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Morphological, biochemical and elemental analysis of *Elaeagnus umbellata,* a multipurpose wild shrub from Pakistan

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Abstract

Elaeagnus umbellata Thunb. is a native multipurpose plant from Himalayan regions of Pakistan. The berries of the plant are rich in vitamins, flavonoides, essential oil, lycopene and other bioactive compounds. In order to compare various populations of *E. umbellata* for morphology and chemical composition, five populations from different areas of district Bagh were compared using plant and fruit characters. Chemical analysis of berries showed variation in vitamin C (13.8-16.9 mg/100 g), seed oil (5.7- 6.1%), oil in pulp (7.6-8.1%), reducing sugar (6.8-8.4%), non-reducing sugar (1.4-2.2%), protein (2.5-5.1%) and chlorophyll content (5.3-6.8%) in leaves, while the mineral element composition revealed high contents of potassium (175-375 ppm), sodium (20-40 ppm), calcium (70-110 ppm), magnesium (70-86.6 ppm), iron (78.5-95 ppm) and phosphorus (110-133 ppm). Significant variation in morphological characters including plant height, number of branches per plant, number and size of thorns, number of leaves, leaf area, plant canopy, stem girth, berry size and berry weight was also observed among different populations. The study established Pakistani *E. umbellata* berries as a good source of chemical and mineral elements. The high variation between different populations shows the potential of selecting desirable types for various defined purposes.

Key words: *Elaeagnus umbellata*, biochemical analysis, vitamin C, oil content, chlorophyll, intra specific variability

Introduction

Elaeagnus umbellata Thunb., a member of Elaeagnacea family also called cardinal olive, atumn olive or autumn elaeagnus (Dirr, 1998) is one of the valuable shrubs with inherent ability to grow under natural conditions of Himalayan regions of Pakistan. It is a common medicinal shrub found in wild at height of 1500 to 2000 m above sea level in Pakistan (Ahmed *et al.*, 2005). *E. umbellata* is a large spreading, spiny-branched shrub often obtaining 3.5 to 5.5 m height and 3.5 to 5.5 m width. The foliage is light green on top and silvery green on the bottom (Dirr, 1998). Leave are alternate and petiolated in small lateral clusters on twigs (Sather and Eckardt, 1987). Leaves are elliptic to ovate-oblong, 4-8 cm long, 1-2.5 cm wide, upper surface sparsely white lepidote, lower surface densely white lepidote, apex acute to sometime obtuse; petioles are 0.5-1.0 cm long, densely white lepidote. The drupes (fruits) are silvery with brown scales when immature, ripening to a speckled red in September to October (Sternberg, 1982). A mature plant can produce 0.9 to 3.4 kg of fruit per year, with the number of seeds ranging from 20,000 to 54,000 (Sather and Eckardt, 1987). *E. umbellata* is valued to prevent soil erosion and to attract wild life (Zarger, 1980).

E. umbellata berry is an excellent source of vitamins and minerals, especially vitamin A, C, E, flavonoids and other bioactive compounds. It is also a good source of essential fatty acids (Chopra *et al.*, 1986). *E. umbellata* fruit contains 69.4 g of moisture, 14.5 g of total soluble solids, 1.15 g of organic acids, 8.34 g of total sugar, 8.13 g of reducing sugars, 0.23 g of non-reducing sugars and 12.04 mg of vitamin C per 100 mg of fruit. The total mineral content of the fruit as represented by its ash is 1.045% (Parmar and Kaushal, 1982). It also contains lycopene, β-carotene, lutein, phytofuluene and phytoene. The lycopene content per 100g ranged from 10.09 to 53.96 mg in fresh fruit from the naturalized plants and from 17.87 to 47.33 mg in the cultivars with red-pigmented fruit. Cultivar with yellow fruit has only 0.82 mg/100 g fresh weight of fruit. In contrast, fresh tomato fruit which is the major dietary source of lycopene, has lycopene content of 0.88 to 4.20 mg 100 g^{-1}. This newly identified source of lycopene may provide an alternative to tomato as a dietary source of lycopene and related carotenoides (Kohlmeier *et al.*, 1997; Fordham, 2001). Lycopene is widely believed to protect against myocardial infection (Kohlmeier *et al.* 1997) and various forms of cancer (Clinton, 1998), including prostrate cancer (Giovannucci, *et al.*, 1995). *E. umbellata* has potential as a deterrent to heart disease, cervix and gastrointestinal tract cancer (Matthews, 1994). Fruits can be used in raw or cooked form (Hedrick, 1972). Fruit is juicy, pleasantly acidic and can also be made into jams or other preserves (Reich, 1991).

