EFFECT OF SALINITY ON GROWTH, ANTIOXIDANT CONTENTS AND PROXIMATE COMPOSITIONS OF SABAH SNAKE GRASS (*Clinacanthus nutans*)

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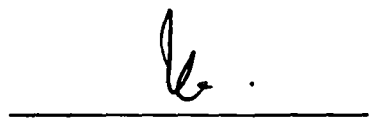

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ABSTRACT

The objective of this study was to determine the effect of salinity on growth, antioxidant contents and proximate compositions of Sabah snake grass (*Clinacanthus nutans*). The experiment was conducted at the rain shelter of Faculty of Sustainable Agriculture, UMS. The soil used in the experiment had electrical conductivity (EC) 1.15 dS m^{-1} and organic carbon 22.02% with pH 5.23. Six salinity levels were used namely 0 (control), 4, 8, 12, 16 and 20 dS m^{-1}. Data on plant height, leaf length, leaf width, relative water content, phenolic content, flavonoids content, and proximate compositions of *C. nutans* were recorded four weeks after application of salinity treatment. The experimental design was Completely Randomized Design (CRD) with five replications. Results were analysed using SAS and the treatment means were compared by Least Significance Differences (LSD) test at the 5% significant level. In this study, highest salinity level, 20 dS m^{-1} significantly increased the phenolic content (1.95 mg GAE/g), flavonoids content (3.84 mg QE/g), and proximate compositions such as ash content (19.83%), crude protein content (16.43%), crude fat content (18.45%) and crude fiber content (10.73%) of *C. nutans* although the plant growth and leaf relative water content were reduced. Therefore, salt-stressed *C. nutans* can be considered as excellent sources of antioxidant contents and protein for human consumption.

Kesan Kemasinan terhadap Pertumbuhan, Kandungan Antioksidan, serta Komposisi Proksimat pada Belalai Gajah (Clinacanthus nutans)

ABSTRAK

Objektif kajian ini adalah untuk membandingkan kesan kemasinan terhadap pertumbuhan, kandungan antioksidan dan komposisi proksimat belalai gajah (Clinacanthus nutans). Eksperimen ini dijalankan di tempat perlindungan hujan Fakulti Pertanian Lestari, UMS. Tanah yang digunakan dalam kajian ini mempunyai kekonduksian elektrik (EC) 1.15 dSm^{-1} dan karbon organik 22.02% dengan pH 5.23. Enam tahap kemasinan telah digunakan iaitu 0 (kawalan), 4, 8, 12, 16 dan 20 $dS\ m^{-1}$. Data mengenai ketinggian tumbuhan, panjang daun, lebar daun, kandungan air relatif, kandungan fenolik, kandungan flavonoid, dan komposisi proksimat C. nutans telah dicatatkan empat minggu selepas rawatan kemasinan. Reka bentuk eksperimen adalah Rekabentuk Rawak Lengkap (CRD) dengan lima ulangan bagi setiap perlakuan. Keputusan telah dianalisis dengan mengguna SAS dan nilai tengah perlakuan telah dibandingkan dengan Uji Beda Nyata Terkecil (LSD) pada aras signifikan 5%. Dalam kajian ini, tahap kemasinan yang paling tinggi, 20 $dS\ m^{-1}$ dapat meningkatkan jumlah kandungan fenolik (1.95 mg GAE/g), jumlah kandungan flavonoid (3.84 mg QE/g), serta komposisi proksimat seperti kandungan abu (19.83%), kandungan protein mentah (16.43%), kandungan lemak mentah (18.45%) dan kandungan fiber mentah (10.73%) dalam C. nutans secara signifikan walaupun pertumbuhan tumbuhan dan kandungan air relatif daun telah dikurangkan. Oleh itu, C. nutans yang telah dirawati garam boleh dianggap sebagai sumber yang sangat baik bagi antioksidan dan protein untuk kegunaan manusia.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

