Introduction

Mounting evidence that dietary polyphenols may modulate dysglycaemia, a major metabolic aberration associated with the development of type 2 diabetes, is rooted in the demonstration by Von Mering in 1886 that high doses of the dihydrochalcone phloridzin (2′-β-D-glucopyranosyloxyphloretin) reduced glucose reabsorption from the renal filtrate, causing glucosuria in dogs and later also confirmed in humans [1]. This earned phloridzin the distinction as “the only compound known to have a definite action in man” as stated in a book chapter on the economic importance of flavonoid compounds in foodstuffs, published in 1962 [2]. Since the discovery of Von Mering, studies on phloridzin have progressed to the development of C-glucosyl analogues, not only with longer pharmacokinetic half-lives and duration of action than O-glucosides, but with high selectivity for SGLT2 over SGLT1 (re-
Johnson R et al. Aspalathin from Rooibos... Planta Med 2018; 84: 568–583

ABBRévIATIONS
AAC antioxidant activity coefficient
ACC acetyl-CoA carboxylase
AKT protein kinase B
AMPK 5’-adenosine monophosphate-activated protein kinase
ApoA apolipoprotein A
ApoB apolipoprotein B
ASP aspalathin
BW body weight
CAM cell adhesion molecules
CD36 cluster of differentiation 36
ChREBP carbohydrate-response element-binding protein
CLP cecal ligation and puncture
CoA coenzyme A
CPT1 carnitine palmitoyltransferase 1
DAG diacylglycerol
DPBS Dulbecco’s phosphate buffered saline
ERK extracellular signal-regulated kinase
FAS fatty acid synthase
FaSSIF fasted state simulated intestinal fluid
FFAs free fatty acids
GLUT2/4 glucose transporter 2/4
GRE aspalathin enriched-green rooibos extract
GSH glutathione
GSK glycogen synthase kinase
HBSS Hank’s balanced salt solution
HMGB1 high mobility group box 1
HUVECs human umbilical vein endothelial cells
IKK IκB kinases
IL interleukin
IRS1/2 insulin-receptor substrate 1/2
JNK c-Jun N-terminal kinase
LDL low-density lipoprotein
M-CoA malonyl-CoA
MCP monocyte chemoattractant protein
nd not detected
NF-xB nuclear factor-xB
Nrf2 nuclear factor (erythroid-derived 2)-like 2
nq not quantified
ORAC oxygen radical absorbance capacity
PI3K/AKT phosphatidylinositols 3-kinase/protein kinase B
PKC protein kinase C
PM plant material
PPAR peroxisome proliferator-activated receptor
ROS reactive oxygen species
SCD stearoyl-CoA desaturase
sEPCR soluble endothelial protein C receptor
SGLT sodium glucose co-transporter
SOD2 superoxide dismutase 2
SREBP1/2 sterol regulatory element-binding protein 1/2
TBARS thiobarbituric acid reactive substances
TEAC Trolox equivalent antioxidant capacity
UCP2 uncoupling protein 2
VLDL very low-density lipoprotein

A recent review of the health effects of phloretin, the aglycone of phloridzin, stated that to date, about 200 dihydrochalcones, isolated from more than 300 plant families, have been identified [12]. *Aspalathus linearis* (Burm.f.) Dahlg., one of more than 270 species of the genus *Aspalathus* (Family Fabaceae, Tribe Crotalariaeae) and endemic to the Cape Floristic Region [13, 14], is the natural and, until recently, only reported source of aspalathin. A closely related species, *Aspalathus pendula* R. Dahlgren, was recently also shown to contain aspalathin [15]. *A. linearis* is an erect to spreading shrub up to 2 m high with green, needle-like leaves on straight, slender branches. The leaves (15–60 mm long; up to 1 mm thick) are densely clustered without stalks and stipules. The small, yellow flowers of the cultivated type appear in spring to early summer and are solitary or arranged in groups at the tips of branches. The fruit is a small lance-shaped pod usually containing one or two hard seeds. The species is exceptionally polymorphic with ecotypes differing in morphology, fire survival strategy (re-seeding or resprouting), geographical distribution, and phenolic composition [16–19]. Van Heerden et al. [19], investigating the phenolic profile of ecotypes of *A. linearis*, as well as that of the closely related *A. pendula*, did not detect aspalathin in the collected *A. pendula* samples, but demonstrated it to be the major compound in most *A. linearis* populations, including the cultivated type. Recently, however, Stander et al. [15], using state-of-the-art mass spectrometric techniques, could detect aspalathin in two populations of *A. pendula*. They concluded that *A. linearis* and viewed by Idris and Donelly [3], Jesus et al. [4], and Blaschek [5]). In silico modelling of the natural C-glucosyl dihydrochalcone aspalathin (Table 1) predicts that it may exhibit antidiabetic effects through inhibition of SGLT2 [6]. The present review provides a short discussion of the link between metabolic syndrome and, respectively, glucose metabolism, lipid metabolism, oxidative stress, and inflammation, as a background to a discussion of the potential of aspalathin in this context. Various in vitro and in vivo studies on aspalathin are highlighted, demonstrating its potential to target underlying metabolic dysfunction relative to the development or progression of serious metabolic diseases such as obesity, type 2 diabetes, and cardiovascular diseases. Studies describing the relevant biological activity of extracts prepared from green rooibos, containing high levels of aspalathin, are included to indicate potential mechanisms of action and provide perspective on the use of an aspalathin-rich extract versus pure compound. Other aspects of aspalathin covered in this review are its natural source, the variation in content and the impact of processing the plant material, physical and chemical properties, chemical synthesis, bioavailability, and potential interference with the metabolism of hypoglycaemic and hypcholesterolaemic drugs. To achieve this, the authors independently and systematically searched major databases, including PubMed, EMBASE, and Google Scholar, for available studies reporting on the chemical and physical properties of aspalathin, its bioavailability profile, as well as its ameliorative potential against various metabolic complications. The systematic search was conducted without any language restrictions, while unpublished and ongoing studies, in addition to review articles, were screened for primary findings.

