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COMPARATIVE BIOCHEMICAL ANALYSIS AND ANTI-OXIDANT PROPERTIES OF LEAVES OF DIFFERENT OLIVE VARIETIES CULTIVATED AT NTHRI SHINKIARI

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Abstract

Olive leaves, a byproduct of olive oil and fruit production, are rich in antioxidants, particularly phenolic compounds. This study investigated the biochemical composition and antioxidant activity of three olive cultivars (Koroneki, Arbequina, and Manzanilla) grown in Pakistan. Koroneki had the highest moisture content (7%), while Arbequina and Manzanilla had similar lower levels (3%). While Arbequina and Manzanilla had higher ash, content (0.65%) compared to Koroneki (0.3%). Koroneki had the highest amino acid content (0.1058%), followed by Arbequina (0.0994%) and Manzanilla (0. 08588%).In Phytochemical Analysis Methanol extracts of all three cultivars contained tannins, Saponin, flavonoids, alkaloids, phenols, glycosides, steroids, and Terpenoids. N-hexane extracts showed minor variations. Arbequina exhibited the highest total polyphenol content (0.082598 mgGAE/g) as well as amino acid (0.0994% per gram) as compared to other varieties (koroneki and Manzanilla). Caffeine levels ranged from 0.461% to 0.596% among all three cultivars. Var.Arbequina shows the highest antioxidant activity (4.000036).

The study highlights the potential of olive leaves as a valuable resource. Their rich phytochemical profile, including antioxidants, suggests various applications in the pharmaceutical, food, and cosmetic industries. By utilizing olive leaves, we can reduce waste and promote sustainable practices in the olive industry.

1. INTRODUCTION

Olea europaea L. is an evergreen plant from the Oleaceae family, also known as olive (Khan *at el.*,2020). Olive leaves can be cut, and it is a waste when harvesting olives. Olive leaves represent about 10% of the total biomass collected during olive oil production and they are considered to be a cheap raw material which can be used as a useful source of high added-value products (phenolic compounds) (Anwar *et al.*,2023).Medicinal plants include biologically active elements that make them effective for treating various ailments Plant extracts are complicated mixtures made from the fruits, leaves,

flowers, wood, roots, resins, or seeds of plants that have been traditionally used or considered as medicinal uses (Sahin *et al.*,2018). Olive leaves, an agricultural waste by-product obtained during the harvesting or fabrication process of olive fruits, contain considerable bio-phenols as the other parts of olive tree. Those relating to the leaves play a part in deciding the color and even the flavor of the fruit according to the quality and quantity of them. Their quantity also differs between several textures (Ghannem *et al.*,2019). The recycling of the olive tree leaf is a useful reality as it relates also to waste management aspects. The leaf is potentially convertible into high value-added products, particularly regarding the target of health benefits owing to its bioactive ingredient rich composition (Ahmad-Qasem *et al.*, 2016; Erbay & Icier, 2009). It is important to research the application of olive leaves in the widest range of industrial branches. Some of its applications include the development of natural drugs, functional foods and natural food preservatives (Ahmad-Qasem *et al.*, 2016; Martiny *et al.*, 2020b). However, the presence of phenolic contents and antioxidant activity in olive leaves have been reported (Abaza *et al.*, 2011; El & Karakaya, 2009; Lee & Lee, 2010).

Phenolic compounds are one of the most common types of active chemicals found in plants, accounting for the majority of plant bioactivity. The bioactivity of olive leaf extracts appears to be linked to antioxidant activity and different phenolic chemicals in the leaves. The composition, concentration, and antioxidant activity of phenolic compounds may influence the use of olive leaves. Several factors can influence the phenolic profiles of olive leaves, such as cultivar/genotype, developmental stage, climate, season, and subsequent processing (Zhang *et al.*,2022). Bio-phenols in leaves are different from those of the flowers, fruits and the branch (Lad Hari *et al.*,2021). Olea europea is the most abundant of all the constituents of olive leaf extract, having anti-oxidative, anti-microbial, antiviral (Markhali *et al.*,2020). Olive leaves are easily obtained from waste products produced by industrial and agricultural activities, as well as from olive orchards (Romani *et al.*,2019). In this research, olive leaves are presented as an unexplored and innovative source of bioactive chemicals, with both fascinating technological and health features, because they have been traditionally used to perform medicinal applications in folk medicine (Ronca *et al.*,2024). Olive leaves have been discovered as having the following phenolic compounds. To begin, olive trees are widely farmed over the world, and their leaves are abundant. It creates an opportunity for more sustainable use of resources and waste reduction. Furthermore, the amount and diversity of phenolic chemicals in olive leaves allow a variety of applications in many fields, including food, beverages, cosmetics and pharmaceuticals (Mir-Cerda *et al.*,2024). The phenolic chemicals found in olive leaves and olive oils in a Mediterranean diet have been linked to a lower incidence of heart disease. As a result, antioxidant-rich foods may mitigate the negative consequences of oxidative metabolism by scavenging free radicals, slowing oxidation, and delaying atherosclerosis. The mechanism is considered to decrease hydrogen peroxide (H2O2) by activating phospholipase C and metabolising arachidonic acid. In our research, an extract of Olea europaea L. leaves was employed. The active phenolic chemicals in this extract are members of the secoiridoid family, which is known for its ability to scavenge H2O2. The findings of this study will contribute to our understanding of the impact of polyphenol antioxidants in olive leaf extract on platelet function (Singh *et al.*,2008). Olive leaves are a byproduct of olive grove farming; they are abundant in the olive oil industry (10% of total olive content) and accumulate during olive tree pruning. Olive leaves are regarded as a low-cost raw resource capable of producing high-value goods. Historically, olive leaves have been used as a folk treatment to treat ailments including malaria. Several studies have found that olive leaf extract can lower blood pressure in animals, enhance blood flow in the coronary arteries, relieve arrhythmia, and avoid intestinal muscular spasms (Garcia, Castillo, Lorente, Ortuno, & Del Rio, 2000) (Bouaziz *et al.*,2008). The olive tree (Olea europea, Oleaceae) has long given major economic and health benefits to the Mediterranean area. Olive bio phenols (OBPs) have best qualities because of their role in the olive tree. OBPs are a large collection of secondary metabolites with a wide range of structural and functional properties. The olive tree (Olea *europeaea*, Oleaceae) has historically

