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MORPHO-BIOCHEMICAL CHARACTERIZATION OF MENTHA SPP. USING CLUSTER ANALYSIS IN GARALA, DISTRICT SUDHNUTI AZAD JAMMU AND KASHMIR

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ABSTRACT

The objective of current study was to explore phenotypic variation and the phytochemical comparative antioxidant potential of Mentha species. The collection sites were different areas of Garala district Sudhnoti. Phenotypic variation was evaluated using quantitative and qualitative traits. While for phytochemical screening, the samples were extracted via two solvent systems viz, Ethanol and Water. Comparative phytochemical analysis was done for estimating total phenolic, total flavonoid content and antioxidant potential. Current results showed that continuous exposure of ethanol as in *Mentha longifolia* (leaves) has a prominent impact on the phytochemical properties. Whereas the qualitative traits showed that *Mentha* species were highly diverse. The Mean values for number of leaves was 78.250, leaf area (9.75), leaf thickness (0.039), stem thickness (3.722), petiole length (0.515), root length (26.925) and for plant height was 54.265. The magnitude of variance ranging from .0001 to 1042.25 and 1042.25 in number of leaves, 14.265 in leaf area and it was 0.117 in stem thickness, 125.789 in petiole length, 831.029 in root length, 1.699 in plant height respectively. The coefficient of variance for stem thickness was 33.6%, leaf thickness (31.8%), number of leaves was 39.0%, leaf area (40.0%), root length (45.3%), plant height (50.2%) and petiole length was (82.7%). Highest antioxidant activity was observed in *Mentha longifolia* leaves (96.67%) and highest phenolic contents were found in *Mentha royleana* leaves (0.164GAE//mg). While considerable flavonoid contents were observed in *Mentha arvensis* roots (0.240GAE/mg). It is concluded that *Mentha* species are phenotypically highly diverse with good potential of antioxidant scavenging reducing power. So the extracts could be utilized for pharmaceutical purpose.

Keywords: Mentha, Phenotype, variance, phyto-constitutes, oxidative stress

INTRODUCTION

The genus *Mentha* belongs to the family *Lamiaceae* consisting about 25 to 30 species, found in temperate regions of the world. Mint habitat ranging from cool to shade moist places. Mints reach 10-120 centimeters in height. The genus *Mentha L.* is rich in many secondary metabolites i.e. alkaloids, antioxidants, phenol and flavonoids (Shinwari et al., 2011). Most *Mentha* species are ecological diverse in their morphology which is reflected on various taxonomic rank during the past 200 years. In addition, the hybridization among mint species contributes towards complex variation characterizing to development of most wild genotypes.

Kohn (1963) classify the mint species on the basis of inflorescence morphology (Erum et al., 2012). He reported morphogenetic characterization of six common *Mentha* species from Pakistan based on morphological traits i.e. leaf area, inflorescence color and shape of leaf among the oldest markers used to identify *Mentha* species (Abasi et al., 2019). *Mentha* leaves have traditionally been used for tea making with the effect of reducing headache, fever and digestive disorders. In modern world, *Mentha* species are commonly used for many gastrointestinal tract diseases (Salehi et al., 2018). *Mentha longifolia* has stolon creeping stem with underground roots and erect stems with average height ranging 30-120 cm with simple, paired, alternate, opposite petiolated leaves which are greyish-green in color.

The flowers are white and may be purple and 3-5 mm long, which are clustred on elongated spikes (Devi et al., 2022). *Mentha pepritica* and *Mentha spitica* are 8-10cm in height and almost 3-5 mm thick

with 3 nodes (Smolik et al., 2007). Phenotypic differences could be finding out by measuring length, diameter, height and width of stem, leaves and roots (Aflatuni et al., 2005; Mahadevappa et al., 2014). In Hindi and Urdu languages it is called Pudeena. It is annual, perennial herbs aromatic in nature (Mahadevappa et al., 2014). The botanical origin of the peppermint plant and its relation to other species of the genus *Mentha* is an exceedingly complicated problem (Shelepova et al., 2021).

In studies on *Mentha* species, different methods are practiced, different secondary metabolites dissolvent and essential oils of plants are used and various pharmacologic effects are determined. In the related studies on species, it is found that *Mentha* species have significant effects such as analgesic, anti-inflammatory, antipyretic, DNA damage protecting activity, antioxidant, anti-androgenic, antimicrobial, cytotoxic, antiviral, anticancer, antiemetic, antibacterial, antiallergic, antiparazitic, sedative, anti-chlamydial, radioprotection, anti-cholinesterase, hepatoprotective, antispasmodic, acute toxicity effect, anti-mutagenic, cardiovascular effects and anti-tumour effects (Sevindik, 2018). Carl Linnaeus was renowned for first describing peppermint from plants and leaves in England during 1753 (Kohn, 1963; Mahadevappa et al., 2014).