Natural source

A recent review of the health effects of phloretin, the aglycone of phloridzin, stated that to date, about 200 dihydrochalcones, isolated from more than 300 plant families, have been identified [12]. *Aspalathus linearis* (Burm.f.) Dahlg., one of more than 270 species of the genus *Aspalathus* (Family Fabaceae, Tribe Crotalariaeae) and endemic to the Cape Floristic Region [13, 14], is the natural and, until recently, only reported source of aspalathin. A closely related species, *Aspalathus pendula* R. Dahlgren, was recently also shown to contain aspalathin [15]. *A. linearis* is an erect to spreading shrub up to 2 m high with green, needle-like leaves on straight, slender branches. The leaves (15–60 mm long; up to 1 mm thick) are densely clustered without stalks and stipules. The small, yellow flowers of the cultivated type appear in spring to early summer and are solitary or arranged in groups at the tips of branches. The fruit is a small lance-shaped pod usually containing one or two hard seeds. The species is exceptionally polymorphic with ecotypes differing in morphology, fire survival strategy (re-seeding or resprouting), geographical distribution, and phenolic composition [16–19]. Van Heerden et al. [19], investigating the phenolic profile of ecotypes of *A. linearis*, as well as that of the closely related *A. pendula*, did not detect aspalathin in the collected *A. pendula* samples, but demonstrated it to be the major compound in most *A. linearis* populations, including the cultivated type. Recently, however, Stander et al. [15], using state-of-the-art mass spectrometric techniques, could detect aspalathin in two populations of *A. pendula*. They concluded that *A. linearis* and...
A. pendula produce similar combinations of main compounds with no diagnostic patterns. Only the selected and improved Nortier type (one of the Red Rocklands types) is used for commercial cultivation of rooibos tea (▶ Fig. 1) in the Cederberg region of the Western Cape.

The aspalathin content of the plant material is known to vary substantially between plants from the same plantation [20,21] due to the fact that the plants are propagated from open-pollinated seeds [22]. ▶ Fig. 2 depicts the variation in the aspalathin content of the leaves of individual plants, harvested on the same date from the same plantation. The largest number of leaf samples contained ca. 8% aspalathin (dry weight basis). ▶ Table 2 summarises ranges and means for aspalathin content in various types of plant material, extracts, and infusions. Aspalathin is present in substantially higher amounts in the leaves (6–13%) [21] than the stems (0.16–0.78%) (▶ Fig. 3) and thus also the whole dried shoots (4–10%) [20]. Rooibos produced in the tradi-

