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Essential oil compounds of three *Centaurea* L. taxa from Turkey and their chemotaxonomy

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The essential oil of three *Centaurea* L. taxa from Turkey (*Centaurea iberica* Trev. ex Spreng.- *Centaurea solstitialis* L. subsp. *solstitialis* and *Centaurea virgata* Lam.) which were collected in the same habitat, have been studied. As a result, thirty nine, forty and forty two components were identified representing 90.8, 91.6 and 92.5% of the oil, respectively. Germacrene D (20.3%), caryophyllene oxide (10.7%), β-caryophyllene (10.5%), β-eudesmol (15.5%), bicyclogermacrene (14.2%), spathulenol (11.3%), and germacrene D (21.4%), β-caryophyllene (16.5%), caryophyllene oxide (9.5%) were detected as the main compounds of *C. iberica*, *C. solstitialis* subsp. *solstitialis* and *C. virgata,* respectively.

Key words: *Centaurea*, essential oil, germacrene D, caryophyllene oxide, chemotaxonomy.

INTRODUCTION

The genus *Centaurea* L. (Asteraceae) is represented by a very large number of species, distributed in particular in southwest, central and east of the Turkey. Furthermore *Centaurea* is a polymorphous genus and comprises 400 to 700 species of annual, biennial and perennial grassy plants, rarely dwarf shrubs predominantly distributed in Europe and Asia (Bancheva and Greilhuber, 2006). *Centaurea* is represented in Turkey with 179 native species out of which which 109 of are endemic (Davis, 1988; Guner et al., 2000), and it is the richest genera in terms of endemic species with the rate of 61.6%, and these are herbaceous perennial herbs grown in mountain slopes and dry lands distributed mainly in Eastern part of Turkey.

The aerial parts of the plant are known as peygamber cicegi, zerdali dikeni, coban kaldiran, timur dikeni in Turkey (Baytop, 1999). *Centaurea* represented in Turkey with 34 sect. *Centaurea iberica*, *Centaurea solstitialis* subsp. *solstitialis* and *Centaurea virgata* belongs to section Calcitrapa DC., Mesocentron (Cass.) DC and Acrolophus (Cass.) DC, respectively. These species are distributed mainly in Eastern Anatolia. They are herbaceous perennial herbs grown in mountain slopes and dry lands (Wagenitz, 1975). Taxonomically, *Centaurea* is very difficult and needs further studies, mainly by using modern cytological and chemical techniques. The unnatural circumscription of *Centaurea* is a very old problem (Wagenitz, 1975) that arises from the large morphological, karyological and palynological diversity (Garcia-Jacas et al., 2001). The principal problems that should be solved are: some sections could be treated as genera, the exact delimitation of many species of some sections should be clarified (Davis, 1988).

Previous chemical studies on the genus *Centaurea* seem to indicate that the sesquiterpene lactones are the most characteristic constituents and systematically important (Nowak et al., 1994). Other secondary metabolites present in plants of this genus include triterpenes (Flamini et al., 2002), steroids (Dumlu and Gurkan, 2006), hydrocarbons, polyacetylenes (Tesevic etal., 2003), flavonoids (Salan and Oksuz, 1999), anthocyanins (Takeda et al., 2005), lignans (Celik et al., 2006) and alkaloids (Shoeb et

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al., 2006). Many species of the genus *Centaurea* have long been used in traditional medicine to cure various ailments and also many members of the genus are reported to be used in Anatolian folk medicine (Sezik et al., 2001). Volatile constituent studies are available in the literature on *Centaurea* species: *Centaurea thessala* subsp. *drakiensis*, *Centaurea zuccariniana*, *Centaurea spruneri*, *Centaurea raphanina* subsp. *mixta* and *Centaurea pelia* (Lazari et al., 2000), *Centaurea calcitrapa* and *C. solstitialis* (Binder et al., 1990), *Centaurea balsamita* Lam. (Bagci and Hayta., 2012), *Centaurea calcitrapa*, *Centaurea gloriosa* and *Centaurea moschata* (Kustrak and Radic, 1985), *Centaurea pseudoscabiosa* subsp. *pseudoscabiosa* and *Centaurea hadimensis* (Flamini et al., 2002), *Centaurea behen* L. (Hayta et al., 2012) and *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. *kotschyi* and *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. *decumbens* Wagenitz (Ertugrul et al., 2003). Chemical compositions of the members of *Centaurea* were also reported by some studies (Dural et al., 2003).

