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Original Research Article

Evaluation of Antioxidant and Antifungal Activities of Pumpkin By-product and Its Application in Banana

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ABSTRACT

Pumpkin peel is a by-product which may contain some bioactive compounds. It should be of interest to investigate the efficacy of pumpkin peel to control postharvest disease. Anthracnose disease caused by *Colletotrichum musae* is a major postharvest disease and causes loss of banana in Thailand. The aims of this research were to investigate antioxidant activity and bioactive compounds in pumpkin peel and to investigate the antifungal activity of pumpkin peel crude extract against anthracnose in banana. Pumpkin peel (*Cucurbita* spp.) was extracted with three different solvents: methanol, ethanol and acetone in ratio of pumpkin peel to solvent at 1:5 (w/v). Methanol was the best solvent for extraction, showing the significant higher ($P \leq 0.05$) values of DPPH (19.57 µmol TE / 100 g DW), FRAP (64.88 µmol Fe(ll) / 100 g DW) and total phenolic content (92.25 mg GAE / 100 g DW), when compared with ethanol and acetone. Methanol at the concentrations of 95%, 70% and 50% were then used for extraction in a subsequent trial. The result showed that 50% methanol gave a significant higher ($P \leq 0.05$) yield, DPPH (40.73 µmol TE / 100 g DW), FRAP (97.15 µmol Fe(ll) / 100 g DW) and phenolic compound (202.43 mg GAE / 100 g DW) when compared with 95% and 70% methanol. The 50% methanol pumpkin peel crude extract was then diluted to 95%, 70% and 50% for antifungal assays, both *in vitro* and *in vivo*. The methanol pumpkin peel extract at all concentrations significantly delayed ($P \leq 0.05$) radial growth of *C. musae* during incubation for 6 days in comparison with the control (dimethyl sulfoxide: DMSO). There was no significant difference between methanol pumpkin peel extracts and control (DMSO) in rot lesion development on banana during storage for 5 days. Although, pumpkin peel extract did not control banana anthracnose, it might be used in combination with fungicide. And hence, the use of chemical would possibly be reduced.

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INTRODUCTION

Pumpkin (*Cucurbita* spp.), a tropical fruit in the family Cucurbitaceae, is a good source of carotene, pectin, vitamins, mineral and other nutrition substances which are good for human health (Saeleaw and Schleining, 2011). Moreover, antioxidant activities and bioactive compounds have been reported in several parts of pumpkin, including flower (Aquino-Bolaños et al., 2013), pulp (Dini et al., 2013), seed (Nawirska-Olszańska et al., 2013) and peel (Asif et al., 2017). Pumpkin peel is considered as a by-product which may contain some bioactive compounds and could be of interest for further utilization.

Anthracnose disease caused by *Colletotrichum musae* is one of the important diseases in banana. Several control methods including chemical, physical and biological methods have been applied to control banana anthracnose. Since fungicide has been concerned over safety and plant pathogen becomes resistant to the chemicals (Nega, 2014), the control has turned into a non-chemical and eco-friendly method. Crude extracts from several plants such as pomegranate, citrus and herbs have been earlier investigated for its efficacy against plant pathogens and have been reported in containing phenolic compounds (Gatto et al., 2016). Extraction and purification of bioactive compounds from plant materials have been investigated by using organic solvents. However, type and concentration of solvent and condition during extraction may affect quantity and chemical compounds in crude extract (Muhamad et al., 2017). The objectives of this study were 1) to investigate antioxidant activity of pumpkin peel crude extract including total phenolic content and total flavonoid content and 2) to study antifungal activity of pumpkin peel extract for control of *C. musae* both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Plant materials

Pumpkin peel used in this study was a by-product obtained from a cafeteria in Mae Fah Luang University, Chiang Rai, Thailand. After collecting, peels were washed under tap water and cut into small pieces before drying in a tray dryer at 40°C for 5 days or until constant weight obtained.

Extraction of pumpkin peel

Three solvents: 95% methanol, 95% ethanol and 95% acetone were initially tested for their efficiency for extraction of phenolic content and antioxidant activity, where the most efficient solvent was then chosen for subsequent trials. For extraction, dried pumpkin peels were soaked in each solvent at the ratio of 1:5 and the mixture was shaken vigorously on a shaker maintained at 200 rpm at ambient temperature (27±2°C for this study) for 5 days (Asif et al. , 2017). The suspended solution was then filtrated through Whatman No.1 filter paper and dried under a vacuum on a rotary evaporator at 40°C for 10 h. The crude extract was collected, kept in air-tight bottles and in the refrigerator at 4°C until used.

