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Received for review December 3, 1973. Accepted January 24, 1974. Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

Oils of *Ocimum basilicum* L. and *Ocimum rubrum* L. Grown in Egypt

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Steam-distilled leaf and flower oils of *Ocimum basilicum* L. and *Ocimum rubrum* L. were subjected to qualitative and quantitative study using one- and two-dimensional tlc, glc, and ir techniques. Many constituents were identified, linalool and methyl chavicol being the most prominent. The physicochemical constants of the investigated oils were also measured.

The oil of *Ocimum basilicum* L. (sweet basil), being employed quite extensively in all kinds of flavors including those for confectionary, baked goods, condimentary products, spiced meats, and sausages, as well as in dental products and certain perfumes (Guenther, 1952), has been the subject of many investigations. Hörhammer *et al.* (1964), using tlc, detected terpineol, linalool, geraniol, citronellol, and possibly nerolidol together with methyl cinnamate and methyl chavicol in Egyptian basil oil. In the case of Bulgarian basil oil, however, Ivanov and Iordanov (1964) made use of ir analysis to identify linalool, methyl chavicol, 1,8-cineole, eugenol, geraniol, *p*-cymene, and myrcene. Pogany (1967) found no qualitative difference between basil oil samples produced from plants grown at different temperatures. Pogany and coworkers (1967) also located benzyl ether in the glc chromatogram of the oil. Nigam and Kameswara (1968) stated that the percentage yield of the oil produced by steam and water distillation of the leaves and soft twigs of *O. basilicum* dried in the shades was 0.8%. They were able to identify methyl cinnamate, methyl chavicol, linalool, cineole, ocimene, borneol, sambulene, and safrole in the oil.

As regards the oil of *O. rubrum*, however, nothing could be traced in the current literature concerning its chemical or physical analysis. The oil was found to occur in a high percentage in the plant and to possess a sweet and fine aroma. On these bases, the oil promises to be of industrial importance.

Accordingly, this work was carried out to present a comparative phytochemical study of the oils produced from *O. basilicum* and *O. rubrum* grown in Egypt.

MATERIALS AND REAGENTS

The following materials and reagents were used during this study: the steam-distilled oils of leaves and flowers of *O. basilicum* L. and *O. rubrum* L. (cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Guiza; the plants were identified through the courtesy of V. Takholm, Faculty of Science, Cairo University, and collected during the flowering stage in March); reference volatile oil constituents; chromogenic spray, 5% w/v vanillin in sulfuric acid; silica gel for column chromatography and silica gel G Merck for tlc.

APPARATUS

A heated dual flame ionization detector programmed chromatograph, Pye series 104, Model 64, fitted with an RE 511 potentiometric recorder adjusted at 5 mV variable, was used. The apparatus was provided with coiled glass columns, 5 ft long and 4 mm i.d., packed with 10% Reoplex 400 (polypropylene glycol adipate) or 3% SE 30 (methyl silicone polymer) on 85–100 mesh Celite; spectrophotometer Unicam SP 200 was employed for ir study.

PROCEDURES

Determination of Physicochemical Constants. The specific gravity, refractive index, optical rotation, alcohol content, ester content, and solubility in 70% alcohol were carried out according to the Egyptian Pharmacopoea (1963) methods. The results together with the percentage yield of the oils (calculated according to moisture-free basis) are shown in Table I.

Tlc. Ten percent (v/v) of the oils under investigation, as well as reference volatile oil constituents in acetone, were chromatographed on silica gel G plates (20 × 20 cm and 0.3 mm thick) using five developing solvent systems: toluene–ethyl acetate (4:1) (TEt), petroleum ether (bp 60–80°)–ether (4:1) (PE), *n*-hexane–ether (4:1) (HE), petroleum ether (bp 60–80°)–ethyl acetate (85:15) (PEt), and benzene–ethyl acetate (95:5) (BEt). Development was carried out in a refrigerator at about 5–10°. The liquid was allowed to run for 15 cm (45 min). In case of BEt, however, development was operated at room temperature (15–20°) to safeguard against the probable crystallization of benzene during development (20–30 min). The chromatograms were then inspected under uv light and located with the chromogenic spray before and after heating at 105° for 5 min.

Two-Dimensional Tlc. This technique was applied to the oils under investigation using, for the first and second developments, the solvent systems BEt and PEt, respectively.

