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The ethnobotany and pharmacognosy of *Olea europaea* subsp. *africana* (Oleaceae)

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Abstract

The ethnobotanical uses of wild olive, *O. europaea* subsp. *africana* (sometimes referred to as subsp. *cuspidata*) in southern Africa and in other parts of Africa are reviewed. Chromatographic analyses of secoiridoids (oleuropein and other oleuropeosides) in 25 wild olive leaf samples from 10 localities in South Africa showed substantial amounts of oleuropein (up to 110 mg/g dry weight) and not trace amounts as reported in the literature. Oleuropein is the main active compound in olive leaf, with demonstrated anti-oxidant, anti-microbial, hypolipidemic and hypotensive activities. A comparison with nine cultivated olive leaf samples (subsp. *europaea*) from six cultivars and two localities showed that commercial olive leaf can be distinguished by the presence of verbascoside, which is absent in wild olive. Extraction methods and solvent systems (TLC and HPLC) were compared, using pure oleuropein (isolated from wild olive leaf and identified by NMR) as an authentic reference sample. The unique peltate scales on the leaves are useful to identify olive leaf raw material (but are the same in both subspecies). The main conclusion is that wild olive leaf is chemically closely similar to cultivated olive leaf and therefore suitable as an alternative source of raw material for olive leaf extract.

Keywords: Ethnobotany; Iridoids; Leaf anatomy; *Olea europaea* subsp. *africana*; Oleaceae; Olive leaf extract

1. Introduction

The commercial olive, *Olea europaea* L. subsp. *europaea* (Oleaceae) is well known for the production of olive oil and for the edible fruits (green and black olives). The closely related African wild olive, previously known as *Olea africana* Mill., is currently regarded as a subspecies of *O. europaea*, namely subsp. *africana* (Mill.) P.S.Green (Klopper et al., 2006). Several wild and cultivated forms are included in the *O. europaea* complex, and alternative names are available, depending on which taxonomy and nomenclature are followed (Hamman-Khalifa et al., 2007). The alternative name for *O. africana* at the subspecies rank is subsp. *cuspidata* (Wall. ex G. Don) Cif. (Klopper et al., 2006; Mabberly, 2008).

In recent years, natural olive leaf or olive leaf extract (OLE) have become popular as commercial herbal medicines, marketed as having anti-ageing, immunostimulant and antibiotic properties. It therefore seems reasonable to consider the leaves of *O. europaea* subsp. *africana* as a suitable alternative to the common olive (subsp. *europaea*) as a source of raw material for product development. The main active compound in olive leaf or OLE responsible for anti-oxidant, anti-microbial, hypolipidemic and especially hypotensive activities is the well-known bitter principle of olives, the secoiridoid called oleuropein (Khan et al., 2007). Oleuropein and other phenolic glycosides (including coumarins) were reported from the bark of subsp. *africana* by Tsukamoto et al. (1984a,b). However, Somova et al. (2003) reported that oleuropein occurs in the leaves of wild olive in trace amounts only, which would suggest that the subsp. *africana* is chemically different from subsp. *europaea* and therefore perhaps not suitable for medicinal purposes.

The aim of this paper is to investigate the presence or absence of oleuropein and other oleuropeosides in wild olive leaf. We also explore the potential of leaf trichomes for the microscopic identification of raw material. Other aspects of relevance to product development are briefly reviewed and presented, namely the ethnobotanical history and pharmacognosy of wild olive leaf (subsp. *africana*).

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2. Materials and methods

2.1. Plant material

Leaf samples of *Olea europaea* subsp. *africana* were collected from 10 natural populations in South Africa (Table 1). In most cases, three different individual trees were sampled. Locality details and quarter degree grid references are: Bainskloof (near Wellington, Western Cape Province; 3319CA); Montagu (Western Cape Province; 3320CC); Riversdale (Western Cape Province; 3421AB); Gamtoos River (Eastern Cape Province; 3325CC); Klipriviersberg (near Johannesburg, Gauteng Province; 2628CA); Sandy Bay (Western Cape Province; 3218CA); Nieuwoudtville (Northern Cape Province; 3119AC); Citrusdal (Western Cape Province; 3219CA); Napier (Western Cape Province; 3419BD); Renosterhoek (near Piquetberg, Western Cape Province; 3218DB). Leaf samples of nine trees of subsp. *europaea* were collected from the farm Vondeling at Paarl in the Western Cape Province (samples 1 to 5) and also from the garden of one of us (B-EvW) (samples 6 to 9). All materials were identified by an experienced taxonomist (B-EvW), following the nomenclature of Coates Palgrave (2002) and Klopper et al. (2006).

For studies of the trichomes, leaf samples of subsp. *africana* (*H Long 12*) and subsp. *europaea* cv. Mission (*ex hort.*, B-EvW) were collected and either air-dried or preserved in formalin:acetic acid:alcohol (FAA).

