

STUDY ON PHYTOCONSTITUENTS AND BIOACTIVE PROPERTIES OF AVOCADO (*Persea Americana* MILL.) SEEDS

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Abstract

Avocado (*Persea americana* Mill.) is well known traditional medicinal plant and one of the most important fruit crops of the local market in Myanmar. According to the phytochemical investigation of avocado seeds, it was observed that phenolic compounds and flavonoids were present in the fruit seeds. Semi-quantitative elemental analysis of avocado seeds was performed by EDXRF method. Potassium and calcium were present as the considerable amounts in the avocado seeds. The nutritional values of avocado seeds were determined by AOAC method. It was observed that carbohydrate was the highest content and protein, fat and fiber were low contents in the sample. Screening of antimicrobial activities from avocado seeds was carried out by agar well diffusion method. In the screening, different crude extracts such as pet-ether, chloroform, ethylacetate, ethanol, methanol and water of avocado seeds were examined with the six microorganisms. According to the antimicrobial screening, avocado seeds possess antimicrobial activity. The antioxidant activity of water and ethanolic extract from avocado seeds was determined by DPPH free radical scavenging activity method using UV spectrophotometer. The ascorbic acid was used as a standard. The IC₅₀ values of standard ascorbic acid, water and ethanolic extract were observed as 37.05, 45.05 and 93.17 µg / mL, respectively. Therefore, the antioxidant activity of water extract from avocado seed was more potent than that of ethanolic extract.

Keywords: avocado seed, nutritional values, antimicrobial activity, antioxidant activity

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Introduction

Persea americana Mill. (Avocado)

The Avocado tree is an evergreen tree that attains heights of 40 to 80 feet and has many branches. The leaves are elliptic or oval in shape and 3 to 10 inches long. Flowers are small, greenish, and perfect (has both male and female parts). The avocado fruit may be round, pear shaped, or oblong, and the skin of the fruit may vary in texture and color. The skin may be pliable to woody, smooth to rough, and green-yellow, reddish-purple, purple, or black in color. The flesh of the fruit is greenish yellow to bright yellow when ripe and buttery in consistency, but inferior varieties may be fibrous. The avocado fruit has one large seed which makes up to 10 - 25% of the fruit weight (Morton, 1987).

Avocado (*Persea americana* Mill.) (Figure 1) is well known traditional medicinal plant and one of the most important fruit crops of the local market in Myanmar. Avocado seeds have more antioxidants than most fruits and veggies on the market and polyphenols like green tea. In fact, avocado seed has 70% of the antioxidants found in the whole avocado fruit, and avocado seed oil is also full of antioxidants, lower cholesterol, and helps fight off diseases.



Figure 1 Plant and flower of Avocado

Scientific Classification

| | |
|----------------|--------------------------------|
| Kingdom | : Plantae |
| Order | : Laurales |
| Family | : Lauraceae |
| Genus | : <i>Persea</i> |
| Species | : <i>P. americana</i> |
| Botanical name | : <i>Persea americana</i> Mill |
| English name | : Avocado |
| Myanmar name | : Htaw-but-thee |

Health Benefits of *Persea americana* Mill (Avocado) Seed

Avocado seed (Figure 2) might offer natural antibiotic and antifungal benefits. In the test-tube study, avocado seed extracts inhibited a variety of pathogens, including *Candida* and other fungi and the mosquito that carries the tropical disease yellow fever. Seed and skin extracts protected against oxidation of fats and proteins in prepared meats, and moderately inhibited some types of pathogenic bacteria.



Figure 2 Fruit and seed of *Persea americana* Mill. (Avocado)

Avocado seed lowered cholesterol levels and may protect against arterial plaque formation (Pahua-Ramos *et al.*, 2012). Then it attributed the cholesterol-lowering benefits to the seed's high content of dietary fiber, which

lowers cholesterol levels by binding to cholesterol in the intestinal tract and preventing absorption. Antioxidant activity of avocado seed might also help prevent cardiovascular disease by inhibiting lipid oxidation, a process that leads to arterial plaque formation .

