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ANTIOXIDANT STUDY OF DIFFERENT PARTS OF STINK BEAN (*PARKIA SPECIOSA*) AND ITS EFFECT ON THE OXIDATIVE STABILITY OF BEEF PATTY

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ABSTRACT

This study investigated the potential of stink bean leaves and peels as natural antioxidants in meat products. The leaves and peels were extracted using water as a solvent. Antioxidant analysis that includes Total Phenolic Compound (TPC), Free Radical Scavenging Activity (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays were carried out to determine the total polyphenols and antioxidant activities in both extracts. TPC was determined using Follin-Ciocalteu reagent and the result indicated that TPC for peel extract was 37.51 mg GAE/g and 3.95 mg GAE/g for leaf extract. The antioxidant activities were compared with synthetic antioxidant which was ascorbic acid. For DPPH, scavenging effects of extracts and ascorbic acid decreased significantly in the order of ascorbic acid, peel extract, and leaf extract with the percentage of 94.93%, 93.64% and 90.35%, respectively at 1000 ppm. Meanwhile for FRAP assay, peel extract at 200 ppm and leaf extract at 800 ppm were comparable to 600 ppm of ascorbic acid. Thus, 200 ppm of peel extract was chosen to be incorporated into beef patty and be compared with ascorbic acid at 600 ppm. Oxidation analysis was performed on beef patties that were prepared using three formulas which were sample without the addition of extract (control), sample with ascorbic acid and sample with peel extract. Lipid oxidation analysis was performed by determining the peroxide value (PV) which varied from 5.89 to 7.56 meq/kg at the end of the storage. Hence, both parts of plants showed antioxidants properties, but peel extract was proven to possess higher antioxidant activity and has a potential to be incorporated into beef patty and as natural antioxidant.

Key Words: stink bean peels, stink bean leaves, antioxidant, beef patty, oxidative stability

1. INTRODUCTION

In food industry, antioxidants are crucial for extending food's shelf life, minimising nutritional losses, and reducing the production of toxic materials (Ko *et al.*, 2014). A study by Fithri *et al.* (2019) stated that stink bean seeds and leaves showed a strong antioxidant activity against the radical effect of superoxide with an approximately 70% inhibition rate. The natural antioxidants regularly consist of substances with numerous OH groups which act as hydrogen donors to retard the lipid oxidation (Mozuraityte *et al.*, 2016). Recently, the natural antioxidant has gained interest from the consumers especially in food industry as the synthetic antioxidant such as butylated hydroxyl anisole (BHA) usage has been linked to a potential toxicity with negative effect, including carcinogenesis (Caleja *et al.*, 2017). Some bakery, dairy, and meat products now incorporate natural extracts from aromatic plants, spices, and fruit powder for antioxidant purposes, hence enhancing the value of end products.

2. METHODOLOGY

2.1 Preparation of plant extract

The extraction of sample was done according to Nurdyansyah & Widyastuti (2020) with slight modifications. Twenty grams of stinky beans powder were extracted with 600 mL of boiling water. The sample was partitioned via filter paper and evaporated using rotary evaporator at 50°C to 60°C. The extracts then were freeze-dried in the freeze dryer until it fully dried into powder. The percentage of plant extracts for each weight of sample were determined.

2.2 Total phenolic compound (TPC)

The Total phenolic content (TPC) in the extracts were determined using Folin–Ciocalteu reagent according to Wonghirundecha (2014) with several modifications. Firstly, gallic acid standard was prepared by using the concentrations of 0.0, 5.0, 10.0, 20.0, 30.0 and 50.0 µg. The solution was inserted into volumetric flask. The flask was mixed with about 0.5 ml of Folin-Ciocalteu reagent. After 3 minutes, 1.5 ml of 20% Na₂CO₃ were added, and the mixture was shaken. About 10 ml of distilled water was added to the mixture, and it was shaken again. The mixture was placed for 2 hours at room temperature and was analysed at 760 nm by UV-vis spectrophotometer. The TPC method was done by dissolving 10 mg of extract in 2 ml of distilled water. About 0.1 ml of the solution was mixed into 10 ml volumetric flask and 0.5 ml Folin-Ciocalteu reagent was added into the solution. After 3 minutes, 1.5 ml 20% Na₂CO₃ was added and shaken. Distilled water was added to the mixture about 10 ml and was shaken again. The mixture was placed for 2 hours at room temperature. The samples were then be read at 760 nm by UV-vis spectrophotometer.

