South African Journal of Botany 90 (2014) 114–117

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Tannin content of leaf extracts of 53 trees used traditionally to treat diarrhoea is an important criterion in selecting species for further work

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ARTICLE INFO

Article history:
Received 23 May 2012
Received in revised form 25 October 2013
Accepted 8 November 2013
Available online 3 December 2013

Edited by J Van Staden

Keywords:
Diarrhoea
Tannin content
Gallic acid
Selecting species
Variation within families

ABSTRACT

Aim of the study: In southern Africa many plant species are used to treat different ailments and diseases related to infections. Diarrhoea is not only an important disease of humans but also causes large losses in the animal production industry. The antimicrobial activity of many of these species may however, be based on their tannin content. Even though high tannin content may be therapeutically effective against pathogens causing diarrhoea, nutritional side effects limit its prophylactic use in animal production. The aims of the study were two-fold. In the first place it was to compile a list of species used traditionally to treat diarrhoea. The second aim was to identify and remove those species where a potential antidiarrhoeal activity may be due to tannins, because it would be counterproductive to use in production animal systems.

Materials and methods: After a literature study, 53 tree species used to treat diarrhoea or dysentery in humans or animals in southern Africa were identified. Plant material was collected and dried powdered leaves were extracted with acetone. To select plants with potential use as prophylactics against diarrhoea, the tannin content was determined by a radial diffusion method of precipitation of bovine serum albumin in agar and expressed as gallic acid equivalents.

Results: Based on our literature research plant species from at least 37 tree families are used traditionally to treat diarrhoea in southern Africa. Most of the species were from the Fabaceae (9), Euphorbiaceae (6), Anacardiaceae (3) and Combretaceae (3). The highest tannin content of 11.3 mg/ml gallic acid equivalents was detected in leaf extracts of *Combretum molle* and *Sclerocarya birrea*. About 40% of the species tested contained no tannin based on the assay used.

Conclusions: As other authors have found, the tannin content within a family varied strongly. Therefore it is dangerous to make any conclusions on the tannin content of the same plant family. About 42% of the species had no detectable tannin content and another 25% contained 0–2 mg/ml gallic acid equivalent tannins. In general it appears that in most cases tannins do not play a major role in treating diarrhoea in southern African ethnomedicine. If aqueous extracts were used, the situation could have been different.

1. Introduction

Diarrhoea often leads to reduced growth of animals or even to their death, as well as to high treatment costs and an economic loss for the farmer. To address this problem, antibiotic feed additives were developed. However selective pressure exerted by the use of antibiotics as growth promoters in food animals appears to have created large reservoirs of transferable antibiotic resistance in these ecosystems (Witte, 2000). This has led to the ban of antibacterial growth promoters that might interfere with human chemotherapy in European Union countries (Witte, 2000). The European Parliament and Council Regulation (EC) No 1831/2003 lays down provisions phasing out the authorisation of antibiotic feed additives as from 1 January 2006 (Anonymous, 2003). Therefore a significant opportunity for the development of alternative feed additives was created. Moreover, following the ban, countries outside the EU will find it difficult to continue exporting animal products such as poultry and pork meat. This opens up new opportunities for developing alternative feed additives.

Diarrhoea causes the death of many children in developing countries of the world, but is also important in animal production Due to weak immune systems, especially in young stock, the occurrence of diarrhoea poses a big threat to animal health and productivity (Houe, 1999). For economical animal production, many obstacles have to be mastered, including the prevention or effective treatment of diarrhoea. One of the most important causes of diarrhoea is infection with bacteria (e.g. *Escherichia coli*), but fungal and parasitic infections can also cause diarrhoea.

