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A convenient thin layer chromatographic technique for chemotaxonomic application in *Maytenus* (Celastraceae)

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The genus *Maytenus* in southern Africa is replete with taxonomic problems, both at specific and supra-specific levels. Chemical data in taxonomy are an important adjunct to morphological evidence as it reflects on relationships of plants at another level of structural organization. In this study fourteen species of *Maytenus* (mainly from the Pondoland Centre of endemism) have been used to test the applicability of an easy-to-use thin layer chromatographic (TLC) technique. Leaf extracts provided chemical 'fingerprints' which were diagnostic for each species. Chemical evidence thus obtained also supports the reinstatement of the segregate genus *Gymnosporia*. The technique described holds considerable promise, not only for resolving classification problems in *Maytenus*, but also for taxonomic application in other groups of plants.

Keywords: Celastraceae, chemotaxonomy, *Gymnosporia, Maytenus*, taxonomy, thin layer chromatography.

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Introduction

Floristically the sandstone region of southern KwaZulu-Natal and Pondoland has been identified as a distinct centre of endemism, called the Pondoland Centre (PC). With thirteen species the PC is particularly rich in species of *Maytenus*; of which at least four are considered endemic to the region (Van Wyk 1990). As currently circumscribed, the genus *Maytenus* is clearly an heterogeneous assemblage, perhaps worthy of splitting into a number of more natural genera. Furthermore, interspecific boundaries in some of the closely-knit species complexes are often vague and in dispute (Van Wyk & Archer 1987).

Hitherto, a wide variety of secondary metabolites such as sesquiterpenoids, triterpenoids, alkaloids and flavonoids have been isolated from members of *Maytenus* in other parts of the world (e.g. Bruning & Wagner 1978; Nozaki *et al.* 1986; Gonzalez *et al.* 1993). Here we test the hypothesis that this variety of secondary metabolites is species specific and could be used as chemical evidence to aid in the identification and classification of the group. Thin layer chromatographic (TLC) fingerprinting of the acidic triterpenoid fraction of leaf extracts in the genus *Combretum* (Combretaceae) has been used extensively to resolve taxonomic problems in this genus (Carr & Rogers 1987; Rogers & Coombes 1999). In the present study we explore the potential taxonomic significance of a similar TLC technique in selected southern African members of the genus *Maytenus*.

Materials and Methods

Fourteen species of *Maytenus*, mostly from the PC, were studied in the summer of 1986/87 (Table 1). Leaf samples were collected from the Umtamvuna Nature Reserve in southern KwaZulu-Natal, except for those of *M. lucida*, which came from the Western Cape Province (Cape Point Nature Reserve). Voucher specimens are deposited in the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria.

Fresh leaves (± 10 g) were immersed in methanol (100 ml) overnight at room temperature. The methanolic solution was decanted from the leaves and concentrated under vacuum; residual water was removed from the extract by azeotropic distillation with benzene (2 × 20 ml). TLC analyses on solutions from each extract [50 mg ml^{-1} chloroform:ethanol (1:1, v/v)] were carried out on Merck silica gel GF_{254} aluminium backed plates using four solvent systems of varying polarity. Solutions were applied to plates in bands 1 cm wide (two or three applications) and allowed to develop for 9 cm. For some species, the composition of the extract is so distinctive that the TLC from a single solvent system (S/S) is sufficient to provide a 'fingerprint' that is diagnostic for that species. For other species extracts may need to be analysed by several S/S's to establish the chemical uniqueness of the species.

In this study the following four solvent systems were used: light petroleum:ethyl acetate (8:3, v/v), for the separation of non-polar constituents; light petroleum:ethyl acetate:chloroform:formic acid (8:7:5:1, v/v), for the separation of constituents of intermediate polarity; and either chloroform:ethyl acetate: formic acid (5:4:1, v/v) or chloroform:methanol:water (12:3:1, v/v-lower layer), for the separation of polar constituents. A spray reagent consisting of *p*-anisaldehyde (5 ml), conc. sulphuric acid (5 ml) and ethanol (90 ml) was used to visualise colourless constituents; these appeared as coloured bands after the sprayed plate had been heated at 110°C for 2–5 minutes. Apart from Rf comparisons, the most diagnostically important information in this technique is provided by variations or similarities

