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Item Type	Article
Authors	Katerere, D.R.;Eloff, J.N.
DOI	http://dx.doi.org/10.1016/S0254-6299(15)30249-0
Publisher	Elsevier
Rights	Attribution-NonCommercial-ShareAlike 4.0 International
Download date	2026-03-11 21:48:47
Item License	http://creativecommons.org/licenses/by-nc-sa/4.0/
Link to Item	https://hdl.handle.net/20.500.14519/955

South African Journal of Botany 2004, 70(2): 303–305
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SOUTH AFRICAN JOURNAL OF BOTANY
ISSN 0254–6299

Short Communication

Variation in chemical composition, antibacterial and antioxidant activity of fresh and dried *Acacia* leaf extracts

DR Katerere and JN Eloff*

Programme for Phytomedicine, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
** Corresponding author, e-mail: kobus.eloff@up.ac.za*

Received 8 April 2003, accepted in revised form 20 October 2003

This study investigated differences in chemical composition and biological activity of fresh and dried leaf extracts of *Acacia galpinii, A. karroo, A. xanthophloea* and *A. sieberiana* occurring in southern Africa. Leaf material was extracted first with acetone and subsequently with chloroform. Drying of leaves led to an increase in the dry mass extracted by acetone and a decrease in the dry mass extracted by the more non-polar chloroform as well as an overall decrease in the dry mass extracted. The chemical profile, determined by TLC, of fresh and dry material of the same species differed. The antibacterial activity was higher in extracts from dried than fresh leaves with the exception of *A. xanthophloea*, which was overall, the most active with an MIC of 78µg ml^{-1} against *Staphylococcus aureus* and 160µg ml^{-1} against *Escherichia coli*. There were at least three free radical scavenging compounds present in acetone leaf extracts of all species. This is the first report of antibacterial and free radical scavenging activity for these species.

Acacia is a member of the Mimosaceae and is an important genus in the dry regions of Africa, being a source of building timber, rope and animal feed (especially during the drought periods) as well as a commercial source of tannins and gums (Watt and Breyer-Brandwijk 1962, Smit 1999, Dube *et al*. 2001).

Despite the widespread occurrence of *Acacia* species, their use in traditional medicine appears to be restricted to a few species used widely in the treatment of abdominal and chest complaints (Gelfand *et al.* 1985, Venter and Venter 1996).

Few reports on the pharmacological activity of *Acacia* species could be found. The presence of acacatechin, catechutannic acid and quercetin has been reported from *A. karroo* (Van Wyk *et al.* 1997). An isoflavone and three known flavonoids apigenin, luteolin and quercetin have been isolated from the leaves of *A. tortilis* (Muhaisen *et al.* 2002) as well as a series of naturally occurring proanthocyanidins from *A. galpinii* and *A. caffra* (Bennie *et al.* 2002). This preliminary study reports on possible antibacterial and antioxidant activity as well as the difference when using fresh or dried leaves as a source.

We examined *A. galpinii* Burtt Davy (monkey thorn), *A. karroo* Hayne (sweet thorn), *A. xanthophloea* Benth. (fever tree) and *A. sieberiana* DC var. *woodii* (paper bark). Voucher specimens of these plants growing on the Onderstepoort Campus, University of Pretoria, were authenticated and stored at the Onderstepoort Herbarium and designated DK-AK-02, DK-AS-02, DK-AX-02 and DK-AG-02 respectively. In each case leaves were divided into two 5g batches and extracted twice by acetone (30ml each time) and then by chloroform (30ml each time) by shaking for 30min on a Labotec orbital shaker. One batch was ground and extracted fresh in a Osterizer homogeniser while the other was dried for several days in a force-ventilated oven (<40°C) and then finely ground before extraction. Each extract was taken to dryness by a rotary evaporator and weighed to determine the mass extracted. Extracts were reconstituted in acetone to a 20mg ml^{-1} stock solution. An aliquot of 5µl (i.e. 100µg) was subjected to TLC to compare the chemical profile of the different extracts. Three different solvent systems (v:v in each case) were used, i.e. hexane:ethylacetate (HE) (2:1); chloroform:ethylacetate: formic acid (CEF) (20:16:4), and ethylacetate:methanol: water (EMW) (40:5.4:4). After development, chromatograms were examined under ultraviolet light at 254nm and 360nm (Camac Universal UV lamp TL-600) and fluorescent compounds marked before spraying with freshly prepared vanillin spray reagent (0.1g in 28ml methanol and 1ml conc. H_2SO_4) (Stahl 1969) and heating at 105°C to optimise colour development.