Since many plant extracts have antimicrobial activities, scientists have focused on testing different species for their antimicrobial activity. However, the selection of these species has been mostly based on traditional use in past decades and a system to recommend species for further in depth investigation could be useful for scientists. An important aspect that should be taken into account when selecting species for

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http://dx.doi.org/10.1016/j.sajb.2013.11.003

further study is that the plant should contain low concentrations of tannin. This is important because tannins bind to proteins. Extracts from these plant species may therefore be useful to treat acute diarrhoea but because the protein binding has an antinutritional effect on growth of the animals, it may not be useful in treating chronic diarrhoea in animal production systems. Tannins generally should not be administered simultaneously with any medication or nutrient because of their potential for reduced absorption and activity (Wynn and Fougère, 2007). Furthermore tannins can cause nausea which is perhaps a result of protein binding within the stomach and duodenum, and high levels of tannins absorbed into the bloodstream can cause serious constipation and hepatotoxicity and damage to other organs. Tannins are also frequently bitter and may affect palatability of stock feed. Ruminants are particularly susceptible to these effects (Wynn and Fougère, 2007).

It is therefore important that plants to be further investigated for their potential therapeutic or prophylactic antibacterial or antiparasitic efficacy should have a low tannin content. In this study leaf extracts of 53 trees traditionally used to treat diarrhoea in southern Africa were selected for evaluation of their tannin content. To promote the sustainable use of resources only leaves of trees were examined in this study. Investigation of species from different families may also indicate which families contain a high concentration of tannins (Bate-Smith and Metcalfe, 1957) and should not be investigated because the traditional use may be based on a high tannin content. The aim of this study is therefore to serve as a preliminary screen for selecting plant extracts that could be potentially used as prophylactic antidiarrheal agents in animal production.

2. Material and methods

Leaves of 53 tree species were selected for this study based on their reported medicinal use in the treatment of diarrhoea (Hutchings et al.,

Table 1
Selected plants for antibacterial screening (Hutchings et al., 1996; Bossard, 1993; Bryant, 1966) percentage of dry powdered leaf material extracted by acetone, gallic acid equivalent content in mg/ml and standard deviation. (PMDB voucher specimen numbers refer to voucher specimens of the Phytomedicine database accessible in PRU.)