The present invesitigation was undertaken to study the variability in morphology and chemical composition of *E. umbellata* populations from different areas of district Bagh of Pakistan in its natural habitat.

Materials and methods

Morphological analysis: Fruits from five morphologically different populations of *E. umbellata* were collected from district Bagh and stored at -20°C for biochemical analysis. The morphology of *E. umbellata* was compared for five different natural populations from District Bagh. There were three replications and five representative plants from each replication for comparison. The plants were randomly selected and tagged indicating the

population and replication. In addition five branches were randomly tagged for other characters *i.e.* average size of main branches on the main stem, average number of thorns on the main branches, average number of fruit bunches per branch, average number of fruit berries on fruiting bunches, average number of berries on main branches, average number of leaves on the main branches and the leaf area. The data was analyzed statistically by following the method of Steel and Torrie (1980).

Biochemical analysis

Ascorbic acid content of berries: Ascorbic acid was determined using phenol indophenol dye method (A.O.A.C., 1984) in 10 g of the fresh berries/fruits, blended with metaphosphoric-acetic acid extracting solution. 5 ml of the filtrate extract were titrated with standard 2,6-dichlorophenol indophenol to pink end point. Experiment was repeated three times.

Lipid content of berries: Oil contents from the berries/fruits of different plants were used for the analysis of lipid content according to the standard methods (AACC, 1983). Samples were dried in an oven at 105 °C for 6-12 hours. 10 g of dried sample was used for extraction of oil in Soxhlet apparatus (30-40 °C) for 6 hours using diethyl ether as solvent. The solvent was removed under vacuum and the residual oil was dried over anhydrous Na_2SO_4. Experiment was repeated three times. analytical grade chemicals were used for extraction of oil.

Chlorophyll content: Chlorophyll was extracted from 1 cm^2 area of fresh leaves of *E. umbellata*. An extraction of chlorophyll was done in 80% acetone and absorbance was measured at 663 and 645 nm with UV-VIS spectrometer for chlorophyll a and b, respectively. Chlorophyll contents were calculated according to the method of Arnon (1949).

Protein content: Crude protein was estimated by Kjeltec system, using sulfuric acid and a mixture of potassium sulphate and copper sulphate for digestion. For distillation 40% NaOH and boric acid were used. N/100 sulphuric acid was used for titration.

Sugar: Reducing and non-reducing sugars were determined by the method given in A.O.A.C. (1984). Diluted juice was titrated against Fehling's solution till the appearance of brick red precipitates. For non-reducing sugar 1N HCl was used for hydrolysis.

Elemental analysis: Mineral content was determined from 5 g of the fruit/berry. Ten ml of the conc. HNO_3 was added to each sample in a digestion flask and allowed to stand overnight. The samples were heated carefully until the production of brown nitrogen (IV) oxide fume has ceased. The flasks were cooled and 2-4 ml of 70% perchloric acid was added. Heating was continued till the solution became colourless. The solutions were then diluted to 50 ml with distilled water. Mineral extract was then used for the estimation of minerals.

The concentrations of calcium, magnesium, iron were determined on Perkin-Elmer atomic absorption spectrometer (Model AA-2380). The accuracy of the measurement was checked by standard addition.

Phosphorus was determined on a spectrophotometer by reacting the sample with ammonium molybdate solution by the method described by Oser (1976). KH_2PO_4 solution was used as a standard. Five ml of mineral extract was treated with H_2SO_4, 0.25% ammonium vandate and 5% ammonium molybdate. The yellow colour complex of molybdenum was measured at 470 nm in Spectronic 20-D (Milton Roy Company) spectrophotometer. Sodium and potassium contents were measured by flame photometer (Model 410) after dilution with deionized water (1:10) .

Statistical analyses: Significance of mean sum of squares due to morphological characters was tested using ANOVA. The results of biochemical analysis were expressed as a mean of three determinations ±SD.

Results and discussion

Morphological characters: Mean values of some morphological characters among the populations of *E. umbellata* are compared in Table 1. Plant height was 261.7(V_4) to 363.68 cm(V_1). V_5 had the lowest (656.16 cm) while V_2 had the highest (727.5 cm) value for plant canopy. Stem girth ranged from 13.17 (V_2) to 17.59 cm (V_5). The size of thorns on stem ranged from 1.47 to 7.86 cm. The number of branches per plant ranged from 47.33 to 54.0. Number of thorns on branches ranged from 3.66 and 8.66. The number of leaves per branch ranged from 98.0 (V_5) to 194.66 (V_1). Leaf area ranged from 6.46 to 9.25 cm^2. 1000-berry pulp weight ranged from 21.56 g (V_5) to 27.56 g (V_4). V_1, V_5 and V_4 had the 1000-berry pulp weight of 22.86, 24.0 and 26.73 g, respectively. The weight of 1000 seeds was found to be in the range of 170 to 183 g. Diameter of berries was in the range of 0.54-0.62 cm whereas berry length was 0.78-0.87 cm.