Avocado seeds have more antioxidants than most fruits and veggies on the market and polyphenols like green tea. In fact Avocado Seed has 70% of the antioxidants found in the whole Avocado, and Avocado Seed Oil is also full of antioxidants, lowers cholesterol, and helps fight off disease. Avocado seed helps to prevent cardiovascular disease, lower cholesterol, and prevent strokes (Gorinstein *et al.*, 2011) .

Materials and Methods

The avocado seed samples were collected from Pyinoolwin Township, Mandalay Region. Firstly, phytochemical investigation of avocado seed was carried out by Test Tube method. Then, semi-quantitative elemental analysis of avocado seed was performed by EDXRF method. The nutritional values of avocado seed sample were determined by AOAC method (AOAC, 2000). The antimicrobial activity of avocado seeds was examined by agar well diffusion method (Mar Mar Nyein, *et al.*, 1991). The antioxidant properties of water and ethanolic extract from avocado seeds were determined by DPPH method using UV visible spectrophotometer.

Results and Discussion

Phytochemical Constituents of Avocado Seed Sample

The phytochemical tests revealed that alkaloids, α -amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugars, saponins, steroids tannins, and terpenoids were present in the sample. Cyanogenic glycosides were absent in the sample. The results are shown in Table 1.

Semi-quantitative Elemental Analysis of Avocado Seed Sample by EDXRF Method

To determine the heavy toxic metals and macronutrient elements in plant samples, qualitative elemental analysis was carried out by EDXRF. Mineral elements present in dried powder samples of avocado seeds were determined by EDXRF spectrometer. The relative compositions of the elements predominantly found in the sample are presented in Table 2 and Figure 1. It was found that K and Ca were major constituents. S, P and Si were observed as the minor constituents and trace mineral elements such as Fe, Ni and Zn were also observed in the avocado seed. These elements play an important role for the nutrition and medicinal formulation of human beings. The high content of K is effective for the persons with hypertension.

Table 1 Results of Phytochemical Investigation of Avocado Seed Sample

| No. | Constituents | Extract | Test reagent | Observations | Remark |
|-----|-----------------------|---------------------------------------|---|----------------|--------|
| 1 | Alkaloids | 1% HCl | Dragendorff's reagent | Orange ppt, | + |
| | | | Mayer reagent | White ppt, | + |
| | | | Sodium picrate solution | Reddish brown | + |
| 2 | α -amino acids | H ₂ O | Ninhydrin | Purple spot | + |
| 3 | Carbohydrates | H ₂ O | 10% α -naphthol, conc: H ₂ SO ₄ | Red ring | + |
| 4 | Cyanogenic glycoside | Dil H ₂ SO ₄ | Sulphuric acid, sodium picrate | No ppt | - |
| 5 | Flavonoids | EtOH | Mg turning, conc: HCl | Pink colour | + |
| 6 | Glycosides | H ₂ O | 10% Lead acetate solution | White ppt | + |
| 7 | Phenolic compounds | H ₂ O | 5% FeCl ₃ solution | Green solution | + |
| 8 | Saponins | H ₂ O | Distilled water | Frothing | + |
| 9 | Steroids | PE | Acetic anhydride, conc: H ₂ SO ₄ | Green | + |
| 10 | Tannins | H ₂ O | 2% gelatin solution | White ppt | + |
| 11 | Terpenoids | CHCl ₃ | Acetic anhydride, conc: H ₂ SO ₄ | Pink colour | + |

(+) present, (-) absent

Table 2 Relative Abundance of Some Elements in Avocado Seed by EDXRF Method

| Elements | Relative abundance (%) |
|----------|------------------------|
| K | 65.51 |
| Ca | 21.38 |
| S | 4.73 |
| P | 2.13 |
| Si | 2.12 |
| Fe | 1.62 |
| Ni | 1.57 |
| Zn | 0.90 |

