

## Study on Chemical Analysis and Antioxidant Activity from Fruit of *Piper retrofractum* Vahl. (Peik-chin-lay)

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### Abstract

In the present work, the fruit of *Piper retrofractum* Vahl. (Peik-chin-lay) which is used for many years as house hold remedies for the treatment of fever, cough and asthma have been selected and screened for antioxidant. The aim of the study is to determine some phytoconstituents, nutritional values and nutrient elements from the fruit of *Piper retrofractum* Vahl. besides to screen the antioxidant activity of crude extract. Some phytoconstituents of Peik-chin-lay fruit such as alkaloids,  $\alpha$ -aminoacids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponin glycosides, steroids, terpenoids and tannins were found to be present and cyanogenic glycosides was absent. The nutritional value such as moisture content (9.19%), ash content (9.28%), protein content (12.82%), fiber content (6.70%), fat content (5.04%), carbohydrate (56.97%) and energy value (325.00 kcal/100g) were examined by using A.O.A.C method. From the nutrient element, the sample had relatively the highest content of K, Na and Ca according to Atomic Absorption Spectrophotometer (AAS). By using direct extraction method, the crude extract of Peik-chin-lay fruit was prepared with petroleum ether extract and 95 % ethanol extract. Then the antioxidant activity of 95 % ethanol extract moderately showed the IC<sub>50</sub> value: 375.89  $\mu$ g/mL by DPPH assay. Nevertheless petroleum ether extract was not found to be antioxidative efficiency. Thus Peik-chin-lay fruit possesses not only the antioxidant activity but also nutrient elements. Since some nutrient elements found in Peik-chin-lay fruit benefit to human health.

**Keywords :** *Piper retrofractum*, fruit, nutritional value, nutrient elements, antioxidant

### Introduction

The study of traditional medicinal plants and their therapeutics play a very important role in health care system of Myanmar because 70 % of its population is in the rural area and they have been using traditional medicine for centuries (Hun Tun, 1993). Therefore, safe, scientific and systematic development of effective drugs are mandatory to ensure the health of Myanmar people. "Let food be your medicine and medicine be your food", wrote Hippocrates in the fifth century, and indeed the very earliest days of civilization, nutrition has formed the backbone of health care (Dahanukar *et al.*, 2000).

In this study, Myanmar medicinal plant, *Piper retrofractum* Vahl. (Peik-chin-lay) was selected to find out some nutrient elements and active principle for antioxidant additives. *Piper retrofractum* Vahl. contains a good amount of minerals like potassium, sodium, calcium, magnesium, iron, cadmium, zinc, lead and copper. These inorganic elements especially the metals play an important role in biological systems. Based on the relative concentrations in the biological systems, the metals are divided into bulk metals (macrominerals) and trace metals (microminerals) which are present in low concentration and are used for biocatalysis. For people, they include dietary trace metals in amounts generally less than 100 mg per day, as opposed to bulk metals which are required in larger quantities. Sodium, potassium, calcium, and magnesium are bulk metals. Trace elements include at least iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, nickel and molybdenum. Some nonmetals are also found in biological systems. Living organisms contain relatively large amount of oxygen, carbon, hydrogen, nitrogen, sulfur, chlorine and phosphorus known as the bulk elements (Upadhyaya, 1998).

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*Piper retrofractum* Vahl. (Peik-chin-lay) moderately showed anti-oxidant activity whereas it had a prominent antibacterial activity. Therefore, it is very popular for the treatment of related to the gastrointestinal tract infection. In fact it could be able to be loss of cholesterol in human body because piperine compound mainly contained in it (Wagner and Bladt, 2005). Peik-chin-lay is composed of health benefiting essential oils besides an alkaloid, piperine gives strong spicy pungent character to the fruit of *Piper retrofractum* Vahl. Therefore, it is medicinally used in arthritis, throat troubles, carminative, colic, gastric ailments, eczema, scabies, alopecia, other skin diseases, asthma, pain, toothache, bronchitis, rheumatism, fever, cough, dysentery, diarrhea, flatulence, and dermatopathy, oedema and obesity. *Piper retrofractum* Vahl. (Peik-chin-lay) has pharmacological activities such as antioxidant, antimicrobial, insecticidal, antipyretic and anti-inflammatory (Upadhyaya, 1998). Therefore, elemental analysis on (Peik-chin-lay) fruit was done by Atomic Absorption Spectrophotometer (AAS) and then antioxidant activity investigation on 95 % ethanol extract from the fruit of *Piper retrofractum* Vahl. was carried out by using DPPH assay method in this study.

