Phytochemistry, Vol. 27, No. 3, pp. 743–751, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

PHENOLICS IN SOME SOUTHERN AFRICAN MEDITERRANEAN SHRUBLAND PLANTS

JOHN P. GLYPHIS* and GILLIAN M. PUTTICK*

Zoology Department, University of Cape Town, Rondebosch 7700, Republic of South Africa

(*Revised received* 25 *May* 1987)

Key Word Index—Phenolics; tannins; polyphenols; proanthocyanidins; astringency; flavanols; chemical defence; mediterranean shrubland.

Abstract—Twelve plant species were collected seasonally and assayed for total phenolics, proanthocyanidins, flavanols and astringency. Results of the different phenol assays correlated with each other, and all correlated with the results of astringency analyses. The use of a two-tier sequential extraction system provided additional information which made some interesting comparisons possible among the different chemical assays. Mean seasonal values for all assays increased from lowest concentrations in winter to highest concentrations in autumn. Total phenols for individual species ranged very widely from 2.0 to 32.0% dry weight. Concentrations in new and old leaves were not significantly different.

INTRODUCTION

Tannin polyphenols occur in a variety of tissues of many plant species particularly in those which become woody during growth [1]. These compounds are generally effective as non-lethal feeding deterrents against a wide range of organisms from insects to mammals, although some exceptions have been reported for insects [e.g. 2]. Among vertebrates there is evidence that they deter feeding in tortoises and lizards [1] and influence the food choice of mountain gorillas [3], chimpanzees [4], howler monkeys [5], vervet monkeys [6] and colobus monkeys [7], snowshoe hares [8], Alaskan ptarmigan, grouse, moose and beavers [9] and Canadian geese [10].

Very little has been published on vertebrate–plant interactions in mediterranean ecosystems [e.g. 11], in contrast with a larger number of studies on insects. In order to examine possible chemical determinants of plant preference by three species of small antelope in a mediterranean shrubland in South Africa, plants were collected for chemical analysis of phenolics, astringency and nutritional quality at the same time as antelope selectivity was studied. In this paper we describe the individual phenolic levels of 15 shrubland species, and seasonal variations in 12 of these. Antelope selectivity and its relationship to overall nutritional quality will be described in later papers.

RESULTS AND DISCUSSION

Plant phenolics and particularly tannin polyphenols appear to be structurally diverse, although the results of only a small number of structural analyses have been published [12]. The relationship between polyphenol structure and ability to complex proteins is more complicated than originally thought [13]. Molecular size, conformational mobility and flexibility in the polyphenol substrate are all important determinants of ability to bind proteins. In order to account for different sizes and classes of phenolics, we analysed for representatives of these (e.g. proanthocyanidins as a representative of condensed tannins), in addition to measuring total phenolics using a Folin reagent.

The chemistry and methodology of phenolic assays have been reviewed in some detail [14]. The 'Improved method' using Folin–Ciocalteu reagent proved to be a more satisfactory method of measuring total phenolics [15] than the more generally used Folin–Denis method [16, 17], despite a number of possibly interfering chemicals [14]. In particular, reaction stability and the absence of carbonate precipitate formation make Folin–Ciocalteu especially suitable for the processing of large numbers of samples [15]. Folin–Ciocalteu test values from a comparison of the two reagents on a subsample of plant extracts in this study were approximately 10% higher than those from Folin–Denis, but this difference was not significant (paired t-test. $D = -10.7$, $t = -1.65$, $df = 21$, $p > 0.1$). Caution should be exercised with both Folin reagents in comparing phenolic levels in different plant genera and/or species since these reagents react with both simple and complex (poly)phenols [16]. There does not yet seem to be a phenolic test reagent which meets the specifications of both chemists and ecologists in being both specific enough yet also being simple to do and permitting comparisons among different plant taxa. However, more recently, tannin analyses have begun to incorporate some measure of functional activity which usually entails the measurable precipitation of some soluble protein [18].

Total phenolic levels [Folin–Ciocalteu tannic acid equivalents (TAE)] and astringency (haemanalysis TAE) in the 100% methanol (A) extracts were significantly correlated both with proanthocyanidin [quebracho tannin equivalents (QTE)] and flavanol [catechin equivalents (CE)] levels in both A and B (50% methanol) extracts

*Present address: Chemistry Department, Northeastern University, Boston MA 02115, U.S.A.

(Table 1A). The highest correlations occurred between the different tests on the A extracts: these correlation coefficients varied between 0.68 and 0.79. In contrast, there were no significant correlations between the different tests on the B extracts, except for a strong correlation between the proanthocyanidins and the flavanols, which was highly significant (Table 1B). Folin–Ciocalteu and haemanalysis on the B extracts produced no significant correlations with any of the other tests on either of the extracts (Table 1A). The significant correlations obtained when comparing each assay result with the A and B extracts totalled (Table 1A) are not unexpected, since the A extract appears to contain from two to five times more phenolics than B extracts in the majority of the plants which were analysed. These results suggest that a relationship exists between structure and function, at least as measured by the assays used in this study.