provided significant economic and health benefits to the Mediterranean region. Olive bio phenols that are OBPs have exceptional characteristics given their function in the leaves of olive trees. OBPs are a vast class of secondary metabolites with a variety of structural and functional characteristics (Japón *et al.*,2006). The antioxidant chemicals found in OL can extend the shelf life of food products by slowing the process of lipid peroxidation. Therefore, olive leaf extracts were investigated as a supplement to improve the quality and stability of meat products and vegetable oils. Moreover, Olive leaves have a high sugar content, particularly mannitol, a bioactive molecule with benefits for health. The extraction of mannitol from olive leaves using ethanol was investigated for medicinal applications (Medina *et al.*,2019).

OBJECTIVES

- To analyze the biochemical composition (moisture, ash, amino acids, and caffeine content) of three olive leaf cultivars (Koroneki, Arbequina, and Manzanilla) grown in Pakistan.
- To evaluate the phytochemical profile of methanol and n-hexane extracts from the selected olive leaf cultivars.
- To determine and compare the total polyphenol content of the three cultivars.
- To assess the antioxidant activity of the olive leaf extracts.

MATERIAL AND METHOD

Plant material

The plant material used in this study consists of olive leaves (*Olea europaea* L.) of three varieties (Koroneiki, Arbeiquna, and Manzanilla) from National tea and high value crops research institute Shinkiari, Mansehra. These varieties were collected in the month of August 2024. The leaves of each variety were washed with water, dried in the shade, then ground to a fine powder, and stored in a closed container before analysis.

Preparations of extract

The extraction of the olive leaf powder was carried out by cold maceration using two organic solvents of different polarity (methanol and hexane). For this, a quantity of 5g of each sample was soaked separately in 50 mL of extraction solvent at room temperature with stirring for 24 h. Then was filtered through Whatman filter paper. The resulting extracts were stored at 4◦C in dark glass bottles until use.

Pic 1 (Drying)

Pic 2 (Powdered Sample)

Pic 3 (Extract Sample)

Moisture Content Determination

The leaves sample (3 g) were weighed into a previously weighed crucible and oven dried to a constant weight at a temperature of 105 °C. The amount of moisture in triplicate samples were carried out and expressed as loss in weight after cool weighing (Irabor *et al.*, 2019).

$$\text{Moisture content (\%)} = \frac{Mo-M1}{Mo} \times 100$$

Ash Content Determination

Crude ash is describing as the mass of a product sample determined after heating at 525°C for 2 hours to burn in a container. 2g of the sample each was measured in replicate into a container, where the sample product was enflamed in a soundproof furnace at virtually 525°C until ash was observed, and an invariable weight realized. The sample product was subsequently placed in the desiccating

dish to remove moisture absorption and weighed to determine the ash content of the material (AOAC,2005).

Amino Acid Analysis

Take 2g of sample and mix in 20ml of distilled water. After that, 1ml of sample in 25ml of volumetric flask and then 0.5ml of 8.0 PH which is make 96.9ml of Na2P2OH and 3ml of Potassium dihydrogen phosphate. After that, add 0.5ml of 2% ninhydrin in the volumetric flask, and dilute up to 25ml with distilled water. When the lay is made inside, then measure in spectrophotometer at 570nm of wavelength. Amino acid percentage are;

$$\text{Amino acid (\%)} = \frac{N \times \frac{L1}{L2} \times 1/1000 \times}{M \times Mr} \times 100$$

Phytochemical Analysis

Phytochemicals taste include; tannins, Saponin, alkaloids, flavonoids, phenols, tannins, steroids, and Terpenoids.

1. Tannins Test

A tiny amount of extract from leaves was put to a test tube containing 1% ferric dichloride. After that, each of the test tubes received to drops of dil. ferric chloride (1%). The present of tannins was shown by changing solution color. The formation of dark green precipitate was the indication for the presence of tannins. (Adegoke *et al.*,2009).

2. Saponin Test

Take 1ml of extracts in a test tube and add 3ml of distilled water to each. For a few minutes, shake the test tube on a homogeneous shaker until foams form. The formation of the fourth was an indication for the presence of Saponin (Evans *et al.*,2009).

