



Original Research Article

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## A Study on the Extraction of Essential Oil from Avocado and its Properties

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### ABSTRACT

Avocado (*Persea Americana*) also known as butter fruit has numerous medicinal properties and is beneficial to health. The pulp and seed were extracted using chloroform and ethanol. The phytochemical analysis determined the presence of alkaloids, flavonoids, tannins and saponins. In avocado pulp, alkaloids and flavonoids were present in a higher concentration and in seeds, cardiac glycoside and tannins showed lower concentration. Macro molecules such as carbohydrates, proteins and fatty acids were determined. In pulp and seed, fatty acid showed higher concentration while proteins showed the lesser concentration in pulp and seed; carbohydrates however were present in the same concentration. Phenolic contents, cholesterol and amino acid were determined. Amino acid and phenolic content showed higher concentration in case of pulp and seed whereas cholesterol was present in small amount. The extraction of oil was carried out by means of cold extraction process. The lipid profile of avocado oil was determined which included the estimation of cholesterol, fatty acid, iodine number and saponification number. Iodine number was high in pulp and seed. Fatty acid value and acid value were the same in pulp and seed. Saponification number was also the same for seed and pulp.

### Introduction

Avocado originated from Central America and hence named "Persea Americana." It provides high quality oil which consists of Vitamin- A, E& D which is beneficial for hair and skin. It contains lecithin, sterolins, and potassium, acts as booster for health as it penetrates and nourishes the skin. It

helps in absorption of vitamins and nutrients, helps in the removal of skin related problems such as eczema, psoriasis, sun-burns, and skin blemishes. The skin of the avocado possesses an antioxidant activity to protect the skin from damage. It also acts as an antibiotic and used to cure dysentery. Avocado pulp helps in dissolving kidney stones which is due to lowering the amount of calcium in

the urinary tract and helps in curing cancer as it induces apoptosis. Avocado contains Vitamin-A, B<sub>6</sub>, C, K protein, copper, potassium and helps in the prevention of cataract in eyes and helps to overcome from Arthritis problems. Avocado seed turns red on exposure to air due to the presence of tannin. The seed contains a milky latex similar to the colour and odour of almond. The seed is used to treat hypercholesterolemia, hypertension, inflammation and diabetes. A small piece of avocado seed, boiled along with its leaves and consumed gives relief from toothaches. Avocado oil is used in the manufacturing of soap and in the cosmetic industry It is used as an anti-oxidant, anti-wrinkle, anti-bacterial agent and helps to lower blood pressure. Avocado seed oil is used against dryness and to prevent wrinkles in the skin. Avocados has lipids which are used in the treatment of Acute Myeloid Leukaemia (AML). The bioactive molecules from avocado help to fight blood cancer. Avocado oil should be stored in air tight container either glass or oil. It should be refrigerated in hot and humid area.

## Materials and methods

### Phyto chemical analysis

The dried extracts were ground into powder and mixed in solvents such as chloroform and ethanol. 10gm of pulp and seed were dissolved in 100ml of chloroform and ethanol respectively in conical flasks and covered with aluminium foil. They were placed in a rotatory shaker for 24 hours. The filtrate obtained from these extracts were used for further phytochemical analysis.

### Phyto chemical screening

#### Qualitative tests

##### 1) Test for carbohydrates

###### Fehling's solution

10ml of Fehling's solution was added to the filtrate and was kept in a boiling water bath for 5-10 minutes.

##### 2) Test for alkaloids

Drangandroff Test:-A small portion of extracts was stirred separately with few drops of concentrated sulphuric acid and it was filtered. Drangandroff reagent was added to the filtrate.

##### 3) Test for proteins and amino acid

(i) Biuret test:-To 3ml of the filtrate, 4ml of 10% NaOH (sodium hydroxide) and few drops of 1% of Copper sulphate.

(ii) Ninhydrin test:-To 1ml of the filtrate, 1% of Ninhydrin was added and heated in a water bath.

##### 4) Test for phyto sterols

The extract was dissolved and 0.05% ferric chloride acetic acid was added followed by adding 1-2 drops of concentrated sulphuric acid along the sides of test tubes.

##### 5) Test for Flavonoids

Alkaline Reagent Method:-To few drops of filtrate, few drops of sodium hydroxide was added. After it became yellow in colour a few drops of concentrated HCl was added.

