CHARACTERIZATION AND ASSESSMENT OF BIOACTIVITIES OF ORGANIC COMPOUNDS OF WILD OLIVE

Aleena Akram[1], Asma Munir [1], Hina Altaf[2], Maryam Shahid[2] and Warda Ahmad[2]

1. Grand Asian University, Sialkot

2. UIBB, University of Arid Agriculture Rawalpindi.

ABSTRACT

This study evaluate the chemical profiling and bioactivities of selective organic compounds from fruits of *Olea ferruginea* and its comparison with *Olea europaea*from three unexplored locations of Pakistan. The oil was characterized into fatty acids by GC-MS and FT-IR. Antioxidant activities were detected by DPPH, ABTS and H_2O_2 scavenging bioassays. For cytotoxicity, Brine-Shrimp-Cytotoxicity-Bioassay was tested. The extracted oil was checked against five bacterial strains. Higher quantity of phytochemicals and yields of oil were present in fruit of *Olea ferruginea* collected from Malakand as compared to other locations.Prominent inhibitory activity against *E.coli* and *S.aureus*was observed during antibacterial assay.Good quality oil with antioxidant properties obtained from fruit of *Olea ferruginea* from Malakand. The comparative studies revealed the same qualitative composition of both species but *Olea europaea* showed greater oil-yield than *Olea ferruginea*. In consequence, the wild species *Olea ferruginea* was found to be economical, wild and drought tolerant.

Keywords Extraction, flavonoids, phenols, methanolic extracts, antioxidant

1. Introduction

Pakistan edible oil sector has drawn increased attention over the past few decades owing to the focus on their qualitative and quantitative analysis and to their health related studies. The government has not only stepped up efforts to fulfill the necessity of edible oil in Pakistan but has also spent rising amount of foreign exchange on edible oil imports (Mirza*et al.,* 2008). Pakistan has become the third largest importer of cooking oil after China and India. Pakistan imports crude and refined cooking oil from Indonesia and Malaysia and soybean oil from North America and Brazil. The import of crude and refined cooking oil has increased to 2.6 million tons per annum

in Pakistan (Chaudhry*et al.,* 1998). The presence of large amount of saturated fatty acids in edible oils (soybean, sunflower, and canola) and high import rates are priority issues for food safety of Pakistan. To fulfill the requirement of edible oil the current study focused on exploring other resources of edible oils especially, olive oil. As the presence of unsaturated fatty acids (Oleic acid, Linoleic acid and Linolenic acid) makes olive oil adequate for edible purposes and is considered most appropriate for human consumption (Zabihullah *et al.,* 2006). The presence of high concentration of tyrosol, hydroxytyrosol and oleuropein in EVOO with scavenging ability suggest that olive oil has beneficial health effects as compared to other edible oils (Tripoli *et al.,* 2005).Olive oil is considered as the healthiest oil among all the edible oils and this is attributed to the presence of healthy monounsaturated fat, which can lower the risk of heart disease by reducing the total and low-density lipoprotein (LDL) cholesterol level in the blood. Of all the edible oils EVOO and VOO are highly recommended for cooking purpose because they passed through less processing and have satisfying taste with minimize risk for diseases (Omar, 2010).The present research was carried out to discover new sources of edible oil. Wild olive species is found in different areas of Pakistan. It does not need any special conditions and is drought tolerant. Moreover, it is economical for the country.The presence of phytochemicals confirms its nutritional value.

An important study was conducted on Italian varieties Tonda di Villacidro, Tonda di Cagliari, Semidana, and Bosana by Tuberoso *et* al., of extra virgin olive oils (EVOOs) to determine their quality parameters which include free acidity, peroxide value and UV absorption values at K_{232} and K_{270} conferring to the analytical methods defined in the European Union Commission Regulations.This research was a good effort towards quantitative analysis of EVOOs but fatty acid determination and triacylglycerols does not clearly distinguish the four varieties.It was discovered by Rodriguez-Morato *et al*., (2015) that the main source of lipids in the Mediterranean Diet is virgin olive oil and contains minor phenolic components particularly hydroxytyrosol. Hydroxytyrosol attracted researchers due to its antioxidant and anti-inflammatory actions which force them to discover its neuroprotective effects. Although this is a great effort but concerning the beneficial effect of a healthy diet to a single compound is disingenuous. Moreover, there is a need to generate information about the practical clinical recommendations. The neuroprotective

effects of olive oil must be analyzed by quantitative analysis of HT (Altaf *et al.,* 2013). Phenolic compounds are responsible for the sensory value of extra virgin olive oil (EVOO). Talhaoui*et al.,*(2015)determinedsix cultivars grown in same orchard under same environmental conditions. The impact of cultivars on the phenolic composition was determined. This was a great contribution but no clear variations were noticed in the transfer rates of simple phenols among the different cultivars (Pourmorad et al., 2006).The research work of Jerman *et al.,* was a great contribution in the phenol analysis and profile characterization of olive matrices. This study also explained the behaviour of phenol during various technological processes under different operative conditions.The research carried out by Gulfraz *et al.,* (2009) reveals that Pakistan imports edible oils from other countries on large scale by using its valuable resources. In different areas of Pakistan *Olea ferruginea* Royle (Oleaceae) wild species of olive, locally known as "*Kahu*", is found. On the basis of chemical composition and quality parameters this study was carried out to discover new source of virgin olive oil.This study was a great motivation for the researchers in Pakistan and there were some discrepancies regarding standard olive oil composition that should be further emphasized to understand the importance of olive oil (Gulfraz *et al.,*2009).