The high endemism ratio (61.6 %) shows that Turkey is one of the gene centers of the *Centaurea*. The research in herbal medicine has increased in developing countries as a way to rescue ancient traditions and as an alternative solution to health problems in cities. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of plants for active compounds has become very important. Various biological activities of the members *Centaurea* were reported elsewhere; cytogenetic (Radic et al., 2005), antimicrobial (Kose et al., 2007), antifungal (Koukoulitsa et al., 2005), antiulcerogenic activities (Yesilada et al., 1999), antioxidant properties (Severino et al., 2007), antiviral (Rusak et al., 1997), antiplasmodial (Medjroubi et al., 2005), antiprotozoal (Karamenderes et al., 2006), anti-colon cancer (Shoeb et al., 2006) and cytotoxic activity (Medjroubi et al., 2005). Furthermore, aerial parts of several *Centaurea* species are used in folk medicine as an antidiarrhoeic, antipyretic, diuretic, choleretic, antiinflammatory and antibacterial (Kargioglu et al., 2010). For example *Centaurea pulchella* Ledeb., *Centaurea drabifolia* Sm. and *C. solstitialis* were reported in Turkish folk medicinal use to treat abcesses, hemorrhoids, peptic ulcers and the common cold (Sezik et al., 2001).

This paper deals with the essential oil composition of three *Centaurea* taxa from Turkey: *C. iberica*, *C. solstitialis* subsp. *solstitialis* and *C. virgata*. We have chosen three taxa growing in the same habitat and with similar ecological needs to evaluate if the pedoclimatic conditions could influence the essential oil composition leading to chemical convergence. And also the aim of the present study is to provide chemical data that might be helpful in chemotaxonomy and to determined qualitative and quantitative essential oil profiles of three *Centaurea* taxa.

MATERIALS AND METHODS

Plant harvesting and analysis of gas exchange

Plant materials (*C. İberica, C. solstitialis* subsp. *Solstitialis, C. virgata*) were collected from the same locality on 5 August, 2011 in Kovancilar province, road side, at an altitude of 1350 to 1500 m, in Elazig/Turkey by O. Kilic. Plant materials were identified with volume five of Flora of Turkey and East Aegean Islands (Wagenitz, 1975). The collected number of samples are 3350, 3351, 3352, respectively.

The extraction of dried aerial part of five grams powder of plant samples were carried out by a (HS-SPME) headspace solid phase microextraction method using a divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fiber, with 50/30 lm film thickness (Supelco, Bellafonte, PA, USA); before the analysis the fiber was preconditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of plant samples, previously homogenized, were weighed into a 40 ml vial; the vial was equipped with a ‘‘mininert’’ valve (Supelco, Bellafonte, PA, USA). The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analytes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al. (2004).

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used with ınjector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 lm film thickness (Chrompack Italy s.r.l., Milan, Italy). The oven temperature was programmed as follows: 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisition range, 40 to 200 m/z; scan rate, 1 us^{-1}. The compounds were identified using the NIST (National Institute of Standards and Technology) library (NIST/EPA/NIH Mass Spectra Library, version 1.7, USA), mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz (1963). The relative amounts were calculated on the basis of peak-area ratios.