Voucher specimens have all been deposited in the University of Johannesburg Herbarium (JRAU).

Table 1
The presence of oleuropein and some other main compounds as revealed by HPLC analyses of 25 samples of wild olive leaf (*Olea europaea* subsp. *africana*) from 10 different localities in South Africa and nine samples of cultivated olive (*O. europaea* subsp. *europaea*) representing five cultivars and two provenances. Results are given in percentage peak area. The positively identified compounds are 1, hydroxytyrosol; 2, tyrosol; 3, verbascoside; 8, oleuropein; 4–7 and 9–11 are unidentified compounds (−=not detected).

COMPOUNDS:	Voucher specimen (in JRAU) or provenance	Yield of crude extract (mg/g)	1	2	3	4	5	6	7	8	9	10	11
R_t in minutes:			2.9	3.6	6.5	8.6	9.0	9.7	10.3	10.6	10.9	11.4	12.3
SAMPLE:													
subsp. africana													
Bainskloof 1	*JS Boatwright 175a*	231	6.1	5.2	–	3.8	18.4	6.7	6.3	22.7	5.5	13.4	2.1
Bainskloof 2	*JS Boatwright 175b*	160	6.0	6.2	–	5.1	23.2	8.1	6.2	11.6	5.0	12.4	3.1
Bainskloof 3	*JS Boatwright 175c*	196	6.4	6.0	–	7.1	20.6	9.3	7.1	8.6	5.0	13.3	4.6
Montagu 1	*B-E Van Wyk 4220a*	223	3.8	4.7	–	4.2	15.4	6.1	5.9	16.1	15.9	10.1	6.0
Montagu 2	*B-E Van Wyk 4220b*	211	3.8	5.4	–	4.5	20.3	5.9	5.9	12.1	5.2	12.3	5.9
Montagu 3	*B-E Van Wyk 4220c*	329	3.2	5.6	–	3.1	16.0	9.9	6.8	17.5	9.1	14.2	7.5
Riversdale 1	*H Long 22a*	253	3.3	4.3	–	8.0	18.2	12.0	6.3	9.1	5.6	18.1	2.6
Riversdale 2	*H Long 22b*	303	2.8	6.6	–	5.7	17.5	9.7	6.9	13.6	10.7	15.6	7.9
Gamtoos River	*H Long 3*	220	2.8	4.6	–	4.4	23.1	6.7	6.2	25.0	5.4	12.4	5.0
Klipriviersberg	*H Long 12*	133	9.4	4.4	–	7.8	11.9	5.9	11.6	9.0	8.2	7.2	9.3
Sandy Bay 1	*B-E* and *M Van Wyk 4314a*	155	5.2	4.5	–	6.0	14.8	11.1	9.0	13.5	5.1	11.8	6.8
Sandy Bay 2	*B-E* and *M Van Wyk 4314b*	197	5.8	3.3	–	5.9	14.6	7.9	5.6	5.4	3.3	13.3	5.4
Sandy Bay 3	*B-E* and *M Van Wyk 4314c*	192	3.5	4.0	–	3.8	17.8	8.9	10.0	17.3	5.8	13.1	7.2
Nieuwoudtville 1	*B-E* and *M Van Wyk 4275a*	217	3.8	4.3	–	3.5	18.8	7.4	6.6	19.7	5.8	10.1	7.3
Nieuwoudtville 2	*B-E* and *M Van Wyk 4275b*	232	4.8	2.0	–	4.0	16.0	6.9	4.5	9.9	10.0	9.0	6.2
Nieuwoudtville 3	*B-E* and *M Van Wyk 4275c*	231	4.6	4.4	–	3.3	22.9	10.6	4.6	21.6	4.7	15.5	7.5
Citrusdal 1	*B-E* and *M Van Wyk 4293a*	166	3.9	3.4	–	3.4	14.0	4.7	8.9	12.1	6.4	9.0	6.4
Citrusdal 2	*B-E* and *M Van Wyk 4293b*	162	4.8	3.4	–	2.9	13.9	5.2	8.8	12.0	8.5	12.9	5.6
Citrusdal 3	*B-E* and *M Van Wyk 4293c*	215	4.2	2.1	–	6.1	19.8	6.7	7.9	16.4	6.2	13.4	9.0
Napier 1	*B-E* and *M Van Wyk 4317a*	113	11.6	2.6	–	6.6	17.0	9.6	6.5	4.3	11.1	15.4	4.6
Napier 2	*B-E* and *M Van Wyk 4317b*	225	3.5	4.3	–	4.3	21.1	7.5	7.7	10.2	7.0	17.0	3.8
Napier 3	*B-E* and *M Van Wyk 4317c*	219	4.2	4.7	–	7.3	18.8	6.8	7.7	9.7	5.2	13.3	4.5
Renosterhoek 1	*B-E* and *M Van Wyk 4322a*	245	4.3	3.3	–	4.5	17.1	9.5	4.0	10.5	5.2	15.2	7.2
Renosterhoek 2	*B-E* and *M Van Wyk 4322b*	224	4.1	4.3	–	4.6	13.1	3.4	7.3	33.9	2.4	9.4	8.6
Renosterhoek 3	*B-E* and *M Van Wyk 4322c*	295	5.3	2.7	–	3.4	17.1	3.9	5.2	13.1	5.4	13.9	10.5
subsp. europaea													
cv. Coratina	Experimental farm, Paarl	130	7.9	3.2	12.6	4.1	19.3	5.8	3.9	17.7	6.7	15.5	3.2
cv. Barouni	Experimental farm, Paarl	104	5.5	2.3	14.0	6.3	16.2	6.0	6.3	11.8	6.3	16.8	8.4
cv. Frantoio	Experimental farm, Paarl	127	7.0	2.9	9.5	4.6	20.4	3.9	3.4	15.9	7.3	15.2	5.2
cv. Kalamata	Experimental farm, Paarl	134	5.5	3.5	23.1	6.7	15.7	3.2	5.1	10.7	5.9	14.9	5.6
cv. Mission	Experimental farm, Paarl	109	5.5	3.8	18.1	5.1	17.2	7.9	4.1	11.1	4.9	16.3	5.9
cv. Mission tree 1	*ex hort.,* B-E Van Wyk	123	9.3	3.9	5.2	4.0	21.8	4.5	2.4	17.3	8.4	15.7	7.4
cv. Mission tree 2	*ex hort.*, B-E Van Wyk	121	7.4	2.4	3.5	3.3	21.9	3.6	2.6	22.4	9.9	14.6	4.3
cv. Mission tree 3	*ex hort.*, B-E Van Wyk	173	4.6	5.6	11.8	5.7	14.9	4.5	3.5	19.9	4.8	19.0	5.6
cv. unknown	*ex hort.*, B-E Van Wyk	158	6.2	4.1	9.8	5.2	12.7	3.0	3.4	21.7	4.7	14.6	5.7