In summary, multiple regression suggests that there are significant correlations, (i) among the different tannin assays and (ii) between the different measures of tannins and the functional haemanalysis test, especially in comparisons among pure methanol extracts. Similarly, Gartlan *et al.* [19], in an extensive study of secondary chemicals in tropical rainforest in Uganda and Cameroon, found strong positive correlations among tests for total phenols, flavanols and proanthocyanidins, and their correlation coefficients are very similar to those reported here. However, they report only a weak correlation between levels of proanthocyanidins and astringency. In a related study of foliage from rainforest trees from the same area and from south India, Choo *et al.* [20] found a positive but weak correlation between the functional assay they used, which was a measure of dry matter digestibility using enzymes, and measures of total polyphenols and condensed tannins. In contrast, Martin and Martin [21] found no correlation between protein-precipitating capacity and either total phenolic or proanthocyanidin content of the mature foliage of six oak species.

Because of possible dependence among seasons based on the general chemistry of each plant species, we also computed correlation matrices for each season individually (Table 2). The overall pattern remained the same (for example, levels of phenolics in the aqueous methanol extracts did not correlate significantly among the different assays, and correlations between levels of proanthocyanidins and flavanols tended to be the highest among all the assays). However, changes in the degree of correlation among the different assays were not consistent from season to season, and the magnitude of correlation coefficients fluctuated considerably with the lowest correlations generally occurring during winter (Table 3). These

Table 1A. Correlation matrix of different tannin polyphenols measures on 62 different plant samples covering approximately 15 different plant species over four seasons

	Phen A	Phen B	Phen Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B
Phen B	0.34*										
Phen Tot	0.97*	0.54*									
Haem A	0.72*	NS	0.70*								
Haem B	NS	NS	NS	NS							
Haem Tot	0.67*	NS	0.67*	0.97*	0.47*						
Pro A	0.68*	NS	0.63*	0.75*	NS	0.68*					
Pro B	0.35†	NS	0.36†	0.39†	NS	0.35†	0.51*				
Pro Tot	0.67*	NS	0.63*	0.74*	NS	0.67*	0.99*	0.59*			
Flav A	0.73*	NS	0.68*	0.70*	NS	0.61*	0.79*	0.46*	0.80*		
Flav B	0.40†	NS	0.42*	0.44*	NS	0.42*	0.46*	0.66*	0.51*	0.51*	
Flav Tot	0.73*	NS	0.68*	0.71*	NS	0.61*	0.80*	0.48*	0.80*	0.99*	0.56*

Table 1B. A subset of data from Table 1A reduced to simplify comparison of each of the extract types and the totals

	Phen A	Haem A	Pro A		Phen B	Haem B	Pro B		Phen Tot	Haem Tot	Pro Tot
Haem A	0.72*			Haem B	NS			Haem Tot	0.67*		
Pro A	0.68*	0.75*		Pro B	NS	NS		Pro Tot	0.63*	0.67*	
Flav A	0.73*	0.70*	0.79*	Flav B	NS	NS	0.66*	Flav Tot	0.68*	0.61*	0.80*

A = 100% methanol extract.
B = 50% methanol extract.
Tot = A + B.
Phen = total phenolics (Folin–Ciocalteu reagent TAE% dry wt).
Haem = Astringency (haemanalysis TAE% dry wt).
Pro = Proanthocyanins [Butanol–HCl hydrolysis, Quebracho Tannin Equivalents (QTE)% dry wt].
Flav = Flavanols [Vanillin-HCl reagent, Catechin (±) Equivalents (CE)% dry wt].
NS = not significant.
* = $p < 0.001$
† = $0.005 > p > 0.001$.
‡ = $0.01 > p > 0.005$.

Table 2. Correlation matrices of different phenolic measures in approximately 15 shrubland plant species during each of four seasons

Winter ($n = 17$)

	Phen A	Phen B	Phen Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B
Phen B	NS										
Phen Tot	0.87*	0.62*									
Haem A	NS	NS	NS								
Haem B	NS	0.43‡	NS	NS							
Haem Tot	NS	NS	NS	0.93*	0.49†						
Pro A	0.47†	NS	0.50†	0.45‡	NS	0.47†					
Pro B	NS	NS	NS	NS	NS	NS	0.72*				
Pro Tot	0.44†	NS	0.48†	0.39‡	NS	NS	0.98*	0.83*			
Flav A	NS	NS	NS	0.52*	NS	0.46‡	0.54*	NS	0.48†		
Flav B	NS	NS	NS	NS	NS	NS	0.72*	0.65*	0.74*	0.43‡	
Flav Tot	NS	NS	NS	0.51†	NS	0.46‡	0.61*	NS	0.55*	0.99*	0.54*

