

Comparitive Study of Antioxidant Activities on the Rhizomes of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb.

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ABSTRACT

Kaempferia parviflora Wall. ex Baker and Curcuma comosa Roxb. belongs to the family Zingiberaceae. Kaempferia parviflora Wall. ex Baker is known as the nannwin net or sannwin net (C_1) in Myanmar. Curcuma comosa Roxb is known as the nannwin khar or sannwin khar (C_2) in Myanmar. Kaempferia parviflora Wall. ex Baker and Curcuma comosa Roxb. were collected from Kyaukse township, Mandalay Region. Morphological, phytochemical and antioxidant activity of Kaempferia parviflora Wall. ex Baker and Curcuma comosa Roxb. were collected from Kyaukse township, Mandalay Region. Morphological, phytochemical and antioxidant activity of Kaempferia parviflora Wall. ex Baker and Curcuma comosa Roxb. were carried out, to get their correct identification. In morphological study, this plants was perennial rhizomatous herbs. Leaves were simple and alternate. The aerial pseudo-stem formed by leaf-sheaths. Inflorescences was tubular spike, axillary, with 1-2 flowers. In the phytochemical studies in the rhizome of Kaempferia parviflora Wall. ex Baker showed the presence of alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, saponin, tannin, terpene, steroid but reducing sugar is absent and alkaloid, glycoside, flavonoid, phenol, polyphenol, polyphenol, lipophenol, saponin, tannin, terpene, steroid, reducing sugar are present in Curcuma comosa Roxb. IC₅₀ values of the standard ascorbic acid was 41.9 µg/mL and ethanol extract of C₁ sample was 20.55 mg/mL and C₂ sample was 3.22 mg/mL. The antioxidant activity of C₁ and C₂ sample were found to be lower than that of standard ascorbic acid.

Keywords : Kaempferia parviflora Wall. ex Baker., Curcuma comosa Roxb., morphological, phytochemical and antioxidant activity

1 INTRODUCTION

Medicinal plants used in India for centuries have an important therapeutic source for treating a variety of ailments. The family Zingiberaceae is represented by 1100 species under 52 genera, distributed in the tropics of the worlds, especially in the Indo-Malayan region. Zingiberaceae are economically important. They are widely used as food, food adjunct, spices, aromatic oils and for medicinal purposes and as cultivated ornamentals (Raul *et al.* 2012).

The family Zingibraceae (ginger family), distributed throughout tropical and subtropical regions of the world, especially Indonesia and Malaysia, has been well studied due to its economic importance. Medicinally, plants of this family are reputed to have value as antihepatotoxic, anti-inflammatory, and bile-expelling agents, for their anti-ulcer, antimicrobial, stomachic, insecticidal and antiprotozoal properties, and as an antidote for cobra venom (Jurgens *et al.* 1994).

The rhizome of ginger has been extensively used with remarkable therapeutic effects for the treatment of inflammations, diarrhea, stomach, fever, flatulence, allergies and poisonous. Powdered rhizome is used to treat ear infection, toothache and to treat stomach disease. The leaves are also used in therapeutics for joint pain (Somchit *et al.* 2005).

Kaempferia parviflora or Krachaidum (in Thai), also known as "Thai ginseng," is a medicinal plant in the family Zingiberaceae. It is found in tropical areas such as Malaysia, Sumatra, Borneo Island, and Thailand. Its rhizome has been long used as folk medicine for many centuries. A number of pharmacological studies of Krachaidum have shown the following properties: anti-inflammatory, antimutagenic, antidepressive, anticholinesterase, antimicrobial, anticancer, anti-peptic ulcer, cardioprotective, antiobesity activity, and aphrodisiac (Saokaew *et al.* 2016).

Kaempferia parviflora Wall. ex Baker (Thai Ginseng) is a herb that has some historical and medicinal usage for treating ailments and improving vitality in Thailand and limited to surrounding region. It is also reported to be an aphrodisiac compound and physical enhancer. Leaves are green with a reddish underside. It has been used as a medicinal plant in Asia (Leardamolkan *et al.*2009).

Curcuma is one of the well known genera of the family Zingiberaceae. The genus consists of about 110 species widely distributed in tropical Asia and the Asia-Pacific region. The greatest diversity occurs in India, Myanmar and Thailand and the distribution extend to Korea, China, Australia and the South Pacific. Several *Curcuma* plant have long been known their uses as food, spices and medicinal plants. It is a species of flowering plant in the ginger family. It is native to much of Asia, including Thailand, Indonesia, and Malaysia. The herb is cultivated in Thailand, especially in the Northern Province, including Petchaboon, and the Northeastern Province (Ravindran *et al.* 2007).

