INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 4, p. 350-358, 2019

RESEARCH PAPER OPEN ACCESS

Impact of harvesting time and geographical region on total phenolics, flavonoids and antioxidant activity of olive (*Olea ferruginea* Royle) leaves, district Zhob

Ayeesha Masood[1], Abdul Kabir Khan Achakzai[1], Rasool Baksh Tareen[1], Misbah Manzoor[2], Shaista Anjum[1], Tariq Ismail[1,4,*], Asad Ullah[3]

[1]*Department of Botany, University of Balochistan, Quetta, Pakistan*

[2]*Sardar Bahadur Khan Women University, Quetta, Pakistan*

[3]*Centre of Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan*

[4]*Department of Plant Protection and Production Kaposvar University, Hungary*

Key words: *Olea ferruginea*, Total phenolic content, Total Flavonoid content, Antioxidant activity.

http://dx.doi.org/10.12692/ijb/15.4.350-358 Article published on October 27, 2019

Abstract

Study area of district Zhob is well known for its naturally occurring population of *Olea ferruginea* .There was emerging need to unveil status of pharmaceutically active compounds in leaves of this locally used abundant species. Present study was conducted to evaluate influence of some geological parameters and harvesting time on concentration of few secondary metabolites and antioxidant activity of leaves extracted in different solvents.Sites selection was made by dividing the area with dense population of olives in north and south facing slopes. During 2017, leaves were collected from three altitudes at each slope 600 feet apart during four harvesting stages (vegetative, green fruiting, purple fruiting and black dried fruiting stage) Leaves were shade dried and extracted in three solvents (*i.e.* acetone, water and 80% ethanol) for quantification of total phenolic contents (TPC), total flavonoid content (TFC) and antioxidant activity (AA) on UV-VIS spectrophotometer by following standard protocols. TPC was significantly high at southern slopes (total mean value 23.73 mg/g) and AA at northern slope (with total mean 41.78%) respectively. Altitude had no significant impact on secondary metabolites and antioxidant activity except for DPPH radical scavenging activity that decreased at the mid altitude (38.19%).TPC, TFC and AA were significantly higher at black (27.99mg/g), green (27.11mg/g) and purple fruiting stage (42.44%) respectively. Solvents also had significant effects on TPC that showed high levels in acetone extracts (23.65mg/g) whereas TFC (42.49mg/g) and AA (42.46%) were higher in leaves extracted in 80% ethanol. Study aids to choose suitable location, harvesting time and extraction solvent to obtain maximum polyphenols and enhance antioxidant activity of *Olea ferruginea* leaves.

* **Corresponding Author:** Tariq Ismail ✉ tariq.ismail@ke.hu

Introduction

Zhob is the district of Balochistan, positioned in agro-ecological zone and covered agricultural area of 126,719 ha (hectares). Geographically, 0.6% area is used for cultivation and 1% area is covered by forest. In winters, weather is cold and areas at higher altitude receive heavy snow (Development profile of Zhob district, 2011). Besides, it's agricultural importance, this is well known for its naturally occurring population of wild olive forest. In Zhob olives are known as "Shnaney" by local community while, in urdu olives are known as "Zaitoon".

Olea ferruginea Royle belongs to family Oleaceae. Olive trees have been reported for their ethno-medicinal and commercial importance in all over the world. Several empirical studies evaluated therapeutic uses of oil and commercially available in the markets. Unfortunately, leaves are considered as agricultural waste (Taamalli *et al.*, 2012) and a byproduct of olive oil industry (Nicolì *et al.,* 2019). Leaves are rich and diverse in phenolic, flavonoids compounds and radical scavenging activity. Furthermore, leaves are widely used as folk medicine and reported for its antioxidant, anticancer (Nicolì *et al,* 2019; Nashwa and Abdel-Aziz, 2014; Fu *et al.*, 2010) and antibacterial activities (anjumSudjana, D'Orazio *et al.* 2009); (Lee and Lee 2010). Presence and compositions of polyphenolic, flavonoids compounds and radical scavenging activities linked with climate, slopes, and altitude of that mountainous or hilly area of Zhob is topic of concern.

