

NOTE

**POLYPHENOLS OF *OCIMUM SANCTUM* FROM SURINAME**

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**ABSTRACT**

*Apigenin, luteolin, apigenin-7-O-β-D-rutinopyranoside, luteolin-7-O-β-D-glucopyranoside, vicenin-2, vitexin, isovitexin, orientin, isoorientin, aesculetin, aesculin, chlorogenic acid and caffeic acid have been isolated from the aerial parts of O. sanctum L.*

**INTRODUCTION**

*Ocimum sanctum* L. (Labiatae), popularly known as “tulsi” in Hindi and “holy basil” in English, is an indigenous Indian plant sacred to Hindus, where is used in folk medicine against cough, bronchitis, fever, bile disturbances and as an anthelmintic (Das, 1959). The leaves of the plant have also been used as an antiemetic, anticancer agent, antiseptic, analgesic, tonic, and rejuvenator or vitalizer to induce longevity and disease-free healthy life (Chopra et al., 1958). Many investigations have been carried out on the biological properties of this plant. For example, preliminary pharmacological and clinical studies with *O. sanctum* show that it possesses anabolic, hypotensive, cardiac depressant, smooth muscle relaxant, hypoglycemic, sedative, antispermatic, antifungal, antifertility, anti-

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inflammatory, analgesic, antipyretic and adaptogenic actions (Krishnamurthy, 1959; Dhar et al., 1968, 1973; Singh et al., 1970; Batta & Santhakumari, 1971; Malviya & Gupta, 1971; Seth et al., 1981; Bhargava & Singh, 1981; Godhawani et al., 1987, 1988). We currently report the isolation and identification of several chemical constituents from *O. sanctum* collected in Suriname.

**MATERIALS AND METHODS**

**Plant Material**

Aerial parts (herba in vegetative stage) of *O. sanctum* L. were collected at Suriname, in 1993, during the flowering period. A voucher specimen (ATHS 093) has been deposited in the Herbarium of the Laboratory of Pharmacognosy, University of Athens.

**Extraction and Isolation**

Shade-dried aerial parts of *O. sanctum* L. (0.40 kg) were finely ground and extracted at room temperature with dichloromethane and methanol, successively. Both extracts were concentrated *in vacuo* to dryness and the methanol residue was extracted at room temperature with petroleum ether, ether, ethyl acetate and *n*-BuOH, successively. The extracts were evaporated under reduced pressure at a temperature below 40°C to obtain a viscous mass. The ether extract (2.5 g) was chromatographed over a silica gel column (Merck, Art. 7736 – 30 × 2 cm; 20 fractions of 50 ml), using CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (75: 25: 2.5) as eluent. Similar fractions were grouped and further purified by preparative TLC: the 1st group (fractions 1–4) on cellulose

plates (Merck, Art. 5716) with 30% aqueous sol. of acetic acid ( $S_1$ ) yielded apigenin and luteolin; fractions 8 and 10, similarly purified with *tert*-BuOH-AcOH- $H_2O$  (3: 1: 1) ( $S_3$ ) and 5% aqueous solutions of acetic acid ( $S_2$ ) yielded caffeic acid (2 mg) and aesculetin (0.5 mg), respectively. The butanol extract (7.5 g) was chromatographed over a Sephadex LH-20 column (33 × 4 cm; MeOH as eluent; 23 fractions of 50 ml). Further purification on prep. TLC of fractions 6 ( $S_3, S_1$ ), 7 ( $S_3$ ), 10 ( $S_3, S_2$ ), 11 ( $S_3, S_2$ ) yielded the following compounds, respectively: chlorogenic acid; apigenin-7-O- $\beta$ -D rutinopyranoside, vicenin-2 and vitexin; luteolin-7-O- $\beta$ -D-glucopyranoside and orientin; isoorientin, isovitexin and aesculin (1.5 mg). The C-glycosides are the main compounds, weighing between 8 and 12 mg each, while the O-glycosides 2–5 mg.