The main inspiration of the present work is to focus on exploring of healthy and cost-effective edible oil from some unexplored areas of Pakistan. The critical advantages of the proposed study are to investigate the wild olives for their oil extraction at Hazara Division and Malakand in order to assure a significant improvement in human health for their nutritional quality at one end and get better the country's economy at the other end. The phenolic contents were determined by using spectrophotometric techniques (acidity, peroxide value, K_{232}, K_{270}).The extracted oil was further subjected by quantification of various fatty acids contents by using GC-MS and FTIR techniques. The oil extracted from *Olea ferruginea* was identified by determining its iodine values as well as saponification number**.** The scavenging ability of olive oil was assessed by DPPH assay and ABTS assay and hydroxyl radical.

2. Materials and methods

2.1. Chemicals Reagents

All chemicals and solvents of analytical grade are used in this study. Methyl alcohol (CH_3OH), ethyl alcohol (C_2H_5OH), *n*-hexane, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, gallic acid, ABTS, potassium hydroxide (KOH), sodium thiosulfate ($Na_2S_2O_3$), hydrocholric acid (HCl), iodine, potassium iodide (KI), acetic acid (CH_3COOH), chloroform ($CHCl_3$), Aluminum chloride hexahydrate ($AlCl_3.6H_2O$) and ether were obtained from Sigma-Aldrich. Starch and phenolphthalein used as indicators in the determination of quality parameters. For antibacterial assay Agar media was prepared from Agar nutrient and Agar technical purchased from Sigma- Aldrich by dissolving them in distilled water.

2.2 Sampling and Sites Selection

Olive fruits of the species *Olea ferruginea*Royle were sampled from three olive orchard locations. The healthy fruit samples were randomly collected (app. 1 kg) at optimum ripening stage from Abbottabad (sample 1), Haripur (sample 2) and Malakand (sample 3) regions between October and November, depending on region's harvest time.

2.3. Physical characterization of olive fruit

Physical characteristics of fruits were determined by following the procedure of International Olive Council Standards (IOC, 2015). The weight of pit and pulp was determined by weighing 100 grains of fruits by using electric balance and average weight per fruit was calculated.

2.4. Estimation of Oil Content through Soxhlet Apparatus

The air-dried and powdered plant samples were extracted with ethanol, methanol and *n*-hexane using the Soxhlet apparatus. The extract obtained was left overnight, so that all the solvent evaporates. Finally percentage of oil yield was noted and extract was then kept in small sterile opaque Eppendorf tubes under refrigerated conditions until further use (Ebrahimzadeh*et al.,* 2008).

2.5. Quality indices

2.5.1. Determination of peroxide value of olive oil

A 3g of sample was added in conical flask and 30mL of Acetic acid-chloroform solution was added in it. 0.5 mL of saturated KI and 30 mL of distilled water was added in the flask. The contents of the flask were titrated against 0.1 N sodium

thiosulfate. Then, 1 mL of starch solution was added as an indicator and the end point was the disappearance of blue grey colour (Gokmen *et al.,* 2014).

2.5.2. Saponification value

A 1 mL of oil was taken in conical flask with the addition of fat solvent (ethanol/ether 1:1). A 25 mL of alcoholic KOH (0.5 mol/L) was added in the flask. Then the flask was heated and 2 drops of phenolphthalein were added as an indicator and the contents were titrated against 0.5 mol/L HCl. The end point was the appearance of pink colour (Tuck & Hayball2002).

2.5.3. Iodine value of oil

A 0.5 g of oil was dissolved in 10 mL of chloroform. A 25 mL of Hanus iodine solution (3.6 g iodine in 825 mL of glacial acetic acid by heating and cooling) was added in the flask. The mixture was allowed to stand in the dark for 30 min with shaking. Then 10 mL of 15% KI and 100 mL of freshly boiled and cooled distilled water was added in the flask. The contents of the flask were titrated against 0.1 N sodium thiosulfate until the yellow solution turned colourless. Then few drops of starch were added as an indicator and titration was continued until the blue colour disappears (Dermis *et al.,* 2012).

2.5.4. Acid value

A 200 mL of fat solvent was prepared by mixing 100 mL of absolute ethanol and 100 mL of diethyl ether in a beaker. Then 1 mL of oil sample was dissolved in 10 mL of fat solvent followed by the addition of 2 drops of phenolphthalein as an indicator and the contents were titrated against 0.01 M KOH until a pale pink end point was reached (Yildiz *et al.,* 2003).

2.5.5. K_{232} and K_{270} values

Determination of K_{232} and K_{270} values provide useful information about the quality of the oil and its degree of oxidation. This test was simply performed by taking the reading of the oil at 232 and 270 nm wavelength (Rao *et al.,* 2009).

2.6. Phytochemical Screening of Plant Extracts

Phytochemical screening of the plant extracts was carried out following the standard procedures.

