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Characterization and Chemical Composition of the Fixed Oil of Fourteen Basil (*Ocimum basilicum* L.) Accessions Grown in Sudan

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Abstract: Oil content, fatty acid composition and lipid profile of seed oils of fourteen basil (*Ocimum basilicum* L.) accessions grown in Sudan were determined. The accessions included wild basil (one accession), two accessions introduced from Germany and one from UAE. The rest (ten) represent basils grown in Sudan as ornamental plants. All were collected as seeds and grown at the University of Gezira farm. The fixed oil components were separated by TLC and GLC. Chemical variability among the fixed oils of Sudanese basils was extremely broad. Seed oil content varied from 8.8 to 30%. The major acylated fatty acids were palmitic (5-13%), stearic (2-3%), oleic (6-10%), linoleic (12-32%) and linolenic acid (49-75%). Linolenic acid, desirable for the potential industrial use of the oil as a drying oil, is high as 75% in seeds of the abundant wild-type of basil; this accession contains high proportion compared to world-wide basils. These results shown basil seed oil especially wild-type appears suitable to be used for industrial purposes. This is promising since seeds of the wild plant, an annual that spontaneously grows during the rainy season in many parts of Sudan, can be cheaply collected in abundance. However, certain accessions e.g., No. 11 contain other constituents such as sterols this may warrant further research.

Key words: Basil, *Ocimum basilicum*, seedoil, fatty acids, Sudanese accessions

INTRODUCTION

The genus *Ocimum* (Lamiaceae formerly Labiatae), collectively called basil has long been recognized worldwide as a diverse, rich source of essential oils and an important culinary herb. Basils show great variation in both morphology and essential oil components (Blank *et al*., 2004; Makri and Kintzios, 2008; Telci *et al*., 2006). *Ocimum* comprised 50 to 150 species of herbs and shrubs from tropical regions of Asia, Africa and Central and South America, particularly regarding plant growth, morphology, physical appearance and essential oil content and composition, most commercial basil cultivars available in the market belong to the species *O. basilicum*. The taxonomy of *O. basilicum* is further complicated by the existence of numerous varieties, cultivars and chemotypes within the species that do not differ significantly in morphology. The composition of volatile oil constituents was used to characterize the diversity among the most economically important *Ocimum* species (Abduelrahman *et al*., 2009; Vieira and Simon, 2006).

Seed oil composition constitutes another characteristic which contributes to the rich diversity of the *Ocimum* genus. Earlier studies on basil seed oils included drying properties of *O. basilicum* reported by Angers *et al*. (1996a). The fixed oils of plant seeds were classically classified as

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non-drying, semi-drying or drying oils. Drying oils are highly unsaturated oils that will oilgomerize or polymerize when exposed to the oxygen in air, usually in the presence of a catalyst (Akpuaka and Nwankwor, 2000). The result is an increase in the molecular weight including cross-linking. The drying index indicates how well or how poorly certain drying oils will oxidize. The drying index of drying oil, semi-drying oil and non-drying oil is >70, 50-70 and <50, respectively.

The essential oil of the plant, which successfully grows in Sudan, has been the subject of studies aimed at chemical characterization as well as testing of certain biological activities attributed to it. Seeds of basil contain a fixed oil with potential uses as a drying oil. In this study, we report on the chemical composition of the fixed oil of seeds of fourteen basil (*Ocimum basilicum* L.) accessions grown in Sudan.

MATERIALS AND METHODS

Plant Material (Seed Sources)

Basil seeds used in these studies were obtained from different parts of Sudan. Commercial basil accession seeds were obtained from Germany and the United Arab Emirates (UAE). Seeds of basil accessions were directly sown on 60 cm wide ridges at the Demonstration Farm (Nishishiba), Fac. Agri. Sciences, University of Gezira, Wad Madani, Sudan. Sowing was done on Feb. 20, 2005. Watering, weeding was carried out as necessary. No chemicals (fertilizers or others) were applied. Observations were made on growth and flowering of the plants weekly, the accessions were given numbers, as earlier reported by Abduelrahman *et al*. (2009).

