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Verbascoside and luteolin-5-*O*-*β*-D-glucoside isolated from *Halleria lucida* L. exhibit antagonistic anti-oxidant properties *in vitro*

Y. Frum [a], A.M. Viljoen [b,*], F.R. Van Heerden [c]

[a] *Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa*
[b] *Department of Pharmaceutical Sciences, Tshwane University of Technology Private Bag X680, Pretoria 0001, South Africa*
[c] *School of Chemistry, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

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Abstract

The purpose of this investigation was to determine and characterise the anti-oxidant activity of the methanol extract of the leaves of *Halleria lucida* utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The methanol extract of the leaves of *H. lucida* displayed promising anti-oxidant activity with an IC_{50} value of 8.49±0.12 μg/ml and was subsequently subjected to activity-guided fractionation resulting in the isolation of a flavone-type flavonoid and phenylpropanoid glycoside, namely luteolin-5-*O*-*β*-D-glucoside and verbascoside (acteoside), respectively. Both compounds displayed promising anti-oxidant activities with IC_{50} values of 6.12±0.40 and 7.18±0.08 μg/ml for luteolin-5-*O*-*β*-D-glucoside and verbascoside, respectively. Furthermore, isobologram construction was undertaken to determine pharmacological interactions between the isolated molecules resulting in a concentration-dependent additive and antagonistic interaction being recognised.

Keywords: Anti-oxidant; DPPH; *Halleria lucida*; Isobologram; Luteolin-5-*O*-glucoside; Verbascoside (acteoside)

1. Introduction

Halleria lucida L. (Scrophulariaceae) is a popular medicinal plant mainly found in the Olifants River Mountains, Western Cape, South Africa, but not confined to this area (Coates-Palgrave, 2002). Ethnobotanical literature indicates that this medicinal plant has been used for a variety of skin diseases. In addition, the Zulu have been reported to use this plant in topical applications primarily by moistening the dried leaf with water and subsequently squeezing the juice into the ear for the relief of earache (Coates-Palgrave, 2002; Hutchings et al., 1996; Watt and Breyer-Brandwijk, 1962).

A full description of the physicochemical criteria and pharmacodynamic interactions between free radicals and anti-oxidant species is complex and beyond the scope of this work. Briefly, the innate chemical nature of free radicals means that they are unstable molecules affiliated towards the interaction with other molecules in order to stabilise their electron configuration. An anti-oxidant is any substance that when present at low concentrations, compared to those of the oxidizable substrate, significantly delays, or inhibits, oxidation of that substrate (Halliwell and Gutteridge, 1989). Given the complexity of medicinal plants as biological entities in their own right, it is not an unreasonable assumption that medicinal plants such as *H. lucida* may potentially contain a variety of anti-oxidant molecules making up their free radical scavenging arsenal. In addition, it is a possibility that medicinal plants used as dermatological agents are anti-oxidants in their mechanism of action because the underlying pathophysiology of many skin diseases (and many other diseases) constitute aberrant anti-oxidant functioning or free radical production (Benavente-Garcia et al., 1997; Vaughan, 1997). Over recent years a number of reports implicated various medicinal plants used for skin wound treatment as anti-oxidant in their mechanism of action (Samuelsen, 2000; Thang et al., 2001; Sumitra et al., 2005). Furthermore, a recent study by our group indicated that South African medicinal plants commonly used for skin diseases possess, by and large, free radical scavenging activity (Frum and Viljoen, 2006). Furthermore, a number of phytochemicals such as flavanoids, isoflavones and polyphenols are known for their anti-oxidant properties (Seifried et al., in press) while

* Corresponding author.
E-mail address: viljoenam@tut.ac.za (A.M. Viljoen).

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their ubiquitous presence in medicinal plants has been well documented (Craig, 1999; Collins, 2005; Thomasset et al., 2007). It was with this in mind that efforts were made to identify a medicinal plant which is commonly used for skin pathologies and to subsequently isolate anti-oxidant compounds.

As a result, the anti-oxidant activity of the methanol extract of the leaves of *H. lucida* was determined. Furthermore, classical chromatographic techniques were employed for the isolation of viable anti-oxidant molecules. Moreover, to the best of our knowledge, limited studies have been performed to determine concentration-dependent interactions between viable anti-oxidant compounds. To this end, isobologram construction was undertaken to determine possible interactions between isolated anti-oxidant molecules in *H. lucida*.

