# PRELIMINARY PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF RHIZOME EXTRACT OF *KAEMPFERIA CANDIDA* WALL.

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## Abstract

Morphological characters of rhizome of *Kaempferia candida* Wall. belonging to the family Zingiberaceae was reported. This specimens had been collected from Mansan Fall, Lashio Township, during July to August 2019.In addition, this rhizome was studied by using phytochemical, antimicrobial tests and then antioxidant activity. The phytochemical tests indicated that alkaloids, glycosides, phenols, polyphenols, reducing sugars and tannins were present in this rhizome. Furthermore, ethanol extract of rhizome has been tested for their antimicrobial activities by using agar-well diffusion method. They were found to be antimicrobial activity against five different types of microbes such as *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, Candida albicans* and *Escherichia coli*. The antioxidant activity of rhizomes extracts of *Kaempferia candida* Wall. was carried by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay. Strong antioxidant activity of *Kaempferia candida* Wall. was shown in this experiment compared with the control ascorbic acid.

**Keywords:** *Kaempferia candida*, Phytochemicals test, Antimicrobial characters, Antioxidant activity, DPPH Free radicals

## Introduction

Human beings are dependent upon the plants and plant products for their basic needs, and in modern civilization, they still need the plants and plant products in daily life, as sources of food, oxygen, wood, drugs, many fibres, fossil fuel, insecticides, biofertilizers, ornamentals, as well as rubber and other products.

Most of the members of the Zingiberaceae have been used worldwide in traditional medicines for the treatment of diseases. Herbal medicine is a traditional medicine or folk medicine based on the use of plant parts and plant extracts. The herbal medicines have been recognized as a valuable and readily available resource of primary health care (Cruickshank, 1970).

The family Zingiberaceae comprises of about 47 genera and 1400 species distributed in tropical and subtropical regions of the world (Hutchinson, 1967; Lawrence, 1964). Hooker (1894) reported that the family comprises of 40 genera and 400-500 species. 42 genera and 750 species were recorded by Rendle (1930). According to Hundley and Chit Ko Ko (1987), 125 species belonging to 18 genera are represented in the Union of Myanmar.

The family is taxonomically characterized by the presence of the leaves which are distichous or in spiral, the sheathing petioles usually opened, rarely closed, the presence of aromatic oils, ligulate; zygomorphic, trimerous flowers with the marked differentiation of the outer perianth series from the inner; the single fertile stamen, the large, usually petaloid staminodium, the inferior ovary and the seeds with copious hard or mealy endosperm.

The phytochemistry has developed with the chemical aspects of various metabolite processes taking place in plants. The phytochemicals are plant chemicals that they have health enhancing effects (British Pharmacopoeia, 1968).

There is an increasing interest in plants secondary metabolites like polyphenols, because of their therapeutic effects. Polyphenols or phenolic compounds form a large group of secondary

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compounds including phenolic acids, carotenoids, flavonoids, tannins, flavones glycosides, etc. These phenolic compounds have the property of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron (Madhu, K., 2013).

Phenolic compounds are responsible for antioxidant property. Antioxidants are classified into two major categories, natural and synthetic antioxidant. Plants are prospective source of natural antioxidants. The natural antioxidants are safer and environment friendly than synthetic antioxidants. Plants are a potential source of natural antioxidants which are secondary metabolites of plant, that exhibit a wide range of biological effects like antibacterial, anti-inflammatory, anti-allergic, anti-hepatotoxic and anti-thrombotic activities and prevention of cardiovascular diseases (Sadeghi, Z., Valizadeh. J., 2015).

Antioxidants are capable of blocking the effect of the Reactive Oxygen Species (ROS). In living organisms, the imbalance in the production and detoxification of free radicals by the biological system causes oxidative stress. Free radicals are generated by different types of exogenous chemicals and a number of endogenous metabolic processes oxidize the bio molecules leading to cell death and tissue damage. The organism must keep free radicals at relatively low concentrations using different defence systems and antioxidant molecules (Bhattacharyya, A., Chattopadhyay, R., Mitra, S, 2014).

Production of high amount of reactive oxygen species overcomes inbuilt antioxidant system and damages the cells, tissues and organs and hence, there is a need to develop new drugs from traditional medicine to protect and support the biological system to avoid serious disorders of liver, cardiac and cancer diseases etc (Shivasharanappa, K., Londonkar, R., 2014).

Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals (Walton and Brown, 1999).