2.3 Free radical scavenging activity (DPPH)

This DPPH assay was conducted according to Ghasemzadeh *et al.* (2018) with several modifications. For both extracts, the sample were prepared in different concentrations. About 0.6 ml of extract of each concentration were transferred to the test tubes. Then 4.5 ml of DPPH was added to every test tubes. Then it were shaken and mixed well. The samples were set in the dark for about half an hour at room temperature. The absorbance was read at 517 nm wavelength.

2.4 Free reducing antioxidant power (FRAP)

Using method performed by Vijayalakshmi & Ruckmani (2016), different concentrations of extracts were prepared in 1 ml distilled water. The solution was mixed with 5.0 ml phosphate buffer and 5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. Then, 5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. Five ml from the upper layer were mixed with 5.0 ml distilled water and followed by 1.0 ml of 0.1% ferric chloride. Absorbance was read at 725 nm by using spectrophotometer.

2.5 Preparation of beef patty

A meat grinder was used to grind the fresh meat. The raw materials were mixed using a mixer includes 750 g lean beef meat, 130 mL iced water, 15g salt, 10g white pepper, and 5 g sugar. The extracts were added into respective parts of the patty's mixture. Then, around 70 g of beef patties were made and placed on a plastic tray. The patties were packed into a seal bag and stored in a chiller at 4°C. The patties were measured for Peroxide value (PV) within three days interval for nine days.

2.6 Lipid extraction

Lipids were extracted by using the chloroform–methanol as described by Holman *et al.* (2019). Ten g of samples were mixed with chloroform: methanol (1:2) and homogenised using homogeniser at 8000 rpm for two minutes. After that, 10 ml of chloroform was added and homogenised again around 30 seconds. Next, the mixture was mixed with 10 ml of distilled water and stirred until homogenous sample formed. Then, the sample was filtered and evaporated using rotary at 60°C until constant weight gained.

2.7 Peroxide value (PV)

Peroxide value was conducted by using the method described in Teye *et al.* (2012) with a few changes. About 5 g beef patty was weighed and 30 mL solvent mixture (glacial acetic acid: Chloroform, 3:2) was added to the sample and shaken until dissolved. Next 1.5 mL saturated potassium iodide solution was added and let it stand for a minute. Then, 30 mL distilled water was added followed by 1 mL of starch indicator. Finally the mixture was titrated with 0.01 M sodium thiosulphate solution.

2.8 Statistical analysis

All data were reported as means and standard deviations. The gathered data was subjected to a one-way analysis of variance (ANOVA) and t-test to do the means comparison. SPSS was used to conduct the analysis. Then, $P > 0.05$ was used as the significance threshold for all comparisons.

3. RESULT AND DISCUSSION

3.1 Extraction Yield

Table 1: Percentage yield of stink bean peels and leaves extract

Sample	% of yield extract
Stink bean peels (SBP)	4.52±0.13 ^a
Stink bean leaf (SBL)	3.38±0.11 ^b

3.2 Total phenolic compound (TPC)

Table 2: Total phenolic content of stink bean peels and leaves extract

Sample	TPC (mg GAE/g extract weight)
Stink bean peels (SBP)	37.51±3.43 ^a
Stink bean leaf (SBL)	3.95±0.22 ^b

The content of TPC for stink bean peels and leaves extract were 37.51 mg GAE/g and 3.95 mg GAE/g, respectively with significant differences ($p < 0.05$). This result indicated that the SBP extract has richer source of phenolic compounds than SBL extract.

3.3 Free radical scavenging activity (DPPH)

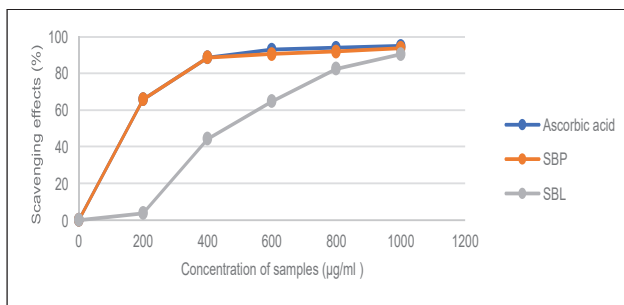


Figure 1. The scavenging activity of stink bean peels, leaves extract and ascorbic acid.

