

CHEMICAL EVALUATION OF THE GENUS ACACIA OF PAKISTAN
Part-V. Composition of Lipid Classes of *Acacia cyanophylla* Lindl., *Acacia tortilis* Hayne and *Acacia victoriae* Benth

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Seed lipids of *Acacia cyanophylla* Lindl. (9.8%), *A. tortilis* Hayne (6.0%) and *A. victoriae* Benth. (5.3%) were examined for lipid classes and their fatty acid compositions. All the seed lipids were found to be rich in neutral lipids (64.1- 72.8%). Triglycerides constituted 75.4, 41.8 and 75.7% of the neutral lipids of *Acacia cyanophylla*, *A. tortilis* and *A. victoriae* respectively. The GC studies showed the presence of lauric (1.9, 0.0, 2.5%), myristic (2.8, 0.0, 1.2%), palmitic (22.1, 16.5, 18.3%), palmitoleic (3.6, 0.0, 2.5%), stearic (5.5, 8.2, 8.7%), oleic (30.4, 37.0, 36.5%), linoleic (29.1, 32.9, 20.5%), linolenic (2.8, 2.3, 4.8%), arachidic (0.8, 1.0, 0.9%) and behenic acids (1.0, 2.1, 4.1%) in total lipids of *A. cyanophylla*, *A. tortilis* and *A. victoriae* respectively. The presence of lignoceric acid was also observed in the seed lipids of *A. cyanophylla*.

Key words: Leguminosae, Genus *Acacia*, Lipid Composition.

Introduction

Acacia cyanophylla Lindl., *A. tortilis* Hayne, and *A. victoriae* Benth. are among the introduced species of *Acacia* in Pakistan. These species show excellent survival characteristics in arid areas and serve as the major fodder for sheep, camel, cattle and other domestic livestock in these areas. Their pods and foliage, both are palatable to stock [1]. *A. victoriae* also exude a clear and tasteless gum which have good qualities for use in foods and industry [2].

The fatty acids composition of the seed oils of *A. tortilis* of Indian and Rhodesian origins were reported by Banerji [3] and Gunstone *et al.* [4] respectively, but the result of these studies were not compatible with each other. There has been no work reported in the literature on the seed oils and their fatty acid compositions of *A. cyanophylla* and *A. victoriae*. The present investigations are in continuation of our previous work [5] in search of new sources of commercially important lipids.

Materials and Methods

The seeds of *A. cyanophylla* Lindl., *A. tortilis* Hayne, and *A. victoriae* Benth. were collected during the year of 1988 from the plantation of Forest Research Institute, Peshawar, Pakistan. The three month old seeds from the date of collection were subjected to the proximate analyses. The results are given in Table 1. The ashes of the seeds were also analyzed for cadmium, copper, lead, potassium, sodium and zinc by the official method of AOAC using Atomic Absorp-

tion Spectrophotometer and the results are given in Table 1. The seed lipids were extracted with a mixture of chloroform-methanol (2:1 v/v) according to the procedure of Folch *et al.* [6]. The physico-chemical characteristics of the seed lipids were determined and tabulated in Table 2. The lipids were fractionated into different lipid fractions by TLC (Table 3) and fatty acid compositions were determined according to the procedure referred in our previous papers [5,7]. Percent peak areas are quoted as composition percent weight in Tables 4 - 6.

TABLE 1. ANALYTICAL DATA ON SEEDS OF *ACACIA* Species.

| | <i>Acacia cyanophylla</i> | <i>Acacia tortilis</i> | <i>Acacia victoriae</i> |
|-----------------------------|---------------------------|------------------------|-------------------------|
| Period of collection (year) | 1988 | 1988 | 1988 |
| Weight of 100 seeds (g) | 3.27 | 4.85 | 3.87 |
| <i>Proximate analysis</i> | | | |
| Moisture (%) | 8.40 | 8.80 | 7.10 |
| Dry matter (%) | 91.60 | 91.20 | 92.90 |
| Ash (%) | 5.30 | 5.20 | 4.71 |
| Fat (%) | 9.65 | 5.63 | 5.10 |
| Crude fibre (%) | 10.89 | 10.50 | 9.63 |
| Crude protein (%) | 27.30 | 32.31 | 25.52 |
| Nitrogen free extract (%) | 46.86 | 46.36 | 55.04 |
| <i>Trace element</i> | | | |
| Cadmium (ppm) | 14.00 | 9.20 | 7.20 |
| Copper (ppm) | 5.60 | 8.60 | 6.30 |
| Lead (ppm) | Nil | 0.50 | Nil |
| Potassium (ppm) | 13,000.00 | 17,300.00 | 14,200.00 |
| Sodium (ppm) | 6.30 | 60.10 | 35.10 |
| Zinc (ppm) | 19.20 | 12.00 | 16.80 |