Plant species	Family	Voucher specimen	Medicinal use in Zulu medicine	Yield	mg/ml gallic acid eq.	SD
Acacia karroo Hayne	Fabaceae	PMDB160	Diarrhoea/dysentery	1.4%	1.67	1.00
Acacia sieberiana var. *woodii* DC	Fabaceae-	421310	Diarrhoea	1.3%	0.00	0.00
Albizia adianthifolia (Schuhmach.) W.F. Wight	Fabaceae	10635	Dysentery	2.7%	0.00	0.00
Annona senegalensis Pers.	Annonaceae	583793	Diarrhoea/dysentery	1.4%	0.00	0.00
Antidesma venosum E. Mey. ex Tul.	Euphorbiaceae	3210	Dysentery	1.8%	0.00	0.00
Berchemia zeyheri (Sond.) Grubov	Rhamnaceae	280012	Dysentery	1.2%	0.00	0.00
Bridelia micrantha (Hochst.) Baill.	Euphorbiaceae	251928	Diarrhoea	2.7%	0.00	0.00
Buddleja salviifolia Lam.	Loganiaceae	335	Diarrhoea	2.9%	0.00	0.00
Capparis tomentosa Lam.	Capparaceae	418913	Diarrhoea	2.0%	0.67	0.58
Cassine aethiopica Thunb.	Celastraceae	114685	Diarrhoea/dysentery	3.1%	0.00	0.00
Cassine transvaalensis (Burtt. Davy) Codd	Celastraceae	PMDB269	Diarrhoea	7.1%	7.33	0.58
Cassinopsis ilicifolia (Hochst.) Kuntze	Icacinaceae	PMDB280	Dysentery	3.1%	3.00	1.73
Cassinopsis tinifolia Harv.	Icacinaceae	114707	Dysentery	2.3%	1.33	1.15
Clausena anisata (Willd.) Hook. F. ex Benth.	Rutaceae	30749	Dysentery	4.2%	4.00	0.00
Clerodendrum glabrum E. Mey.	Verbenaceae	PMDB728	Diarrhoea	3.6%	0.00	0.00
Combretum molle R. Br. ex G. Don	Combretaceae	3770	Diarrhoea	2.9%	11.33	0.58
Combretum zeyheri Sond.	Combretaceae	324770	Diarrhoea	3.5%	4.33	1.53
Curtisia dentata (Burm. F.) C.A. Sm.	Cornaceae		Diarrhoea	6.6%	3.33	1.53
Deinbollia oblongifolia (E. Mey. ex Arn.) Radlk.	Sapindaceae	PMDB410	Diarrhoea/dysentery	2.5%	0.00	0.00
Dichrostachys cinerea subsp. *africana* (L.) Wight et Arn.	Fabaceae-Mimosoidae	563160	Diarrhoea	1.3%	0.33	0.58
Dombeya rotundifolia (Hochst.) Planch.	Sterculiaceae	754696	Diarrhoea	1.4%	0.00	0.00
Ekebergia capensis Sparrman	Meliaceae	3141	Dysentery	2.1%	0.00	0.00
Elephantorrhiza elephantina (Burch.) Skeels	Fabaceae	391207	Diarrhoea/dysentery	2.9%	0.00	0.00
Faidherbia albida (Del.) A. Chev.	Fabaceae	423557	Diarrhoea	2.4%	1.33	1.15
Faurea saligna Harvey	Proteaceae	371255	Diarrhoea	13.7%	1.67	1.00
Ficus sur Forssk.	Moraceae	36664	Diarrhoea/dysentery	1.9%	2.67	1.15
Flueggea virosa (Roxb. Ex. Willd.) Pax & K. Hoffm.	Euphorbiaceae	PMDB48	Diarrhoea	1.4%	0.00	0.00
Hippobromus pauciflorus (L. F.) Radlk.	Sapindaceae	273761	Diarrhoea/dysentery	1.4%	8.00	1.15
Jatropha curcas L.	Euphorbiaceae	3186	Diarrhoea	2.7%	1.00	0.00
Kigelia africana/Kigelia pinnata (Jacq.) DC	Bignoniaceae	566356	Dysentery	1.3%	0.00	0.00
Lannea discolor (Sond.) Engl.	Anacardiaceae	PMDB565	Diarrhoea	2.0%	4.00	0.00
Lippia javanica (Burm. f.) Spreng.	Verbenaceae	113001	Diarrhoea/dysentery	1.9%	0.00	0.00
Lonchocarpus capassa Rolfe	Fabaceae	PMDB613	Dysentery	1.4%	0.00	0.00
Olea eurpoea subsp. *africana* (Mill.) P.S. Green	Oleaceae	PMDB306	Diarrhoea	5.7%	0.00	0.00
Oncoba spinosa Forssk.	Flacourtiaceae	PMDB62	Dysentery	3.7%	1.33	1.15
Ozoroa obovata (Oliv.) R. & A. Fernandes	Anacardiaceae	PMDB248	Dysentery	3.9%	3.33	1.53
Peltophorum africanum Sonder	Fabaceae	PMDB301	Diarrhoea	1.1%	6.00	1.73
Pittosporum viridoflorum Sims	Pittosporaceae	3702	Dysentery	2.3%	0.00	0.00
Ricinus communis L.	Euphorbiaceae	PMDB	Diarrhoea	2.3%	3.00	1.73
Schotia brachypetala Sond.	Fabaceae	114741	Diarrhoea/dysentery	1.2%	4.67	1.00
Sclerocarya birrea (A. Rich.) Hochst.	Anacardiaceae	PMDB130	Diarrhoea/dysentery	3.7%	11.33	0.58
Spirostachys africana Sond.	Euphorbiaceae	271399	Diarrhoea/dysentery	3.3%	0.00	0.00
Strychnos spinosa Lam.	Loganiaceae	841522	Diarrhoea/dysentery	2.2%	0.00	0.00
Syzygium cordatum Hochst. Ex Krauss	Myrtaceae	PMDB77	Diarrhoea	3.6%	4.00	0.00
Tecomaria capensis (Thunb.) Spach	Bignoniaceae	PMDB362	Diarrhoea/dysentery	2.9%	5.67	2.08
Terminalia phanerophlebia Engl. & Diels	Combretaceae	3086	Diarrhoea	2.2%	0.67	0.58
Tetradenia riparia (Hochst.) Codd	Lamiaceae	573263	Diarrhoea	1.6%	0.00	0.00
Thespesia acutiloba (Bak. F.) Exell & Mendonca	Malvaceae	114692	Dysentery	3.9%	0.33	0.58
Trema orientalis (L.) Blume	Ulmaceae	362949	Dysentery	4.0%	5.67	0.58
Trichilia emetica Vahl	Meliaceae	PMDB87	Dysentery	0.8%	0.33	0.58
Vangueria infausta subsp. *infausta* Burchell	Rubiaceae	PMDB89	Diarrhoea	1.2%	4.08	1.04
Ximenia caffra Sond.	Olacaceae	PMDB586	Diarrhoea	3.9%	4.00	0.00
Ziziphus mucronata Willd.	Rhamnaceae	850284	Diarrhoea/dysentery	1.2%	0.67	0.58

