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# The comparative characteristics of snake and kiwi fruits

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ABSTRACT

In the time of globalization many of the tropical fruits can be find at the markets of Europe and North America. Most customers are not familiar with the nutritional and proliferative values of these fruits. Therefore, a less known snake fruit was compared with better known kiwi fruit, using fluorometry, FT-IR spectroscopy, several radical scavenging and proliferative assays and statistical evaluation.

It was found similarity between snake fruit (cultivar Sumalee) and kiwi fruit (cultivar Hayward) in the contents of polyphenols (8.15–7.91, mg GAE $g^{-1}$ DW), antioxidant values by DPPH (11.28–10.24, μMTE $g^{-1}$ DW), and antiproliferative activities on both human cancer cell lines (Calu-6 for human pulmonary carcinoma, and SMU-601 for human gastric carcinoma, 90.5–87.6 and 89.3–87.1%, cell survival, respectively).

In conclusion, snake fruit cultivar Sumalee is comparable with kiwi fruit cultivar Hayward. Two fruits can be used as supplements to the normal diet. Consumption of a combination of both fruits could be recommended in order to receive the best results.



## 1. Introduction

Nowadays a variety of tropical fruits are common ingredients of different diets in Europe and North America (Kondo et al., 2005; Luximon-Ramma et al., 2003; Murcia et al., 2001; Yuka et al., 2003). The high nutritional value of the subtropical and tropical fruits led to significant increase in their consumption. However, most customers are not familiar with the nutritional values of these fruits. Therefore, in this investigation a less known and less investigated snake fruit (*Salacca edulis* Reinw, cultivar Sumalee) was compared with the better known kiwi fruit (*Actinidia chinensis*, cultivar Hayward) and the main nutritional components and the antioxidant and proliferative potentials of both fruits were determined and compared.

In order to receive reliable results it was decided to use snake and kiwi fruits of the same ripeness and to determine their antioxidant potentials by four different assays: 1. FRAP, ferric-reducing/antioxidant power assay (Ozgen et al., 2006). 2. ABTS−+, 2,2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt assay (Ozgen et al., 2006); 3. DPPH, 1,1-diphenyl-2-picrylhydrazyl method (Ozgen et al., 2006); and 4. CUPRAC, cupric reducing antioxidant capacity assay (Apak et al., 2004). The role of ascorbic acid in the total antioxidant potentials (AP) of fruits is controversial. Some authors claim that the AP of fruits might be attributed mainly to the content of phenols (Wang et al., 1996; Rapisarda et al., 1999), and the contribution of ascorbic acid to the total AP is less than 15% (Wang et al., 1996). On the other hand, there are investigators who claim that ascorbic acid plays a major role in the total AP (Vinson et al., 2002). It was decided to determine the content of ascorbic acid in the studied samples and its contribution to AP.

As far as we know there are no published results of such investigations.

## 2. Materials and methods

### 2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate,

*Abbreviations:* Kiwi OHE, kiwi fruit organic Hayward ethylene treated; Kiwi CHE, kiwi fruit conventional Hayward ethylene treated.

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$FeCl_3 \times 6H_2O$, $CuCl_2 \times 2H_2O$, 2,9-dimethyl-1, 10-phenanthroline (neocuproine), butylated hydroxyanisole (BHA), were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

### 2.2. Samples and preparation

Snake fruit (*S. edulis* Reinw) is originated from Southeast Asia. The fruit is egg like in shape and the skin of the mature fruit is brown. The fruit has pineapple-, pear-, and banana-like aroma. The weight is up to 70 g at the last maturation stage. The fruit contains three pieces of seeds covered with white flesh. Most of this fruit is freshly consumed and some are processed into fruit juice, canned fruit or jam.

The kiwi fruit (*A. chinensis*) is native to the Yangtze River valley of northern China and Zhejiang Province on the coast of eastern China. When sliced, the fruit yields an attractive emerald green flesh with rows of small, dark, edible seeds, and a light cream colored center. Its flavor is similar to a blend between strawberry and pineapple.

Hayward cultivars of kiwi fruits (conventional and organic) at their commercial maturity stage were harvested in the orchard (Heanam County, Jeonnam province, Korea, 2008).

The kiwi fruit samples [organic 'Hayward' ethylene treated (OHE) and conventional 'Hayward' ethylene treated (CHE)] were treated with 100 ppm of ethylene for 24 h at 20° C in a growth chamber (Percival Scientific Inc., Perry, IA, USA). The samples were put into an 18 l glass jar and ventilated with humidified flow of ethylene at 300 ml $min^{-1}$. Then the ethylene-treated kiwi fruits were ripened at 20° C in the same growth chamber for 10 days (Park et al., 2008).

Two cultivars of snake fruits Noen Wong (old variety, 2006) and Sumalee (new variety, 2008) were sampled from orchard (Muang district, Chantaburi province, Thailand).

All fruits were cleaned with tap water and dried, using five replicates of five fruits each. The edible parts of the above-mentioned fruits were prepared for this investigation manually without using steel knives. The peeled fruits were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20° C until the bioactive substances were analyzed.

### 2.3. Determination of bioactive compounds, antioxidant potentials, basic nutritional compounds, minerals and trace elements

The bioactivity in snake and kiwi fruits such as antioxidant potentials, dietary fibers, proteins, fats, carbohydrates, minerals and trace elements determined by a Perkin–Elmer 5100 ZL atomic absorption spectrometer [Perkin–Elmer Ltd., Beaconsfield, Buckinghamshire, England, using the flame and flameless method] were determined as previously described (Leontowicz et al., 2006, 2007; Haruenkit et al., 2007; Gorinstein et al., 2009).

