Determination of Phenolic Content and Chemical Compositions of Essential Oil from Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

Mya Mu Aye, Zin Mar Win, Khaing Khaing Kyu

Abstract

In the present study, the rhizomes of *Hedychium flavescens* Carey ex Roscoe (Zingiberaceae) collected from Kayin Chaung village, Hpakant Township, Kachin State were investigated. Firstly, the qualitative and quantitative determination of phenolic content in dried rhizomes of *Hedychium flavescens* Carey ex Roscoe was conducted by Folin-Ciocalteu reagent using UV spectrophotometer. Gallic acid was used to make calibration curve. Moreover, the essential oil contents in the dried rhizomes of *Hedychium flavescen*s Carey ex Roscoe were determined by steam distillation method. Then the phytochemical analyses were done by gas chromatography coupled to mass spectrometry (GC-MS). The major compounds found in the oil of dried rhizomes were α-terpineol (34.84%) and retinal (40.63%). α-terpineol is a monoterpene compound described in the literature that shows a wide range of biological applications.

Keywords: *Hedychium flavescens*, Folin-Ciocalteu, UV, gallic acid, GC-MS

Introduction

The genus *Hedychium* consists of 80 species in worldwide and is one of the most popular genera of Zingiberaceae because of its attractive foliage, diverse and showy flowers, and sweet fragrance (He, 2000). The essential oil can be extracted from leaves, flowers and rhizomes of these plants. In addition, *Hedychium* species were widely cultivated for their perfume essences, and the constituent in the aerial stems is a useful raw material for manufacturing paper (Hartati, *et al*., 2014). Composition of essential oils from *Hedychium* species is characterized by the abundance of monoterpenes with major percentage of 1,8-cineole and pinene derivatives. *Hedychium*, popularly called ginger lily is a rhizomatous flowering plant belonging to family Zingiberaceae. The plant is native to tropical Asia and the Himalayas.

Essential oils are complex mixtures of volatile and odoriferous substances from various plant tissues. They are composed primarily of terpenoids, predominantly monoterpenes, sesquiterpenes and their oxygenated derivatives such as alcohols, ketones, aldehydes, esters, phenols and oxides. These metabolites play essential biological functions for survival and adaptation of the plant to the environment, as well as present pharmacological activities of medical-scientific interest (Da Silva, 2012).

The essential oils concentration in the different parts of the plants varies qualitatively and quantitatively in relation to several factors, mainly the soil, the climate, the period of the day and the seasons of the year (Gobbo-Neto, 2007). Essential oils obtained in different organs of the same plant can have significantly different chemical composition, odor and physicochemical characteristics (Cook, *et al*., 2007 & Ikan, 2008).

Hedychium flavescens Carey ex Roscoe (yellow ginger) belongs to the family Zingiberaceae (ginger family). The rhizome of this plant is used by certain tribal groups of Bihar (India) as a febrifuge and is considered as an anti-rheumatic, tonic (Ajaikumar, *et al*., 2008).

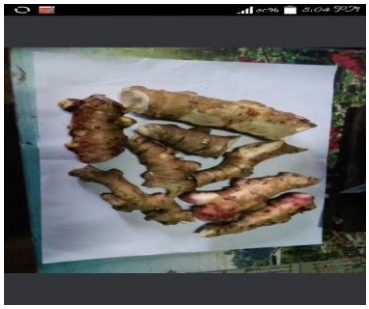
The rhizome of this plant is used in various skin infections. Boiled extract of the rhizome with common salt is used as gargle in throat swellings and also prescribed orally in abdominal swellings, colic and piles. Crushed extract of the rhizome is used with honey in chest congestion, cough, asthma and bronchitis (Singh, 2018).

The essential oil of rhizomes exhibited antimicrobial activity. It also showed effect on central nervous system, tranquilising effect and showed anthelmintic activity (Dixit, 1979). Based on review of literature, a few reports were available on the study of phenolic content determination and chemical compositions of essential oil from the rhizomes of *Hedychium flavescens* Carey ex Roscoe. Hence, rhizomes of *Hedychium flavescens* Carey ex Roscoe were selected for the present study.

The objectives of this study were determination of the preliminary phytochemical constituents, the mineral contents, phenolic content and the chemical compositions of the essential oil from the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe.