Curcuma comosa Roxb. (Nanwin-ga) (family- Zingiberaceae) grows widely on Myanmar and Thailand. In Myanmar traditional medicine, this plant is known to be useful in the treatment of diabetes mellitus, hypertension, fever and stress. It also contains in some Myanmar traditional medicine formulations for hypertension and diabetes mellitus (Khin Khin Lwin *et al.* 2008).

Curcuma comosa Roxb. is a perennial herb belonging to the Zingiberaceae family. The rhizome has been traditionally used for the treatment of postpartum uterine bleeding and inflammation in Thailand. Medicinal plants contain both organic and inorganic constituents and many medicinal plants are found to be rich in one or more individual elements, thereby providing a possible link to the medicinal value of the medicines (Winuthayanon *et al.* 2009).

The term "antioxidant" is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet. Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes produced internally, or the dietary antioxidants vitamin C, and vitamin E.

The aim of this research is to study the medicinal value of plant drugs. The objectives are to investigate the plant constituents and their action. The objective of this research was to study the effect of altitude and different shading conditions on the vegetative growth of *Curcuma comosa* Roxb. and *Kaempferia parviflora* to determine the best cultivation practice of this plants.

2 MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb.

The specimens of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb. were collected from Moe Kaung village, Sintgaing Township, Kyaukse District, Mandalay Region. The collected plants were taxonomically identified with the help of references literature such as Hooker 1885 and Dassanayake 1987. The fresh specimens were pressed, dried and preserved for morphological studies.

2.2 Preliminary Phytochemical Characterization of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb.

Preliminary phytochemical investigation was carried out at Department of Chemistry, University of Mandalay, according to Harbone 1984. It was carried out for the rhizome of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb. with a view to determine the constituents of alkaloid, glycoside, flavonoid, phenolic compound, polyphenol, reducing sugar, saponin, steroid, tannin and terpene.

2.3 Determination of Antioxidant Activity

1-1 diphenyl-2- picryl-hydrazyl (DPPH) powder was used as stable free radical. Ascorbic acid was used as standard antioxidant and ethanol was used as solvent. The absorbance was determined at 517 nm wavelength.

2.4 DPPH Assay

DPPH free radical method is an antioxidant assay based on electron- transfer that produces a violet solution in ethanol.

This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution.

The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry.

2.5 Preparation of Reagents

Four solutions were prepared. They are DPPH solution, standard solution and various concentration of two sample solutions.

2.6 Preparation of DPPH Solution

2.346 mg of DPPH powder was dissolved in 100 mL of ethanol. This solution was thoroughly mixed at room temperature and it was stored in brown colored bottle. This solution kept for no longer than 24 hours.

2.7 Preparation of Standard Solution

2 mg of ascorbic acid was dissolved in 20 mL of ethanol. This solution was thoroughly mixed at room temperature to obtain 100 μ g/mL of standard solution. The concentration of standard solution (50, 25, 12.5, 6.25 and 3.124 μ g/mL) was determined by using two fold dilution methods. 1 mL of ascorbic acid and 2 mL of DPPH solutions were thoroughly mixed for about 15 min at room temperature. The absorbance of mixture was measured at 517 nm.

2.8 Preparation of Test Sample Solution

0.200 g of ethanol crude extract of rhizome of *Kaempferia parviflora* Wall. ex Baker was dissolved in 10 mL of ethanol. The solution was thoroughly mixed at room temperature for 15 minutes to obtain 20 mg/mL of sample solution. The concentration of test sample solutions (20, 16, 12, 8 and 4 mg/mL) were prepared by serial dilution method and denoted as C₁.

0.200 g of ethanol crude extract of rhizome of *Curcuma comosa* Roxb. was dissolved in 10 mL of ethanol. The solution was thoroughly mixed at room temperature for 15 minutes to obtain 20 mg/mL of sample solution. The concentration of test sample solutions and (4, 2, 1, 0.5 and 0.25 mg/mL) were prepared by two fold dilution method and denoted as C₂.

2.9 Determination of Absorbance of Sample Solutions

1 mL of sample solution and 2 mL of DPPH solutions were thoroughly mixed for about 15 minute at room temperature. The absorbance of the mixture was measured at 517 nm.

% inhibition= $\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$

This formula is the calculation of percent inhibition of (IC_{50}) value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

2.10 Determination of Half Maximal Inhibitory Concentration

(IC₅₀) values were obtained from the best-fit line plotted concentration verses inhibition.