Determination of total phenolic content

Total phenolic content (TPC) in pumpkin peel extracts was determined by using Folin-Ciocalteu method as described by Asif et al. (2017) using gallic acid as a standard. Two hundred µL of pumpkin peel extract was mixed with 1 mL of 10% (v/v) Folin-Ciocalteu reagent, then 800 µL of 7.5% (w/v) sodium carbonate was added. The mixture was kept at room temperature for 1 h and the absorbance was measured at 765 nm with a spectrophotometer (G10S UV-Vis, Thermo Scientific, USA). The total phenolic content were calculated and expressed as mg gallic acid equivalent (GAE) per 100 g dry weight (DW).

Determination of total flavonoid content

Total flavonoid content (TFC) in pumpkin peel extracts was determined by aluminium chloride colorimetric method as described by Kim et al. (2003). A 0.5 mL of pumpkin peel extract was mixed with 2.2 mL of distilled water, 0.15 mL of 5% sodium nitrite and 0.15 mL of aluminium chloride. After 6 min, 2 mL of 4% sodium hydroxide was added and the mixture was then incubated at room temperature for 15 min. The absorbance was measured at 510 nm with a spectrophotometer (G10S UV-Vis, Thermo Scientific, USA). A calibration curve was prepared using a standard solution of catechin. Results were expressed in mg catechin equivalent (CAE) per 100 g dry weight (DW).

Determination of antioxidant activity

DPPH radical scavenging activity: the free radical scavenging activity was determined by following the method of Molyneux (2004). Fifty µL of pumpkin peel extract was mixed with 1,950 µL of ethanoic solution of DPPH (DPPH 0.00236 g in 100 mL ethanol). The mixture was then shaken thoroughly and kept in dark at room temperature for 30 min. The absorbance was measured at 517 nm with a spectrophotometer (G10S UV-Vis, Thermo Scientific, USA). Trolox was used as a standard and the results were expressed as µmol trolox equivalent (TE) per 100 g dry weight (DW).

Ferric reducing antioxidant power assay (FRAP): the FRAP assay was determined according to the method described by Benzie and Szeto (1999). The FRAP solution was prepared by mixing 100 mL of acetate buffer (pH 3.6) with 10 mL of 10 mM TPTZ solution and 10 mL of 20 mM Iron Trichloride ($FeCl_3$). The 400 µL of peel extract was mixed with 2.6 mL of FRAP solution and the mixture was incubated at 37°C for 30 min. The absorbance was measured at 595 nm. Ferrous sulfate was used as standard and the results were expressed as µmol Fe(ll) per 100 g dry weight (DW).

Determination of antifungal activity

The fungus *Colletotrichum musae* was isolated from diseased banana and its pathogenicity was conducted before the trials. A 14-day-old culture was used throughout this study, both *in vitro* and *in vivo*.

Radial growth: the radial growth of *C. musae* was determined by using a disc diffusion method. A 0.1-mL drop of spore suspension (10^5 spore/mL) was placed in a 5.0-mm-diameter hole previously made on Potato Dextrose Agar (PDA) plate and a 0.1-mL drop of pumkin peel extract (either 50%, 70% or 95%) was placed in an opposite hole. A 0.1% carbendazim was used as a positive control and dimethyl sulfoxide (DMSO) was used as a negative control. Inoculated plate was incubated at ambiance temperature and the diameter of radial growth was measured every day for 6 days. There were 6 replications per treatment.

Control of anthracnose in banana: the efficacy of pumpkin peel extract for control of anthracnose in banana was determined by measuring rot lesion diameters as described by Meng et al. (2015). Mature yellow banana were washed with 0.05% sodium hypochlorite, rinsed with distilled water and air-dried at room

temperature. Banana were wounded at the center of the fruit by using a sterile needle and inoculated with mycelial plugs (5-mm diameter) of a 14-day-old culture of *C. musae* on the wound. The inoculated banana were incubated in a moist plastic box at ambient temperature, 90±5% RH for 24 h before dropping a 0.5-mL of peel extract (50%, 70% or 95%) on each wound. A 0.1% carbendazim was used as a positive control and DMSO was used as a negative control. After treatment, banana were incubated in a moist plastic box at ambient temperature, 90±5% RH. The lesion diameters were measured horizontally and vertically everyday, and the mean for lesion diameters was calculated and recorded in cm. There were 8 replications of 1 fruit for each treatment.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Packages for the Social Science (SPSS) version 20. Duncan's multiple range test was used to compare significant differences among treatments. Differences were considered significant at $P \leq 0.05$. All the analyses were carried out in triplicates.