RESULTS

The chemical composition of the essential oil of dried aerial parts of *C. iberica, C. solstitialis* subsp. *solstitialis* and *C. virgata* were analyzed by HS-SPME/GC-MS (Headspace Solid Phase Microextraction Method) extraction technique combined with the GC-MS (Gas chromatography–mass spectrometry) system. The yield of oils are ca. 0.30, 0.35 and 0.40 ml/100 g, respectively. Thirty nine, forty and forty two compounds were identified in *C. iberica, C. solstitialis* subsp. *solstitialis* and *C. Virgata,* respectively, accounting from 90.8, 91.6 and 92.5% of the whole oil. Germacrene D (20.3%), caryophyllene oxide (10.7%) and β-caryophyllene (10.5%), β-eudesmol (15.5%), bicyclogermacrene (14.2%) and spathulenol (11.3%), germacrene D (21.4%),

(21.4%), β-caryophyllene (16.5%) and caryophyllene oxide (9.5%) were detected as the main compounds of *C. iberica*, *C. solstitialis* subsp. *solstitialis* and *C. virgata,* respectively.

The identified components of studied *Centaurea* taxa are listed in Table 1. The table also includes their retention indices and the percentage composition. And the main constituents of *Centaurea* taxa from literature and studied taxa are listed in Table 2. In both cases, chemical composition consisted of complex mixtures of different substances, with sesquiterpenes as the dominating constituents.

DISCUSSION

Composition of the three *Centaurea* species resulted poor in monoterpenes. The essential oil of three taxa were characterized, either numerically or quantitatively by sesquiterpenes. Among these, the main ones were germacrene D followed by caryophyllene oxide, β-caryophyllene, spathulenol and β-eudesmol (Tables 1 and 2). Among the sesquiterpenes, germacrene D was detected as one of the major compound in *C. iberica* (20.3%) and in *C. virgata* (10.2%) (Table 1). Similarly, germacrene D was found as a major compound of the essential oils of *C. pseudoscabiosa* subsp. *pseudoscabiosa* and *C. hadimensis* (Flamini et al., 2002), *C. mucronifera* and *C. chrysantha* (Dural et al., 2003), *C. kotschyi* var. *kotschyi* and *C. kotschyi* var. *decumbens* (Ertugrul et al., 2003), *C. cineraria* subsp. *umbrosa* (Senatore et al., 2003), *C. drabifolia* Sm. subsp. *detonsa* (Bornm.) Wagenitz (Zengin et al., 2012) and ten *Centaurea* species (Flamini et al., 2006).

In the composition of *C. aucheri* (DC.) Wagenitz., germacrene D was observed to be lower in our study (Asadipour et al., 2005). Furthermore, germacrene D (21.6%) was determined as the principal component of *Gundelia tournefortii* var. *armata* Freyn. and Sint. which belongs to Asteraceae like *Centaurea* (Bagci et al., 2010). Whereas, germacrene D (3.3 to 1.7 to 0.2%) resulted in very low amounts in study pattern of *C. armena* Boiss. (Yayli et al., 2005)*, C. euxina* Velen. (Rosselli et al., 2009) and *C. napifolia* L. (Senatore et al., 2003), respectively. On the other hand, this compound has not been found in *C. sessilis* Willd. (Yayli et al., 2005).

In this study, caryophyllene oxide (25.2%), β-eudesmol (12.5%), and germacrene D (10.2%) were the main compounds of *C. saligna* (K.Koch) Wagenitz and germacrene D (27.4%), β-caryophyllene (19.3%), β-eudesmol (7.7%), and caryophyllene oxide (6.2%) were the main compounds of *C. kurdica* Reichardt from Turkey.