3. Flavonoids Test

In the test tube, 1ml of extract was mixed with 2ml. A few drops of concentrated ammonia are applied. The arrival of the yellow tint at the end indicated the presence of flavonoids. (Adegoke *et al.*,2009).

4. Phenol Test

In a test tube, combine 1ml of extract and a few drops of 10% Fecl3. The blue-green color suggests the presence of phenol in leaves. The blue green at the end confirmed the color presence of phenol. (Evans *et al.*,2009).

5. Alkaloids Test

The method for the detection of alkaloids was elaborated by (Okwu, 2005). 1 ml of extract was taken and, afterward, 2.5ml of 2% hydrochloric acid was added in extracts. After added HCL, the test tubes kept on water bath for heating. After heating, 0.5ml of Wagner reagent are added. The reddish brown is show that, the alkaloids are present in the extracts (Adegoke *et al.*,2009).

6. Phlobatannins Test

One ml of each extracts was taken in test tube. 2% of HCL are taken drop by drops then boiled for few minutes. Formation of red color show that the presence of Phlobatannins (Adegoke *et al.*,2009).

7. Steroids Test

1ml of extract is pipetted into a test tube. Add a few drops of 10% Fecl3 to the test tube. Add 3 drops of concentrated H2SO4, slowly from the side wall of test tube. The presence of red color shows the presence of steroids. (Evans *et al.*,2009).

8. Terpenoids Test

One ml of each extracts in test tube and treated with 2ml of chloroform. Afterwards 3ml of concentrated H2SO4 are added in each extract. The reddish brown color appeared at interface. (Adegoke *et al.*,2009).

9. Glycoside Test

1 ml of the chloroform, ethanol and distilled water extract was pipetted out in a test tube.5ml of concentrated H2SO4.This was then boiled for almost 15 minutes using a water bath. After that test tubes were cooled, and 20% KOH was added to neutralize the mixtures. At the end few drops of FeCl3 were added, the formation of green to reddish brown precipitates were appeared, this shows the presence of glycosides (Evans *et al.*,2009).

10. Carotenoids Test

One ml of extract in test tube.5ml of chloroform was added in each test tube. After adding chloroform in test tube and the test was, shake very well. This mixture was filtered out treated with 85% Sulphuric acid. The blue green color at the end confirms the presence of carotenoids (Evans *et al.*,2009).

Polyphenol Analysis

Sample Preparation

One gram of extract weight is then diluted with 20 ml of methanol, resulting in a final concentration of 1000 ppm. Around 7.5 g is prepared in 100 ml of distilled water to form a 7.5% solution.

Method

A glass reaction (500, 250, 125, 62.25, 31.25, and 15.625 ppm) of sample is combined with 1 ml of FC reagent. The effect lasted for two mints. 1 mL of sodium carbonate is added to the solution and kept for 30 minutes. The sample is reacted at 765nm by the spectrophotometer. The blank is prepared without extract.

Gallic Acid Calibration

Gallic acid is made for different concentrations (500, 250, 125, 62.5, 31.25, and 15.625 ppm). After take different ml in test tube add 1 ml FC reagent in all sample, after 2 minutes add 1 ml of sodium carbonate in all extract. The Gallic acid sample are reacted at 765nm by the spectrophotometer.

Antioxidant Analysis

Take 1g of sample in 25 ml of methanol, placed in shaking water bath 100 rpm at room temperature for 2.5 hours.

DPPH Solution

Used methanol to make a solution of 2,2-diphenyl-1,1-picryl hydrazyl (DPPH). Take 0.004 g/4 mg DPPH and add 100 ml of methanol. Mix 1 ml of DPPH solution with 2 ml of methanol extracts (1 and 2 mg ml−1). Place the mixture at room temperature for 30 minutes in the dark. Filter the supernatant. Make a solution series using the extracted solution and methanol in the amount of 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL, or 50µL, 100µL, 150µL, 200µL, 250µL, and 300µL. Add 3 mL of the DPPH solution to each concentration. Dilute each measured sample solution with 99% methanol to a total of 10 ml. Leave it in the dark for 30 minutes before taking the reading at 517 nm. Measure the absorbance of the resultant solution at 517 nm against the reagent blank using a UV-vis spectrophotometer (UV-1602, Biotechnology Medical Services, USA). Calculate the DPPH radical scavenging activity using the following equation:

$$\% RSA = \frac{(ABS of\ control) - (ABS of\ sample)}{ABS\ of\ control} \times 100$$

Where (ABS of control) is the absorbance of the blank (solvent+DPPH) and (ABS of sample) is the absorbance of the solvent extracts.

Caffeine Analysis

Take 5 g of each sample in 50 mL of distilled water. Next, we boiled and filtered each sample. After filtering, combine 10 mL of HCL and 2 mL of lead acetate in 20 mL of extract. After adding the lead acetate and HCL, then the dilute 250 mL with distilled water and shake thoroughly and evenly, lay aside for clarification. Again, filter the dilute extract/sample, and then take 50 ml from the filtered sample. After that, add 0.2 mL of 9N sulfuric acid. Finally, examine the spectrophotometer measurement at 420 nm wavelength. Then look for the caffeine content from standard curve as per optical density E.