RESULTS AND DISCUSSION

Effect of different solvents on total phenolic content, total flavonoid content and antioxidant activity

Under the same extraction time and temperature, the solvent extraction of phytochemical from plant is mainly influenced by solvent polarity and composition of the sample (Do et al., 2014). In this study, different solvents used for extracting pumpkin peel resulted in different total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (Table 1). The yields of 95% methanol, 95% ethanol and 95% acetone extracts of pumpkin peels were 16.97%, 11.26% and 8.48%, respectively, showing that the extraction yield increases with increasing polarity of the solvent used in extraction. Similarly, the highest yield of extractable compounds was obtained in methanol extract from peel and seed of pomegranate (Singh et al., 2002) and from pineapple (Hossain and Rahman, 2011) compared to other solvents tested. In addition, methanol or aqueous methanol commonly achieved in extraction of phenolic compounds from fruit (Hossain and Rahman, 2011). In pumpkin peels, among the three solvents, 95% methanol (92.25 mg GAE / 100 g DW) indicated the best solvent for extracting TPC in comparison with 95% ethanol (73.44 mg GAE / 100 g DW) and 95% acetone (57.41 mg GAE / 100 g DW). However, 95% acetone pumpkin peel extract contained the significant highest ($P \leq 0.05$) TFC (15.73 mg CAE / 100 g DW) followed by 95% methanol (7.18 mg CAE / 100 g DW) and 95% ethanol (5.22 mg CAE / 100 g DW).

The antioxidant capacity of pumpkin peel extracts was examined through DPPH and FRAP assays. The 95% methanol extract of pumpkin peels exhibited significant higher ($P \leq 0.05$) DPPH and FRAP values than 95% ethanol and 95% acetone extracts did. This can be interpreted that 95% methanol pumpkin peel extract had a higher antioxidant capacity among the three extracts. Bussaman et al. (2012) and El Zawane Kamarudin et al. (2014) explained that different solvents may give different antioxidant activities due to solvent polarity, pH, temperature, extraction method, extraction time, composition of the sample and age of plant. Moreover, different concentrations of the same solvent may also give different antioxidant activities and bioactive compounds, which is probably because of solubility of the phenolic compounds in the solvent (Do et al., 2014).

Table 1. Total phenolic content, total flavonoid content and antioxidant capacity of pumpkin peel extracts obtained from different solvent extractions.

Solvents	Total Phenolic Content (mg GAE / 100 g DW)	Total Flavonoid Content (mg CAE / 100 g DW)	DPPH (µmol TE / 100 g DW)	FRAP (µmol Fe(ll) / 100 g DW)
95% methanol	92.25±0.81 a	7.18±0.09 b	19.57±0.07 a	64.87±0.27 a
95% ethanol	73.44±0.13 b	5.22±0.02 c	14.85±0.94 b	54.87±1.06 b
95% acetone	57.41±0.39 c	15.73±0.73 a	12.72±0.54 c	53.92±0.42 c

Values are mean ± standard deviation of 3 replicates. Means followed by the different letters in the same column indicate significant differences among treatments according to Duncan's multiple range test at $P \leq 0.05$.

Effect of different concentrations of methanol on yield, total phenolic content, total flavonoid content and antioxidant activity

Table 2 shows efficacy of methanol at three different concentrations: 95%, 70% and 50% on extraction of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity from pumpkin peels. The yields of 95%, 70% and 50% methanol extracts of pumpkin peels were 14.05%, 15.75% and 20.00%, respectively. Similar result was obtained by Do et al. (2014) that increasing the water concentration in the solvent enhances extraction yield of *Limnophila aromatica*. It was suggested that other compounds apart from phenolic compounds may have been extracted and contribute to higher yield. Among the three concentraions of solvent used, 50% methanol extract contained the signifciant highest ($P \leq 0.05$) amount of TPC (202.43 mg GAE / 100 g DW) followed by 70% methanol extract (155.27 mg GAE / 100 g DW) and 95% methanol extract (143.86 mg GAE / 100 g DW). The total flavonoid contents varied from 1.50 – 7.08 mg CAE / 100 g DW. The highest TFC was obtained in 95% methanol extract (7.08 mg CAE / 100 g DW) followed by 70% methanol (2.61 mg CAE / 100 g DW) and 50% methanol (1.50 mg CAE / 100 g DW).

The free radical scavenging potentials of the pumpkin peel extracts examined through DPPH method were found to be in the order of 50% methanol extract (40.72 µmol TE / 100 g DW) > 70% methanol (37.59 µmol TE / 100 g DW) > 95% methanol (35.48 µmol TE / 100 g DW). Likewise, in FRAP assay, the antioxidant capacity of the pumpkin peel extracts was found to decrease in the order, 50% methanol extract (97.14 µmol Fe(ll) / 100 g DW) > 70% methanol (80.88 µmol Fe(ll) / 100 g DW) > 95% methanol (43.82 µmol Fe(ll) / 100 g DW. This order is similar to total phenolic content of the extract in which it showed the extent of antioxidant capacity of the extract is in accordance with the amount of total phenolic content present in the extract. The phenolic compounds are the dominant antioxidants that exhibit scavenging efficiency on free radicals (Prior and Cao, 2000) and numerous reports have conclusively shown a close relationship between total phenolic content and antioxidant activity of fruits and vegetables.