Consequently, Southern slopes receive more sunlight and become more xeric and warmer, favors drought resistant vegetation and less conducive for tree growth and in contrast, northern slopes are cold, humid and supporting moisture loving plants (Måren, Karki *et al.* 2015). Several empirical studies reported that the solar radiation received at different levels tend to increase the differences between two contrasting aspects (Northern and Southern slope) like, in North America (Cantlon 1953), in the Middle East (Kutiel and Lavee 1999), Australia (Kirkpatrick, Fensham *et al.* 1988), in east Africa (Vetaas 1992), the Himalaya (Ghimire, Mainali *et al.* 2010) , (Paudel and Vetaas 2014), and even within the same elevation (Shank and Noorie 1950).

Due to small sized fruit as compared to commercially important species of olive, *Olea ferruginea* Royle could never catch attention of pharmaceutical and food industry as only fruit is considered valuable for oil extraction. But area surveys and interviews from local people and nomads unveil extensive use of leaves as decoction to cure various gastrointestinal and respiratory tract related ailments and there is a need to investigate phytochemistry of leaves. There was no specific information that could show relationship between fluctuating polyphenols and antioxidant activity with respect to opposite faces of two slopes, altitude, and different developmental stages of *Olea ferruginea* Royle leaves extracted in different solvents. Present work provides a baseline study that could be extended in future.

Materials and methods

Site selection for plant sampling

Zhob district was surveyed for altitudinal variation with dense population of *Olea ferruginea*. Study area was further marked with northern and southern slopes and each slope was visited at three altitudes with 500 feet difference.

The highest measured point was 6370 feet with ((31°16.637 N, 069°32.033 E). Second site at northern slope was 5770 feet high (31°29 57.98 N, 069°22 29.76 E). The lowest site was at 5170 feet in (31°30 22.15 N, 069°20 58.66 E). With same difference of 500 feet, southern slope was also divided in to three strata; top site was 6380 feet (31°17.0690 N, 069°32.836 E) middle site was 5770 feet high (31°17.42 N, 069°34 43.19 E) and the lowest site was 5170 feet high (31°27 05.49 N, 069°35 48.88 E).

Sampling

Stratified randomized sampling was done at each point. Randomly twenty trees were selected at each point for collection and leaves were kept in zip lock bags.

Processing

All leaves were washed thoroughly with water thrice to remove any traces of dirt and foreign particles. After that, leaves were spread separately on clean white cloth in dark for four weeks until crispy dried. These leaves were powdered in electrical grinding machine and were stored in air tight plastic jars for further use.

Extraction

Dry material was extracted against three different solvents i.e., (80%)ethanol, acetone and distilled water. Extraction method of Abideen *et al.*, (2015) was followed with few modifications. Plant material was mixed with ratio of 1:2 respectively for each solvent, then mixture was kept on shaking water bath for 3 hours at 40°C. After 3 hours flasks were cooled at room temperature and then centrifuged at 4500rpm for 15 minutes. Then clear supernatant was collected. Sample was kept in refrigerator for further analysis**.**

Total Flavonoid Content (TFC)

Procedure of (Dewanto, Wu *et al.* 2002)) was followed with few modifications, for quantification of total flavonoids. In 10mlvolumetric flask, 1 ml extract was added and diluted with 5ml distilled water. After that, 0.3 mL of $NaNO_2$ was mixed. After 5 minutes,0.6 mL of 10% $AlCl_3$ was added. After waiting for another 5 min 2 mL of 1M NaOH was added. Then whole mixture was diluted with distilled water. Absorbance was measured at 510 nm. All results were expressed as mg/g Catechin.