##### 6) Test for Saponins

Foam test:-To few drops of the filtrate, 5ml of distilled water was added and it was shaken vigorously.

##### 7) Test for Tannins

i) Gelatin test:-Small quantity of extracts were taken and dissolved in water which was tested with 1% of Gelatin and 10% of Sodium chloride.

(ii) Lead acetate test:-Small quantity of extracts were taken and dissolved in water which was tested with 10% of lead acetate.

(iii) Ferric chloride test:-Small quantity of extracts was taken separately in water with dilute ferric chloride.

## 8) Test for Cardiac Glycoside

Keller-Killani test:-To 2 ml of the filtrate, few drops of glacial acetic acid, 5% of ferric chloride and concentrated sulphuric acid were added.

## 9) Test for fixed Fats and Oil

Sudan black test:-To the few ml of the extracts, Sudan black was added.

## Quantitative Tests

### Estimation of Carbohydrate (Anthrone Method)

The pulp and seed samples was weighed and taken into boiling test tubes. 2ml of 1N of HCl was added to the boiling test tube which was kept in a water bath for 3 hours or till the appearance of pink colour. Sodium carbonate was added to the test tube to obtain effervescence. Then added distilled water and made up the volume to 100 ml.

The supernatant was collected. The Blank (B) was prepared by adding water and Anthrone reagent. Standards were taken in the range of 0.2ml-1 ml. The volume was made up to 1ml in all the test tubes. The test tubes were placed in a boiling water bath and it was cooled rapidly till it turned from green to dark blue colour. Optical density was obtained at 630 nm.

### Estimation of Alkaloid Content (Harborne Method)

The samples (pulp and seed) were weighed and added to the beakers. Two hundred ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. It was filtered. Extracts were kept in a water bath till it was reduced to one-fourth of original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was obtained. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide which was then filtered.

**Alkaloid content=**

$$\frac{\text{Weight of Alkaloid extract}}{\text{Weight of sample (mg)}} \times 100$$

### Estimation of proteins (Lowry's method)

Standard solution (0.2 to 1.0ml) was taken in test tubes. The extracted samples (pulp and seed) were taken in test tubes. The volume of water was made up to 1ml including the blank. 5ml of alkaline copper tartrate reagent was added to the all the test tubes. It was mixed and allowed to stand for 10 minutes. 0.5 ml of Folin's reagent was added to test tubes including the blank. The test tubes were incubated at room temperature in dark for 30 minutes. The Optical density was read at 660nm.

### Estimation of Flavonoids

The samples (pulp and seed) were taken and 100ml of 80% of aqueous methanol was added. The whole solution was filtered through Whatman filter paper. The filtrate obtained was transferred into a crucible and evaporated into dryness over a water bath.

### Estimation of Amino acid (Ninhydrin method)

Standards were taken as 0.2ml to 1.0ml in the test tubes. Extracts of samples (pulp and seed) were also taken. The volume of distilled water was made up to 2ml in all the test tubes including the blank. 2ml of Ninhydrin was added to all the test tubes. The test tubes were kept in boiling water bath for 15 minutes. 3 ml of ethanol was added to all the tubes. The optical density was read at 570nm.

### Estimation of Tannin (Poly Vinyl Pyrolindine method)

0.5 ml of the extracts (Pulp and seed) were taken in the test tubes. 0.05ml of PVP (Poly vinyl pyrolindine) was added to the test tubes. 0.05 ml of distilled water was added to both the extracts. It was incubated for 4 hours and it was centrifuged at 5000 rpm for 5 minutes. The supernatant was removed and OD was taken at 725 nm in colorimeter.

**Tannin (mg/g extract) = Total phenolic substance (mg/g) - Free phenolic substance (mg/g).**

### **Estimation of total phenolic contents**

Standards were taken as 1.25ml to 6.75ml in the test tubes. The sample extracts (pulp and seed) were taken in test tubes. 0.5ml of Folin-Ciocalteu reagent was added to all the test tubes including the blank. 2.5 ml of 20% of sodium carbonate was added to all the test tubes. The tubes were made up to 5.75 ml with distilled water. The test tubes were incubated at room temperature for 2 hours. The optical density was determined at 760nm.

