Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [11]: 38-46
[http://scholarsresearchlibrary.com/archive.html]

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Antiproliferative and Antioxidant Properties of *Ananas comosus* L. Merr., Patavia and Nanglae, From Northern Thailand

Manee Khamenkhetkarn[1*], Churdsak Jaikang[2], Thanapat Sastraruji[3]

[1]*Toxicology Section, Regional Medical Science Center, Chiang Mai, Department of Medical Science, Ministry of Public Health, Mae Rim District, Chiang Mai, Thailand*

[2]*Toxicology Section, Faculty of Medicine, Department of Forensic Medicine, Chiang Mai University, Thailand*

[3]*Faculty of Dentistry, Dentistry Research Center, Chiang Mai University, Chiang Mai 50200 Thailand*

***Corresponding author**: Khamenkhetkarn M, Toxicology Section, Regional Medical Science Center, Chiang Mai, Department of Medical Science, Ministry of Public Health, Mae Rim District, Chiang Mai, Thailand*
E-mail: Manee707@hotmail.com

ABSTRACT

Pattavia and Nanglae are famous pineapple species (Ananas comosus L. Merr) from Northern Thailand. The objective of this study was carried out antiproliferative activity, phytochemical content and antioxidant of both ethanolic Pattavia and Nanglae extracts. The results showed that Pattavia and Nanglae extracts were effective to growth inhibiton of Hep G2 cells lines with IC50 at 22.40 and 24.28 µg/ml, respectively. Total phenolic contents in Pattavia and Nanglae were 8.20 ± 0.10 and 9.66 ± 0.18 mg gallic acid equivalent/g extract. Total flavonoid in in Pattavia and Nanglae were 1.17 ± 0.08 and 1.15 ± 0.02 mg quercetin equivalent/g extract, respectively. The extract of Pattavia showed scavenge hydrogen peroxide with IC50 value at 106.75 µg/ ml better than Nanglae with IC50 value at 109.10 µg/ ml. Pattavia and Nanglae extracts could change Fe3+ to Fe2+ by using ferric reducing antioxidant power (FRAP) with value of 33.74 ± 0.14 and 40.50 ± 0.64 mM/ g extract, respectively. Both the ethanolic extracts of Pattavia and Nanglae had antioxidant properties. Phenolic and flavonoid compounds might have the important roles in antiproliferative and antioxidant activities of the pineapple extracts.

Keywords: *Ananas comosus L. Merr*; Antiproliferative; Antioxidant; Pattavia; Nanglae

INTRODUCTION

Reactive oxygen species (ROS) are a term which encompasses all highly reactive, oxygen-containing molecules, including superoxide radical, hydroxyl radical and hydrogen peroxide [1]. Excessive ROS can be lead to oxidative stress that be involved with development of many diseases such as autoimmune disorders, rheumatoid arthritis, aging, cardiovascular, neurodegenerative diseases and especially cancer [2].

Cancer is the major causes of morbidity and mortality problem in human worldwide. Since 1990, the data have been shown that incidence of cancer in Thailand is increasing, especially liver cancer [3]. Environmental factors especially carcinogens are major cause of cancers. The carcinogens induce initiation, promotion and progression process which can change normal cells to cancer cells [4]. While, antioxidant plays a major role in inhibiting and scavenging radicals. It involves a variety of components, both endogenous and exogenous in cells, that function interactively and synergistically to neutralize free radicals [5]. Inhibition of ROS generation and acceleration of their elimination as a result of reducing the oxidative stress can be done by consumption of natural antioxidant from beverage and fruits. [6].

Fruits are the major sources of active phytochemicals could act as protective agents with respect to human carcinogenesis. A relationship between the presence of phytochemical substance, such as phenolic content and flavonoid with the reduced risk of cancer have been reported [7-9].

Pineapple, Ananus Comosus (L) Merr, is tropical fruit that is gained popularity worldwide. Previous research with antioxidant activity of pineapple in other countries reported that highest total phenolic compound [10]. Pattavia (Sriracha) and Nanglae are mostly cultivated in the Northern Thailand, especially in Lampang and Chiang Rai province. However, the data of antioxidant and antiproliferative activity of the pineapple are lack of report. Then, in this study aims to determine antiproliferative activity, antioxidant and phytochemical contents in ethanolic extracts of the pineapple.

