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Antioxidant capacity and total phenol content of peanut butter fruit (*Bunchosia armenica*)

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Abstract
Bunchosia armenica being a member of family "*Malpighiaceae*" is an edible red-coloured fruit and a source of bioactive compounds. In this study, fruit of *Bunchosia armenica* was analysed to investigate antioxidant properties and total polyphenol content. DPPH assay (IC_{50} value) and Folin-Ciacalteu method calculated as Gallic acid equivalents (GAE) were carried out. Reducing power of peanut butter fruit extract was checked as a qualitative test to confirm presence of antioxidant substances in peanut butter fruit extract.Total phenol content of flesh of peanut butter fruit was obtained as a mean of 33.27mg Gallic acid equivalents per L of fruit extract. In DPPH radical scavenging assay, the IC_{50} value in peanut butter fruit variety was obtained as 13.44±0.29mg/ml. In the analysis of reducing power in peanut butter fruit extract, colour changed into green by confirming the presence of antioxidant substances.

Keywords: antioxidant activity, *Bunchosia armenica*, bioactive compounds, total phenolic compounds

1. Introduction
Bunchosia armenica or peanut butter fruit is an evergreen shrub or tree 12 to 40 feet tall. Flowers are small, yellow, in elongated clusters, giving rise to the fruits, about an inch long. The fruit's shape recalls the common apricot (*Prunus armeniaca*), and hence the species was named in 1789 *Malpighia armeniaca*, then *Bunchosia armeniaca* in 1824. The seed or pit is said to be poisonous [1] Fruit of *Bunchosia armenica* is eaten fresh or processed form. When the red fruits soften, they taste like strawberry jam with the consistency of peanut butter. It can be demonstrated that methanol extract of ripen and unripen fruits of *Bunchosia armeniaca* plant contained different phytochemical compounds and possessed diverse bioactive properties [2]. Although the *Malpighiaceae* family has a large number of species, only few has been studied under the chemical aspect Members of *Malpighiae* family have been reported as having antibacterial and anti-inflammatory effects [3].The phytochemical analysis of methanol extract of peanut butter fruit flesh showed the presence of bio-active compounds such as antimicrobial, antifungal, antibacterial, antiviral, antioxidant, anticancer, anti-inflammation activities and flavour compounds[2].Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Vegetables and Fruits are sources of different antioxidants, such as, tocopherol, glutathione, carotenoids, ascorbic acid and anthocyanins which protect against oxidative damage. Researches revealed that methanol extract of ripen fruit of *Bunchosia armeniaca* showed promising antioxidant activity [2].

Major antioxidant capacity assays can be divided into two categories based on their chemical reactions as Hydrogen Atom transfer (HAT) assays and Single Electron Transfer (SET) assays [4]. SET assays detect the ability of a potential antioxidant to transfer one electron to reduce a species including metals, carbonyls and radical. They are based on deprotonation and ionization potential. SET reactions are usually slow and antioxidant capacity is measured in percent decrease in products. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay are some single electron transfer assays.

The DPPH radical decolourisation method is strongly steady and the IC50 value correlates with the total antioxidants in the sample [5]

Phenolic compounds are natural antioxidants. The antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. In addition, they have a metal chelating potential [6]. TPC is determined by the Folin-Ciocalteu (F-C) reagent assay.

The F-C method is an electron transfer-based assay and gives reducing capacity, which has normally been used to estimate phenolic contents of biological materials. The F-C method is simple, sensitive and precise and can be useful in characterizing and standardizing sample.

Reducing power is one mechanism of action of antioxidant and may serve as a significant indicator of potential antioxidant activity. The reducing power work on mechanism of transformation of ferric ion to the ferrous ion and is estimated spectrophotometrically by the potassium ferricyanide reduction method. The presence of reductants such as antioxidant substances in the fruit extracts cause the reduction of Fe^{3+} and the yellow colour of the solution changes into various shades of green and blue in a dose dependent manner. It has been shown that the reducing power is related with antioxidant activity and may give a significant indication of the antioxidant activity [7]. Compounds with reducing power indicate that they are electron donors and can thus act as primary and secondary antioxidants [8].

2. Materials and Methods

2.1 Sample preparation

For the extraction, the procedure followed the method of [9] with some modifications.The extraction of fresh peanut butter fruit pulp was carried out using methanol as a solvent. Two grams of pulp were mixed with 8ml methanol, followed by centrifugation at 6000rpm for 10 min. The supernatant was collected and was used for the analysis of antioxidant activity.

