Authentication of Barks Used in South African Traditional Healthcare with Thin Layer Chromatography

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Abstract

Bark products are an important source of medicine in South African traditional healthcare. They are difficult or impossible to identify in the dried state in which they are sold, and misidentification or adulteration increasingly affect their appropriate use. Thin Layer Chromatography was investigated for its potential to authenticate medicinal bark products. Our preliminary study focused on eight bark species, identified by a traditional medical practitioner, that present problems of identity. TLC-generated fingerprints of three reference samples of each species were compared. At the intraspecific level, TLC was useful in confirming the relationship of hexane bark extracts, but was less meaningful in distinguishing between fingerprints of different species at the interspecific level. Three medicinal bark products of each study species were purchased and their fingerprints were compared to a reference. The technique proved useful in confirming the identity of several medicinal bark products. TLC may be a useful tool for the authentication of medicinal bark products used in South African traditional healthcare.

INTRODUCTION

In South Africa, the trade and use of traditional herbal medicines is largely unregulated. The integrity of the traditional healthcare sector, and the safety of its patients, who comprise the majority of South Africans (Mander, 1998), are threatened by increasing numbers of untrained practitioners and traders. Dried bark products sold in the medicinal plant trade are generally difficult to identify, as many useful diagnostic characters are lost through desiccation. Inadvertent misidentification or purposeful substitution affect the appropriate use of bark medicines. The single existing guide to some commonly traded bark products in South Africa is that of Tait and Cunningham (1988). Authentication of traditional herbal medicines would facilitate accurate documentation of taxa traded, medicinal usage and assist in identifying material implicated in poisoning cases.

Despite the development of advanced chromatographic techniques for the elucidation of compounds from natural products, Thin Layer Chromatography (TLC) remains effective and favoured for its simplicity and affordability (Gibbons and Gray, 1998; Kirchner, 1967). Terminology implies that phytochemical ‘fingerprints’ are unique but, in the absence of fingerprints of all plant species, this cannot yet be guaranteed. However, due to the diagnostic value of TLC-generated fingerprints, the technique has largely replaced microscopy in plant drug authentication and quality assessment (Jackson and Snowdon, 1990).

Manana and Eloff (2001) compared TLC fingerprints of reference plant material to those of medicinal products traded in the Pretoria area of South Africa, and found a good correlation between reference and medicinal samples. In this study, we focused on medicinal bark products traded in KwaZulu-Natal. TLC-generated fingerprints were investigated for their potential to distinguish between the bark of eight extensively used species. According to a local traditional healer, the species are frequently misidentified and/or substituted for other bark products by traditional healers and plant traders. The study aimed to establish fingerprint references for each species, against which medicinal
bark products purportedly of the same species could be authenticated.

MATERIALS AND METHODS

Eight study species were grouped in two ‘complexes’ according to the patterns of substitution or misidentification that affect their usage (Table 1). Reference bark samples of each species were harvested from three specimens growing in different localities up to ca. 100 km apart, to allow comparison of genetically unrelated material subjected to different environmental variables. Fresh material was gently scraped with a blade, and dried overnight in an oven (50°C). Three dried bark products of each species, traded under local isiZulu vernacular names recorded in the literature, were purchased from different herbal retailers in Pietermaritzburg. Voucher specimens were deposited in the Bews Herbarium at the University of Natal.

Ethanol and hexane plant extracts (50 mg ml\(^{-1}\) concentration) of each specimen were submitted to TLC with a stationary phase of silica gel on pre-coated plastic sheets (Merck 60 F\(_{254}\)). Sufficient extract to yield 0.5 mg plant material was applied in 0.8 cm bands to the origin of the chromatography plate. Glass chromatography chambers were pre-washed with the mobile phase and allowed to equilibrate for approximately 2 min before plates were placed in the tank and run over a migratory distance of 8 cm. A solvent system comprising 95% petroleum spirit: ethyl acetate: chloroform: formic acid (8:7:5:1) was selected as suitable for analyses of both ethanol and hexane bark extracts. Chromatograms were viewed in visible and UV light (254 nm and 366 nm) prior to, and following, treatment with either anisaldehyde-sulphuric acid reagent or vanillin-sulphuric acid reagent (Wagner and Bladt, 1995).

RESULTS AND DISCUSSION

The most diagnostically important information in TLC analyses such as these are colouration and R\(_f\) values of compound bands, which together provide the fingerprint of a particular species (Rogers et al., 2000). A high degree of consistency was evident between phytochemical fingerprints at the intraspecific level. Despite differing habitats, maturity, and other variables known to influence bark characteristics, fingerprints of three bark specimens compared closely for study species in both complex 1 and complex 2. *Acacia xanthophloea* was the single species to require comparison of an additional three bark samples to determine a common fingerprint profile. It is postulated that the abundance of characteristic yellow powdery flakes on the bark of *A. xanthophloea* may be responsible for observed differences in fingerprints.

