

PHYTOCHEMICALS ANALYSIS OF VARIOUS PARTS OF THE AVOCADO PLANT (PERSEA AMERICANA)

Pavitra Shyam & S. Kauvery Bai

Research Scholar, Smt. V. H. D. Central Institute of Home Science, Bangalore University, Karnataka, India

ABSTRACT

*Phytochemical screening was conducted on its leaves, bark, seed, and fruit skin of the Avocado. The active ingredients in the avocado plant part were extracted in three solvents- Distilled water, 70% ethanol and acetone. Qualitative tests were conducted on the supernatant extracted from the different plant parts of the Avocado using different chemical tests to detect and identify the various classes of phyto chemicals present. The screening revealed presence of Alkaloids, Flavonoids, Terpenoids, Saponins, tannins etc. The phenolics and flavonoids were further studied to analyse them quantitatively. As seen by the phyto chemical tests conducted on the various parts of the avocado (*Persea Americana*), the plant parts of the avocado showed good results for the presence of tannins and hence this study ascertains the use of these plant extracts as a dye on fabrics. Most extracts also showed some amount of phenolic and flavonoid content, so they could also play a significant role in enhancing the performance of textiles as health benefiting eco-friendly finishes.*

KEYWORDS:

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INTRODUCTION

Plants and plant parts have been used in textiles since time in memorial. Before 1850, Natural dyes from plant, animal and minerals were the main sources of obtaining colour on the various fabrics. Traditional approach of using natural dyes was one that improved by repeated trial and error methods conducted over many years, with the progress in science, now empirical approaches have been created to build an understanding of a relevant lead molecule that can then be further studied and thereby obtain more standardised methods of obtaining coloured compounds from plant sources.

Phytochemical analysis is an empirical method used to detect and document the various phyto constituents present in the plant. Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. The name comes from the Greek word 'phyton', meaning plant. Phenolic compounds are the most abundant secondary metabolites in plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens, parasites and predators as well as contribute to the plant colour. There are more than 8000 phenolic structures currently known. They are aromatic benzene rings compounds with one or more hydroxyl group. Flavonoids are one of the important contributing compounds of colour to the plant and hence also important for the use as dye. Flavonoids are very effective antioxidants (Yanishlieva-Maslarova-2001) other phenolic compounds are Alkaloids, Tannins, Saponins and Terpenoids.

In this study the researcher, carried out phytochemical analysis on the various plant parts of the Avocado (*Persea Americana*), to determine which part of the plant is best suited to be used as a fabric dye.

Avocado (*Persea Americana*) is a plant that bears a very large fruit, resembling a huge berry. Avocado, Butterfruit, or Alligator pear is a native tree of Mexico and belongs to the family of flowering plants lauraceous. There are several wild species of *Persea* in Central America. Varieties being cultivated today had been developed by people in the region of modern Mexico and Guatemala thousands of years ago. (Seeds found in the caves of the Tehuacán Valley, south of Mexico City, have been determined to be nearly 10,000 years old and are cited as proof of the early use of the avocado fruit by humans.)

The British introduced the Avocado to India from Sri Lanka, in the early part of the 20th Century. They planted it in hill stations of South and central India like Tamilnadu, Kerala, Karnataka and Maharashtra, as it grew well in Tropical or semi tropical areas experiencing some rainfall in summer. Avocado was not a commercial fruit crop until a few years ago, with the advent of Mexican restaurants and juice bars around cities the produce of the avocado plant has been in demand. The three varieties being grown in India are 'Western India', 'Guatemalan' and 'Mexican'.