2.6.1. Detection of Flavonoids

The detection of flavonoids was based on procedure reported by(Makkar 2003). The obtained absorbance was analyzed against standard curves prepared withQuercetin for total flavonoids (Chang *et al.,* 2018).

2.6.2. Detection of Total Phenolic Content

Determination of total phenolic contents of olive oil was based on procedure reported by (Sathianathan *et al.,* 2014), using method of Folin- Ciocalteu reagent. The absorbance of solution was recorded at 760 nm versus the blank on UV-Visible Spectrophotometer. Final results were expressed as standard Gallic acid equivalent.

2.6.3. Detection of Tannins

Detection of tannins was carried by the method already reported by (Ozen *et al.,*2002). The absorption was determined at a wavelength of 725 nm via UV- visible Spectrophotometer. The measured absorbance values were expressed as mgEq/g of plant extract.

2.7. Structural Elucidation

Characterization of the isolated compounds were governed by spectroscopic techniques. Gas chromatography was used to determine the fatty acid composition of oil by a PerkinElmer gas chromatograph from Clarus600 and Clarus600c MS in EI mode detector. Fourier transform infrared (FT-IR) spectroscopy has been developed for the analysis of functional groups present in the sample.

2.8. Determination of Biological Activities

The antioxidant effect of hydrophilic phenols and tocopherols on oxidative stability in olive oil was also evaluated.

2.8.1. Antioxidant Assays

The scavenging ability of oil was assessed by using 1, 1 diphenyl 1-2 -picryl-hydrazyl (DPPH) assay, 2, 2′-azinobis (3-ethylbenzothiazoline-6 sulfonic acid) (ABTS) assayand H_2O_2 assayas reported by (Moon&Shibamoto*et al.,* 2009).

2.8.2. Brine Shrimp Cytotoxicity Bioassay

The bioassay was carried out by the method reported by (Shobha&Onkarappa*et al.,* 2010). A 32 mg of each of the test samples were taken and dissolved in 200 μL of dimethyl sulfoxide (DMSO). The volume was made upto 20 mL with seawater. The concentration of the stock solution became 1600 μg/mL. Then the solution was diluted to 600, 300, 100, 10 *etc.* μg/mL with seawater. After this, a 2.5 mL of plant extract solution was added to 2.5 mL of seawater containing 10 nauplii.

2.8.3. Determination of Antibacterial Activity

Test Organisms: Bacterial Strains

The *Olea ferruginea* extracts were screened against five bacterial strains by the method already described [Upadhyay et al., 2010]. The strains of Gram +ive bacteria such as *Staphylococcus aureus*(KX262674) and *Bacillus cereus* (KX262674) and Gram –ive bacteria *Escherichia coli* (ATCC 10536), *Salmonella typhi*(ATCC 6539) and *Pseudomonas aeruginosa*(ATCC 9027) were examined in this protocol.

3. Results and Discussion

3.1. Physical Index and Sensorial Parameters of *Olea ferruginea* Fruit

The difference was observed in the physical characteristics of *Olea ferruginea* fruits collected from the three locations due to various agronomical and environmental aspects. The physical index of olive fruit is given in Table 1.According to the results fruits of *Olea ferruginea* obtained from Malakand areas have higher pit size, weight, dry matter, water content and total yields of oil as compared to the fruits analyzed from the other two locations. Results of physicochemical parameters of *Olea ferruginea* fruits obtained in the current study were more or less similar to the results already reported for *Olea europaea*.

Table 1

Physical and Chemical Parameters of *Olea ferruginea* Fruits from Various Locations

Cultivar	Weight of pit (g) 100 grains	Weight of pulp (g) 100 grains	Weight of Total 100 grains	Dry matter	Water content	Total oil (%)
Malakand	25.6±3.5	64.5 ± 6.34	130.5±5.68	10.8 ± 1.8	36.7 ± 3.60	55.5 ± 5.7
Haripur	23.6 ± 2.5	59.2 ± 5.6	125.3±2.64	6.4 ± 1.6	22.3 ± 5.82	45.6 ± 1.6
Abbottabad	22.4 ± 1.8	58.4 ± 5.2	124.4±2.45	5.8± 1.2	18.2± 2.55	32.2 ± 1.6

Mean ± SD (n=3)

3.2.Quality Index of Olive Oil

The geographical features of the production area like altitude, climatic conditions, cultivar *etc*, greatly affect the quality of the oil. The exceptional flavour of olive oil is attributed to the volatile compounds that develop during oil extraction from the fruit. The presence or absence of particular volatile compounds partly explains the differences in the quality of oil (Psomiadou&Tsimidou 1998). Different parameters are required to maintain the quality of edible oil. Therefore, various parameters of olive oil were determined in the present study and are presented in Table 2.