<table>
<thead>
<tr>
<th>Property (units)</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC name</td>
<td>3-(3,4-Dihydroxyphenyl)-1-(2,4,6-trihydroxy-3-[(25,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl]phenyl)propan-1-one</td>
<td>[7]</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{21}H_{24}O_{11}</td>
<td>[7, 8]</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>452.412</td>
<td>[7, 8]</td>
</tr>
<tr>
<td>Melting point</td>
<td>152–154 °C</td>
<td>[9]</td>
</tr>
<tr>
<td>Polar surface area (Å²)</td>
<td>208</td>
<td>[7]</td>
</tr>
<tr>
<td>Log P (predicted)</td>
<td>2.07</td>
<td>[8]</td>
</tr>
<tr>
<td>Log D (pH 7.4) (experimental)</td>
<td>0.13</td>
<td>[10]</td>
</tr>
<tr>
<td>Log D (pH 5.5) (experimental)</td>
<td>−0.347</td>
<td>[11]</td>
</tr>
<tr>
<td>H bond acceptors</td>
<td>11</td>
<td>[7, 8]</td>
</tr>
<tr>
<td>H bond donors</td>
<td>9</td>
<td>[7, 8]</td>
</tr>
<tr>
<td>Freely rotating bonds</td>
<td>6</td>
<td>[7, 8]</td>
</tr>
<tr>
<td>“Rule of 5′″ violations</td>
<td>2</td>
<td>[8]</td>
</tr>
<tr>
<td>Solubility at pH 2 (µM)</td>
<td>153</td>
<td>[10]</td>
</tr>
<tr>
<td>Solubility at pH 6.5 (µM)</td>
<td>123</td>
<td>[10]</td>
</tr>
<tr>
<td>Solubility in FaSSIF at pH 6.5 (µM)</td>
<td>119</td>
<td>[10]</td>
</tr>
</tbody>
</table>
tional manner that includes the “fermentation” (oxidation) step for development of the characteristic red-brown leaf colour and “woody”, “fynbos-floral”, and “honey” flavour [23, 24], and contains very little aspalathin (0.02–1.2%) [20], while the “unfermented product”, green rooibos, has an aspalathin content between 2.5 and 4.5% [25]. Hot water extracts prepared from fermented and green rooibos are used as food and/or cosmetic ingredients. If a high aspalathin content is required, a hot water extract (aspalathin content > 8%) of green rooibos [25] is preferable.

On the other hand, a hot water extract (aspalathin content < 2%) of fermented rooibos [26] is more economical to produce and it is preferred in food products, such as rooibos iced tea, when the flavour is important [27]. Similarly, green rooibos infusions at “cup-of-tea” strength have a much higher aspalathin content (78–251 mg/L) [28] than fermented rooibos infusions (not detected-16 mg/L) [29]. The bulk of rooibos production is processed to supply the demand for the traditional “fermented” herbal tea product [22].

Quantification of aspalathin

Several HPLC methods, based on UV-Vis detection, have been published through the years since the first HPLC method was developed to quantify the change in aspalathin content of the plant material with fermentation [31]. The need to quantify the phenolic content of a cup of fermented rooibos tea and extracts used in biological studies resulted in the development of a variety of methods [32–36]. The method developed by Beelders et al. [34] to characterise the phenolic content of infusions of fermented rooibos could, besides aspalathin, quantify the content of the dihydrochalcone nothofagin, the phenolic acid ferulic acid, and (Z)-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid, an enolic glucoside of phenylpyruvic acid, as well as the major flavones and flavonols. Quantitative data for rutin and quercetin-3-O-robinoiose indicate that these compounds most likely co-eluted when using previous methods. Comprehensive analysis of the phenolic composition of fermented rooibos is problematic since a number of critical peak pairs need to be resolved. The most recent method was shown to be suitable for quantification of the flavanone oxidation products of aspalathin [37]. A need to screen large numbers of green rooibos samples led to the development of a rapid HPLC method to quantify aspalathin, nothofagin, isoorientin, and orientin [30]. Kazuno et al. [38] used a triple quadrupole MS detector in a selected reaction monitoring mode to quantify a number of rooibos phenolic compounds, including aspalathin. The use of mass spectrometric detection increases the sensitivity and specificity of the method. The suitability of capillary zone electrophoresis for quantification of aspalathin and other phenolic compounds in rooibos was investigated by Arries et al. [39]. It was deemed less sensitive and a smaller number of compounds could be quantified with suitable reproducibility, but has potential as a rapid, inexpensive alternative method, especially for quantification of the major compounds.

Physical and chemical properties

Aspalathin (PubChem CID: 11282394; Chemsider ID: 9457391) is a natural C-glucosyl dihydrochalcone (3′-β-D-glucopyranosyl-2′,3,4,4′,6′-pentahydroxydihydrochalcone) with the molecular formula C21H24O11 (MW 452.412 g/mol) (Table 1) [7, 8]. The compound was first described by Koeppe [40, 41], originally designated “compound J”, and tentatively identified as a flavanone based on its chromatographic properties, chromogenic reactions with a variety of reagents, UV-Vis and infrared spectra, spectral shifts, and hydrolysis products. Aspalathin was eventually identified as a dihydrochalcone based on its oxidation products [9] and NMR data acquired at 60 MHz [42]. Renewed interest in the phe-
nolic composition of rooibos more than 30 years after the first isolation of aspalathin resulted in full elucidation of its structure via $^1$H NMR data acquired at 300 MHz [43].