AOAC	Association of Analytical Communities
Ca	Calcium
CAT	Catalase
CC_{50}	50% Cytotoxic Concentration
CHN	Carbon/Hydrogen/Nitrogen
Cl	Chloride
CMIR	Cell-mediated Immune Response
CRD	Completely Randomized Design
dS m^{-1}	Deci Siemens per meter
EC	Electrical Conductivity
FAMA	Federal Agricultural and Marketing Authority
FAO	Food and Agriculture Organization of the United Nations
FSA	Faculty of Sustainable Agriculture
FW	Fresh Weight
GAE	Gallic Acid Equivalent
GTX	Glutathione Peroxidase
HSV	Herpes Simplex Virus
IC_{50}	50% Inhibitory Concentration
IL	Interleukin
K	Potassium
KDa	Kilodalton
LSD	Least Significant Differences
MPO	Myeloperoxidase
Na	Sodium
NCC	Nitrogen-Containing Compounds
PMA	Phorbol Myristate Acetate
PMBCs	Peripheral Blood Mononuclear Cells
QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
rpm	Revolutions per Minute
RWC	Relative Water Content
SAS	Statistical Analysis System
SOD	Superoxide Dismutase
TW	Turgor Weight
UMS	Universiti Malaysia Sabah
USD	United State Dollar
VZV	Varicella Zoster Virus
w/v	Weight per Volume
WHO	World Health Organisation

LIST OF FORMULAE

$$\text{Crude fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100\%$$

$$\text{Crude fiber content (\%)} = \frac{(m_3 - m_1 - m_4 - m_5)}{m_2} \times 100\%$$

Blank value, $m_5 = m_7 - m_6$

Where:
m_1 = Fiberbag (g)
m_2 = Initial sample weight (g)
m_3 = Incinerating crucible and dried fiberbag after digestion (g)
m_4 = Incinerating crucible and ash (g)
m_5 = Blank value of the empty fiberbag (g)
m_6 = Incinerating crucible (g)
m_7 = Incinerating crucible and ash of the empty fiberbag (g)

CHAPTER 1

INTRODUCTION

1.1 Introduction

Recently, interest in pharmaceutical products and drugs derived from medicinal plants has been increasing tremendously. World Health Organisation (WHO) has defined medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO, 2008). Cultivation of medicinal plants especially high value medicinal plants is creating new dimension in the field of agriculture as they serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Globally, herbal medicine industry is projected to worth USD five trillion (RM 17.70 trillion) by 2050 from USD 200 billion (RM 707.60 billion) in 2008 (WHO, 2008). In Malaysia, local herbal market industry has reached RM10 billion in 2008 and is poised to grow of 15% annually for upcoming 10 years (FAMA, 2008) based on increasing acceptance of herbal products as traditional medicine, functional foods, health food supplements, care products themselves natural and organic foods.

Clinacanthus nutans (Burm. f.) Lindau, belonging to the family of Acanthaceae, is a medicinal herb that can be found throughout Southeast Asia, primarily indigenous to Thailand, Indonesia and Malaysia (Nesheim *et al.*, 2006). It is locally known as Sabah snake grass or belalai gajah in Malaysia. *Clinacanthus nutans* has gained high popularity among Malaysians because of its high medicinal value in treating cancer. Moreover, *C. nutans* is broadly used to treat uric acid, gout, urinates neuropathies, liver cancer, kidney syndrome, nasal cavity cancer and uterine fibroid in Malaysia and other Asian countries (Arullappan *et al.*, 2014). This plant also has been endorsed to treat skin rashes, insect and snake bite as well as Herpes Simplex Virus (HSV), and Varicella Zoster Virus (VZV)

lesions in the Primary Health Care Programme (Wanikiat *et al.*, 2008). *Clinacanthus nutans* had been proved to have positive effect on antiviral activity against HSV type-2 (Jayavasu *et al.*, 1992b) and VZV (Thawaranantha *et al.*, 1992), immunomodulatory activity (Sriwanthana *et al.*, 1996), antioxidant activity (Pannangpetch *et al.*, 2007; Yong *et al.*, 2013), and anti-inflammatory (Wanikiat *et al.*, 2008; Satayavivad *et al.*, 1996). Due to its medicinal properties, *C. nutans* was chosen as my focus of research.