Table 2

Quality index of the oil extracted from fruit of *Olea ferruginea*

Quality parameters value*	Malakand	Haripur	Abbottabad	Ref.
Free acidity (% oleic acid)	8.65 ± 0.02	8.43 ± 0.01	7.42 ± 0.01	0.1-1.5
Peroxide value (meq O_2 kg^{-1})	12.3 ± 0.15	15.36 ± 0.35	13.42 ± 0.5	3.6-29.6
Saponification number mg/g	218.7 ± 3.7	215.5 ± 6.5	212.3 ± 3.5	197.8
Iodine value (%)	82.67 ± 2.5	74.56 ± 2.6	72.52 ± 1.5	87.3
K_{232}	0.95 ± 0.01	0.85 ± 0.03	0.84 ± 0.04	1.2-2.7
K_{270}	1.13 ± 0.01	1.15 ± 0.00	1.14 ± 0.00	0.09-0.2
Carotenoids	2.65 ± 0.15	2.54 ± 0.1	2.36 ± 0.08	2.3-14.0
Chlorophyll	11.24 ± 0.12	8.36 ± 0.16	7.25 ± 0.2	1.7-27

Mean $\pm$ SD (n=3); *= Standard values of *Olea europaea*

The percentage of grams of free fatty acids in 100g of oil refers to free acidity and it is an important parameter that defines the quality of olive oil. When the lipolytic enzymes present in the pulp and the seed come into contact with the oil, they give rise

to free acidity. High values of free acidity in the olive oil can be due to different factors such as production from an unhealthy fruit, delayed harvesting and storing before processing (Tan *et al.,* 1994). FFA are a source of flavours and aromas. Their breakdown products such as aldehydes, ketones, alcohols and organic acids provide characteristic flavour and aroma. Some of them are short chain FFA which are volatile while others are long chain FFA which may be saturated or unsaturated and are more susceptible to oxidation in free form. In most cases these flavours and aroma are considered a defect in the oil, fats and food that contain those (Zeleke *et al.,* 2012). The free acidity of oil extracted from the fruit of *Olea ferruginea* was determined and found to be 8.65±0.01% for the Malakand sample followed by the sample from Haripur 8.43 ± 0.01% and Abbottabad 7.42 ± 0.01% respectively. The increase in free acidity value of olive oil extracted from Malakand sample is due to the presence of free fatty acids mostly owing to oleic acid. The results of acidity of oil are comparable with the results of olive oil as reported by (Tan *et al.,* 1994).

3.2.1. Peroxide value

Determination of peroxide value is an important test which is related to the storage of oil and it measures the degree of rancidity of the oil. The process of oxidation happens very quickly after it has started. Higher peroxide value means less stable oil with a shorter shelf life. There are many factors that influence peroxide value including temperature, visible light, oxygen, contact with metal surfaces and delay between harvesting and processing period of the fruit (Zeleke *et al.,* 2012). The peroxide value of the oil extracted from the fruit of *Olea ferruginea* collected from Malakand was 12.3±0.15 (meq O_2 kg^{-1}), which was higher than the peroxide value of the oil extracted from fruit collected from Abbottabad that was 13.42 ± 0.5 (meq O_2 kg^{-1}), followed by fruit from Haripur which was 15.36 ± 0.35 (meq O_2 kg^{-1}). These current results show that, the more stable oil is the oil extracted from sample collected from Malakand. The lower peroxide value of Malakand sample is due to the presence of greater percentage of unsaturated fatty acids including oleic acid, as compared to the other two samples. The present study is in good agreement with already reported work by (Psomiadou&Tsimidou1998).

3.2.2. Saponification Number

Saponification is refers to the hydrolysis of fats or oils to form glycerol and salt of corresponding fatty acid. It is very important for the industries to determine the amount of FFAs present in the oil. The quantity of alkali required to neutralize the fat, determines the amount of free fatty acids. Saponification number gives information about the nature of the fatty acids present, the longer is the chain of fatty acids, the less will be the acid liberated per gram of fat hydrolyzed. It is also explained as a measure of the average molecular weight (or chain length) of all the fatty acids present. It is reported that the saponification value of fats having long chain fatty acids is low because they have less number of carboxylic groups and higher molecular weight (Dag*et al.,* 2011). In the current study, the saponification number of olive oil was also determined and it was found to be 218.7 ± 3.7mg/1g of oil for sample collected from Malakand followed by the sample from Abbottabad and Haripur. High saponification values indicate that the oil is normal and is edible. Therefore, the oil extracted from sample collected from Malakand division is more feasible for edible purposes.

3.2.3. Iodine value

The iodine value is the measure of degree of unsaturation of fat or oil. The unsaturation is in the form of double bonds which reacts with iodine compounds. It is often used to guide fractionation processes in order to achieve the desired product quality at optimum operating conditions. The more unsaturated the fatty acids, the greater will be the iodine value (Leon 2012). In the present study, the iodine value of olive oil was also determined and presented in Table 7. The iodine value of the oil extracted from Malakand fruit was found to be 82.67± 2.5% followed by the iodine value of oil extracted from Haripur fruit 74.56 ± 2.6% and Abbottabad 72.52 ± 1.5. The current results greatly supports the work already reported by (Psomiadou&Tsimidou1998). As iodine value determines the degree of unsaturation of oil, so the higher iodine value in the oil of *Olea ferruginea* collected from Malakand indicates high degree of unsaturation which supports the fact that olive oil can be used for edible purposes and is easily digestable.

