Biological Sciences

Pak. J. Sci. Ind. Res. 2009 **52** (5) 260-263

Seed Oils of Pakistani Wild species of Umbelliferae Family: *Ducrosia anethifolia, Bunium persicum, Bunium cylindricum* and *Ammi majus*; as Potential Industrial Raw Material

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(received February 27, 2008; revised August 26, 2009; accepted August 26, 2009)

Abstract. Seed oils from *Ducrosia anethifolia* (8.7%), *Bunium persicum* (16.1%), *Bunium cylindricum* (15.1%) and *Ammi majus* (7.78%) growing wild in Pakistan were studied for their fatty acid composition. GC and degradative oxidation techniques revealed that the seed oils contained 58.8%, 43.2%, 27.3% and 38.56% petroselinic acid, respectively. Fatty acids mainly consisted of oleic, linoleic, linolenic and palmitic acids with minor amounts of other saturated fatty acids. Petroselinic acid was determined by degradative oxidation of the C_{18} monoenoic ester and GC of the oxidized product esters. These species with high oil content and high percentage of petroselinic acid can be used as raw material source in soap and chemical industries.

Keywords: umbelliferae, seed oil, fatty acids, petroselinic acid, *Ducrosia anethifolia*, *Bunium persicum*, *Bunium cylindricum*, *Ammi majus*

Introduction

Ducrosia anethifolia, Bunium persicum, Bunium cylindricum and *Ammi majus* are herbs of the family Umbelliferae. These plants abundantly grow wild in areas such as Gilgit, Swat, Hazara, Zargon and the province of Baluchistan, while *Ammi majus* has been successfully cultivated in the Punjab, Baluchistan and North West Frontier Province of Pakistan. These plants and their roots have been widely used in medicines as carminative, astringent, diuretic, expectorant, anthelmintic, in dental preparations, cosmetics and as insecticides. They are also used in many food products as condiments, component of various flavouring agents used in beverages and find extensive use in curry powders. The seeds are also reported to possess hi-cough curing properties. The medicinal values of *A. majus* have also been described in the old Arabic literature, where the seeds of the plant have long been used for the treatment of leucoderma (Dymock, 1972).

The oils extracted from the cultivated species of *Celery, Daucus, Apium graveolens, Feniculum vulgare* and *Cuminium cyminum* have already been studied for their fatty acid compositions (Hilditch and Williams, 1964).

The seed oils of *D. anethifolia*, *B. persicum*, *B. cylindricum* and *A. majus* wild species have already been extracted and chemically evaluated. The fatty acid profiles of these seed oils are supposed to be similar to that of other cultivated members of this family as indicated in the earlier studies on Celery, *Daucus carota, A. graveolens, F. vulgare* and *C. cyminum* (Waheed *et al*., 2000, Mallet *et al*., 1990).

Predominant occurence of petroselinic acid, as the major constituent of triglycerides, is established in both wild and cultivated species but the percentage of this particular fatty acid has considerable variations in the seed oils of different members of the Umbelliferae family. These variations may be due to changes in climatic conditions, geographical variations of the soil and the health of the plants (Waheed *et al*., 2000).

Kleiman *et al*. (1969) and Hilditch and Williams (1964) reported that the celery seeds contained 20% oil with 66% petroselinic acid in it. Further studies of Mallet *et al*. (1990) on *D. carota, A. graveolens, F. vulgare* and *C. cyminum* showed that the seeds contained 17.3%, 16.7%, 13.9% and 18.4% oil, respectively. These seed oils also contained a high percentage of petroselinic acid, being 63.5%, 61.6%, 60.6 and 49.0%, respectively. These four species are expected to be cultivated as crops and have the potential of becoming a source of essential and fixed oil-bearing crops.

In view of the high percentage of oil with high percentage of petroselinic in the members of Umbelliferae family, it was planned to study the seed oil compositions of wild growing species of *Ducrosia anethifolia*, *Bunium persicum*, *Bunium cylindricum* and *Ammi majus*.

Materials and Methods

Fresh seeds of *D. anethifolia*, *B. persicum*, *B. cylindricum* and *A. majus* were obtained from the market in the months of

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May and June for extraction of oil. The chemicals used for this study were of analytical grade and the reagents used were prepared according to the standard methods of AOAC (2000).

Extraction of oil. Dried seeds of *D. anethifolia* (Gwartkh), *B. persicum* (Zira siyah), *B. cylindricum* (Siah zira khar) and *A. majus*, after extraction of essential oils, 100 g each (steam distillation), were powdered and subjected separately to extraction of oil in Soxhlet extractor (AOAC, 2000) with normal hexane as the solvent. The oils so obtained were dried over anhydrous sodium sulphate and the solvent was removed under vacuum. Oil percentage was determined on dry weight basis.