2.2. Extraction

Air-dried leaves were powdered and extracted for 15 min at 60 °C in methanol, or overnight at room temperature in ethyl acetate or boiling water, at a ratio of 1 g/10 ml. After filtration, the solvents were evaporated (on a rotary evaporator or in the case of water, by freeze-drying).

2.3. Chromatographic analysis

Thin layer chromatography (TLC) was performed on pre-coated silica plates (Sil G-25 UV_{254} 0.25 mm thick, Macherey-Nagel). Two solvent systems were used: 1, ethyl acetate:dioxane: water (30:10:0.3) (Wagner and Bladt, 1996) and 2, chloroform: methanol:water:acetic acid (60:30:8:6). Leaf extracts were dissolved in methanol. Spots were visualized by spraying with $FeCl_3$ or vanillin-sulphuric acid reagent and heated at 110 °C for 10 min. Authentic samples of oleuropein, verbascoside and isoverbascoside were used as reference compounds (see later).

High pressure liquid chromatography (HPLC) was done on a Beckman System Gold apparatus with programmable Solvent Module 126 and a Beckman Diode Array Detector Module 168, using a Luna 5 µm C18 (2) 100 A column (250×4.6 mm). The flow rate was 1 ml/min and the absorbance changes were read at 340 nm (channel A) and 280 nm (channel B). The mobile phase was acetic acid and water (1:99, solvent A) and methanol (solvent B) in a linear gradient from 60% A and 40% B to 0% A and 100% B over 20 min (held for 2 min), then decreased to 95% A and 5% B over 2 min (held for 2 min) and then back to the initial conditions. The dried sample extract was dissolved in a methanol: water (50:50) solution and filtered through a 55 µm C18 (100 mg/ 1 ml) solid phase extraction column (SPE) before loading onto the HPLC. Oleuropein was identified by comparison with authentic oleuropein isolated by column chromatography and identified by NMR spectroscopy (see later). Authentic reference samples of verbascoside and isoverbascoside were a gift from Dr. Sandra Combrink (Tshwane University of Technology, Pretoria).

2.4. Isolation of oleuropein

Column chromatography was performed with flash silica gel 60 under nitrogen gas pressure to isolate the main compound in wild olive leaf (visible on TLC at R_f 0.62). The eluent system used was chloroform:methanol:water:acetic acid (60:30:8:6). The leaf sample from Montagu (sample 3, see Table 1) was chosen for the isolation of oleuropein because it showed relatively high levels of the compound (TLC and HPLC). Fractions were monitored by TLC and those showing a zone at R_f 0.62 were pooled and concentrated with a rotary evaporator under vacuum and then under a stream of nitrogen at 25 °C. A sample of 110 mg of pure oleuropein was obtained from 1 g of dry leaves (giving a yield of 11% dry wt).