2. Materials and methods

2.1. Plant collection and extraction

Leaves of *H. lucida* were harvested from the Walter Sisulu Botanical Garden (WSBG) in June 2003. Specimens were identified by Mr. A. Hankey (WSBG, Johannesburg, South Africa). A voucher specimen (AV 1251) is housed in the Department of Pharmaceutical Sciences, Tshwane University of Technology, South Africa. Plants were air dried at room temperature for 3 days and macerated. Approximately 5 g of the macerated sample was subjected to extraction procedures. Cold extraction with methanol took place for 8 h and the sample was re-extracted for an additional 8 h. Methanol extracts were subjected to rotary vacuum evaporation.

2.2. Isolation of anti-oxidant compounds

Approximately 1 g extract per 100 g silica was weighed (1200 mg) and applied to a wet packed column (600 mm × 20 mm ∅) utilizing silica gel (size 0.063–2 mm, Macherey-Nagel). Gradient elution of increasing polarity was initiated consisting of successive elutions of hexane and dichloromethane (9:1); dichloromethane and methanol (6:1); and methanol. Six fractions were collected (F1–F6). Anti-oxidant activity was monitored by DPPH (2,2-diphenyl-1-picrylhydrazyl) atomisation (0.04% in HPLC grade methanol from Ultrafine Limited) on a TLC plate developed in a mobile phase comprising methanol:water:ethyl acetate (16.5:13.5:100). A total of 79.6 mg of F4 was dissolved in methanol and applied as a band to two TLC plates (size 20 × 20 cm, thickness 0.5 mm, SIL G-50 UV_{254} from Macherey-Nagel). The plates were then developed in a mobile phase comprising methanol:water: ethyl acetate (16.5:13.5:100) for approximately 120 min. Preparative TLC and DPPH atomisation yielded 7.8 mg and 9 mg of two anti-oxidant molecules.

2.3. DPPH assay

Although a large number of anti-oxidant assays are available, the DPPH-free radical is extremely stable and thus allows for easy handling and manipulation. Furthermore, its stability implies that a potential anti-oxidant will react with other well-known free radical entities which are more unstable and therefore more reactive. Thus, an anti-oxidant candidate which proves promising in the DPPH anti-oxidant assay would provide an optimistic scaffold for prospective *in vivo* studies. The anti-oxidant activity of the extract/compound was determined based on the colorimetric method described by Shimada et al. (1992) to test for DPPH radical scavenging activity with modifications. A 96-well microtitre plate was used to generate a quantitative measure of radical scavenging activities of extract/compound. All concentrations refer to final concentrations in each well. The reaction mixture contained 50 µl extract/compound dissolved in DMSO (Saarchem) and 200 µl DPPH (0.077 mmol/l) (Fluka) dissolved in HPLC grade methanol (Ultrafine Limited). Reaction mixtures for negative controls contained extract/compound dissolved in DMSO, and methanol without DPPH. Each extract/compound was tested at an initial concentration of 100 µg/ml. The plate was shaken for 2 min and stored in the dark for an additional 30 min. Percentage decolourisation was obtained spectrophotometrically at 550 nm using a Labsystems Multiskan RC microtitre plate reader. Each assay was performed in triplicate. Extract/compound which displayed promising activity (≥ 50% decolourisation at 100 µg/ml) was retested at lower concentrations using serial dilutions. Analytical grade ascorbic acid constituted the positive control. A maximum concentration of 100 µg/ml was chosen because the limitations of transdermal drug delivery (TDD) imply the usage of concentrated topical formulations which could lead to toxicity problems during prospective scale-up studies. The concentration of the extract/compound that caused 50% decolourisation (IC_{50}) was determined using Enzfitter® version 1.05 software.

2.4. Nuclear magnetic resonance (NMR)

NMR spectroscopy experiments on the compounds were performed on a Varian Inova 2000 300 MHz spectrophotometer. All spectra were recorded at 25 °C in deuterated chloroform. Chemical shifts were recorded in ppm referenced to tetramethyl silane (TMS) as internal standard.