It is distributed wildly and can be seen in almost all environmental conditions especially wet and moist soil (Mahadevappa et al., 2014). When the mint leaves are crushed, ground and steam distilled, the volatile distillate thus obtained is said to be mint oil. It is a carminative naturally occurring. Numerous minerals and nutrients including manganese, iron, magnesium, calcium, folate, potassium and copper are present in peppermint oil. It also contains omega-3 fatty acids, Vitamin A and Vitamin C (Mahadevappa et al., 2014). Release stress, mental exhaustion and depression and it helps in strengthening the immune system. It also acts as mosquito repellent (İşcan et al., 2002; Mahadevappa et al., 2014).

Morphological tools i.e. leaf area, inflorescence color and shape of leaf among the oldest markers used to identified *Mentha* species (Abasi et al., 2019; İşcan et al., 2002; Yousef et al., 2015) because genetic differences, in *Mentha* species due to environmental changes that cause variations in phenotype of individuals are considerable (Abasi et al., 2019; Singh, 1986). Understanding the genetic multiplicity of *Mentha* species is required for crops improvement (Yousef *et al*., 2015). Morphological traits, cellular biochemical, and isozyme polymorphism has been used to detect the genetic variability among the *Mentha* specimens (Abasi et al., 2019).

In this study we addressed the phenotypic variation and total phenolics, flavonoids, antioxidant properties in four species of Mentha that were collected from the Garala, district suduhnoti, Azad Kashmir. Due to lack of information on mentha species identification keys and phytoconstitutes from their natural habitat, it is necessary to document and explore species on their natural habitat.

MATERIAL AND METHODS

Study Area

Sudhnoti is Northern district of Azad Jammu & Kashmir state (Figure 1). Topography of Sudhnoti is mainly hilly and mountainous. The ecosystems mountain are relatively unstable and have low intrinsic productivity, within this fragile environment there is a great diversity of ecological niches upon which people base their livelihood. The area is full of natural beauty with thick rest, fast flowing river and winding streams and main river is Jhelum. Some areas of Sudhnoti have cold weather and some have moderate weather.

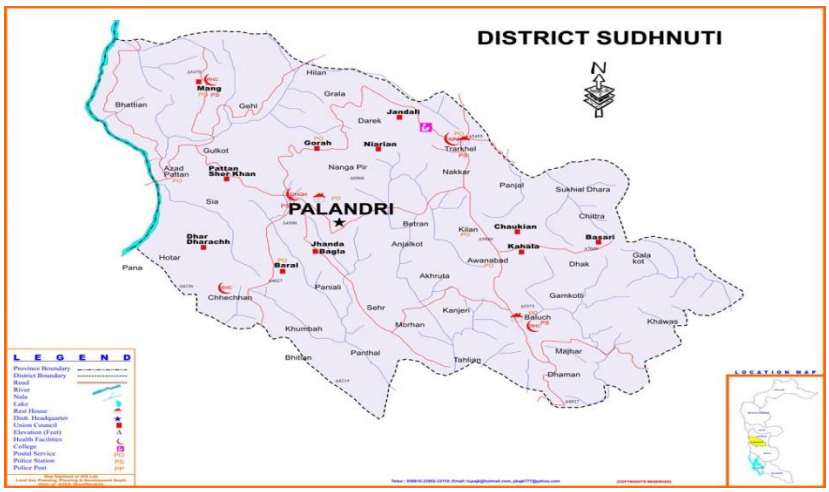

Figure 1: Map of the study area.

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Collection of Plant Material

Four *Mentha* species were collected from different areas of Garala (Elevation.5187, Latitude $33^0$47'36"E, Longitude $73^0$44'12"E) District Sudhnoti and evaluated for various agro-morphological traits (Table 1).

Identification of *Mentha* Species

All collected species were identified on the base of morphology. Identification was completed by evaluation of the morphology by identification keys and published literature (Erum *et al*., 2012). Scientific naming and citations were further confirmed by online database from plant net and by expert taxonomist Dr Sajad Hussian (Herbarium incharge) department of Botany, University of Poonch Rawalakot.

Agro-Morphological Studies

The data was recorded in triplicate and observations were documented for following traits.

Quantitative Traits

Leaf area: Leaf area was measured using formula (Leaf area = Length of leaf x width of leaf) in centimeter.

Leaf Thickness: Leaf Thickness was measured using screw gauge in centimeter.

Stem Thickness: Stem Thickness was measured using screw gauge in centimeter.

Stem length: Stem length was measured by measuring tape in centimeter.

Petiole length: Petiole length was measured by measuring scale in centimeter.

Plant height: Plant height was measured by measuring tape in centimeter.

Number of leaves: Number of leaves were count by visual observation.

Table 1. Selected site for sample collection of study area.

Area	*Mentha spicata*	*Mentha royleana*	*Mentha arvensis*	*Mentha Longifolia*
1	Dari	Kaknihel	Gorah	Mara
2	Ora	Bara	Nakar	Ora
3	Mara	Tridamora	Junjalhel	Devan Gorah
4	Malvani	Devan Gorah	Dana	Junjalhel

Qualitative Traits

All qualitative traits (Leaf margin, Leaf apex, Leaf base, Leaf color, Leaf arrangement, Leaf venation, Leaf odor, Stem color, Flower color, Shape of leaf blade) were observed visually.