Total phenolic content

Total polyphenol content of all three solvent extracts were determined by following the procedure of (Folin and Denis 1912) with slight modifications. From each stored extract 0.5ml sample was taken in test tube and diluted with 16.5ml distilled water. Then 1ml of 1:10 Folin Reagent and 2ml of 7% solution of Sodium Carbonate was added. After 30 minutes of incubation its absorbance was measured at 765nm by using Shimadzu UV-Visible Spectrophotometer (UV 160). Standard curve was prepared with Gallic acid. Results were expressed as milligrams Gallic acid equivalent per gram dried weight of sample.

DPPH radical scavenging assay

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) assay are widely used to investigate the scavenging ability of antioxidants (Nabavi *et al.*, 2009).Method of DPPH was selected to investigate antioxidant activity of selected plantextracts in different solvents. Protocol of (Queiroz *et al.*, 2009) was followed with slight modifications. Extract and its polar fractions were added at an equal volume in 500µl ethanolic solution of DPPH (0.1mM). After 30 minutes incubated at room temperature the absorbance was recorded at 517 nm. BHT was used as standard. Inhibition of free radical by DPPH was calculated in the following way:

$I\ (\%) = 100 \times (A_{blank} - A_{sample} / A_{blank})$.

Where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. Percentage scavenging can be calculated.

Statistical Analysis

Results obtained from chemical analysis were processed in MINITAB software to obtain main effect plots that revealed relation of all parameters *i.e.,* total phenolics, total flavonoids and antioxidant activity with changing slopes, altitudes , growth stages and extraction solvents. “STATISTIX 10” version software was used to analyze factorial analysis of variance (ANOVA) and least significance difference(LSD). A statistical probability (p-value), if less than 0.05, shows a statistically significant difference between groups.

Results and discussion

Slope

Slopes also had a significant effect on the AA and TPC content however TFC remain unaffected with respect to slopes (Table.5). Higher average antioxidant activity was reported in leaves of *Olea ferruginea*collected from northern slopes (data values ranged between 28 – 54%) as compared to (22 – 52%) southern slopes. TPC showed a reverse gradient

and it was significantly higher in leaves collected from southern slopes.

Slope:
1= North facing
2= South facing,

Altitude:
1 = Top
2 = Middle
3 = Bottom

Growth :
stages :
1 = Vegetative
2 = Green raw fruiting stage
3= Purple ripened fruiting stage
4 = Black wrinkled fruiting stage

Solvents :
1 = Acetone
2 = Water
3 = 80% Ethanol

Altitude

Altitudinal variations have very strong impact on antioxidant capacity of olive leaves though there is no uniform pattern for it i.e., maximum DPPH radical scavenging activity was recorded at top most sampling site (28 – 53%) that suddenly dropped after 600 feet (26 – 53%) and again an average boost was noticed at lower most site (28 – 52%). Same results were obtained by Pandey *et al.* (2018), they confirmed high DPPH scavenging activity at high altitude as compared to lower altitude. Whereas, Jothiramshekar *et al.* (2013) reported lowest DPPH activity with increasing altitudinal gradient. TPC and TFC showed least response toward changing altitudes and results remained nearly insignificant (Fig. 1, Table 5).

Table 1. Mean comparison for total phenolic contents of *Oleaferruginea* leaves extract collected from two slopes, three altitudes, four growth stages and extracted in three solvents.