#### **Botanical Aspects of *Piper retrofractum* Vahl.**

Scientific name	: <i>Piper retrofractum</i> Vahl.
English name	: Balinese pepper
Myanmar name	: Peik-chin-lay
Family	: Piperaceae
Fruit (part used)	: Fruits are tiny berries, rod-like structure and merge to a single. (Kress, <i>et al.</i> , 2003)



Figure (1). Plant of *Piper retrofractum* Vahl.

#### **Distribution**

The *Piper* species are of South Asian origin, whereas the closely related *Piper retrofractum* Vahl. comes from South East Asia and is mostly cultivated in Indonesia, Bangladesh, China, Phillipines and Thailand. It is found in many other parts of the Myanmar as a run wild. The plant of *Piper retrofractum* Vahl. (Peik-chin-lay) was shown in figure (1).

#### **Chemical Constituents**

In fruit of *P. retrofractum* Vahl. (Peik-chin-lay) contained piperine, piperlonguminine, sylvatine, guineensine, piperlongumine, filifiline, sistosterol, methyl piperate and a series of piperine-analog retrofractum. Moreover, it also contained sesquiterpene hydrocarbons and ethers, bisabolene,  $\beta$ -caryophyllene, caryophyllene oxide,  $\alpha$ -zingiberene, and saturated aliphatic hydrocarbons: 18 % pentadecane, 7 % tridecane, 6 % heptadecane (Manske, 1952).

## Materials and Methods

**Plant Material:** The healthy fresh fruit sample of *P. retrofractum* Vahl. (Peik-chin-lay) was collected from Kyauk-ey village, Hinthada Township, Ayeyawady Region. It was identified by a botanist from Botany Department, Hinthada University. The fruit of *P. retrofractum* Vahl. was washed, cleaned and the sample was dried in air, shade and a dust free environment. Then the dried sample was powdered and stored in air-tight container to study phytoconstituents, nutritional values, nutrient elements and antioxidant activity.

**Instruments:** AAS, Aanalyst-800 (Perkin Elmer Co. Ltd.), URC (Yangon), UV spectrophotometer

**Chemicals:** Phytoconstituents; Conc. sulphuric acid, sodium hydroxide, toluene, hydrochloric acid, antioxidant; petroleum ether (PE), 96 % ethanol (EtOH), DPPH

### Physicochemical Properties of *P. retrofractum* (Peik-chin-lay) Fruit

#### Determination of nutritional value from fruit of *P. retrofractum* (Peik-chin-lay)

Some physicochemical properties from fruit of *P. retrofractum* (Peik-chin-lay) were determined by using A.O.A.C method.

#### Extraction from Fruit of *Piper retrofractum* (Peik-chin-lay) by cold extraction method

The powdered sample of fruit of *P. retrofractum* (50 g) was extracted with petroleum ether (500 mL) and 95 % ethanol, respectively in conical flasks for three weeks at room temperature and then filtered. The filtrate was evaporated to dryness at normal pressure on a water bath and desiccated. The yield % of petroleum ether and 95 % ethanol extract were determined.

#### Screening of Antioxidant Activity of Crude Extracts from Fruit of *P. retrofractum* by DPPH Assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system. Therefore, petroleum ether extract and 95 % ethanol were subjected to study antioxidant activity by DPPH free radical scavenging assay and the test procedure was as follow. DPPH (2 mg) was thoroughly dissolved in ethanol (100 mL). This solution was freshly prepared in the brown colored flask. Then, it must be stored in the fridge for no longer than 24 hours. Each 2 mg of test sample and 10 mL of methanol (EtOH) were thoroughly mixed by vortex mixer. The mixture solution was filtered and the stock solution was obtained. Desired concentration 400 µg/ mL, 200 µg/ mL, 100 µg/ mL, 50 µg/ mL, 25 µg/ mL and 12.5 µg/ mL of solution were prepared from this stock solution of dilution with appropriate amount of ethanol. Blank solution was prepared by mixing the test sample solution (1.5 mL) with ethanol (1.5 mL). As the standard solution, 2 mg of butylated hydroxyl toluene (BHT) was dissolved in methanol and made up the volume to 100 mL. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of ethanol using vortex mixer. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Each experiment was done triplicate.