MATERIALS AND METHODS

Materials

Bark, Leaf, Seeds and Fruit Skin of Avocado (*Persea Americana*). Ethanol, Acetone and Distilled Water. Wagner's reagent- An aqueous solution of iodine and potassium iodide, Sodium Hydroxide (NaOH) Dilute Hydrochloric acid (HCl), Ferric chloride (FeCl₃), Gelatine, Lead Acetate (Pb(CH₃COO)₂) Chloroform and Concentrated Sulphuric acid (H₂SO₄), Folin- Ciocalteu reagent, Sodium Carbonate (Na₂CO₃), Gallic acid standard. Aluminium Chloride, Rutin Standard, Sodium Nitrite (NaNO₂). All chemicals used for the study were of analytical grade.

Methods

Plant Preparation: The leaves of the Avocado plant were plucked just before the shedding season, the leaves were washed in distilled water, air dried at room temperature and then ground into a fine powder. During this period fruit bearing trees are also pruned, for better yield, the bark obtained from these cut branches was used in the research. The Seeds and fruit skin which were collected from various eateries around the city were scrapped, cleaned, washed and air dried. Then they were ground into fine powder.

Extraction: this is the first important step to extract the active ingredients from the plant parts. 2gms of the ground powders of each plant part was taken and dissolved in 25 ml of 3 different solvents- Distilled water, 70% ethanol and acetone. These samples were transferred into centrifuge tubes and incubated for 24 hours at room temperature (27°C). Then they were agitated in a shaker for 3 hours at 150 rpm at 27^o centigrade. The mixtures were then placed in a centrifuge machine for 20 minutes at 4^o centigrade at 4000 rpm. Supernatant liquid that separated out was filtered from the extract residue using Whatman filter paper. To the residue 25ml more, of each of the solvents was added once again and the shaking, centrifugal process repeated. This was to ensure complete extraction of the active ingredients from the plant parts. The Supernatant fluid from the two extractions was mixed and stored in refrigerated conditions at 4^o centigrade, for further analysis as suggested by Yun Shen et al, (2008).

Qualitative Photochemical Screening

Qualitative tests were conducted on the supernatant extracted from the different plant parts of the Avocado using different chemical tests to detect and identify the various classes of phenolics present in the extract- eg. Alkaloids, Flavonoids, Terpenoids, Saponins etc,

Tests for Phenol Compounds and Tannins: Tannin produce colour as well as are anti-microbial in nature. Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink).

Gelatine Test: 2ml of 1% solution of gelatine containing 10% NaCl is added to 1 ml of the extract, presence of white precipitate confirms presence of phenolic compounds

Lead Acetate Test: 3ml of 10% lead acetate solution was mixed with the extract. Bulky white precipitate confirms presence of Phenolic compounds.

Ferric Chloride Test: 1ml of extract was separately stirred with 10 ml of distilled water and then filtered. A drop of 5% FeCl_3 was added to the filtrate. Blue-Black or blue-Green colouration or precipitation indicates the presence of Tannins.

Tests for Alkaloids: Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), the use of alkaloids containing plants as dyes can be traced back almost to the beginning of civilization.

Wagner's Test: Few drops of Wagner's reagent are added to 1 ml of the extract, a reddish brown precipitate confirms presence of Alkaloid.

Tests for Flavonoids: Flavonoids are water soluble Polyphenolic compounds responsible for the colour of fruits and vegetables.

Ammonia Test: a few drops of ammonia NH_3 were added to 1ml of the extract, yellow colouration shows presence of Flavonoids.

Sodium Hydroxide Test: Few drops of 20% NaOH solution were added to 1ml of the extract, it turns yellow, then HCl is added to this mixture and if the mixture turns colourless, it depicts the presence of flavonoids.

Tests for Terpenoids: They are a combination of 5 carbon precursors called isoprene and are used for moth proofing, especially in the art of textile conservation. Terpenoids are commercially interesting because of their use as flavours and fragrances

Salkowski Test: 1 ml of the extract was mixed with 0.5 ml of chloroform, 1ml of concentrated H_2SO_4 was slowly added to this to form a layer, if a reddish brown colouration at the interface is formed, it shows the presence of Terpenoids.