3.2.4. K_{232} and K_{270} Value

The quality of oil can also be determined by spectrophotometric examination of the sample between 200 and 300nm. A low absorption in this region is indicative of a

high quality extra virgin olive oil, whereas adulterated or refined oils show a greater level of absorption in this region (Escuderos,2011). The K_{232} and K_{270} values of oil extracted from *Olea ferruginea* are also presented in Table 7. The oil extracted from Malakand sample indicated low absorbance found to be 0.95 ± 0.05 and 1.13 ± 0.01 respectively, which is indication of high quality of the oil as compared to the oil extracted from the samples collected from Haripur and Abbottabad. The low absorbance is due to the presence of unsaturated fatty acids in the sample.

3.2.5. Chlorophyll

The chlorophylls are the pigments which are responsible for characteristic green colour of olive fruit. After processing olive oil is obtained in three forms extra virgin olive oil, regular olive oil and light olive oil. Extra virgin olive oil is of high quality because it is obtained after the first pressing of freshly prepared olives. It has a greenish yellow colour and has a fruity taste due to the presence of volatile compounds obtained after extraction. Regular olive oil has lower percentage of volatile compounds due to which it has pale yellow colour and slight aroma. The light olive oil is obtained under pressure, so volatile compounds and chlorophyll is removed from it (Sharma *et al.,* 2015). According to the results presented in Table 7, the amount of chorophyll in the oil of *Olea ferruginea* fruit collected from Malakand was found to be 11. $24 \pm 0.12\%$ followed by Haripur extract 8. $36 \pm 0.16\%$ and Abbottabad extract $7.25 \pm 0.28\%$ respectively, due to difference in the climatic conditions and harvesting time which supports the results already reported for *Olea europaea* with minor differences (Zamora *et al.,* 2001).

3.2.6. Carotenoids

Pigments are responsible for the colour of olive oil and affect the quality of the oil. The concentration of the pigments can vary depending on the climatic conditions, harvesting time and olive cultivar (Banu&Prasad 2017). Unrefined edible oils due to the presence of ß-carotenes possess slow oil oxidation (da Silva *et al.,* 2012). The current results for carotenoids presented in Table 7, for oil extracted from Malakand sample were $2.65 \pm 0.15\%$, from Haripur $2.54 \pm 0.12\%$ and from Abbottabad $2.36 \pm 0.08\%$ respectively. The minor difference between the values of samples collected from three locations indicate that there is slight difference in quality of oil from three locations due to variations in the climatic conditions.

3.3. Profiling of Fatty Acid Composition

The chemical constituents of olive oil were obtained through investigation of oil by GC-MS and results are presented in Table 8. The percentage (%) which indicates the concentration of oleic acid is (2.2 %), stearic acid is (0.6%), palmitic acid is (0.1 %) and palmitoleic acid is (0.1 %) for the sample. The olive oil and relative percentage of the total chromatogram is summarized in the respective Table 3.
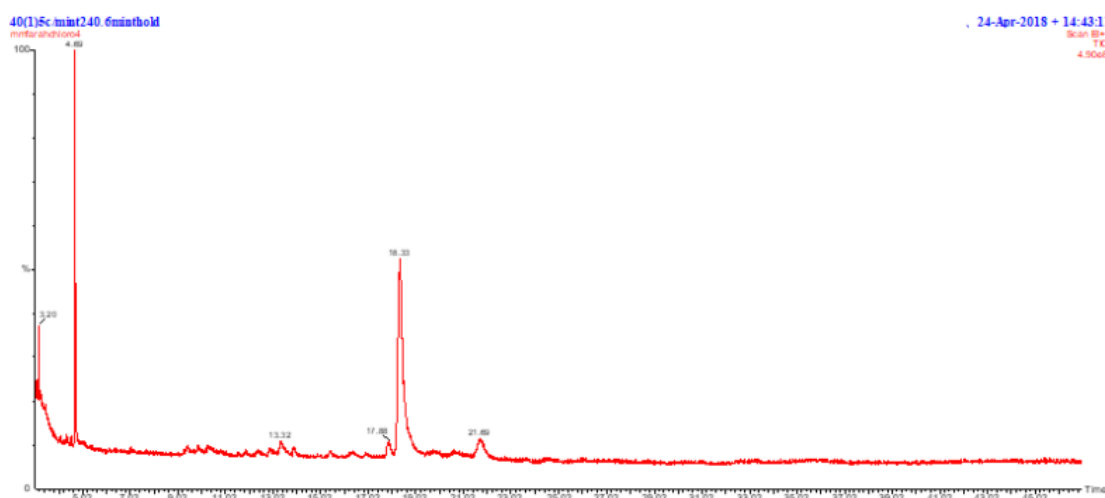

Fig.1: Chromatogram showing GC-MS analysis of *Olea ferruginea* extract

Table 3

Chemical composition of *Olea ferruginea* extract based on total ion chromatogram of GC-MS.

Comp.no	Name	t_R (min)	Area	%Composition
1.	Oleic acid	35.762	19351182	2.201441
2.	Stearic acid	45.621	5620912.5	0.63945
3.	Palmitic acid	40.954	881360.688	0.100266
4.	Palmitoleic acid	40.954	881360.688	0.100266
5.	Linoleic acid	39.553	681227.625	0.077498
6.	Linolenic acid	31.885	685402.563	0.077973
7.	Arachidic acid	3.669	1008801.5	0.587509

3.4. FT-IR Analysis

FT-IR Analysis was conducted to investigate the structural aspects of the extracts obtained from Malakand and Abbottabad samples. The prominent peaks are attributed to carboxylic acid O-H stretch, N-H functional group and ester (R-COOR). The absorbance peak for aldehyde (CHO) and C-O stretch was also observed and shown in Table 4.