The present research deals with the phytochemical and antimicrobial tests and antioxidant activity of rhizomes of *Kaempferia candida* Wall. (Pan-u) belonging to family Zingiberaceae.

Finally, the aims of this research were to provide the information of morphological characters; to observe the preliminary phytochemical findings; to interest the pharmacological active compounds and antimicrobial effects; to use the ethno medicine for treatment of bacterial infections.

## Materials and Methods

### Plant Material Collection

The specimens had been collected from Mansan Fall, Lashio Township, during July to August 2019. The collected specimen was recorded by photographs while flowering and fruiting periods. The specimens were collected during the extended field study by GPS (Global Positioning System). The collected specimens were identified by referring to Flora of British India (Hooker, 1894), Flora of Java (Backer, 1968), Flora of Ceylon (Dassanayake, 1976).

### Preparation of Plant Extract

The collected specimens were washed to remove dust and rinsed again with distilled water. The plant samples were air dried at room temperature 35ºC-40ºC for one month. After drying the samples, they were ground to get powder and stored in air-tight containers for further chemical analysis.

**Preparation of Ethanolic Extract**

The dried powder of rhizomes 100g were percolated with 500 ml of ethanol for one week and filtered with filter paper for three times respectively. The filtrates were evaporated by removing the solvent under reduced pressure using rotary evaporators at 50ºC. Then, the filtrates were dried in a beaker placed on a water bath at 60ºC. The dried extract was stored in the desiccator for further analysis.

**Phytochemical Screening**

The preliminary phytochemical screening for bioactive compounds was carried out by the standard methods (Harbone, 1998). The preliminary phytochemical screening of *Kaempferia candida* rhizome extracts was carried out for the presence or absence of alkaloids, flavonoids, glycosides, phenols, polyphenols, saponins, reducing sugars, carbohydrates and tannins.

**Test for Alkaloids**

Two grams of dried powdered sample was boiled with few ml of 1% HCL for 10 min, allowed to cool and filtered. The filtrate was tested with Wagner's reagent, Dragendroff's reagent, and Mayer's reagent. The reddish brown, orange and the white of creamy precipitate indicated as positive according to the test in the respective reagent.

**Test for Flavonoids**

Two grams of dried powdered sample was boiled with ethanol (5ml) for 10 min and filtered. The filtrate was placed in a test tube. Then, 0.5 ml of concentrated hydrochloric acid and a few pieces of magnesium (Mg) were added and the mixture was boiled for a few minutes. If pink or red colour develops, presence of flavonoids is noted.

**Test for Glycosides**

One gram of dried powdered rhizomes was boiled with distilled water for about 10 minutes, allowed to cool and filtered. The filtrate was treated with 10 % lead acetate solution. If the white precipitates were traced, it showed the presence of glycosides.

**Test for Phenolic Compounds**

Two grams of dried powdered sample was boiled with distilled water for 10 min and filtered. The filtrate was tested with 5% $FeCl_3$ solution. The dark green colour indicates the presence of phenolic compounds.

**Test for Polyphenols**

Two grams of dried powdered sample was boiled with distilled water for 10 min and filtered. The filtrate was tested with 5% $FeCl_3$ solution and 1% potassium ferricyanide ($K_3Fe(CN)_6$). The dark green blue colour indicates the presence of phenolic compounds.

**Test for Saponins**

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. The formation of froth or persistent foam indicated that saponins were present.

**Test for Reducing Sugars**

Two grams of dried powdered sample was boiled with distilled water for 10 min, the solution was cooled and filtered. The filtrate was tested fehlling A and B solution. The red precipitate indicates the presence of reducing sugars.

**Test for Carbohydrates**

The water extract of the sample was put into a test tube and 2 drops of 10 % napthanol was added to it. Then concentrated sulphuric acid was gradually poured down into the side of the test tube and allowed to stand. The formation of violet red ring indicates the presence of carbohydrates.

**Test for Tannins**

Dried plant materials (about 2g) were introduced into a test tube and was boiled with distilled water (10 $cm^3$) for about 20 minutes and filtered. The filtrate was treated with 3ml of 10 % lead acetate solution. The white precipitate shows the presence of tannins.

**Antimicrobial Tests**

Tested microorganisms: The antimicrobial screening was carried out, using the following organisms; *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, Escherichia coli* and a fungus *Candida albicans*.