Biochemical Studies

The whole plants of Mentha species were dried in shade for about 2-3 weeks and then powder with a mechanical grinder. The powder was stored in a labeled air tight container for further studies. After preparing the samples were rinsed thoroughly with tap water followed by sterilized distilled water. Afterwards the leaves were dried in sunlight for 5 to 10 days, then homogenized into a fine coarse powder with the aid of mortal and pistle. About 5g of the healthy plant parts (leaf and root) of each species were grind in order to obtain fine powder. The prepared samples were shifted in test tubes. All test tubes were filled with 100ml of hot water, shake well and prepared extract of root and leaf of different samples. Then the sample were screened for following biochemical traits.

Estimation of Total Phenol Content

The procedure of determining the quantity of phenolic content in samples is termed as TPC activity. Plant-derived phenolic compounds have redox characteristics, which allow them to act as antioxidants. Total phenolic content was determined using Folin-reagent Ciocalteu's test.

Estimation of Total Flavonoid Content

Aluminum chloride colorimetric assay was used to assess the total flavonoid content (Bibi et al., 2022).

Antioxidant Activity Using (DPPH) Radical

The free radical scavenging activity of Mentha arvensis leaf extracts was assessed using the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (Bibi et al., 2022). The svavenging activity was calculated as per formula;

$$Scavenging\ activity\ (\%) = \frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Statistical Analysis

Computer software, Microsoft excel was used to compute mean comparison of various plant samples by plotting graph. The phenotypic data were statistically subjected for Mean separation, Standard deviation, variation and coefficient of variance and cluster analysis is done for evaluating the diversity among Mentha species based on biochemical traits using SPSS 2.0 computer software.

RESULTS AND DISCUSSION

Quantitative Traits

In the present study 4 genotypes of *Mentha* species were studied. The quantitative traits which

are mainly considered as the economic/ agronomic traits were leaf area, leaf thickness, stem thickness, stem length and petiole length for 4 genotypes of *Mentha*. Table 3 shows that leaf area ranged from 4.09 to 13.122 with 40.0% Coefficient of Variation. High variation ranging from 15.6 to 85.26 cm was observed for stem length with 50.2% Coefficient of Variation. In root length the mean ranged from 19.8 cm to 30.5 cm with 45.3% Coefficient of Variation. While in petiole length the mean ranged from 0.2 cm to 1 cm with 82.7% Coefficient of Variation. Moreover in thickness of stem mean ranged from 1.93 cm to 5.002 cm with 33.6 Coefficient of Variation. Similarly in the thickness of leaf mean ranged from 0.024 cm to 0.05 cm with 31.8 % Coefficient of Variation. While number of leaves ranged from 31 to 99 with 39.0% Coefficient of Variations (Table 2).

Table 2. List of Quantitative traits of *Mentha* species.

Variety	No of leaves	Leaf area	Leaf Thickness	Stem thickness	Petiole length	Root length	Plant height
Mentha arvensis	31	4.09	0.024	1.93	0.46	19.8	15.6
Mentha royleana	99	13.12	0.048	3.736	1	30.5	56.1
Menthe spicata	99	8.79	0.05	5.002	0.4	16.3	85.26
Mentha longifolia	84	10.3	0.032	4.22	0.2	41.1	60.1
Mean	78.250	9.075	.039	3.722	.515	26.925	54.265
Variance (V)	1042.2	14.26	.0001	.117	125.789	831.02	1.699
Std. Deviation	32.284	3.777	.013	1.304	.342	11.216	28.828
Range	68.000	9.030	.026	3.072	.800	24.800	69.660
Coefficient of Variation(CV)	39.0%	40%	31.8%	33.6%	82.7%	45.3%	50.2%

Correlation Analysis for Morphological Traits

Table 3 shows that Leaf area positively correlated (0.876) with number of leaf Thickness of leaf positively correlated (0.698) with number of leaf (0.889) and leaf area (.698). Petiole length positively correlated with number of leaf (0.256), leaf area (0.486) and thickness of leaf (0.475). Root length positively correlated with number of leaf (0.250) and leaf area (0.554) and negatively correlated with thickness of leaf (-0.182) and petiole length (-0.097). Plant height positively and significantly correlated with number of leaf (0.910) and positively correlated with leaf area (0.608) ,thickness of leaf (0.824), root length (0.037).Plant height negatively correlated with petiole lengths (-0.081).Stem diameter positively and significantly correlated with number of leaf (0.906). Stem diameter. Positively correlated with leaf area (0.625) and thickness of leaf (0.764). Stem diameter negatively correlated with petiole length (-0.149). Stem diameter positively and highly significant to plant height (.993). (Abasi et al., 2019; Erum et al., 2012) investigated quantitative traits of *Mentha* species by using Coefficient of Variation.