2.5. Isobologram construction

The synergistic, antagonistic or additive interaction between the two isolated molecules was determined. Different concentrations of one isolated molecule were prepared using serial dilutions and these were combined with serial dilutions of the second isolated molecule. The DPPH assay was used and IC_{50} values for each combination determined. Isobolograms were constructed by plotting fractional IC_{50} values for one compound against fractional IC_{50} values for the second compound. The different fractional IC_{50} values for each combination were calculated and plotted. Points lying above the straight diagonal line (convex in shape) and those lying below the straight diagonal line (concave in shape) indicated an antagonistic or synergistic interaction, respectively (Berenbaum, 1978). Points lying on the straight line indicated that the

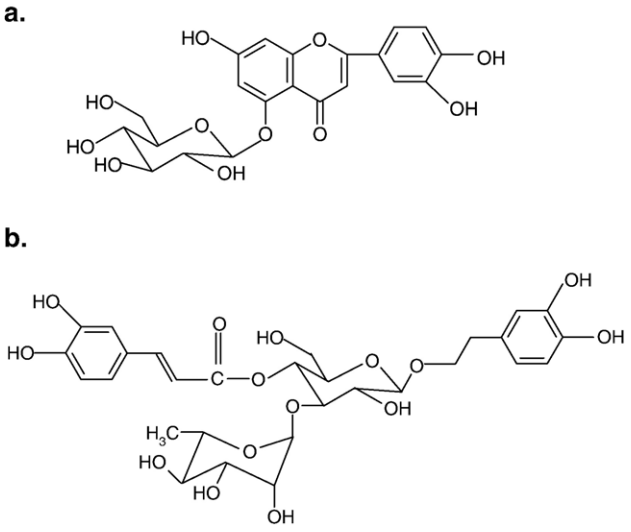

Fig. 1. Chemical structures of anti-oxidant compounds isolated from the leaves of *H. lucida*: (a) luteolin-5-*O*-*β*-D-glucoside, (b) verbascoside.

combination of two compounds in question have an additive interaction.

2.6. Statistical analysis

Student's *t*-test was used to determine statistical differences in biological activity with a significance level of $p \leq 0.05$. All analyses were performed with GraphPad Prism® version 4.03 software.

3. Results

The yield of the methanol extract expressed as a percentage of fresh plant material weighed before extraction procedures were initiated was approximately 19.52%. The more polar compound (7.8 mg, R_f 0.51) was identified as luteolin-5-*O*-*β*-D-glucoside (Fig. 1). NMR spectra correlated to that given in literature (Lin et al., 2001). The less polar compound (9.0 mg, R_f 0.57) was identified as verbascoside (acteoside) (Fig. 1). NMR spectra correlated to that given in literature (Andary et al., 1982).

The DPPH anti-oxidant activities of the methanol extract of *H. lucida*, luteolin-5-*O*-*β*-D-glucoside and verbascoside are 8.49±0.12, 6.12±0.40 and 7.18±0.08 μg/ml, respectively.

Investigation of the interaction between luteolin-5-*O*-*β*-D-glucoside and verbascoside resulted in an isobologram which was convex in shape with points lying above the straight diagonal line (not shown). This indicated an antagonistic interaction. Raw data generated for the construction of the isobologram are presented in Table 1 and are indicative of concentration-dependent additivity and antagonism.

4. Discussion

Both luteolin-5-*O*-*β*-D-glucoside and verbascoside have been previously isolated from *H. lucida* (Messana et al., 1984). Verbascoside is widely distributed as a phytochemical and has been reported to be present in a number of different plants (Franzyk et al., 2004; Lin et al., 2004; Pinar et al., 2004). Furthermore, the anti-oxidant activity of verbascoside has previously been reported and has been associated with accelerated wound healing (Mensah et al., 2001). While little is known about the distribution of luteolin-5-*O*-*β*-D-glucoside, related flavonoid compounds luteolin and corresponding glycosides are also widely distributed (Agati et al., 2002; Wang et al., 2005). Furthermore, approximately 8000 polyphenolics have been identified thus far and the types of interactions described in this report may well hold true for many of these given the presence of strong structural similarities (Opara and Rockway, 2006).

Anti-oxidant activities were marginally greater for the isolated compounds than for the crude extract ($p \geq 0.05$). The proximity in anti-oxidant activity between the crude plant extract and pure compounds is surprising given that the yield of both isolated compounds is <1% of the crude plant extract. As a result one would expect a far greater IC_{50} value obtained for the crude extract compared to the IC_{50} values of the two isolated compounds. Possible additivity or synergism between luteolin-5-*O*-*β*-D-glucoside and verbascoside was hypothesized which could explain the observed anti-oxidant IC_{50} value of the crude plant extract. As a result isobologram construction was utilized to test this hypothesis.