The caffeine content (mg/ml) checked out from the standard curve as below

$$\text{Caffeine } (\%) = \frac{\frac{C}{1000} \times L \times 250/20 \times 100/50}{M \times M1} \times 100$$

RESULTS AND DISCUSSION

Moisture content

The moisture content analysis of olive leaves is critical since it determines shelf life and quality. The moisture level of Olive leaves samples obtained at NTHRI Mansehra ranged between 3% to 7%. The moisture content of the olive leaves samples Koroneki, Arbequina, and Manazanilla was 7%, 3%, and 3%, respectively (Table 01). The substantial range between these samples is attributable to

differences in collection time, weather and environment, and processing delays.

Table No: 1 moisture content in different varieties of Olive leaves

Varieties	Koroneki	Arbequina	Manzanilla
Moisture %	7%	3%	3%

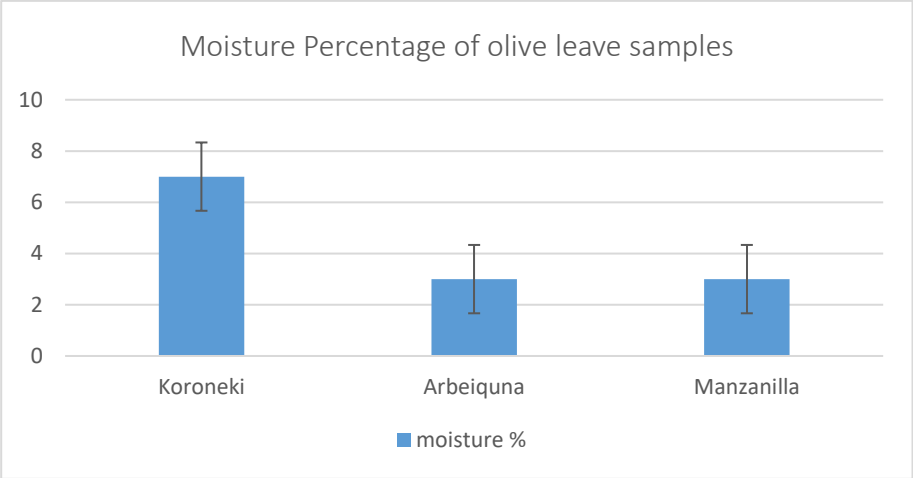

Fig 5 moisture content of olive leaves samples

Ash Content Determination

Ash content in olive leaves were found to range from 3.00 to 4.38%, with the cultivar Koroneki leaves having the highest percentage. When analyzing the cultivar of Arbequina from Caçapava do Sul (Antunes *et al.*,2021). As a result, all three types of olive leave have ash content. Ash content is higher in the Arbequina and Manazanilla samples, at 0.65%. In contrast, the presence of the Koroneki sample is 0.3%.

Table No. 2: Ash content of three different varieties

Varieties	Wt. of sample(g)	Wt. of china dish(g)	Wt. of sample+china dish(g)	1st weight of sample(g)	2nd weight of sample again heating(g)	Ash content (%)
Koroneiki	2	53.341	55.341	53.394	53.388	0.3
Arbeiquna	2	57.945	59.945	58.000	57.987	0.65
Manzanilla	2	42.480	44.480	42.532	42.519	0.65

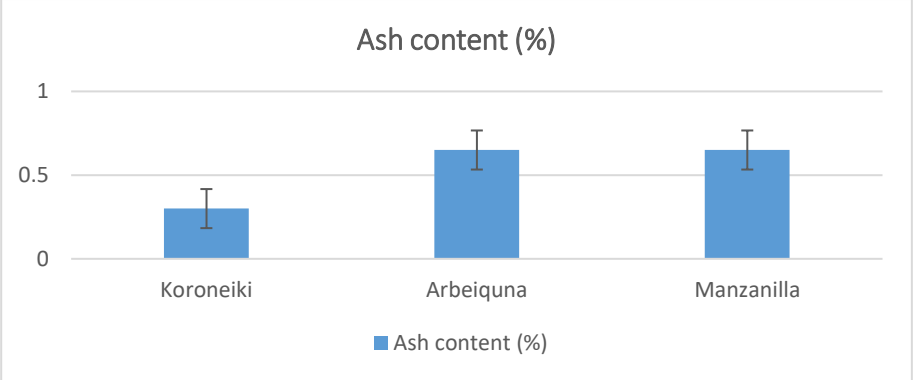

Fig 6 Ash content of olive samples

Phytochemical analysis

I.Tannins Test

Tannins in olive leaves are mostly hydrolysable and condensed tannins. Hydrolysable tannins make up a core structure of glucose or galactose coupled to Gallic or ellagic acids. Tannins may protect leaves against insect herbivores by despair and/or toxicity. In insect herbivores, tannins have no impact on protein digestion, despite early notions to the contrary. On the other hand, tannins can hinder the digestion of proteins in vertebrate herbivores. In insects with high pH guts, tannins are particularly vulnerable to oxidation, which can result in the formation of quinones, semiquinone radicals, and other reactive oxygen species (Barbehenn *et al.*,2011).In Arbeiquna, Koroneki, and Manzanilla, the tannins are present in methanol extract in all three extract. But in n-hexane extract the tannins are absence in all three varieties.