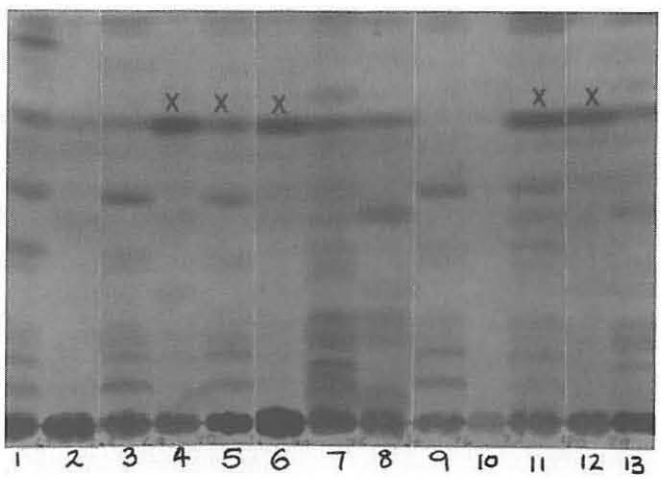

Figure 1 Representative TLC fingerprints of extracts developed in the non-polar S/S: light petroleum:ethyl acetate (8:3 v/v).

Table 1 Species of *Maytenus* investigated: TLC track No's; voucher specimen numbers (all collected by A.T.D. Abbott); and percentage yield of extract

TLC Track No.	Collectors No.	Species	% yield of extract (fresh leaves)
1	5766	*M. abbottii* Van Wyk	18.9
2	5767	*M. acuminata* (L.f.) Loes	24.7
3	5768	*M.* sp. *A* (= *Gymnosporia filiformis* Davison p.p.)	12.8
4	5769	*M.* sp. *B* [= *Gymnosporia heterophylla* (Eckl. & Zeyh.) Loes]	17.9
5	5770	*M. mossambicensis* (Klotzsch) Blakelock	24.8
6	5771	*M.* sp. *C* (= *Gymnosporia uniflora* Davison)	21.9
7	5772	*M. undata* (Thunb.) Blakelock	22.5
8	5775	*M. procumbens* (L.f.) Loes	16.4
9	5774	*M. cordata* (E. Mey. ex Sond.) Loes.	23.2
10	5776	*M. peduncularis* (Sond.) Loes.	9.8
11	5777	*M. heterophylla* (Eckl. & Zeyh.) N.K.B. Robson s.l. [= *Gymnosporia buxifolia* (L.) Szyszyl.]	21.7
12	5788	*M. bachmannii* (Loes.) Marais [= *Gymnosporia bachmannii* (Loes.) Szyszyl.]	12.0
13	5789	*M. oleosa* Van Wyk & Archer	24.8
14	4432	*M. lucida* (L.) Loes.	dried leaves extracted; yield not relevant

in the colour of these bands, which provide the fingerprint for the particular species. The TL chromatograms were recorded photographically. As it is almost impossible to capture the true colours on the chromatograms by photographic means, it is preferable to record them as colour photocopies or on a flat bed scanner. Certain species contained UV active compounds; these were detected using UV light at 366 nm.

Results and Discussion

An analysis of the chromatograms from all four solvent systems shows that the leaf extracts for each species provide a unique chemical fingerprint that supports the specific distinctness of all the species investigated. Not all the chromatograms could be reproduced. Consequently Figures 1–3, which show representative chromatograms developed in non-polar, intermediate and polar S/S's respectively, do not provide all the TLC evidence used in this study. In Figure 4 a more detailed illustration of the fingerprints for the polar constituents of seven of the species was obtained by increasing the width of the applied band and placing the bands closer together; the fingerprints in this Figure show how effective the technique can be. Jordaan (1995) proposed the re-instatement of the segregate genus *Gymnosporia* for the spiny members of *Maytenus*. Five of the species studied belong to this group, namely *Maytenus bachmannii* (12), (the numbers refer to the TLC Tracks shown in Figures 1–4), *M. heterophylla* (11), *M. mossambicensis* (5), *M.* sp. *B* (4) and *M.* sp. *C* (6). The close relationship between these five species is evident from the chromatograms and this is supported by the fact that they all share at least one major chemical constituent that distinguishes them from all

