin dryness due to the compromised skin barrier [61, 62] and elevation of melanin production due to chronic sunlight exposure [63].

The integrity of human skin is contributed by extracellular matrix (ECM) protein which composed of collagen, elastin, fibronectin and proteoglycan in which dermal collagen plays the major role [62]. Skin aging develops as one ages or is exposed to ultraviolet radiations (UVR) with no treatment is adopted [60]. UVR and ROS potently induce matrix metalloproteinase-1 (MMP-1) which is an enzyme that has the ability to fragment the collagen that will make cleavage to the collagen fibrils and cause skin problems such as aging, lesion and cancer [62]. Therefore, the ingredients used for anti-aging effect are stated to avoid oxidative reactions thus the formation of free radicals and eventually reduction in the fragmentation of collagen.

Numerous compounds including plant extracts, anti-oxidants, proteins, retinoids, sunscreens and hydroxy acids are known to avert oxidative reactions by inhibiting the production of free radicals thus support the collagen and elastin synthesis [64]. One of the ingredients usually used in the anti-aging product is antioxidant [58, 60, 64]. Various entities have been studied to discover their beneficial effect against ROS-causing injury thus exert notable anti-aging effect on skin including the extracts from *C. nutans* [60]. Petroleum ether extract of the *C. nutans leaves* (4mg/mL) together with its methanol stem extracts (10mg/mL) exhibited high radical scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) by 82% and 70% respectively [1]. In another study, chloroform extract of *C. nutans* leaves (major compound was 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester) capable of exerting potent anti-oxidant effect by the high scavenging activity for DPPH as well as galvinoxyl which were 7852.63 ± 449.90 μg Trolox eq/g extract and 12248.82 ± 173.50 μg Trolox eq/g extract. In the same study, nitric oxide scavenging activity of 32.33 ± 0.97% had been exhibited by 100 μg/mL of *C. nutans* aqueous extract [23]. By benefiting the anti-oxidant effect of *C. nutans* extracts such as the phenolic compounds [7], cosmetics such as anti-aging can be developed since anti-oxidant topical application is one of the approaches for anti-aging [58].

Protocatechuic acid (PCA)-enrich extract had been recovered from this plant by using maceration method followed by liquid-liquid extraction [24]. This compound, PCA, has the potential to have cosmeceutical values which are potent anti-oxidant and skin lightening agent [65]. Both skin lightening effect and anti-oxidant are parts of the approaches widely used in the anti-aging cream since they can correct the feature of aging skin which are patchy skin tone and wrinkles [64, 66].

Furthermore, the toxicity study also had been conducted and revealed that the extracts from the *C. nutans* showed no toxicity [3]. Both acute and sub-acute toxicological studies done also showed no toxicity towards the tested subjects (mice) [4, 67–69]. Therefore, due to the properties of non-toxicity as well as the existence of beneficial

phenolic compounds, this plant can be potentially used in cosmetics that exert beneficial cosmeceutical effects such as antioxidant, lightening and anti-aging.

Potential anti-acne effect of C. nutans

Acne development is a problematic skin condition that caused by multiple factors in which one of them is abnormal sebum overproduction. Other than that, the modification of lipid composition, hyperkeratinisation, sebaceous follicles obstruction by abnormal keratinization and microbial colonization of pilosebaceous units by Propionibacterium acnes that encourages perifollicular inflammation will cumulatively cause the development of acne [70, 71].

The pathogenesis of acne development is begun when the minute holes also called as pore on skin surface become clogged. Each of the pore on the skin surface opens to a follicle that comprises a hair and an oil gland (sebaceous gland) (see Figure 6). However, when the glands secrete overly amount of oil, the pores become clogged. The blocked pores lead to the build-up of cell, dirt and bacteria (Propionibecteria acne) [72]. Eventually caused increased inflammation occurred primarily due to the activation of the adaptive immune system [73]. The blockage is known as comedone which can be in two types; closed comedone (whitehead) and opened comedone (blackhead) [74]. Whitehead is characterised by the whitish raised bumps due to the collection of oil and skin in pores. While, blackheads are small, dark spots on the skin surface which are caused by a small plug in the follicle opening (pore) [75]. Comedone is said to be the first stage of acne progression before the infection by *Propionibacteria acnes* which cause the inflammation to be worsen. The swelling and red bumps of acne occurs after the breaking open of the comedone [73].

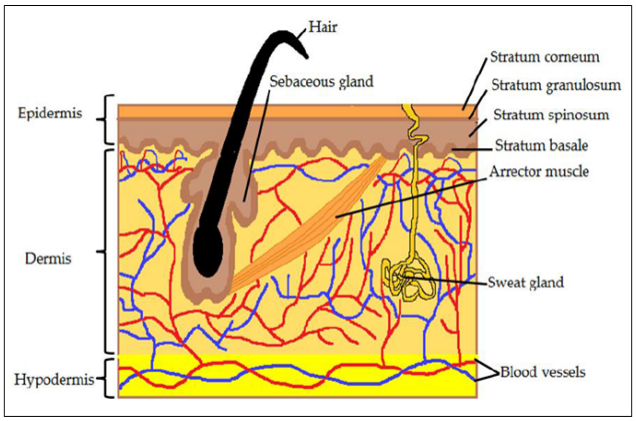

Figure 6: Illustration of sebaceous gland and other skin structures.

