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Chemical Characterization, Antioxidant Activity and GC-MS Analysis of the Extracted Oil from Seeds of *Persea americana* Mill. (Avocado)

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Abstract

From ancient times plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contribution to human health and well being. There is a long tradition of using the pits for various medicinal purposes. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. In this research work, Avocado seed was selected for chemical analysis. Avocado (Htaw-Bat-Thee) was collected from Pyin-Oo-Lwin Township, Mandalay Region. Firstly, phytochemical screening for the Avocado seed was performed. According to phytochemical screening, alkaloid, glycoside, saponin, phenolic compound, polyphenol, reducing sugar, terpene, lipophenol and tannin compound were found to be present in the Avocado seed. The oil extracted from Avocado seed was done with petroleum ether by using Soxhlet extraction method. The physicochemical parameters, such as specific gravity, viscosity, acid value, saponification value, iodine value and unsaponifiable matter were determined by conventional method. Then, elemental analysis of Avocado seed was performed by using Energy Dispersive X-ray Fluorescence (EDXRF) method. Moreover, the fatty acid composition of Avocado seed oil was analyzed by Gas Chromatography Mass Spectrometry (GC-MS) method. Moreover, antioxidant activities of Avocado seed evaluated on the basis of their scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical. IC_{50} value was 180.06 μg/mL compare to that of ascorbic acid which was 0.65 μg/mL.

Keywords: Avocado seed, Phytochemical, Physico-chemical, EDXRF, GC-MS, Antioxidant activity

Introduction

Fats and oils are triesters of glycerols with saturated and unsaturated fatty acid. They are also known as glycerides or triglycerids. Fats and oils liberate much heat energy when digested, and they can be stored in the body and used as required. Fats give flavor to our foods and vitamins to our body. It is fat that carries vitamins A, D, E and K from our food to our tissues. Fats and oils contribute to our life, health and well-being but when taken in excess can be dangerous. Too much fat in the diet can lead to obesity which is linked with blood pressure and diabetes. Dietary fat is implicated as a cause of heart diseases and cancer of breast, colon and prostate (Alione Bailey, 1945). Vegetable oils are known to be natural products with vegetable origin that contained mixtures of esters derived from glycerol that have chains of fatty acid with 14 to 20 carbon atoms that have different degrees of unsaturation. Vegetable oils have an important functional and sensory role in food products because of their fatty acids composition and the fat-soluble vitamins (A, D, E and K). They are also sources of energy and essential fatty acids like linoleic and linolenic that are responsible for growth and the health of organisms (Botriestean, Hadaruga and Jionu, 2012).

Aim and Objectives

Aim

The aim of the present work is to investigate the fatty acid composition in extracted oil from Avocado seed.

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Objectives

The objectives of the present research work are as follows:

- To collect the sample
- To screen the phytochemical constituents of Avocado seed
- To extract Avocado seed oil by using Soxhlet extraction method
- To measure specific gravity, viscosity, acid value, saponification value, iodine value and unsaponifiable matter of Avocado seed oil
- To measure the mineral contents of Avocado seed
- To identify the fatty acid composition from the oil of Avocado seed by GC-MS method

Botanical Description

Scientific Name	:	*Persea americana* Mill.
Family Name	:	Lauraceae
English Name	:	Avocado
Local Name	:	Htaw-Bat-Thee
Genus	:	*Persea*
Species	:	*P. americana*
Part Used	:	Seed

l.

Sample Collection

The seed of Avocado was collected from Pyin-Oo-Lwin Township, Mandalay Region. Firstly, the samples were cut into small pieces and air-dried in the shade. The samples were ground thoroughly and then from these powders, oil was extracted by using Soxhlet extraction method.

Preliminary Phytochemical Tests for Seed of Avocado

Preliminary detection of phytochemical compounds present in Avocado seed was carried out according to the phytochemical methods.

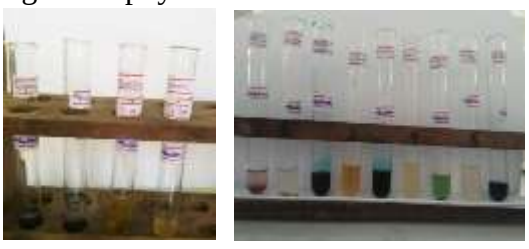

Figure 2. Phytochemical tests of *Persea americana* Mill.