1996; Bossard, 1993; Bryant, 1966). For this project, only the leaves of trees were used due to their availability and being a renewable source. A list of the investigated plant species and their families as well as their use is presented in Table 1.

2.1. Plant collection and storage

The leaves were collected at the Pretoria National Botanical Garden, the Lowveld National Botanical Garden, the Manie van der Schijff Botanical Garden of the University of Pretoria and the Onderstepoort Campus of the University of Pretoria in the summer months. The plants were identified by labels on the trees and the identity was confirmed by taxonomists in the different botanical gardens. Voucher specimens were lodged in the HGJW Schweickert Herbarium (PRU) at the Main Campus of the University of Pretoria.

The plant samples were dried in a dark room under a constant flow of air. The dried leaves were ground with a Macsalab mill to a fine powder and then stored in sealed glass containers.

2.2. Preparation of samples

Eloff (1998) showed that acetone is the most suitable extractant for the screening of antimicrobial components in plants. Acetone may not be the best extractant for tannins; frequently 70% acetone is used, but it is very efficient in extracting antimicrobial compounds (Kotze and Eloff, 2002). If it extracts antimicrobial compounds without extracting tannins it represents no problem in the application to treat diarrhoea. Therefore the ground plant material was extracted with acetone by placing one gramme (1.0 g) of the ground plant material of each of the species listed in Table 1 and adding 10 ml acetone (technical grade-MERCK) in 30 ml glass tubes. The mixture was shaken for 20 min on a Labotec Model 20.2 shaking machine at high speed and left to settle. The extracts were filtered through Whatman No 1 filter paper into pre-weighed glass vials. This extraction process was repeated once on another sample of each species to get average values. Acetone was removed in a stream of cold air at room temperature from an aliquot of the extract, the mass extracted determined and then made up to 10 mg/ml in acetone in tightly closed containers stored in a refrigerator if not used immediately. This procedure removes the difficulty of resolubilizing dried extracts (Eloff, 2004).