Column Chromatography. Five-tenths milliliter of

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Table I. Physical and Chemical Constants of Oils of *O. basilicum* and *O. rubrum*

Value	*O. basilicum*	*O. rubrum*
% (on free-moisture basis)	0.82	0.92
Specific gravity at 20°	0.916	0.930
Refractive index	1.470	1.485
Optical rotation	−6° 44′	−5° 6′
Alcohol % (as linalool)	39.00	35.00
Ester % (as linalyl acetate)	4.90	4.30
Acid value	0.50	0.45
Solubility in 70% alcohol	1:1 v/v	1:1 v/v

each of the oils under investigation was fractionated into less polar and more polar constituents on a 10-g column of silica gel using *n*-hexane and ethanol, respectively, as eluents. Both fractions were concentrated under reduced pressure and reexamined by tlc and glc.

Isolation of Pure Methyl Chavicol by Plc. A comparatively thick layer of silica gel G plate (3 mm thick) was used. Five-tenths milliliter amounts of the oil were fractionated on each plate, as described in a previous communication (Karawya *et al.*, 1971) using any one of the aforementioned developing systems. By this method, methyl chavicol could be isolated from the oil of *O. basilicum*, which was found to contain a high percentage of this constituent. The purity and identity of this compound were confirmed through its physicochemical characters (Guenther, 1952) as well as by comparing its infrared spectrum with that of an authentic sample; absorption characteristics were shown at 1650, 1620, 1590, 1310, 1250, and 1180 cm^{-1}. Moreover, the isolated methyl chavicol showed one spot by tlc and one peak by glc using the previously mentioned different solvent systems and liquid phases, respectively.

Glc. The use of one type of stationary phase is likely to be inadequate for good separation and identification of the oil components. Therefore, Reoplex 400 representing a polar liquid phase and SE 30 representing a nonpolar liquid phase were chosen for the glc study of the oils under investigation, adopting the following operating conditions: column temperature, 80°, kept isothermal for 5 min and then programmed by increasing the temperature at the rate of 12°/min to 200° in the case of Reoplex 400 and to 225° in the case of SE 30, and kept isothermal at the maximum temperature for 15 min in both columns; detector oven temperature, 240°; carrier gas, nitrogen at flow rate of 60 ml/min; hydrogen flow rate, 50 ml/min; air flow rate, 390 ml/min; attenuation, 10×10^{-2} and 20×10^{-2} for the oils and 2×10^{-4} for the authentic samples; chart speed, 1 cm/min; sample size, 0.2 μl of the 10% v/v solution of the oil in acetone.

To identify the components thus separated, the relative retention time (t_R rel) with respect to linalool, which produced a sharp peak in all the chromatograms, was determined and compared with that of pure components and by using the enrichment technique. This last technique was comprised of adding to the tested oil a certain amount of the compound assumed to be present so that the concentration thereof was increased. The quantitative determination was based on the peak area measurement (height × width at half height). The results are compiled in Table II.

Ir. A thin film of the oil under investigation was made between two disks of sodium chloride to obtain the respective ir spectrum.

RESULTS AND DISCUSSION

From the data presented in Table I, one can conclude that the oils of *O. basilicum* and *O. rubrum* are rich in alcohols (35 and 39%, respectively, calculated as linalool) and relatively poor in esters (4.3 and 4.9%, respectively, calculated as linalyl acetate). They are levorotatory and freely soluble in 70% ethanol (1:1 vol).

Using the chromatographic techniques, the leaf and flower oils of both species show a qualitative resemblance. Maximum tlc resolution of the oils of *O. basilicum* (ten spots) and *O. rubrum* (11 spots) was attained by developing with TEt and HE, respectively, in case of one-dimensional development. On adopting the two-dimensional tlc, however (using BEt and PEt), the number of spots increased to 15 and 26 in both oils, respectively. Using glc, on the other hand, up to 40 and 37 peaks on SE 30 and 31 and 23 peaks on Reoplex 400 could be located in the chromatograms of oils of *O. basilicum* and *O. rubrum*, respectively. Identification of any constituent was confirmed by its location by tlc (one- and two-dimensional) using different developing systems and by glc using both polar and nonpolar liquid phases. The last technique, in fact, offered good means for the identification of the peaks; an identified peak should be identical to that of the authentic isolate in the case of both phases. Othe peaks were considered unidentified and further work for their separation and identification is being carried out.