Determination of Oil Content of Avocado Seed

Oil content was determined by using Soxhlet extraction method.

Procedure

About 30.00 g of sample was weighed, placed in a clothe bag and the bag was then placed in a Soxhlet extractor. Petroleum ether (300 mL) was

poured into the extractor until some of it overflowed into the flask. The flask was heated on a water-bath. The extraction was assumed to be complete when a small amount of extract placed on a watch glass did not leave any residue on evaporation of solvent. A duration of about 8 hr was required for complete extraction. The pet-ether was removed by simple distillation until the volume of the pet-ether was remained to about 10 mL. The last trace of the solvent was then removed by placing the content in an oven at about 100°C until the constant weight was obtained. The oil content of sample was calculated by the following equation.

Figure 3. Determination of oil by soxhlet extractor

$$\text{Oil (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

Determination of the Composition of Extracted Oil from Seed of Avocado by Gas-Chromatography Mass Spectrometry (GC-MS)

The extracted oil from seed of Avocado was analyzed by Gas-Chromatography Mass Spectrometry (GC-MS). It was measured at Department of Chemistry, University of Mandalay.

Figure 4. GC-MS spectrophotometer

The GC-MS is composed of two major building blocks: the gas chromatography and the mass spectrometer. A capillary column is utilized in gas chromatography, which depends on the column's dimensions such as length, diameter, film thickness and the phase properties. The difference in the chemical properties between different molecules in a mixture will separate the molecules because of the sample travels the length of the column. The molecules take different amounts of time to come out of (elute from) the gas chromatography is called the retention time.

Preliminary Screening of Radical Scavenging Activity by Spectrophotometric Method

Preparation of 60 μM DPPH Solution

DPPH powder 0.0024 g (2.4 mg) was weighed and it was thoroughly and gently dissolved in 100 mL of 95 % ethanol and stored in brown coloured reagent bottle. It must be kept in the fridge for no longer than 24 hours before use.

Preparation of Standard Ascorbic Acid Solution

0.01 g (10 mg) of ascorbic acid was weighed and dissolved in 100 mL 95 % ethanol. It was diluted with 95 % ethanol in various ratios to obtain five ranges of concentration, such as 0.25 μg/mL, 0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL and 4.0 μg/mL respectively and the some volume 5.0 mL of standard ascorbic acid solution was prepared for each concentration.

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Preparation of Test Sample Solution

The finely Avocado powder were mixed with 95 % ethanol in various ratios to obtain five ranges of concentration, such as 15.62 μg/mL, 31.25 μg/mL, 62.5 μg/mL, 125 μg/mL and 250 μg/mL respectively. Then, 5.0 mL of ethanol solution was prepared for each concentration.

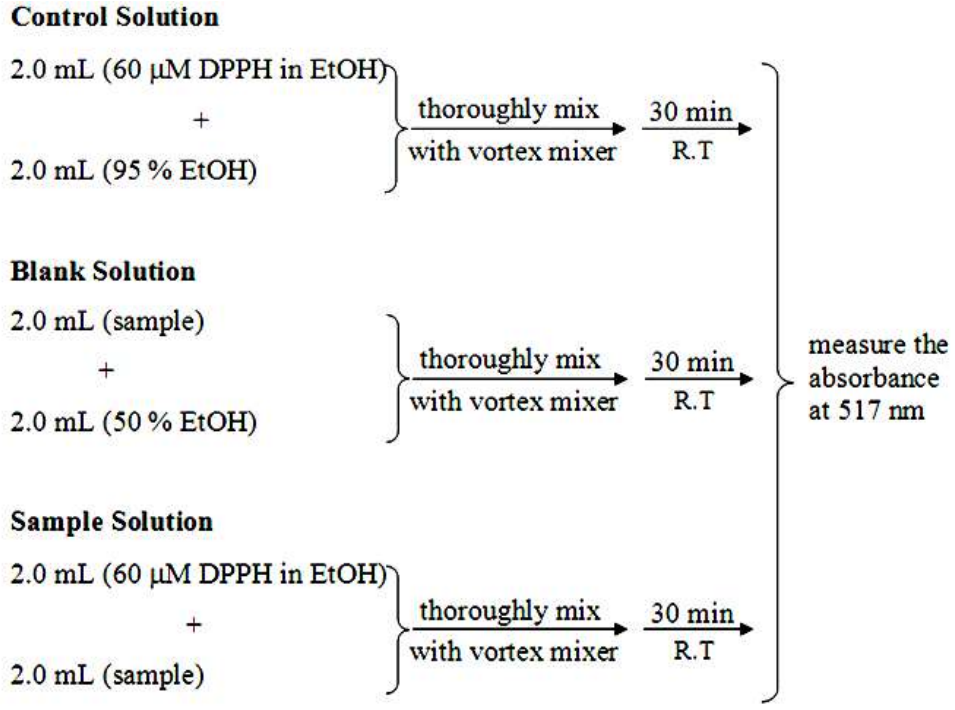
Measurement of DPPH Radical Scavenging Activity by Spectrophotometric Method