Qualitative Traits

In the present study, great diversity was expressed within four genotypes in table 4 for qualitative traits including shape of leaf blade, leaf margin, leaf apex, leaf base, leaf color, leaf arrangement, leaf venation, leaf odor, stem color and flower color. Flower color of *Mentha royleana* and *Menthe spicata* is whitish in appearance. Flower of *Mentha Longifolia* is Lilac in color and *Mentha arvensis* is purple. However, stem color showed Dark purple light green and green color among the genotypes under study observations were recorded for leaf color, that are in three shades (green, light green, dark green). Arrangement of leaf was opposite. Likewise, Arcuate, pinnate types of leaf venation was observed in *Mentha* spp. The odor of *Mentha* leaves is a characteristic of each genotype due to the presence of unique bioactive compounds. Consequently, four special aroma was smelled even in the four samples i.e., mint gum, camphoraceous, pungent and acrid. *Mentha* species are characterized by different aroma due to the presence of bioactive compounds and essential oils evident from the literature (Zviniene and Pank, 1996). Moreover, shape of leaf also exemplify great variation with diverse patterns including leaf blade are ovate, elliptic and lanceolate among four *Mentha* spp. Leaf margin is mostly serrate. Leaf apex are acute or broadly acute mostly. Obtuse leaf bases were observed mostly but in horse and field mint cuneate base is present Moreover, similar observations was reported by (Shinwari et al., 2011) on the diversity of *Mentha* species that showed taxa maintained high levels of genetic polymorphism among species but not among population (Erum et al., 2012). It investigated the morphological diversity and relationship within 17 genotypes of *Mentha* species. (Abasi et al., 2019)

investigated morphological diversity among *Mentha* species in AJK. The polymorphism within populations depicted genotype richness, recombination and gene flow. Higher levels of diversity support the concept that mint have a long history of independent evolution.

Total Plenolic Contents

All the tested samples contained different amount of phenolic contents. The phenolic contents varied mostly in relation to the plant parts i.e., leaf and root of various samples. While comparing different samples of Mentha, higher content was reported in leaf and root sample (Table 5).

Among the tested samples, leaf extract of Mentha *royleana* has the highest amount of phenolic contents (0.164GAE/mg). In leaf extract of *Mentha longifolia* the amount of phenolic contents was 0.143GAE/mg. In leaf extract of *Mentha arvensis* the amount of phenolic contents was 0.113GAE/mg. The lowest amount of phenolic contents was in leaf extract of *mentha spicata* that was 0.088GAE/mg. Among the root samples *Mentha spicata* has the highest amount of phenolic contents (0.146GAE/mg). In root sample of *Mentha arvensis* the amount of phenolic contents was 0.139GAE/mg. In root sample of *Mentha royleana* the amount of phenolic content was 0.109GAE/mg. The root sample of *Mentha longifolia* has the lowest amount of phenolic contents (0.040GAE/mg).

On analyzing the total phenolic content of the sample extratct of Mentha possessed the higher content of total phenolic content in leaf wheareas the root extract of Mentha also contained the considerable amount of phenolic. Based on previous research, it has been found that there is variation in phenolic contents in different parts of the plant by Araghi et al. (2019). Similarly Yang et al. (2018) reported that phenolic contents varied among the plant samples collected from different locations. Variation of phenolic content was due to various environmental factors, soil texture and altitude differences. It could be said that phenolic content variation was the result of a plant interaction to its environment. Change in phenolic directly affects the medicinal quality of the plant main cause for the variation in the phenolic contents was due to difference in soil texture, environment and altitude, stem, leaf and inflorescent characters were classified for the identification of *Mentha* species Salehi et al. (2018).

Flavonoid Contents

The flavonoids content varied mostly in relation to the plant parts i.e., leaf and root of various samples. While comparing different samples of Mentha, higher flavonoid content was reported in *Mentha arvensis* roots. Among the tested samples, leaf extract of *Mentha arvensis* has the highest amount of flavonoids contents (0.200GAE/mg). In leaf extract of *Mentha royleana* the amount of flavonoids contents was 0.139GAE/mg. In leaf extract of *Mentha longifolia* the amount of flavonoids contents was 0.124GAE/mg. The lowest amount of flavonoid contants was in *Mentha spicata* (0.099GAE/mg) (Table 6). Among the root samples *Mentha arvensis* has the highest amount of flavonoid contents (0.240GAE/mg). In root sample of *Mentha spicata* the amount of flavonoid contents was 0.103GAE/mg. In root sample of *Mentha longifolia* the amount of flavonoid contents was 0.233GAE/mg. The root sample of *Mentha royleana* has the lowest amount of flavonoid contents (0.078GAE/mg).

Flavonoids possess health promoting effects, mainly because of their antoxidative properties (Gracindo et al., 2006). Flavonoid are naturally occurring in plants and are thought to have positive effect on human health. Studies on flavonoid derivatives have shown a wide range of antibacterial, antioxidant and anti-inflammatory activity. In a previous study, Chang et al. (2002) have shown that the real content of total flavonoids must be the sum of flavonoid contents determined by the aluminum chloride method and that using the aluminum chloride method alone, it is possible to underestimate the content of total flavonoids and the total amount of phenolic compounds could be affected by the presence of some amino-acids and proteins in honey that can react with Folin-ciocalteu reagent (Wabaidur et al., 2020).