## Results and Discussion

### Preliminary Phytochemical Examination from Fruit of *P. retrofractum* (Peik-chin-lay)

Phytochemical screening serves as an initial step in expanding the knowledge about plant constituents and interest of phytochemists trying to recover new sources of phytochemical. Out of the twelve tests, cyanogenic glycosides were absent. Cyanogenic glycosides were hydrolyzed to generate hydrogen cyanide. From the result, poisonous cyanogenic glycosides were not found in Peik-chin-lay fruit. Therefore, Peik-chin-lay fruit is suitable for human health. The results obtained were described in the Table 1.

Table (1). Preliminary Phytochemical Constituents from Fruit of *P. retrofractum* (Peik- chin-lay)

No	Test	Extract	Reagent Used	Observation	Remark
1	Alkaloids	1% HCl	Mayer's reagent Dragendroff's reagent Wagner's reagent	White ppt. Orange ppt. Reddish brown ppt.	(+) (+) (+)
2	$\alpha$ -amino acids	D/W	Ninhydrin reagent	violet spot	(+)
3	Carbohydrates	D/W	10 % $\alpha$ -naphthol and 1 cm <sup>3</sup> of Conc. H <sub>2</sub> SO <sub>4</sub>	Red ring	(+)
4	Cyanogenic Glycosides	D/W	Sodium picrate and Conc. H <sub>2</sub> SO <sub>4</sub>	No brick-Red ppt.	(-)
5	Flavonoids	80% EtOH	1 % HCl and Mg Turning	Red colour	(+)
6	Glycosides	D/W	10 % lead acetate Solution	White ppt.	(+)
7	Phenolic Compounds	D/W	5 % FeCl <sub>3</sub> solution	Deep blue colour	(+)
8	Reducing Sugar	Dil H <sub>2</sub> SO <sub>4</sub> and NaOH	Benedict's Solution	Brick-Red ppt.	(+)
9	Saponin Glycosides	D/W	Distilled water	Marked Frothing	(+)
10	Tannins	D/W	2 % NaCl and 1 % gelatin	White ppt.	(+)
11	Steroids	Toluene	Acetic anhydride and Conc. H <sub>2</sub> SO <sub>4</sub>	Green colour	(+)
12	Terpenoids	EtOH	Acetic anhydride and Conc. H <sub>2</sub> SO <sub>4</sub>	Pink colour	(+)

(+) = presence

(-) = absence

### Physicochemical Properties from Fruit of *P. retrofractum* (Peik-chin-lay)

Some physicochemical properties from fruit of *P. retrofractum* (Peik-chin-lay) were determined by using A.O.A.C method. The powder of Peik-chin-lay fruit was investigated in the content of moisture, ash, protein, fiber, fat, carbohydrate and energy value. The obtained results are shown in table (2).

Table (2). Nutritional Values from Fruit of *P. retrofractum* (Peik-chin-lay)

No.	Parameter	Content (%)
1	Moisture	9.19
2	Ash	9.28
3	Protein	12.82
4	Crude fiber	6.70
5	Crude fat	5.04
6	Carbohydrate	56.97
7	Energy value (kcal/100 g)	325.00

### Elemental Analysis from fruit of *P. retrofractum* (Peik-chin-lay)

From the AAS analysis, the nutrient elements (K, Na, Ca, Mg, Fe, Cd, Zn, Pb and Cu) content were reported in Table 3. According to results K was the highest abundance in Peik-chin-lay fruit. And then, Na and Ca were relatively high. K is extremely important to cells. Without K we could not survive and the nerve cell could not send message to our brain. Sodium is very important part of blood plasma and to digest the food. Addition, Ca need to use in cell wall, building bones and initiating DNA synthesis. So Peik-chin-lay fruit is compatible for human health.

Table (3) Elemental Contents from Fruit of *P. retrofractum* (Peik-chin-lay) by Atomic Absorption Spectrophotometer (AAS)

No.	Element	Content (ppm)
1	K	31.13
2	Na	23.55
3	Ca	22.63
4	Mg	9.14
5	Fe	3.56
6	Cd	3.42
7	Zn	1.65
8	Pb	0.17
9	Cu	0.16

### Extraction from Fruit of *P. retrofractum* (Peik-chin-lay) by cold extraction method

Extraction from fruit of *P. retrofractum* (Peik-chin-lay) was carried out by different solvents such as petroleum ether and 95 % ethanol at room temperature for three weeks. Petroleum ether extract (4.20 %) and 95 % ethanol extract (10.60 %), respectively were obtained. These extracts were used to test antioxidant activity from Peik-chin-lay fruit.