The control solution was prepared by mixing 2 mL of 60 μM DPPH solution and 2.0 mL of 95 % ethanol using vortex mixer. Moreover, the blank solution could be prepared by mixing 2.0 mL of test sample solution and 2.0 mL of 50 % ethanol thoroughly in the vortex mixer. Furthermore, the prepared standard ascorbic acid solutions and the test sample were also prepared by gently mixing each of 2.0 mL of 60 μM DPPH solution and 2.0 mL of test sample solution with various concentrations by applying vortex mixer. After that, the solutions were allowed to stand for 30 minutes at room temperature. Then, the absorbance value of each solution at 517 nm was measured by UV spectrophotometer.

The absorbance values obtained were applied to calculate percent inhibition by the following formula.

$$\%\,\text{inhibition} = \frac{\text{DPPH}_{\text{alone}} - (\text{Sample} - \text{Blank})}{\text{DPPH}_{\text{alone}}} \times 100$$

% inhibition = percent inhibition of test sample
Sample = absorbance of test sample solution
DPPH = absorbance of control solution
Blank = absorbance of blank solution



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Results and Discussion

The Results of Phytochemical Tests for the Seed of *Persea americana* Mill. (Avocado)

The seed of Avocado was tested by phytochemical screening and the results are shown in Table (1).

Table 1. The Results of Phytochemical Tests for the Seed of *Persea americana* Mill. (Avocado)

No.	Tests	Extracts	Reagents	Observation	Remark
1	Alkaloids	1 % HCl	Wagner's reagent	No reddish-brown ppt.	+
2	Glycosides	H_2O	10 % lead acetate	White ppt.	+
3	Flavonoids	EtOH	conc: HCl & Mg ribbon	No pink color solution	–
4	Steroids	pet-ether	Acetic anhydride, $CHCl_3$, conc: H_2SO_4	No greenish color solution	–
5	Reducing sugars	H_2O	Benedict's solution	Brick-red ppt.	+
6	Saponins	H_2O	Distilled water	Frothing	+
7	Tannins	H_2O	10 % $FeCl_3$, conc: H_2SO_4	Yellowish color solution	+
8	Terpenes	EtOH	Acetic anhydride, $CHCl_3$, conc: H_2SO_4	Pink color solution	+
9	Phenolic compound	H_2O	10 % $FeCl_3$	Deep blue color solution.	+
10	Polyphenols	EtOH	1 % $FeCl_3$ and 1 % $K_3[Fe(CN)_6]$	Greenish blue color solution	+
11	Lipophenols	H_2O	0.5 N KOH, 4 drops of NaOH	Deep color solution	+

(+) = presence of constituents (–) = absence of constituents

According to Table (1), glycosides, reducing sugars, saponins, tannins, terpenes, phenolic compound, polyphenols and lipophenols were present in the seed of Avocado.

Oil Content of Avocado Seed

The seed of Avocado was determined by using Soxhlet extraction method. These results are shown in Table (2).

Table 2. The Results of Oil Content of Avocado Seed

No. of experiment	Weight of sample (g)	Weight of oil (g)	% of oil
1	30	1.3	4.33
2	30	1.5	5.00
3	30	1.4	4.67

According to this Table (2), the oil content of Avocado seed was found to be 4.67 %.