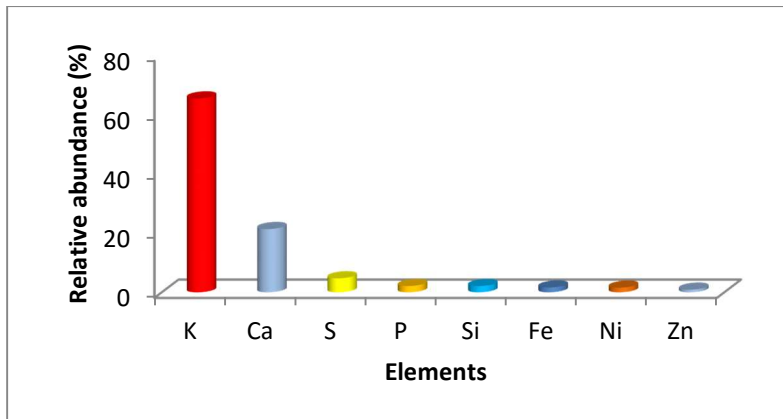


Figure 1 The histogram of elemental contents from avocado seed sample by EDXRF method

Nutritional Values of Avocado Seed

According to the method of AOAC (2000), it was found that the amount of carbohydrate and moisture were highest in the sample. Protein, fat and fibre were observed as minor nutrients. The high content of carbohydrate and low content of fat are required for the human health. The results are shown in Table 3 and Figure 2.

Table 3 Nutritional Values (%) in Avocado Seed Sample

| No. | Nutrients | Contents (%) |
|-----|----------------------------|--------------|
| 1 | Moisture | 10.90 |
| 2 | Ash | 2.38 |
| 3 | Crude Protein | 7.84 |
| 4 | Crude fiber | 1.54 |
| 5 | Crude fat | 5.81 |
| 6 | Water soluble carbohydrate | 71.53 |

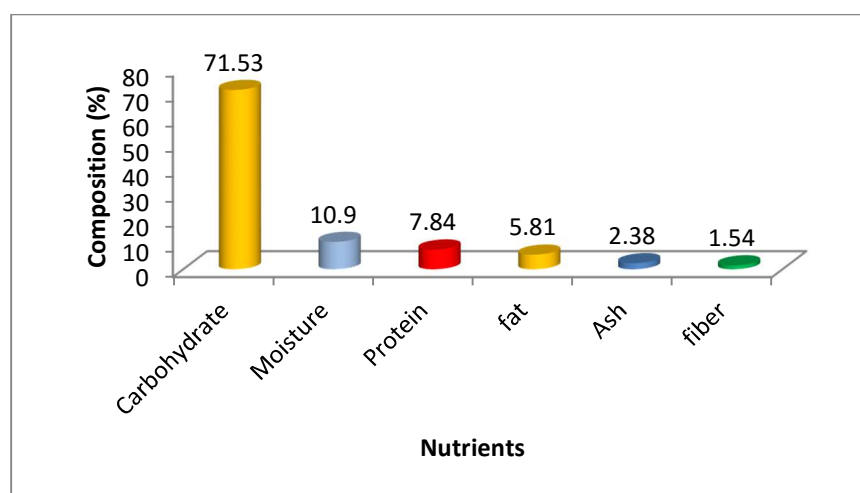


Figure 2 The histogram of nutritional values in avocado seed sample

Screening of Antimicrobial activity

Antimicrobial activities of Pet-ether, CHCl_3 , MeOH, EtOAc, EtOH and H_2O extracts were screened by agar well diffusion method (Table 4). In this screening, the crude extracts were tested on six species of microorganisms; *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E.coli* species.

In the sample of *Persea americana* Mill (avocado) seed, all the extracts are active on all organisms except MeOH and EtOH extracts which are inactive against *Pseudomonas aeruginosa*. The activities of EtOAc extract on all microorganisms are considerably high. The sensitivity of EtOH and H₂O extract are suitable for the medicinal formulation of antimicrobial drugs.