		Northern slopes			Southern slopes		
Sampling		Total Phenolic Content (mg/g)			Total Phenolic Content (mg/g)		
	Growth stages	Extraction solvents			Extraction solvents		
		Acetone	Water	80% Ethanol	Acetone	Water	80% Ethanol
Top	vegetative	25 ±0.81	25 ±0.81	22 ± 0.81	27 ±0.81	28 ±1.25	24 ±0.00
	Green fruit	27 ±0.81	24 ±0.81	18 ±0.00	25 ±0.00	29 ±0.81	22 ±0.81
	Purple fruit	28 ±2.16	27 ±0.81	22 ±0.81	27 ±0.81	27 ±0.81	24 ±9.34
	Black fruit	19 ±1.63	16 ±0.00	14 ±0.95	21 ±2.5	22 ±0.00	18 ±0.81
Mid	Vegetative	17 ±0.00	18 ±0.81	17 ±0.50	22 ±0.81	25 ±0.81	17 ±0.00
	Green fruit	19 ±0.81	16 ±1,41	13 ±1.50	22 ±0.00	25 ±0.81	18 ±0.81
	Purple fruit	12 ±0.95	14 ±1.63	12 ±0.00	19 ±1.63	17 ±0.50	14 ±0.81
	Black fruit	14 ±2.16	12 ±1.25	11 ±0.81	18 ±0.00	16 ±1.41	16 ± 0.00
Bottom	vegetative	12 ±1.63	12 ±1.70	12 ±0.81	22 ±0.81	17 ±1.70	14 ±0.81
	Green fruit	29 ±0.95	22 ±0.81	24 ±0.81	37 ±0.81	30 ±0.81	25 ±1.41
	Purple fruit	27 ±0.00	24 ±0.00	23 ±0.81	37 ±0.00	31 ±0.00	27 ±0.81
	Black fruit	27 ±0.81	24 ±1.70	24 ±0.00	36 ±0.81	30 ±0.81	27 ±0.00

Growth stages

Considering the effect of growth stages only, it was observed that *Olea ferruginea* leaves exhibit significantly higher total flavonoid content (13mg/g - 45mg/g) at the time when green unripe fruit appeared and lower ranges were seen (9 – 43mg/g) at purple fruiting stage.(Table. 2).

Results of growth stages irrespective of solvents, indicated an average antioxidant activity in leaves extracts of *O. ferruginea* collected at vegetative stage while slight decline in antioxidant activity was noticed at onset of green fruit that turned in to sudden and intense rise when fruit changed the color in to purple. The activity again dropped drastically wen fruit color

turned black. Gull *et al.* (2012) also reported that DPPH activity was higher in semi ripe fruiting plants as compared to fully ripe fruits. Maximum antioxidant activity, measured as DPPH% (22 -52%) at the time when fruit turned purple and minimum activity (24 – 54%) was noted in leaves extracts when fruit turned black and wrinkled (Table 3).

Table 2. Mean comparison for Total Flavonoid content of *Oleaferruginea* leaves extract collected from two slopes, three altitudes, four growth stages and extracted in three solvents.

		Northern slopes			Southern slopes		
Sampling		Total Flavonoids Content (mg/g)			Total Flavonoids Content (mg/g)		
		Extraction solvents			Extraction solvents		
	Growth stages	Acetone	Water	80% Ethanol	Acetone	Water	80% Ethanol
Top	Vegetative	16 ± 0.17	13 ±0.46	40 ±0.14	6 ±0.51	20 ±0.30	35 ±0.27
	Green fruit	18 ± 0.00	22 ±0.34	39 ±0.13	20 ±0.17	24 ±0.17	44 ±0.13
	Purple fruit	16 ± 0.00	13 ±0.34	37 ±0.13	11 ±0.45	9 ±0.45	37 ±0.14
	Black fruit	13 ±0.34	10 ±0.69	47 ±0.13	11 ±0.34	20 ±0.17	43 ±0.14
Mid	Vegetative	12 ±0.17	13 ±0.51	43 ±0.41	13 ±0.52	14 ±0.45	43 ±0.42
	Green fruit	14 ±0.06	19 ±0.00	40 ±0.06	24 ±0.08	16 ±0.41	45 ±0.17
	Purple fruit	10 ±0.46	20 ±0.30	35 ±0.23	12 ±0.00	11 ±0.38	39 ±0.37
	Black fruit	11 ±0.14	12 ±0.48	49 ±0.30	13 ±0.18	21 ±0.00	44 ±0.44
Bottom	vegetative	15 ±0.30	17 ±0.34	45 ±0.27	12 ±0.19	16 ±0.07	44 ±0.06
	Green fruit	18 ± 0.10	21 ±0.06	40 ±0.13	23 ±0.09	13 ±0.06	45 ±0.04
	Purple fruit	16 ± 0.51	18 ±0.34	43 ±0.00	12 ±0.09	12 ±0.14	40 ±0.07
	Black fruit	13 ±0.14	12 ±0.40	49 ±0.05	12 ±0.04	22 ±0.08	44 ±0.07