Table 3. Correlation among Mentha species based on quantitative traits.

Traits	Number of leaves	Leaf area	Leaf Thickness	Petiole length	Root length	Plant height	Stem diameter
Number of leaves	1						
Leaf areas	.876	1					
Leaf Thickness	.889	.698	1				
Petiole length	.256	.486	.475	1			
Root length	.250	.554	-.182	-.097	1		
Plant height	.910*	.608	.824	-.081	.037	1	
Stem diameter	.906*	.625	.764	-.149	.143	.993**	1

*. Correlation is significant at the 0.05 level; **. Correlation is significant at the 0.01 level.

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Table 4. Qualitative variations among four Mentha species.

Sr. No	Scientific name	Leaf blade	Leaf base	Leaf color	Leaf arrangement	leaf apex	leaf venation	Leaf odor	stem color	Leaf margin	flower color
1	*Mentha arvensis*	Ovate-Elliptic	Oblique	Dark green	Opposite	Broadly acute	Arcuate	Lemon	Dark purple	Serrate	Purple
2	*Mentha royleana*	Lanceolate	Cuneate	Light green	Opposite	Narrowly Acute	Pinnate	Pungent	Light green	Slightly serrate	White
3	*Menthe spicata*	Long elliptic	Obtuse (rounded)	Green	Opposite	Acute	Pinnate	Acrid	Green	Serrate	White
4	*Mentha Longifolia*	Ovate-Lanceolate	Cuneate	Green	Opposite	Broadly acute	Arcuate	Camphraceous	Green	Serrate	Lilac

Figure 2. Morphological variation in leaf among *Mentha* specie.

Table 5. Phenolic contents in leaf and root samples of Mentha species.

Sr. No.	Name of sample	Leaves	Roots
1.	Mentha arvensis	0.11385±0.03	0.13915 ±0.1
2.	Mentha *royleana*	0.16445 ±0.01	0.10925 ±0.33
3.	Mentha longifolia	0.14375 ±0.07	0.04025 ±0.03
4.	Mentha spicata	0.08855 ±0.22	0.14605 ±0.07

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Table 6. Flavonoid contents in leaf and root samples of Mentha species.

Sr No.	Sample name	Leaves	Roots
1.	Mentha arvensis	0.2003 ±0.44	0.2408 ±0.03
2.	Mentha spicata	0.0996±0.24	0.1354 ±0.34
3.	Mentha longifolia	0.1244 ±0.01	0.1032±0.04
4.	*Mentha royleana*	0.1397 ±0.56	0.0781±0.02

Antioxidant activity

Among the tested samples leaf extract of *Mentha longifolia* has the highest amount of antioxidant contents (96.67%). In leaf extract of *Mentha spicata* the amount of antioxidant contents was 92.09%. In leaf extract of *Mentha royleana* the amount of antioxidant contents was 82.74%. The lowest amount of antioxidant contents was in leaf extract of *Mentha piperita* that was 81.70%. Percentage inhibition in term of IC50 value for menthe species ranging from 4.67µl/ml-12.56µl/ml. highest IC50 value was observed in *Mentha longifolia* 12.5667µl/ml indicating the strong potential towards oxidative stress (Table 7). All menthe species are good sources of scavenging power with considerable amount of phytoconstitutes (Figure 5).

Antioxidant Activity in Root Samples of *Mentha* Species

Among the root samples *Mentha spicata* has the highest amount of antioxidant contents (93.55%). In root sample of *Mentha longifolia* the amount of antioxidant contents was 92.51 %. In root sample of Mentha *royleana* the amount of antioxidant contents was 86.48% (Table 8). The root sample of *Mentha arvensis* has the lowest amount of antioxidant contents (83.99%). Percentage inhibition in term of IC50 value for *Mentha* species in roots extract ranging from 5.78µl/ml-17.45µl/ml. Highest IC50 value was observed in *Mentha spicata* 17.45µl/ml indicating the significant oxidative potential (Figure 6). All menthe species are excellent in scavenging power with considerable role in oxidative stress.

Table 7. Antioxidant activity in leaf samples of Mentha species.

S. No.	Mentha species	Sample	Standard	IC50
1.	Mentha arvensis	81.70	90.111	4.67
2.	Mentha *royleana*	82.74 -----	90.111	6.54
3.	Mentha longifolia	96.67	90.111	12.56
4.	Mentha spicata	92.09	90.111	9.67

Table 8. Antioxidant activity in root samples of *Mentha* species.

Concentration mg/ml	Mentha species	Sample	Standard	IC50 values
1.	Mentha arvensis	83.99	90.111	5.78
2.	Mentha *royleana*	86.48	90.111	9.56
3.	Mentha longifolia	92.51	90.111	12.23
4.	Mentha spicata	93.55	90.111	17.45

In the current study, antioxidant activity of leaf and root extracts of Mentha was determined. Considerable amount of variation was observed among the tested samples. Leaf extract of Mentha longifolia exhibited the highest antioxidant activity.similarly strong DPPH radical scavenging avtivity of Mentha was reported by Singh et al. (2017). They made comparision on different plant parts and observed the highest antioxidant activity in leaves of Mentha species. DPPH is a stable organic free radical and present the ability to accept hydrogen radical or an electron. Mentha is the best antioxidant as compared to ascorbic acid and many other plants (Mahadevappa et al., 2014). Antioxidant activity among the different parts of the plant and among different plants varies depending on the function and mechanism of phenolic compounds (Smolik et al., 2007).