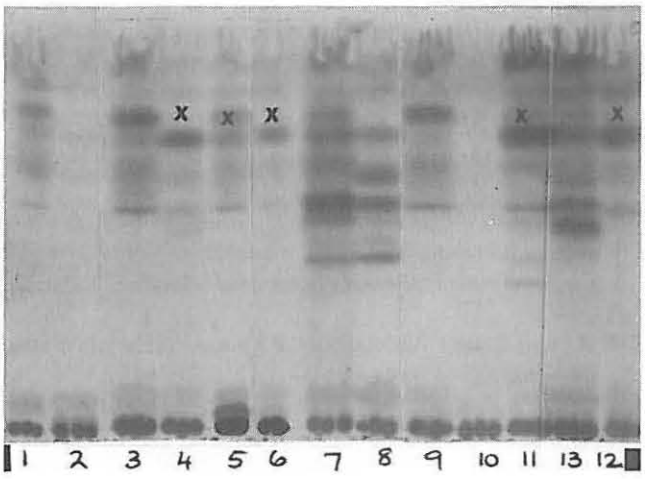

Figure 2 Representative TLC fingerprints of extracts developed in the S/S of intermediate polarity; light petroleum:ethyl acetate:chloroform:formic acid (8:7:5:1, v/v).

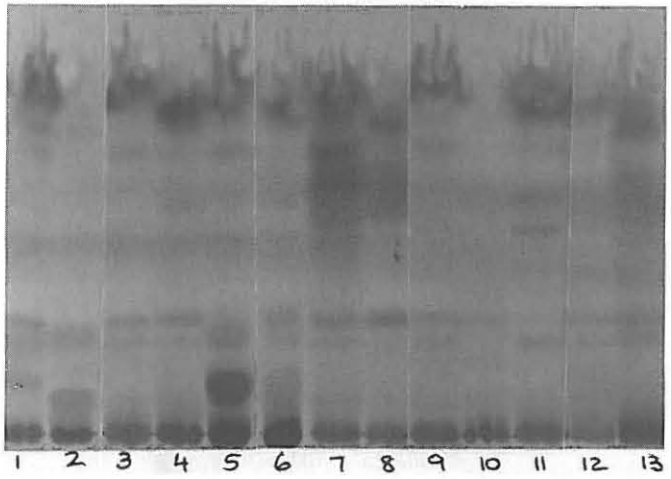

Figure 3 Representative TLC fingerprints of extracts developed in the the polar S/S; ethyl acetate:chloroform:formic acid (5:4:1, v/v).

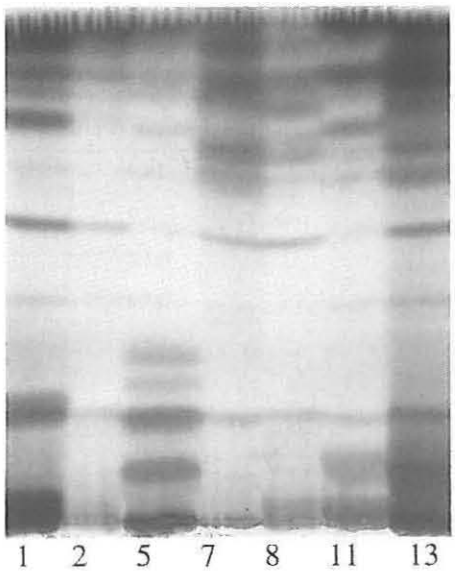

Figure 4 Detailed fingerprints of seven species developed in the polar S/S: chloroform:methanol:water (12:3 :1, v/v lower layer).

the other members of *Maytenus* studied. For example see the compound marked 'X' in Figures 1 and 2. Whilst this compound does occur in *M. undata* (7), *M. procumbens* (8) and *M. oleosa* (13), it is obvious from their fingerprints that these three species are quite different from the five *Gymnosporia* species.

Three species, namely *M. acuminata* (2), *M. bachmannii* (12) and *M. oleosa* (13), contain compounds that are UV active at 366 nm. The presence of these compounds is useful to distinguish *M. oleosa* (13) from the closely related and chemically rather similar *M. undata* (7). *M. lucida*, a species confined to the Western Cape, was included in the study to establish whether chemistry supports an aggregate species comprising this species, *M. undata* (7) and *M. procumbens* (8) (the so-called *M. undata* complex), or three distinct taxa as currently recognised. Chemical evidence clearly supports the latter view. Although chemically clearly