Extraction of Plant Material

The fixed oil was prepared from seeds of different accessions according to AOCS (1993) official method No. Aa 4-38 (Angers *et al*., 1996a). Extraction was continued for 6 h in a Soxhlet extractor using n-hexane.

Thin Layer Chromatography (TLC)

n-Hexane/diethyl ether/acetic acid (70:30:1), methanol/diethyl ether (1:19) and diethyl ether/hexane (1:2) were used as TLC developing solvent for separation of lipid closes of basil seed fixed oil. Different ratios of conc. H_2SO_4/H_2O (e.g., 1:9, 1:4 or 1:2) were used for identification of individual separated triglycerides. After spraying, the plate was heated on a hot plate at 120°C until spots were visible. Weak spots may be intensified by heating for longer times or at somewhat higher temperatures.

GLC Separation Conditions

The oils composition was determined by Gas Liquid Chromatography (GLC) for fixed oils of basil selected accessions. A PYE UNICAM Gas chromatograph GCD, with a Flame Ionization Detector (FID) and computing integrator, was employed in all analyses. Hydrogen was used for the flame; oxygen-free nitrogen was the carrier gas. Column (1.2 m) was packed with the liquid phase polyethylene glycol succinate (PEGS) on celite (100-120 mesh).

Analysis of fatty acid methyl esters was carried out isothermally at a column oven temperature of 198°C and column inlet and detector oven temperature of 220°C; the carrier gas flow was 1 mL sec^{-1}. The identification of fatty acids was carried out by comparison of the retention time of each fatty acid ester with that of the esters of authentic standards (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid).

Transmethylation of Fatty Acid (For GLC Analysis)

Crude extracts of basil lipid were dried in a steam of nitrogen in the presence of benzene to remove traces of water. The methylation solution (methanol/benzene/conc. H_2SO_4 [20:10:1] by volume) was added in two portions (2×2 mL) to dissolve the lipid. The solution was transferred to 10 mL methylation ampoules; after flame-sealing the ampoules was heated in an oven at 80°C for 90 min. After cooling the seal was broken and the content transferred to glass stoppered test tubes. Four milliliter of saturated aqueous sodium carbonate were added and the fatty acid methyl esters were extracted twice in 4 mL portions of hexane after gentle shaking. The combination methyl ester extracts were washed with a further 4 mL of sodium carbonate solution and finally with 4 mL distilled water and dried over anhydrous sodium sulphate. The extracts were incubated at 0°C and analysed within 24 h.

RESULTS AND DISCUSSION

Oil Content of Seeds of Basil Accessions

Table 1 shows the fixed oil content of seeds of 14 accessions of basil. The accessions included wild basil (one accession), the two accessions introduced from Germany and one accession introduced from UA Emirates; the rest (10) represent basils grown in Sudan as ornamental plants. The seed oil content was quite variable among the 14 accessions and ranged between 8.82% (accession No. 10) to 30% (accession No. 17).

World-wide the fixed oil content of basil seeds varied between 18 and 34% (Earle *et al*., 1960; Angers *et al*., 1996b). If we set oil content of 14.38% as the minimum required for qualification of an oilseed to the states of feasible commercial exploitation, then 9 out of the 14 accessions would qualify, including seeds of wild basil (No. 20). This is promising since seeds of the wild plant, an annual that spontaneously grows during the rainy season in many parts of Sudan, can be cheaply collected in abundance.

Fatty Acid Composition of Seed Oils of Basil Accessions

Fatty acids of seeds of 10 basil accessions were quantified by GLC. All basil seed oils contained palmitic acid (5-13%), stearic acid (2-3%), oleic acid (6-10%), linoleic acid (12-32%) and linolenic acid (49-75%) in differing proportions. Total oil unsaturation was reflected in the so called unsaturation index calculated from the fatty acid composition. The value (Table 2) ranged from 218 (accession 19, UAE) to as high as 256 in wild basil seedoil (No. 20).