Composition of seed meals and physicochemical value of the oils. The seed meals, obtained after oil extraction, were freed from the solvent at 70 °C under vacuum and were then analyzed for protein, moisture, ash, fibre and carbohydrate contents by the method of AOAC (2000) and Solelo *et al*. (1995). Physicochemical values such as refractive index, specific gravity, saponification value and iodine value of the oil were also determined by standard methods (AOAC, 2000). Mineral composition of ash was estimated by using atomic absorption spectrophotometric technique (El-Gendi, 1988; Chowdhury *et al*., 1983).

Chromatographic analysis. The seed oils were separately saponified with 0.5 N alcoholic potash and extracted with diethyl ether to remove the unsaponifiable matter. Fatty acids were then obtained from the soap by acidification with 4 N sulphuric acid followed by extraction with petroleum ether. The ether extract was washed thrice with distilled water, and dried over anhydrous sodium sulphate (AOAC, 2000). Methyl esters of the liberated fatty acids were prepared by adding 1-2 ml of methylated mixture of borontrifluoride in screwed test tubes containing fatty acids. The tubes were heated in boiling water bath for half an hour, followed by cooling and extraction of esters with *n*-hexane and analysed by GC and thin layer chromatography (Kumar and Sunoda, 1978; Morrison and Smith, 1964). Fatty acid esters were separated on glass plates (20 × 20 cm) coated with 0.5 mm thick layer of 20% $AgNO_3$ in silica gel using 50:50 benzene/chloroform mixture as the developing solvent. The ester bands scraped from the plates, were recovered from the adsorbent by extraction with diethyl ester. The solvent was removed by rotary evaporator on water bath.

The esters obtained were oxidized by the modified Von-Rudloff's method, (Hamilton and Raie, 1972). The short chain methyl esters of adipic and lauric acids were estimated by gas chromatography (GC). GC analysis was carried out with a GC-14A gas chromatograph (Shimadzu) fitted with hydrogen gas flame ionization detector and data processor. A PEG capillary column (25 m x 0.2 m.m. i.d.) was used and the column temperature was maintained at 180 °C for fatty acid methyl esters; for adipic and lauric acid methyl esters, the column was operated with temperature programming from 150 to 180 °C. The injection and detector temperatures were maintained at 250 °C and 300 °C, respectively. Flow rate of carrier gas (nitrogen) was 20 ml/min at split ratio of 1:50. Identification of components was based on their retention times as compared with those obtained for methyl esters of known fatty acids analysed under the same conditions.

Results and Discussion

The seeds of *D. anethifolia, B. persicum, B. cylindricum* and *A. majus* species yielded about 8.7%, 16.1%, 15.4 % and 7.78% oil, respectively. Seed meals were subjected to chemical treatment for evaluation of protein, moisture, ash, fibre and carbohydrate contents (Table 1).

The physicochemical characteristics, refractive index, specific gravity, iodine value, saponification value, saponifiable and unsaponifiable matter of the four oils as determined experimentally are presented in Table 2. Data for the fatty acid composition of the four species is presented in Table 3. GC data of these esters revealed the presence of high percentage of petroselinic acid in the analysed four species. The results are almost similar to those reported for other species of the plants of the family Umbelliferae, (Klieman and Spencer, 1982; Hilditch and Williams, 1964). Ash content was further evaluated for the mineral profile; results are shown in Table 4.

Physicochemical characteristic of *D. anethifolia, B. persicum, B. cylindricum* and *A. majus* seed oils have been found to be similar in all the species studied with a little variation in the oil percentage. The residual meals obtained after extraction of the fixed oils were analysed to determine their chemical composition for protein, moisture, ash, fibre and carbohydrate contents (AOAC, 2000) and the results are shown in Table 1. Seed meals of *D. anethifolia, B. persicum, B. cylindricum* and *A. majus* contained 17.40%, 18.40%, 22.84% and 18.94% protein and 11.89%, 8.05%, 7.85% and 10.74% fibre, respectively, whereas carbohydrates were present in high amounts being 47.73%, 48.48%, 45.05% and 49.12%, respectively.

Table 1. Chemical composition of seed meals of the plants

Species	Protein	Moisture	Ash	Fibre	Carbohydrate	Total
D. anethifolia	17.40	7.38	8.9	9.89	47.73	91.3
B. persicum	18.40	5.10	3.86	8.05	48.48	83.89
B. cylindricum	22.84	4.95	3.91	7.85	45.05	84.6
A. majus	18.94	5.02	8.39	10.74	49.12	92.21

Table 4 shows the mineral contents of these species determined by atomic absorption photometry, with high quantities of K, Mg and Ca, whereas other elements Na, Fe, Cu, Mn, Zn and P were present in moderate quantities. The results indicate that *D. anethifolia, B. persicum, B. cylindricum* and *A. majus* meals are rich sources of proteins, carbohydrates and minerals. These can also be used as cattle feed. These results are similar to those reported by the earlier workers (El-Gendi, 1988; Hans, 1969; Miller, 1951). Many of the Umbelliferae plants have already been cultivated for food and food additives and the wild species under study have also been cultivated successfully (Bhatty *et al*., 1977). These species have the potential for use as industrial crops as they have appreciable amounts of oil contents with high petroselinic acid content.