Table 4

FTIR analysis of *Olea ferruginea* extract.

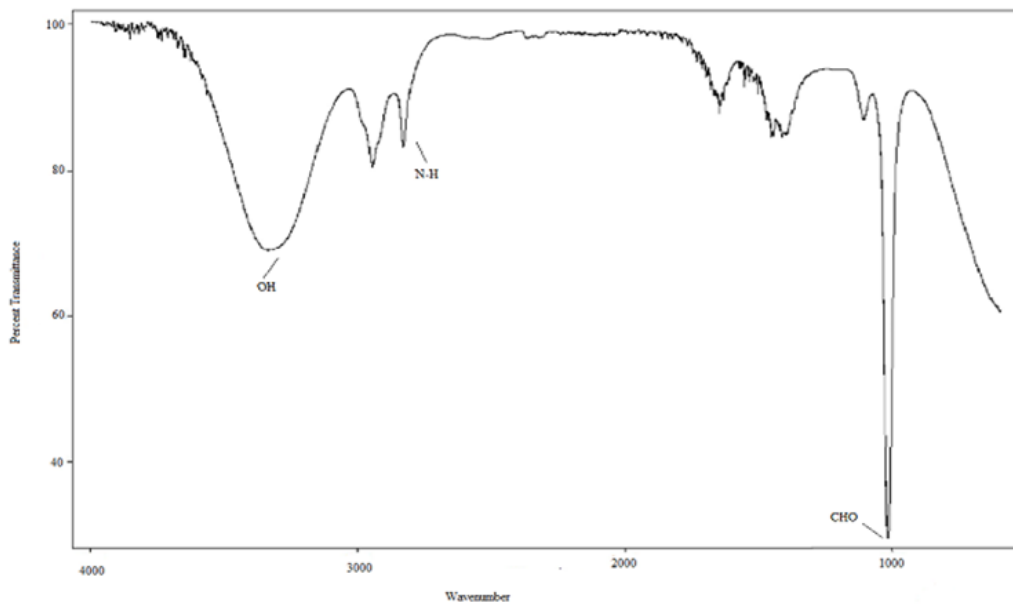| S.No	Wavelength (cm^{-1})	Bond	Functional Group
1.	3000-2500 cm^{-1}	O-H stretch	Alcohols
2.	3500-3300 cm^{-1}	N-H stretch	Amine
3.	1750 cm^{-1}	R-COOR	Ester
4.	1740-1690 cm^{-1}	CHO	Aldehyde
5.	1300 cm^{-1}	C-O stretch	Carboxylic acid, Ethers,
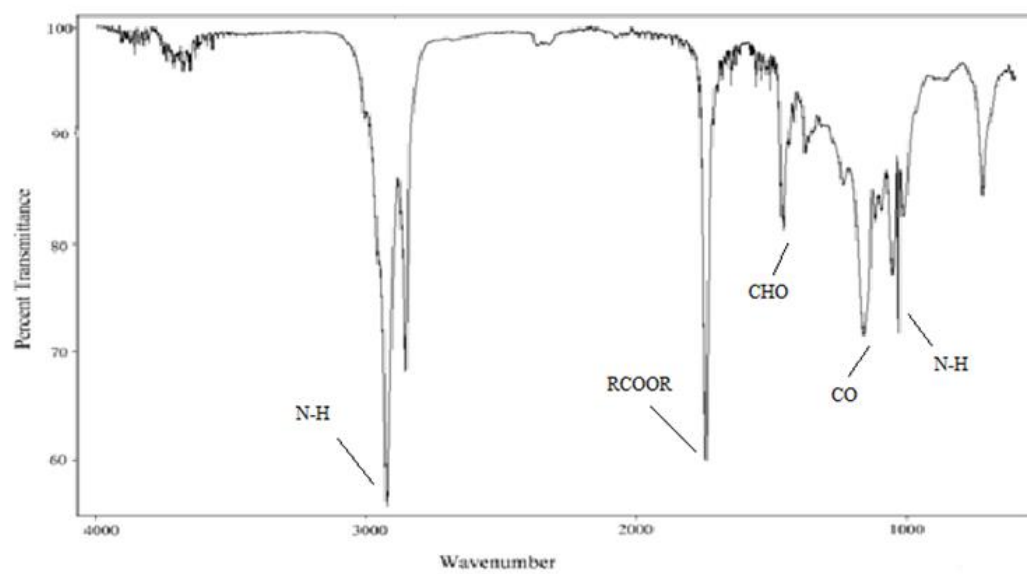
Figure 2:FT- IR analysis *O. ferruginea* oil

