Chemical constituents and biological studies of *Origanum vulgare* Linn.

Gottumukkala Venkateswara Rao, T. Mukhopadhyay, T. Annamalai, N. Radhakrishnan, M. R. Sahoo

CavinKare Research Centre, Ekkattuthangal, Chennai – 600 032, India

Submitted: 19-10-2010 Revised: 08-12-2010 Published: 08-06-2011

ABSTRACT

Bioassay-guided isolation of methanolic extract of the leaves of *Origanum vulgare* Linn., yielded two protocatechuic acid ester derivatives, origanol A (1) and origanol B (2) along with ursolic acid (3), oleanolic acid (4), *β*-sitosterol (5), and triacontanol (6). Structures of the compound were established based on physical and spectral data (UV, IR, ^{1}H and ^{13}C NMR and mass). Origanol A (1) showed significant mushroom tyrosinase inhibition activity.

Key words: *Origanum vulgare* Linn., origanol A and B, triterpene acids, tyrosinase inhibition, β-sitosterol

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.81964

Quick Response Code:

PLANT SOURCE

The genus *Origanum* (Labiatae) is very small, perennial herbs or shrubs distributed in the Mediterranean region and extra tropical Asia. Only one species, *Origanum vulgare*, occurs in India. The herb possesses an aromatic, thyme-like flavor. The leaves and tops before blooming have been used to flavor foods. This plant has been employed to flavor alcohol and beer. The *Origanum* oil possesses carminative, stomachic, diuretic, and emmenagogue properties; it is also applied in chronic rheumatism, tooth ache, and ear ache. The oil is also being used in veterinary liniments and also stimulates the growth of hair. In homoeopathy, it has been used for hysteric condition. It is also being used in healing lotions for wounds along with other herbs as an external application.[1,2]

PREVIOUS STUDY

Earlier various groups from different regions worked on this plant and reported variety of compounds, viz., phenolics, phenolic acids, esters and its glycosides, flavonoids, steroids, and some volatile compounds from different parts of the plant. Nineteen polar constituents were reported from the leaves of *O. vulgare* collected from Greece. These are apigenin, luteolin, chrysoeriol, diosmetin, quercetin, eriodictyol, cosmocide, vicenin-2, caffeic acid, rosmarinic acid, p-menth-3-ene-1,2-diol 1-*O*-β-glucopyranoside, thymoquinol 2-*O*-β-glucopyranoside, thymoquinol 5-*O*-β-glucopyranoside, thymoquinol 2,5-*O*-β-diglucopyranoside, 12-hydroxyjasmonic acid and its β-glucopyranoside, lithospermic acid B, epi-lithospermic acid B, and 10-epi-lithospermic acid.[3] Two anti-fungal active compounds, thymol and carvacrol, were reported from the oil.[4] Five antioxidant phenolic compounds, rosmarinic acid derivative, caffeic acid, protocatechuic acid, phenyl glucoside, and 2-caffeyloxy-3-[2-(4-hydroxybenzyl)-4, 5-dihydroxy]-phenyl propionic acid were reported from the same plant collected in Japan.[5] About 40 terpenoids were reported from the essential oil of *O. vulgare* collected from Germany. The major compounds are sabinene, 1,8-cineole, β-ocimene, β-caryophyllene, germacrene D, bicylcogermacrene, β-bisabolene, and spathulenol.[6] One new antioxidant, 4'-*O*-β-D-glucopyranosyl-4'-hydroxybenzyl protocatechuate, had been reported from the leaves of *O. vulgare*.[7,8] Three DPPH radical scavengers – 4'-*O*-β-D-glucopyranosyl-3', 4'-dihydroxybenzyl protocatechuate, 4'-*O*-β-D-glucopyranosyl-3',4'-

Address for correspondence:
Dr. G. Venkateswara Rao, CavinKare Research Centre, 12, Poonamalle Road, Ekkattuthangal, Chennai – 600 032, India.
E-mail: rao.gv@cavinkare.com

dihydroxybenzyl 4-*O*-methylprotocatechuate, 4'-*O*-β-D-glucopyranosyl-4'-hydroxybenzyl protocatechuate – were reported from *O. vulgare* collected from Japan.[9]