Scientific Classification of *Hedychium flavescens* Carey ex Roscoe





(A) **(B)** **(C)**

Figure 1- (A)-Plant, (B)-Flowers and (C)-Rhizomes of *Hdychium flavescens* Carey ex Roscoe

Family Name	:	Zingiberaceae (Ginger family)
Scientific Name	:	*Hedychium flavescens* Carey ex Roscoe
English Name	:	Yellow ginger, Cream garland-lily, Cream ginger
Myanmar Name	:	Shwe-Pan

Materials and Methods

Plant Material

Hedychium flavescens Carey ex Roscoe (Zingiberaceae) were collected from Kayin Chaung Village, Hpakant Township, Kachin State, Myanmar. The plant was identified by the Taxonomist at the Department of Botany, University of Mandalay. The rhizomes of this plant were washed in tap water and then rinsed in distilled water. The rhizomes were cut into small pieces, dried at room temperature. These samples were blended by using grinder to obtain a powdered form.

Chemicals

Analytical grade chemicals were used during experiments. (Folin-Ciocalteu reagent, standard gallic acid, sodium carbonate)

Preliminary Phytochemical Screening

Preliminary phytochemical tests were carried out qualitatively on each extract using standard method to identify the phytochemical constituents.

Determination of Mineral Contents

The mineral contents of the rhizomes of *Hedychium flavescens* Carey ex Roscoe were determined by applying EDXRF (Energy Dispersive X-ray Fluorescense) spectrometer at Department of Chemistry, Monywa University.

Determination of Total Phenolic Content

Preparation of Gallic Acid Solution for Calibration Curve

10 mg of the gallic acid was taken in a test tube. 10 mL of distilled water was added to the standard compound. 1 mL of this standard solution was taken in another test tube. The volume of the solution was made up to 10 mL with distilled water. The solution was taken by micropipette into a series of test tubes 20 µL, 40 µL, 60 µL, 80 µL and 100 µL respectively. The volume was made up to 1.6 mL with distilled water in each tube. And then 100 µL of Folin-Ciocalteu reagent and 300 µL of standard Na_2CO_3 (20%) solution were added. After each standard solution were heated in the water bath at 40 ºC for 30 min. The absorbance of prepared sample solution was measured at 765 nm using a UV spectrophotometer with respect to the blank solution. The gallic acid standard calibration curve was established by plotting concentration versus absorbance.

Folin-Ciocalteu Reagent Method

The total phenolic content of the extract of dried rhizomes was measured by using the Folin-Ciocalteu reagent following Singleton, *et al.*, 1999. 1 mL of sample extract was diluted with 9 mL of distilled water. The mixture was centrifuged at 5000 rpm for 30 minutes. The supernatant was decanted to a small beaker. The procedure was repeated for two times. The supernatant was poured to the same container and used for quantitative analysis of flavonoids. Firstly, 20 µL of diluted sample was taken in a test tube. It was made up to 1580 µL with distilled water. 100 µL of Folin-Ciocalteu reagent was mixed, then 300 µL of saturated Na_2CO_3 (20%) was added. The mixture was heated in a water bath at 40 ºC for 30 minutes and then cooled in an ice bath. The absorbance of this prepared sample solution was measured at 765 nm using a UV spectrophotometer. The total phenolic content in the rhizomes extract was expressed as gallic acid mg/g.

Extraction of Essential Oil by Steam Distillation Method

2 L of distilled water was poured into the still body and perforated cone was set over it, 150 g of the air dried rhizomes was placed on the perforated cone of the still. It was heated carefully without decomposition of oil.

After heating for 2 hours, a mixture of volatile oil and steam was passed into the condenser. The oil colleting on the surface of the water was separated by using the solvent n-hexane. And then n-hexane was evaporated in air at room temperature. The obtained essential oils were dried over anhydrous sodium sulphate and stored at 4 ˚C until for further analysis. The yield of the pale yellow oil from dried rhizomes was 0.018% (w/w).

GC-MS Analysis

The extracted essential oils from the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe were analyzed by GC-MS using pet-ether as solvent for the identification of different compounds, at the Department of Chemistry, University Research Center (URC), University of Mandalay.

Structural assignments were based on analysis of fragmentation pattern of mass spectra and comparison of mass spectral data with profiles in the National Institute of Standards and Technology (NIST) Library.

Results and Discussion

Preliminary Phytochemical Screening of the Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

Preliminary phytochemical screening of the selected sample was done by using standard method and the results revealed the presence of phenolic compounds, glycoside, reducing sugar, tannin, polyphenol, terpene, flavonoid, saponin and lipophenol.

The results were shown in Table 1.