2.2 Determination of reducing powder

The reducing power of the fresh peanut butter fruit extracts was determined according to the method of Oyaizu (1986) [10]. About 2.5 ml of the extract in each case was mixed with phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%), the mixture being incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (TCA, 10%) was added to the mixture which was then centrifuged at 1,500 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.3 Determination of total polyphenols

The total phenolic content (TPC) was determined using Folin-Ciocalteu's reagent as described by [11].

Martials and apparatus

- Centrifuge – Spectrafuge 6C
- Magnetic stirrer – HP 3100
- Vortex Mixer – VELP SCIENTIFICA
- Spectrophotometer – UVmini – 1240
- Distilled water

Reagents

- Gallic acid – Analytical reagent
- Acetone – Analytical reagent
- Folin-Ciocalteu (FC) reagent – analytical reagent
- 7.5 % w/v Sodium carbonate solution – Laboratory reagent

a. Preparation of standards

Initially 20 mg of the gallic acid powder was measured to a watch glass and transferred to a 100 mL volumetric flask by using glass funnel and dissolved in 100.00 mL of distilled water to prepare 200 ppm gallic acid standard solution. Then 10, 20, 40, 60, 80, 100 ppm gallic acid standard solutions were prepared by pipetting 5, 10, 20, 30, 40 and 50 mL separately from above and each of them were diluted up to the level of 100 mL with distilled water in 100 mL volumetric flasks.

b. Preparation of standard curve

Initially 0.3 mL of each standard gallic acid solution was pipetted into cleaned, dried test tubes. Then 1.5 mL of 10% FC reagent and 1.2 mL of 7.5% w/v Na_2CO_3 solution was also added and mixed well by using Vortex mixer at a speed of 20 Hz. The solution was allowed to stand at room temperature for 30 minutes and then the absorbance was recorded at a wavelength of 765 nm using UVmini-1240 Spectrophotometer. Distilled water was used as a blank to replace gallic acid. By using the absorbance values, corresponding to gallic acid standard solution a standard curve was drawn.

Then 0.3 mL of the extract was added to a cleaned and dried test tube. Then 1.5 mL of 10% F-C reagent and 1.2 mL of 7.5% w/v Na_2CO_3 solution was also added and mixed well using Vortex mixer. The mixture was allowed to stand for 30 minutes at room temperature. Then the absorbance was measured by using UVmini-1240 Spectrophotometer at 765 nm wave length.

2.4 DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

For the DPPH assay, the procedure followed the method of Chen *et al* (2016) [8] with some modifications. The stock solution was prepared by dissolving 3 mg of DPPH in 100 ml methanol to obtain an absorbance of 0.900±0.02 units at 517 nm using UV-Vis Spectrophotometer. 3ml of methanol was used as the blank sample. Control sample was prepared by mixing 2ml DPPH solution in 2ml methanol. For each peanut butter fruit sample a serial dilution was prepared and 1.5 ml of each diluted sample was mixed with 1.5 ml DPPH solution and incubated at room for 30 minutes in a dark place.

The absorbance was read at 517 nm. The percentage inhibition of absorbance was calculated according to the following equation.

% Inhibition =

$$\frac{\text{Absorbance of control}(517) - \text{Absorbance of sample}(517)}{\text{Absorbance of control}(517)} \times 100$$

The calculated percentage inhibition of absorbance at 517nm was plotted as a function of concentration of samples and the sample concentration which gives the 50% inhibition activity was estimated as the IC50 value from regression analysis using the software MINITAB R 17. Gallic acid was used as the standard antioxidant. Six different concentrations of Gallic acid; 1, 2, 3, 4, 5, 6 mg/L were prepared and from each Gallic acid solution 2 ml was mixed with 2 ml DPPH solution and the absorbance was measured at 517 nm after 10 minutes of incubation at room temperature in a dark place. The percentage inhibition was calculated for each dilution of Gallic acid solution and was plotted as a function of concentration of standard antioxidant. The concentration which gives the 50% inhibition activity was estimated as the IC_{50} value from regression analysis using the software MINITAB R 17 for standard reference.

3. Discussion

3.1 Result of reducing power of peanut butter fruit extract

Reducing power is one mechanism of action of antioxidants and may serve as a significant indicator of potential antioxidant activity. The presence of reductants such as

antioxidant substances in the extracts of the peanut butter fruit causes the reduction of Fe^{3+} and the yellow colour of the solution changes into various shades of green and blue in a dose-dependent manner.
Reducing power of peanut butter fruit extract was checked as a qualitative test to confirm whether antioxidant substances contain in the peanut butter fruit extract by observing the colour change.

Fig 1: Reducing power of peanut butter fruit extract

According to figure 1, colour changed into green by confirming the presence of antioxidant substances. Therefore this test was confirmed that antioxidant substances contain in the peanut butter fruit extract.