Tests for Saponins: Saponins are glucosides with foaming characteristics. The foaming ability is formed by a hydrophobic (fat part) and a hydrophilic (sugar part). Saponins are bitter to taste.

Foam Test: 1 ml of extract was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of this filtrate was mixed with 5ml of distilled water and mixed vigorously for 15 minutes. Formation of a persistent foam after 5 minutes shows the presence of Saponins.

Quantitative Analysis: to Analyse the Quantitative Content of Phenolics and Flavonoids in the Given Plant Parts.

Total Phenol Content or TPC is determined using Folin Ciocalteu assay method (Singleton and Rossi 1965) with a little modification of using Gallic acid as the reference standard. All the solvents extracted were diluted to appropriate volumes and 100ul of Folin Ciocalteu Reagent was added to the mixture. This was then incubated for 3 minutes then 2 ml of 10% Na₂CO₃ solution was added. This mixture was incubated under dark conditions at room temperature for 60 minutes. The absorbance is measured at 765nm using a UV-Vis spectrophotometer. TPC is expressed as a Gallic acid equivalent (GAE) in milligrams per gm of sample.

Total Flavonoid Content or TFC is determined by a colorimetric method with minor modification. 1 ml of each Aliquots from the diluted plant extracts is pipetted into a 15ml polypropylene conical flask separately, this is mixed with 2ml double distilled water, 0.15ml of 5% Sodium Nitrite(NaNO₂). After incubating for 5 minutes 0.15ml of Aluminium Chloride Hexahydrate (AlCl₃.6H₂) is added and the mixture is incubated for another 5 minutes, after which 1 ml of 1M Sodium Hydroxide(NaOH) was added. The mixture was stirred well to ensure through mixing and kept aside for 15 minutes. The absorbance was measured using UV-Vis Spectrophotometer. TFC is calculated using Rutin as the standard. A Rutin curve is formed and expressed as 'mg' Rutin equivalent (mg RE) per gram of sample.

RESULTS AND DISCUSSIONS

Qualitative Photochemical screening tests conducted on various extracts of leaf, bark, and seed and fruit skin of Avocado plant are recorded in Table 1 and explained thereafter.

Phenolics and Tannins: the leaf extracted in Acetone showed the maximum presence of tannins and phenolics using Ferric Chloride (FeCl₃) test. Using gelatin test, the acetone leaf extract showed the least amount of tannins and phenolics but the other two extracts showed high presence of the Tannins and phenolics, Lead acetate test also showed high presence of tannins and phenolics in all three solvents.

The bark extracted in all three solvents in the Ferric chloride test showed marginal presence of tannins and phenolics. Only in the lead acetate test did the bark extract in the three solvents showed a high presence of tannins or phenolics, there was no change in reaction using gelatin for the bark extract.

The seed extract in water as solvents showed minor presence of phenolics and tannins in the ferric chloride test and Lead acetate test, no presence with gelatin test. Ethanol and acetone solvent extracts of the seed showed more amounts of phenolics and tannins in ferric chloride test and lead acetate test and no presence using gelatin.

Water extract of the skin showed the least, yet good amount of tannins and phenolics in all three tests and skin extracted in ethanol and acetone showed largest presence of tannins and phenolics. Presence of tannins indicates that they could be used as dyes and anti-microbial agents; the presence of Phenolic compounds indicates good anti- microbial, anti-fungal properties.

Alkaloids: As seen there is a more presence of Alkaloids in the leaf and Fruit skin of the plant as compared to that in the bark or seed, extracted using any solvent. Presence of alkaloids indicates good antibacterial and antifungal activities.

Flavonoids: the leaf extracted with ethanol showed change in colour and precipitation with Ammonia test. And also when tested with HCl and NaOH it showed more presence of flavones.

Bark extracted in water and ethanol showed good presence of flavonoids when tested with ammonia and lesser amount in the acetone extraction. The bark extracted showed more presence of flavones in the ethanol extract when tested with HCl and NaOH as compared to when extracted in water and acetone.