Table 1. The Results of Phytochemical Test for the Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

No	Constituent	Reagent used	Observation	Results
1.	Alkaloid	Dragendroff reagent Wagner reagent	No orange ppt No reddish brown ppt	-
2.	Phenolic	10% $FeCl_3$	Deep blue color solution	+
3.	Glycoside	10% Lead acetate	White ppt	+
4.	Reducing sugar	Benedict solution	Yellow ppt	+
5.	Tannin	10% $FeCl_3$, dil. H_2SO_4	Yellowish brown ppt	+
6.	Steroid	Acetic anhydride, conc. H_2SO_4	No green color solution	-
7.	Polyphenol	1% $FeCl_3$, 1% K_3 $[Fe(CN)_6]$	Greenish blue ppt	+
8.	Terpene	Acetic anhydride, conc. H_2SO_4, $CHCl_3$	Pale red solution	+
9.	Flavonoid	conc: HCl, Mg coil, conc. H_2SO_4	Yellowish brown solution	+
10.	Saponin	Distilled H_2O	Frothy	+
11.	Lipophilic	0.5 N KOH, NaOH	Deep color solution	+

(+) = presence of constituents
(–) = absence of constituents

Determination of Mineral Content in the Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

The elemental compositions of the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe were determined by using EDXRF method at the Department of Chemistry, Monywa University. The results were described in Table 2.

From the analysis of mineral contents, it was found that the amount of potassium is the highest in the dried rhizomes of *Hedychium flavescens* Corey ex Roscoe. Potassium takes part in ionic balance of the human body and maintains tissue excitability. Potassium is important for diuretic nature (Chen, *et al*., 2005).

No	Element	Symbol	Relative Abundance (%)
1	Potassium	K	0.866
2	Silicon	Si	0.212
3	Phosphorus	P	0.206
4	Calcium	Ca	0.158
5	Sulfur	S	0.105
6	Iron	Fe	0.020
7	Manganese	Mn	0.006
8	Copper	Cu	0.002
9	Nickel	Ni	0.001
10	Chromium	Cr	0.001
11	Zinc	Zn	0.001

Table 2. Mineral Compositions in the Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

Estimation of Total Phenolic Content in the Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

Folin-Ciocalteu Colorimetric Method

This colorimetric assay measures the total concentration of phenolic hydroxyl groups in the sample. Polyphenols in the sample react with specific redox reagent (Folin-Ciocalteu reagent) to from a blue complex that can be quantitatively measured by UV/VIS spectrophotometer. The absorbance of prepared sample solution was measured at 765 nm with respect to the blank solution for two times. From these results, the amount of total phenolic content of analyzed sample was obtained by using the standard graph. The total phenolic content present in the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe was found to be 133.7 mg gallic acid equivalent/gram.

Table 3. Concentrations and Absorbance of Standard Gallic Acid Solution

No	**Test Sample**	**Concentration (µg/mL)**	**Absorbance at 765 nm**
1	Blank	0	0.000
2	Std GA 1	2	0.322
3	Std GA 2	4	0.520
4	Std GA 3	6	0.722
5	Std GA 4	8	1.004
6	Std GA 5	10	1.191

Figure 2. Standard Calibration Curve for Gallic Acid (GA) for the Determination of Phenolic Content

Table 4. The Results of Concentration and Absorbance of Selected Sample

No	Test Sample	Concentration (µg/mL)	Absorbance at 765 nm
1	Sample (20 µL)	2.684	0.354
2	Sample (40 µL)	5.367	0.692

The amount of total phenolic in the extract of the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe was determined by using Folin-Ciocalteu reagent method. Gallic acid was used as standard compound and total phenolic content was described as gallic acid equivalents. Therefore, the total phenolic content of the extract of the dried rhizomes of selected plant is 133.7 mg gallic acid equivalent/g. According to the results, high phenolic content is present in the extract of the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe.

Chemical Compositions of Essential Oils from the Dried Rhizomes of *Hedychium flavescens* Carey ex Roscoe

The dried rhizomes were steam distilled to obtain a pleasant smelling, pale yellow colored oil at a yield of 0.018% w/w. The rhizome essential oils of *Hedychium flavescens* Carey ex Roscoe were analyzed by means of GC/MS technique. The GC chromatogram indicates the presence of more than 30 compounds, of which 7 compounds were identified by NIST library data of the peaks with those reported in literature. The results are collected and the chemical structures, respective area, retention time of main constituents are shown in Table 5. Analysis of volatiles from rhizomes of *Hedychium flavescens* Carey ex Roscoe showed the presence of monoterpenes as the major constituents followed by sesquiterperne. The analyzed oil contained monoterpenes (42.62%), sesquiterpene (4.06%) and linear triterpene (5.49%) and others (47.83%). The majority of the essential oil components were monoterpenes and the major constituents were retinal (40.63%), α-terpineol (34.84%), N,N-dimethyl-1-dodecanamine (7.20%), squalene (5.49%), 9-cedranone (4.06%), borneol (3.93%) and terpinen-4-ol (3.85%).