Cluster Analysis

Cluster analysis for 4 Mentha species was displayed in figure 3. Results showed that species were fall into two main clusters at Euclidean distance of 14. Cluster 1 consisted of Mentha longifolia and Mentha spicata. These two species shows close genetic distance based on phyto constitutes. Similarly cluster 2 fall under two species e.g Mentha arvensis and Mentha *royleana*. These two species have phytochemically close genetic distance. The species

fall in cluster 1 have great diversification in bioconstitutes compared to species in cluster 2. Variation among species based on phytoconstitutes would helpful in screening of best mentha species having potential toward oxidative stress. It helps to understand multidimensional datasets by observing similarities and dissimilarities in other clusters. Cluster analysis methods have a long history (Abramo et al., 2013). However, the first systematic work on clustering was performed by R. C. Tryon. Tryon's major area of interest concerned individual differences. Tryon had worked with Thurstone and was influenced by his important work in developing the methodology of factor analysis. However many studies investigate the relationships between bibliometric indicators on an individual level (Boczek et al., 2021; Costas et al., 2010). However, an analysis of both bibliometric output (Sabatier and Chollet, 2017) and grants budget data mainly uses data at an aggregated level (Wildgaard, 2016).

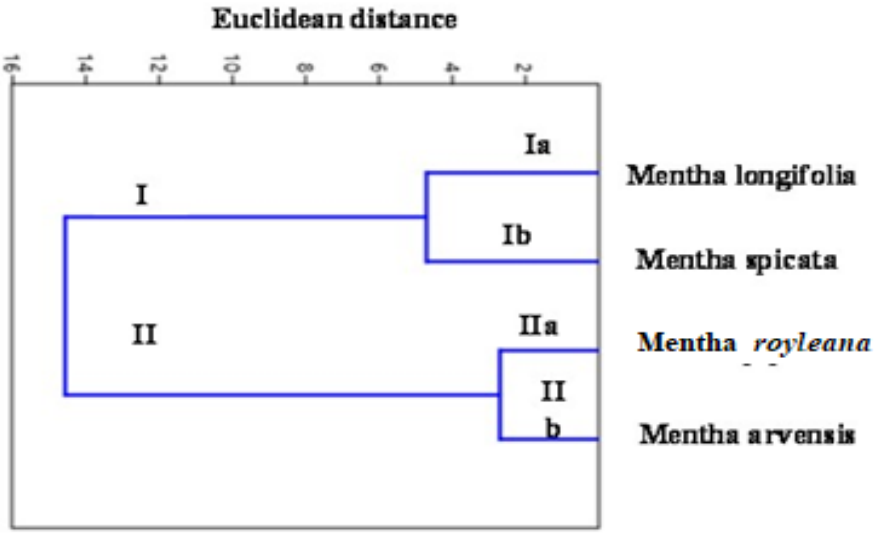

Figure 3. Cluster analysis of *Mentha* species based on phytoconstitutes.

Screeplot on the Basis of Cluster Analysis

Screeplot analysis of Mentha species were displayed in figure 4. Results showed that the studied species displayed variability fall into two main components based on phytochemical constitutes. Mentha longifolia and Mentha *royleana* found in compoment 1 while Mentha spicata and Mentha arvensis are found in component 2. These species dispersed away from genetic origin and showing great genetic magnitude. The falling of species apart from each other showing high magnitude of variability among them which confirmed species are highly diverse based on phytoconstitutes and has various amounts of phytoconstitutes in them. The result concluded the presence of variable amount of phytoconstitutes in mentha species which might be helpful for medicinal aspects. To calculate the appropriate genotypic variability existing among all studies clusters, a Ward's dendrogram was constructed (Sharif et al., 2020).The statistical implements could be used for the designation of other potential resources. The dendrogram resulting from Wards clustering demonstrates variation among the cluster. Many methods for comparing the number of groups do not allow the consideration of the simplest clustering solution. However, when empirically determining the number of clusters it is essential that the one-group solution be considered. Rules derived from maximum likelihood theory will allow this. An alternative approach is the GAP statistic (Tibshirani et al., 2001), which compares the quality of cluster solutions for different numbers of groups based on a given (heuristic) cluster criterion.