Biochemical analysis: The vitamin C content ranged from 13.8 to 16.9 mg 100 g^{-1} among different samples of *E. umbellata* (Table 2). The oil content in the seeds of *E. umbellata* was in the range of 5.91 to 6.1% (Table 2). Maximum oil was reported in V_1 (6.1%) while minimum oil in V_2 (5.91%). The oil in pulp was also extracted and was found in the range of 7.60 to 8.06% (Table 2). Maximum oil was reported in V_1 (8.06%) while minimum in V_2 (7.60%). Thus, the pulp of *E. umbellata* had greater quantity of fatty oil as compared to the seed. The amount of chlorophyll in the leaves of *E. umbellata* ranged from 5.3 to 6.8 mg cm^{-2}. Maximum chlorophyll was reported in V_1 (6.8 mg cm^{-2}) while minimum in V_5 (5.3 mg cm^{-2}).

E. umbellata berries were found sweet with reducing sugar content of 6.8-8.4 g 100g^{-1}. Maximum amount of reducing sugar was reported in V_2 while minimum in V_1. Quantity of non-reducing sugars content was reported in the range of 1.4-2.2 g 100g^{-1}. Protein content of *E. umbellata* berries was found in the range of 2.5 to 5.1%. V_5 had the lowest (2.5%) while V_1 had the highest amount of protein (5.1%) in pulp.

When the populations of *E. umbellata* were compared on the basis of mineral elements a wide range of variation was observed (Table 3). Potassium was the most abundant of all the elements investigated in berries or juice. Mineral element composition revealed high content of potassium (175-375 ppm), sodium (20-40 ppm), calcium (70-110 ppm), magnesium (70-86.6 ppm), iron (78.5-90 ppm) and phosphorus (110-133 ppm).

Table 1 indicated significant variation ($P < 0.05$) among all of the traits but for berry length the difference was non-significant. It implies that the populations of *E. umbelatta* from different areas of district Bagh vary greatly among themselves. Plant height and width (canopy) of 3.5 to 5.5 m, described in earlier studies from

Table 1. Comparison of mean values and their standard deviation for some morphological traits in *E. umbellata* populations

Traits	MSS	V_1	V_2	V_3	V_4	V_5
Plant height (cm)	7338.4*	261.79±16.60	307.66±8.67	310.13±10.10	363.68±6.12	351.6±13.70
Plant canopy (cm)	2424.0*	713.6±25.10	727.5±18.20	721.26±13.10	702.66±23.60	656.16±35.10
Stem girth (cm)	0.856*	16.19±0.71	13.17±0.72	14.33±1.10	15.55±0.61	17.59±0.65
Number of thorns per stem	12.76*	8.33±0.01	8.66±0.07	5.66±0.10	3.66±0.15	6.0±0.09
Size of thorns on stem (cm)	0.236*	5.16±0.04	2.91±0.02	7.86±0.05	3.19±0.03	1.47±0.01
Number of branches per plant	25.067*	47.33±10.10	54.0±9.50	52.0±7.10	54.0±5.60	54.0±9.00
Size of branches per plant (cm)	101.054*	64.46±3.84	52.73±6.64	50.26±4.15	60.91±4.96	57.51±11.10
Number of leaves per branch	5019.3*	194.66±16.10	106.66±12.10	107.33±9.10	102.33±5.10	98.00±15.70
Leaf area (cm^2)	3.866*	9.25±0.20	7.84±0.11	6.43±0.15	7.46±0.12	6.56±0.21
Number of berries per branch	2441.433*	39.67±0.51	28±1.10	100±6.50	74.67±3.10	58.33±2.10
Number of berries per bunch	108.93*	2.66±0.01	3.0±0.02	4.0±0.10	3.66±0.03	3.0±0.04
1000 berries pulp weight (g)	18.981*	28.60±5.10	24.00±4.10	26.70±10.20	7.40±2.50	21.56±1.50
1000 seed weight(g)	94.132*	173.60±17.10	169.64±15.20	176.73±14.60	183.03±13.02	181.83±11.10
Diameter of fruit berries (cm)	0.0029*	0.55±0.04	0.58±0.02	0.62±0.05	0.54±0.02	0.60±0.01
Length of fruit berries (cm)	0.0008^{NS}	0.78±0.20	0.87±0.10	0.84±0.15	0.82±0.24	0.79±0.10