II.Saponin Test

Olive leaves (Olea europaea) have been studied for their possible therapeutic effects, with saponins identified as one of the bioactive molecules responsible for these benefits. Apart from the possible surfactant application, saponins have a wide range of potential applications, including the pharmaceutical industry, food, and cosmetics. Their antibacterial, antioxidant, anti-inflammatory, antidiabetic, anticancer, cholesterol-lowering, and hemolytic effects, which enhance the immune response of organisms, link to pharmaceutical applications (Rai *et al.*,2021). Methanol extracts contain saponins. However, saponins are absent in both n-hexane extracts.

III.Flavonoids Test

Olive leaves are high in flavonoids, which are plant-derived chemicals with antioxidant characteristics and potential health benefits. Researchers have widely studied the roles of oleuropein, hydroxytyrosol, and apigenin in numerous physiological processes due to their chemical properties (Ronca *et al.*,2024). The flavonoids' hydroxyl group is very reactive, causing free radicals to be inactivated. Some flavonoids directly scavenge superoxide, while others scavenge highly reactive oxygen-derived radicals such as peroxynitrite ions (Khan *et al.*, 2021). In Methanol extract flavonoid are present in all three varieties. But, absence in n-Hexane extract. In methanol, the color change from light green to dark green.

Fig 7 Extract of sample in n-hexaneand methanol extract

IV.Alkaloids Test

Alkaloids are a type of naturally occurring organic nitrogen-containing molecule found mostly in botanicals. Their diverse actions give plant alkaloids a wide range of pharmacological applications. The disease treatment sector has raised current and potential applications for these compounds (Sharma *et al.*, 2019). Alkaloids are present both extract methanol and n-Hexane in all three varieties. The color change color from green to reddish brown.

Fig 8 extract of sample in methanol and n-hexane extract

V.Phlobatannins Test

Phlobatannins, also known as condensed tannins, are a kind of polyphenol found in plant tissues. Possible health advantages, including antioxidants and anti-inflammatory qualities, have been intensively researched (Khan *et al* 2020). In both extract methanol and n-Hexane, Phlobatannins are absence in all three varieties. There is no color change in all extract.

Fig 9 extract of sample in methanol and

n-hexane extract

VI. Phenol Test

Plant phenols are one of the most prevalent and significant classes of defensive secondary metabolite chemicals that protect against herbivores, including insects. Insect attacks or defense mechanisms alter and enhance the qualitative and quantitative parameters, as well as the oxidative enzyme's activity (Misra *et al.*, 2023). In methanol extract phenol are present in all three varieties. But, in n-Hexane phenol are absence in all three varieties. In methanol extract, the color change was change light green to dark green.

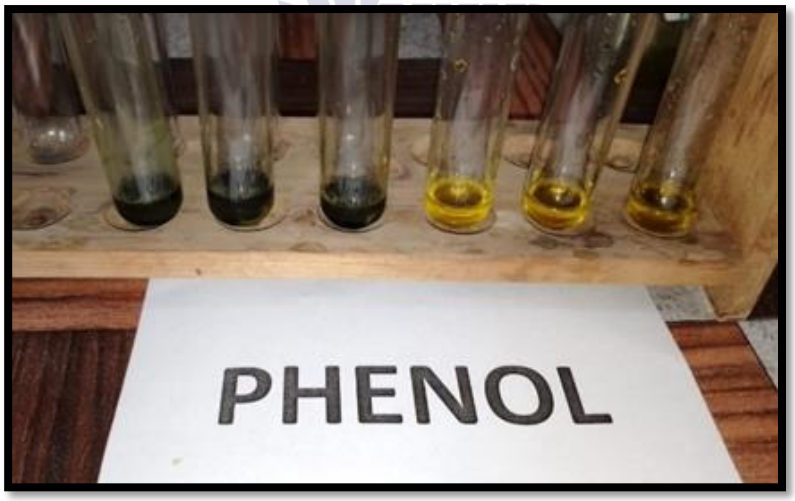

Fig 10 extract of sample in methanol and n-hexane extract

VII. Steroid Test

Steroids are chemical molecules that serve important roles in a variety of biological processes, such as hormone production, cell signaling, and membrane formation. They are commonly connected with synthetic anabolic steroids used in sports, but they also exist naturally in plants (Tarkowská *et al.*,2019). In methanol and n-Hexane extract, steroids are present in all three extract. In methanol extract, the color is change in dark green to light green. Also n-Hexane extract color are change from yellow to light green.

Fig 11 extract of sample in methanol and n-hexane extract

VIII.Terpenoids Test

Terpenoids are the biggest class of plant secondary metabolites, and their biosynthesis and regulation are exceedingly complex. Terpenoids play an important role in plant-microorganism-animal interactions and defense reactions. Terpene molecules are highly important to both plants and the surrounding ecosystem. While plants receive protection, they can also influence the environment, affecting the evolution of plant groups and even entire ecosystems (Li, C *et al.*,2023). In methanol extract, terpenoids are present in all three varieties. The color change of methanol extract from green to reddish brown. As well as, in n-Hexane extract terpenoids are also present in all three varieties. The color change was yellow to light green.