Aspalathin is readily soluble in water and polar solvents, but insoluble in nonpolar solvents. Other physical properties, relating specifically to bioavailability, are discussed later. It is highly susceptible to oxidation in the presence of oxygen in solution [30, 42, 44–47] and rooibos plant material [31]. In the latter matrix, enzymes catalyse the reaction (unpublished data). Bruising of the fresh leaves results in rapid browning. The oxidation products identified in solution are shown in Fig. 4. Oxidation commences by cyclisation of aspalathin (1) to form the diastereomeric flavanone mixture (S)- and (R)-6-$\beta$-D-glucopyranosyleriodictyol (2 and 3) as major products and the diastereomeric mixture (S)- and (R)-8-$\beta$-D-glucopyranosyleriodictyol (4 and 5) as minor products. The flavanones 2 and 3 are oxidised to form the flavone isoorientin (6). The latter compound is susceptible to a Wessely-Moser-type rearrangement, i.e., hydrolysis of the enolic ether functionality and subsequent recyclication of a 1,3-diketo intermediate via the alternative o-hydroxy group, to form orientin (7) irreversibly, although the major oxidation products are unidentified brown material. The flavanones 4 and 5 are not oxidised directly to orientin (7), but these compounds are postulated to be reversibly transformed into the thermodynamically more stable flavanones 2 and 3 via the intermediate quinone methide. Further oxidation products of aspalathin include 8 and 9 atropo-diastereomeric phenols formed via phenol oxidative A- to B-ring coupling. These dimers can undergo a second phenol oxidative coupling to form the dimer 10. The 9H-fluorene 11 is postulated to form via a two-step oxidation process. The dimers are also susceptible to further oxidation into unidentified brown products. A study to investigate the formation of the brown colour upon oxidation identified aspalathin as the most important substrate for the formation of coloured products [47].

Degradation of aspalathin is pH dependent (Fig. 5). Aspalathin stability is highest at a low pH and substantial degradation (47%) occurs over 24 h at pH 7 in a citric acid-phosphate buffer at room temperature (Fig. 5A) [30]. Other factors such as buffer type, ascorbic acid, and environment also have an effect (Fig. 5B, C; unpublished results). A phosphate buffer (pH 7.4) without citric acid showed up to 97% degradation over 24 h at room temperature (Fig. 5B), indicating that citric acid has a protective effect. The metal chelating properties of citric acid may be a contributing factor. Ascorbic acid, on the other hand, was able to completely prevent aspalathin degradation in a phosphate buffer (pH 7.4) over 24 h at room temperature (Fig. 5B).
phenolic antioxidants in the diet could assist in maintaining redox balance, and might also have a role as an exogenous antioxidant, due to the hypothesis that synthetic antioxidants in food, but later the focus shifted to its potential as a model for oxidation in a membrane system. For better comprehension of its interaction with the membrane to protect against oxidation, in particular, in comparison to its 3-deoxy- and co-occurring analogue, nothofagin, the minimum energy conformations of these dihydrochalcones were considered to explain their relative affinity to interact with the membrane [54]. According to their most likely conformers, aspalathin would have a more “open” structure, postulated to improve accessibility of its catechol group to interact with the polar heads of the lipid bilayers for radical scavenging at the membrane interphase [54]. Aspalathin shares the 2,6-dihydroxyacetophenone antioxidant pharmacophore with phloretin [59]. The oxidation products of aspalathin, namely (S)- and (R)-6-β-D-glucopyranosylerydicitol, isoorientin and orientin, generally had lower antioxidant activity than aspalathin [52,54,56]. The two exceptions are that aspalathin and orientin showed similar DPPH radical scavenging activity [52], while isoorientin and orientin showed higher activity than aspalathin in the ORAC assay [57].

Chemical and potential biocatalytic syntheses

The emphasis to obtain a process for chemical synthesis of aspalathin stems from its relatively low content in the bulk of rooibos produced (i.e., fermented rooibos tea), the cost and challenge of isolation from the plant material, and the limited supply of plant material, especially green rooibos. Synthesis of aspalathin would represent a sustainable option if economically feasible. An eight-step process using tri-O-benzylglucal, tri-O-benzylphloroglucinol, and 3,4-bis(benzyloxy)phenylacetylene as starting materials was reported by Yepremyan et al. [60]. This process involved a stereoselective Lewis acid-promoted coupling of 1,2-di-O-acetyl-3,4,6-tri-O-benzylglucose with tri-O-benzylphloroglucinol, leading to the synthesis of aspalathin.