The seed, in all the three solvents showed marginal presence of flavonoids when tested with ammonia and with HCl and NaOH.

The fruit skin extracted in the three solvents showed large presence of flavonoids using the ammonia test and the skin extracted in water and ethanol showed large presence of flavonoids.

Terpenoids: In the leaf extracted in water no presence was detected of Terpenoids, moderate amount in the ethanol extract and large amounts in the acetone extract.

The bark too showed no presence of Terpenoids in water extract, but moderate amounts in the ethanol and acetone extracts.

Seed also did not show any presence in water extracted solvent, but marginal amounts in ethanol and acetone.

Skin showed maximum amount of presence of Terpenoids when extracted in ethanol, little less in acetone and the least amount when extracted in water.

Presence of Terpenoids means the extract could be used as a fragrance provider to the textile.

Saponins: The leaf in all three solvents showed high presence of Saponins, Bark showed negligible amounts.

Seed showed a large amount when extracted using ethanol, and moderate amounts when extracted using water and acetone.

Skin too showed maximum Saponins when extracted with ethanol and negligible amounts when extracted with water and acetone. Many Saponins are known to be antimicrobial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems.

Table 1

Chemical Screening Test	Results Expected	Avocado Leaf			Avocado Bark			Avocado Seed			Avocado Fruit Skin		
		Aq Aqueous AVL1	Ethanol AVL2	Acetone AVL3	Aq Aqueous AVB1	Ethanol AVB2	Acetone AVB3	Aq Aqueous AVS1	Ethanol AVS2	Acetone AVS3	Aq Aqueous Avsk1	Ethanol Avsk2	Acetone Avsk3
Alkaloids													
Wagner's Reagent test	Reddish brown precipitate	+++	+++	+++	++	++	++	+	+	++	+++	+++	+++
Flavonoids													
Ammonia test	Yellow colouration and darker with precipitate	++	+++	++	+	++	+	+	+	+	+++	+++	++
Sodium Hydroxide test	Changes from Yellow to colourless	+	++	+	+	++	+	+	+	+	++	++	+
Tannins and Phenolics													
Ferric Chloride test	Dark green	++	++	+++	+	+	++	+	++	++	++	+++	+++
Gelatine Test	white precipitate	+++	+++	++	-	-	-	-	-	-	++	++	++
Lead Acetate test	Bulky white precipitate	+++	+++	+++	+++	++	+++	+	++	++	++	+++	+++
Saponins													
Foam Test	persistent foam	++	++	+++	+	+	+	+	+++	++	+	+++	+
Terpenoids													
Salkowski test	Reddish brown precipitate	-	++	+++	-	++	++	-	+++	++	+	+++	+++

Key '+'= Presence of compound, '++'= Dark colour, '+++'= Dark colour and precipitate, '-'= absence of compound.

Total Phenolic Content: substance which shows good phenolic properties might have good anti-bacterial properties.

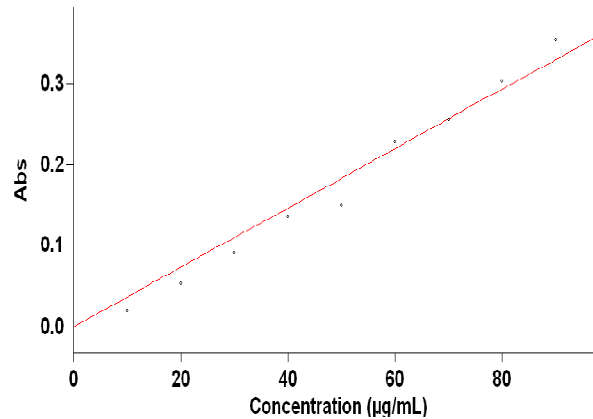


Figure 1: Total Phenolic Content.