3.5. Analysis of Plant Extract for Phytochemicals

Olea ferruginea extract from Malakand, Haripur and Abbottabad is rich in phytochemicals such as phenols, flavonoids and tannins. The methanolic extract from Malakand showed greater percentage of phytonutrients. The percentage of phytochemicals from the three locations is shown in Fig 2.
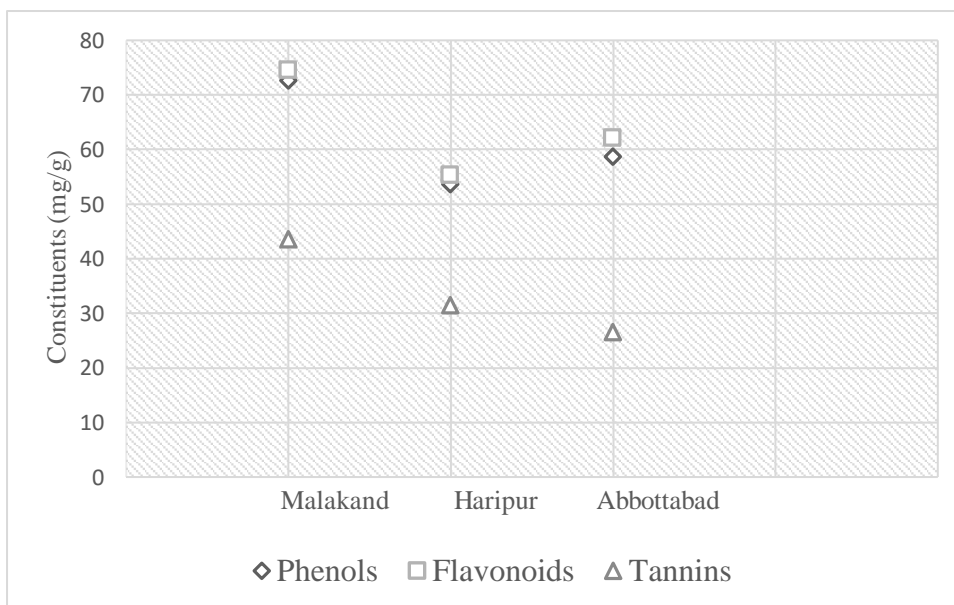

Fig. 2: Percentage of Phytochemicals from the Three Locations

3.6. Biological activities

3.6.1. DPPH, ABTS and H$_2$O$_2$ Scavenging Potential

For the evaluation of antioxidant activities of *Olea ferruginea* extracts, DPPH, ABTS and H$_2$O$_2$ scavenging bioassays were employed. The radical scavenging activity of plant extract with methanol was much enhanced for all the three locations. The scavenging activity of the extracts was greater in DPPH scavenging assay as compared to H$_2$O$_2$ and ABTS assays (Fig. 3). While the scavenging activity was lower in case of H$_2$O$_2$ scavenging assay for all the three locations.
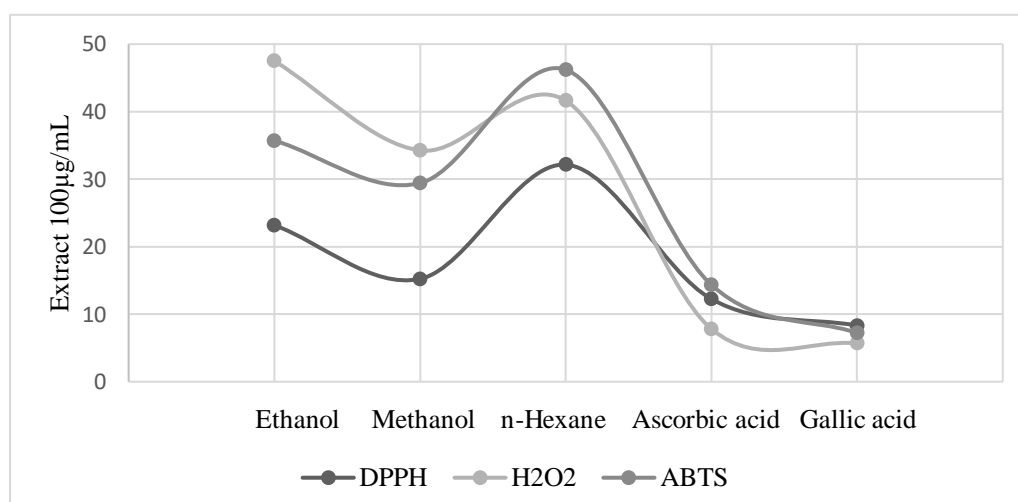

Fig. 3: Antioxidant effects of fruit extracts of olive fruits (µg/mL).

3.6.2. Brine Shrimp Cytotoxicity Bioassay

The brine shrimp cytotoxicity bioassay of *Olea ferruginea* extract indicated that the cytotoxicity is increased with the increasing concentration of the plant extract. However, the maximum percentage was observed at the concentration of 600 µg/mL. The values of the brine shrimp lethality obtained for extracts of *Olea ferruginea* extract are shown in Table 5.