For a study on the permeability of aspalathin in a Caco-2 cell model [10], the stability of aspalathin as a pure compound and when present in an aspalathin-rich green rooibos extract was determined under cell culture conditions (pH 7.4) over 2 h in HBSS (Fig. 5 C). Under these conditions, 14 and 27% degradation of the compound occurred when pure aspalathin and aspalathin-rich extract, respectively, were tested. At pH 6 under cell culture conditions, the aspalathin concentration slightly increased, which was attributed to evaporation of the medium.

Antioxidant and pro-oxidant properties

Research interest in natural phenolic antioxidants began to escalate from 1995 as evidenced by the increase in the number of papers published [49]. Prior to this date, Japanese interest in rooibos produced a few papers relating to the free radical scavenging ability of fermented rooibos (as reviewed by Joubert et al. [50]). The latter research led to the association of rooibos with anti-ageing properties [22]. These findings, together with the demonstration of high aspalathin levels in the “unfermented” plant material and the susceptibility of aspalathin to oxidation [31], prompted research into the antioxidant properties of aspalathin. This research focussed at first on aspalathin as a potential replacement for synthetic antioxidants in food, but later the focus shifted to its potential role as an exogenous antioxidant, due to the hypothesis that phenolic antioxidants in the diet could assist in maintaining redox homeostasis in the cell gaining traction [51].

The in vitro antioxidant activity of aspalathin (Table 3) has been assessed in a variety of assays including radical scavenging and lipid peroxidation assays [52–56]. Aspalathin generally had lower antioxidant activity than the well-known radical scavenger quercetin [52,54,55,57], although similar activity was reported in the ABTS radical cation [54,56], Frenzy’s radical [56] and superoxide radical anion [52] scavenging assays. Aspalathin also showed pro-oxidant activity in the deoxyribose assay [53]. In this assay, hydroxyl radicals are generated in a Fenton reaction model system containing FeCl3-EDTA and H2O2, where aspalathin was able to reduce Fe3+ to Fe2+, thereby increasing formation of hydroxyl radicals. In this system, potent antioxidants act as potent pro-oxidants. In a recent paper, the dual antioxidant and/or pro-oxidant role of rooibos polyphenol constituents, in particular aspalathin, was postulated to play a role in the prevention of UVB-induced skin carcinogenesis [58]. With regard to lipid peroxidation, the activity of aspalathin was compared to that of isoquercitrin, a quercetin glucoside, in the Rancimat and LDL oxidation assays. Aspalathin showed higher and lower inhibitory activity than isoquercitrin in these assays, respectively. In the β-carotene bleaching and Fe(II)-induced microsomal lipid peroxidation assays, aspalathin showed lower activity than quercetin [54,55]. The Fe(II)-induced microsomal lipid peroxidation assay was chosen as a model for oxidation in a membrane system. For better comprehension of its interaction with the membrane to protect against oxidation, in particular, in comparison to its 3-deoxy- and co-occurring analogue, nothofagin, the minimum energy conformations of these dihydrochalcones were considered to explain their relative affinity to interact with the membrane [54]. According to their most likely conformers, aspalathin would have a more “open” structure, postulated to improve accessibility of its catechol group to interact with the polar heads of the lipid bilayers for radical scavenging at the membrane interphase [54]. Aspalathin shares the 2,6-dihydroxyacetophenone antioxidant pharmacophore with phloretin [59]. The oxidation products of aspalathin, namely (S)- and (R)-6-β-D-glucopyranosylerydicitol, isoorientin and orientin, generally had lower antioxidant activity than aspalathin [52,54,56]. The two exceptions are that aspalathin and orientin showed similar DPPH radical scavenging activity [52], while isoorientin and orientin showed higher activity than aspalathin in the ORAC assay [57].