### Identification

All compounds were identified by comparison of their UV and  $^1H$ -NMR spectra with those reported in the literature (Mabry et al., 1972; Harborne et al., 1975; Harborne & Mabry, 1982; Murray et al., 1982). In addition to their spectroscopic data, their  $[\alpha]_D$  were measured and some of them were also subjected to co-chromatography (TLC, HPLC) with authentic samples (Roth) or previously isolated substances from other plants. The TLC was done with three different solvent systems ( $S_1, S_2, S_3$ ) and also HPLC was performed ( $\mu$ Bondapak 10  $C_{18}$  eluted with MeOH: AcOH 5% 4: 6; UV absorbance detection at (340 nm). UV spectra were recorded on a Shimadzu UV-160A spectrophotometer, according to standard procedures (Mabry et al., 1972; Harborne et al., 1975; Harborne & Mabry, 1982);  $^1H$ -NMR spectra were taken on a Bruker-AC 200 spectrometer in DMSO- $d_6$  and  $CD_4O$  solutions and chemical shifts were recorded in  $\delta$ -values;  $[\alpha]_D$  were measured on a Perkin-Elmer polarimeter 341 in  $CH_3OH$  solutions.

Spots on TLC were detected directly under UV light and after evaporation with Neu's reagent (Neu, 1957).

The nature and the position of sugar moiety in the O-glycosides were determined through acid (5% HCl, 1 h) and enzymatic ( $\beta$ -glucosidase, Sigma, G-8625) hydrolysis. The apigenin-7- $\beta$ -O-D-rutinoside was subjected to partial hydrolysis (5% HCl, 30 min; AcOH-10%, 4 h; HCOOH, cyclohexanol, 4 h) (Harborne, 1984). The resulting sugar moiety of each hydrolysis was subjected to co-chromatography with authentic carbohydrates (Fluka) on TLC (Merck, Cellulose plates Art. 5716) with EtOAc- $C_5H_5N$ - $H_2O$  (12: 5: 4), using aniline oxalate (Liskens, 1959) for detection.

### RESULTS AND DISCUSSION

Previous studies on *O. sanctum* from India revealed the presence of the following polyphenolic compounds: apigenin, luteolin, apigenin-7-glucuronide, luteolin-7-glucuronide, orientin, molludistin (= 8-C- $\beta$ -D-glucopyranosyl-genkwanin), galuteolin (= luteolin-5-O-glucoside) (Nair & Gunasegaran, 1982); vicenin-2, cirsilineol (= 5,4'-dihydroxy-6,7,3'-trimethoxyflavone) (Nörr & Wagner, 1992), apigenin-7-glucoside, luteolin-7-glucoside (Nörr, 1993), rosmarinic acid, gallic acid, gallic acid methyl ester, gallic acid ethyl ester, protocatechuic acid, vanillic acid, 4-hydroxybenzoic acid, vanillin, 4 hydroxybenzaldehyde, caffeic acid and chlorogenic acid (Nörr & Wagner, 1992).

The present study revealed a similar, but not identical chemical profile, since we did not find any methylated flavonoid. Concerning the flavonoids of this genus, previous studies on *O. basilicum* revealed the presence of flavonol aglycones and glycosides, of flavanones, of vicenin-2 (Skaltsa & Philianos, 1986, 1990) and of methylated flavones (Grayer et al., 1996). Only 4-methyl ethers of apigenin derivatives were detected in *O. minimum* L., while the flavonoid profile of *O. citriodorum* Vis. is very similar to those of *O. basilicum* (Grayer et al., 1996). Phytochemical studies on *O. canum* Sims. resulted in the isolation of two methylated flavones (Xaasan et al., 1980).