2.5. Nuclear magnetic resonance spectroscopy

The column chromatography samples of purified oleuropein were freeze dried at −80 °C (Virtis "K" series benchtop freeze dryer with an Edwards No. 5 vacuum pump) before preparation for NMR. ^{13}C and ^{1}H NMR spectra were obtained at 300 MHz using the NMR Inova series with vnmr software. Prof. Fanie Van Heerden, School of Chemistry, University of KwaZulu-Natal, kindly confirmed the identity of the isolated compound as oleuropein (Fig. 1) by direct comparison of the NMR spectra with literature data (Tsukamoto et al., 1984b; Gariboldi et al., 1986; Kuwajima et al., 1988; Montedoro et al., 1993).

2.6. Trichome studies

2.6.1. Light microscopy

Epidermal peels of portions of fresh leaves were prepared using Jeffrey's maceration fluid (Kiger, 1971). Some were stained with either safranin or toluidine blue before viewing. In addition, permanent slides of leaf transverse sections were prepared using the paraffin wax method and staining with safranin and fast green (Johansen, 1940). Photographs were taken with a JVC KY-F1030 digital camera. Milled dried leaf powder was also observed with a light microscope after mounting in a drop of distilled water on a slide.

2.6.2. Scanning electron microscopy

Portions of air-dried leaves (with adaxial and abaxial surfaces uppermost) as well as milled dried leaf powder were mounted on stubs and coated with gold. They were viewed with a Jeol JSM 5600 scanning electron microscope and compared.

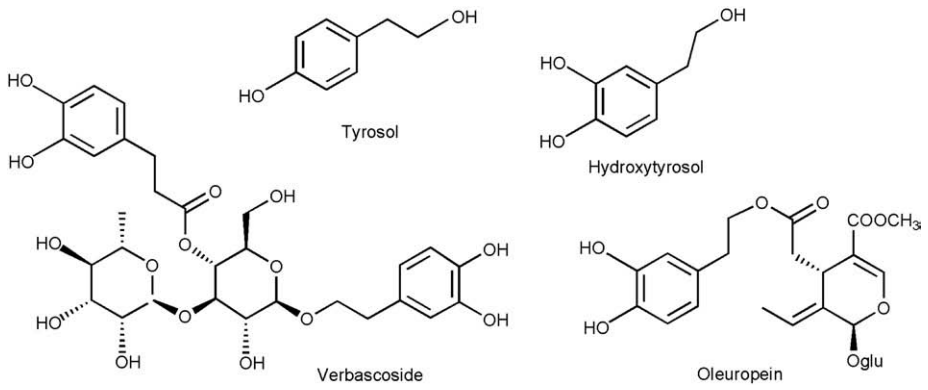

Fig. 1. Chemical structures of oleuropein and structurally related phenolic compounds in *Olea europaea* leaves.

3. Results and discussion

3.1. Ethnobotany

A summary of the recorded medicinal uses of *Olea europaea* subsp. *africana* in southern Africa as well as other parts of Africa is presented in Table 2. Only original anecdotes are listed — those that were obviously cited from other sources were omitted. These include Neuwinger (2000), Von Koenen (2001), Van Wyk and Gericke (2000), Coates Palgrave (2002) and Van Wyk et al. (2009). In southern Africa, the leaves or leaf extracts of subsp. *africana* are mainly used as an eye lotion for humans and animals, as a styptic and to treat colds, sore throat, enlarged tonsils and diphtheria (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Van Wyk and Gericke, 2000; Van Wyk et al., 2009). The earliest record of the medicinal use of wild olive leaves in southern Africa is that of Pappe (1857) who reported their use as a styptic on fresh wounds. An early Cape Dutch use, namely the treatment of eye injuries with a cold poultice of the leaves, was given by Dykman (1908). Roots and bark are commonly used in various parts of Africa for urinary ailments, colic, tapeworms, rheumatism and other ailments (Table 2).

In Europe and the Mediterranean region, *Olea europaea* is widely used as a diuretic, hypotensive, emollient, febrifuge, tonic and an anti-inflammatory agent (Hutchings et al., 1996; Van Wyk and Wink 2004; Khan et al., 2007). The leaf, bark and wood have been used as an anti-febrile, the leaf for healing sores and the flower as an anti-diarrhoeal. The wild form of *Olea europaea* is used in the traditional treatment of diabetes and hypertension in south-eastern Morocco, where the leaf is used in the form of a decoction (Tahraoui et al., 2007).

It has been widely reported that *Olea europaea* leaf extracts show anti-viral, anti-microbial, anti-fungal, anti-diabetic, hypolipidemic and anti-oxidant activities (Benavente-Garcia et al., 2000; Khan et al., 2007; Pereira et al., 2007). Hansen et al. (1996) found that various secoiridoids, including oleuropein

Table 2
Ethnobotanical uses of Olea europaea subsp. africana in southern Africa and in the rest of Africa.