Aspalathin from Rooibos... Planta Med 2018; 84: 568–583
corresponding \(\beta\)-D-glucopyranosylchlorogluconol derivative that was subsequently transformed to aspalathin. Nothofagin was also synthesised in a similar manner. Since the aforementioned synthetic protocol resulted in a low yield (20\%), an alternative sequence was developed by Van der Westhuizen and coworkers (Fig. 6) [61]. Glucosylation of the di-\(O\)-benzylacetophenone 14 with the \(\alpha\)-fluoroglucosyl derivative 13 under Lewis acid-catalysis afforded the \(\alpha\)-glucopyranosylxoy analogue 15 in an 86\% yield. Increasing the temperature from −40 to −15° C permitted a facile \(\text{BF}_3\)-catalysed rearrangement to the \(\beta\)-glucopyranosyl derivative 16 in an 84\% yield. Aldol condensation of 16 with 3,4-dibenzyl-\(\text{oxybenzaldehyde 17 afforded the protected chalcone 18 (96\% yield), which upon hydrogenation gave aspalathin in an 80\% overall yield. Based on the excellent overall yield and its scalability potential, the process was subsequently patented [62]. Recently, a number of \(\text{C-}\)-glycosyl chalcone analogues of aspalathin were synthesised by a simple three-step process [63]. These analogues displayed good DPPH radical scavenging activity and inhibited proliferation of liver and breast cancer cells.

Table 3 Summary of in vitro antioxidant and pro-oxidant activity of aspalathin.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Antioxidant mechanism</th>
<th>Antioxidant measure</th>
<th>Relative activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH radical scavenging</td>
<td>Synthetic radical scavenging</td>
<td>% Inhibition</td>
<td>Higher activity than BHT, BHA, (\alpha)-tocopherol, luteolin, vitexin, rutin, and phenolic acids Lower activity than quercetin, catechin, and isoquercitrin</td>
<td>[55]</td>
</tr>
<tr>
<td>DPPH radical scavenging</td>
<td>Synthetic radical scavenging</td>
<td>% Inhibition</td>
<td>Higher activity than isoorientin, catechin, epicatechin, rutin, Trolox, vitexin, and chrysoeriol Similar activity to orientin, luteolin, isoquercitrin, and propyl gallate Lower activity than quercetin and procyanidin B1, B2, B3, and B4</td>
<td>[52]</td>
</tr>
<tr>
<td>ABTS radical cation scavenging</td>
<td>Synthetic radical scavenging</td>
<td>IC(_50)</td>
<td>Higher activity than Trolox, catechin, orientin, isoorientin, luteolin, chrysoeriol, rutin, isoquercitrin, hydroxide, nothofagin, vitexin, and isovertixin Similar activity to quercetin and epigallocatechin gallate</td>
<td>[54]</td>
</tr>
<tr>
<td>ABTS radical cation scavenging</td>
<td>Synthetic radical scavenging</td>
<td>TEAC</td>
<td>Higher activity than nothofagin, isoorientin, orientin, isovitexin, vitexin, (S)- and (R)-6-(\beta)-D-glucopyranosyleriodictyol, rutin, isoquercitrin, and hydroxide Similar activity to quercetin</td>
<td>[56]</td>
</tr>
<tr>
<td>Fremy’s radical scavenging</td>
<td>Synthetic radical scavenging</td>
<td>Mole radicals scavenged per mole compound</td>
<td>Higher activity than nothofagin, isoorientin, orientin, isovitexin, vitexin, (S)- and (R)-6-(\beta)-D-glucopyranosyleriodictyol, rutin, isoquercitrin, and hydroxide Similar activity to quercetin</td>
<td>[56]</td>
</tr>
<tr>
<td>Superoxide radical scavenging</td>
<td>Oxygen radical scavenging</td>
<td>% Inhibition</td>
<td>Higher activity than orientin, luteolin, isoquercitrin, isoorientin, catechin, epicatechin, rutin, Trolox, vitexin, and chrysoeriol Similar activity to quercetin Lower activity than propyl gallate and procyanidin B1, B2, B3, and B4</td>
<td>[52]</td>
</tr>
<tr>
<td>ORAC with fluorescein</td>
<td>Peroxyl radical scavenging</td>
<td>ORAC index</td>
<td>Higher activity than nothofagin Lower activity than quercetin, orientin, isoorientin, vitexin, isoquercitrin, and hydroxide</td>
<td>[57]</td>
</tr>
<tr>
<td>ORAC with pyrogallol red</td>
<td>Peroxyl radical scavenging</td>
<td>ORAC index</td>
<td>Higher activity than nothofagin, orientin, isoorientin, vitexin, isoquercitrin, and hydroxide Lower activity than quercetin</td>
<td>[57]</td>
</tr>
<tr>
<td>Deoxyribose degradation in Fenton system</td>
<td>Hydroxyl radical scavenging/ generation</td>
<td>TBARS vs. control</td>
<td>Pro-oxidant activity in the absence of ascorbic acid</td>
<td>[53]</td>
</tr>
<tr>
<td>Rancimat lard oxidation</td>
<td>Lipid peroxidation</td>
<td>Induction period</td>
<td>Higher activity than (p)-hydroxybenzoic acid, ferulic acid, and (p)-coumaric acid Lower activity than quercetin, BHT, (\alpha)-tocopherol, catechin, luteolin, rutin, isoquercitrin, caffeic acid, and protocatechuic acid</td>
<td>[55]</td>
</tr>
<tr>
<td>Coupled (\beta)-carotene bleaching and linoleic acid oxidation</td>
<td>Lipid peroxidation</td>
<td>AAC after 120 min reaction time</td>
<td>Higher activity than catechin, vitexin, rutin, isoquercitrin, and phenolic acids Lower activity than quercetin, BHT, BHA, (\alpha)-tocopherol, and luteolin</td>
<td>[55]</td>
</tr>
<tr>
<td>LDL oxidation</td>
<td>Lipid peroxidation</td>
<td>Lag time</td>
<td>Higher activity than isoorientin, nothofagin, and (S)-6-(\beta)-D-glucopyranosyleriodictyol Lower activity than isoquercitrin</td>
<td>[56]</td>
</tr>
<tr>
<td>Fe(II)-induced microsomal lipid peroxidation</td>
<td>Lipid peroxidation</td>
<td>IC(_50)</td>
<td>Higher activity than Trolox, orientin, isoorientin, luteolin, chrysoeriol, rutin, isoquercitrin, hydroxide, nothofagin, vitexin, and isovertixin Similar activity to catechin Lower activity than quercetin and epigallocatechin gallate</td>
<td>[54]</td>
</tr>
</tbody>
</table>