### **Estimation of cardiac glycoside**

The powdered samples (pulp & seed) were mixed in 50ml of 35% ethanol in conical flasks. The conical flasks were covered and placed in shaker for 6 hours. It was filtered to another flask. 10 ml of distilled water and 20ml of 6.4% lead acetate were added. 45 ml of distilled water was added and shaken well by placing it in a shaker for 10 minutes. To the precipitate formed 7.5ml of 2.38% distilled sodium hydrogen phosphate solution was added. The resultant solutions were filtered. To the filtrate added 1mg of dried extract, 1ml of glacial acetic acid and 2 drops of 1% of Ferric chloride solution.

**Cardiac glycoside=**

**Weight of the dried extracts × 100**  
**Weight of dried ground sample**

### **Estimation of cholesterol (Zak's method)**

Standard solutions were taken in the range of 0.5 to 2.5ml in different test tubes. 0.2ml and 0.4ml of seed sample were taken in separate test tubes. 0.05% ferric chloride acetic acid reagent was added to make up the volume to 5ml. 5 ml of ferric chloride was used as the blank. 3ml of concentrated sulphuric acid was added to all the test tubes. The test tubes were incubated at room temperature for 10-20 minutes. The optical density was read at 570nm.

### **Estimation of fatty acid**

The sample (pulp and seed) were taken in a conical flask. 50ml of neutral solvent was added along with a few drops of phenolphthalein indicator. The pH was maintained at 8 by adding drops of NaOH. Potassium hydroxide was taken in the burette and it was titrated against the sample. Appearance of pale pink colour was the end point.

### **Thin layer chromatography**

#### **Detection of phenolic compound**

Chloroform: Methanol in ratio of 9:1 served as mobile phase. Folin-Ciocalteu reagent was used as spraying reagent. The sample (pulp and seed) were spotted on TLC plate.

#### **Detection of phyto-sterol**

The Thin layer chromatography was done using 10×20 cm. silica plate. Benzene and Methanol were used as a mobile phase. 5% of alcoholic sulphuric acid was used as spraying agent. Appearance of bluish green colour indicated the presence of steroids.

#### **Detection of saponins**

Chloroform: Glacial acetic acid: Methanol: Water (8:4:1.5:6.5) was used as mobile phase. The plates were developed using Iodine vapours.

#### **Extraction of pulp and seed oil**

The pulp and seed were removed from Avocado. They were cut and ground into small pieces. They were made into juice using blender. After straining, they were sieved. The thick juice of pulp and seed were kept for overnight. After 24 hours, the top creamy layer was removed. In a clean pan, it was kept on a low flame and was allowed to heat until the proteins turned brown.

**Lipid profile** - 2.5 ml of oil was diluted with 25ml of chloroform and used to analyses.

### Estimation of cholesterol method (Zak's method)

Standard solutions were taken in the range of 0.5 to 2.5 ml in different test tubes. 0.2 ml and 0.4 ml of seed sample were taken in separate test tubes. 0.05% ferric chloride acetic acid reagent was added to make up the volume to 5 ml. 5 ml of ferric chloride was used as the blank. 3 ml of concentrated sulphuric acid was added to all the test tubes. The test tubes were incubated at room temperature for 10-20 minutes. The optical density was read at 570 nm.

### Estimation of fatty acid

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### Acid value of oil

The samples (pulp and seed) were taken in conical flask. 25 ml of ethanol was added into the conical flask. It was shaken well and phenolphthalein indicator was added. The samples were titrated against 0.1 N KOH which was taken in a burette. The faint pink colour was the end point which was persistent for 20-30 seconds. The Blank which was prepared without the test solution was titrated with 0.1 N KOH.

$$\text{Acid Value (mg KOH/g of fat)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample (gm.)}}$$

### Iodine value of oil

5 ml of Wiji's solution was dissolved with a known amount of oil. It was shaken thoroughly and allowed it to stand in dark for 1 hour. Blank solution was prepared without the oil. After 1 hour, the stopper and the neck of the Iodine flask was rinsed with 10 ml of water and 2 ml of Potassium Iodide was added. The liberated Iodide was titrated against

sodium thiosulphate till the contents of the flask turned straw yellow in colour. The solution was continuously titrated against sodium thiosulphate solution till the blue colour disappeared.

$$\text{Iodine value} = (x-y) = \frac{12.7 \times 100}{1000 \times \text{weight of the sample}}$$

### Saponification of Oil

One gram of pulp and seed oil was weighed accurately and dissolved in 3 ml of fat solvent. 25 ml of 0.5 N alcoholic KOH was added. It was attached into a reflux condenser and the contents of boiling water bath for 30 minutes. It was cooled at a room temperature and few drops of phenolphthalein were added. The blank was refluxed with 25 ml or 0.5 N alcoholic KOH without the fat sample. The contents of the flask were titrated till the pink colour disappeared.

$$\text{Saponification value} = \frac{28.05 \times \text{Titre value}}{\text{Weight of the sample (g)}}$$

### Identification of saturated and unsaturated fats

The pulp and seed oil was kept in refrigerator to check for solidification to determine the level of saturation.