	Reference
Traditional uses in southern Africa	
Dried and powdered leaf used as a styptic on fresh wounds.	Pappe (1857)
Powdered leaf used as snuff to stop nosebleeds.	Atherstone ined., cited in Smith (1966)
Pounded leaves are used (as a cold poultice) to treat eye injuries.	Dykman (1908)
A preparation used for headaches (Lesotho).	Phillips (1917)
Fresh bark infusion taken to relieve colic; leaf infusions are used as an eye lotion for humans and animals. A leaf decoction is gargled to treat diphtheria and other sore throats (Queenstown area). A strong decoction of the root and bark is taken before breakfast for urinary ailments.	Watt and Breyer-Brandwijk (1962)
Unspecified parts are used as protective charms against lightning (Lesotho). A leaf preparation is used for headaches. Other uses cited from Watt and Breyer-Brandwijk (1962).	Jacot Guillarmod (1971)
Uses cited from Watt and Breyer-Brandwijk (1962).	Palmer and Pitman (1973)
Leaves used as tea. Other uses cited from Watt and Breyer-Brandwijk (1962).	Palmer (1985)
Cold water infusions are used to rinse "painful red eyes with discharge".	Hedberg and Staugård (1989)
Bark and leaves used to treat headache as well as lung and kidney ailments.	Pooley (1993)
The leaves of "tjoubie" are boiled with sugar and used for colds (Bitterfontein area). Tincture of the stem tips is used to treat enlarged tonsils (Montagu).	Rood (1994)
Leaf infusions taken for a sore throat, kidney complaints and a bad back.	Montagu Museum (1998)
Decoctions of grated root and scraped bark are taken to treat urinary and bladder infections and for headaches. The Tswana use juice of ripe fruit to soften corns. Other uses cited from Watt and Breyer-Brandwijk (1962).	Roberts (1983)
Inhaling the smoke from a fire made with the timber is used to treat a hangover (it "will clear the head and the blood"). A fresh leaf extract is used for eye ailments (as eye bath or by application with a cloth). Root decoctions are used against headache, influenza, fever and rheumatism. Dried fruit powder is mixed with oil and rubbed into aching joints. The Tswana used a wad of leaves for washing in the belief that it will purify the body. Other uses cited from Watt and Breyer-Brandwijk (1962) and Smith (1966).	Roberts (1992)
Debittered inner bark used as an ingredient when brewing honey beer (mead) (Richtersveld).	Archer (1994)
Uses cited from the literature given here.	Hutchings et al. (1996)
Leaves used as tea substitute. Other uses cited from Watt and Breyer-Brandwijk (1962).	Venter and Venter (1996)
Ripe fruits are boiled with sugar and used as a cough syrup (Cederberg area).	Willem Hanekom (pers. com. to B-EvW)
Traditional uses in other parts of Africa	
The Masai drink a tea made from the heart-wood (Kenya).	Hollis (1905), cited in Watt and Breyer-Brandwijk (1962)
Leaf used as taenifuge (Ethiopia); twig and leaf burnt as fumigant (Tanzania); root used to treat rheumatism.	Brenan and Greenway (1949), cited in Watt and Breyer-Brandwijk (1962)
Decoction of bark and roots is taken to treat tapeworm, ascariasis, diarrhea and intestinal infections (Ethiopia).	Lemordant (1971)
Bark maceration is taken against tapeworm (East Africa).	Kokwaro (1976)
Bark decoction (taken or applied as vapour bath) is used to treat an itching rash (Kenya). Other uses cited from Watt and Breyer-Brandwijk (1962) or Kokwaro (1976).	Lindsay (1978)
Bark decoctions are used internally and topically for the treatment of dermatitis, itches and rashes (Kenya).	Kokwaro (1988)
Stems are used in soup to treat backache and painful joints (Kenya). Roots used for rheumatism (mainly backache). Leaves are used for eye ailments.	Gachathi (1989)

have hypotensive activity partly due to the inhibition of the angiotensin converting enzyme (ACE). Olive leaf extracts may also possess anti-spasmodic, vasodilator and anti-arrhythmic properties (Khan et al., 2007). Olive leaves are the richest known source of oleuropein and its derivatives (Luque de Castro and Japón-Luján, 2006). These bitter secoiridoids are considered to be responsible for the biological activities of olive leaf. Somova et al. (2003) however, showed that triterpenoids isolated from leaves of both the European olive and from the wild African olive prevented the development of severe hypertension and atherosclerosis and improved insulin resistance in experimental animals.