Spring ($n = 21$)

Phen B	0.57*										
Phen Tot	0.98*	0.71*									
Haem A	0.86*	0.57*	0.86*								
Haem B	NS	NS	NS	NS							
Haem Tot	0.73*	0.62*	0.77*	0.92*	0.63*						
Pro A	0.77*	0.51*	0.77*	0.67*	NS	0.48†					
Pro B	0.43†	0.44†	0.46†	0.49†	NS	0.40‡	0.76*				
Pro Tot	0.73*	0.52*	0.74*	0.66*	NS	0.48†	0.99*	0.83*			
Flav A	0.79*	0.58*	0.81*	0.69*	NS	0.52*	0.91*	0.73*	0.91*		
Flav B	NS	NS	NS	NS	NS	NS	NS	0.85*	0.46*	NS	
Flav Tot	0.76*	0.58*	0.78*	0.69*	NS	0.53*	0.91*	0.80*	0.93*	0.99*	0.44†

Summer ($n = 15$)

Phen B	NS										
Phen Tot	0.92*	0.56†									
Haem A	0.68*	0.54†	0.77*								
Haem B	NS	0.68*	NS	NS							
Haem Tot	0.64*	0.63*	0.77*	0.98*	NS						
Pro A	0.88*	NS	0.80*	0.66*	NS	0.64*					
Pro B	NS	NS	NS	NS	NS	0.49‡	0.51†				
Pro Tot	0.87*	NS	0.79*	0.67*	NS	0.65*	0.99*	0.58*			
Flav A	0.63*	NS	NS	NS	NS	NS	0.68*	NS	0.67*		
Flav B	NS	NS	NS	NS	0.46‡	0.42*	0.51‡	0.52†	0.75*	NS	
Flav Tot	0.64*	NS	NS	NS	NS	NS	0.70*	NS	0.69*	0.99*	NS

Autumn ($n = 20$)

Phen B	NS										
Phen Tot	0.98*	NS									
Haem A	0.55*	NS	0.51*								
Haem B	NS	NS	NS	NS							
Haem Tot	0.45†	NS	0.41‡	0.98*	0.48*						
Pro A	0.63*	NS	0.56*	0.79*	NS	0.76*					
Pro B	NS	NS	NS	NS	NS	NS	0.47†				
Pro Tot	0.63*	NS	0.56*	0.77*	NS	0.74*	0.99*	0.56*			
Flav	0.81*	NS	0.73*	0.62*	NS	0.53*	0.85*	0.43‡	0.85*		
Flav B	NS	NS	NS	NS	NS	NS	0.43‡	0.72*	0.49†	0.45†	
Flav Tot	0.80*	NS	0.73*	0.62*	NS	0.53*	0.85*	0.46†	0.85*	0.99*	0.50†

A = 100% methanol extract.
B = 50% methanol extract.
Tot = A + B.
Phen = total phenolics (Folin–Ciocalteu reagent TAE % dry wt).
Haem = Astringency (haemanalysis TAE % dry wt).
Pro = Proanthocyanins (Butanol–HCl hydrolysis, Quebracho Tannin Equivalents (QTE) % dry wt).
Flav = Flavanols (Vanillin-HCl reagent, Catechin (±) Equivalents (CE) % dry wt).
NS = not significant.
* = $p < 0.001$.
† = $0.005 > p > 0.001$.
‡ = $0.01 > p > 0.005$.

Table 3. A subset of the correlations from Table 2 to simplify comparison on a seasonal basis (W = winter, Sp = spring, S = summer and A = autumn) of each of the extract types and the totals

	Phen Tot				Haem Tot				Pro Tot			
	W	SP	S	A	W	Sp	S	A	W	Sp	S	A
Haem Tot	0.28¶	0.77*	0.77*	0.41‡								
Pro Tot	0.48†	0.74*	0.79*	0.56*	0.39¶	0.48†	0.65*	0.74*				
Flav Tot	0.20¶	0.78*	0.41¶	0.73*	0.46‡	0.53*	0.34¶	0.53*	0.55*	0.93*	0.69*	0.85*

A = 100% methanol extract.
B = 50% methanol extract.
Tot = A + B.
Phen = total phenolics (Folin–Ciocalteu reagent TAE% dry wt).
Haem = Astringency (haemanalysis TAE % dry wt).
Pro = Proanthocyanins [Butanol–HCl hydrolysis, Quebracho Tannin Equivalents (QTE)% dry wt].
Flav = Flavanols [Vanillin-HCl reagent, Catechin (±) Equivalents (CE) % dry wt].
* = $p < 0.001$.
† = $0.005 > p > 0.001$.
‡ = $0.01 > p > 0.005$.
¶ = not significant.

differences must obviously be due to changes in plant biochemistry and physiology during seasonal growth, particularly in winter. Such differences are not unexpected since winter rainfall, a characteristic of mediterranean ecosystems, mobilises soil nutrients, and this is followed by growth in late winter and early spring.