Straight Graph Description of Phytoconstitutes using Cluster Analysis

Straight graph representing that Strong antioxidant activity is found in *Mentha arvensis* (Figure 5). After *Mentha arvensis* the activity is located in Mentha *royleana* then in *Mentha longifolia*. *Mentha* spicata showed the lowest activity. The main frameworks for cluster analysis are probabilistic, partitioning, hierarchical and hybrid clustering (Wildgaard, 2016). In previous study, the two-step hierarchical method was adopted, which allows for the clustering of cases and variables and enables the simultaneous analysis of mixed scale data, e.g. nominal, ordinal and interval data. Nowadays, carrying out cluster analyses is relatively

straightforward. Most general-purpose statistical packages contain procedures for hierarchical and optimization clustering. Routines for model-based clustering are available in some general-purpose packages (e.g., mclust in R, (Fraley and Raftery, 2003), specialized latent classes and finite mixtures programs (e.g., LatentGOLD and MIXMOD), or modeling packages such as Mplus. In addition, there are a number of packages solely devoted to cluster analyses.

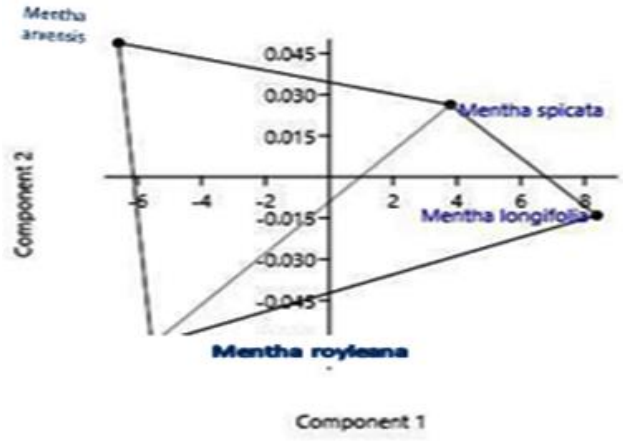

Figure 4. Screeplot representing diversity among mentha species based on cluster analysis.

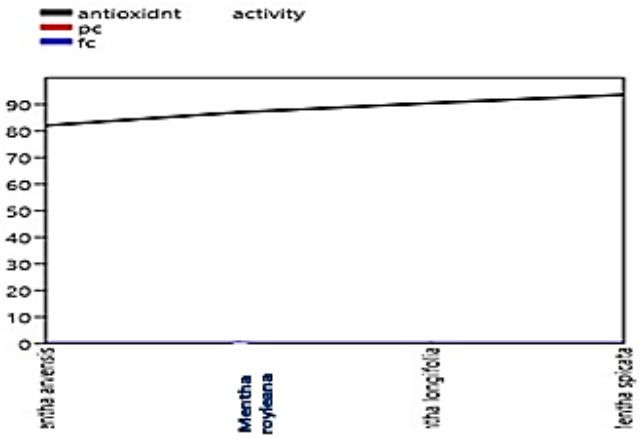

Figure 5. Antioxidant activity, PC, FC on the basis of cluster analysis.

CONCLUSIONS

This study presents sufficient and reliable information for successful identification and morpho-biochemical characterization of the *Mentha* species found in their natural habitat. The screening outcomes of Mentha spp also depicted that mentha spp possessed the considerable phytoconstitutes with good potential for oxidative reducing power that might be utilized for pharmaceutical and medicinal purpose**.**

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Author's contributions

TR supervise and design experiment, MS, IK and AE do experiment and wrote the final manuscript, MS interpret the results, AM Planning , SN Analyzing, BM, AA and DH preparing final draft. MFK revise the manuscript. All authors have read and permitted the published version of the manuscript.

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Conflicts of Interest

The authors declare there are no conflicts of

interest

REFERENCES

Abasi, F., Ahmad, I., khan, S.U., Ahmad, K.S., Ulfat, A., Khurshid, R., 2019. Estimation of genetic diversity in genus mentha collected from Azad Jammu and Kashmir, Pakistan. BioRxiv, 866111.

Abramo, G., Cicero, T., D'Angelo, C.A., 2013. Individual research performance: A proposal for comparing apples to oranges. Journal of Informetrics 7, 528-539.

Aflatuni, A., Uusitalo, J., Ek, S., Hohtola, A., 2005. Variation in the amount of yield and in the extract composition between conventionally produced and micropropagated peppermint and spearmint. Journal of Essential Oil Research 17, 66-70.

Araghi, A.M., Nemati, H., Azizi, M., Moshtaghi, N., Shoor, M., Hadian, J., 2019. Assessment of phytochemical and agro-morphological variability among different wild accessions of *Mentha longifolia* L. cultivated in field condition. Industrial Crops and Products 140, 111698.

Bibi, N., Shah, M.H., Khan, N., Al-Hashimi, A., Elshikh, M.S., Iqbal, A., Ahmad, S., Abbasi, A.M., 2022. Variations in total phenolic, total flavonoid contents, and free radicals' scavenging potential of onion varieties planted under diverse environmental conditions. Plants 11, 950.

Boczek, M., Hovana, A., Hutník, O., Kaluszka, M., 2021. New monotone measure-based integrals inspired by scientific impact problem. European Journal of Operational Research 290, 346-357.

Chang, C.-C., Yang, M.-H., Wen, H.-M., Chern, J.-C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10, 73-79.