Isobolograms are useful not only in displaying synergism, antagonism and additivity but also in allowing one to correlate such activity over a range of concentrations for two compounds. The starting point for isobologram construction involves testing each isolated compound on its own. Unfortunately, testing each compound on its own at the extrapolated concentration in the crude plant extract could not generate IC_{50} values as free radical scavenging activity was too low. Consequently, a decision was made to test for possible interaction between the two isolated compounds using a conservative range of arbitrary concentrations applicable for prospective scale-up studies given the physicochemical limitations of TDD and possible toxicity problems. Raw data presented in Table 1 reveal the existence of two patterns of interaction. Luteolin-5-*O*-*β*-D-glucoside (*X*) and verbascoside (*Y*) appear to interact in an additive manner when

Table 1
Data generated for the construction of an isobologram of the interaction between luteolin-5-*O*-*β*-D-glucoside and verbascoside

	Concentrations (μg/ml)		IC_{50} values (μg/ml)				Ratio values [a]	
Plate	*X* [b]	*Y* [c]	*X*	SD [d]	*Y*	SD [d]	*X*	*Y*
1	50	0	9.75	0.24	0.00	0.00	1.00	0.00
	50	1	9.65	0.37	0.19	–	0.99	0.02
2	35	5	20.24	0.27	2.89	–	2.08	0.30
	25	10	13.23	0.39	5.29	–	1.36	0.55
3	10	25	3.62	–	9.05	0.26	0.37	0.94
	5	35	1.28	–	8.94	0.19	0.13	0.93
4	1	50	0.20	–	9.75	0.24	0.02	1.01
	0	50	0.00	0.00	9.65	0.37	0.00	1.00

[a] Ratio values denote the quotient of fractional IC_{50} values and are therefore unitless.
[b] Luteolin-5-*O*-*β*-D-glucoside.
[c] Verbascoside.
[d] Standard deviation ($n=3$).

one of the two compounds is present in low concentrations. Such low concentrations of either compound could presumably negate any antagonism. However, additivity is more apparent for low concentrations of luteolin-5-*O*-*β*-D-glucoside than for low concentrations of verbascoside. It is of interest to note that low concentrations of the two isolated compounds most probably mimic the concentrations in the crude extract more accurately which could explain its respective anti-oxidant activity. The greatest antagonism was observed for the combinations in plate 2, i.e., 35 μg/ml of luteolin-5-*O*-*β*-D-glucoside with 5 μg/ml of verbascoside and 25 μg/ml of luteolin-5-*O*-*β*-D-glucoside with 10 μg/ml of verbascoside. Antagonism was therefore found to be concentration-dependent. A number of explanations can be proposed to explain the antagonistic phenomena observed. It is possible that luteolin-5-*O*-*β*-D-glucoside and verbascoside interact chemically to form a complex resulting in lower anti-oxidant potential. Furthermore, the pK_a values could be similar which would indicate an equal likelihood, and therefore competition, for scavenging the DPPH free radical. In addition, the concentrations of luteolin-5-*O*-*β*-D-glucoside and verbascoside might be reaching saturation level with regards to DPPH free radical scavenging activity. However, such a scenario is unlikely as raw data generated by the DPPH anti-oxidant assay for the combination tests did not indicate saturation (all combinations ≤n90% DPPH-free radical scavenging/decolourisation) at the highest concentration tested. Another plausible possibility is that hydrogen bonding between the two isolated compounds might be taking place thus reducing the number of potential electron donating sites on the polyphenolic ring systems.

The marginal differences in anti-oxidant activity between the methanol extract of the leaves of *H. lucida* and the two isolated compounds could also possibly indicate that there are additional compounds in the crude extract which might act synergistically with the two compounds isolated. This could involve the prevention of decomposition or autoxidation of luteolin-5-*O*-*β*-D-glucoside or verbascoside (Palozza and Krinsky, 1992) or alternatively by a mechanism involving recycling of luteolin-5-*O*-*β*-D-glucoside and verbascoside free radicals.