Caryophyllene oxide was found as principal constituents of *C. iberica* (10.7%) and in *C. virgata* (9.5%) (Table 1). Like our results, this compound also has been detected as a principal constituent in *C. chrysantha* (9.5%) (Dural et al., 2003), *C. euxina* (6.2%) (Rosselli et al., 2009), *C. helenioides* (18.2%) (Yayli et al., 2009), *C. amanicola* Hub.-Mor. (12.0%), *C. consanguinea* DC. (7.3%) and in *C. ptosimopappa* (4.3%) Hayek. (Formisano et al., 2008). On the other hand, this compound was not a principal constituent in *C. cuneifolia* Sibth. (Rosselli et al., 2009) and was of low amounts in *C. pseudoscabiosa* subsp. *pseudoscabiosa* Boiss. et Buhse (4.4%), *C. hadimensis* Wagenitz (3.1%) (Flamini et al., 2002) and in *C. solstitialis* subsp. *solstitialis* (5.2%) (Table 1). β-Eudesmol was of high percentage in *C. solstitialis* subsp. *solstitialis, C. cariensis* subsp. *niveotomentosa* (Ugur et al., 2010), *C. cuneifolia* (Rosselli et al., 2009) and *C. mucronifera* (Dural et al., 2003). It is interesting that this compound has not been detected in *C. chrysantha* (Dural et al., 2003), *C. pseudoscabiosa* subsp. *pseudoscabiosa* and *C. hadimensis* (Flamini et al., 2002). Hexadecanoic acid was the most abundant component of *C. aladagensis* Wagenitz (Kose et al., 2007), *C. luschaniana*, *C. tossiensis*, *C. wagenitzii* (Kose et al., 2008), *C. saligna* (Altintas et al., 2009), *C. paphlagonica* (Kose et al., 2009) from Turkey, three *Centaurea* taxa from Italy (Senatore et al., 2008) and *C. eryngioides* Lam., *C. iberica* Trev. var. *hermonis* Boiss. Lam. from Lebanon (Senatore et al., 2005). Whereas in our study, hexadecanoic acid was determined in low percentages in three *Centaurea* taxa (Table 1).

The main differences between under studied samples are high percentages of bicyclogermacrene (14.2%), β-eudesmol (15.5%) and spathulenol (11.3%) detected only in *C. solstitialis* subsp. *solstitialis* (Table 1). β-Caryophyllene was detected as the main compound in the essential oil of *C. iberica* (10.5%), *C. virgata* (9.5%) (Table 1), *C. pseudoscabiosa* (8.1%), *C. hadimensis* subsp. *pseudoscabiosa* (9.8%) (Flamini et al., 2002), *C. kotschyi* var. *kotschyi* (12.1%)*, C. kotschyi* var. *decumbens* (11.2%) (Ertugrul et al., 2003) and in *Anthemis cretica* subsp. *pontica* (20.26%) (Asteraceae) (Kilic et al., 2011). Whereas β-caryophyllene was not determined in the essential oil of *C. cuneifolia* and *C. euxina* (Rosselli et al., 2009). Moreover, β-caryophyllene has been reported to be of low percentages in volatile constituents of *Tripleurospermum parviflorum* (Willd.) Pobed (3.2%) (Asteraceae) essential oil from Turkey (Kilic and Bagci, 2012) and in *C. solstitialis* subsp. *solstitialis* (5.3%) (Table 1).

Spathulenol was detected as a major compound in *C. cuneifolia* (6.3%) and *C. euxina* (10.8%) (Rosselli et al., 2009). It is interesting that spathulenol was not detected in *C. napifolia* and detected in trace amounts in *C. cineraria* (Senatore et al., 2003). Antimicrobial activity of germacrene D (Mishra et al., 2011), antibacterial properties of caryophyllene oxide (Ulubelen et al., 1994), pharmaceutical use of β-caryophyllene have been revealed previously.

Sesquiterpenes are the main class and among these include β-caryophyllene, germacrene D,

Table 1. Identified components of *Centaurea* taxa (%).