Fig 12 extract of sample in methanol and n-hexane extract

IX.Carotenoids Test

Carotenoids are essential for both photosynthesis and photoprotection in plants. As pigments that capture light and as structural elements of photosystems, they are essential. Additionally, carotenoids supply building blocks for the synthesis of strigolactones (SLs) and abscisic acid (ABA), two phytohormones (Sun, T *et al.*,2022). In methanol

extract, carotenoids are present in all three varieties. The color methanol extract from green to yellow. While, in n-Hexane carotenoids are absence in all three varieties.

Fig 13 extract of sample in methanol and n-hexane extract(right

X.Glycoside Test

Olive leaves contain glycosides because of the plant's secondary metabolism and built-in defenses. These substances are essential for
defending the plant against a variety of environmental stresses, including infections, herbivory, and oxidative stress (Acar-Tek *et al.*,2020)

In methanol extract, glycoside is present in all three varieties of olive leaves. The color change of methanol extract is green to reddish brown. Also, in n-Hexane glycoside are also present in all three varieties. The color change from yellow to pale green.

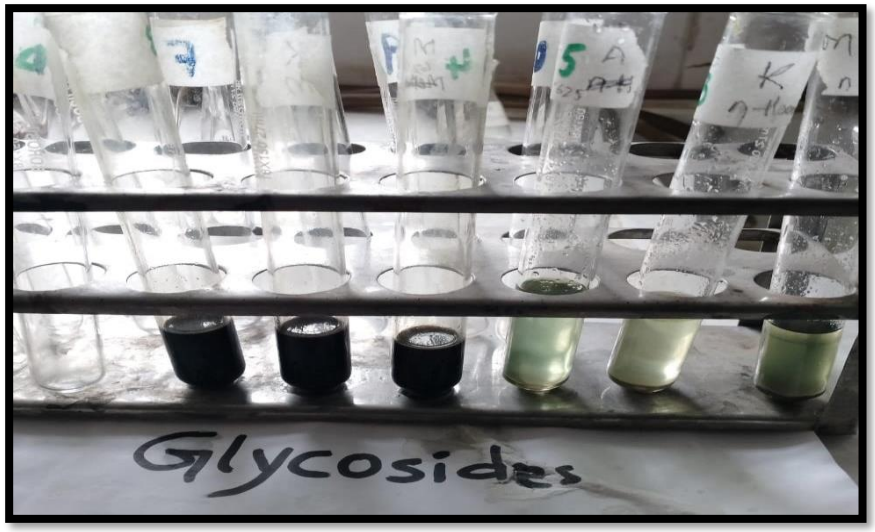

Fig 14 extract of sample in methanol and n-hexane extract

Table NO. 3: Phytochemicals analysis of different varieties of Olive

Test	Methanol	n-Hexane
Saponins	+	-
Tannin	+	-
Alkaloids	+	+
Phalobatanins	-	-
Phenol	+	-
Glycosides	+	+
Steroids	+	+
Carotenoids	+	-

Polyphenol Analysis

In olive leaves, polyphenol compounds are present in all three varieties in different concentrations. Koroneki was range of 0.262625 µg /ml to 0.011195 µg/ ml of polyphenol compound at different concentrations. As well, Arbeiquna was also content polyphenol compound from 0.26721µg/ml to 0.011195µg/ml. Also, Manazanilla was also content; the range of polyphenol compounds of the Manazanilla variety is 0.2594µg/ml to 0.00272 µg /ml.

Heights total polyphenol content was present in var.Arbequina (0.08259 mg GAE/g)

Table NO. 4: Polyphenol analysis of olive leaves var. Koroneki

Serial No.	Concentration (µg/ml)	Absorbance (GAE)	Absorbance of sample (Y) Koroneki	Polyphenol content (µg/ml)	Total Polyphenol conc. in Koroneki (mg/ml)
1.	500	1.98	1.501	0.52525	0.262625
2.	250	1.07	0.506	0.16989	0.084945
3.	125	0.52	0.393	0.12954	0.06477
4.	62	0.266	0.251	0.0789	0.03945
5.	31	0.156	0.146	0.04132	0.02066
6.	15	0.084	0.093	0.02239	0.011195
7.	Average polyphenol conc. in Var.Koroneki (mg GAE/g)				0.080608

Table NO.5: Polyphenol analysis of Olive leaves Var.Arbequina

Serial No.	Concentrations (µg/ml)	Absorbance (GAE)	Absorbance of sample (Y) Arbeiquna	Polyphenol content (mg/ml)	Total phenolic conc. in Arbeiquna mg/ml)
1.	500	1.98	1.415	0.53442	0.26721
2.	250	1.07	0.493	0.17981	0.08991
3.	125	0.52	0.369	0.13212	0.06606
4.	62	0.266	0.273	0.09519	0.0476
5.	31	0.156	0.123	0.0375	0.01875

6.	15	0.084	0.057	0.01212	0.00606
7.	Average polyphenol conc. in Var. Arbeiquna (mgGAE/g)				0.082598