### REFERENCES

- Batta SK, Santhakumari G (1971): The antifertility effect of *Ocimum sanctum* and *Hibiscus rosasinensis*. *Indian J Med Res* 59: 777–781.
- Bhargava KP, Singh N (1981): Anti-stress activity of *Ocimum sanctum* Linn. *Indian J Med Res* 73: 443–451.
- Das SK (1959): Medicinal, economic and useful plants of India, 63 pp. Calcutta, Bally Seed Store.
- Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C (1968): Screening of Indian plants for biological activity Part I. *Indian J Exp Biol* 6: 232–247.
- Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Simal RC, Tandon JS (1973): Screening of Indian plants for biological activity Part VI. *Indian J Exp Biol* 11: 43–54.
- Godhawani S, Godhawani JL, Vyas DS (1987): *Ocimum sanctum*: an experimental study evaluating its anti-inflammatory, analgesic and antipyretic activity in animals. *J Ethnopharmacol* 21: 153–163.
- Godhawani S, Godhawani JL, Vyas DS (1988): *Ocimum sanctum* – a preliminary study evaluating its immunoregulatory in albino rats. *J Ethnopharmacol* 24: 193–198.
- Grayer R J, Bryan SE, Veitch NC, Goldstone FJ, Paton A, Wollenweber E (1996): External flavones in sweet basil,

- Ocimum basilicum* and related taxa. *Phytochemistry* 43: 1041–1047.
- Harborne JB, Mabry TJ, Mabry H (1975): *The Flavonoids*, Chapman & Hall, London.
- Harborne JB, Mabry TJ (1982): *The Flavonoids: Advances in Research*. London N.Y., Chapman & Hall.
- Harborne JB (1984): *Phytochemical Methods*, 222–235 pp. London N.Y., Chapman & Hall.
- Harborne JB (1994): *The Flavonoids: Advances in Research Since 1986*. London, Glasgow, N.Y., Tokyo, Melbourne, Madras, Chapman & Hall.
- Krishnamurthy TR (1959): Some pharmacological action of an extract of *Ocimum sanctum*. *Indian J Phys Pharmacol* 3: 92–94.
- Liskens HF (1959): *Papierchromatographie in der Botanik*, 91 pp. Berlin, Göttingen, Heidelberg, Springer-Verlag.
- Mabry TJ, Markham KR, Thomas MB (1970): *The Systematic Identification of Flavonoids*, Berlin, Heidelberg, N.Y., Springer-Verlag.
- Malviya BK, Gupta PL (1971): Growth prompting properties of *Ocimum sanctum*. *Indian J Pharm* 33: 126–127.
- Murray RDH, Méndez J, Brown SA (1982): *The Natural Coumarins*. Chichester, New York, Brisbane, Toronto, Singapore, Wiley J & Sons.
- Nair AGR, Gunasegaran R (1982): Chemical investigation of certain south Indian plants. *Indian J Chem* 21B: 979–980.
- Neu R (1957): *Die Naturwissenschaften* 44: 181–2.
- Nörr H, Wagner H (1992): New Constituents from *Ocimum sanctum*. *Planta Med* 58: 574.
- Nörr H (1993): Phytochemische und pharmakologische Untersuchungen der Adaptogendrogen *Eleutherococcus senticosus*, *Ocimum sanctum*, *Codonopsis pilosula*, *Rhodiola rosea* und *Rhodiola crenulata*. Thesis, University of Munich.
- Seth SD, Johri N, Sundaram SR (1981): Antispermatic effect of *Ocimum sanctum*. *Indian J Exp Biol* 19: 975–976.
- Singh TJ, Gupta PD, Khahn SY, Misra KC.(1970): Preliminary pharmacological investigations of *Ocimum sanctum*. *Indian J Pharm* 32: 92–94.
- Skaltsa H, Philianos S (1986): Contribution à l'étude chimique d'*Ocimum basilicum* L. – 1ère communication. *Plant méd et Phyt* 20: 291–299.
- Skaltsa H, Philianos S (1990): Contribution à l'étude chimique d'*Ocimum basilicum* L. – 2ème communication. *Plant méd et Phyt* 25: 193–196.
- Xaasan Cusmaan C, Xaasan Ciilmi C, Faarax MX, Passananti S, Piozzi F, Paternostro M (1980): Unusual flavones from *Ocimum sanctum*. *Phytochemistry* 19: 2229–2230.

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