The above results suggest that the interaction between the anti-oxidant compounds isolated from the methanol extract of the leaves of *H. lucida* is antagonistic over a number of concentration combinations. This holds enormous implications in a therapeutic clinical setting. It might be advisable not to co-administer luteolin-5-*O*-*β*-D-glucoside and verbascoside but rather allow for a lag period of time between sequenced doses. Furthermore, the above investigation also revealed that the reductionist approach for the identification and isolation of biologically active compounds is not necessarily the best approach and may, in certain circumstances, be short-sighted given the possibility of other phytochemicals being responsible for the anti-oxidant activity observed. In addition, the oxidation cascade in a living biological system is extremely complex and many potential targets for pharmacological intervention exist. For example, the phenylpropanoid glycoside verbascoside has been reported to display radical scavenging activity against DPPH, hydroxyl and superoxide anion (Gao et al., 1999; Kyriakopoulou et al., 2001) while luteolin derivatives have been shown to inhibit lipid peroxidation (Lee et al., 2002). Therefore, the anti-oxidant activity and interactions of *H. lucida*, luteolin-5-*O*-*β*-D-glucoside and verbascoside might potentially be different depending on the biological assays employed. Furthermore, correlation between *in vitro* and *in vivo* anti-oxidant activities needs to be established and this warrants further investigation.

The additive and antagonistic interactions revealed by the isobologram construction in this report also hold important implications for the use of *H. lucida* in a traditional setting. It has been reported that medicinal plants are often used in combination with other traditional remedies and in polyherbal formulations (Ram, 2001). This practice is probably aggravated in a clinical setting due to inevitable co-morbidity. Furthermore, medicinal plants are also used in combination with conventional medicines (Delgoda et al., 2004; Cappuzzo, 2006). Such scenarios are likely to replicate the patterns of interaction observed in this study given the wide distribution of anti-oxidant phytochemicals. This could possibly result in a concomitant loss of anti-oxidant efficiency as observed in this study with subtherapeutic results.

In conclusion, the DPPH anti-oxidant activity of *H. lucida* was found to be due to luteolin-5-*O*-*β*-D-glucoside and verbascoside. Although the two isolated compounds displayed significantly greater DPPH anti-oxidant activity as compared to the crude plant extract, this could not explain the potency of activity of the crude plant extract given the low percentage yield of the two isolated compounds in this plant. This displayed the limitations of the reductionist approach in certain cases given the time and effort utilized in isolating such molecules. Concentration-dependent additivity and antagonism were shown to be present between the two compounds. This holds important implications in polypharmaceutical approaches in a clinical setting. Clearly, the investigation of such interactions could yield important information with regards to developing superior protocols for anti-oxidant therapy.

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Copies of the original NMR spectra are available on request from the author of correspondence.

References

Agati, G., Galardi, C., Gravano, E., Romani, A., Tattini, M., 2002. Flavonoid distribution in tissues of *Phillyrea latifolia* L. leaves as estimated by microspectrofluorometry and multispectral fluorescence microimaging. Photochemistry and Photobiology 76, 350–360.

Andary, C., Wylde, R., Laffite, C., Privat, G., Winternitz, F., 1982. Structures of verbascoside and orobanchoside, caffeic acid sugar esters from *Orobanche rapum-genistae*. Phytochemistry 21, 1123–1127.

Benavente-Garcia, O., Castillo, J., Marin, F.R., Ortuno, A., Del Rio, J.A., 1997. Uses and properties of *Citrus* flavonoids. Journal of Agricultural and Food Chemistry 45, 4505–4515.

Berenbaum, M.C., 1978. A method for testing for synergy with any number of agents. The Journal of Infectious Diseases 37, 122–130.

Cappuzzo, K.A., 2006. Herbal product use in a patient with polypharmacy. The Consultant Pharmacist 21, 911–915.

Coates-Palgrave, K., 2002. Trees of Southern Africa. Struik, South Africa.

Collins, A.R., 2005. Antioxidant intervention as a route to cancer prevention. European Journal of Cancer 41, 1923–1930.

Craig, W.J., 1999. Health-promoting properties of common herbs. The American Journal of Clinical Nutrition 70(S), 491–499.

Delgoda, R., Ellington, C., Barrett, S., Gordon, N., Clarke, N., Younger, N., 2004. The practice of polypharmacy involving herbal and prescription medicines in the treatment of diabetes mellitus, hypertension and gastrointestinal disorders in Jamaica. The West Indian Medical Journal 53, 400–405.