### Study on DPPH Radical Scavenging Activity from Fruit of *P. retrofractum* by DPPH Assay

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. This method is based on the reduction of coloured free radical DPPH in ethanol solution by different concentration of the samples. The present study was carried out to investigate the radical scavenging activity of petroleum ether extract and 95 % EtOH crude extract from selected medicinal plant by using DPPH assay according to the spectrophotometric method.

From these results, it can be clearly seen that  $IC_{50}$  value was found to be 375.89  $\mu\text{g/mL}$  for 95 % EtOH extract of Peik-chin-lay fruit shown in Table 4. Nevertheless,  $IC_{50}$  value of petroleum ether extract of Peik-chin-lay fruit was greater than 400  $\mu\text{g/mL}$ . Thus, it was remarked that PE extract may be impossible to show antioxidant activity. From these experimental results, it was found that the concentrations were increased. It means that increase in concentration and increase in radical scavenging activity of samples usually expressed in term of % inhibition,  $IC_{50}$  (50 % inhibition concentration) values in  $\mu\text{g/mL}$ .

Table (4). DPPH Radical Scavenging Activity of Petroleum ether extract and 95 % Ethanol Extract from Fruit of *P. retrofractum* (Peik-chin-lay)

Tested samples	Percent Oxidative Inhibition in Different Concentration ( $\mu\text{g/mL}$ )						$IC_{50}$ ( $\mu\text{g/mL}$ )
	400	200	100	50	25	12.5	
95 % EtOH Ext.	53.67	24.77	12.61	4.97	2.52	0.54	375.89
PE Ext.	-	-	-	-	-	-	ND

ND = not detected

### Conclusion

In the present study, some phytochemical constituents used in medicine such as alkaloids,  $\alpha$ -aminoacids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponin glycosides, steroids, terpenoids and tannins were found to be present and cyanogenic glycosides was absent in this fruit. Therefore, it may be assured that Peik-chin-lay fruit could be convenient for human health. In addition, the nutritional values of Peik-chin-lay fruit was observed to be moisture content (9.19 %), ash content (9.28 %), protein content (12.82 %), fiber content (6.70 %), fat content (5.04 %), carbohydrate (56.97 %) and energy value (325.00 kcal/100 g) respectively. The nutrient elements of this fruit was determined by using Atomic Absorption Spectrophotometer (AAS). The results showed that potassium (31.13 ppm), sodium (23.55 ppm), calcium (22.63 ppm), magnesium (9.14 ppm), iron (3.56 ppm), cadmium (3.42 ppm), zinc (1.65 ppm), lead (0.17 ppm) and copper (0.16 ppm) respectively. Among these elements, potassium, calcium and sodium concentration are distinctly higher than that of other elements. By cold extraction method, two crude extracts were prepared from fruit of *Piper retrofractum* Vahl. (Peik-chin-lay) using petroleum ether and 95 % ethanol as the different solvent polarity. It was indicated that 4.20 % of ethyl acetate extract and 10.60 % of 95 % ethanol extract were included in Peik-chin-lay fruit.

The screening of antioxidant activity on petroleum ether extract and 95 % ethanol extract were done by using DPPH assay. In this experiment, the  $IC_{50}$  value of 95 % ethanol crude extract was found to be 375.89  $\mu\text{g/mL}$  that moderately indicated DPPH radical scavenging activity. But petroleum ether extract ( $IC_{50} > 400 \mu\text{g/mL}$ ) didn't show antioxidant activity. So it could be assigned that 95 % ethanol extract possessed synergetic effect. Whatever it is the selected plant possesses not only the antioxidant activity but also nutrient elements that show anti-secretory activity in gastrointestinal infections. Based on the finding of present study it is concluded that the selected Myanmar medicinal plant (Peik-chin-lay) may be used as "natural antioxidant additives" in both therapeutic and food industry in place of "synthetic antioxidant additives". Since some nutrient elements found in Peik-chin-lay fruit benefit to human health, it could be denoted to be safely and widely used in food industry.

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