### Cytotoxicity studies-(MTT ASSAY)

#### Stock concentration

1 mg of sample was dissolved in 1 ml of serum free MEM/DMSO (Dimethyl sulphoxide). The stock was freshly prepared and filtered through 0.45 µm filter before each assay.

#### Preparation of working standard

To 1 ml of MEM (Minimal Essential Media) without serum concentration, DMSO (dimethyl sulphoxide) was added to 1 ml of sample as a working concentration. The sample concentration ranging from 1000 µg to 7.8 mg can be prepared from the working stock was made up to 1 ml.



Vero Cell lines were plated in 24 well plates and incubated at 37°C with 5% CO<sub>2</sub> conditions. After the cell reached the confluences, media was removed without disturbing the cells. The procedure was repeated at various concentration of the samples. After incubation of the sample, it was washed with phosphate buffered saline at pH of 7.4. 100 µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-phenyl-tetrazoliumbromide (MTT) was added.

After incubation, 1ml of DMSO was added to all the wells. The absorbance was measured at 570nm with UV spectrophotometer using DMSO. Measurements were performed and the concentration required for 50% inhibition was determined graphically. The percentage of viability of cells were determined by -

$$\% \text{ Cell Viability} = \frac{\text{A570 of treated cells} \times 100}{\text{A570 of controlled cells}}$$

### Results



**Fig.1:** The powdered form of seed.



**Fig.2:** The powdered form of pulp.

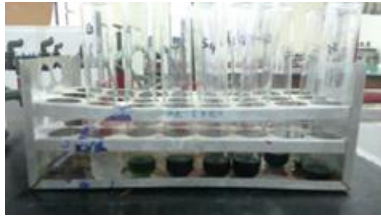


**Fig. 3:** Shows the results for the qualitative analysis of phytochemicals conducted on the pulp and seed of avocado.

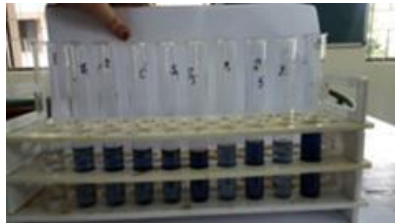
**Table 1.** The qualitative analysis of phytochemicals in pulp and seeds of avocado.

Phytochemical tests	Chloroform (Pulp)	Chloroform (Seed)	Ethanol (Pulp)	Ethanol (Seed)
Carbohydrates	+	+	+	+
Proteins and amino acid	+	+	+	+
Fats and oil	+	+	+	+
Tannins	-	-	+	+
Alkaloids	+	+	+	+
Cardiac glycoside	+	+	+	+
Flavonoids	+	+	+	+
Phenolic contents	+	+	+	+
Phyto sterols	+	+	+	+
Saponins	+	+	+	+

The phytochemical such as carbohydrates, proteins, amino acid, fats and oils, phenolic contents, saponins, flavonoids, Cardiac glycosides, and Alkaloids were present in pulp and seed except for tannins which was absent in chloroform extract.



**Fig. 4:** Results for estimation of carbohydrate



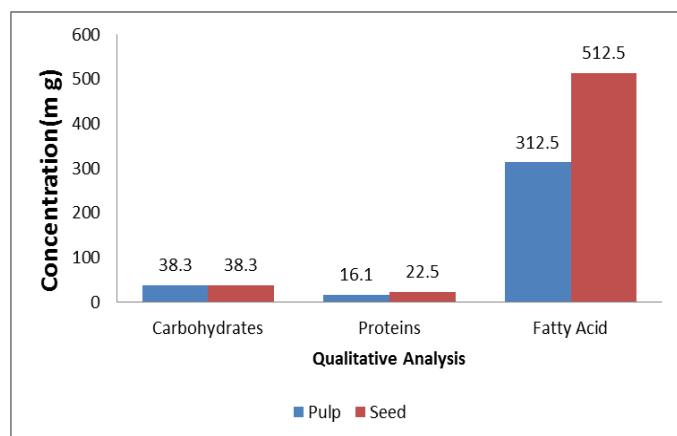
**Fig. 5:** Results for estimation of protein