Plants are good source of minerals, vitamins and phytochemicals. Two groups of phytochemical, namely phenolic and flavonoid compounds, are known as antioxidant agents (Landrum and Bone, 2001). These two phytochemical compounds had also been identified in *C. nutans* (Sathisha, 2013). Several researches have shown that many medicinal plants have therapeutic potentials as natural antioxidants due to their phenolic components (Cook and Samman, 1996). The presence of phenolic and flavonoids plays an important role in reducing free radical induced tissue damage (Mimica-Dukic *et al.*, 2004) by activated oxygen species (Rice-Evans and Packer, 1998) and in the maintenance of health and protection from some age-related degenerative disorders such as cancer and coronary heart diseases (Hill, 1952). *Clinacanthus nutans* also contain high amount of phenolic and flavonoids, thus this study may provide some information on the changes of these antioxidants under saline condition.

Changes in the environment may represent stress situations in plants (Schwarzlander *et al.*, 2008). Salt stress in soil or water is one of the major abiotic stresses especially in arid and semi-arid regions which refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. Soil salinity has become a global problem posing major threat to sustainable agriculture in the world. Globally, about 400 million hectares of land throughout the world are affected by salinity (FAO, 2000), constitutes about 23% of the world's cultivated lands (Khan and Duke, 2001). Salinity affects plant growth because the high concentrations of soluble salts through their high osmotic pressures restrict the uptake of water by the roots and interferes with balanced absorption of essential nutritional ions by plants (Tester and Devenport, 2003).

Besides, salinity affects both the primary and secondary metabolism of the plant and hence giving to the different bioactivity of the plants (Hong *et al.*, 2008). Salinity causes water deficit, resulting in the generation of oxidative stress in plant tissues by

impairing the cellular electron transport within different subcellular compartments, leading to the generation of Reactive Oxygen Species (ROS) such as Singlet Oxygen, Superoxide Anion, Hydrogen Peroxide and Hydroxyl Radicals (Sreenivasulu *et al.*, 2000). Plants respond to oxidative stress by ROS scavenging through activation of the antioxidant system, which includes both enzymatic and non-enzymatic defence mechanisms. The non-enzymatic system involves the synthesis of several secondary metabolites of the phenylpropanoid pathway, such as flavonoids, phenolic acids, tannins and phenolic diterpenes. In this case, phenolic compounds play an important role in adsorbing and neutralizing free radicals, quenching Singlet Oxygen or decomposing Peroxide (Ksouri *et al.*, 2007; Oueslati *et al.*, 2010). In short, salinity or salt stress are closely related to the accumulation of polyphenol constituents such as flavonoids and phenolic acids. Hence, it is very important to evaluate the effect of salinity on growth and antioxidant contents of *C. nutans*.

1.2 Justification of Study

Clinacanthus nutans is a popular medicinal plant in Southeast Asia with reported bioactivities, but the effects of salinity on *C. nutans* physiology have been little studied. Considering the increase of salinization on arable lands, this study was carried out to test the effect of salinity on growth, antioxidant content, and proximate compositions of *C. nutans*.

Besides, *C. nutans* has very high content of phenolic and flavonoids content which are known for its antioxidant ability in inhibiting and preventing cancer and cardiovascular diseases. However, so far there is no similar research about the effect of salinity on these phenolic and flavonoids content of *C. nutans*. Thus, this study may contribute for a better understanding of the responses of *C. nutans* on phenolic and flavonoid content to different level of salinity and improve knowledge of the physiology and ecology of this important species.

Furthermore, there is still limited scientific information about the properties of the *C. nutans* leaves which contain many essential nutrients that provide beneficial medicinal value. Through this research, more information regarding the effect of salinity on proximate compositions in *C. nutans* such as ash content, crude protein content, crude fiber content and crude fat content can be studied. In addition, the result of this

research can also be a reference to the *C. nutans* grower in future. The effects of different salinity level will be important for improving the efficacy of *C. nutans* and conservation of highly bioactive varieties.

1.3 Objective

The objective of this research was to determine the effect of salinity on growth, antioxidant contents and proximate compositions of *C. nutans*.

1.4 Hypothesis

H_O: There was no significant difference among the effect of salinity on growth, antioxidant contents and proximate compositions of *C. nutans*.