MATERIALS AND METHODS

Plant collection and extraction

Ananas comosus L. Merr., Pattavia was purchased from local Chiang Mai market and Nanglae was purchased from local Nanglae Subdistrict, Meung Chiang Rai, Chiang Rai during the session of June to July 2015. The fresh pulp was extracted with 70% ethanol and continuously stirred at 25°C for 3 hour. The extract was filtered with filter cloth and centrifuged at 3,500 rpm for 15 min. Supernatant was evaporated and the residue was collected at 4°C.

Cell culture and treatment

Hep G2 cells were cultured in Dulbecco's Modified Eagle Medium(DMEM) medium which supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ ml of streptomycin and 2.5 µg/ ml fungizone and maintained at 37°C in humidified atmosphere of 5% CO_2. The cells were treated with various concentration of Pattavia and Nanglae solution (0-400 µg/ml).

Cell viability assay

Cell viability assay was measured using (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay according to the method of Mark [11]. After treating the cells with the pineapples concentration for 48 h, 20 µl of 0.5% MTT solution was added and stood at room temperature for 4 h in the dark. The medium with MTT was removed and then 200 µl of dimethyl sulfoxide (DMSO) was added for dissolving formazan crystals. The absorbance was measured at 550 nm and 630 nm by using microplate reader. The % cell inhibition was calculated using equation below:

$$\% \text{ Cell inhibition} = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100$$

Determination of total phenolic contents

Total phenolic contents in pineapple extracts were measured by using Folin-Ciocaltue reagent according to Kasempitakpong et al. [12]. Briefly, 0.5 ml of the pineapple extract was mixed with 5% Folin-Ciocaltue solution and 1M sodium carbonate. The mixture was stood at room temperature for 15 min. The absorbance was measured at 765 nm and the total phenolic content was calculated using gallic acid as standard curve. The result was expressed as gallic acid equivalent/ dried weight extract.

Determination of total flavonoid contents

Total flavonoid content was determined using aluminium chloride according to the method of Kasempitakpong et al. [12]. The ethanolic extract of pineapples was added in the reaction solution containing 0.1 ml of 10% aluminium chloride and 1 M potassium acetate. The solution was stood for 15 min at room temperature and the absorbance was measured at 415 nm. The results was calculated and expressed as quercetin equivalent/ dried weight extract.

Determination of phytochemical content in the extracts

The isolation of phytochemicals from ethanolic pineapple extract was performed by solid phase extraction (SPE) and GC-MS according to Kusirisin et al. [13]. Briefly, 0.1 g of the extract was dissolved with distilled water then HCl was dropped and boiled for 20 minutes. The C18 Cartridge column was eluted with ethyl acetate, methanol and mili Q water, the sample solution and methanol, respectively. The saluted solution was injected in GS-MS for phytochemical analysis. A gas chromatography machine (Varian star 3400 CS) was included a column (DB-5, 30 m × 0.25 mm ID. × 0.25 µM film thick) and conditioned with inlet of 220°C, oven temperature of 80°C held for 1 min and then increased to 300°C at 10°C/ min held for 5 min, a flow rat of helium gas carrier at 1.0 min/ min. A mass spectrometry detector (Varian Saturn 2000, MS ion trap 150°C, MS source 230°C) was used for analysing samples which were compared with standard compounds as stored in a database library.

Hydrogen peroxide scavenging

The ability of the ethanolic pineapple extracts to scavenge hydrogen peroxide was measured according to the method of Sharma et al. [14]. The extracts were extracted in 40 mM hydrogen peroxide solution and stood at 25°C for 10 min. The absorbance of solution was measured at 230 nm against a blank solution containing phosphate buffer pH 7.4 and the extracts. The percentage of hydrogen peroxide scavenging was calculated followed equation

$$\% H_2O_2 \text{ scavenging} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Ferric reducing antioxidant power assay

FRAP assay was carried out according to Wong et al. [15] with slight modification. Briefly, the freshly FRAP reagent was composed of acetate buffer pH 3.6, 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol Iron (III) chloride in ratio 10: 1: 1 (v/ v), respectively. The sample was added in 1.5 ml the FRAP reagent and incubated in room temperature for 4 min. The absorbance of the reaction mixture was read at 593 nm. The results were expressed as µmol Fe (II)/ g plant extract by using Iron (II) sulfate as standard curve.