**Determination of Antimicrobial Activity**

The agar well diffusion method was used for antimicrobial activity, evaluation by modifying the method described by Schlegel. Tested microorganisms were inoculated in Muller Hinton Broth at 37ºC for overnight. On the next day, the overnight broth culture was diluted with Normal saline to obtain the $OD_{600}$ at 0.08 to 0.1 with the approximate cell density of $1.5X10^8$ CFU/ml. Muller Hinton Agar plates were prepared and sterilized by autoclaving at 121ºC for 15 min. The broth inoculums were evenly spread out with sterile cotton swabs on the Muller Hinton Agar plates to obtain the uniform inoculums. After the plate was inoculated, 8-mm diameter wells were made on the agar medium by using a sterile cork borer. Each 50ul of plant extract (500ug/50ul) was introduced into each well labelled. Chlorempheniсol 30ug/well was used as the positive control. Then, the plates were placed in an incubator at 37ºC for 16 to 18 hours. After incubation, the plates were examined and zone diameters of complete inhibition were measured and recorded to the closest milimeter.

Positive control : Chlorempheniсol

Negative control : 70% EtOH

**Antioxidant Activity**

The ethanolic extract of Pan-u was determined its antioxidant activity by DPPH Scavenging Assay.

Chemical and materials: Reagents and chemical used in this experiment were of the highest analytical grade. The antioxidant activity of plant extracts were determined by the DPPH free radical scavenging assay according to Lee *et.al*. The samples were dissolved in DMSO (10mg/ml) and the dissolved samples were diluted with 50% EtOH for various concentrations. Briefly, the reaction mixture containing 50µl of diluted test sample of various concentrations and 50 µl of DPPH (300 µml) dissolved in ethanol, was taken in a 96-well microliter plate and kept standing at 37ºC for 30 min. The absorbance was measured at 517 nm by using 96-well microplate reader (Spectrostar Nano, BMG Labtech Microplate reader). Ascorbic acid was used as positive control.

50% EtOH was used as negative control and added to the 96-well plate instead of the sample. Percent Radical Scavenging Activity (% RSA) was calculated by using the following formula:

%RSA = [1-(ABS test compound/ABS control)] x100

ABS = Absorbance

RSA = Radical Scavenging Activity

**Statistical Analysis**

The experimental work was performed by triplicate test. The results were reported as mean±standard deviation (SD). Calibration curve was obtained by plotting percentage inhibition against standard concentration. The $IC_{50}$ value was calculated from linear regression analysis using Microsoft excel.

## Results

**Morphological Characters**

***Kaempferia candida*** Wallich, Pl.Asiat. Rar.1:47.1830.

Myanmar Names : Padatsa; Pan-u; Pan-u-phyu

English Name : Narrow-leaf peacock ginger

Family : Zingiberaceae

Flowering period : June to November.

Perennial erect shrubs, 6.0-9.0 cm tall. Rhizomes tuberous, ovoid, brown without and white within. Leaves mostly 2, alternate, broadly elliptic, 10-12 cm long and 6.0-10 cm wide, obtuse at the bases, more or less undulate the margins, acute at the apex. Inflorescences terminal spikes; bracts variable, the two outer ones large, enclosing the inflorescences, ovate to lanceolate, the two inner floral ones linear. Flowers infundibuliform, 4.0-6.0 cm long and 2.3-3.0 cm wide, white. Calyx tubular, 1.5-2.0 cm long and 3-5 mm wide; tubes 1.5-1.7 cm long and 3-5 mm wide; lobes tooth-like, about 1 mm long and wide. Corolla infundibuliform; tubes 2.0-2.4 cm long and 2.0-3.0 mm wide, white; lobes linear, 1.5-1.9 mm long and wide. Fertile stamen one, erect, 0.5-1.0 cm long and 1.0-2.0 mm wide, pale yellow; filament flattened; anther lobes linear-oblongoid; lateral staminodes obovate, 1.5-1.7 cm long and 1.0-1.3 cm wide, white; basal staminodes two, linear-acicular, 2-5 mm long and 0.4 mm wide, white; labellum obovate, 1.7-2.2 cm long and 2.0-2.3 cm wide, white. Ovary oblongoid, 2-4 mm long and 2-3 mm wide, trilocular, one ovule in each locule on the axile placenta, style filiform; stigma globoid.

Specimen examined: Mansan Fall, Lashio Township, Northern Shan State, 20 July, 2019, Khin Myo Aye, Collection No.1.