3.2. Chromatographic analysis

The TLC solvent system 2 developed in our laboratory (Fig. 2) was found to give a much better separation than solvent system 1 (the system recommended by Wagner and Bladt, 1996). The large dark brown zone observed at R_f 0.62 using solvent system 2 was confirmed to be oleuropein by comparative HPLC and TLC using the authentic reference standard (Fig. 2). The size and intensity of this zone on TLC and the peak size on HPLC chromatograms at R_t 10.6 min showed limited variation, both between localities and within localities (i.e. the three different individuals sampled). The samples contained at least 11 major and minor compounds (Table 1). With the exception of verbascoside, no qualitative variation was observed, and only limited quantitative variation. Oleuropein is almost always a major compound in both wild olive and cultivated olive (Table 1; Fig. 2). Examples of typical HPLC chromatograms are shown in Fig. 3. The presence of oleuropein as major compound in both wild and cultivated olive leaf (R_t 10.6; absorption maxima at UV 280 and 340 nm, recorded by the diode array detector) is confirmed. Only one sample of wild olive leaf (Napier sample 1 — see Table 1) showed a low concentration of oleuropein (but it had high levels of hydroxytyrosol, a precursor and derivative of oleuropein).

It was possible to identify three other compounds by direct comparison with an authentic sample (verbascoside, Fig. 1) or comparisons with literature data (hydroxytyrosol and tyrosol, see Fig. 1) (Benavente-Garcia et al., 2000; Ryan et al., 2001; Owen et al., 2003; De Marco et al., 2007; Japón-Luján and Luque de Castro, 2007; Savarese et al., 2007; Jemai et al., 2008). It is interesting to note that verbascoside was only detected in cultivated olive leaf and not in wild olive leaf (Table 1; Figs. 2 and 3). Verbascoside can easily be detected on TLC, even at relatively low concentrations (Fig. 2, the zone at R_f 0.34). Hydroxytyrosol and tyrosol (Figs. 1 and 3) are characteristic of *Olea* leaf extracts. Hydroxytyrosol is a precursor of oleuropein (Benavente-Garcia et al., 2000) but is also considered to be a degradation product of the latter (Ryan et al., 2001; De Marco et al., 2007; Japón-Luján and Luque de Castro, 2007; Jemai et al., 2008). Despite their low concentrations, these two compounds were easily detected by HPLC (but not TLC).

There are obvious differences in the clarity and resolution of the main zones (Fig. 2) using different extraction methods. Extraction with ethyl acetate appears to be superior in terms of the detection of the two marker compounds of interest, namely oleuropein (O in Fig. 2, at R_f 0.62) and verbascoside (V in Fig. 2, at R_f 0.34).

Our results clearly show that both subspecies of *O. europaea* contain substantial quantities of oleuropein. Somova et al.

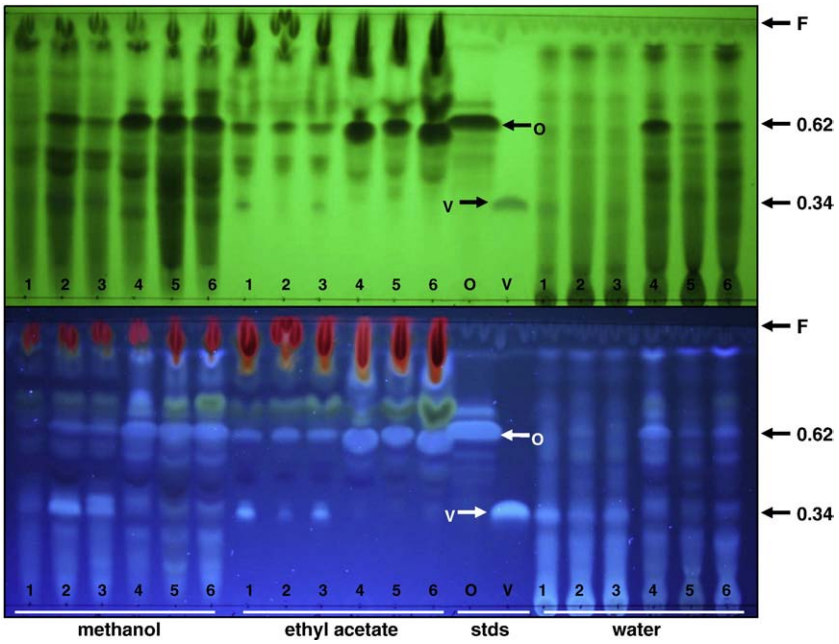

Fig. 2. Thin layer chromatography plate of *Olea europaea* crude leaf extracts (methanol left; ethyl acetate centre; boiling water right) as observed under short wavelength ultra-violet light (top, UV 254 nm) and long wavelength (bottom, UV 365 nm). Tracks 1–3, *O. europaea* subsp. *europaea*; tracks 4–6, *O. europaea* subsp. *africana*: 1, cv. Kalamata; 2, cv. Mission; 3, cv. Barouni; 4, Nieuwoudtville (tree 3); 5, Citrusdal (tree 3); 6, Sandy Bay (tree 3). O = oleuropein reference standard; V = verbascoside reference standard. Note the prominent zones of oleuropein at $R_f \sim 0.62$ and the absence of the verbascoside zone ($R_f \sim 0.34$) in subsp. *africana* (clearly observed especially in the ethyl acetate extracts).