Below the Table 2 the amount of TPC, estimated using Folin Ciocalteu assay (FC) method (Singleton and Rossi 1965) with a little modification of using Gallic acid as the reference standard (Table 2). Total Phenolic content ranged from 0.491 to 4.790 µg Gallic Acid Equivalent. (GAE). Leaf samples extracted in Acetone had the highest GAE reading second in leaf extracted in 70% ethyl alcohol. The Skin samples extracted in Acetone and Ethyl Alcohol showed the next best readings, followed by Seed samples in both these solvents. Most extracts showed some amount of phenolic content, so they could be used as eco-friendly anti-microbial agents.

Table 2: Total Phenol Content (TPC) Values in GAE (Mg / G) of Various Parts of the Avocado Plant

Sample	Solvent	Concentration GAE* (mg / g)
Leaf	Water	1.460
Leaf	70 % Ethyl alcohol	4.631
Leaf	Acetone	4.790
Bark	Water	0.491
Bark	70 % Ethyl alcohol	0.645
Bark	Acetone	1.499
Seed	Water	0.846
Seed	70 % Ethyl alcohol	1.890
Seed	Acetone	2.481
F.Skin	Water	1.871
F.Skin	70 % Ethyl alcohol	3.579
F.Skin	Acetone	3.829

(GAE):Gallic Acid Equivalent.

Total Flavonoid Content: Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory

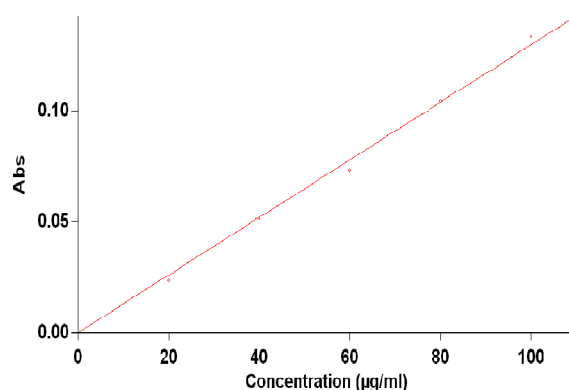


Figure 2: Total Flavonoid Content.

Table 4: Total Flavonoid Content (TFC) Values Inre*(Mg / G) of Various Parts of the Avocado Plant

Sample	Solvent	Concentration RE* (Mg / G)
Leaf	Water	1.460
Leaf	70 % Ethyl alcohol	5.971
Leaf	Acetone	2.432
Bark	Water	0.869
Bark	70 % Ethyl alcohol	1.379
Bark	Acetone	1.379
Seed	Water	0.961
Seed	70 % Ethyl alcohol	2.068
Seed	Acetone	1.253
F.Skin	Water	3.406
F.Skin	70 % Ethyl alcohol	3.722
F.Skin	Acetone	2.666

RE*:Rutin Equivalent

Below the Table 4 Total Flavonoid content of the solvent extracted from the leaf, bark, seed and Skin of the avocado were as shown in table 4, the range of readings got was from 5.971 mg / g RE for leaf extracted in 70 % ethyl alcohol, followed by 3.722 mg / g RE skin extracted in 70 % ethyl alcohol. Acetone and ethyl alcohol solutions used as extracting solvents showed better results as compared to water as a solvent, other than for the fruit skin samples where water too gave high readings. TFC values were more than the TPC values.

Substance that have Phenol and Flavonoid content are seen to be good anti-microbial agents as they not only curb Microbial growth on the fabrics but also add additional healing powers to the fabric.

CONCLUSIONS

Worldwide the need of the hour is to find newer eco-friendly plant products for textile applications as there would lower adverse reactions and would be sustainable too. As seen by the phyto chemical tests conducted on the various parts of the avocado (*Persea Americana*), the plant parts of the avocado showed good results for the presence of tannins and hence this study ascertains the use of these plant extracts as a dye on fabrics. Most extracts also showed some amount of phenolic and flavonoid content, so they could also play a significant role in the performance of fabrics as eco-friendly health finishes.

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