A serial dilution microtitre method was used to determine minimum inhibitory concentrations (MIC) of the plant extracts against test bacterial cultures (Eloff 1998a). The growth indicator used was *p*-iodonitrotetrazolium violet (INT, Sigma Chemicals) and the bacterial cultures were *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli*

(ATCC 27853). The crude extracts were reconstituted to stock solutions of 20mg ml^{-1} in 5% dimethylsulphoxide in water for use in the assay. Ampicillin and neomycin were used as positive controls made up as stock solutions of 100µg ml^{-1}.

The bioautography procedure as employed by Eloff (1998b) was used. TLC plates were developed using the three solvent systems, exhaustively dried, sprayed with a dense *S. aureus* culture, incubated overnight at 37°C and sprayed with 2mg ml^{-1} INT. Clear bands indicated antibacterial activity.

The qualitative DPPH assay used by Braca *et al.* (2002) was employed to assay for free radical scavenging activity. Extracts were separated by TLC using the three solvent systems described above. Once developed and dried, the plates were sprayed with 0.2% DPPH in MeOH and examined for colour change over 30min.

It appeared that there was a change in the polarity of the constituents of the leaves upon drying. The average mass extracted from fresh leaves by acetone was nearly two times higher than the average mass extracted from dried leaves. Concomitantly the average mass extracted by the less polar chloroform from the acetone marc of fresh leaves was nearly four times lower than the average mass extracted from the acetone marc of dried leaves (Table 1).

The moisture content of the leaves was low and varied from 2–4%. The quantitative and qualitative differences between compounds extracted from dry and fresh leaves could therefore hardly be explained by dilution of the acetone extractant by the water present in the fresh leaves. With the ratio of leaf material to acetone the moisture present in the leaves reduced the acetone concentration to 99.3%.

Table 1: Mass (mg) extracted from 5g dry and 5g fresh leaves of the four *Acacia* species by acetone followed by chloroform

	Fresh leaves		Dried leaves	
Plant species	Acetone	Chloroform	Acetone	Chloroform
A. galpinii	290	12	130	36
A. karroo	136	20	52	29
A. sieberiana	169	27	156	100
A. xanthophloea	185	12	61	65
Average	195	18	100	58

Drying of leaves did not have a major impact on the chemical composition of highly non-polar compounds separated by the HE system (Table 2). Dry leaf *A. galpinii* acetone and dry leaf *A. sieberiana* acetone extracts had at least seven components. Along with dry leaf *A. karroo* extracts, they shared three major components (after vanillin spray reagent) with R_f 0.53 (purple), 0.46 (purple) and 0.32 (green). Dry *A. xanthophloea* showed a similar TLC profile but lacked the compound at R_f 0.53.

There was poor separation with CEF for all the extracts, in this moderately polar acidic TLC solvent system. All extracts had at least five distinct components when separated with the more polar system, EMW.

Fresh leaf extracts contained fewer discrete spots compared to extracts of dry leaves. The bands were also separated better. Fresh leaves may thus be more useful in analysing interspecies chemical variation.