Typical GLC separations of accession No. 19 and 20 were shown in Fig. 1A and B. The percentage of the industrially important linolenic acid was highest in wild basil (No. 20) reaching 75% of the seed oil total fatty acids and low in accession No. 19 in which case the somewhat lower content of this acid was associated with an increase in linoleic acid. A reverse reciprocal relation was observed in wild basil (No. 20) i.e., high 18:3 and low 18:2. This may reflect the relative efficiency of the

Table 1: Fixed-oil content of seeds of basil accessions grown in Nishishiba Farm, Wad Medani

Accession No.	Percentage of oil (w/w)
1	15.00
3	16.38
9	18.01
10	8.82
11	15.82
12	9.94
13	16.72
14	12.02
15	11.46
16	11.06
17	30.01
18	23.96
19	20.08
20	14.38

Table 2: Fatty acid composition (%) of the seed oils of 10 accession of basil grown in Nishishiba, Wad Medani, U.I. unsaturated index

Accession No.	16:0 (Palmitic)	18:0 (Stearic)	18:1 (Oleic)	18:2 (Linoleic)	18:3 (Linolenic)	U.I
1	8	2	10	22	58	228
3	10	2	6	20	62	232
11	5	3	10	26	56	230
14	9	3	8	29	51	219
15	13	2	7	22	56	219
16	8	3	7	24	58	229
17	10	2	9	21	58	225
18	8	2	9	22	59	230
19	8	2	9	32	49	218
20	4	2	7	12	75	256

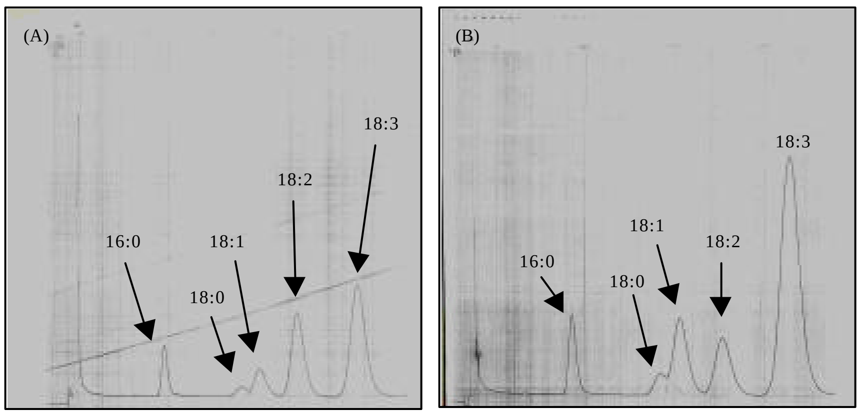

Fig. 1: Typical GLC chromatograms of the fatty acids of two basil-seed oils (Fatty acid identity is indicated above each peak), (A) accession No. 19 and (B) accession No. 20

enzymatic machinery involved in 18:2 to 18:3 conversions in wild basil and basil of German origin (No. 17 and 18). Thus wild basil could be a cheap commercial crop for seeds rich in linolenic acid. It is noteworthy that the plant inflorescence is non-shattering and the seed could be collected long after maturity. High linolenic acid oil, such as that found in accession No. 20 (wild) and accession (No. 3) could be used in the paint, varnish and ink industries.

Angers *et al.* (1996a, b) studied the fatty acids compositions of some basils oils and reported the presence of palmitic acid (3.3-10%), stearic acid (0.2-6.4%), oleic acid (5.3-15.4%), linoleic acid (14-66%) and linolenic acid (15.7-65%). Prakash and Gupta (2000) reported that the seeds of sacred basil contained a fixed oil containing five fatty acids, including about 17% linoleic acid and just over 50% linolenic acid.

Thus, the fixed oil of wild Sudanese basil contains extremely high proportions of 18:3 compared to world- wide basils. Linolenic acid is used in making soaps, emulsifiers and quick-drying oils and has become increasingly popular in the beauty products industry because of its beneficial properties on the skin. Research points to linoleic acids affective properties when applied topically on the skin, i.e., anti-inflammatory, acne reduction, moisture retention properties.