The goals of therapy of the treatment of acne are the recovery of inflammatory lesions, avoidance of insistent inflammation and prevention of relapse of comedo formation. Thus, agents with both antimicrobial and anti-inflammatory activities are highly efficacious for acne treatment [76]. There are many plant extracts that had been proven to have the anti-microbial and anti-inflammatory activities in which the extracts can be utilized to be included in the anti-acne cosmetics since they have the potential to cover the goals of therapy in acne treatment. Some of the examples of medicinal plants in which their extracts possess both desired activities are *Aristolochia indica*, *Melilotus indicus*, *Tribulus terrestris* and *Cuscuta pedicellata* [77]. Other than that, 70% ethanolic extract of stem bark and leaves of *Pterygota macrocarpa* and *Cola gigantean* showed anti-microbial activity and significant anti-inflammatory effect [78].

As for *Clinacanthus nutans*, there were several studies that investigated the capability of its extracts for the anti-microbial as well as anti-inflammatory capacities. A study conducted by Yang et al. had revealed that the methanolic extract of *C. nutans* had been screened to have several phytochemical groups of saponin, phenolics, phytosterols, diterpenes and flavonoids. This methanolic extract of *C. nutans* leaves had been shown to have anti-microbial effect against several bacteria strains which are *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Escherichia coli* [79]. As mentioned before, *Propionibacterium acnes* had contributed in the development of acnes and aggravate the pro-inflammatory mediators which leads to persistent inflammation at the site of the acnes [80]. Furthermore, aside of having anti-microbial capacity towards specific microbe contributing to acne development, *C. nutans* extracts had been discussed preciously to have anti-inflammatory activity which can be benefited for the inflammatory lesion of acnes. Thus, via the inclusion of *C. nutans* in face cream, an anti-acne cream can be possibly developed. The summary of cosmeceutical value, the bioactive compounds or extract involved and mechanism of action is outlined in Table 5.

Table 5: Mechanism of action of cosmeceutical value of *C. nutans*.

Cosmeceutical value	Bioactive compound / extract	Mechanism of action / receptor involved	Reference
Whitening effect	i. Caffeic acid[a] ii. p-Coumaric acid (p-CA)[a] iii. Cinnamic acid[a] iv. Quercetin[b]	Structural similarity with tyrosine makes them able to compete with tyrosine for the active site of tyrosinase and directly inhibit its activity[a].	Che Sulaiman et al. 2015[a,b]; Chang 2012[a]; Kim 2015[a]; Choi & Shin 2016[b]
	i. Linoleic acid[a,b]	Specifically increase the ubiquitin-dependent degradation which eventually lead to the acceleration of enzyme (tyrosinase) degradation[b]	Che Sulaiman et al. 2015[a]; Kim 2015[b]
	i. Vannilic acid[a,b] ii. Protocatechuic acid[a,b,c]	Inhibition of tyrosinase gene expression[a]	Chang 2012[a]; Che Sulaiman et al. 2015[b]; Sarega et al., 2016[c]
Anti-aging	i. Petroleum ether leaves[a] ii. Methanol stem[a] iii. Chloroform leaf[b] iv. Methanol leaf[b]	Avoid oxidative reactions thus inhibit formation of free radicals that can potently induce MMP-1 (an enzyme that has the ability to fragment the collagen) and cause skin problems such as aging.	Arullappan et al. 2014[a] Yong et al. 2013[b]
Anti-acne	Methanol leaves (saponin, phenolics, phytosterols, diterpenes and flavonoids)	Anti-microbial effect against several bacteria strains especially *Propionibacterium acnes* which promote the development of acne and pro-inflammatory mediators.	(Yang et al. 2013)
	(Refer to Table 4 on Anti-inflammatory activity)		

CONCLUSION

It is well known that *Clinacanthus nutans* had been widely used traditionally in Asia especially in the tropical regions and even had been adopted as a primary therapy by Public Health Ministry of Thailand. Its popularity had triggered a number of research groups to thoroughly study on its phytochemicals and pharmacological effects that may contribute to the discovery of new alternatives in medicinal field. This article had reviewed several studies about this plant's phytochemical screenings as well as studies that focused on the pharmacological properties of *C. nutans* that had been widely studied namely, anti-herpes simplex virus, anti-inflammatory and anti-cancer activities from the extracts of this plant. However, there is no study that isolate any compounds from this plant which help to discover the true compound that actually contribute to its pharmacological effects. Therefore, the study that isolate and screen for the compound's pharmacological activity from this plant may highly contribute to the discovery of alternatives in medicinal industries. Other than pharmacological benefits, *Clinacanthus nutans* also can be potentially used for cosmetics although there is no study that focuses on its cosmeceutical effect. Further study should be done to investigate the cosmeceutical benefits that this plant may potentially have since many discovered phytochemicals from this plant had been studied to own cosmeceutical values such as anti-oxidants that can be exploited to develop anti-aging products as well as phenolic acids (caffeic acid, vannilic acid and protocatechuic acid) that can be used as whitening agents.

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