Table 4 Results of Antimicrobial Screening of *Persea americana* Mill (avocado) Seed against Six Microorganisms

| Solvent extracts | <i>B. subtilis</i> | <i>S. aureus</i> | <i>P. aeruginosa</i> | <i>B. pumilus</i> | <i>C. albicans</i> | <i>E. coli</i> |
|-------------------|--------------------|------------------|----------------------|-------------------|--------------------|----------------|
| Pet-ether | 13 mm (+) | 18 mm (++) | 12 mm (+) | 19 mm (++) | 18 mm (++) | 16 mm (++) |
| CHCl ₃ | 18 mm (++) | 13 mm (+) | 13 mm (+) | 14 mm (+) | 12 mm (+) | 13 mm (+) |
| EtOAc | 23 mm (+++) | 22 mm (+++) | 20 mm (+++) | 23 mm (+++) | 24 mm (+++) | 22 mm (+++) |
| EtOH | 18 mm (++) | 21 mm (+++) | - | 17 mm (++) | 15 mm (++) | 16 mm (++) |
| MeOH | 18 mm (++) | 12 mm (+) | - | 16 mm (++) | 16 mm (++) | 15 mm (++) |
| H ₂ O | 14 mm (+) | 13 mm (+) | 25 mm (+++) | 13 mm (+) | 12 mm (+) | 12 mm (+) |

Agar well : (10 mm), 10 mm-14 mm (+), 15 mm-19 mm (++) , 20 mm-above (+++)

Antioxidant Activity

The DPPH radical scavenging activity of water and ethanolic extracts from avocado seed was compared with ascorbic acid. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which was induced by antioxidants. Determination of radical scavenging by DPPH method base on the change in absorbance of crude extracts solutions in various concentrations. Six kinds of concentrations 400 µg / mL, 200 µg / mL, 100 µg / mL, 50 µg / mL, 25 µg / mL and 12.5µg / mL were prepared by dilution with ethanol as solvent. Ascorbic acid (tablets) was used as standard

sample and ethanol was employed as control. Blank solution was also prepared by mixing sample and ethanol. The absorbance values were measured at wavelength 517nm for different concentration of extracts and the control. These values are used to calculate the percentage inhibition of DPPH radical against the samples. The IC₅₀ values of various extracts were calculated from the percentage inhibitions at various concentrations. The results of the free radical scavenging activity of avocado seed assessed by DPPH assay was summarized by IC₅₀ using method of linear regression. The lower the value of IC₅₀ the higher is the antioxidant property. From the screening, IC₅₀ value of standard ascorbic acid was 37.05 µg / mL (Table 5 and Figure 3). It was observed that water and ethanolic extract of avocado seed have the radical-scavenging activity with IC₅₀ values of 45.05 µg / mL (Table 6 and Figure 4) and 93.17 µg / mL (Table 7 and Figure 5), respectively. According to the IC₅₀ values, water and ethanolic extracts of avocado seeds possess the rich antioxidant properties. It can be seen that the antioxidant activity of water extract is more potent than that of ethanolic extract.

Table 5 % RSA and IC₅₀ Value of Standard Ascorbic Acid (Tablet) at 517 nm

| Concentration (µg/mL) | %RSA | Absorbance | IC ₅₀ (µg/mL) |
|--------------------------|-------|------------|-----------------------------|
| 12.5 | 36.37 | 0.636 | 37.05 |
| 25.0 | 45.89 | 0.658 | |
| 50.0 | 56.63 | 0.634 | |
| 100.0 | 65.36 | 0.611 | |
| 200.0 | 76.63 | 0.550 | |
| 400.0 | 92.26 | 0.497 | |