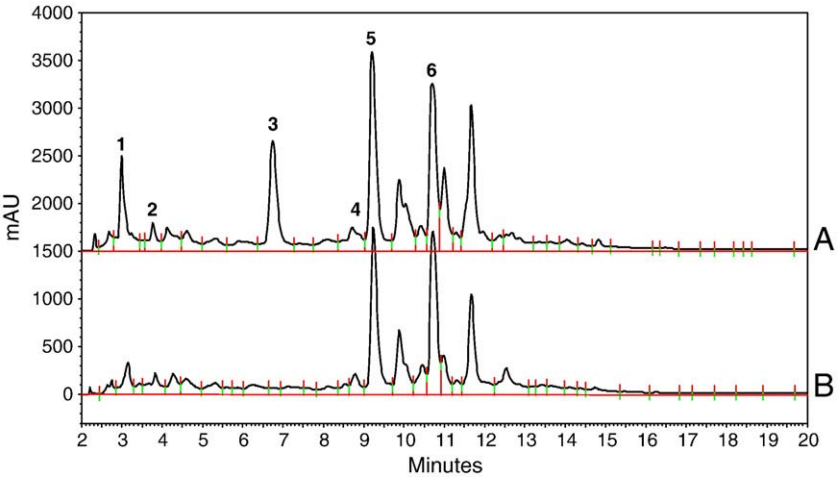

Fig. 3. Examples of HPLC chromatograms of *Olea europaea* crude leaf extracts (diode array detection, only the UV-280 channel shown here). (A) *O. europaea* subsp. *europaea* (cv. Coratina) and (B) subsp. *africana* (Nieuwoudtville, tree 3). Oleuropein (peak 6) and verbascoside (peak 3) were identified by co-chromatography using authentic reference standards (see also the TLC plate in Fig. 2). Other major compounds were tentatively identified according to their chromatographic behaviour and UV–VIS spectra as described in several published chromatographic studies (see Section 2): hydroxytyrosol (peak 1); tyrosol (peak 2); isoverbascoside (peak 4) and an unidentified flavone (peak 5).

(2003) reported that wild olive leaf had only trace amounts of oleuropein but their result, based on analysis of a single tree, was clearly not representative of the natural variation in the subspecies. The results reported here are more in agreement with those of Tsukamoto et al. (1984a,b), who found no differences in the lignans, coumarins and secoiridoids produced by the bark of the two subspecies.

3.3. Trichomes (peltate scales)

The trichomes are easily recognisable under low magnification, either *in situ* (Fig. 4A, B) or after separation from the leaf surface, as in a powder (Fig. 4C, D). They occur on both the adaxial and the abaxial leaf surfaces but have a much higher density on the abaxial surface. This contributes to the markedly discolorous nature of the leaves which are dark green above and pale silvery below. It is interesting to note that the scales on the abaxial side (Fig. 4E) differ from those on the adaxial side (Fig. 4F) in having more deeply scalloped margins and more prominent radiating markings. No obvious differences were noted between the two subspecies. These unique non-glandular, multicellular trichomes are present on the leaves of all *Olea* species but occur in a much greater density on those of *O. europaea*. This provides an easy visual method to distinguish this species from possible adulterants.

The trichomes of *O. europaea* have substantial UV-B absorbing capacity due to the presence of flavonoids and other phenolic compounds (Liakoura et al., 1997; Liakopoulos et al., 2006). Since these compounds are known to have anti-microbial properties, the trichomes may also function as a defensive barrier against pathogenic microorganisms (Liakopoulos et al., 2006).

4. Conclusions

Olea europaea subsp. *africana* has several recorded medicinal uses in southern Africa. However, it appears to be much less important in traditional medicine when compared to the large number of uses recorded for *Olea europaea* subsp. *europaea* in Mediterranean Africa and Europe. The modern uses of olive leaf or leaf extract are not clearly related to traditional uses in southern Africa. Tea made from the leaves is traditionally ingested so there is at least some indication that the extract may be safe.

Oleuropein is present in both subspecies and at high concentrations (up to 110 mg/g dry wt or more). Other characteristic compounds include the structurally related tyrosol and hydroxytyrosol that co-occur with oleuropein in almost all the samples.

The difference in verbascoside is of potential diagnostic value in distinguishing between cultivated and wild olive leaf. The compound is readily separated and easily detected on TLC using ethyl acetate as extraction solvent and chloroform:methanol: water:acetic acid (60:30:8:6) as eluent. This seems to be a practical and reliable method for routine quality control work.