Physical and Chemical Characteristics of *Persea americana* Mill. (Avocado Seed Oil)

Physicochemical properties of Avocado seed oil were studied by conventional method. These results are shown in Table (3).

Table.3. Physical and Chemical Characteristics of *Persea americana* Mill. (Avocado Seed Oil)

No.	Characteristics	Avocado Seed Oil
1	Specific gravity (at 30°C)	0.878
2	Viscosity (cP)	0.357
3	Acid value (mg KOH/g)	2.805
4	Saponification value (mg KOH/g)	234.61
5	Iodine value	127.64
6	Unsaponifiable matter (%)	1.53

According to Table (3), the viscosity of Avocado seed oil was found to be 0.357 cP. Therefore, Avocado seed oil could be used in the production of body cream. The acid value of Avocado seed oil was found to be 2.805 and which is used for edible purpose. The saponification value was found to be 234.61 and this value indicates that the presence of low proportion of higher fatty acids in Avocado seed oil. The iodine value of Avocado seed oil was found to be 127.64 and this value indicates that the oil sample was highly unsaturated fatty acid.

Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF)

Energy dispersive X-ray fluorescence (EDXRF) spectrometry (shimadzu EDX-700) can analyze the elements from Na to U under vacuum condition. Relative abundance of elements present in sample was determined by EDXRF spectrometry. The results are shown in Table (4).

Table 4. Relative Values of Elements Present in Avocado Seed by EDXRF Method

No.	Element	Symbol	Quantitative Results (%)
1	Potassium	K	1.3730
2	Calcium	Ca	0.1518
3	Silicon	Si	0.1132
4	Phosphorus	P	0.0818
5	Aluminum	Al	0.0458
6	Chlorine	Cl	0.0302
7	Sulfur	S	0.0228

According to Table (4.4) potassium was found to be present in the seed of Avocado 1.3730 % as the highest value.

Composition of Extracted Oil from Seed of Avocado

The extracted oil from seed of Avocado was analyzed by using GC-MS method and these results are shown in Table (5). Identified chemical compounds with their molecular weight, retention time, molecular formula and structure are tabulated in Table (5).

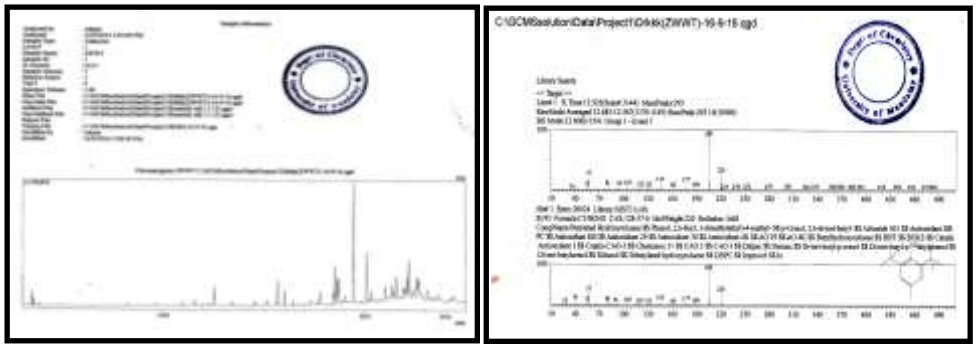

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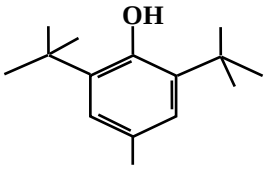
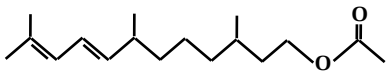
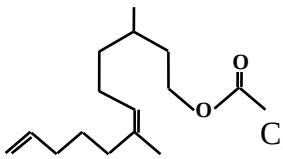
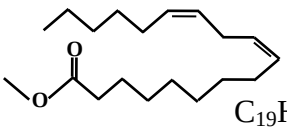
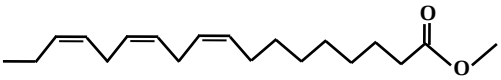
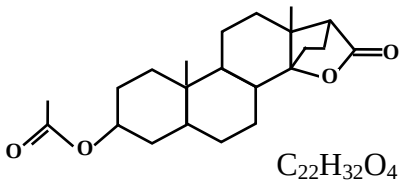
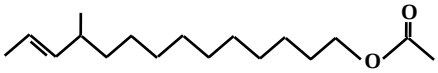
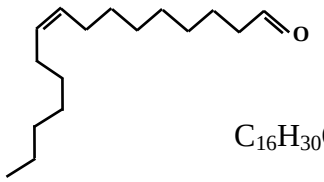
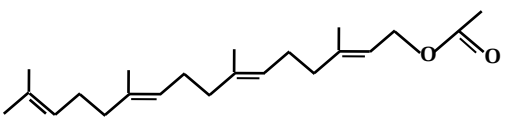
Table 5. Identified Compounds in Avocado Seed Oil by GC-MS