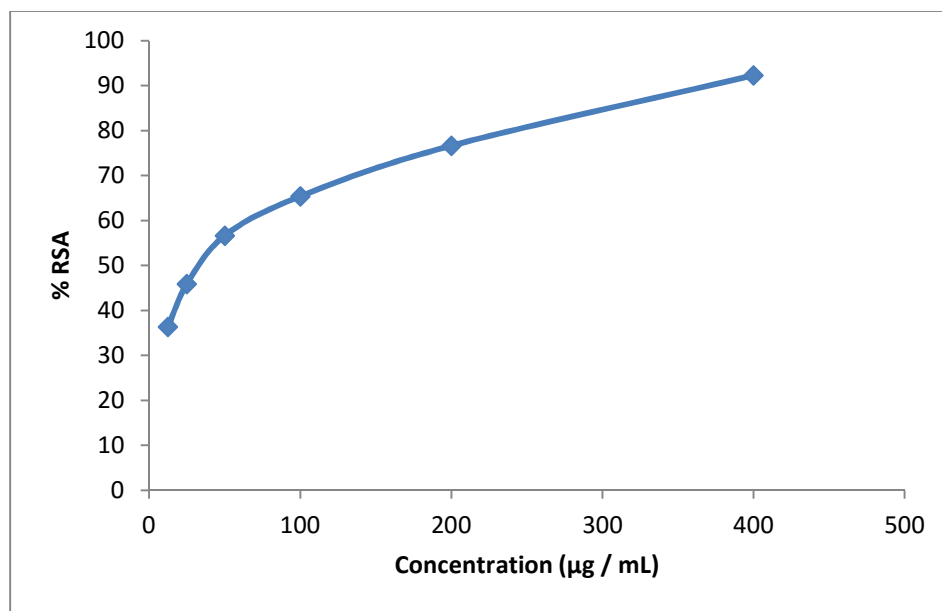


Figure 3 Correlation between DPPH radical scavenging activity and concentration of standard ascorbic acid (tablet)

Table 6 % RSA and IC₅₀ Value of Water Extract of Avocado Seed

| Concentration (µg/mL) | %RSA | Absorbance | IC ₅₀ (µg/mL) |
|-----------------------|-------|------------|--------------------------|
| 12.5 | 41.01 | 0.693 | 45.05 |
| 25.0 | 47.97 | 0.658 | |
| 50.0 | 52.61 | 0.634 | |
| 100.0 | 56.67 | 0.611 | |
| 200.0 | 70.41 | 0.550 | |
| 400.0 | 80.27 | 0.497 | |

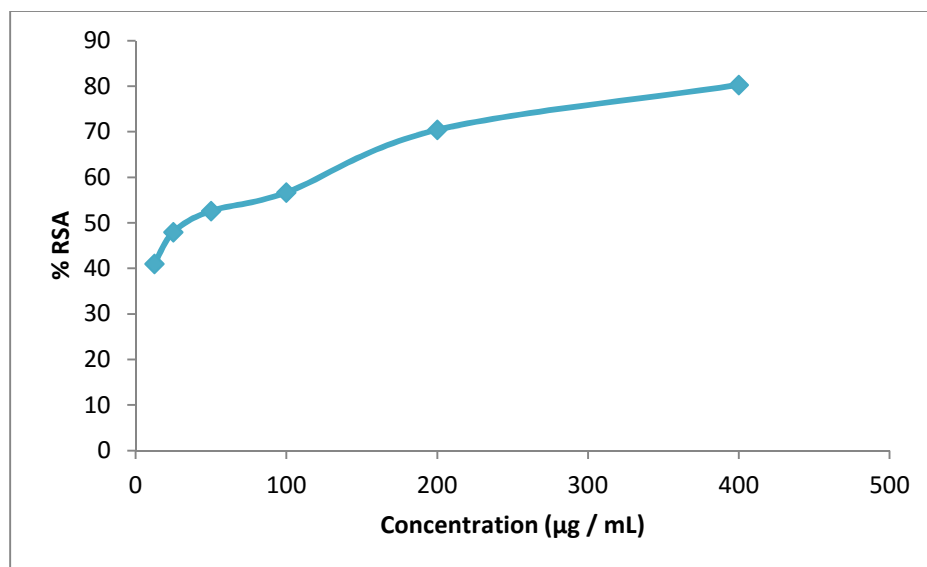


Figure 4 Correlation between DPPH radical scavenging activity and concentration of water extract of avocado seed