2.3. Tannin assays

The tannin content in the plant extracts was determined using the radial diffusion method developed by Hagerman (1998). Briefly, 8 μl of each of the plant extracts (at a concentration of 10 mg/ml in acetone) was transferred into a well in a bovine serum albumin (BSA)-containing agar slab in a Petri dish. The agar slab was prepared from 1 g of agarose type 1 to which 0.1 g of bovine serum albumin was added. There were four wells per Petri dish. Afterwards the plates were sealed with parafilm and placed in an incubator at 30 °C for 96 h. The detection limit of the method is 0.025 mg tannic acid or condensed tannin with a variability coefficient of 6%. The diameter of the ring was measured with a plastic ruler. The accuracy of the values was 0.5 mm. The square of the diameter is proportional to the tannin content in the sample (Hagerman, 1998). There was also a solvent control (acetone) included in the tannin determination assay, as well as 8 μl gallic acid (10 mg/ml) (Hagerman and Butler, 1989) as a positive control to quantify the amount of tannin in the plant samples. The mg/ml gallic acid equivalent content was calculated by dividing the precipitation area of the sample extract with the precipitation area of the gallic acid solution.

3. Results and discussion

3.1. Extract yield

The extraction process was repeated to determine any possible variations in the extract yield. Quantities varying from 0.68% to 6.2% were extracted in a single extraction from the different species. In the case of *Faurea saligna* a much higher percentage (13.7%) was extracted (Table 1). In general, substantially higher quantities were extracted from different members of the Combretaceae (Eloff, 1999). With values as high as 22% for *Terminalia sericea* and a higher value for *Combretum zeyheri* (6.6% compared to the 2.9% found here). This can be explained by three repeated extractions of the same plant material compared to a single extraction in this contribution.

3.2. Tannin assays

About 42%, i.e. 22 of the 53 investigated species had no detectable tannin content in their acetone leaf extracts (Table 1). Another 25% (13 spp.) had a tannin content of between 0 and 2 mg/ml gallic acid equivalent, followed by 19% (10 spp.) between 2 and 4, 8% (4 spp.) between 6 and 8 and 6% (3 spp.) between 8 and 10 mg/ml gallica acid equivalent. Two species had a tannin content higher than 10 mg/ml gallic acid equivalent units.

The families with the highest average tannin content were Anacardiaceae, Ulmaceae and Combretaceae (Table 2). Species from the following families had no measurable tannin content in the acetone leaf extracts: Annonaceae, Lamiaceae, Loganiaceae, Oleaceae, Pittosporaceae, Sterculiaceae and Verbenaceae (Table 2). Because only one or two species were examined, no generalizations can be made at this stage. There was substantial variation within families as well. In the Anacardiaceae with the highest average tannin content of 6.2 gallic acid equivalent units, the standard deviation was 4.4. This again shows that it would be risky to extrapolate from a few results to characterize different plant families' tannin content. When looking at these results it can be assumed that the results are in line with the study of the systematic distribution of tannins by Mole (1993). In this study previous

Table 2
Average tannin content in mg/ml gallic acid equivalent of acetone leaf extracts, standard deviation and number of species examined in different families investigated.

Family	Tannin content	Standard deviation	Number of spp.
Anacardiaceae	6.2	4.4	3
Annonaceae	0.0	0.0	1
Bignoniaceae	2.8	4.0	2
Capparaceae	0.7	0.0	1
Celastraceae	3.7	5.2	2
Combretaceae	5.4	5.4	3
Cornaceae	3.3	0.0	1
Euphorbiaceae	0.7	1.2	6
Fabaceae	1.6	2.3	9
Flacourtiaceae	1.3	0.0	1
Icacinaceae	3.7	5.2	2
Lamiaceae	0.0	0.0	1
Loganiaceae	0.0	0.0	2
Malvaceae	0.3	0.0	1
Meliaceae	0.2	0.0	2
Moraceae	2.7	0.0	1
Myrtaceae	4.0	0.0	1
Olacaceae	2.0	0.0	1
Oleaceae	0.0	0.0	1
Pittosparaceae	0.0	0.0	1
Proteaeceae	1.7	0.0	1
Rhamnaceae	0.7	0.2	2
Rubiaceae	4.1	0.0	1
Rutaceae	4.0	0.0	1
Sapindaceae	4.0	4.2	2
Sterculiaceae	0.0	0.0	1
Ulmaceae	5.7	0.0	1
Verbenaceae	0.0	0.0	2

assumptions that tannins are characteristic plant compounds within families (Bate-Smith and Metcalfe, 1957) were overturned and Mole (1993) found that there was much more variability in the presence or absence of tannins within a family than was previously presumed. Even though Mole's data was based on different methods for the detection of tannins and Bate-Smith and Metcalfe mostly used protein-precipitation to detect tannins as we did in our study, our results support the conclusions reached by Mole (1993).