No.	Compound	Retention time	Molecular formula and structure
1	Butylated Hydroxytoluene MW: 220	12.525	 $C_{15}H_{24}O$
2	3,7,11, Trimethyl-8, 10-dodecedienylacetate MW: 266	15.675	 $C_{17}H_{30}O_2$
3	(6Z)-3,7-Dimethyl-6,11-dodecadienyl acetate MW: 252	15.675	 $C_{16}H_{28}O_2$
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (or) linoleic acid, methyl ester MW: 294	18.625	 $C_{19}H_{34}O_2$
5	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) MW: 292	18.700	 $C_{19}H_{32}O_2$
6	3.beta., 14-dihydroxy-, gamma-lactone, acetate MW: 360	20.067	 $C_{22}H_{32}O_4$
7	E-11-Methyl-12-tetradecen-1-ol acetate MW: 268	20.992	 $C_{17}H_{32}O_2$
8	cis-9-Hexadecenal MW: 238	21.550	 $C_{16}H_{30}O$
9	2,6,10,14-Hexadecatetraen-1-ol,3,7,11,15-tetramethyl-acetate, (E,E,E) MW: 332	23.100	 $C_{22}H_{36}O_2$

According to this Table (5), the chemical components in the oil seed of Avocado contain such as butylated hydroxytoluene; 3,7,11, trimethyl-8,10-dodecedienylacetate; (6Z)-3,7-dimethyl-6,11-dodecadienyl acetate; 9,12-octadecadienoic acid (Z,Z)-, methyl ester (or) linoleic acid, methyl ester; 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z); 3.beta., 14-dihydroxy-, gamma-lactone, acetate; E-11-Methyl-12-tetradecen-1-ol acetate; cis-9-

hexadecenal and 2,6,10,14-hexadecatetraen -1-ol,3,7,11,15-tetramethyl -acetate, (E,E,E) respectively.

Determination of Antioxidant Activities of CAvocado Seed

Antioxidant activities of the finely Avocado seed powder were determined by the DPPH radical scavenging method. In DPPH scavenging assay, the antioxidant activity was measured by the decrease in absorbance as the DPPH radical received and electron or hydrogen radical from an antioxidant compound to be become a stable diamagnetic molecule. In the study, ascorbic acid was used as a standard antioxidant.

Antioxidant activities of finely Avocado seed powder were expressed as percentage of DPPH radical inhibition and IC_{50} values (μg/mL). IC_{50} values of the samples were calculated from the concentration Vs percent inhibition curve.

Table 6. Absorbance Values and % Inhibition of Standard Ascorbic Acid

Ascorbic Acid	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5
Concentration (μg/mL)	0.25	0.5	1	2	4
Absorbance	0.3459	0.3422	0.3257	0.2915	0.2639
Inhibition (%)	48.25	48.80	51.27	56.39	60.82

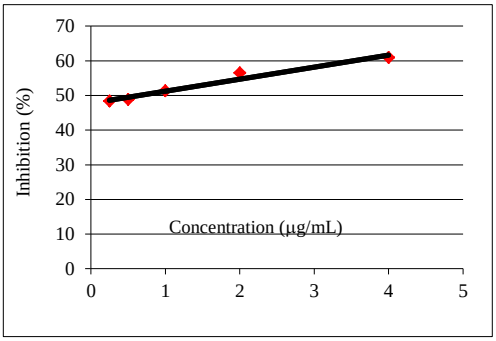


Figure 5. Plot of % inhibition Vs concentration of standard ascorbic acid

Table 7. Absorbance Values and % Inhibition of Finely Avocado Seed Powder

Ascorbic Acid	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5
Concentration (μg/mL)	15.62	31.25	62.5	125	250
Absorbance	0.3559	0.3499	0.3462	0.3352	0.3291
Inhibition (%)	46.75	47.65	48.20	49.85	50.76