Franzyk, H., Olsen, C.E., Jensen, S.R., 2004. Dopaol 2-keto- and 2,3-diketoglycosides from Chelone oblique. Journal of Natural Products 67, 1052–1054.

Frum, Y., Viljoen, A.M., 2006. *In vitro* 5-lipoxygenase and anti-oxidant activities of South African medicinal plants commonly used topically for skin diseases. Skin Pharmacology and Physiology 19, 329–335.

Gao, J.J., Igalashi, K., Nukina, M., 1999. Radical scavenging activity of phenylpropanoid glycosides in *Caryopteris incana*. Bioscience, Biotechnology and Biochemistry 63, 983–988.

Halliwell, B., Gutteridge, J.M.C., 1989. Free Radicals in Biology and Medicine. Clarendon Press, Oxford.

Hutchings, A., Lewis, G., Cunningham, A., Scott, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg, South Africa.

Kyriakopoulou, I., Magiatis, P., Skaltsounis, A.L., Aligiannis, N., Harvala, C., 2001. Samioside, a new phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from *Phlomis samia*. Journal of Natural Products 64, 1095–1097.

Lee, J.Y., Chang, E.J., Kim, H.J., Park, J.H., Choi, S.W., 2002. Antioxidative flavonoids from leaves of *Carthamus tinctorius*. Archives of Pharmacal Research 25, 313–319.

Lin, J., Lin, Y., Huang, Y., Wen, K., Chen, R., Ueng, T., Liao, C., 2001. Isolation and cytotoxicity of flavonoids from *Daphnis genkwae* Flos. Journal of Food and Drug Analysis 9, 6–11.

Lin, L.C., Chiou, W.F., Chou, C.J., 2004. Phenylpropanoid glycosides from *Orobanche caerulescens*. Planta Medica 70, 50–53.

Mensah, A.Y., Sampson, J., Houghton, P.J., Hylands, P.J., Westbrook, J., Dunn, M., Hughes, M.A., Cherry, G.W., 2001. Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. Journal of Ethnopharmacology 77, 219–226.

Messana, I., Sperandei, M., Multari, G., Galeffi, C., Marini Bettolo, G.B., 1984. A cyclohexadienone and a cyclohexenone from *Halleria lucida*. Phytochemistry 23, 2617–2619.

Opara, E.C., Rockway, S.W., 2006. Antioxidants and micronutrients. Disease-a-Month 52, 151–163.

Palozza, P., Krinsky, N.I., 1992. beta-Carotene and alpha-tocopherol are synergistic antioxidants. Archives of Biochemistry and Biophysics 297, 184–187.

Pinar, S.F., Ezer, N., Calis, I.L., 2004. Three acylated flavone glycosides from *Sideritis ozturkii* Aytac & Aksoy. Phytochemistry 65, 2095–2099.

Ram, V.J., 2001. Herbal preparations as a source of hepatoprotective agents. Drug News and Perspectives 14, 353–363.

Samuelsen, A.B., 2000. The traditional uses, chemical constituents and biological activities *Plantago major* L. A review. Journal of Ethnopharmacology 71, 1–21.

Seifried, H.E., Anderson, D.E., Fisher, E.I., Milner, J.A., in press. A review of the interaction among dietary antioxidants and reactive oxygen species. The Journal of Nutritional Biochemistry.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40, 945–948.

Sumitra, M., Manikandan, P., Suguna, L., 2005. Efficacy of *Butea monosperma* on dermal wound healing in rats. The International Journal of Biochemistry and Cell Biology 37, 566–573.

Thang, P.T., Patrick, S., Teik, L.S., Yung, C.S., 2001. Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. Burns 27, 319–327.

Thomasset, S.C., Berry, D.P., Garcea, G., Marczylo, T., Steward, W.P., Gescher, A.J., 2007. Dietary polyphenolic phytochemicals-promising cancer chemopreventive agents in humans? A review of their clinical properties. International Journal of Cancer 120, 451–458.

Vaughan, M., 1997. Oxidative modification of macromolecules minireview series. The Journal of Biological Chemistry 272, 18513.

Wang, X., Cheng, C., Sun, O., Li, F., Liu, J., Zheng, C., 2005. Isolation and purification of four flavonoid constituents from the flowers of *Paeonia suffruticosa* by high-speed counter-current chromatography. Journal of Chromatography 1075, 127–131.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, Edinburgh.

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