PRESENT STUDY

General experimental procedure

Melting points reported are uncorrected. The 400 MHz NMR spectra were recorded on a Bruker AMX 400 in CD_3OD with TMS an internal standard. The ^{13}C NMR spectra were recorded at 100 MHz in CD_3OD. IR spectra were recorded on a Shimadzu IR prestige 21; UV spectra were recorded on Shimadzu UV spectrophotometer; mass spectra were on a Jeol SX 102/DA 6000 mass spectrometer. TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck) and the spots were visualized by exposure to iodine vapor or spraying with 5% sulfuric acid in methanol followed by heating the plate at 110ºC for 5 min. Kojic acid was purchased from the M/s. Sigma-Aldrich, USA.

Plant material

The leaves of *O. vulgare* were collected in April, 2006, from Ooty, Tamilnadu, India, and was authenticated by Dr. P. Santhan, botanist, M/s. Durva Herbal Centre, Chennai. A voucher specimen (CK-15/06/OV) was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and isolation

The air-dried leaves of *O. vulgare* (700 g) were powdered coarsely, subjected to an extraction with methanol through soxhlet and the extract concentrated under reduced pressure to get crude methanol extract (92 g). Part of the extract (89.0 g) was subjected to vacuum liquid chromatography (VLC) on silica gel using hexane, hexane:ethyl acetate (98:2, 95:5), and methanol to get corresponding fractions 16.69 (Fr.1), 7.55 (Fr.2), 3.30 (Fr.3), and 39.65 g (Fr.4), respectively. The fraction 2 showed two spots on TLC, one major, and one minor compound along with some green color pigments. Fraction 2 was repeatedly purified over silica gel column, obtained compound (1, 240 mg)[9] and compound (2, 50 mg)[9] as amorphous powders [Figure 1]. The greenish residue (Fr.1) obtained from hexane was purified over silica gel column, followed by recrystallization to obtain four pure compounds – ursolic acid (3, 800 mg),[10] oleanolic acid (4, 20 mg),[11] β-sitosterol (5, 40 mg),[12,13] and triacontanol (6, 120 mg).[12,14] These structures were elucidated by physical and spectral data and by comparison with the previously published data