Statistical analysis

All experiments were measured in triplicates and data were expressed as mean ± standard deviation. The difference between groups were analysed by one-way ANOVA. *p*-value of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Extraction

All fresh samples were extracted by ethanol maceration and the residue was re-extracted in triplicate. The extracts were concentrated using rotary evaporator and % yield extract of Pattavia and Nanglae was 2.59% and 1.43% w/w, respectively and results are showed in Table 1. Pattavia gave higher yield extract than Nanglae about two times may be from different of pulp texture of Pattavia that contains high fibers but less water than Nanglae.

Table 1: Type of pine apple and % yield extract, total phenolic contents, total flavonoid contents and FRAP values.

Type of pineapple	**% yield extract (w/w)**	**Total Phenolic (mg gallic acid/ g extract)**	**Total Flavonoid (mg quercetin/ g extract)**	**FRAP Assay (mM/ g extract)**
Pattavia	2.59	8.20 $\pm$ 0.10	1.17 $\pm$ 0.08	33.74 $\pm$ 0.64
Nanglae	1.43	9.66 $\pm$ 0.18	1.15 $\pm$ 0.02	40.50 $\pm$ 0.64

Antiproliferative assay

The MTT assay was used for evaluating the antiproliferative effects of the ethanolic pineapple extracts on HepG2, human liver cancer cell line. The cells were exposed to various concentrations and the result is shown in Fig. 1. Inhibition concentration at 50 percent (IC_{50}) value of Pattavia and Nanglae extract were 22.40 and 24.28 µg/ml, respectively. The Pattavia extract was more toxic to HepG2 cells than Nanglae. Phenolic compounds are a large group of natural compounds which contain hydroxyl groups, and have been demonstrated to exhibit anticancer properties [16]. The potential application of these phenolic compounds in the development of therapeutic agents for cancer treatment has gained increasing importance. Sun and his colleagues showed pineapple had no effect on HepG2 antiproliferative acitivity [3]. However, in this study found that pineapple from Norther Thailand high effective to suppress HepG2 proliferation. Different of phenolic and flavonoid contents in pineapple may cause different the effectives.

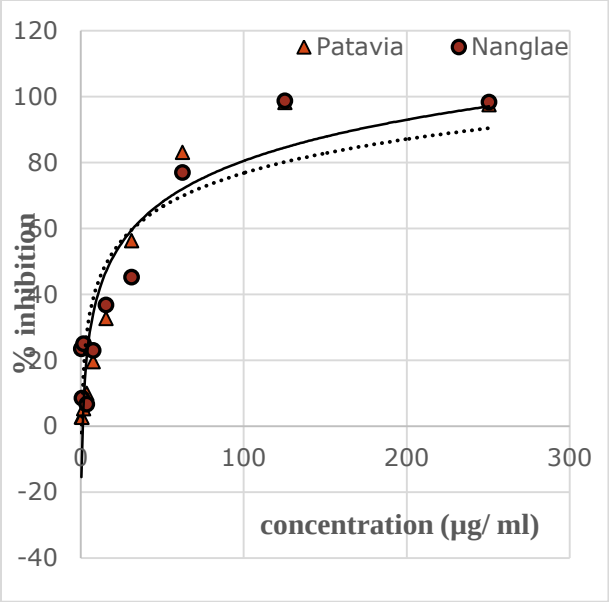

Figure 1: %Inhibition of HepG2 cells proliferation (full line – Pattavia and dash line --- Nanglae)

Antioxidant assay

Total phenolic and flavonoid extraction of Pattavia and Nanglae are expressed as mg of gallic acid and quercetin equivalent/g extracts, respectively and the result are summarized in the Table 1. The result showed that total phenolic of Nanglae was higher than Pattavia while total flavonoid of both pine apple were not significantly difference.

Identification of Pattavia and Nanglae extracts using GC-MS which each peak were identified using library search program and results are shown in Fig. 2A and Fig. 2B, respectively.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is based on ability of antioxidant power to reduce ferrous ion to ferric ion. The reducing power is an indicator for potential antioxidant activity. The Table 1 showed the FRAP value of Pattavia and Nanglae extract. The result showed that the antioxidant potential of Pattavia was more effective reduce TPTZ-Fe (III) to TPTZ-Fe (II) complex. FRAP assay was used for antioxidant activity assessment in food and their product samples. The secondary metabolites in food are act as redox-active compounds that will be donated electron in the FRAP assay [17].