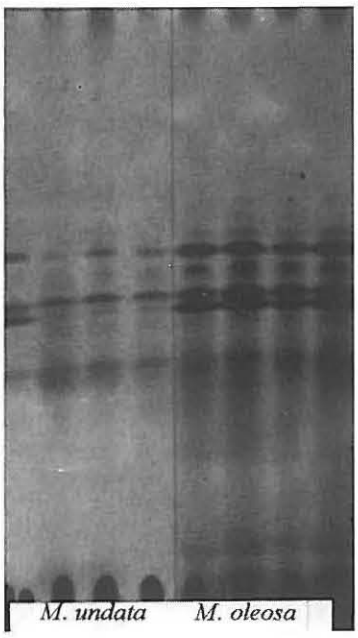

Figure 5 TLC fingerprints of extracts from specimens of *M. undata* and *M. oleosa* collected from four different localities S/S; chloroform:methanol:water (12:3:1, v/v lower layer).

related (see TLC tracks 7 and 8), the TLC fingerprints for all three species were also significantly different (the TLC track for *M. lucida* does not appear in any of the Figures reproduced here).

M. peduncularis (10), a species morphologically quite different from the other investigated taxa, is distinctive in that its extract has insufficient constituents to give a meaningful fingerprint. Our results also support the separate specific status of *M. abbottii* (1), *M. acuminata* (2), *M. cordata* (9) and *M.* sp. *A.* (3), all of which at one stage were lumped under *M. acuminata* (Van Wyk 1983).

TLC fingerprints of extracts from different samples of the same species from different localities showed a remarkable degree of congruency; the TLC analysis of extracts from four different specimens of *M. oleosa* (13) and *M. undata* (7) are shown in Figure 5. Similar results were reported for *Combretum* species collected from vastly different geographical locations (Carr & Rogers 1987), which helps validate this technique. Although not all plant taxa lend themselves to TLC fingerprinting, this study shows the technique can be applied with confidence to the genus *Maytenus*. Chemotaxonomic evidence thus obtained proved useful at specific and in some cases also supraspecific levels.

It must be stressed that the conclusions arrived at in this study were done on many more TLC plates than those shown in Figures 1–5, which are not meant to represent a comprehensive record of all the TLC results. Consequently the differences between certain species such as *M. cordata* (9) and *Gymnosporia filiformis* (3) may not be apparent from the limited evidence illustrated in these Figures.

References

BRUNING, R. & WAGNER, H. 1978. **Review**. Übersicht Über die Celasraceen-inhaltsstoffe: chemie, chemotaxonomie, biosynthese, pharmakolgie. *Phytochem.* 17: 1821–1858.

CARR, J.D. & ROGERS, C.B. 1987. Chemosystematic studies of the genus *Combretum* (Combretaceae). I. A convenient method of identifying species of this genus by a comparison of the polar constituents extracted from leaf material. *S. Afr. J. Bot.* 53: 173–176.

GONZALEZ, A.G.; JIMENEZ, I.A.; RAVELO, A.G.; SAZATORNIL & BAZZACCHI, I.L. 1993. New sesquiterpenes with antifeedant activity from *Maytenus canariensis* (Celastraceae). *Tetrahedron.* 49: 697–702.

JORDAAN, M. 1995. A taxonomic revision of the spiny members of subfamily Celastroideae (Celastraceae) in southern Africa. M.Sc. thesis, University of Pretoria, Pretoria.

NOZAKI, H.; SUZUKI, H.; HIRAYAMA, T.; KASAI, R.; WU, R-Y. & LEE, K-H. 1986. Antitumour triterpenes of *Maytenus diversifolia*. *Phytochem.* 25: 479–485.

ROGERS, C.B. & COOMBES, P.C. 1999. Acidic triterpene glycosides in trichome secretions differentiate subspecies of *Combretum collinum* in South Africa. *Biochem. Systematics & Ecology.* 27(3): 321–323.

VAN WYK, A.E. 1984. A new species of *Maytenus* (Celastraceae) from southern Natal. *S. Afr. J. Bot.* 3: 115–119.

VAN WYK, A.E. & ARCHER, R.H. 1987. *Maytenus oleosa* (Celastraceae), a new species from southern Natal and Pondoland. *S. Afr. J. Bot.* 53: 155–160.

VAN WYK, A.E. 1990. The sandstone regions of Natal and Pondoland: remarkable centres of endemism. In: Palaeoecology of Africa, ed. K. Heine, Vol. 21, pp. 243–257. A.A. Balkema, Rotterdam.