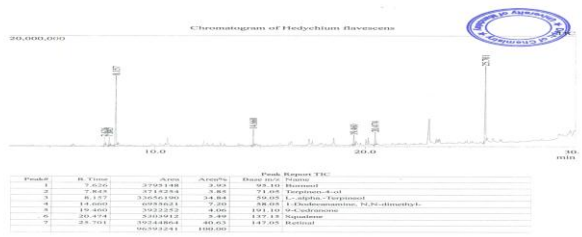
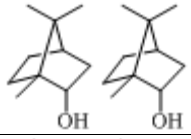
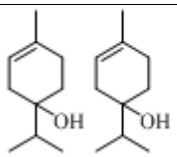
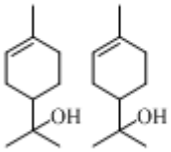
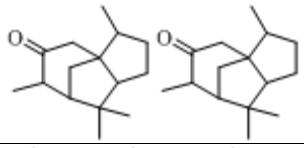
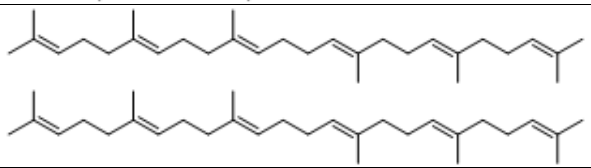
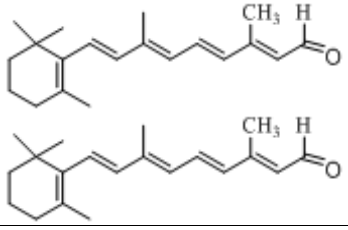


Figure 3. GC-MS Chromatogram of the essential oil of the dried rhizomes of *Hedychium flavescens* Carey ex Roscoe

α-terpineol, a volatile monoterpene compound, is the major component of essential oils of several species of aromatic plants which are widely used for medicinal purposes. α-terpineol proved to possess a potent antioxidant activity against

free radicals causing injury. It showed an anti-proliferative activity, which could be used in the prevention or even treatment of cancer, as it was found that α-terpineol demonstrated a potential antioxidant capacity effect against different human cancer cell lines (breast, lung, prostate, ovarian and leukemia). Several studies have been reported that α-terpineol also possesses antiulcer and anti-inflammatory activities (Christina, *et al*., 2018). Terpinen-4-ol is a potent bactericidal agent that possesses antifungal properties (Shapira, *et al*., 2016). Borneol has a therapeutic potential for painful and inflammatory disorders (Liu, *et al*., 2011).

Table 5. Structures of Chemical Constituents of Essential Oil and their respective area, Retention Time and Similarity Indices

Name	Structure	RT[a]	Peak area (%)	SI[b]
Borneol		7.625	3.93	91
Terpinen-4-ol		7.845	3.85	96
α-terpineol		8.157	34.84	96
N,N-dimethyl-1-dodecanamine		14.660	7.20	98
9-cedranone		19.460	4.06	78
Squalene		20.474	5.49	79
Retinal		25.701	40.63	71

[a] Retention time [b]Similarity Indices

Conclusion

In this research, the rhizome of *Hedychium flavescens* Carey ex Roscoe (Zingiberacear family) which is rich sources of essential oil was selected for chemical compositions of essential oil. Phytochemical screening of the dried rhizome of *Hedychium flavescens* Carey ex Roscoe showed the presence of phenolic compounds,

glycoside, reducing sugar, tannin, polyphenol, terpene, flavonoid, saponin and lipophenol respectively. Additionally, the total phenolic content of the extract of the selected rhizomes could also be evaluated by the Folin-Ciocalteu reagent using UV spectrophotometer at 765 nm. According to the test, it was found that the total phenolic content of the rhizomes extract is 133.7 mg gallic acid equivalent per gram. GC-MS profiling of essential oil of the rhizomes of *Hedychium flavescens* Caery ex Roscoe revealed that the extract contains diversified terpenoids, especially monoterpenes, sesquiterpene and linear triterpene. These compounds have their medicinal importance that can encourage tofind out new therapeutic uses in health care system. The rhizome of *Hedychium flavescens* Carey ex Roscoe is high rich source of phenolic compounds. Therefore, this plant is needed for isolation and screening individual phyto-compounds is required for the further activity.

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