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.
Another possibility for the synthesis of aspalathin may be the biotransformation by enzymatic C-glycosylation of the aglycone, as was achieved for nothofagin [64, 65]. A C-glycosyltransferase from rice, which was perfectly selective, was used to glucosylate phloretin using uridine diphosphate-glucose, giving an 80% yield of nothofagin [64]. In further work, the same group increased the efficiency of conversion by complexing phloretin with β-cyclodextrin to improve its solubility [65].

De novo synthesis of nothofagin and a number of other related dihydrochalcones was recently accomplished using metabolically engineered Saccharomyces cerevisiae [66]. This was done by expressing the full-length biosynthetic pathways, consisting of between four and nine genes, in the microbial host. Although the authors note that the process is not yet economically viable, metabolically engineered microbes have great potential for targeted sustainable production of high-value phenolic compounds such as aspalathin.

Bioavailability
Aspalathin, normally ingested by drinking rooibos or consuming food supplemented with rooibos extract, requires absorption through the gut into the blood stream to reach the systemic site of action in adequate concentrations to be bioefficient, apart from its beneficial gastrointestinal luminal effects. Considering that various physical properties of aspalathin (▶Table 2) related to membrane permeability fail to meet various criteria, limited absorption is to be expected. Whilst its molecular weight and log P value do not exceed the limits of 500 and 5, respectively, the number of H-bond donors and acceptors violate Lipinski’s “Rule of 5” [67]. The hydrophilic nature of aspalathin not only indicates poor permeability for gastrointestinal penetration, but also susceptibility to renal clearance [68]. Additionally, the polar surface area of aspalathin exceeds 140 Å², indicating a high probability of poor oral bioavailability [69].

Low membrane permeability of aspalathin has been reported in Caco-2 monolayer cell model studies. Relevant details of these studies are summarised in ▶Table 4. Notably, Huang et al. [11] found that aspalathin absorption increased when present in green rooibos extract as opposed to the pure compound, indicating that other plant components present in the extract may assist in its transport across the membrane. In contrast, Bowles et al. [10] found absorption of pure aspalathin to be similar to that when present in green rooibos extract. Experimental differences may account for this disparity in results, in particular the substantially higher aspalathin concentration, as well as the higher permeability of the monolayer used by Huang et al. [11]. Courts and Williamson [70] postulated that the passive diffusion of aspalathin is most likely responsible for its absorption across the intestinal epithelial monolayer. Insight into the mechanism of aspalathin transport, using the Caco-2 monolayer cell model, was gained from the study by Bowles et al. [10]. Following inhibition of aspalathin transport in the presence of a high glucose concentration (20.5 mM), the role of active glucose transporters such as SGLT1 and GLUT2 was investigated by performing experiments in the presence and absence of SGLT1 (phloridzin), GLUT2 (phloretin), and efflux (Pg) (verapamil) inhibitors. No effect on aspalathin transport was observed, leading to the conclusion that aspalathin is transported paracellularly, especially given its physical characteristics. Transport of aspalathin across the intestinal epithelial monolayer occurred without evidence of deglucosylation [70] or formation of other metabolites [10].