Constituent	RRI*	*C. iberica*	*C. solstitialis* subsp. *solstitialis*	*C. virgata*
Hexanal	820	0.5	0.2	0.4
Heptanal	882	-	0.1	0.2
Tricyclene	927	0.2	0.1	-
α-pinene	935	0.8	-	0.3
Benzaldehyde	962	0.1	1.1	0.3
2-pentylfuran	980	0.3	-	0.1
Octanal	1003	-	0.5	0.2
α-phellandrene	1006	1.5	0.1	-
p-cymene	1015	0.7	0.5	0.1
Limonene	1025	0.1	-	0.2
β-phellandrene	1032	0.4	0.5	0.2
Terpinene	1052	0.3	1.1	2.2
Octanol	1063	2.3	3.5	4.1
Nonanal	1076	1.2	0.5	0.1
Linalool	1102	-	0.9	0.1
α-terpineol	1180	0.1	0.1	-
Decanal	1203	1.8	2.1	1.4
β-sesquiphellandrene	1223	2.5	2.4	3.1
α-cubebene	1350	-	0.2	0.1
α-copaene	1375	2.3	3.1	-
β-cubebene	1390	1.8	0.5	0.6
β-caryophyllene	1418	10.5	5.3	16.5
A-ylangene	1420	0.1	-	0.3
(E)-β -Farnesene	1452	-	0.9	1.1
α-humulene	1455	1.3	-	1.5
Aromadendrene	1462	-	0.4	0.2
γ-muurolene	1475	0.5	0.3	-
Germacrene D	1480	20.3	6.3	21.4
β-selinene	1488	0.9	-	0.3
Bicyclogermacrene	1496	4.2	14.2	4.8
β-bisabolene	1512	1.3	1.4	0.3
δ-cadinene	1520	-	0.5	0.2
Nerolidol	1525	0.2	-	0.3
Spathulenol	1575	5.4	11.3	7.5
Caryophyllene oxide	1580	10.7	5.2	9.5
Globulol	1605	0.4	0.1	0.7
α-cadinol	1640	2.7	0.3	-
B-eudesmol	1650	5.3	15.5	4.8
α-bisabolol	1680	2.5	-	0.3
Hexadecanoic acid	1692	3.2	4.1	0.6
Pentadecanal	1710	-	2.2	0.2
Hexadecanal	1815	1.3	4.2	5.6
Pentadecanoic acid	1865	1.1	0.5	0.4
Nonadecane	1905	-	0.2	0.8
Cis-phytol	2110	0.7	0.1	0.1
Tricosane	2295	-	0.4	0.3
Pentacosane	2495	0.2	0.3	-
Heptacosane	2650	0.3	-	0.7
Nonacosane	2700	0.8	0.5	0.4
Total		90.8	91.6	92.5

RRI*: relative retention indices. - : not detected.

Table 2. Main constituents of *Centaurea* taxa from literature and studied samples (%).

Main constituent	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
β-caryophyllene	8.1	9.8	11.2	12.1	0.7	0.9	6.0	1.0	4.3	1.3	5.4	7.3	4.2	-	-	8.6	2.8	10.5	5.3	16.5
Germacrene D	36.0	44.3	29.4	44.2	-	-	-	-	61.0	-	3.3	29.3	27.4	-	1.7	22.0	0.2	20.3	6.3	21.4
Bicyclogermacrene	4.2	7.9	4.1	5.5	-	-	-	-	7.2	-	-	4.8	5.4	-	-	1.7	-	4.2	14.2	4.8
Caryophyllene oxide	4.1	3.1	1.9	3.0	7.8	6.2	10.3	0.3	-	10.0	4.7	5.2	9.5	2.9	6.2	3.2	-	10.7	5.2	9.5
Hexadecanoic acid	-	-	-	-	7.4	6.5	6.7	0.1	-	-	-	-	-	17.6	20.3	-	-	3.2	4.1	0.6
B-eudesmol	-	-	1.9	-	-	-	5.6	2.9	-	12.4	19.3	17.4	-	0.8	-	1.2	-	5.3	15.5	4.8
Spathulenol	-	-	-	-	3.8	4.2	3.9	0.9	-	4.9	3.9	1.5	3.8	6.3	10.8	-	-	5.4	11.3	7.5