Levels of total phenolics (Folin–Ciocalteu TAE), flavanols (CE), proanthocyanidins (QTE) and astringency (TAE) increased from winter through to autumn (Table 4). Levels varied significantly both among species and across the different seasons (Table 5). (Phenolic concentrations for individual plant species in each of four seasons are tabulated in Table 6). Total phenolics increased two-fold while proanthocyanidins and flavanols increased three-fold; however, since the standards used in each assay are different, these increases were all relative rather than absolute. Changes in astringency were not synchronised with changes in the three phenol measures: astringency was lowest on average in spring and highest in autumn. Nevertheless, astringency showed the closest approximation to total phenolics in all seasons except winter. Proanthocyanidin concentrations appeared to be

Table 4. Means and standard deviations (% dry wt) for four different phenolic tests on some shrubland plant species measured in each of four seasons

	Winter (n = 12)		Spring (n = 12)		Summer (n = 12)		Autumn (n = 21)	
	X	SD	X	SD	X	SD	X	SD
Phen A	3.3	2.5	4.4	4.1	6.5	3.5	10.8	11.2
Phen B	2.4	1.8	1.4	0.9	2.0	1.4	2.2	1.5
Phen Tot	5.8	3.2	5.8	4.8	8.2	3.8	11.8	8.1
Haem A	5.7	4.1	4.9	3.4	7.3	5.4	10.5	7.4
Haem B	2.5	1.2	1.6	2.2	1.2	1.1	1.9	1.8
Haem Tot	8.2	4.5	6.4	4.6	8.5	5.7	12.4	7.8
Pro A	15.2	17.1	22.1	30.9	56.3	64.5	70.2	70.8
Pro B	4.4	4.2	5.0	7.1	5.0	6.2	7.9	8.1
Pro Tot	19.3	21.0	27.0	36.0	61.5	67.1	78.1	75.4
Flav A	5.9	8.6	6.4	8.8	11.7	13.6	20.6	22.7
Flav B	1.1	1.0	0.6	1.2	0.6	0.7	1.5	1.5
Flav Tot	7.0	9.3	7.0	9.2	12.4	13.9	22.2	23.7

A = 100% methanol extract.
B = 50% methanol extract.
Tot = A + B.
Phen = total phenolics (Folin–Ciocalteu reagent TAE% dry wt).
Haem = Astringency (haemanalysis TAE % dry wt).
Pro = Proanthocyanins [Butanol–HCl hydrolysis, Quebracho Tannin Equivalents (QTE) % dry wt].
Flav = Flavanols [Vanillin-HCl reagent, Catechin (±) Equivalents (CE) % dry wt].

Table 5. Results of a two-way analysis of variance comparing changes in phenolic levels in the sampled plant species during four seasons.

		F	df	p
Total phenolics	species	8.08	11,33	< 0.010
	season	9.20	3,33	< 0.001
Total proantho-cyanidins	species	7.82	11,33	< 0.001
	season	8.51	3,33	< 0.001
Total flavanols	species	6.33	11,33	< 0.001
	season	6.18	3,33	< 0.005
Total astringency	species	4.41	11,33	< 0.002
	season	4.29	3,33	< 0.050

particularly high, in some cases exceeding 100% dry wt. This is most likely to be due to the presence of impurities in the Quebracho tannin standard, which may not have been removed by purification.

The use of a two-tier sequential extraction procedure separates lower M_r phenolics from the more complex and more tightly bound compounds: Hillis and Swain [22] showed that simple low M_r phenolics occurred in the absolute methanol phase while proanthocyanidins occurred in the 50% aqueous methanol. Goldstein and Swain [17] suggested that the water breaks hydrogen bonds holding these compounds to other cellular structures and cell wall and they are not necessarily 'insoluble' in absolute methanol. In general, extractability remains a central problem in phenolic chemical analysis [1]. In this study, the use of two-tier extractions made it possible to make some interesting comparisons of different chemical assays on each of the extracts. The levels of astringency measured by the functional test of haemanalysis [18] in the pure methanol extracts correlated significantly with the levels of total phenolics (Folin–Ciocalteu TAE), condensed tannins and flavanols in the same extracts. However, in the aqueous methanol extracts, astringency (TAE) was not significantly correlated with phenolic concentrations measured by the other three tests, and was generally lower in the aqueous methanol than in the pure methanol. This result is consistent with those of Bate-Smith [18]. The aqueous methanol extracts had some astringency varying from 1.3 to 2.3% TAE. In contrast, levels of condensed tannins and flavanols in the aqueous methanol extracts were significantly correlated. This is perhaps explained by the close relationship between the biosynthetic pathways of these two types of compounds [1]. It appears to be superfluous to do the additional aqueous methanol extracts in herbivory studies since, in this study at least (i) levels of phenolics in the aqueous extracts comprised a small percentage of total levels (Table 4) and (ii) astringency (the functional test) did not correlate with levels of phenolics in these extracts (Table 1). On the other hand, since the lower molecular weight and less tightly bound phenolics are extracted by pure methanol, performing a two-tier extraction enables us to infer that these molecules, rather than those extracted by aqueous methanol, are the ones responsible for astringency.