TLC Lipid Profiles of Basil Seed Oils

Seed oils of 14 basil accessions were subject to argentation TLC analysis to determine the species of triacylglycerols. Ground nut and sesame oils were included for comparison. The results are shown

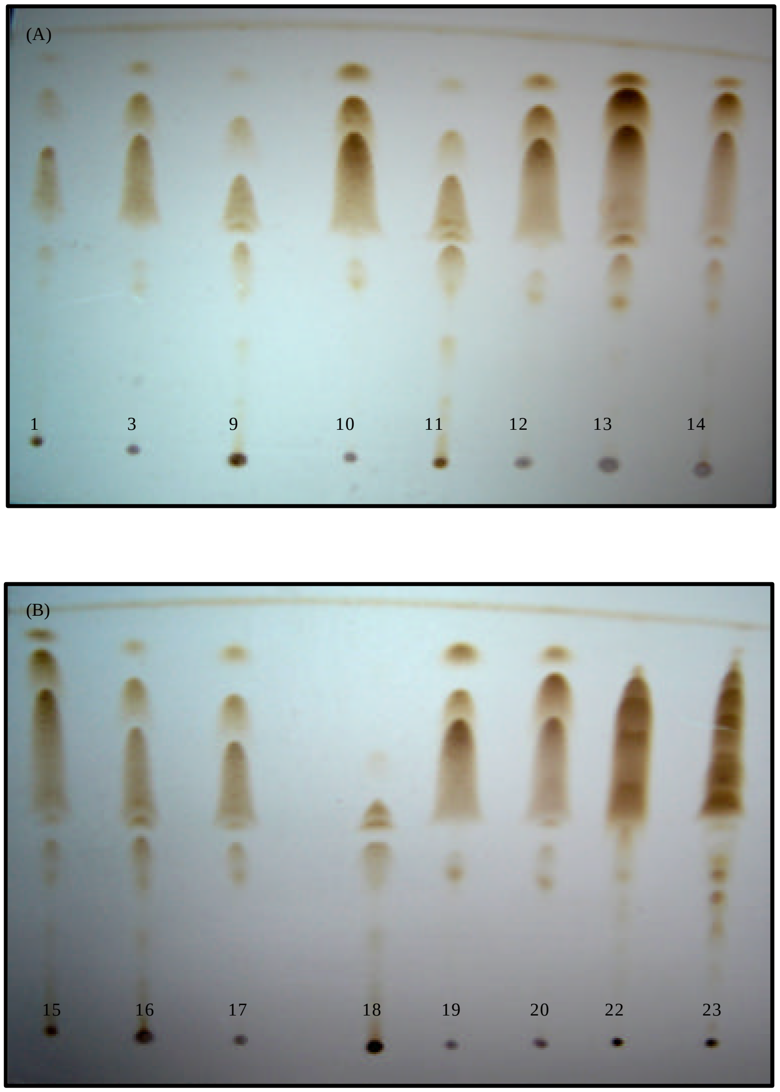

Fig. 2: TLC separation of seed oils of basil accessions grown in Nishishiba. Adsorbent: Silica gel incorporating 5% silver nitrate, solvent: n-hexane/diethyl ether/acetic acid, 70:30:1. Lane: 1-20: Basil accessions, Lane 22: Sesame, Lane 23: Grounut, (A) Lane 1-14 and (B) Lane 15-23

in Fig. 2A and B. Up to 7 species could be observed in the oils of some accessions. All basil fixed oils showed some similarity but their patterns were somehow different from the oils of sesame and ground nut. Accessions No. 18, of German origin, showed a fewer number of triacyl glycerol species compared to richer accessions such as No. 16. Knowledge of the distribution of triacyl glycerol species is important for research on oil biochemistry (biosynthesis), genetical engineering and breeding. It may also serve as a finger- point for oil source identification in quality control.