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Results and Discussion

The seeds of *A. Cyanophylla*, *A. tortilis* and *A. victoriae* were analyzed for their proximate composition. The moisture content of the seeds ranged from 7.1 to 8.8%. All the seeds had good percentage of proteins. The highest percentages of fat and crude fibre were found in *A. cyanophylla* seeds. The analyses of the ashes of the seeds for trace elements showed the seeds to be rich in potassium (13,000-17,300 ppm) but low in sodium. The presence of negligible amount (0.5 ppm) of lead was noted in *A. tortilis* seeds. The results of the proximate analysis are comparable to the results of Chowdhury *et al.* [8], who reported the protein, ash and mineral contents of 8 *Acacia* species. However, the amounts of sodium found in local species were lower than the reported by the Banerji *et al.* [3].

The seeds of *A. cyanophylla*, *A. tortilis* and *A. victoriae* contained 9.8, 6.0 and 5.3% total lipids (on dry basis) respectively. The total lipids were fractionated into different lipid

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF SEED LIPIDS OF ACACIA SPECIES.

| | <i>Acacia cyanophylla</i> | <i>Acacia tortilis</i> | <i>Acacia victoriae</i> |
|----------------------|---------------------------|------------------------|-------------------------|
| Lipids contents (%) | 9.8 | 6.0 | 5.3 |
| Iodine value | 91.3 | 99.1 | 85.5 |
| Saponification value | 197.4 | 192.7 | 195.2 |

TABLE 3. PERCENTAGE OF LIPID FRACTIONS OF ACACIA SPECIES.

| | <i>Acacia cyanophylla</i> | <i>Acacia tortilis</i> | <i>Acacia victoriae</i> |
|-------------------|---------------------------|------------------------|-------------------------|
| Neutral lipids | 64.1 | 72.8 | 69.1 |
| Polar lipids | 35.9 | 27.2 | 30.9 |
| Hydrocarbons | 0.4 | 2.2 | 0.9 |
| Wax esters | 0.7 | 0.6 | 0.9 |
| Triglycerides | 48.3 | 30.4 | 52.3 |
| Free fatty acids | 8.7 | 14.8 | 2.0 |
| 1, 3-Diglycerides | 1.2 | 4.7 | 4.5 |
| 1, 2-Diglycerides | 1.0 | 13.3 | 6.8 |
| Monoglycerides | 3.8 | 6.8 | 1.7 |

TABLE 4. PERCENT FATTY ACIDS COMPOSITION OF THE TOTAL LIPIDS AND LIPID CLASSES OF ACACIA CYANOPHYLLA SEEDS.

| | 12:0 | 14:0 | 16:0 | 18:0 | 20:0 | 22:0 | 24:0 | 16:1 | 18:1 | 18:2 | 18:3 | Percent saturated acids | Percent unsaturated acids |
|-------------------|------|------|------|------|------|------|--------|------|------|------|------|-------------------------|---------------------------|
| Total lipids | 1.9 | 2.8 | 22.1 | 5.5 | 0.8 | 1.0 | Traces | 3.6 | 30.4 | 29.1 | 2.8 | 34.1 | 65.9 |
| Wax esters | 3.7 | 4.2 | 22.9 | 12.5 | 2.9 | 5.2 | 2.1 | 8.3 | 31.2 | 2.5 | 4.5 | 53.5 | 46.5 |
| Triglycerides | 1.4 | 2.5 | 19.3 | 9.7 | 2.5 | 5.5 | 3.8 | 5.2 | 37.7 | 9.4 | 3.0 | 44.7 | 55.3 |
| Free fatty acids | 1.8 | 2.9 | 23.6 | 15.7 | 3.4 | 2.4 | 0.5 | 7.9 | 34.0 | 3.1 | 4.7 | 50.3 | 49.7 |
| 1, 3-Diglycerides | 1.4 | 2.8 | 28.5 | 14.2 | 6.4 | 2.5 | 3.6 | 7.1 | 28.5 | 1.5 | 3.5 | 59.4 | 40.6 |
| 1, 2-Diglycerides | 4.7 | 5.7 | 23.4 | 13.1 | 4.9 | 3.1 | 3.3 | 7.8 | 27.4 | 2.4 | 4.2 | 58.2 | 41.8 |
| Monoglycerides | 1.7 | 2.4 | 25.9 | 17.6 | 4.5 | 4.1 | 2.1 | 10.3 | 20.7 | 5.5 | 5.2 | 58.3 | 41.7 |
| Polar lipids | 1.4 | 1.1 | 24.8 | 9.6 | 5.0 | 3.0 | 1.1 | 6.9 | 33.0 | 11.1 | 3.0 | 46.0 | 54.0 |