The peltate trichomes of *O. europaea* have considerable potential as a diagnostic character in pharmacognosy. However, no differences could be found between the two subspecies.

Our work clearly showed that wild olive leaf is chemically similar to cultivated olive leaf and that it is therefore suitable as an alternative source of raw material for olive leaf or olive leaf extract.

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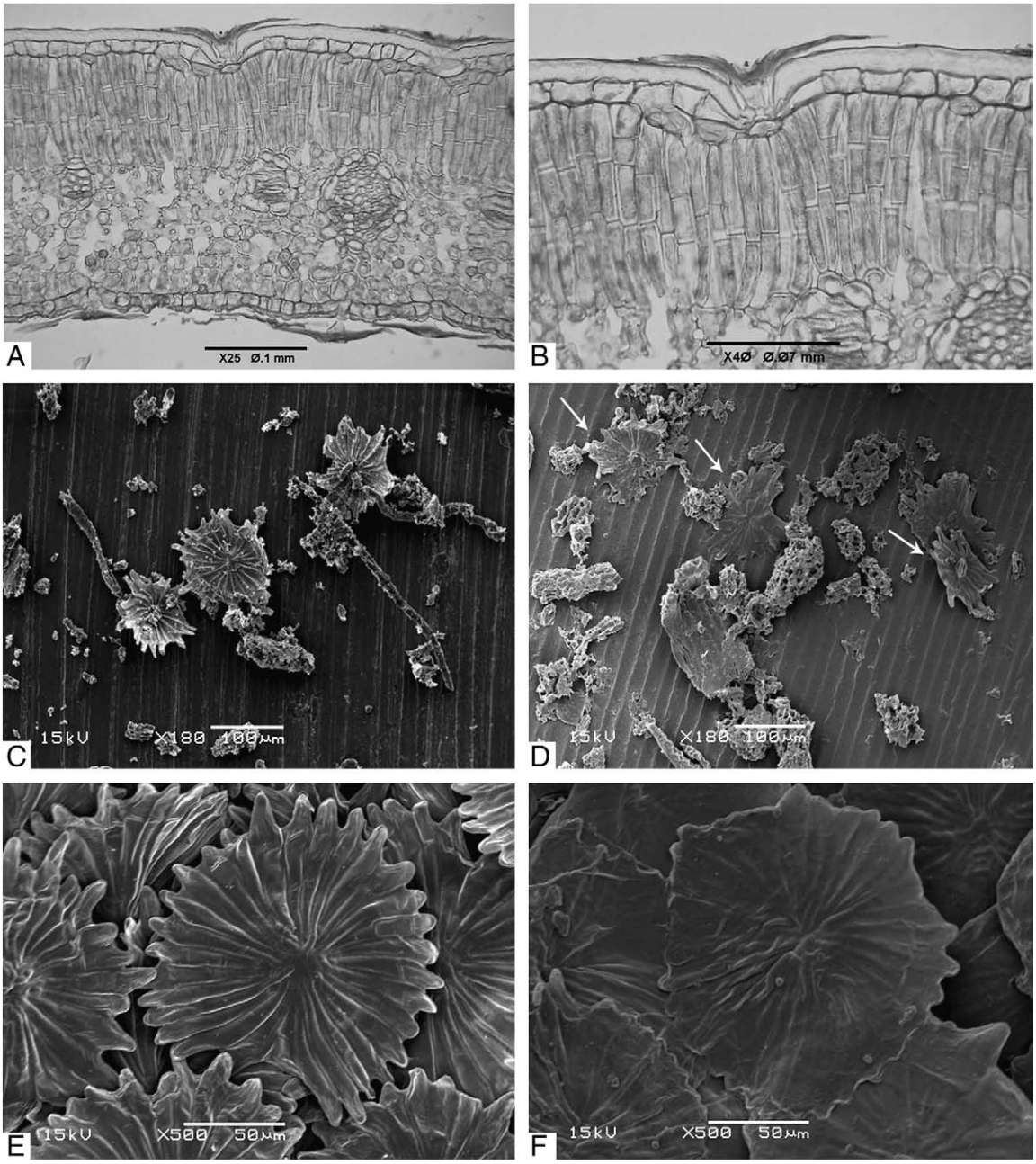

Fig. 4. Light microscope (A and B) and SEM micrographs (C–F) showing the trichomes (peltate scales) of *Olea europaea* subsp. *africana* and subsp. *europaea* leaves. A and B, transverse sections through a leaf to show the structure, position and density of the trichomes; C and D, leaf powder of subsp. *africana* (C) and subsp. *europaea* (D) showing the easily identifiable trichomes; E and F, surface view of the leaf of subsp. *africana* showing the characteristic overlapping trichomes on the abaxial (E) and adaxial (F) sides.

compounds, verbascoside and isoverbascoside. Dr. Emmy Reinton, ARC Infruitec-Nietvoorbij supplied leaf samples from various olive cultivars.

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