Table No. 6: Polyphenol analysis of leaves of Var. Mnazanilla

Serial No.	Concentration (µg/ml)	Absorbance (GAE)	Absorbance of sample (Y) Manzanilla	Polyphenol content (µg/ml)	Total phenolic conc. in Manzanilla (mg/ml)
1.	500	1.98	1.195	0.5188	0.2594
2.	250	1.07	0.518	0.1964	0.0982
3.	125	0.52	0.398	0.1394	0.0697
4.	62	0.266	0.287	0.0864	0.0432
5.	31	0.156	0.178	0.0345	0.01725
6.	15	0.084	0.117	0.00543	0.00272
Average polyphenol conc. in Var.Manzanilla (mgGAE/g)			0.081745		

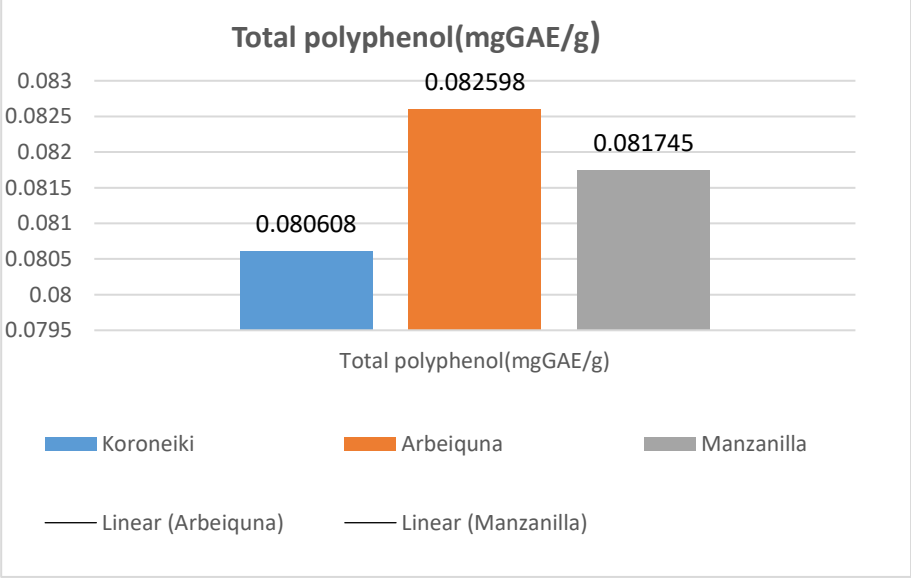

Fig 15 Total polyphenol content

Antioxidant Analysis

Several of these compounds were tested separately, and the antioxidant effects were linked to functional group characteristics, quantity, and hydroxyl position in their structures, which conferred redox qualities. A sample's antioxidant activity can be measured using a variety of chemical analysis techniques. In vitro tests generally assess a compound's scavenging ability against free radicals or other reactive species. Synthetic DPPH radicals are typically utilized to assess antioxidant activity in vitro (Lins *et al.*,2018).

All three varieties of olive leaves contain antioxidants, but in varying amounts. Comparing Arbeiquna to Koroneki and Mnazanilla, the former has more antioxidant compounds. Arbeiquna absorbance ranges from 0.308 to 0.812 nm.

Additionally, Manzanilla has 0.209 to 0.541 nm and Koroneki has 0.208 to 0.4211 nm. Arbeiquna ≥ Koroneiki≥ ≥ Manzanilla possesses antioxidants of various concentrations.

Table no 7: Antioxidant analysis of Koroneki olive leaves

Serial no	Control	Conc.(µg/ml)	Absorbance (Koroneki)	%RSA	IC_{50}
1	1.054	5000	0.812	22.9601518	3.999993
2	1.054	4000	0.756	28.2732448	
3	1.054	3000	0.671	36.3377609	
4	1.054	2000	0.425	59.6774194	
5	1.054	1000	0.308	70.7779886	

Table NO 8. Antioxidant analysis of var. Manzanilla olive leaves

Serial no	Control	Conc.(µg/ml)	Absorbance (Manzanilla)	%RSA	IC_{50s}
1	1.054	5000	0.812	22.9601518	3.999993
2	1.054	4000	0.756	28.2732448	
3	1.054	3000	0.671	36.3377609	
4	1.054	2000	0.425	59.6774194	
5	1.054	1000	0.308	70.7779886	
6	Average of IC_{50} values				

Table No. 9: Antioxidant analysis of var. Arbequina olive leaves

Serial No.	**Control**	**Concentration (µg/ml)**	**Absorbance (Arbeiquna)**	%RSA	IC_{50}
1	1.054	5000	0.514	51.2333966	4.000036
2	1.054	4000	0.436	58.6337761	
3	1.054	3000	0.337	68.0265655	
4	1.054	2000	0.29	72.4857685	
5	1.054	1000	0.209	80.170778	
6	Average of IC_{50} values				