### 2.4. Determination of the contents of the main bioactive compounds, Fourier transform infrared (FT-IR) spectra of polyphenols and fluorometry

The presence of polyphenols in the investigated fruit samples was studied by Fourier transform infrared (FT-IR) spectroscopy. A Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisk was prepared from finely ground lyophilized powder of 2 mg of fruit samples with 100 mg of KBr (Sinelli et al., 2008).

Fluorescence measurements were done using a model FP-6500, Jasco Spectrofluorometer, serial N261332, Japan. Fluorescence emission spectra for all fruit samples at a concentration of 0.25 mg/ml were taken at emission wavelength (nm) of 330, and recorded from wavelength of 265 to a wavelength of 310 nm, at emission wavelengths of 685 nm from 300 to 750 nm; and at excitation of 350 nm from 370 to 650 nm. Standards of quercetin of 0.01 mM in methanol were used.

Phenols were extracted from lyophilized fruits with methanol (concentration 25 mg/ml) at room temperature twice during 3 h and then were determined by Folin-Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett–Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW. Phenols were extracted as well in the same concentration with ethanol and water.

Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE). Total ascorbic acid was determined by CUPRAC assay (Ozyurek et al., 2007). Water extract was prepared from 100 mg of lyophilized sample and 5 ml of water. This extract (1 ml) was mixed with 2 ml of $3.0 \times 10^{-3}$ M of lanthanum (III) chloride heptahydrate. Ethylacetate (EtAc) was used for extraction of flavonoids in order to avoid the interference. One millilitre of Cu(II)–neocuproine (Nc), in ammonium acetate-containing medium at pH 7, added to 1 ml of the obtained extract. The absorbance of the formed bis (Nc)-copper(I) chelate was measured at 450 nm.

### 2.5. Determination of the antioxidant potentials

The following antioxidant assays were used: (1) ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the samples to reduce ferric tripyridyltriazine ($Fe^{3+}$-TPTZ) to a ferrous form ($Fe^{2+}$) which absorbs light at 593 nm. The antioxidant activity was determined at constant concentration and also with different concentrations of the fruits from 5 to 25 mg/ml; (2) 2,2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$) was generated by the interaction of ABTS (mmol/l) and $K_2S_2O_8$ (2.45 mmol/l). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm; (3) 1-diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 ml, 25 mg/l) in methanol was mixed with the samples extracts (0.1 ml). The reaction progress was monitored at 515 nm until the absorbance was stable; (4). Cupric reducing antioxidant capacity (CUPRAC) is based on utilizing the copper(II)–neocuproine [Cu(II)–Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 ml of Cu(II), Nc, and $NH_4Ac$ buffer solution, extract of durian sample (or standard) solution ($x$ ml) and $H_2O$ [$(1.1 - x)$ ml] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank.

### 2.6. Chemometrical processing

Samples with different portion of fruit extracts (5, 10, 15, 20 and 25 mg/ml) were analyzed by FRAP antioxidant activity assay. The data set consisted of a $150 \times 3$ matrix in which rows represented the different extracts of fruit species and columns the three FRAP variables (several readings of absorbance A1–A3 and Trolox equivalent values). Basic chemometric characterization of the investigated fruit extracts according to their ability to reduce the $Fe^{3+}$ ions was carried out by descriptive (normal probability, box/whisker and dot plots) and multivariate (principal component, factor and discriminant analysis) statistics. All statistical procedures were realized by statistical programme Unistat® (Unistat, London, United Kingdom) and basic descriptive statistics by Microsoft® Office Excel 2003.

### 2.7. Determination of the antiproliferative activity

Anticancer activity of methanol extracts of the studied plants on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell lines (suspended) were purchased from Korean Cell Line Bank (KCLB) for MTT assay. Cells were grown in RPMI-1640 medium at 37 °C under 5% $CO_2$ in a humidified incubator. Cells cultured were harvested, counted ($3 \times 10^4$ cells/ml), and transferred into a 96-well plate, and incubated for 24 h prior to the addition of fruit ethanol, methanol and water extracts. Serial dilutions of the extracts were prepared by dissolving compounds in dimethyl sulfoxide (DMSO) followed by dilution with RPMI-1640 medium to give final concentration at 125, 250, 500, 1000, and 2000 μg $ml^{-1}$.

To give final concentration of extracts from 125 to 2000 μg $ml^{-1}$, solutions were prepared for cell lines at 90 μl and samples (plant extracts) at 10 μl, and incubated for 72 h. MTT solution at 5 mg/$ml^{-1}$ was dissolved in 1 ml of phosphate buffer solution (PBS), and 10 μl of it was added to each of the 96 wells. The wells were wrapped with aluminum foil and incubated at 37° C for 4 h. The solution in each well containing media, unbound MTT, and dead cells were removed by suction, and then 150 μl of DMSO was added to each well. Final concentration of DMSO was 10%. The plates were then shaken and optical density was recorded using a microplate reader at 540 nm. Distilled water was used as positive control and DMSO as solvent control. The effect of the fruit extract on the proliferation of cancer and normal cells was expressed as cell viability: percent viability = OD of fruit extract of treated sample/OD of none treated sample) × 100, where OD is an optical density (Boivin et al., 2008; Chon et al., 2009). Three different extracts were used (methanol, ethanol and water).

### 2.8. Statistical methods

The results of the investigation are mean ± SD of five measurements. Where it was appropriate, differences between groups were tested by two-way analysis of variance (ANOVA). The *P*-values of $< 0.05$ were considered significant.

## 3. Results

### 3.1. Nutrients in soil, minerals and trace elements and basic nutritional compounds

The soil where the investigated fruits were grown was a sandy loam with the following data: pH 5.4, EC 296 μS $cm^{-1}$, organic