One of the predictions of plant apparency theory [23] is that in general older leaves should contain higher levels of quantitative digestibility-reducing defensive compounds such as tannin polyphenols, than younger leaves. In this study, the differences were equivocal for four different measures of phenolics. Comparisons between new and old leaves were possible in eight species and in three of these there was a replicate in a different season (Table 7). Slightly more than half of the total of 44 ratios calculated for the four measures of phenolics indicated that new leaves had higher levels than old leaves, while the remainder indicated the reverse. These results, therefore, did not indicate any consistent pattern. However, an increasing number of studies have shown that new leaves have higher levels of digestibility reducing compounds than old leaves [24].

Although the mean concentration of total phenolics was high in the mediterranean shrubland plants in this study, the range was large. This meant that some plant had negligible levels of these compounds while others were extremely high, e.g. *Colpoon compressum* with 33% TAE (dry wt) total phenolics in autumn.

The biosynthesis of phenolics has been linked to plant stress in experimental tissue culture and whole plant studies as well as in field experiments [25]. In particular, a number of studies highlight the importance of substrate fertility conditions, and report high polyphenol levels associated with low nutrient levels [26]. Mediterranean ecosystems have characteristically low soil nutrient levels, especially in Australia and South Africa [27]. The results of this study support these observations: soil nutrient levels in this shrubland are particularly low (nitrogen levels range between 0.012 and 0.014% and phosphorus between 0.008 and 0.010% [27]), while phenolic levels were fairly high.

EXPERIMENTAL

Study site. Plant material was collected on 'Rondeberg' farm (33°25'S, 18°16'E) on the Atlantic ocean coast 65 km north of Cape Town, South Africa. This site is located in strandveld vegetation which is a mediterranean shrubland analogous to garrigue and maquis in southern France and chaparral in California. A more complete site description appears elsewhere [28].

Collection and extraction of plant material. Approximately 300 g of leaf and soft twig material was clipped from a minimum of five individual plants from each of 12 plant species. Identifications were checked using ref. [29]. In addition, ecologists and taxonomists more familiar with strandveld vegetation were regularly consulted about all identifications and updated nomenclature. Collections were made during October 1979, January, April and July 1980. Individual plants were tagged when sampled so that no plant was subsequently sampled a second time. Plant material which had noticeable insect or mechanical damage was not collected. Samples were immediately placed in an insulated box in shade and refrigerated without freezing within a couple of hours. At the earliest opportunity (within 36 hr), the leaves from each species sample were separated from the woody twig and petiole/midrib material. A 3.0 g thoroughly mixed subsample of leaf material was then mechanically macerated in pure MeOH using a high speed electric homogeniser (Ultra-turrax). This homogenate was made up to *ca* 45 ml with additional MeOH and a two-tier extraction process was then performed [16]. This was done using four aliquots of 100% MeOH followed by a further three with aqueous (50%) MeOH. Each fresh aliquot was added to the homogenate and brought to the boil briefly in a water bath. The two types of extract were decanted separately and kept separate for later analysis. Their individual vols were adjusted to *ca* 20 ml by evapn or by addition of solvent. Each

Table 6. Concentrations of phenolics (% dry wt) in leaf material of some South African shrubland plant species measured during four seasons (family in parentheses)