3.2 Total polyphenol content of flesh of peanut butter fruit

Total polyphenol content of flesh of peanut butter fruit was expressed comparatively Gallic acid standard. Standard curve of Gallic acid is in following figure 2.
The standard curve for determination of total polyphenols [11]

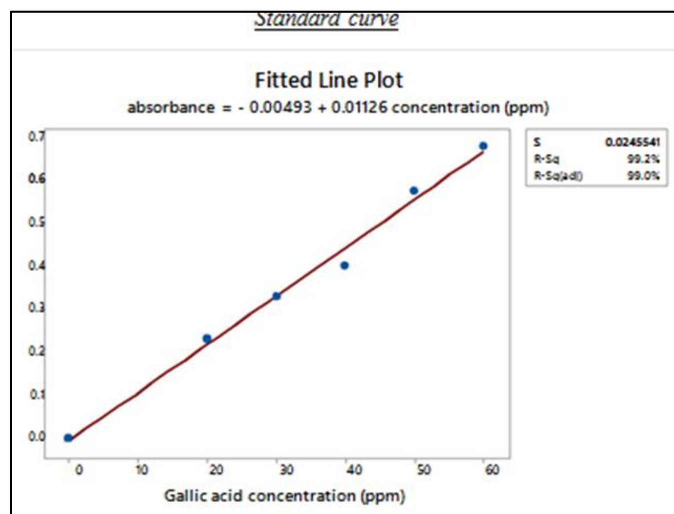

Fig 2: Standard curve of Gallic acid

Total polyphenol content of flesh of peanut butter fruit was obtained as a mean of 33.27mg Gallic acid equivalents per L of fruit extract. According to previous research, Total phenolic content of the sample was recorded as 870.80± 8.28 mg/GAE/100g [2]. This difference in values may occur due to the difference in extraction method. The data obtained in the this study indicated that the phenoliccompound level is high in the ripe fruit of *Bunchosia armeniaca.*

3.3 Results of DPPH radical scavenging assay

Results of the DPPH radical scavenging assay based on IC50 values in peanut butter fruit extract. According to the DPPH radical scavenging assay, IC50 value is the antioxidant concentration in the peanut butter fruit that shows 50% inhibition activity of the DPPH free radical and it is indicated as mg of Gallic acid equivalents per ml of extract. Low IC50 value indicates higher antioxidant activity whereas high IC50 value indicates low antioxidant capacity.

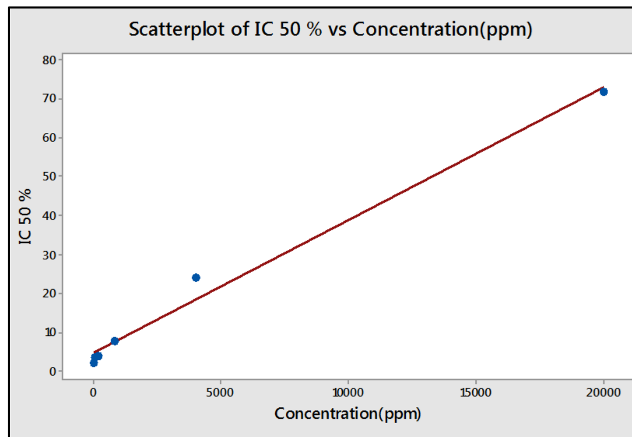

Fig 3: IC_{50} value of peanut butter fruit extract

According to above graph, the IC50 value in peanut butter fruit variety was obtained as 13.443±0.29mg/ml. The IC_{50} value of the Gallic acid standard was recorded as 0.0036 mg/ml. According to previous research done by [2] average antioxidant activity was 0.981±0.002 mg/mL. The IC50 value in peanut butter fruit is comparatively higher than the IC_{50} value of the Gallic acid standard. Due to thegreater hesitancy when donating hydrogen atoms in natural antioxidants, Synthetic antioxidants may show a higher activity. Results revealed that methanol extract of ripen fruit of *Bunchosia armeniaca* showed antioxidant activity.

4. Conclusion

Total polyphenol content of flesh of peanut butter fruit was obtained as a mean of 33.27mg Gallic acid equivalents per L of fruit extract. In DPPH radical scavenging assay, the IC_{50} value in peanut butter fruit variety was obtained as 13.443±0.29mg/ml. The IC50 value in peanut butter fruit is comparatively higher than the IC_{50} value of the Gallic acid standard. But these essays have shown that possibility of free radical scavenging activity. Therefore fruit of *Bunchosia armeniaca* can be identified as a significant source of bioactive compounds.

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