Development with tlc was carried out at a low temperature (5–10°) to minimize the evaporation of any component and to keep the atmosphere of the jar always satu-

Table II. Identified Constituents of Oils of *O. basilicum* and *O. rubrum* and Their Percentages

Compound	t_R rel[a]		*O. basilicum* oil		*O. rubrum* oil	
	SE 30	Reoplex	L	Fl	L	Fl
α-Pinene	0.41	0.12	1.12	1.32	0.34	0.30
Camphene	0.44	0.19			1.00	1.30
β-Pinene	0.51	0.23	0.44	0.80	1.60	1.00
Ocimene	0.54	0.24	7.44	5.64	12.50	11.20
Cineole	0.82	0.24				
Δ^3-Carene	0.58	0.24			1.11	0.84
Linalool	1.00	1.00	37.20	37.60	16.50	14.30
Linalyl acetate	2.21	1.10	2.30	2.40	8.50	6.00
Terpineol	1.35	1.42	0.50	0.45	0.48	0.42
Methyl chavicol	1.70	1.52	27.92	33.80	38.00	50.00
Benzyl acetate	1.14	1.63	1.00	1.20		
Phenyl ethyl alcohol	0.72	2.13	1.21	1.36	2.50	2.04
Nerolidol	4.44	1.82	0.16	0.08	0.18	0.20
Farnesol	3.84	2.20	0.70	0.65	0.75	0.64
Undecyl aldehyde	1.21	1.18			0.30	0.25
Geranyl acetate	2.02	1.68	0.84	0.72	0.12	0.10
Eugenol	3.27	2.45	1.02	0.85	0.50	0.75
Isoeugenol	3.50	2.45	0.75	0.54	1.20	1.15

[a] t_R rel = relative retention time according to linalool; L = leaf oil; Fl = flower oil.

rated with the solvent system, thus allowing better separation and formation of the spots.

On fractionating the oils by column chromatography, terpene and sesquiterpene hydrocarbons, together with some methyl chavicol (weakly polar), were eluted with *n*-hexane. The other oxygenated constituents, together with the remaining methyl chavicol, were eluted with 95% ethanol.

Plc, however, was successfully applied for the isolation of methyl chavicol in a pure noncontaminated form as confirmed by tlc, glc, and ir analysis, as well as its bp (213–215°).

Comparing the R_f values and t_R rel of the resolved spots and peaks with those of authentic isolates, the following observation could be concluded. Leaf and flower oils of both species contained the following constituents: α-pinene, β-pinene, ocimene, 1,8-cineole, linalool, nerolidol, farnesol, phenyl ethyl alcohol, terpineol, geranyl acetate, methyl chavicol, eugenol, and isoeugenol. In addition, the oils of *O. rubrum* contained camphene, Δ^3-carene, and undecyl aldehyde and the oils of *O. basilicum* contained benzyl acetate.

As regards the ir study, the spectra of the leaf oils of both species showed nearly the same pattern given by the respective flower oils, although those of the flowers are comparatively weaker in intensity. The band corresponding to the OH stretching frequency of hydroxylic compounds (3360–3450 cm^{-1}) was found predominating in both oils. The methylene groups were detected in their spectra as a shoulder at their respective band position (3085, 2978 cm^{-1}). The CH stretch characteristic for aliphatics (2800–2960 cm^{-1}) in the ocimum oils was very distinct. The bands corresponding to aromatic compounds, on the other hand, showed prominent peaks in their spectra (1500–1600 cm^{-1}) (probably due to methyl chavicol). Alcohols, phenols, esters, carbonyls, and ethers exhibited their characteristic bands between 1000 and 1300 cm^{-1}. Saturated tertiary and saturated secondary alcohols (absorbing at 1170 and 1120 cm^{-1}) were very distinct in the spectra of both oils. The unsaturated primary and secondary alcohols (1000 and 1080 cm^{-1}, respectively) were also of common occurrence. The acetate esters exhibited characteristic bands between 1235–1250 cm^{-1} and at about 1025 cm^{-1}; bands from 700–1000 cm^{-1} representing monosubstituted and ortho, meta, and para compounds and trisubstituted benzene derivatives were detected in the oils under investigation.

From the quantitative point of view (Table II) it is clear that both leaf and flower oils of *O. rubrum* can be distinguished from those of *O. basilicum* by possessing higher contents of β-pinene, methyl chavicol, linalyl acetate, and phenyl ethyl alcohol and by containing undecyl aldehyde. *O. basilicum* oils, however, could be signified by containing benzyl acetate.

CONCLUSION

From the aforementioned results, one can conclude that the physical and chemical characteristics of the Egyptian basil oil are in accordance with those reported by Guenther (1952). Moreover, owing to its fine odor, *O. rubrum* oil could be satisfactorily used in the perfume industry and as a flavoring agent provided that it does not possess any objectionable property.

Supplementary Material Available. Thin-layer and gas–liquid chromatograms of *O. basilicum* and *O. rubrum* oil and an ir spectrum of *O. basilicum* oil will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-74-520.

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Received for review July 7, 1972. Accepted October 26, 1973.