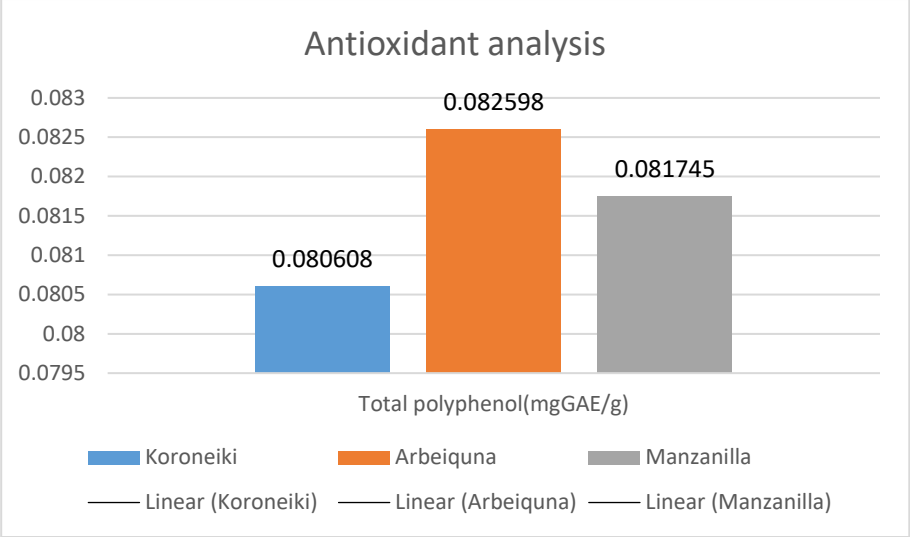

Fig 16 Antioxidant analysis

Table no.10: Amino acid analysis of different three varieties

Varieties	Absorbance(nm)	Amino Acid(%)
Koroneki	0.158	0.1058
Arbeiquna	0.169	0.0994
Manzanilla	0.146	0.0859

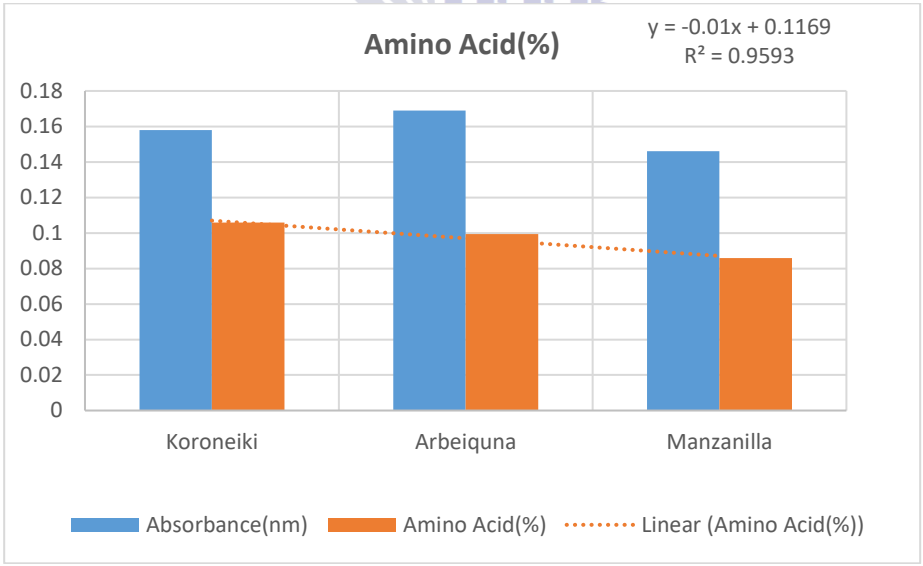

Fig 17 Amino acid content of different varieties of olive leaves

In olive leaves, amino acids were abundantly present in all three varieties. Koroneiki was more present of amino acid, which was 0.158%. Like that, Arbeiquna and Manzanilla have amino acids, which were 0.0994 and 0.08588%.

Caffeine Analysis

Table no 11: Caffeine analysis different varieties of olive leaves

Varieties	Absorbance of sample (%)	Caffeine (%)
Koroneiki	0.461	0.980
Arbeiquna	0.596	1.27
Manzanilla	0.567	1.206

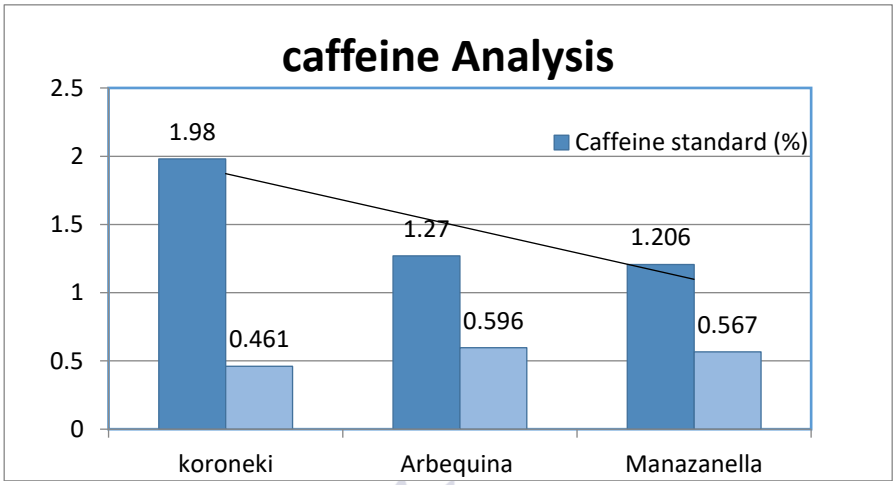

Fig 18 Caffeine analysis of three different varieties of Olive

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