**Figure 1** *Kaempferia candida* Wall., natural habit of a plant

**Figure 2** *Kaempferia candida* Wall., flower as seen

**Figure 3** *Kaempferia candida* Wall., plant rhizomes

## Preliminary Phytochemical Analysis

The preliminary phytochemical studies for the ethanol extract of rhizomes of *Kaempferia candida* Wall., show the presence of alkaloids, glycosides, phenols, polyphenols, reducing sugars and tannins and the absence of flavonoids, saponins, and carbohydrates. The phytochemical constituents of the plants investigated are summarized in Table 1.

**Table 1 Preliminary phytochemical test of rhizomes of ethanol extract for *Kaempferia candida* Wall.**

| No | Chemical Constituents | Chemical Reagents | Observations | Results (+/-) |
|---|---|---|---|---|
| 1. | Alkaloids | Wagner's | Reddish brown | + |
| | | Dragendroff's | Orange | + |
| | | Mayer's | Creamy ppt | + |
| 2. | Flavonoids | Mg + HCL | No Pink | - |
| 3. | Glycosides | 10% Lead acetate | White ppt | + |
| 4. | Phenolic Compounds | 10 % $FeCL_3$ | Dark green | + |
| 5. | Polyphenols | 10% $FeCL_3$+ 1% $K_3Fe(CN)_6$ | Dark green blue | + |
| 6. | Saponins | Water ($H_2O$) | No Foam | - |
| 7. | Reducing Sugars | Fehlling A+ B | Red ppt | + |
| 8. | Carbohydrates | 10 % Napthanol | No Red ring | - |
| 9. | Tannins | 10 % Lead acetate | White ppt | + |

+ = presence of chemical constituents, - =absence of chemical constituents

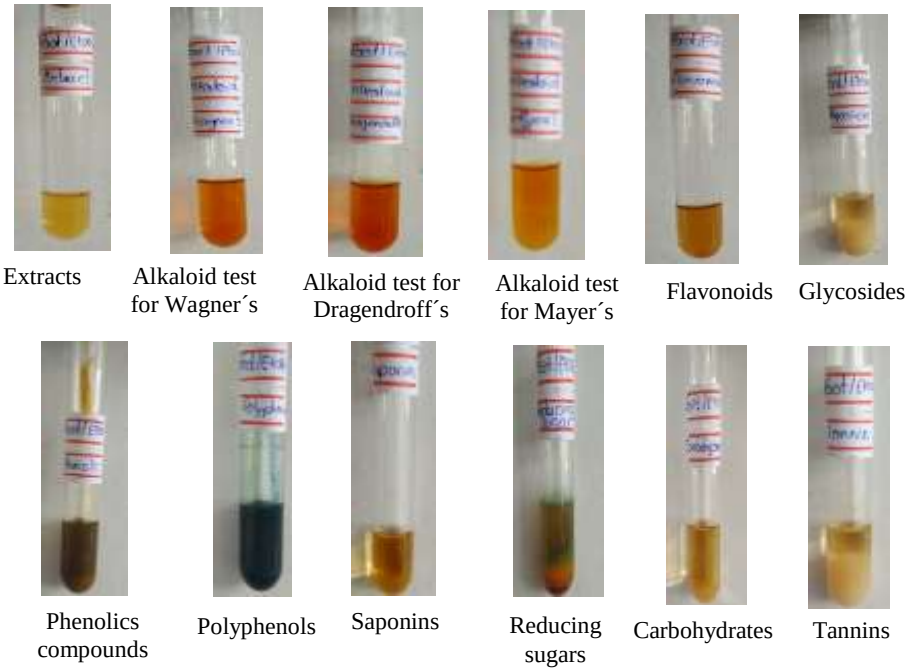


**Figure 4** Preliminary Phytochemical tests of rhizomes of ethanol extract for *Kaempferia candida* Wall.

## Antimicrobial Activity

One Gram-negative bacterium (*Escherichia coli*), three Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis and Bacillus cereus*) and one fungal strain (*Candida albicans*) were used as the tested microorganisms for this experiment.

**Table 2 Antimicrobial activity of ethanol extract from powdered rhizomes of *Kaempferia candida* Wall.**

| Sample | Inhibition Zone Diameter(mm) | | | | |
|---|---|---|---|---|---|
| | ***Escherichia coli*** | ***Enterococcus faecalis*** | ***Staphylococcus aureus*** | ***Bacillus cereus*** | ***Candida albicans*** |
| Panu | 0 | 11 | 16 | 17 | 14 |
| Chloremphenicol | 40 | 35 | 40 | 28 | 32 |

The ethanol extract of *Kaempferia candida* (Pan-u) shows antimicrobial activities against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* but it does not show inhibition zone against *Escherichia coli*.