3 **RESULTS**

3.1 Morphological Studies of Kaempferia parviflora Wall. ex Baker

Scientific name	-	Kaempferia parviflora Wall. ex Baker
Myanmar name	-	Nannwin net, Sannwin net
Family	-	Zingiberaceae
Flowering period	-	September to December

Perennial rhizomatous herbs, 0.6-0.8 m high, rhizomes fleshy, branched, black. The aerial pseudo-stem formed by leaf-sheaths. Leaves simple, alternate, tuft base, 10.0-15.5 cm long, 5.0-12.5 cm wide; petioles long, 8-22 cm long, 0.3-1.0 cm wide, pale green, glabrous; leaf blade elliptic, acute at apex, green, glabrous on both surfaces; upper surface is green and lower surface is pale green. Inflorescence tubular spike, axillary, substanded by two bracts; lanceolate 3-4cm long. Flower bisexual, zygomorphic, epigynous, 4-6 cm long, white, center purple; pedicles 3.5-4.5 cm long. The most conspicuous part of the flower is two or three lobed lip labellum formed by the fusion of two staminode. Calyx tubular, glabrous or pubescent, apex acuminated split into equally lobes. Corolla tube thin and slender, white. Fertile stamen short; filament very short, flattened, anther dithecous, crested orbicular entire, about 1.5 cm long, yellow, basified connective prolonged, spur absent. Labellum emarginated, bright violet; Ovary inferior, pale yellow, pubescent, trilocular; style filiform, white. Fruit unknown.

Specimen examined: Kyaukse Township, Mandalay Region, Put Thaing Village

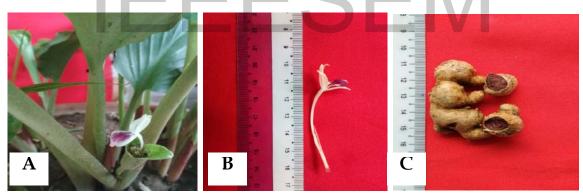


Figure 1.Morphological Studies of Kaempferia parviflora Wall. ex Baker
A. HabitC. FlowerC. Rhizome

3.2 Morphological Studies of Curcuma comosa Roxb.

Scientific name	-	Curcuma comosa Roxb.
Myanmar name	-	Nannwin khar, Sannwin khar
Family	-	Zingiberaceae
Flowering period	-	October to December

Perennial rhizomatous herbs, 0.8-1.2 m high, rhizomes fleshy, cylindrical branched, smell and bitter taste, pale yellow; the stem cylindrical branched. Leaves simple, alternate

and distichous, tuft basal: petiole 13-17.5 cm long, reddish brown, ligulate distinct, leaf blades green with reddish brown midrib; lanceolate,20.0-35.4 cm long and 5.0-10.0 cm wide; attenuate at the base, entire along the margin, acuminate at the apex, glabrous on both surfaces. Inflorescence terminal, cylindric spikes; bract broadly ovate, 4.0-6.2 cm long, white, apex purple, connate to each other, glabrous. Flowers bisexual, zygomorphic, epigynous, 3-4 cm long, white; pedicelsterect, about 2.0 cm long. Calyx tubular, 3-lobed, lobe 1.5-2.0 cm long and 1.0-1.4 cm wide, membranous, white, glabrous. Corolla funnel shaped, 3 lobed, 1.8-2.5 cm long, and 1.0-1.3 cm wide, white, membranous, glabrous. Fertile stamen 1; filament flat, glabrous; anther dithecous, about 7 mm long, basifixed, spur present; labellum obovate, 3 lobed, central lobe retuse, yellow. Ovary inferior, oblongoid, pale yellow; trilocular with many ovule in each locule,axile placentation, pubescent;, style filiform, white, glabrous; stigma 2 -fids, yellow. Fruits not available in this study period.

Specimen examined:Kyaukse Township, Mandalay Region, Put Thaing Village



Figure 2. Morphological Studies of *Curcuma comosa* Roxb. A. Habit B. Rhizome

3.3 Preliminary Phytochemical Properties of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb.

Preliminiary phytochemical properties were carried out for the *Kaempferia parviflora* Wall. ex Baker with a view to determine the presence of the constituent of alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, saponin, tannin, terpene and steroid. However, reducing sugar is absent. The results were present in Table 1. Similarly,*Curcuma comosa* Roxb. with a view to determine the presence of the constituent of alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, reducing sugar ,saponin, tannin, terpene and steroid. However, reducing sugar is absent. The results were present in Table 2.