**Fig. 6:** End point for estimation of fatty acid

**Table 2.** Shows the result obtained for the estimation of Carbohydrate, Proteins and Fatty acid.

Estimation	Pulp (mg)	Seed (mg)
Carbohydrate	38.3±04	38.3±0.4
Proteins	16.1±3.62	22.5±2.27
Fatty acid	312.5±4.4	512.5±6.4



**Graph 1.** Illustrates the quantitative analysis of Carbohydrates, Proteins and Fatty acid indicating almost equal quantities of Carbohydrates in pulp and seed slightly higher concentration of proteins in seeds and very high in quantity in seed in comparison to pulp.



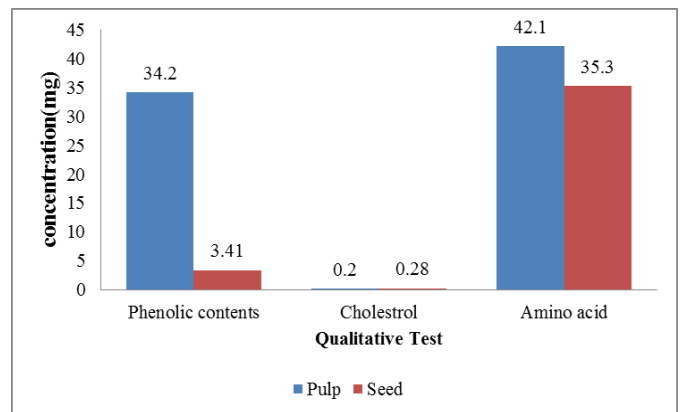
**Fig. 7:** Results for estimation of phenolic contents.



**Fig. 8:** Results for estimation of cholesterol.

**Table 3.** Shows the results for the estimation of Phenolic contents, Cholesterol, Amino acid.

Estimation	Pulp (mg)	Seed (mg)
Phenolic contents	34.2±5.4	34.1±2.5
Cholesterol	0.2±0.08	0.2±0.07
Amino acid	42.1±0.6	35.3±0.2



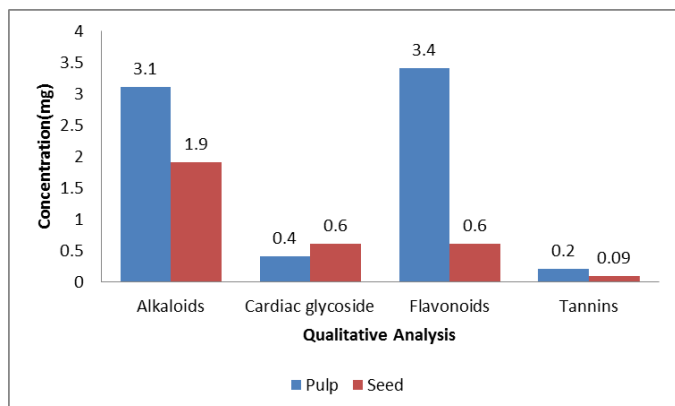
**Graph 2.** From graph-2: Cholesterol content was very low in both pulp and seed while the phenolic and amino acid contents were much greater in comparison to seed.



**Fig. 9:** Cardiac glycoside powder.

**Table 4.** The results of estimation of alkaloids, flavonoids, cardiac glycoside and tannins in avocado.

Estimation	Pulp (mg)	Seed (mg)
Alkaloids	3.1±0.8	1.8±0.08
Flavonoids	3.4±0.2	0.68±1.5
Cardiac glycoside	0.4±0.1	0.68±1.2
Tannins	0.2±0.1	0.09±0.5



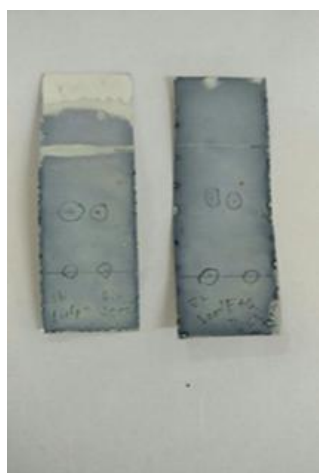
**Graph 3.** Alkaloids, Flavonoids, tannins, the pulp showed higher amount of concentration. In cardiac glycoside, the seed showed lesser amounts.