Seed oils of the four species contained 69.72-84.50% unsaturated, 11.48-21.96% saturated and 4.01-5.29% unidentified fatty acids. Petroselinic acid (PA) is the general characteristic acid of the seed oils of species of Umbelliferae, as reported by Mallet *et al*. (1990) and Prasad *et al*. (1987). These acids were separated on thin layer chromatographic plates into unreacted methyl esters of monoenoic acid and short chain fatty acids; nonanoic and dodecanoic acids gave percentages of positional isomers as petreroselinic acid (PA) and oleic acid (Table 3). It is indicated that the amounts of monoenoic acid 66.89%, 55.05%, 44.7% and 61.46% in the seed oil of *D. anethifolia, B. persicum, B. cylindricum* and *A. majus*, respectively, are the sum of petroselinic and oleic acids, being 58.8% and 8.09%, 44.2% and 10.85%, 33.3% and 11.4% and 38.56% and 22.9% in their seed oils, respectively. These results are almost similar to those reported in the literature determined by other techniques (Mallet *et al*., 1990). Lenoleic acid is present in significant amounts in the four species (13.37%, 15.02%, 22.02% and 21.27%, respectively), while in three species linolenic acid is present in small amounts (1.67%, 2.67% and 1.77% in *D. anethifolia, B. persicum* and *A. majus*, respectively). But an appreciable amount of linolenic acid (11.0%) was present in *B. cylindricum*. These results are very close to the results reported earlier by Klieman *et al*. (1969).

Significant amounts of palmitic acid and other short chain saturated fatty acids were present in all the seed oils of these four species, but distribution is different in all the individual species (Table 3). In the earlier studies, $C_{16:1}$ was reported in

Table 2. Physicochemical evaluation of seed oils

Species	Oil content (on dry weight basis)	Refractive Index	Specific gravity	Iodine value	Saponification value	Unsaponifiable matter
D. anethifolia	8.70	1.4693	0.9075	92.50	138.9	1.99
B. persicum	16.10	1.4738	0.8992	112.1	152.5	2.15
B. cylindricum	15.40	1.4694	0.9199	120.6	137.85	2.51
A. majus	7.78	1.4660	0.9087	98.6	175.5	2.01

Table 3. Fatty acid composition of seed oils obtained by GC

Species	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}6$*	$C_{18:1}9$**	$C_{18:2}$	$C_{18:3}$	Unidentified
D. anethifolia	—	3.34	2.17	7.36	1.00	58.8	8.09	13.37	1.67	4.21
B. persicum	2.25	3.38	5.63	9.01	1.69	44.2	10.85	15.02	2.67	5.25
B. cylindricum	2.58	2.92	2.75	7.34	1.83	33.3	11.4	22.02	11.0	4.85
A. majus	1.18	1.77	1.44	5.91	1.18	38.56	22.9	21.27	1.77	4.01

* = petroselinic acid; ** = oleic acid

Table 4. Mineral contents of seed meals

	Elements on dry weight basis (%)								
	K (g)	Mg (g)	Ca (g)	Na (mg)	Cu (mg)	Fe (mg)	Mn (mg)	Zn (mg)	P (mg)
D. anethifolia	1.21	1.30	0.41	33.85	28.03	11.67	0.15	3.12	0.43
B. persicum	0.99	1.13	0.38	37.78	21.49	13.04	0.22	2.6	0.26
B. cylindricum	1.38	1.24	0.43	29.07	19.26	12.29	0.28	3.01	0.48
A. majus	1.02	1.39	0.32	0.36	20.12	10.98	0.31	2.9	-

trace amounts in some Umbelliferae species by Rankova *et al*. (1957) and Pearson (1970), but the present study did not show its presence in any of these species. The presence of higher percentage of petroselinic acid (PA) as compared to oleic acid is in agreement with the earlier investigations showing PA as a characteristic fatty acid of this family (Hamilton and Raie, 1972). The percentages of petroselinic acid (PA) in seed oils of these species as determined here are lower than those claimed by Kleiman and Spencer (1982), which may be attributed to the differences in climatic conditions. However, it is observed that, by and large, the occurrence of PA is the dominant feature of the fixed oil of members of Umbelliferae. Minor variations in its content as the constituent of the glycerides is attributed to the climatic as well as soil conditions of various regions. It can be thus inferred that these plants with high quantity of petroselinic acid may be used as potential raw material for the industry (Merck, 2006; National Research Council US, 2000).

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