No	Constituents	Extract	Test Reagent	Observation	Result
1	Alkaloid	Water	Dragendroff's reagent Wagnar reagent	Greenish Green- ish	+
2	Glycoside	Water	10%(CH ₃ CO) ₂ Pb	Brick red ppt.	+
3	Flavonoid	Ethanol	Conc. HCl, Mg	Reddish brown	+
4	Phenol	Water	10%FeCl ₃	Reddish brown	+
5	Polyphenol	Ethanol	1%FeCl ₃ ,K ₃ Fe(CN) ₆	Greenish brown	+
6	Lipophenol	Water	0.5N KOH	Red solution	+
7	Reducing Sugar	Water	Benedict's solution	Green yellow	_
8	Saponin	Water	Shake	Froth	+
9	Tannin	Water	10%FeCl ₃	Reddish brown	+
10	Terpene	Ethanol	(CH ₃ CO) ₂ O,CHCl ₃ , Conc H ₂ SO ₄	Reddish	+
11	Steroid	Ethanol	CHCl _{3,} Conc.H ₂ SO ₄	Two layer red ppt.	+

Table 1. Preliminary Phytochemical Tests for Rhizome of Kaempferia parviflora Wall. ex Baker

(+) = present (-) = absent	
Table 2. Preliminary Phytochemical Tests for Rhizome of Curcuma comosa Roxb.	

No	Constituents	Extract	Test Reagent	Observation	Result
1	Alkaloid	Water	Dragendroff's reagent Wagnar reagent	Greenish blue Reddish brown	+
2	Glycoside	Water	10%(CH ₃ CO) ₂ Pb	Brick red ppt.	+
3	Flavonoid	Ethanol	Conc. HCl, Mg	Red solution	+
4	Phenol	Water	10%FeCl ₃	Brown ppt.	+
5	Polyphenol	Ethanol	1%FeCl ₃ ,K ₃ Fe(CN) ₆	Greenish blue	+
6	Lipophenol	Water	0.5N KOH	Red solution	+
7	Reducing Sugar	Water	Benedict's solution	Red ppt.	+
8	Saponin	Water	Shake	Froth	+
9	Tannin	Water	10%FeCl ₃	Reddish brown ppt.	+
10	Terpene	Ethanol	$(CH_3CO)_2O,CHCl_3,$ Conc. H_2SO_4	Two layer red ppt.	+
11	Steroid	Ethanol	CHCl ₃ ,Conc.H ₂ SO ₄	Two layer reddish brown	+

(+) = present

3.4 Antioxidant Activity Using DPPH Assay in Standard Ascorbic Acid

The result of antioxidant activity using DPPH assay in standard ascorbic acid was shown in Table (3).

Sample	Mean	Mean %	IC ₅₀
Concentration (µg/mL)	Absorbance	inhibition	(µg/mL)
50	0.260	58.7	
25	0.426	32.4	
12.5	0.551	12.5	41.9
6.25	0.614	2.5	
3.125	0.626	0.6	

Table 3. % Inhibition of Various Concentration of Standard Ascorbic Acid

Absorbance of control =0.63

 C_{50} value was calculated by using linear regressive equation.

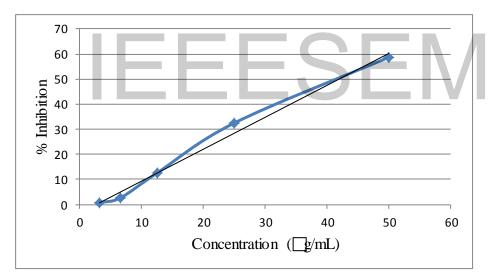


Figure 3. % Inhibition of Different Concentration of Standard Ascorbic Acid

Determination of Antioxidant Activity of Ethanol Extract of C₁ Sample

The result of antioxidant activity using DPPH assay in ethanol extract of sample was shown in Table (4).

Sample	Mean	Mean%	IC ₅₀
Concentration (µg/mL)	Absorbance	inhibition	(mg/mL)
20	0.324	48.6	
16	0.354	43.9	
12	0.371	41.2	20.55
8	0.411	34.9	
4	0.453	28.2	

Table 4. % Inhibition of Various Concentration of C₁ Sample

Absorbance of control =0.63

 IC_{50} value was calculated by using linear regressive equation.

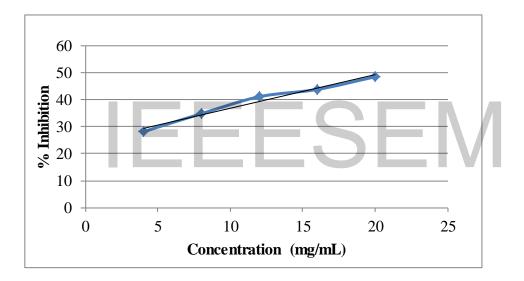


Figure 4. % Inhibition of Different Concentration of C₁ Sample

According to table (3) and (4) , IC_{50} value of the standard ascorbic acid was 41.9 $\mu g/mL$, ethanol extract of C_1 sample was 20.55 mg/mL. The antioxidant activity of C_1 sample was found to be lower than that of standard ascorbic acid.