	Phen A	Phen B	Phen Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B	Flav Tot
Chrysanthemoides incana (Burm. F.).) T. Norl. (Asteraceae)												
Winter N	0.7	1.3	2.0	4.0	3.0	7.0	1.9	2.9	4.8	0.3	0.6	0.9
O	4.1	2.7	6.8	4.0	2.7	6.7	1.6	5.2	6.8	1.2	0.8	2.0
Spring N	1.6	3.6	5.2	1.7	1.9	3.6	1.1	0.0	1.1	0.5	0.0	0.5
O	2.0	1.2	3.2	1.9	1.4	3.3	1.1	0.9	2.0	0.1	0.0	0.1
Summer	3.7	3.1	6.8	2.8	2.1	4.9	1.6	0.0	1.6	1.1	0.0	1.1
Autumn	3.8	1.6	5.4	2.5	1.7	4.2	1.3	1.0	2.3	1.3	0.7	2.0
Colpoon compressum Berg. (Santalaceae)												
Winter	8.8	4.6	12.8	2.6	2.0	4.6	40.9	13.0	53.9	10.8	1.1	11.9
Spring N	17.8	3.1	20.9	11.4	0.0	11.4	123.6	15.2	138.8	36.0	0.1	36.1
O	12.0	2.6	14.6	9.8	0.0	9.8	63.5	13.0	76.5	18.4	0.7	19.1
Summer	13.0	4.3	17.3	20.5	2.2	22.7	192.9	14.0	206.9	10.2	1.8	12.0
Autumn	30.2	1.8	33.0	24.2	0.0	24.2	200.6	15.8	216.4	66.1	1.2	67.4
Eriocephalus africanus L. (Asteraceae)												
Winter	6.9	2.1	9.0	5.9	4.6	10.5	2.7	0.0	2.7	1.0	0.5	1.5
Spring	6.0	1.3	7.3	9.2	2.1	11.3	1.1	0.0	1.1	0.7	0.4	1.1
Summer	4.5	2.0	6.5	2.9	2.3	5.2	1.1	0.0	1.1	1.1	0.2	1.3
Autumn	8.1	2.3	10.4	11.2	2.6	13.8	1.8	0.0	1.8	1.4	1.5	2.9
Euclea racemosa Murr. (Ebenaceae)												
Winter	6.3	2.2	8.5	16.1	2.1	18.2	48.2	7.0	55.2	25.9	1.7	27.6
Spring N	5.3	1.4	6.7	2.8	4.0	6.8	21.1	2.6	23.7	7.0	0.0	7.0
O	7.6	2.4	10.0	8.1	1.8	9.9	34.9	7.4	42.3	25.5	0.6	26.1
Summer	3.8	1.0	4.8	12.8	0.0	12.2	23.5	3.8	27.3	11.1	0.5	11.6
Autumn	12.7	2.9	15.6	13.1	0.0	13.1	70.2	10.3	80.5	33.6	1.8	35.4
Euphorbia burmannii E. Mey. (Euphorbiaceae)												
Winter	2.5	1.2	3.7	4.3	3.6	7.9	0.0	2.0	2.0	1.1	0.0	1.1
Spring	4.4	0.8	5.2	6.1	0.0	6.1	55.2	3.3	58.5	6.9	0.0	6.9
Summer	9.7	0.2	9.9	6.5	0.0	6.5	58.6	3.8	64.4	11.1	0.0	11.1
Autumn	8.1	0.2	8.3	14.0	6.7	20.7	128.6	3.8	132.4	14.6	0.1	14.7
Nylandtia spinosa DC (Polygalaceae)												
Winter	3.4	0.8	4.2	3.8	0.8	4.6	0.6	0.0	0.6	0.3	1.1	1.4
Spring	1.1	0.1	1.2	1.1	0.0	1.1	0.6	0.0	0.6	0.1	0.1	0.2
Summer	6.0	2.4	8.4	3.9	0.0	3.9	0.7	0.0	0.7	2.3	0.3	0.6
Autumn	1.8	0.2	2.0	1.3	0.8	2.1	0.9	0.0	0.9	0.4	0.1	0.5
Passerina vulgaris (Meisn.) Thoday (Thymelaeaceae)												
Winter	0.1	5.1	5.2	6.8	2.0	8.8	10.4	6.2	16.6	1.7	0.8	2.5
Spring	1.7	1.3	3.0	2.8	1.8	4.6	2.7	4.6	7.3	0.5	0.9	1.4
Summer	1.9	1.6	3.5	3.3	2.6	5.9	22.8	19.9	42.7	4.2	1.6	5.8
Autumn	5.0	4.8	9.8	3.1	2.0	5.1	36.7	25.6	62.3	8.4	2.5	10.9
Pterocelastrus tricuspidatus (Lam.) Sond (Celastraceae)												
Winter	1.2	1.1	2.3	2.0	1.4	3.4	6.2	2.8	9.0	0.9	0.9	1.8
Spring N	1.1	0.8	1.9	0.0	0.0	0.0	8.2	3.1	11.3	2.8	0.0	2.8
O	2.3	1.9	4.2	2.9	0.0	2.9	36.4	11.1	47.5	11.9	1.1	13.0
Summer	8.2	0.4	8.6	2.8	0.0	2.8	133.8	1.5	135.3	21.4	0.0	21.4
Autumn	6.3	2.0	8.3	9.0	2.5	11.5	83.5	11.6	95.1	18.4	1.0	19.4
Rhus incana Ait (Anacardiaceae)												
Winter	3.5	1.1	4.6	10.6	2.5	13.1	33.8	8.4	42.2	5.7	2.4	8.1
Spring N	1.7	0.6	2.3	4.0	0.9	4.9	4.7	2.2	6.9	1.8	0.0	1.8
O	0.7	0.6	1.3	1.3	1.1	2.4	0.7	1.9	2.6	0.1	0.2	0.3
Summer	7.3	1.5	8.8	7.0	1.4	8.4	66.8	6.7	73.5	16.3	1.5	17.8
Autumn N	9.1	2.9	12.0	11.4	1.6	13.0	113.9	12.5	126.4	33.8	3.3	37.1
O	13.2	2.3	15.5	17.7	2.0	19.7	153.3	6.4	159.7	41.0	2.0	43.0