H_A: There was a significant difference among the effect of salinity on growth, antioxidant contents and proximate compositions of *C. nutans*.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical Description of *Clinacanthus nutans*

Clinacanthus nutans (Burm. f.) Lindau, belonging to the family of Acanthaceae, is a medicinal herb that can be found throughout Southeast Asia, primarily indigenous to Thailand, Indonesia and Malaysia (Neshim *et al.*, 2006). It is locally known as Sabah snake grass or Belalai Gajah in Malaysia. *Clinacanthus nutans* has gained high popularity among Malaysians of its high medicinal value in treating cancer as published in Guang Ming Daily Newspaper (2011) although it is not supported by scientific evidence. The entire *C. nutans* can be taken as vegetable and it can be propagated easily using stem cuttings.

According to Panyakom (2006), *C. nutans* is a shrub or perennial herbs that can grow about one to three meter in height with pubescent branches. The stem of *C. nutans* is torete, striate and glabrescent. Meanwhile, the leaves are simple, opposite, narrowly elliptic-oblong or lanceolate, with measurement of 2.5-13 cm long and 0.5-1.5 cm wide. Hu and Daniel (2011) further described that the leaves of *C. nutans* have apex acute or acuminate and exsculptate, dentate or subentine margins. Furthermore, the leaf blades are lanceolate-ovate, lanceolate or linear-lanceolate. Both surfaces of leaves are pubescent when young then glabrescent except abaxially pilose along veins. The leaves consist of four to six secondary veins on each side of midvein and abaxially elevated. The leaf base are cuncate, obtuse rounded or truncate, often oblique, while the petioles are 0.3-2 cm long, sulcate and bifariously pubescent.

In addition, the flowers of *C. nutans* are in dense cymes at the top of the branches and branchlets, and always covered with 5-alpha cymules. The cymes consist of five to

eight flowers that are dull red with green base, and are often terminating with drooping horizontal branches but the branches themselves are erect, subsecund, and hence combined into a large lax and leafy panicle (Hu and Daniel, 2011). Each flower has calyx densely patently glandular-pubescent of about 1 cm long, corolla glandular-pubescent of about 3.5 cm, lower lip that is turned upwards with yellow streaks, apically sordidly yellow or greenish yellow, and two stamens that are inserted in the throat, more or less appressed against the upper lip. In addition, the flower has ovary that is compressed and consists of two ovules in both cells, style filiform that is shortly bidentate, and 4-seeded capsule that is oblong, with a short basal stalk (Backer and Bakhuizen, 1965).

The morphological features of *C. nutans* are illustrated in Figure 2.1.

Figure 2.1 (a) Matured plants; (b) leaves; and (c) flower of *C. nutans*
Source: (a) Anonymous, 2014; (b) Ng, 2013; (c) Globinmed, n.d.

2.2 Properties and Characteristics of *Clinacanthus nutans*

Clinacanthus nutans possesses many good characteristics and desired planting properties. As for purity test, the loss on drying of *C. nutans* is not more than 14%, the

total ash properties is less than 21% while the acid-insoluble ash in *C. nutans* is less than 4% (Kharnngan, 1991). Besides, the extractive values of alcohol-soluble extract, water-soluble extract and ether-soluble extract was found to be more than 27%, 26% and 1% respectively as reported by Kharnngan (1991).

In addition, *C. nutans* contains only a few of heavy metals, which makes it to be safe for human consumptions. According to Globinmed (n. d.), *C. nutans* contains not more than 5.0 mg/kg of Arsenic, not more than 0.5 mg/kg of Mercury, less than 10.0 mg/kg of Lead, and less than 0.3 mg/kg of Cadmium.

2.2.1 Chemical Constituents of *Clinacanthus nutans*

In general, Sathisha (2013) proved that some phytochemical compounds such as alkaloids, phenolic acids, tannins, flavonoids, cardiac glycosides, diterpenes and phytosterols were found in *C. nutans*.

In 1976 and 1977, Dampawan *et al.* reported the isolation of stigmasterol, lupeol, and β-sitosterol from a light petroleum extract of the leaves, stems, and roots of *C. nutans*. Lin *et al.* reported the isolation of betulin, lupeol, and β-sitosterol from the roots of *C. nutans* in 1983.