Based on these results it appears that the activity of the majority of plants used to treat diarrhoea in southern Africa may not be related to a high tannin content. It has to be kept in mind however, that the method used to determine tannin content has limitations and also that using an aqueous extract as traditional healers do, may have led to extracts with a higher tannin content.

4. Conclusions

In this study the tannin content of 53 plant species traditionally used for antidiarrhoeal treatment in southern Africa was assessed. The different species were ranked according to their tannin content (Table 2). In general there was hardly any correlation between the family to which a species belonged and its tannin content. There was a substantial variation in tannin content of species within the same family. It is therefore not rational to eliminate a family from further investigation unless many more species were examined. The results does however allow for the elimination of certain species without carrying out expensive and time consuming animal feeding experiments.

The fact that a plant has a high tannin content does not mean that it is not useful in treating acute attacks of diarrhoea, but it would probably not be useful as a replacement of antibacterial feed additives in large scale animal production.

A limitation of this study was that only one assay for tannins was used. The intention was however, to decrease the large number of species originally selected and not to do an exhaustive study of tannin content of a large number of species.

About half of the species used traditionally to treat diarrhoea can now be eliminated from the list. The next aspect to be taken into account to determine the most promising species for in depth investigation is to determine the in vitro antibacterial activity of plant extracts against Gram-positive and Gram-negative nosocomial agents. This aspect is currently being investigated to determine selective activity against the Gram-negative bacteria which are more commonly associated with diarrhoea. This approach may help scientists to make a more rational decision when selecting plant species for the potential development of alternative drugs for the treatment of diarrhoea.

Acknowledgements

The Medical Research Council and the Faculty of Veterinary Science, University of Pretoria, South Africa provided financial support. The curators of the Lowveld National Botanical Garden, the Pretoria National Botanical Garden and the Manie van der Schijff Botanical Garden at the University of Pretoria allowed us to collect leaves from the trees.

References

Anonymous, 2003. Regulation (EC) no. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. European Commission — Official Journal of the European Communities L268, 29–43.
Bate-Smith, E.C., Metcalfe, C.R., 1957. Leuco-anthocyanins. 3. The nature and systematic distribution of tannins in dicotyledonous plants. Journal of the Linnean Society of London, Botany 55, 669–705.
Bossard, E., 1993. Angolan medicinal plants used also as piscicides and/or soaps. Journal of Ethnopharmacology 40, 1–19.
Bryant, A.T., 1966. Zulu Medicine and Medicine-Men. C. Struik, Cape Town.
Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.
Eloff, J.N., 1999. The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95, 148–152.
Eloff, J.N., 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine 11, 370–371.
Hagerman, A.E., 1998. The Tannin Handbook. http://www.users.muohio.edu/hagermae/ (accessed 25.10.2013).
Hagerman, A.E., Butler, L.G., 1989. Choosing appropriate methods and standards for assaying tannin. Journal of Chemical Ecology 1795–1810.
Houe, H., 1999. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. Veterinary Microbiology 64, 89–107.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.
Kotze, M., Eloff, J.N., 2002. Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). South African Journal of Botany 68, 62–67.
Mole, S., 1993. The systematic distribution of tannins in the leaves of angiosperms: a tool for ecological studies. Biochemical Systematics and Ecology 21, 833–846.
Witte, W., 2000. Ecological impact of antibiotic use in animals on different complex microflora environment. International Journal of Antimicrobial Agents 14, 321–325.
Wynn, S.G., Fougère, B.J., 2007. Veterinary Herbal Medicine. Mosby Elsevier, USA.