Determination of Antioxidant Activity of Ethanol Extract of C2 Sample

The result of antioxidant activity using DPPH assay in ethanol extract of sample was shown in Table (5).

Sample	Mean	Mean %	IC ₅₀
Concentration (µg/mL)	Absorbance	inhibition	(mg/mL)
4	0.315	50.5	
2	0.325	48.9	
1	0.377	40.8	3.22
0.5	0.498	21.8	
0.25	0.554	13	

 Table 5. % Inhibition of Various Concentration of C2 Sample

Absorbance of control = 0.63

IC₅₀ value was calculated by using linear regressive equation.

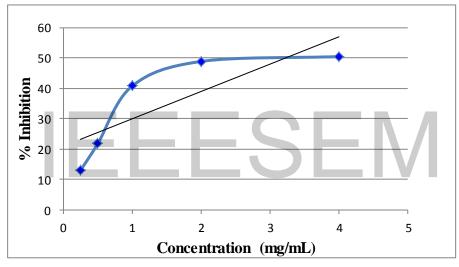


Figure 5. % Inhibition of Different Concentration of C₂ Sample

According to table (3) and (5) , IC₅₀ value of the standard ascorbic acid was 41.9 μ g/mL, ethanol extract of C₂ sample was 3.22 mg/mL. The antioxidant activity of C₂ sample was found to be lower than that of standard ascorbic acid.

4 DISCUSSION AND CONCLUSION

Kaemperia parviflora Wall. ex Baker and *Curcuma comosa* Roxb. are widely cultivated throughout the tropical region of Myanmar. It is one of the species in Zingiberaceae family. In the present work, the morphological and histological characters of *Kaemperia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb. were presented.

In morphological studies, *Kaemperia parviflora* Wall. ex Baker is perennial rhizomatous herbs. The stems are aerial pseudo-stem formed by leaf-sheaths. The leaves are alternate, simple, leaf blade, elliptic, acute at apex, green glabrous on both surface. These characters are similar to those given by Lawrence 1965.

In morphological studies, Curcuma comosa Roxb. was perennial rhizomatous herbs. The

stems are aerial pseudo-stem formed by leaf-sheaths. The leaves are alternate, simple, leaf blade, elliptic, acute at apex, green glabrous on both surfaces. These characters are similar to those given by Lawrence 1965.

For *Kaemperia parviflora* Wall. ex Baker Inflorescences are tubular spike axillary. Flowers are white, center purple, bisexual, zygomorphic. Ovary is inferior, pubescent, trilocular, axile placentation. Style filiform white. These characters are similar to those given by Dassanayake 1987.

For *Curcuma comosa* Roxb. Inflorescences are tubular spike axillary. Flowers are white, center purple, bisexual, zygomorphic. Ovary is inferior, pubescent, trilocular, axile placentation. Style filiform white. These characters are similar to those given by Dassanayake 1987.

According to the results, phytochemical studies on the rhizome of *Kaemperia parviflora* Wall. ex Baker showed the presence of a wide of secondary metabolites such as alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, saponin, tannin, terpene and steroid. Reducing sugar are absent.

According to the results, phytochemical studies on the rhizome of *Curcuma comosa* Roxb. showed the presence of a wide of secondary metabolites such as alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, reducing sugar, saponin, tannin, terpene and steroid.

IC₅₀ value of the standard ascorbic acid was 41.9 μ g/mL and ethanol extract of C₁ sample was 20.55 mg/mL and C₂ sample was 3.22 mg/mL. The antioxidant activity of C₁ and C₂ sample were found to be lower than that of standard ascorbic acid. From the comparitive data of same family of rhizome of two different plants, *Curcuma comosa* Roxb.(Myanmar name - Nannwin khar) show the greater antioxidant activity than *Kaempferia parviflora* Wall. ex Baker (Myanmar name - Nannwin net).

In Myanmar, a large number of medicinal plants are found as natural resources. Local people are identifying plants based mostly on morphological characters, but they cannot be able to identify the dry parts of the medicinal plants.

In this research work, the morphological and histological characters of *Kaempferia parviflora* Wall. ex Baker and *Curcuma comosa* Roxb. were examined clearly and systematically and agree with those of references.

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