Table 2. Total phenolic content, total flavonoid content and antioxidant capacity of methanol extracts of pumpkin peel obtained from different concentrations of methanol extraction.

Solvents	Total Phenolic Content (mg GAE / 100 g DW)	Total Flavonoid Content (mg CAE / 100 g DW)	DPPH (µmol TE / 100 g DW)	FRAP (µmol Fe(II) / 100 g DW)
95% methanol	143.86±1.20 c	7.08±0.05 a	35.48±0.49 c	43.82±0.00 c
70% methanol	155.27±0.87 b	2.61±0.01 b	37.59±0.19 b	80.88±0.28 b
50% methanol	202.43±0.24 a	1.50±0.02 c	40.72±0.31 a	97.14±0.66 a

Values are mean ± standard deviation of 3 replicates. Means followed by the different letters in the same column indicate significant differences among treatments according to Duncan's multiple range test at $P \leq 0.05$.

Effect of methanol extract of pumpkin peels on *in vitro* growth of *Colletotrichum musae*

In the treatments with pumpkin peel extracts, the readial growth of *C. musae* was significant lower ($P \leq 0.05$) than that in the DMSO treatment (as a negative control) (Figure 1). There was no statistical difference among the concentrations of methanol extract of pumpkin peels on growth of *C. musae*. The result indicated that pumpkin peel extracts delayed an *in vitro* mycelial growth of *C. musae*, compared to the control. The inhibitory effect of the extract might be due to some bioactive compounds, such as TPC and TFC, present in pumpkin peel extract.

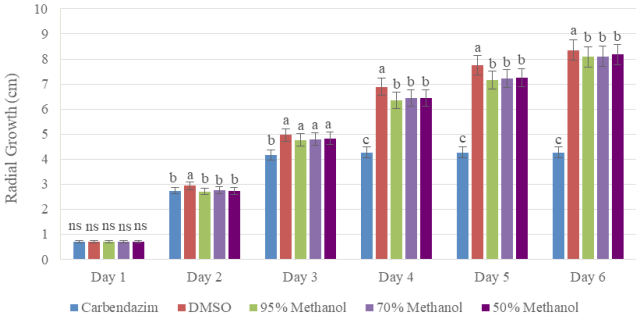

Figure 1. Effect of methanol extract of pumpkin peels at different concentrations on radial growth of *Colletotrichum musae* during incubation for 6 days. Values are means ± standard deviation from $n = 6$.

Effect of methanol extract of pumpkin peels for control of anthracnose in banana

There was no lesion development on artificially inoculated banana during storage for up to 3 days (Figure 2). The rot lesion was observed in all treatments, except carbendazim, after storage for 4 days. The severity as measured by rot lesion development of anthracnose disease on banana treated with pumpkin peel extract at all concentrations were slightly lower than that on banana treated with DMSO, with no significant difference. This is probably due to the presence of secondary metabolites, e.g. phenolic compound, in the pumpkin peel extract which could delay growth of the fungus. However, several factors such as inoculum level, amount of extract applied, stability of extract and rapid degradation of bioactive components in the extracts may influence the antifungal efficacy of the extracts.

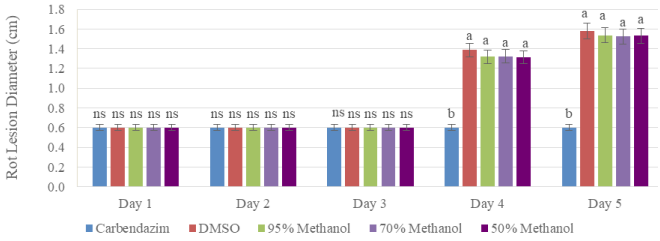

Figure 2. Effect of methanol at different concentrations on anthracnose rot lesion on artificially inoculated banana during storage at 25±2°C, 90±5% RH for 5 days. Values are means ± standard deviation from $n = 6$.

CONCLUSIONS

This present study revealed that pumpkin peels, a by-product, contained some bioactive compounds, e.g., total phenolic content and total flavonoid content and contained antioxidants. However, the amount of bioactive compounds may vary due to cultivar of pumpkin in which different cultivars may contain different compounds. Among the solvents tested, methanol showed the best solvent for extracting total phenolic content and antioxidant activity from pumpkin peels. The methanol extract of pumpkin peels delayed mycelial growth of *C. musae in vitro*. In the *in vivo* treatment, pumpkin peel extracts did not achieve in controlling anthranose disease in banana. The results obtained in this study, however, suggests that pumpkin peel extracts could possibly be used in combination with other control methods in order to reduce the use of synthetic chemical fungicides.

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