Generally there is a rise in total phenolics in plants grown in the sunny situations relative to the shady ones (Waterman and Mole 1994) Total phenolic content were noted at their peak in leaves extract when fruit turned black and wrinkled (22 – 37mg/g) in autumn season while minimum ranges of TPC were recorded in leaves collected at fruit ripening stage (11 – 22mg/g).

Table 3. Mean comparison for DPPH radical scavenging activity of *Oleaferruginea* leaves extract collected from two slopes, three altitudes, four growth stages and extracted in three solvents.

		Northern slopes			Southern slopes		
Sampling		DPPH radical scavenging activity (%)			DPPH radical scavenging activity (%)		
	Growth stages	Extraction solvents			Extraction solvents		
		Acetone	Water	80% Ethanol	Acetone	Water	80% Ethanol
Top	vegetative	43 ±0.17	43 ±0.07	42 ±0.00	47 ±0.17	46 ±0.62	30 ±0.30
	Green fruit	32 ±0.17	44 ±0.00	43 ±0.17	43 ±0.17	26 ±0.31	39 ±0.17
	Purple fruit	45 ±0.35	39 ±0.05	39 ±0.18	43 ±0.10	35 ±0.00	40 ±0.16
	Black fruit	45 ±0.35	53 ±0.04	32 ±0.17	42 ±0.00	32 ±0.00	40 ±0.35
Mid	Vegetative	40 ±0.19	53 ±0.09	32 ±0.13	40 ±0.06	26 ±0.01	42 ±0.00
	Green fruit	45 ±0.39	45 ±0.15	31 ±0.18	38 ±0.54	36 ±0.01	42 ±0.39
	Purple fruit	46 ±0.52	42 ±0.17	38 ±0.17	43 ±0.17	46 ±0.08	50 ±0.35
	Black fruit	39 ±0.17	43 ±0.18	41 ±2.05	43 ±0.13	22 ±0.14	51 ±0.57
Bottom	vegetative	46 ±0.17	34 ±0.03	44 ±1.94	42 ±0.04	38 ±0.00	52 ±0.00
	Green fruit	30 ±0.17	47 ±0.20	51 ±4.58	28 ±0.17	32 ±0.10	42 ±0.17
	Purple fruit	28 ±0.28	45 ±0.05	46 ±0.70	27 ±0.00	24 ±0.93	41 ±0.07
	Black fruit	31 ±0.08	40 ±0.00	54 ±0.08	28 ±0.03	35 ±0.00	43 ±0.61

Total polyphenol content of leaves extracts of *O. ferruginea* irrespective of solvents, showed a gradual decrease from vegetative to green fruiting and very low levels at purple stage which a sudden boost with maximum polyphenol content was seen in leaves extracts collected at black fruiting stage. (Fig. 1, Table 1).

Table 4. Factorial ANOVA (Analysis of variance) for TFC, TPC and antioxidant activity.