In most cases, the extracts prepared from fresh leaves appeared to be less complex than the extracts of dry leaves. This may be due to the concentration of minor constituents during desiccation or reduced extractability of certain compounds contained within some cellular organelles. Homogenising of fresh leaves may not be as efficient as the extraction of finely ground dry material, but the average mass extracted by acetone and chloroform from fresh leaves (213mg) was still higher than the value for dried leaves (158mg) (Table 1). It is possible that during the drying, oxidation occurs leading to chemical modification and that using dried leaves may produce artifacts.

The influence of drying on the biological activity of extracts in this study was not the same for the different species (Table 2). In the case of the *A. xanthophloea*, the acetone extract of fresh leaf was significantly more active than the extract of dry material in the microtitre assay against both *E.*

Table 2: Minimum inhibitory concentration (mg ml^{-1}) towards *S. aureus* and *E. coli* and chemical complexity of extracts of *Acacia* species. Complexity was evaluated by counting the number of distinct bands seen on TLC under UV (350nm) and after treatment with vanillin spray reagent

Plant species	Extractant	State	*S. aureus*	*E. coli*	CEF	HE	UV-CEF
A. galpinii	Acetone	Fresh	5.00	>5.00	4	4	4
		Dried	5.00	>5.00	3	7	0
	Chloroform	Fresh	5.00	>5.00	6	7	2
		Dried	2.50	2.50	3	6	0
A. karroo	Acetone	Fresh	0.63	5.00	3	2	0
		Dried	0.63	0.63	3	3	1
	Chloroform	Fresh	>5.00	5.00	5	5	0
		Dried	5.00	0.63	4	6	1
A. xanthophloea	Acetone	Fresh	0.08	0.16	11	5	0
		Dried	1.25	5.00	5	7	1
	Chloroform	Fresh	5.00	2.50	7	4	0
		Dried	5.00	5.00	4	5	1
A. sieberiana	Acetone	Fresh	2.50	2.50	5	5	5
		Dried	2.50	>5.00	5	8	3
	Chloroform	Fresh	5.00	>5.00	8	8	4
		Dried	0.63	2.50	2	7	3

coli and *S. aureus*. While both fresh leaf extracts and dry leaf extracts were generally equally active in the microtitre assay, the former showed no activity against *S. aureus* in the bioautography assay in contrast to the extracts of dried leaves. Bioautography of the more polar compounds (eluted with EMW) showed a single constituent (R_f 0.68) common to both acetone and chloroform extracts of dry *A. karroo* and *A. xanthophloea* to possess activity against *S. aureus*. In the case of *A. sieberiana* and *A. galpinii* the chloroform extracts from dried leaves had only one or two very polar compounds whereas chloroform extracts from fresh leaves had several less polar antibacterial compounds.

The chloroform extracts generally had less antibacterial activity than acetone-extracted fractions. Of the fresh acetone extracts, *A. xanthophloea* was the most active against both test organisms (MIC 78µg ml^{-1} for *S. aureus* and 156µg ml^{-1} for *E. coli*), while the *A. galpinii* extract was the least active (MIC > 5 000µg ml^{-1}) (Table 2). The significance of this is that in bioassay-guided work, using fresh material may lead to different results.

All acetone fractions had some free radical scavenging activity according to the DPPH assay with at least three bands of activity (results not shown). No such activity was shown by any chloroform extracts. Anti-oxidant moieties are frequently phenolic and hence more polar and likely to be extractable in acetone rather than in chloroform. The extracts from fresh leaves were also more active than those from dry leaves.

In most ethnopharmacological and phytochemical work, extracts of dried plant material are used mainly because of ease and purported stability. The possibility that drying may change the phytochemical profile and pharmacological activity of the extracts has not been thoroughly explored and documented. These results show that there is a need to revisit the almost exclusive use of dry leaf material for bioassay-guided isolation. The use of fresh plant material at least with these *Acacia* spp. resulted in higher extraction yields especially of intermediary polar compounds although it is not as convenient because of the distances between the laboratory and the area of collection.

Acknowledgements — The National Research Foundation funded this study.

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Edited by AK Jäger and J van Staden