Table 5

The brine shrimp cytotoxicity bioassay of *O. ferruginea* and *O.europaea* extract

Sample	Conc (µg/mL)	Total Nauplii	Live	Dead	% Mortality

O. ferruginea	10	18	16	2	11.1
	100	18	13	5	27.8
	300	19	9	10	55.6
	600	19	6	13	72.2
O. europaea	10	18	17	1	5.6
	100	19	16	3	15.8
	300	18	15	3	16.7
	600	19	13	6	31.6

It was observed that the methanolic extracts of *Olea ferruginea* showed prominent activity at a concentration of 10 µg/mL. At the concentrations of 100 and 300 µg/mL, the plant extract exhibited significant brine shrimp lethality. At a concentration of 600 µg/mL maximum mortalities took place, whereas, at 10 µg/mL concentration least mortalities were observed. While the extract obtained from *Olea europaea* showed less mortalities as compared to *Olea ferruginea* extracts and minimum mortality was observed at 10 µg/mL which is found to be 5.6 %. This significant lethality of plant extract revealed the presence of potent cytotoxic components.

3.6.3. Antibacterial Activity

The antibacterial assay of *Olea ferruginea* extracts were performed against the strains of Gram +ve and Gram –ve bacteria. The results showed that Malakand extracts exhibited significantly higher antimicrobial activity in the presence of methanol, as evidenced by larger inhibitory zones.It was observed that, methanolic extracts from Malakand showed prominent inhibitory activity against *E. coli* and *S. aureus*.The data

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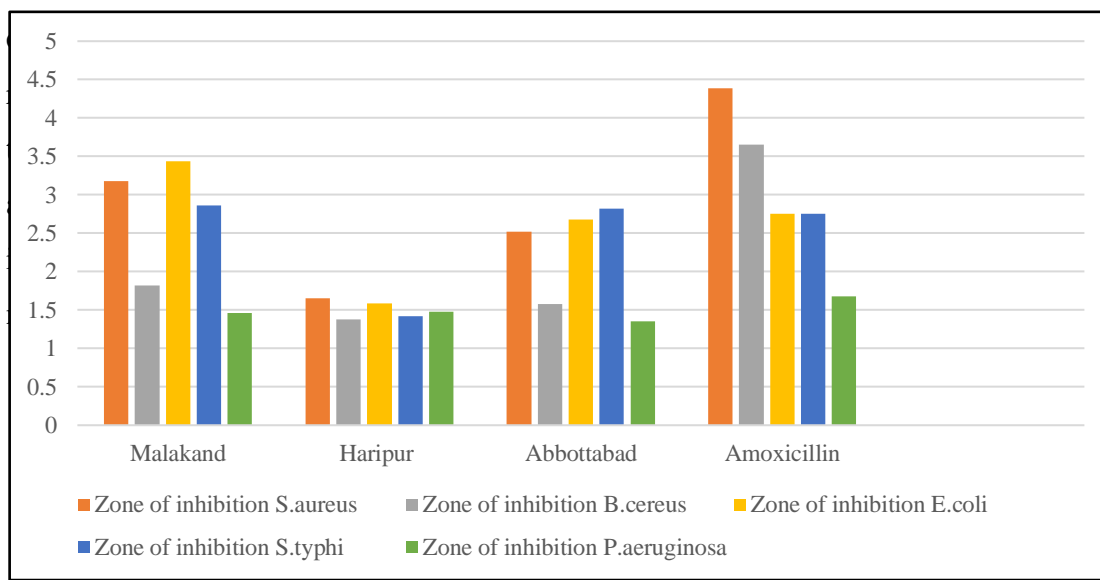
ing to the antimicrobial potential of the *Olea ferruginea* extracts is given in the Fig 4.

Fig. 4: Inhibitory action of methanolic extracts.

3.7.   Comparsion of *Olea ferruginea* with *Olea europaea*

The comparison of oil yields and fatty acid contents of fruits of *Olea ferruginea* from the three locations revealed similarity of various parameters. However, oil yields and level of saturated and unsaturated fatty acids was comparatively lower than *Olea europaea* which might be due to larger fruit size and different climatic conditions for the growth of *Olea europaea* trees. The large size of the fruit confirms greater percentage of phytochemicals which is related to the greater antioxidant and antibacterial activity of cultivated olives. The fruits of *Olea europaea* was greater in size and has the large size of seed due to which the oil yield was greater as compared to oil extracted from *Olea ferruginea.Olea europaea* is a cultivated species which is grown under adjusted conditions to obtain greater oil yield. Various quality parameters of *Olea ferruginea* were also compared with the already reported values of *Olea europaea* which indicated that the oil extracted from *Olea ferruginea* exhibits quality parameters which are in good agreement with the standard values of *Olea europaea*. Moreover, *Olea ferruginea* is more economical for Pakistan because it is wild species and it is drought tolerant.

4.   Conclusion

In order to discover healthy and cost-effective source of edible oil in Pakistan,the oil was extracted from the fruits of *Olea ferruginea* (collected from three regions) and compared with *Olea europaea.*The fruit extracts were found to contain phenols, flavonoids and tannins which is the scientific justification of medicinal use of the olive oil. Moreover, the confirmation of important fatty acids indicates the high degree of unsaturation in olive oil as equated to other edible oils supports its health benefits. Additionally, the plant extracts had showed the antibacterial potency when it was compared with the standard antibiotic. Through comparison,qualitatively the oils extracted from the two species had the same composition while the oil yield was greater in case of *Olea europaea*forlarge fruit size. The wild species *Olea ferruginea* was found to be economical as compared to the cultivated one.

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