(A) *Escherichia coli*


(B) *Enterococcus faecalis*


(C) *Staphylococcus aureus*


(D) *Bacillus cereus*


(E) *Candida albicans*

**Figure 5** Antimicrobial activities of ethanol extract of *Kaempferia candida* Wall.

## Antioxidant Activity

The antioxidant activity of Pan-u was carried out by DPPH scavenging assay. Strong antioxidant activity of ($IC_{50}$: 96.8 ±4.15) µg/ml was shown in this experiment compared with the control ascorbic acid. ($IC_{50}$: 84.78 ± 0.39) µg/ml.

**Table 3 The antioxidant activity of Pan-u was carried out by DPPH scavenging assay**

| **Sample (Concentration ug/ml)** | **1000** | **500** | **250** | **125** | **62.5** | **31.25** | **15.63** | **IC(ug/ml)±SD** | **Method** |
|---|---|---|---|---|---|---|---|---|---|
| DPPH Scavenging (%)±SD | >100 | 86.64±3.60 | 84.68±1.18 | 64.64±3.28 | 32.42±1.23 | 1.77±0.68 | - | 96.8±4.15 | DPPH Radical Scavenging Assay |

All data were represented as Mean±SD from triplicate experiments. Ascorbic acid was used as a positive control for DPPH Radical Scavenging Assay. Ascorbic acid shows 94.63± 0.34% inhibition at 500µg/ml and $IC_{50}$ of Ascorbic acid is 84.78± 0.39µg/ml. The concentration of DPPH used for this experiment was 0.3mM.

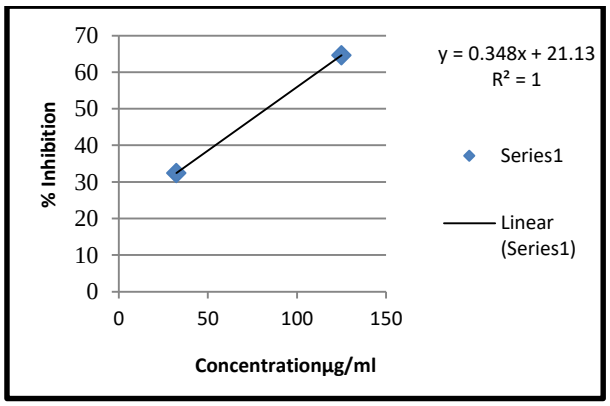


**Figure 6** Calibration Curve

## Discussion

*Kaempferia candida* Wall. are characterized by perennial underground stems (rhizomes), simple and distichous leaves. Petioles are long and the sheathing petioles are usually opened, rarely closed, ligulate. The flowers are irregular, trimerous, with single fertile stamen and two to five petaloid staminodes. The ovary was inferior, trilocular, axile placentation. These morphological characteristics were in accordance with those described by Rendle(1930), Lawrence (1964), Nyunt Nyunt San (1992).

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer (Hossain MA, Nagooru, MR,2011).

In the present study,the phytochemical screening carried out on the ethanol extract of rhizomes of *Kaempferia candida* Wall.,show the presence of alkaloids, glycosides, phenolic compounds, polyphenols, reducing sugar and tannins and the absence of flavonoids, saponins, and carbohydrates.

The ethanol extract of rhizomes of *Kaempferia candida* (Pan-u) shows antimicrobial activities against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* but it does not show inhibition zone against *Escherichia coli.*

Cruickshank (1970) stated that soft tissues infections and skin diseases are caused by *Bacillus cereus. Staphylococcus aureus* is the cause of inflammation,burns and wound infections. *Candida albicans* can also cause sores, many skin diseases.

According to the results of this research,ethanol extract of rhizome of *Kaempferia candida* Wall.,will be useful in curing the diseases caused by the microbes mentioned above.

The antioxidant activity of Pan-u was carried out by DPPH scavenging assay. Strong antioxidant activity of ($IC_{50}$: 96.8 ±4.15) µg/ml was shown in this experiment compared with the control ascorbic acid ($IC_{50}$: 84.78 ± 0.39) µg/ml.

## Conclusion

The extracts of rhizomes of *Kaempferia candida* plant contains chemical compounds such as phenolic compounds and polyphenols that are responsible for its antioxidant and antimicrobial activity. This result showed that rhizomes of *Kaempferia candida* extracts have interesting pharmacological active compounds and antimicrobial effects, and such could be used in ethno medicine for treatment of bacterial infections and ailments.

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