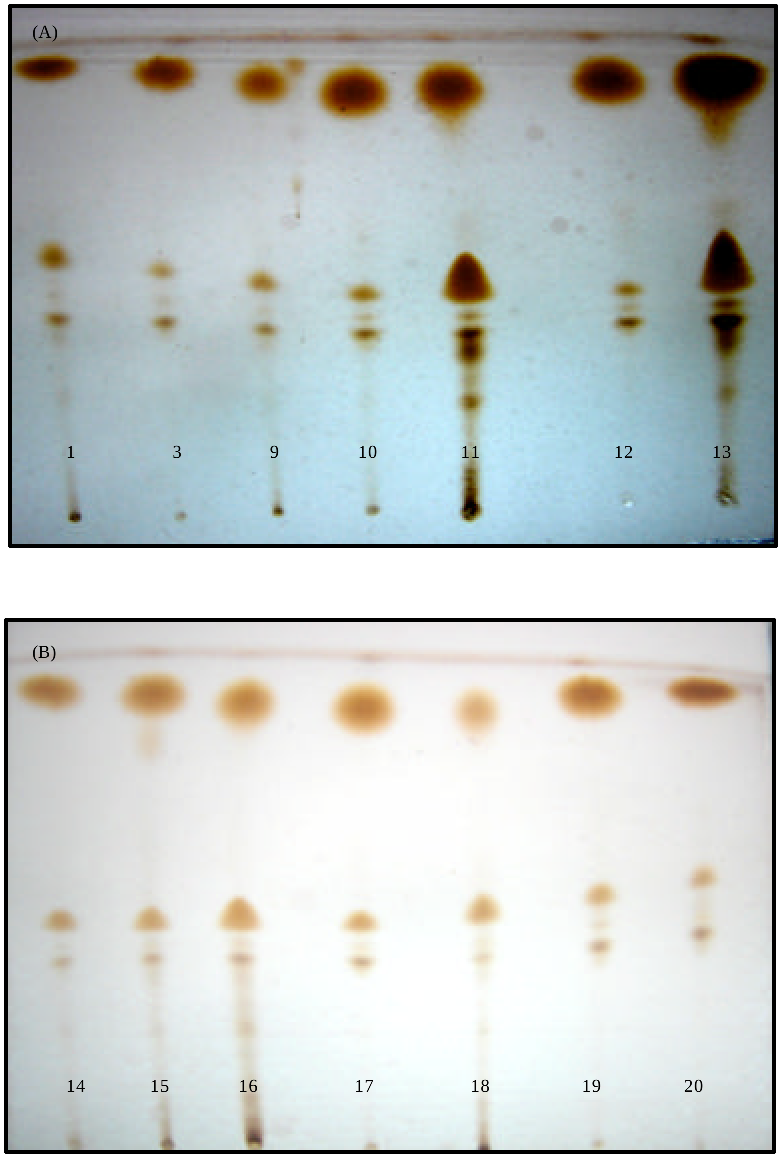

Fig. 3: TLC separation of seed oils of basil accessions grown in Nishishiba. Adsorbent: Silica gel (without silver nitrate), solvent: n-hexane/ diethyl ether/ acetic acid, (70:30:1) (A) Lane 1-13 and (B) Lane 14-20

Figure 3A and B show TLC separations of the fixed oils of the same 14 basil accessions using normal (non-argentation) TLC. Included for record purposes, all oils showed similar constituents with the major spots corresponding to total triacylglycerols, free fatty acids and mono- and di-acylglycerols. However, certain accessions e.g., No. 11 contain other constituents such as sterols. This may warrant further research.

CONCLUSION

Chemical variability among the fixed oils of Sudanese basil accessions was extremely broad. And 9 of 14 consider being good source of fixed oil. The wild basil with high percentage of linolenic acid reaching 75%, this accession contains high proportion compared to world-wide basils, therefore, this basil could be very promising and good natural source of linolenic acid for industries. Thus, these results could encourage the search for new suitable natural sources for fixed oils in other plants in Sudan.

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