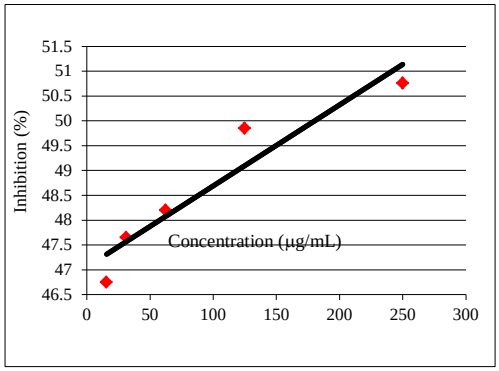


Figure 6. Plot of % inhibition Vs concentration of Avocado powder

Table 8. IC_{50} Values of Standard Ascorbic Acid and Finely Avocado Seed Powder

Test samples	IC_{50} values (µg/mL)
Ascorbic Acid	0.65
Finely Avocado Seed Powder	180.06

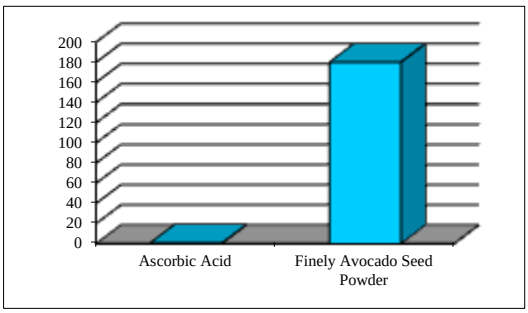


Figure 7. IC_{50} values of ascorbic acid and finely Avocado seed powder

In the above Figure (7), IC_{50} value of finely Avocado seed powder compare with standard ascorbic acid. The lower IC_{50} value, the greater the antioxidant activity becomes. The antioxidant potential of sample powder can be determined by IC_{50} (50 % Inhibition concentration). It means that the concentration of sample can inhibit the oxidation in 50 %. The IC_{50} value of Avocado seed powder indicate the high antioxidant activity.

Conclusion

In this study, the phytochemical screening of Avocado seed indicated that the presence of alkaloids, glycosides, reducing sugars, saponins, tannins, terpenes, phenolic compound, polyphenols and lipophenols.

In addition, mineral contents of this sample were analyzed by EDXRF method. From elemental analysis, the amount of potassium (1.3730 %) was found to be the highest in the seed. Potassium is essential for maintaining the cells membrane potential together with sodium, which enables nerve impulse transmission. Deficiency of potassium causes hypokalemia, constipation, abnormal heart, muscle weakness, fatigue and muscle spasms.

According to GC-MS data, the chemical components in the oil of seed of Avocado contain linoleic acid methyl ester, linolenic acid methyl ester, very little concentration of other compounds, etc.

According to the results, oil content was found to be (4.67 %) in the avocado seed. In extracted oil sample, the low viscosity prevents the dryness of the skin when Avocado seed oil

used as body cream. The acid value is an indicator for edibility oil and suitability for industrial use. Therefore, Avocado seed oil is suitable because of its low acid value. Avocado seed oil was within the limit of saponfication value of most common edible oil.

Iodine value is a measure of the concentration of fats and oils. The higher iodine value, the greater will be the degree of unsaturation. Therefore Avocado seed oil is suitable to use as edible oil. The unsaponifiable matter of Avocado seed of were found to be 1.53 %. The more the iodine value, the better the quality oil and Avocado seed oil in the research work is high quality. Therefore, it was obtained from the extracted Avocado seed oil was found to be suited for human consumption. The IC_{50} value of finely Avocado seed powder were found to be 180.06 μg/mL. Finely Avocado seed powder might be a potential source of natural antioxidants.

These results indicated that Avocado seed oil can be applied for edible, medicinal, cosmetic, antioxidant and industrial purposes.

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