**Fig. 12:** Pulp oil of avocado.



**Fig. 13:** Seed oil of avocado.



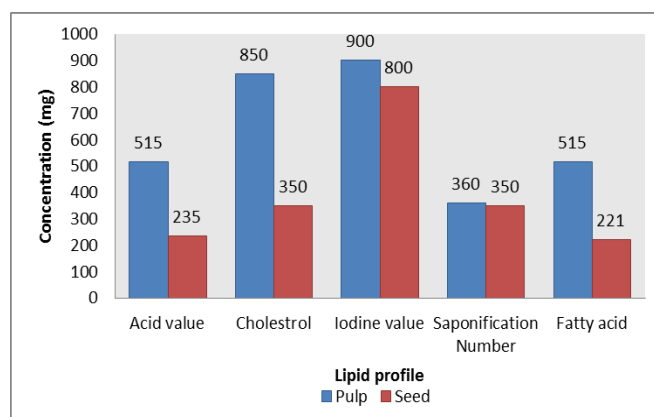
**Fig. 10:** Chromatogram showing the presence of phenolic compound



**Fig. 11:** Shows the chromatogram for presence of phytosterols

**Table 5.** The results for the lipid profile of oil extracted from avocado.

Estimation	Pulp (mg)	Seed (mg)
Acid value	515±5	235±5
Cholesterol	850±5	350 ±4
Saponification value	360±2.5	350±1
Iodine number	900±0.1	800±0.1
Fatty acid	515±5	221±4



**Graph 4.** From Graph-4, the Pulp showed high concentration of Cholesterol, Acid value, Iodine value. The Saponification value was almost the same for pulp and seed.



## Cytotoxicity test

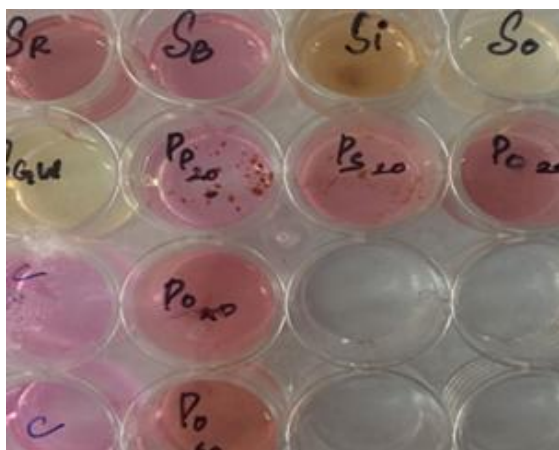


Fig. 14: Titre plates showing cytotoxicity studies.

Table 6. Cytotoxicity studies of avocado.

Samples	20µl	40µl	60µl
Pulp	92.8%	-	-
Seed	41.8%	-	-
Pulp oil	35%	40%	42.5%

From the results obtained for cytotoxicity studies, it was seen that the pulp, seed, and oil were good cytotoxic agents. Pulp was showing the highest of 92.8% while oil shows effective cytotoxicity in 20, 40, and 60µl of sample.

## Conclusion

Various parameters were analysed on extracts of avocado seed and pulp. Macromolecules such as Carbohydrates, Protein, and Fatty acids showed appreciable concentration in both avocado seed and pulp. Fatty acid showed higher concentration while proteins showed the least concentration in pulp and seed; carbohydrates were present in the same concentration. Phytochemical analysis showed the presence of alkaloid, flavonoids, saponins, tannins which were also quantified. Presence of Phenolic compounds, cholesterol and amino acid were determined. Amino acid and phenolic content showed higher concentration in case of pulp and seed whereas cholesterol was present in small amount. Oil was extracted from both avocado pulp and seed. Lipid profile analysis conducted showed significant results for the estimation of cholesterol,

fatty acid, Iodine number, Saponification number. Iodine number was high in pulp and seed. Fatty acid value and acid value were the same in pulp and seed. Saponification number was also the same for seed and pulp. Cytotoxicity studies done with Avocado pulp and oil showed 35%, 40%, and 42.5% cytotoxic effect at 20, 40 and 60 µl with pulp showing the highest toxicity of 92.8%. In conclusion, Avocado is a whole some nutritive fruit which is not much explored but contains a number of benefits for the consumer.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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