Source Variation	Total Flavonoid Content mg/g					Total Polyphenolic content mg/g				Antioxidant activity (DPPH %)			
	DF	SS	MS	F	P- value	SS	MS	F	P	SS	MS	F	P-value
Replicate	2	0.6	0.3			3.02	1.51			1.07	0.533		
Slope	1	0.2	0.2	0.02	0.8769	740.41	740.41	225.49	0.0000	391.22	391.218	19.88	0.0000
Altitude	2	44.7	22.4	2.72	0.0687	4.62	2.31	0.70	0.4965	431.73	215.867	10.97	0.0000
Growth Stages	3	739.6	246.5	29.99	0.0000	5163.99	1721.33	524.23	0.0000	421.84	140.612	7.14	0.0002
Solvents	2	21675.2	10837.6	1318.42	0.0000	539.13	269. 56	82.10	0.0000	334.53	167.267	8.50	0.0003
Slopes × Altitudes	2	44.8	22.4	2.73	0.00	0.42	0.21	0.06	0.9380	108.20	54.101	2.75	0.0669
Slopes× Growth stages	3	264.0	88.0	10.71	0.0684	156.71	52.24	15.91	0.0000	470.86	156.952	7.97	0.0001
Slopes× Solvents	2	10.3	5.2	0.63	0.5348	38. 50	19.25	5.86	0.0035	1517.08	758. 542	38.54	0.0000
Altitude × Growth stages	6	121.1	20.2	2.46	0.0266	30.28	5.05	1.54	0.1690	117.67	19.612	1.00	0.4295
Altitude× Solvent	4	69.0	17.3	2.10	0.0831	8.58	2.14	0.65	0.6255	475.92	118.980	6.04	0.0001
Growth × Solvents	6	812.0	135. 3	16.46	0.0000	217.01	36.17	11.01	0.0000	3464.25	577.375	29.33	0.0000
Error	166	1364.5	8.2			545.06	3.28			3267.33	19.683		
Total	199												
	Grand Mean 24.512 CV 11.70					Grand Mean 21.611			CV 8.38	Grand Mean: 40.246			CV: 11.02

DF = Degree of freedom, SS = Sum of square, MS = Mean square .CV = Coefficient of variation.

Table 5. Least significance difference (LSD) test of total flavonoid content total phenolic content and antioxidant activity (DPPH%) in relation to slope, altitude, growth stages and extraction solvents.

Source	Variation	Mean antioxidant activity (DPPH%)	Homogenous groups	Mean total phenolics (mg/g)	Homogenous groups	Mean total flavonoids (mg/g)	Homogenous groups
Slopes	North facing	41.787	A*	19.491	B*	24.546	A
	South facing	38.705	B*	23.731	A*	24.477	A
Altitude	Top	41.569	A	21.500	A	24.014	B
	Mid	38.196	B*	21.469	A	24.236	AB
	Bottom	40.973	A	21.863	A	25.285	A
Growth stages	Vegetative	40.522	B	24. 996	B*	23.399	C*
	Green fruiting	39.635	BC	18.691	C*	27.119	A*
	Purple ripe fruiting	42.442	A*	14.805	D*	22.012	D*
	Black wrinkled fruit	38.390	C	27.996	A*	25.516	B*
Solvents	Acetone	39.375	B	23.653	A*	14.514	C*
	Water	38.903	B	22.208	B*	16. 528	B*
	80% Ethanol	42.461	A*	18.971	C*	42.493	A*

* means significant difference between the groups (P< 0.05).

Extraction solvents

Acetone extracts showed high levels of total polyphenol content. Aqueous extracts were second best and 80% ethanol extracts showed least efficient for polyphenol extraction.

Maximum TFC was recorded in leaves extracted in 80% ethanol while water and acetone were respectively less efficient to extract TFC from leaves of *O.ferruginea*. Antioxidant activity of 80% ethanol extract was higher followed by acetone and aqueous extracts. (Fig. 1, Table 3).

Our results are in favor of Mehmood and Murtaza, (2018) findings in response of higher antioxidant activity in leaves of *O. ferruginea* extracted in ethanol as compared with other organic solvents.