Table 7 % RSA and IC₅₀ Value of Ethanolic Extract of Avocado Seed

| Concentration (µg/mL) | %RSA | Absorbance | IC ₅₀ (µg/mL) |
|-----------------------|-------|------------|--------------------------|
| 12.5 | 30.25 | 0.260 | |
| 25.0 | 39.35 | 0.254 | |
| 50.0 | 45.81 | 0.141 | 93.17 |
| 100.0 | 54.52 | 0.002 | |
| 200.0 | 69.19 | 0.0721 | |
| 400.0 | 98.67 | 0.025 | |

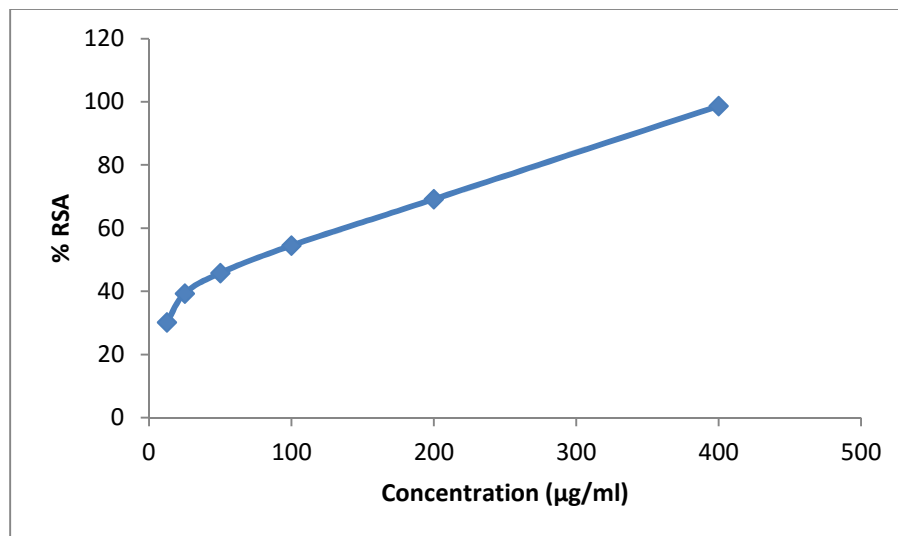


Figure 5 Correlation between DPPH radical scavenging activity and concentration of ethanolic extract of avocado seed

Conclusion

Phytoconstituents such as alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, steroids, tannins, and terpenoids were present in the avocado seed sample. These phytoconstituents are applicable for the use of human health. But cyanogenic glycosides were not found in the sample. The observed phytochemical constituents are essential compounds for the metabolism and nutrition of human body.

In the study of elemental analysis, the high contents of K (65.51%) and Ca (21.38%) were observed. The high content of K is effective for the persons with hypertension. Ca helps the teeth and bones to be strong for human.

On antimicrobial screening of avocado seed sample, the various crude extracts were examined with six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E.coli* species. From the screening, EtOAc extract showed the highest activities against all microorganisms. With *Pseudomonas aeruginosa*, MeOH and EtOH extract were inactive. The sensitivities of EtOH and H₂O

extracts are very important for the medicinal formulation of antimicrobial drugs.

The antioxidant activity of avocado seed was continued to determine by DPPH free radical scavenging activity method using UV spectrophotometer. IC₅₀ values for the antioxidant activity of the standard ascorbic acid, water and ethanolic extract from avocado seed were 37.05, 45.05 and 93.17 µg / mL, respectively. According to the IC₅₀ values of avocado seed, the antioxidant activity of water extract was more potent than that of ethanolic extract. The antioxidant compounds reduce risk for chronic diseases including cancer and heart diseases. Therefore, avocado seeds may be used in the medicinal formulation of human health.

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