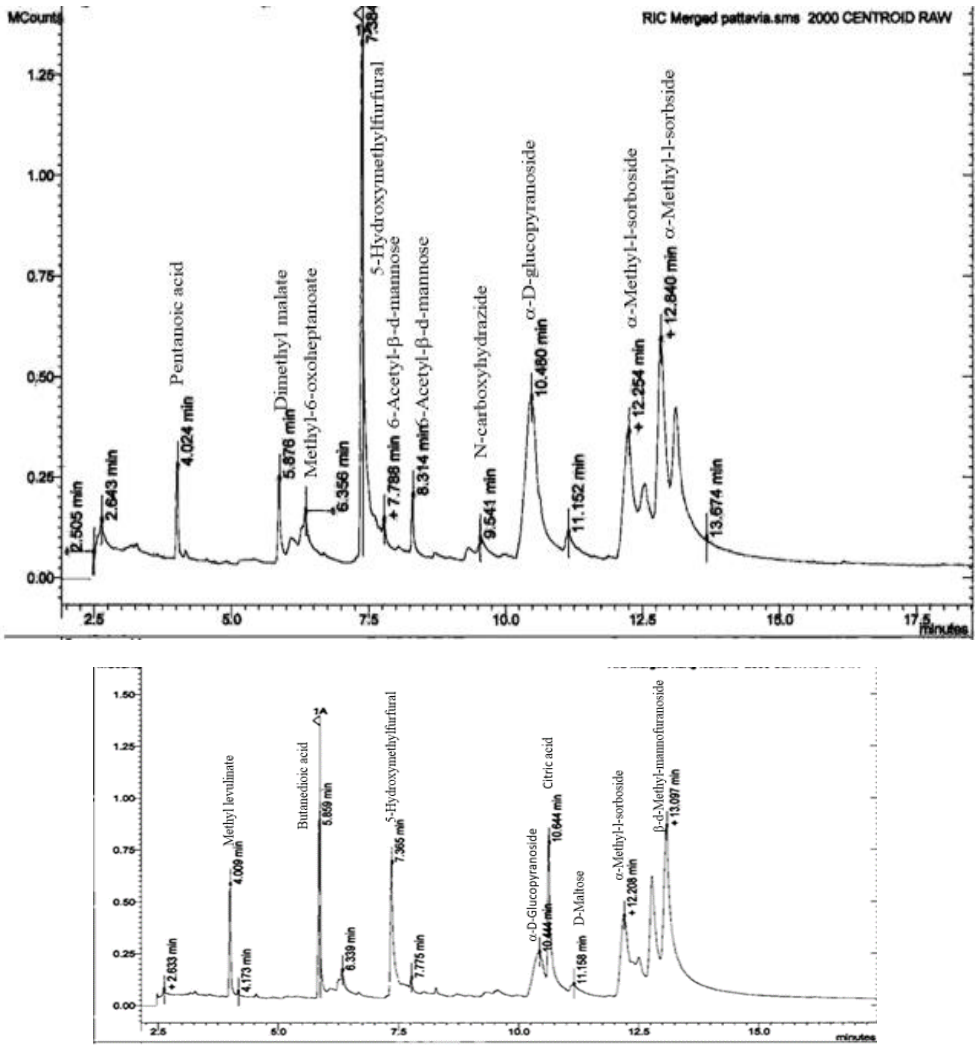

Figure 2: GC-MS chromatogram of Pattavia extract (A) and Nanglae (B)**.**

Hydrogen peroxide scavenging activity

The scavenging activity of ethanolic extracts of Pattavia and Nanglae on hydrogen peroxide is shown Figure 3. The Pattavia and Nanglae extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. The results showed that the hydrogen peroxide scavenging activity concentration at 50 percent (IC_{50}) value of Pattavia and Nanglae extract were 106.75 and 109.10 µg/ml, respectively. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cell because it may change to hydroxyl radical in the cells. Thus, the scavenging of hydrogen peroxide is very important for antioxidant defense in the cell systems [18].