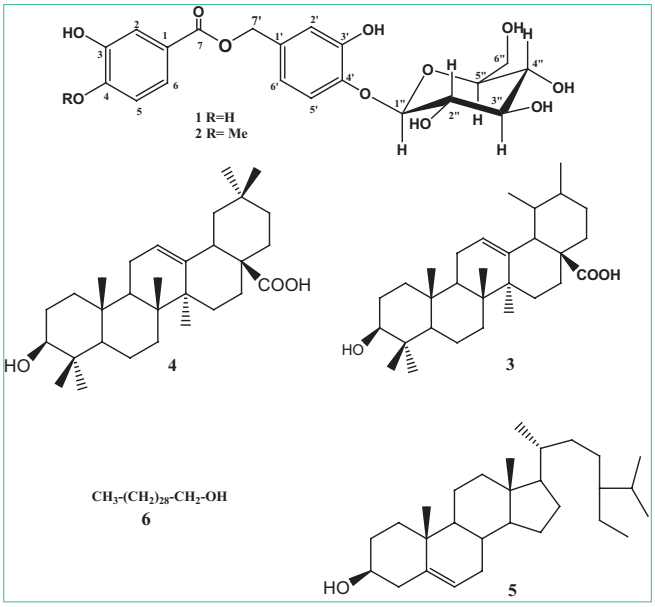

Figure 1: Chemical constituents of *O. vulgare*

RESULTS

Compound 1: white powder; mp: 171–72ºC; IR:3392, 1718, 1606, 1033, and 759 cm^{-1}; UV (nm): 222, 263, 295; ^1H NMR (CD_3OD): δ 3.30–3.52 (4H, m, H-2" to 5"), 3.71 (1H, m, H-6"), 3.88 (1H, d, *J* = 12.08 Hz, H-6"), 4.77 (1H, d, *J* = 7.2 Hz, H-1"), 5.17 (2H, s, H-7'), 6.79 (1H, d, *J* = 8.7 Hz, H-5), 6.83 (1H, dd, *J* = 8.7, 1.9 Hz, H-6′), 6.93 (1H, d, *J* = 1.7 Hz, H-2'), 7.18 (1H, d, *J* = 8.3 Hz, H-5'), 7.42 (1H, s, H-2), 7.44 (1H, m, H-6). ^{13}C NMR (CD_3OD): δ 122.5 (C-1), 117.3 (C-2), 146.2 (C-3), 151.6 (C-4), 115.7 (C-5), 123.5 (C-6), 167.6 (C-7), 133.2 (C-1'), 116.5 (C-2'), 148.1 (C-3'), 145.4 (C-4'), 118.5 (C-5'), 120.6 (C-6'), 67.0 (C-7'), 104.0 (C-1"), 78.3 (C-2"), 77.4 (C-3"), 74.7 (C-4"), 71.2 (C-5"), 62.3 (C-6"); ESIMS (rel.int.): *m/z* 438 (M^+).

Compound 2: white powder; mp: 150–55ºC; IR: 3373, 1701, 1602,1072, 765 cm^{-1}; UV(nm): 222, 264, 295; ^1H NMR (CD_3OD): δ 3.28–3.43 (4H, m, H-2′ to 5′), 3.61 (1H, br d, *J* =12.1 Hz, H-6"), 3.77 (1H, d, *J* =12.1 Hz, H-6"), 3.81 (3H, s), 4.69 (1H, d, *J* =7.3 Hz, H-1"), 5.09 (2H, s, H-7'), 6.79 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'), 6.84 (1H, d, *J* = 2.0 Hz, H-2′), 6.88 (1H, d, *J* = 8.5 Hz, H-5), 7.09 (1H, d, *J* = 8.3 Hz, H-5'), 7.34 (1H, d, *J* = 2.0 Hz, H-2), 7.43 (1H, dd, *J* = 8.4, 2.0 Hz, H-6). ESIMS (rel. int.): *m/z* 452 (M^+).

BIOLOGICAL ACTIVITY STUDIES

Tyrosinase inhibition activity was determined by dopachrome method using L-tyrosine as substrate.[15,16] Fresh solutions of L-tyrosine (3 mM), buffer (*p*H 6.8, 50 mM), and a stock solution of 500 units/mL of mushroom

Table 1: Comparison of tyrosinase inhibition values

Compound	Tyrosinase inhibition
Crude methanolic extract	45% @150 µg/mL
Fraction 2	IC_{50} = 4.57 µg/mL
Kojic acid	IC_{50} = 1.75 µg/mL
Origanol A	IC_{50} = 2.47 µg/mL

tyrosinase were prepared. The reaction mixture constitutes 235 µL of L-tyrosine, 365 µL of buffer, 90 µL of enzyme, and 10 µL of inhibitor. The assay mixture was incubated at 37°C for 30 min. The dopachrome was measured spectrophotometrically at 475 and percentage of inhibition was calculated. It was found that the methanolic extract, fraction 2, and origanol A (1) showed significant tyrosinase inhibition activity whereas origanol B showed very weak activity. IC_{50} value of origanol A (1) was found to be 2.47 µg/mL while the IC_{50} value of the control (Kojic acid) was found to be 1.75 µg/mL [Table 1].

CONCLUSION

In conclusion, we have reported the isolation of six known compounds from this plant. The tyrosinase inhibition activity of crude extract, fractions, and two of isolated compounds were analyzed and one of the compound showed significant activity. Out of the six compounds mentioned in this paper, ursolic acid (3), oleanolic acid (4), β-sitosterol (5), and triacontanol (6) were isolated from the plant *Origanum vulgare* for the first time. The tyrosinase inhibition studies of the extract and the compound origanol A was reported for the first time. The present study of screening of bioactive secondary metabolites revealed *O. vulgare* as a source for the production of tyrosinase inhibitory compounds. These metabolites can be further exploited in cosmetic applications.