Costas, R., Van Leeuwen, T.N., Bordons, M., 2010. A bibliometric classificatory approach for the study and assessment of research performance at the individual level: The effects of age on productivity and impact. Journal of the American Society for Information Science and Technology 61, 1564-1581.

Devi, A., Iqbal, T., Wani, I.A., Sharma, G., Verma, S., Noureldeen, A., Darwish, H., 2022. Assessment of variability among morphological and molecular characters in wild populations of mint [*Mentha longifolia* (L.) L.] germplasm. Saudi Journal of Biological Sciences 29, 3528-3538.

Erum, S., Naeemullah, M., Masood, S., 2012. Phenotypic variation among *Mentha* spp. Pakistan Journal of Agriculture Research 25, 55-62.

Fraley, C., Raftery, A.E., 2003. Enhanced model-based clustering, density estimation, and discriminant analysis software: MCLUST. Journal of Classification 20, 263-286.

Gracindo, L., Grisi, M., Silva, D., Alves, R., Bizzo, H., Vieira, R., 2006. Chemical characterization of mint (*Mentha* spp.) germplasm at Federal District, Brazil. Revista Brasileira de Plantas Medicinais 8, 5-9.

İşcan, G., Kirimer, N., Kürkcüoğlu, M.n., Başer, H.C., Demirci, F., 2002. Antimicrobial screening of *Mentha piperita* essential oils. Journal of Agricultural and Food Chemistry 50, 3943-3946.

Kohn, A.J., 1963. Type specimens and identity of the described species of Conus. I. The species described by Linnaeus, 1758–1767. Zoological Journal of the Linnean Society 44, 740-768.

Mahadevappa, N., Hanumantharaju, D.K., Suvarna, P.V., Moses, V., Chandrashekar, S., Gowda, S., 2014. Study on advanced application of mint oil. Journal of Advanced Scientific Research 5, 1-3.

Sabatier, M., Chollet, B., 2017. Is there a first mover advantage in science? Pioneering behavior and scientific production in nanotechnology. Research Policy 46, 522-533.

Salehi, B., Stojanović-Radić, Z., Matejić, J., Sharopov, F., Antolak, H., Kręgiel, D., Sen, S., Sharifi-Rad, M., Acharya, K., Sharifi-Rad, R., 2018. Plants of genus *Mentha*: From farm to food factory. Plants 7, 70.

Sevindik, M., 2018. Pharmacological properties of *Mentha* species. Journal Tradit Med Clin Nature 7, 259.

Sharif, I., Nazir, A., Shahzadi, E., Chohan, S.M., Sarwar, G., 2020. Influence of Sowing Dates on Cotton Growth (*Gossypium hirsutum*), Yield and Fiber Quality. Pakistan Journal of Agricultural Research 33, 866-871.

Shelepova, O.V., Tkacheva, E.V., Golosova, E.V., 2021. The history of the introduction of peppermint (*Mentha× piperita* L.) in Imperial Russia, BIO Web of Conferences. EDP Sciences, p. 00115.

Shinwari, Z.K., Sultan, S., Mehmood, T., 2011. Molecular and morphological characterization of selected *Mentha* species. Pakistan Journal of Botany 43, 1433-1436.

Singh, T., 1986. Mentha-Taxonomic studies as interpreted through cytology, genetics and phytochemistry. Indian Journal of Genetics and Plant Breeding 46, 198-208.

Singh, V., Thenua, O., Shivay, Y., 2017. Effect of phosphorus management on productivity of sunflower (*Helianthus annuus* L.). Progressive Research International Journal 12, 348-352.

Smolik, M., Rzepka-Plevneš, D., Jadczak, D., Sękowska, A., 2007. Morphological and genetic variability of chosen *Mentha* species. Herba Polonica 53, 90-97.

Tibshirani, R., Walther, G., Hastie, T., 2001. Estimating the number of clusters in a data set via the gap statistic. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 63, 411-423.

Wabaidur, S.M., Obbed, M.S., Alothman, Z.A., Alfaris, N.A., Badjah-Hadj-Ahmed, A.Y., Siddiqui, M.R., Altamimi, J.Z., Aldayel, T.S., 2020. Total phenolic acids and flavonoid contents determination in Yemeni honey of various floral sources: Folin-Ciocalteu and spectrophotometric approach. Food Science and Technology 40, 647-652.

Wildgaard, L., 2016. A critical cluster analysis of 44 indicators of author-level performance. Journal of Informetrics 10, 1055-1078.

Yang, X.-J., Dang, B., Fan, M.-T., 2018. Free and bound phenolic compound content and antioxidant activity of different cultivated blue highland barley varieties from the Qinghai-Tibet Plateau. Molecules 23, 879.

Yousef, R.M., El-Leel, O.F.A., Mohamed, S.Y., 2015. Comparative study for some *Mentha* species and varieties under sandy soil conditions. Journal of Applied Sciences 15, 355-366.

Zviniene, N., Pank, F., 1996. Data processing for numerical taxonomy in genus *Mentha* L. growing in Lithuania, Proc. Intl. Symp. Breeding research on medicinal and aromatic plants, Quedlinburg, Germany, pp. 103-107.