Table 6. *Continued*

	Phen A	Phen B	Phen Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B	Flav Tot
Rhus lucida L. (Anacardiaceae)												
Winter N	2.3	4.0	6.3	5.7	5.5	11.2	49.5	16.9	66.4	14.2	5.1	19.4
O	1.2	1.8	3.0	5.2	1.1	6.3	1.0	0.0	1.0	26.2	1.6	27.8
Spring N	5.5	2.9	8.4	6.0	2.6	8.6	59.7	25.7	85.4	17.8	4.6	22.4
O	4.1	0.8	4.9	6.9	2.1	9.0	47.3	20.8	68.1	12.5	3.7	16.2
Summer	11.4	2.0	11.4	11.3	1.7	13.0	135.3	5.8	141.1	49.0	1.2	50.2
Autumn	13.6	3.2	16.8	20.4	2.1	22.5	161.2	14.8	176.0	56.5	5.5	62.0
Salvia aurea L. (Labiatae)												
Winter	2.7	5.7	8.4	5.5	4.3	9.8	2.4	0.0	2.4	1.6	0.2	1.8
Spring	7.7	2.7	10.4	8.7	7.9	15.6	1.4	0.0	1.4	0.9	0.5	1.4
Summer	4.5	4.5	9.0	10.1	2.4	12.5	1.9	0.0	1.9	0.7	0.1	0.8
Autumn	9.9	4.0	13.9	10.7	2.7	13.4	2.0	0.0	2.0	1.5	0.6	2.1
Willdenowia striata Thunb. (Restionaceae)												
Winter	1.9	0.5	2.4	1.4	0.9	2.3	6.5	0.8	7.3	1.1	0.0	1.1
Spring	1.0	0.1	1.1	1.1	0.0	1.1	1.2	0.4	1.6	0.2	0.0	0.2
Summer	3.4	0.4	3.8	4.2	0.0	4.2	36.7	4.4	41.1	14.4	0.3	14.7
Autumn	3.4	0.4	3.8	2.0	0.0	2.0	22.2	2.4	24.6	8.1	0.3	8.4
Aspalathus hispida L. (Leguminosae)												
Autumn	13.6	7.3	21.0	5.7	0.0	5.7	2.2	0.6	2.8	1.8	0.1	1.9
Asparagus capensis L. (Liliaceae)												
Autumn	2.0	0.5	2.5	2.8	0.0	2.8	1.1	0.0	1.1	1.7	0.1	1.8
Cotyledon orbiculata L. (Crassulaceae)												
Autumn	3.8	0.5	4.3	17.3	3.1	20.4	63.2	3.7	66.9	11.0	0.4	11.4
Haemanthus sp. (Amaryllidaceae)												
Spring	0.4	0.4	0.8	0.0	0.0	0.0	2.3	0.0	2.3	0.2	0.0	0.2
Olea exasperata Jacq. (Oleaceae)												
Spring N	7.3	0.5	7.8	3.7	0.0	3.7	0.7	0.7	1.4	2.9	0.2	3.1
O	4.0	0.4	4.4	2.5	0.9	3.4	1.1	0.0	1.1	1.4	0.2	1.6
Summer	2.9	0.6	3.5	3.1	1.0	4.1	1.2	0.0	1.2	2.4	0.0	2.4
Phyllica stipularis L. (Rhamnaceae)												
Autumn	9.7	2.3	12.0	8.5	1.9	10.4	69.1	21.6	90.7	25.5	4.0	29.5
Protea repens (L.) L. (Proteaceae)												
Winter	3.1	1.4	4.5	2.2	0.0	2.2	19.0	15.3	34.3	4.2	2.2	6.4
Putterlickia pyracantha (L.) Endl. (Celastraceae)												
Summer N	9.4	2.3	11.7	10.8	2.0	12.8	139.2	12.4	151.6	19.6	2.1	21.7
Autumn N	18.8	1.1	19.9	2.9	0.0	2.9	93.3	4.4	97.7	55.0	0.8	55.8
O	21.6	1.7	23.3	11.7	1.6	13.3	80.0	8.3	88.3	57.3	1.6	58.9
Senecio elegans L. (Asteraceae)												
Winter	1.8	2.1	3.9	8.1	0.0	8.1	4.4	11.3	15.7	2.1	0.0	2.3
Thesium aggregatum A. W. Hill (Santalaceae)												
Winter	7.1	2.3	9.4	3.2	2.0	5.2	23.2	9.7	32.9	1.9	2.7	4.6
Summer	3.1	1.1	4.2	1.8	1.3	3.1	6.4	0.0	6.4	0.5	1.5	2.0
Autumn	11.3	2.4	13.7	2.2	1.6	3.8	20.5	10.1	30.6	2.2	2.4	4.6