ACKNOWLEDGEMENT

We thank Mr. C. K. Ranganathan, CMD of CavinKare Pvt. Ltd., Chennai for providing the necessary facilities and constant encouragement. We also thank to Dr. P. Santhan for identification of the plant material and M/s. Suven Life Sciences, Hyderabad for providing the spectral data.

REFERENCES

1. Anonymous. The wealth of India. Vol 7. New Delhi: CSIR; 2001. p. 105.
2. Kirthikar KR, Basu BD. Indian medicinal plants. Vol 3. Allahabad: Bishan Singh Mahendra Pal Singh; 1994. p. 1986.
3. Koukoulitsa C, Karioti A, Bergonzi C, Pescitelli G, Bari LD, Skaltsa H. Polar constituents from the aerial parts of *Origanum vulgare* L. ssp *hirtum* growing wild in Greece. J Agric Food Chem 2006;54:5388-92.
4. Vijaya M, Cass I, Judy G, Talpur MA, Echard BW, Bagchi D, *et al*. Anti-fungal activities of *Origanum* oil against *Candida albicans*. Mol Cell Biochem 2001;228:111-7.
5. Kikuzaki H, Nakatani N. Structure of a new antioxidative phenolic acid from oregano (*Origanum vulgare* L.). Agric Biol Chem 1989;53:519-24.
6. Mockute D, Asa JJ, Bernotiene G. Volatile constituents of cultivated *Origanum vulgare* L., inflorescences and leaves. Chemija 2004;15:33-7.
7. Nakatani N, Kikuzake H. A new antioxidative glucoside isolated from oregano (*Origanum vulgare*). Agric Biol Chem 1987;51:2727-32.
8. Nakatani N, Kaneko K, Kojo K. 4-[3,4-(Dihydroxybenzoyloxy) methyl] phenyl-D-glucoside from oregano leaves as antioxidant. Jpn Kokai Tokkyo Koho 1987;6202-93.
9. Matsura H, Chiji H, Asakawa C, Amano M, Yoshihara T, Mizutani J. DPPH radical scavengers from dried leaves of Oregano (*Origanum vulgare*). Biosci Biotech Biochem 2003;67:2311-6.
10. Moghaddam FM, Farimani MM, Salahvarzi S, Amin G. Chemical constituents of dichloromethane extract of cultivated *Satureja khuzistanica*. Evid Based Complement Alternat Med 2007;4:95-8.
11. Gohari AR, Saeidnia S, Hadjiakhoondi A, Abdoullahi M, Nezafati M. Isolation and quantificative analysis of oleanolic acid from *Satureja mutica* Fisch. and C. A. Mey. J Med Plants 2009;8:65-9
12. Maria YR, Angelina GM, Ma LV. Sterols, triterpenes and biflavonoids of *V. jucundum* and cytotoxicity activity of ursolic acid. Planta Med 2001;67:683-4.
13. Boonyaratavej S, Petsom A. Chemical constituents of *Bridelia tomentosa*. J Sci Soc Thailand 1991;17:61-9.
14. Chauhan JS, Sultan M, Srivastava K. Chemical constituents from *Salmalia malbaricum*. Can J Chem 1980;58:328-30.
15. Lee KT, Kim BJ, Kim JH, Heo MY, Kim HP. Biological screening of 100 plant extracts for cosmetic use (I): Inhibitory activities of tyrosinase and DOPA auto-oxidation. Int J Cosmet Sci 1997;19:291-8.
16. Piao LZ, Park HR, Park YK, Lee SK, Park JH, Park MK. Mushroom tyrosinase inhibition activity of some chromones. Chem Pharm Bull (Tokyo) 2002;50:309-11.

Cite this article as: Rao GV, Mukhopadhyay T, Annamalai T, Radhakrishnan N, Sahoo MR. Chemical constituents and biological studies of *Origanum vulgare* Linn.. Phcog Res 2011;3:143-5.

Source of Support: Nil, **Conflict of Interest:** None declared.