According to Teshima *et al.* (1997), methanol extracts of *C. nutans* stems and leaves have been found to contain six known C-glucosyl flavones such as vitexin, isovitexin, shaftoside, isomollupentin 7-*O*-β-glucopyranoside, orientin, and isoorientin. In addition, five sulfurous glucosides such as clinacosides A, clinacosides B, clinacosides C, cycloclinacosides A1, and cycloclinacosides A2 have also been isolated from the butanol and water soluble portions of the methanol extract of stems and leaves of *C. nutans* (Teshima *et al.*, 1998).

In 2001, Satakhum *et al.* reported the isolation of two glycoglycerolipids which are 1,2-Odilinolenoyl-3-O-β-D-galactopyranosyl-glycerol and 1-O-palmitoyl-2-Olinolenoyl-3-O-[α-D-galactopyranosyl-(1" 6')-O-β-D-galacctopyranosyl]-glycerol from the leaves of *C. nutans*. Further investigation was done by Suwanborirux *et al.* in 2003 and anti-HSV activity of both compounds was found.

According to Tuntiwachwuttikul *et al.* (2004), a mixture of nine cerebrosides and a monoacylmonogalactoylglycerol have been found from the ethanol extracts of *C. nutans* leaves. The structures of the cerebrosides were characterized as 1-O-b-D-glucosides of phytosphingosines, which comprised a common long-chain base, (2S, 3S, 4R, 8Z)- 2-amino-8(Z)-octadecene-1,3,4-triol with nine 2-hydroxy fatty acids of varying chain lengths (C16, C18, C20-26) linked to the amino group. The glycosylglyceride was characterized as (2S)-1-O-linolenoyl-3-O-b-D-galactopyranosylglycerol.

In addition, chloroform extracts of *C. nutans* leaves was found to contain chlorophyll derivatives such as 13^2-hydroxy-(13^2-R)-phaeophytin b, 13^2-hydroxy-(13^2-S)-phaeophytin a, 13^2-hydroxy-(13^2-R)-phaeophytin a, 13^2-hydroxy-(13^2-S)-phaeophytin b, 13^2-hydroxy-(13^2-S)-chlorophyll b, 13^2-hydroxy-(13^2-R)-chlorophyll b, purpurin 18 phytyl ester, and phaeophorbide a. Fatty acids such as n-pentadecanol, eicosane, 1-nonadecene, heptadecane, dibutylphthalate, n-tetracosanol-1, heneicosane, behenic alcohol, 1-heptacosanol, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl)-ester, nonadecyl heptafluorobutyrate, eicosayl trifluoroacetate, dinonyl ester, phthalic acid and dodecyl nonylester were also found in the chloroform extracts of *C. nutans* as reported by Sakdarat *et al.* in 2006 and 2009.

2.3 Medicinal uses of *Clinacanthus nutans*

Clinacanthus nutans is an important herbal medicine in Thailand, China and Malaysia (Teshima *et al.*, 1997). It had been proved to have positive effect on antiviral activity (Sangkitporn *et al.*, 1993a Jayavasu *et al.*, 1992b; Thawaranantha *et al.*, 1992), immunomodulatory activity (Sriwanthana *et al.*, 1996), antioxidant activity (Pannangpetch *et al.*, 2007; Yong *et al.*, 2013), and anti-inflammatory (Wanikiat *et al.*, 2008). *Clinacanthus nutans* is widely used as folklore medicine although some of the traditional medicinal uses are not supported by experimental or clinical data.

Malaysia and other Asian countries broadly use *C. nutans* to treat uric acid, gout, urinates neuropathies, liver cancer, kidney syndrome, nasal cavity cancer and uterine fibroid. In Indonesia, *C. nutans* is used to treat diseases such as diabetes, dysuria and dysentery by consuming the decoction from fresh leaves boiled with hot water as reported by Hariana in 2008.

In Thailand, fresh leaves of *C. nutans* has long been used traditionally to treat inflammation (Wanikiat *et al.*, 2008), viral infection (Yoosook *et al.*, 1999; Charuwichitratana, 1996), skin psoriasis and insect bites (Janwitayanuchit *et al.*, 2003) due to its anti-inflammatory property. Daduang and Uawonggul (2008) recommended the uses of *C. nutans* in treating insect bites especially for ant, bee, catfish, centipede, hornet, jellyfish, millipede, mosquito, scorpion and wasp. Furthermore, this plant has been used as anti-venom for snake and scorpion bites and also removes nettle rashes due to its anti-cell lysis property (Watson and Preedy, 2008).