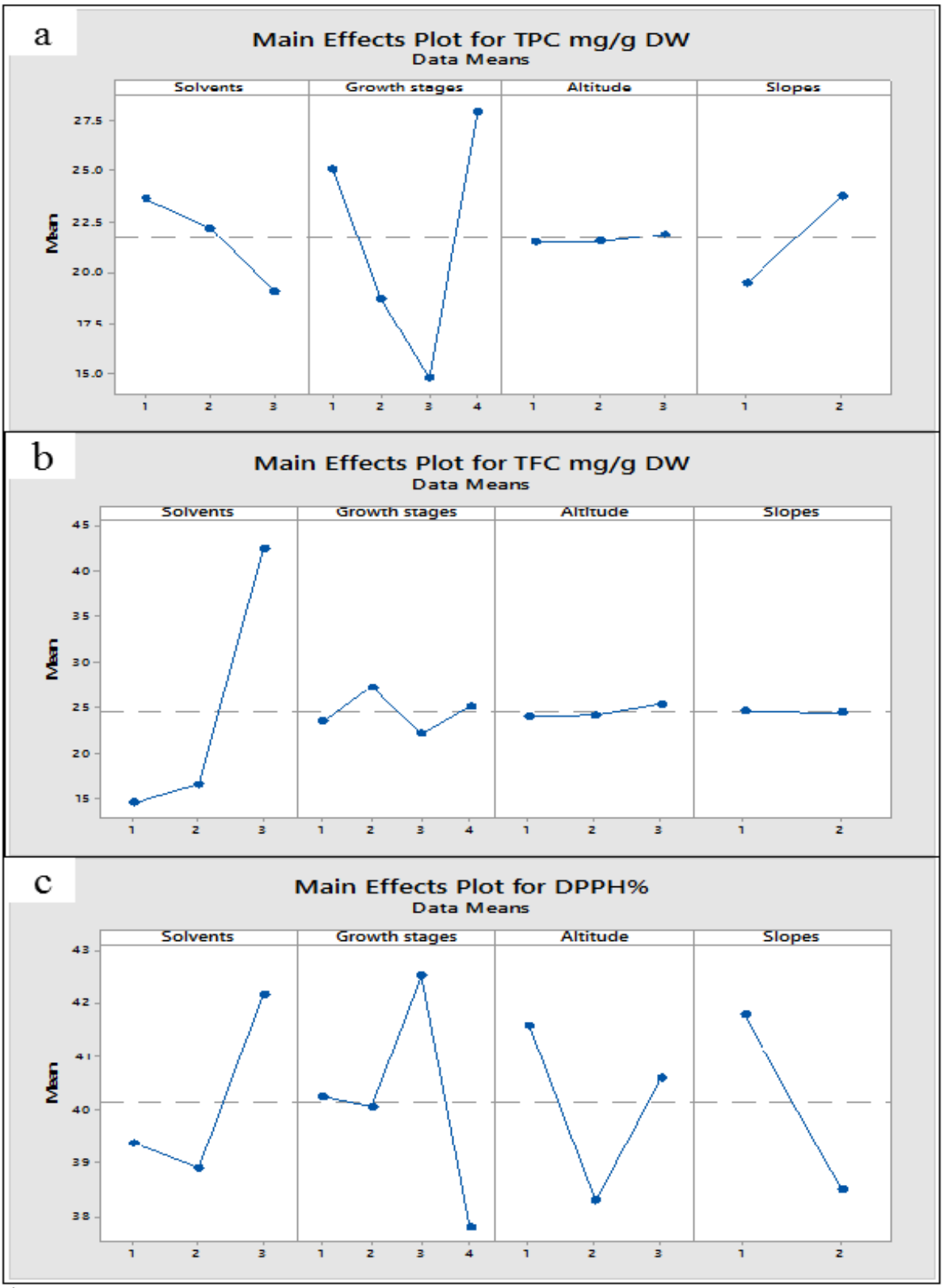

Fig. 1. Impact of slopes, altitude, growth stages and solvents on a) total phenolics , b) total flavonoids and c) antioxidant activity.