At the interspecific level, three out of four species in complex 1 were readily distinguishable by their phytochemical fingerprint. Hexane extracts of *E. capensis*, *H. caffrum* and *S. brachypetala* yielded fingerprints that were readily separable after development. *R. melanophloeos* fingerprints failed to show diagnostic compound bands unique to that species. Whereas interspecific differences were pronounced in species complex 1, three species in complex 2 (*A. xanthophloea*, *A. adianthifolia* and *C. sylvaticus*) failed to show diagnostic compounds at that level. *Acacia sieberiana* was distinguishable by a pale pink band in both ethanol and hexane extracts at R\(_f\) 0.9 on undeveloped chromatograms. Since *A. xanthophloea* and *A. adianthifolia* belong to the same family (Mimosaceae), similarities in their phytochemical fingerprints may be attributed to their chemotaxonomic relationship, and provide an indicator of close usage relationships.

Co-chromatography of a standard reference with the test extract(s) is necessary to afford accurate comparison of qualitative data (Stahl and Schorn, 1969). Following earlier results, hexane extracts were used and detected with anisaldehyde-sulphuric acid reagent for species in complex 1 and vanillin-sulphuric acid reagent for complex 2. As a technique with which bark products may be authenticated, TLC was shown to be more reliable than expected, considering the anatomical and morphological variability of bark characters.

In complex 1, *E. capensis*, *H. caffrum* and *S. brachypetala* showed convincing
similarities between the medicinal and reference specimens. Fingerprints of \textit{R. melanophloeos} specimens showed similarities under UV 366 nm prior to development, but following development, the medicinal lanes bore no resemblance to the reference lane. The observation may correspond to the trend noted in intra- and interspecific analyses, where \textit{R. melanophloeos} failed to show consistent and distinctive fingerprints. Alternatively, since each medicinal product was purchased by a different vernacular name, the possibility exists that each represents a different species.

In complex 2, similarities were noted between the medicinal specimens and respective references of \textit{A. sieberiana}, \textit{A. xanthophloea} and \textit{A. adianthifolia}. It is unclear if the medicinal bark specimens compared to the \textit{C. sylvaticus} reference were indeed the same species, since each medicinal specimen deviated from the reference differently. Although medicinal and reference lanes of \textit{A. xanthophloea} and \textit{A. adianthifolia} showed similarities following development, differences in fluorescence were noted under UV 366 nm.

In the case of \textit{A. xanthophloea}, one medicinal specimen exhibited unusual quenching and fluorescent bands at R$_f$ 0.85 and 0.9 under UV light. It was purchased by a vernacular name different to the other two products, and in fact consisted entirely of wood. The value of TLC for bark authentication may, therefore, be extended to identifying the presence of wood adulterants in bark medicines. Processed bark products may be easily adulterated with wood to increase product volume. Additionally, removal of dried bark from underlying wood is sometimes difficult, and medicinal bark products may include a large amount of attached wood.

From our initial investigation, it is apparent that TLC may be used to assist in the authentication of medicinal bark products. The usefulness of the technique may depend largely upon the inherent variability of a plant species: some taxa show highly variable phytochemical properties between populations and chemical races that would require thorough documentation before TLC authentication would be reliable. However, considering the anatomical and morphological variability of bark characters, TLC was shown to be more reliable than expected in the authentication of medicinal bark products. TLC may indeed fulfil the requirements for simple, affordable methods to authenticate medicinal bark products traded in South Africa.

**ACKNOWLEDGEMENTS**

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**Literature Cited**


Tables

Table 1. Study species and grouping in species complexes according to usage patterns resulting from confusion of medicinal bark products.

<table>
<thead>
<tr>
<th>Family</th>
<th>Taxon</th>
<th>Taxa with which bark products are confused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species complex 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meliaceae</td>
<td><em>Ekebergia capensis</em> Sparrm.</td>
<td><em>Harpephyllum caffrum</em>; <em>Schotia brachypetala</em></td>
</tr>
<tr>
<td>Anacardiaceae</td>
<td><em>Harpephyllum caffrum</em> Bernh.</td>
<td><em>Ekebergia capensis</em>; <em>Rapanea melanophloeos</em></td>
</tr>
<tr>
<td>Myrsinaceae</td>
<td><em>Rapanea melanophloeos</em> (L.) Mez</td>
<td><em>Harpephyllum caffrum</em></td>
</tr>
<tr>
<td>Fabaceae – Caesalpiniaceae</td>
<td><em>Schotia brachypetala</em> Sond.</td>
<td><em>Ekebergia capensis</em></td>
</tr>
<tr>
<td>Species complex 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabaceae – Mimosaceae</td>
<td><em>Acacia sieberiana</em> DC.</td>
<td><em>Albizia adianthifolia</em>; <em>Croton sylvaticus</em></td>
</tr>
<tr>
<td>Fabaceae – Mimosaceae</td>
<td><em>Acacia xanthophloea</em> Benth.</td>
<td><em>Croton sylvaticus</em></td>
</tr>
<tr>
<td>Fabaceae – Mimosaceae</td>
<td><em>Albizia adianthifolia</em> (Schumach.) W.F. Wight</td>
<td><em>Acacia sieberiana</em>; <em>Croton sylvaticus</em></td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td><em>Croton sylvaticus</em> Meull. Arg.</td>
<td><em>Acacia sieberiana</em>; <em>Acacia xanthophloea</em>; <em>Albizia adianthifolia</em></td>
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