Laboratory investigations in Thailand have indicated that *C. nutans* extract exhibits anti-viral properties against the HSV (Sangkitporn et al., 1993a; Jayavasu *et al.*, 1992b) and VZV (Thawaranantha *et al.*, 1992). Clinical trials in patients with genital herpes are also reported (Sangkitporn *et al.*, 1993b; Jayavasu *et al.*, 1992a). In the Primary Health Care Programme, *C. nutans* has been promoted for treatment of HSV, VZV, and skin pruritus (Ministry of Public Health, 1999).

In addition, *C. nutans* topical cream or lotion is a good natural product for the relief of minor skin inflammation and insect bites (Satayavivad *et al.*, 1996) due to its excellent and rapid acting, anti-inflammatory property (Chuakul, 1986; Tanasomwong, 1986; Satayavivad *et al.*, 1996). *Clinacanthus nutans* cream was later clinically shown to be as efficacious as acyclovir in relieving pain, and healing HSV and VZV without causing a burning sensation which is a side effect experienced by some patients using acyclovir (Jayavasu *et al.*, 1992a; Jayavasu *et al.*, 1992b; Sangkitporn *et al.*, 1993a; Sangkitporn *et al.*, 1993b; Charuwichitratana *et al.*, 1996). Hence, topical *C. nutans* products are currently being used in many Thai hospitals to replace topical acyclovir in treating the two diseases.

In China, the whole plant of *C. nutans* is used in various manner to treat inflammatory conditions such as haematoma, contusion, strains and sprains of injuries and rheumatism. This plant is also used to treat inflammation such as hematoma, bruises on eye, anxieties, injuries and rheumatism. Besides, Chinese healers believe that this plant is effective in regulating menstrual function, relieving pain, anaemia, repairing of fractured bones and jaundice (Pieroni and Vandebroek, 2009).

2.4 Biological and Pharmacological Study

2.4.1 Antiviral Activity against Herpes Simplex Virus (HSV)

A study was done by Jayavasu *et al.* (1992b) to compare the ability of the ethanol extract of *C. nutans* leaves to inactivate HSV-2 with that of acyclovir. They found that the extracts of the leaves of *C. nutans* was able to inhibit plaque formation by HSV-2 in baby hamster kidney cell line. Jayavasu *et al.* (1992a) also reported on clinical trial in the treatment of genital herpes patients with *C. nutans* extract. It showed that both *C. nutans* and acyclovir could help patients to develop crust lesion within three days and heal within seven days while the placebo group had crusting time in four to seven days and healing time in 7-14 days or more. As a result, *C. nutans* and acyclovir have good efficacy in shortening the duration of infection and reducing severity. Besides, *C. nutans* has no sticky, burning, stinging pain and side effects. Hence, *C. nutans* is believed to be suitable for the treatment of genital herpes patients.

In addition, Sangkitporn *et al.* (1993b) reported the results of treatment of recurrent Genital Herpes Simplex virus infection with *C. nutans* extract. *C. nutans* extract-treated group and the acyclovir-treated group have significantly increased the numbers of patients with lesion crusting within three days and with lesion crusting within seven days compared with the placebo-treated group. Mild, transient burning or pains associated with application of acyclovir were found in 21 male patients (24.7%) and in 18 female patients (23.1%) but there was no side effect of *C. nutans* during treatment. Thus, *C. nutans* is concluded to be the most suitable for the treatment of genital herpes patients.

Vachirayonstien *et al.* have done a research in 2010 on molecular evaluation of extracellular activity of medicinal herb *C. nutans* against HSV-2. The study showed that ethanol extract of *C. nutans* leaves did reduced quantity of HSV-2's DNA and reduced the quantity of eight HSV-2's proteins which are 20, 40, 44, 55, 69, 78, 125 and 146 KDa proteins. However, the study done by Yoosook *et al.* in 1999 on the anti HSV-2 stains against oragnic solvent extracts of *C. nutans* did not show any anti-HSV-2 viral activity. This contradictory results may be attributed to differing laboratory factors and plant material as clinical trial done on the extracts of *C. nutans* did show significant antiviral properties.

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