Conclusion

High phenolic contents in *Olea ferruginea* leaves is indication of abiotic stress at southern slopes of Zhob that receives much sun light and is an open access area as compared to northern slopes that are naturally less exposed to solar radiation

comparatively. Mean total flavonoid content remained same at both slopes while pattern of antioxidant activity showed an increase from southern to northern slopes. There is no uniform scheme of sample collection based on altitudinal and seasonal variation for maximum recovery of penolics, flavonoids and antioxidant capacity together. For each activity there are separate sampling priorities. For high flavonoid content, and antioxidant activity, 80% ethanol was found highly effective while phenolics were extracted best in acetone.

These findings can support the pharmaceutical and food industry specially and cosmetic industry in general to encourage the sampling at right time, place and processing in most suitable solvents to obtain maximum concentrations of desired compounds.

Recommendations and future plans

In future there is need to isolate pharmaceutically active compounds in from *Olea ferruginea* leaves that can be supposed to contribute in ethnomedicinal and pharmaceutical uses. Work on antibacterial activity of leaves is in progress by the same researcher and it is also recommended to investigate antiviral and antifungal activities that are expected due to good levels of total phenolics and total flavonoids and high antioxidative properties of leaves. Conservation practice by government is also need of time as locally strict measures are taken up by tribal men to avoid over exploitation of forest but this practice is limited to a small area of north facing slopes.

References

Cantlon JE. 1953. "Vegetation and microclimates on north and south slopes of Cushetunk Mountain, New Jersey." Ecological Monographs **23(3),** 241-270.

Dewanto V. 2002. "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity." Journal of agricultural and food chemistry **50(10),** 3010-3014.

Folin O, Denis W. 1912. "On phosphotungstic-phosphomolybdic compounds as color reagents." Journal of Biological Chemistry **12(2),** 239-243.

Ghimire B. 2010. "Regeneration of Pinus wallichiana AB Jackson in a trans-Himalayan dry valley of north-central Nepal." Himalayan Journal of Sciences **6(8),** 19-26.

Kirkpatrick J. 1988. "Vegetation-radiation relationships in the wet-dry tropics: granite hills in northern Australia." Vegetatio **76(3),** 103-112.

Kutiel P, Lavee H. 1999. "Effect of slope aspect on soil and vegetation properties along an aridity transect." Israel Journal of Plant Sciences **47(3),** 169-178.

Lee OH, Lee BY. 2010. "Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract." Bioresource technology **101(10),** 3751-3754.

Måren IE. 2015. "Facing north or south: Does slope aspect impact forest stand characteristics and soil properties in a semiarid trans-Himalayan valley?" Journal of arid environments **121,** 112-123.

Paudel S, Vetaas OR. 2014. "Effects of topography and land use on woody plant species composition and beta diversity in an arid Trans-Himalayan landscape, Nepal." Journal of Mountain Science **11(5),** 1112-1122.

Shank R, Noorie E. 1950. "Microclimate vegetation in a small valley in eastern Tennessee." Ecology **11,** 531-539.

Sudjana AN. 2009. "Antimicrobial activity of commercial Olea europaea (olive) leaf extract." International journal of antimicrobial agents **33(5),** 461-463.

Vetaas OR. 1992. "Gradients in field-layer vegetation on an arid misty mountain plateau in the Sudan." Journal of Vegetation Science **3(4),** 527-534.

Waterman PG, Mole S. 1994. Analysis of phenolic plant metabolites, Blackwell Scientific Oxford District Development Profile 2011. july 18th 2012. Zhob. Prepared by planning and Development Department, Government of Balochistan, Quetta (in collaboration with) UNICEF United Nationn Children's fund provincial office in Balochistan, Quetta, p **81.**