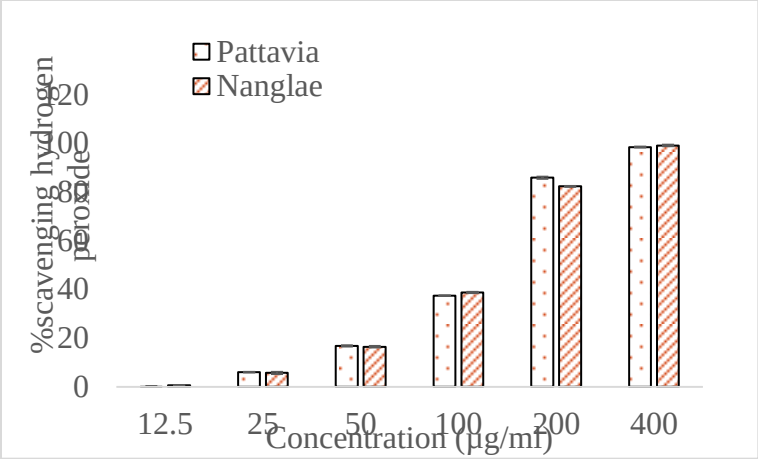

Figure 3: Hydrogen peroxide scavenging

CONCLUSION

Ethanolic extracts of Pattavia and Nanglae demonstrated antiproliferative on HepG2 cells line and antioxidant activities. Phenolic and flavonoid conpounds may be the important constituent's substances for antiproliferative and antioxidant activities of the pineapple extracts.

ACKNOWLEDGEMENTS

This work was supported by Thailand Research Fund (RDG5420054). We are also grateful to Toxicology section, Regional Medical Science Centre 1 (Chiang Mai) for instruments and chemicals.

REFERENCES

1. Zorov, D.B., Juhaszova, M., and Sollott, S.J., Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol. Rev,* **2014**. 94: 909-950.
2. Ebrahimzadeh, M.A., et al. Antioxidant and free radical scavenging activity of h. Officinalis l. Var. Angustifolius, v. Odorata, b. Hyrcana and c. Speciosum. *Pak. J Pharm Sci.,* **2010.** 23: 29-34.
3. Annal Report 2015 Bruere of Non Communicable Disease p.21.
4. Wattenberg L.W., Chemo prevenction of cancer, *Cancer Res.,* **1985.** 45: 1-8.
5. Jacob, RA., Quality evaluation of the bread added *Toona sinensis* and its antioxidant activity analysis. *Nutri. Res.,* **1995.** 15:755-766.

6. Lobo, V., et al. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev,* **2010.** 4: 118-126.
7. Sun, J., et al. Antioxidant and antiproliferative activities of common fruits. *J Agric Food Chem,* **2002.** 50: 7449-7454.
8. Pandey, K.B., and Rizvi, S.I., Plant polyphenols as dietary antioxidants in human health and disease. *Oxi. Med. Cell Longev*, **2009.** 2: 270-278.
9. Russo, M., Phytochemicals in cancer prevention and therapy: Truth or Dare?. *Toxins (Basel),* **2010.** 2: 517-551.
10. Alotman, M., Bhat, R., Karim, A.A., *Food Chem*, **2009.** 115: 785-788.
11. DC Mark, L. Belov, M.W. Davey, R.A. Davey, *Leuk. Res.*, **1992**, 16, 1165-1173.
12. Kasempitakpong, B., Antioxidant and acetylcholinesterase inhibitory potential of Thai Medicinal plants, *Curr. Nutri. Food Sci.,* **2015.** 11: 99-104.
13. Kusirisin, W., et al. Synthesis and preclinical evaluation of novel PET probes for P-glycoprotein function and expression, *Med. Chem,* **2009.** 5: 583-538.
14. Sharma, J.K., and Singh, AP., Complementary and alternative medicine in the treatment of refugees and survivors of torture: A review and proposal for, *J Acupunc. Meridian Stud.* **2012.** 5: 112-118.
15. Wong, CC., et al. *Food Chem.*, **2006.** 97: 705-711.
16. Karabin, M., Hudcora, T., and Jelinek, L., P. Toasting ingredients in fermented and distilled beverages. Dostalek. *Compr. Rev. Food Sci. Food Safety*. **2016.** 15: 542-567.
17. Stratil, P., Klejdus, B., and Kuban, V., Determination of total content of phenolic compounds and their antioxidant activity in vegetables. Evaluation of spectrophotometric methods, *J. Agric. Food Chem.*, **2006**, 53, 607-616.
18. Kumarappan, CT., Thilagam, E., and Mandal, SC., *Saudi. J. Biol. Sci*, **2012.** 19: 349-355.