1. C. pseudoscabiosa subsp. *pseudoscabiosa* and 2. *C. hadimensis* (Flamini et al., 2002). 3. *C. kotschyi* var. *decumbens* and 4. *C. kotschyi* var. *kotschyi* (Ertugrul et al., 2003). 5. *C. thessala* subsp. *drakiensis* and 6. *C. zuccariniana* (Lazari et al., 2000). 7. *C. raphanina* subsp. *mixta* and 8. *C. spruneri* (Lazari et al., 1999). 9. *C. solstitialis* (Binder et al., 1990). 10. *C. sessilis* and 11. *C. armena* (Yayli et al., 2005). 12. *C. mucronifera* and 13. *C. chrysantha* (Dural et al., 2003). 14. *C. cuneifolia* and 15. *C. euxina* (Rosselli et al., 2009). 16. *C. cineraria* subsp.*umbrosa* and 17. *C. napifolia* (Senatore et al., 2003). 18. *C. iberica*, 19. *C. solstitialis* subsp. *solstitialis* and 20. *C. virgata* (studied samples).

bicyclogermacrene, caryophyllene oxide, followed by β-eudesmol and spathulenol (Table 2). Two fatty acids were present in all taxa 9.9% with hexadecanoic acid being the most abundant (4.1%) in *C. solstitialis* subsp. *solstitialis* followed by pentadecanoic acid with low percentanges in all taxa (Table 1). *C. pseudoscabiosa* subsp. *pseudoscabiosa*, *C. hadimensis*, *C. kotschyi* var. *decumbens*, *C. kotschyi* var. *kotschyi*, *C. solstitialis, C. mucronifera, C. chrysantha* and *C. cineraria* subsp. *umbrosa* contained high concentrations of germacrene D (36.0, 44.3, 29.4, 44.2, 61.0, 29.3, 27.4 and 22.0%, respectively) and β-caryophyllene (8.1, 9.8, 11.2, 12.1, 4.3, 7.3, 4.2 and 8.6%, respectively). *C. thessala* subsp. *drakiensis, C. zuccariniana*, *C. raphanina* subsp. *mixta*, *C. spruneri*, *C. sessilis* and *C. cuneifolia* were characterized by their being free of germacrene D. Furthermore, *C. armena, C. euxina and C napifolia* were characterized by their lower content of germacrene D (3.3, 1.7 and 0.2%, respectively); *C. cuneifolia* and *C. euxina* showed a very different chemical behavior from all the other species, producing high amounts of hexadecanoic acid (17.6 to 20.3%) and spathulenol (6.3 to 1 0.8%), followed by no percentages of β-caryophyllene and bicyclogermacrene (Table 2).

Conclusion

These species synthesized many similar compounds in their essential oils that could be justified by the similar ecological conditions of their habitat (biochemical convergence). However, taking into account the differences referred to some constituents, also the taxonomic distance of these species could be confirmed by our chemical data. The comparison between the two taxa evidenced a similarity, at least with reference to the presence of the main constituents: in fact germacrene D, caryophyllene oxide and β-caryophyllene were among the principal ones in both studied taxa. Also, the percentages of β-spathulenol and β-eudesmol were comparable. The only differences between the three taxa were substantially due to bicyclogermacrene which was found only in high percentages in *C. solstitialis* subsp. *solstitialis* (14.2%) (Table 1). It is possible to say that *C.iberica* showed germacrene D/caryophyllene oxide/ β-caryophyllene, *C. solstitialis* subsp. *solstitialis* comprised β-eudesmol/bicyclogermacrene/spathulenol and *C. virgata* germacrene D/β-caryophyllene/ caryophyllene oxide chemotype in Eastern Anatolian region of Turkey. Some of the *Centaurea* species showed different type of essential oil, like germacrene D in *C. mucronifera* and *C. chrysantha* (Dural et al., 2003), β-eudesmol in *C. sessilis* and *C. armena* (Yayli et al., 2005), β-eudesmol/hexadecanoic acid in *C. cuneifolia* (Rosselli et al., 2009) and hexadecanoic acid/spathulenol chemotype in *C. euxina* (Rosselli et al., 2009). Besides, chemical composition of the essential oil of *Centaurea* taxa showed differences, similarities and different qualitative and quantitative profiles. These differences could be due to the local, climatic and seasonal factors (Perry et al., 1999).

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