* Significant (*P*=0.05), NS= Non-significant, MSS=Mean sum of squares

Table 2. Comparison of vitamin C, oil content, sugar, protein and chlorophyll content in *E. umbellata* populations

Biochemicals	V_1	V_2	V_3	V_4	V_5
Vitamin C (mg $100g^{-1}$)	16.9 ±0.10	15.6±0.10	13.8±0.12	16.2±0.05	14.4±0.30
Oil in seed (g $100g^{-1}$)	6.1±0.21	5.91±0.31	6.06±0.01	5.84±0.15	5.7±0.50
Oil in pulp (g $100g^{-1}$)	8.06±0.15	7.60±0.08	7.63±0.17	8.11±0.01	7.62±0.90
Chlorophyll in leaves (mg cm^{-2})	6.8±1.10	5.4±0.6	7.1±1.05	6.5±1.10	5.3±0.60
Reducing sugar (g $100g^{-1}$)	7.4±1.23	8.4±0.12	8.1±0.21	7.9±0.14	6.8±0.52
Non-reducing sugar (g $100g^{-1}$)	2.2±0.14	1.7±0.04	1.4±0.15	1.8±0.17	2±0.53
Protein (g $100g^{-1}$)	5.1±0.30	3.9±0.04	4.12±0.09	3.2±0.01	2.5±0.02

Table 3. Elemental analysis among different populations of *E. umbellata* populations

Elements (ppm)	V_1	V_2	V_3	V_4	V_5
K	175±1.60	375±1.65	240±0.14	340±0.23	185±0.10
Na	30±0.01	25±0.01	30±0.02	20±0.01	40±0.03
Ca	80±0.20	100±0.50	98±0.15	70±0.03	110±0.16
Mg	86.6±0.15	70±0.20	72.4±0.90	83.9±0.24	75.5±0.31
Fe	78.5±0.21	85±0.14	86.1±0.21	95±0.13	90.05±0.05
P	131±0.11	128±0.04	133±0.12	115±0.05	110±0.12

North America (Dirr, 1998), is little more than the present investigation. However, such variation may be due to population specificity and the adaptation of plants in different environmental or soil conditions (Szafoni, 1991). Dirr (1998) measured the length and width of the leaves as 4-8 cm long, 1-2.5 cm wide. His results are similar to our results with some variation among the populations (Table 1). Our results indicated less number of seeds/ kg of berries compared to the results of earlier investigations (Sather and Eckardt, 1987), where 1 kg fruits had about 20,000 seeds. This variation may be due to the larger size of fruits having more pulp in our investigation. The populations compared were also variable in the characters indicating the potential for future improvement.

Earlier studies indicated 12.04 mg of vitamin C 100 g-1 of fruit (Parmar and Kaushal, 1982), which was little lower as compared to this investigation. This variation may be due to harvesting of fruits at different time or the climatic factors. The presence of essential oils in fruits and flowers has been indicated in the earlier literature (Potter, 1995) but the actual quantity has not been mentioned. The present investigation indicated large amount of fatty oil in seed as well as in the pulp of the fruit. The therapeutic value of *E. umbellata* against heart diseases and others may be due to the presence of high amount of oil in fruits. The plant oil and the phytosterols are known to have anticoagulant properties which are highly suitable for lowering the blood cholesterol and angina (Fordham, 2001). Parmar and Kaushal (1982) reported reducing and non-reducing sugar content of berries as 8.13 and 0.23%, respectively. This study reported almost equal amount of reducing sugar (8.4%). However, the amount of non-reducing sugar was found quite high as 2.2%. The high amount of sugar makes this berry equally good for eating as well as its use in other food products like jams, jellies, chocolates etc. *E. umbellata* berry was also found rich in protein (5.1%) and there use should therefore, be encouraged.

Most of the fruits contain low amount of minerals but *E. umbellata* berry was found excellent source of minerals (Table 3). Potassium was most abundant among all the minerals analyzed. Variations in content of all the elements studied were wide. Differences may originate from the natural contents of elements in the soil as well as contamination in both soil and air.

The investigation although was of preliminary nature but provided a source of basic information and in the establishment of *E. umbellata* in the mountainous areas of Kashmir where the rapid soil erosion has become an alarming threat. This shrub can effectively control soil erosion and its biochemical significance can make it suitable plant of economic importance to the poor

masses of the region. The medicinal components of berries will provide very cheep raw material for national and international pharmaceutical industries benefiting the humanity at large.

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