At 1000 ppm, the decreasing order in scavenging effect were observed as follows: Ascorbic acid (94.93%) > SBP (93.64%) > SBL (90.35%). At each concentration, SBP extract exhibited higher DPPH scavenging activity compared to SBL extract. This might be related to the TPC obtained in the result earlier whereby SBP extract with high in phenolic compounds increased in its antioxidant activity. A study from Nurdyansyah (2020) reported the scavenging effects of aqueous extract of SBP were 9.12%, 16.30%, 21.97%, 30.58% and 37.13% for concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm, respectively. However, the scientific literature provides limited information regarding the radical scavenging activity of SBL with water extract.

3.4 Ferric reducing antioxidant power (FRAP)

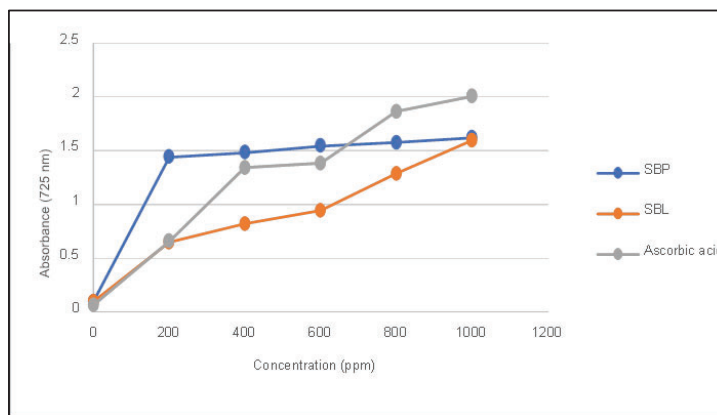


Figure 2. Ferric reducing antioxidant power of stink bean peel and leaf extracts with ascorbic acid

Increase in absorbance showed a reducing power of the samples. At 200 ppm, reducing power of samples decreased in the order: SBP > Ascorbic acid > SBL. However, at 1000 ppm, the reducing capability changed in order where: Ascorbic acid > SBP > SBL. SBP at 200 ppm with absorbance equal to 1.442 is comparable to 600 ppm of ascorbic acid. SBL extract needs to be in high concentration (800 ppm) to be able to obtain absorbance that is near to SBP and ascorbic acid, (1.288). Owing to the highest absorbance (indicating high antioxidant activity) at 200 ppm, SBP extract was chosen to be incorporated into beef patty.

3.5 Peroxide value (PV)

Table 3: The peroxide values of beef patties during nine days of storage

Storage (days)	Control	Ascorbic acid	SBP
0	2.56 ± 0.19 ^a	1.89 ± 0.38 ^b	2.44 ± 0.19 ^a
3	4.89 ± 0.51 ^a	2.00 ± 0.33 ^b	2.56 ± 0.20 ^b
6	5.33 ± 0.34 ^a	2.55 ± 0.39 ^c	4.11 ± 0.7 ^b
9	7.56 ± 0.51 ^a	5.89 ± 0.77 ^b	6.22 ± 0.51 ^b

At the end of the storage, the value varied from 5.89 to 7.56 meq/kg. PV was non-significant ($p > 0.05$) at day zero, day three and the last day of chilled storage. The value only became significant ($p < 0.05$) at day six between control and treated samples. However, in this study, PV for all formulations were below 10 meq/kg throughout nine days of storage. According to Ali *et al.* (2019), food product with peroxide value between 5 to 10 meq/kg is considered moderately oxidised, while above 10 meq/kg is considered highly oxidised.

4. CONCLUSION AND RECOMMENDATION

The study found that both parts of stink bean have antioxidant properties, with peel has higher antioxidant activity than leaf extract. The TPC of SBP extract was significantly higher than SBL extract, and it exhibited higher scavenging effects compared to SBL but slightly lower than ascorbic acid. SBP extract also had higher reduced power, leading to higher antioxidant activities compared to SBL extract. The SBP extract at 200 ppm was observed to act as an antioxidant against beef patty oxidation during nine days of chilled storage at 4°C. Therefore, it is suggested that SBP extract could be used in the food industry as an antioxidant. Additionally, further study on the application of different parts of stink bean extract into other food products should be conducted to observe more research about its effectiveness as natural antioxidant.

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