classes by thin layer chromatography (Table 1). The ratio of neutral to polar lipids was found to be 1.8:1 in case of *A. cyanophylla*, 2.7:1 in case of *A. tortilis* and 2.2:1 in case of *A. victoriae*. Triglycerides were the main fractions which constituted 48.3, 30.4 and 52.3% of total lipids of *A. cyanophylla*, *A. tortilis* and *A. victoriae* seed lipids respectively. Free fatty acids fractions were also the major fractions in the seed lipids of *A. cyanophylla* (8.7%) and *A. tortilis* (14.8%). Corresponding to its high free fatty acids contents, the lipids of *A. tortilis* had high percentages of 1:3- diglycerides and 1:2-diglycerides (Table 3).

The fatty acid compositions of total lipids and lipid fractions of the species showed that the total seed lipids of the species were rich in unsaturated fatty acids (64.3 - 72.2%). The iodine values and saponification values of the total seed lipids have been calculated and are given in Table 2. The iodine values are within the range of 85-103 and are similar to the values reported by Grindley [10] for seed oils of *A. albida*, *A. sieberiana*, *A. verec*, *A. mellifera*, *A. seyal* and *A. arabica*. The saponification values are also in close proximity with the values reported by Banerji [3] and Grindley [10] for different *Acacia* seed oils.

Among unsaturated acids oleic and linoleic acids were the predominating acids. The sum of these acids amounted 59.5, 69.9 and 57.0% of the total fatty acids in the seed lipids of *A. cyanophylla*, *A. tortilis* and *A. victoriae* respectively. Gunstone [4] and Chowdhary *et al.* [8] also reported the seed oils of the *Acacias* of African and Indian origin to be rich in unsaturated fatty acids. They found palmitic, oleic and linoleic acids predominating in these seed oils. The present study also showed these acids to be high in the lipids of the species studied. The sum of these acids were found to be 81.6, 86.4, and 75.3% of the total fatty acids in *A. cyanophylla*, *A. tortilis* and *A. victoriae* seed lipids respectively. Stearic, arachidic, behenic and linolenic acids were also present in all these seed lipids. Lauric, myristic and palmitoleic acids were present in *A. cyanophylla* and *A. victoriae* seed lipids. Lignoceric acid was detected in *A. cyanophylla* seed lipids only. This is the first

TABLE 5. PERCENT FATTY ACID COMPOSITIONS OF THE TOTAL LIPIDS AND LIPID CLASSES OF *ACACIA TORTILIS* SEEDS.

| | 16:0 | 18:0 | 20:0 | 22:0 | 18:1 | 18:2 | 18:3 | Percent saturated acids | Percent unsaturated acids |
|-------------------|------|------|------|--------|------|------|------|-------------------------|---------------------------|
| Total lipids | 16.5 | 8.2 | 1.0 | 2.1 | 37.0 | 32.9 | 2.3 | 27.8 | 72.2 |
| Wax esters | 29.4 | 14.7 | 3.0 | 3.5 | 38.2 | 5.9 | 5.3 | 50.6 | 49.4 |
| Triglycerides | 21.8 | 19.1 | 1.9 | 4.1 | 46.3 | 2.7 | 4.1 | 46.9 | 53.1 |
| Free fatty acids | 26.5 | 18.1 | 1.6 | 2.2 | 44.1 | 4.0 | 3.5 | 48.4 | 51.6 |
| 1, 3-Diglycerides | 29.6 | 16.9 | 2.5 | 2.8 | 42.3 | 1.7 | 4.2 | 51.8 | 48.2 |
| 1, 2-Diglycerides | 33.0 | 20.7 | 6.6 | Traces | 24.8 | 8.3 | 6.6 | 60.3 | 39.7 |
| Monoglycerides | 31.4 | 13.7 | 2.7 | 1.6 | 35.3 | 11.8 | 3.5 | 49.4 | 50.6 |
| Polar lipids | 18.5 | 8.1 | 3.4 | 2.1 | 37.0 | 25.4 | 5.5 | 32.1 | 67.9 |

TABLE 6. PERCENT FATTY ACID COMPOSITIONS OF THE TOTAL LIPIDS AND LIPID CLASSES OF *ACACIA VICTORIA* SEEDS.