New (N) and old (O) material were distinguished from each other when both were present in a particular species.
A = 100% methanol extract. B = 50% methanol extract. Tot = A + B.
Phen = total phenolics (Folin–Ciocalteu reagent TAE % dry wt). Haem = Astringency (haemanalysis TAE % dry wt).
Pro = Proanthocyanins [Butanol–HCl hydrolysis, Quebracho Tannin Equivalents (QTE) % dry wt].
Flav = Flavanols [Vanillin–HCl reagent, Catechin (±) Equivalents (CE) % dry wt].

Table 7. Ratio of total phenolics, flavanols, proanthocyanidins and astringency in new: old leaves

Species	Season	Ratio			
		Phenolics	Flavanols	Proanthocyanidins	Astringency
Chrysanthemoides incana	Winter	0.3	0.5	0.7	1.1
	Spring	1.6	5.0	0.1	1.1
Colpoon compressum	Spring	1.4	1.8	1.8	1.2
Euclea racemosa	Spring	0.7	0.3	0.6	0.7
Olea africana	Spring	1.7	1.9	13.6	1.1
Pterocelastrus tricuspidatus	Spring	0.5	0.2	0.2	0.1
Putterlickia pyracantha	Autumn	1.1	1.1	1.3	0.7
Rhus incana	Spring	1.9	9.0	0.8	2.0
	Autumn	0.8	0.9	0.8	0.7
Rhus lucida	Winter	2.1	0.7	66.4	1.8
	Spring	1.8	0.8	1.3	0.9

A ratio greater than 1.0 indicates that a particular concentration is higher in the new leaves while a ratio of less than 1.0 indicates the reverse.

extract was filtered through Whatman's No. 1 cellulose filter paper into volumetric flasks, accurately made up to 25 ml and then transferred into glass bottles with Teflon-lined rubber caps. for refrigerated storage. The remaining leaf material was weighed and oven-dried at 60° to constant mass and then reweighed in order to determine moisture content. This dry material was then ground in a Wiley Mill to pass through 20 mesh, and analysed for nutritional components at a later date. These results will be reported elsewhere.

Analyses. The concentrations of three classes of compounds were measured colorimetrically: total phenolics, and two of the condensed tannins—proanthocyanidins and flavanols. Optimum wavelength for these readings was determined from absorbance versus wavelength curves which were generated using the most concentrated standard as a sample. The standards used for each test are given below. Each extract was tested in duplicate or triplicate for each of the three classes of compounds. The concentrations determined from the pure MeOH and aqueous MeOH extracts for each species were added together to give a final concentration for each test. For total phenolics, all pure MeOH extracts for each species were added together to give a final concentration for each test. For total phenolics, all pure MeOH and aqueous MeOH extracts were tested using the 'Improved method' of Singleton and Rossi [15]. Tannic acid (Sigma corp.) was used as a standard and absorbances were read at 750 nm after a 2 hr colour development period. All results were calculated and reported as tannic acid equivalents (TAE). Proanthocyanidins were measured using butanol–HCl [16]. A standard curve was generated using Quebracho tannin ('Bark Tan', Van Dyke Supply Company, Woonsocket, SD, U.S.A.) and absorbances were read at 550 nm. The concentration of flavanols in the extracts was determined using a simpler version [30] of an earlier method [16]. Catechin (±) was used as a standard and absorbances were read at 498 nm after 25 min colour development at 25°. Relative astringency of extracts was measured using haemanalysis [18] modified as follows: fresh blood (20 ml) was drawn from the cannulated jugular of an adult sheep into a heparinised tube. Within 20 min, the blood was diluted 1 : 50 with cold distilled H_2O and kept refrigerated. This solution was usable for about 36 hr before the formation of any suspensions. Aliquots (2 ml) of the dilute blood were mixed with 2 ml of pure or, if necessary, diluted plant extract. This was centrifuged at 3000 rpm for 10 min and the absorption of the supernatant was measured at 578 nm. Tannic acid was again used as a standard with dilutions from 0.3 mg to 0.8 mg per ml. All astringency values were calculated and expressed as tannic acid equivalents (TAE).

Statistical analyses. Multiple linear regression analysis [31] was used to examine the correlation between the results of the various phenolic tests. Each plant sample was considered independent for statistical purposes since, although species were sampled seasonally, individual plants were sampled only once.

Acknowledgements—We thank the South African C.S.I.R. C.S.P. Fynbos Biome Programme for financial support. Mr F van der Riet Duckitt very kindly permitted us to use his farm 'Rondeberg'. An unknown reviewer provided some very helpful comments.

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