| | 12:0 | 14:0 | 16:0 | 18:0 | 20:0 | 22:0 | 16:0 | 18:1 | 18:2 | 18:3 | Percent saturated acids | Percent unsaturated acids |
|-------------------|------|------|------|------|------|------|------|------|------|------|-------------------------|---------------------------|
| Total lipids | 2.5 | 1.2 | 18.3 | 8.7 | 0.9 | 4.1 | 2.5 | 36.5 | 20.5 | 4.8 | 35.7 | 64.3 |
| Wax esters | 3.1 | 7.3 | 24.9 | 12.9 | 2.3 | 6.6 | 6.2 | 22.8 | 4.6 | 9.3 | 57.1 | 42.9 |
| Triglycerides | 0.9 | 2.2 | 22.2 | 12.5 | 0.1 | 4.4 | 4.4 | 44.5 | 2.1 | 6.7 | 42.2 | 57.7 |
| Free fatty acids | 4.1 | 8.6 | 20.6 | 10.3 | 4.1 | 6.9 | 6.5 | 24.1 | 6.2 | 8.6 | 54.6 | 45.4 |
| 1, 3-Diglycerides | 3.5 | 3.0 | 23.2 | 11.6 | 3.2 | 6.4 | 10.2 | 29.1 | 1.1 | 8.7 | 50.9 | 49.1 |
| 1, 2-Diglycerides | 1.6 | 3.2 | 20.8 | 10.4 | 4.2 | 5.4 | 4.7 | 31.2 | 10.4 | 8.1 | 45.6 | 54.4 |
| Monoglycerides | 4.6 | 6.1 | 17.6 | 10.5 | 3.9 | 4.4 | 7.9 | 28.1 | 10.2 | 6.7 | 47.1 | 52.9 |
| Polar lipids | 5.3 | 3.5 | 18.4 | 8.7 | 6.1 | 12.2 | 8.7 | 21.8 | 6.6 | 8.7 | 54.2 | 45.8 |

report of the study of *A. cyanophylla* and *A. victoriae* seed lipids and their fatty acid compositions. However, the fatty acid composition of the seed oils of *A. tortilis* of Indian and Rhodesian origin were reported by Banerji [3] and Gunstone [4] respectively. The seed oil of *A. tortilis* of Indian origin was reported to contain 71.7% linolenic acid as compared to 2.0% reported by Gunstone *et al.* [4] and was also 2.3% determined in the present study. Gunstone [4] reported the fatty acid

TABLE 7. CODEX RANGES OF FATTY ACIDS COMPOSITION OF COTTON SEED OIL.

| Fatty acid | Percentage |
|------------|------------|
| < 14 | < 0.1 |
| 14:0 | 0.4-2.0 |
| 16:0 | 17.0-31.0 |
| 18:0 | 1.0-4.0 |
| 20:0 | 0.7 |
| 22:0 | 0.5 |
| 24:0 | 0.5 |
| 16:1 | 0.5-2.0 |
| 18:1 | 13.0-44.0 |
| 18:2 | 33.0-59.0 |
| 18:3 | 0.1-2.1 |
| 20:1 | < 0.5 |
| 22:1 | < 0.5 |

composition of the seed oil of *A. tortilis* of Rhodesian origin as palmitic 2.0%, stearic 3.0%, oleic 19.0%, linoleic 60.0%, Linolenic 2.0%, arachidic 2.0% and behenic (2.0) acids. Whereas, Banerji *et al.* [3] recorded the presence of lauric 2.5%, tridecanoic 2.5%, myristic 0.1%, C_{14:1} 0.1% pentadecanoic 0.3%, palmitic 9.5%, palmitoleic 0.1%, margaric (Traces), stearic 2.4%, oleic 6.1%, linoleic 2.0%, linolenic 71.7%, arachidic 1.1%), epoxy C_{18:1} 2.1% and C_{20:1} 0.2% acids in the seed oil of *A. tortilis* of Indian origin.

The comparison of the fatty acid composition of free fatty acids, 1:3-diglycerides and 1:2-diglycerides of *A. tortilis* showed that free fatty acid and diglycerides had higher percentages of oleic acid (44.1 and 42.3% respectively), than 1:2-diglycerides (24.8%). This indicates that the terminal positions of *A. tortilis* seed glycerides are rich in unsaturated fatty acids specially oleic acid.

The comparison of the fatty acid patterns of total seed lipid of *A. cyanophylla*, *A. tortilis*, and *A. victoriae* with codex ranges of fatty acid composition of fats and oils of commercial importance, (Table 7) reported by Gunstone [9], revealed that these lipids resembled cotton seed oil. As all the studied seed lipids were found to be rich in unsaturated fatty acids such as oleic and linoleic acids, therefore, the seed oils or whole seeds of these species can be utilized in animal feeds as an energy or energy and protein source respectively.

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