In vitro phase II metabolism of aspalathin with microsomal and cytosolic subcellular rat liver fractions and added cofactors produced two glucuronidated and one sulphated metabolite [71]. Methylation of aspalathin was demonstrated when treated with human liver and intestinal cytosolic fractions, following the addition of the cofactor [72]. Conjugates of aspalathin with glucuronic acid, sulphate, a methyl group, or a combination were detected in animal and human urine [10, 33, 36, 72, 73]. Deglucosylation of aspalathin is thus not a prerequisite for its absorption. Traces of methyl conjugates were found in the plasma of Vervet monkeys after being fed a single bolus containing green rooibos extract delivering 25 mg aspalathin per kg body weight [28]. Unconjugated aspalathin was detected in human plasma after ingesting a green rooibos beverage containing 287 mg aspalathin (▶Table 5). In all of the in vivo studies, except the mouse study, aspalathin was in-
gested as part of a rooibos extract, either mixed into the feed of the animals or consumed as a rooibos beverage by human subjects (Table 5). Major outcomes of these studies were that aspalathin metabolites reached maximum concentration in the plasma in 3 h or less [33] and that only a small quantity of the ingested amount was bioavailable [33,36, 72].

Of interest is the presence of the conjugated aspalathin aglycone, observed in mouse [10] and human [33] urine. The resistance of the C-C bond to the action of lactase phloridzin hydrolase, present in the brush-border of the small intestinal epithelial cells, as well as the action of cytosolic β-glucosidase [74], requires liberation of the aglycone by colonic microflora. Human colonic bacteria able to hydrolyse the C-C bond have been identified for a number of C-glucosyl compounds [75–77]. Eubacterium cellulosolvens, isolated from mice, are able to deglucosylate orientin and isoorientin [78]. Using oxidation products of aspalathin as a starting point and outcomes of studies on the anaerobic catabolism of these flavones, Muller et al. [28] proposed a microbial biotransformation pathway for aspalathin, ultimately leading to the formation of dihydrocaffeic acid and organic acids in the colon.

### Rising burden of metabolic syndrome and preventative potential of aspalathin

The metabolic syndrome describes a cluster of metabolic anomalies that underlie the development of serious metabolic disease such as type 2 diabetes, obesity, and cardiovascular disease, contributing to the rising burden of noncommunicable diseases [79]. Perturbations of glucose and lipid metabolism as a result of insulin resistance play a major role in the development of the metabolic syndrome. Individuals with this condition exhibit multiple risk factors including elevated fasting plasma glucose, high serum triglycerides, and high blood pressure, which increase the probability of developing cardiovascular complications [80]. Urbanisation with accompanying lifestyle changes, namely, excessive energy intake and lack of physical activity, contributes to these metabolic diseases [79]. Current global estimates show that more than 2 billion children and adults are overweight or obese, whereas a total of 107.7 million children and 603.7 million adults were recorded to be obese in 2015 [81]. Furthermore, the International Diabetes Federation has reported that 415 million adults have diabetes at present and this number is expected to rise to 642 million by 2040 [82]. Cardiovascular diseases, major comorbidities of type 2 diabetes, greatly contribute to global mortality [79]. In addition to cardiovascular diseases, the metabolic syndrome increases the risk of organ damage to the liver (nonalcoholic fatty liver disease), muscle (muscle deterioration), and pancreas (pancreatic β-cell dysfunction) [83,84]. The generation of excessive oxidative stress, a consequence of depleted antioxidant systems due to the overproduction of ROS, and accelerated inflammation driven by elevated proinflammatory cytokine production, are both implicated in metabolic syndrome-induced organ damage [85]. Nevertheless, there is an increase in evidence that dietary interventions may reduce oxidative stress and inflammation associated with the metabolic syndrome, thereby decreasing cardiovascular risk [86, 87]. For example, antioxidant transcriptional factors such as Nrf2, as well as its associated downstream target genes including those coding for GSH, have emerged as essential targets in the amelioration of oxidative stress-induced cardiovascular and liver damage [88–90]. Similarly, effective modulation of protein kinases such as JNK, IKK, ERKs, and AMPK by certain natural products has been correlated with a reduced inflammatory response and cell damage [91–93]. Evidence demonstrating the ameliorative potential of aspalathin against the metabolic syndrome and its associated complications is attracting a lot of interest. The following sections provide a brief overview of the metabolic processes involved in altered glucose and lipid metabolism, leading to exacerbated oxidative stress and inflammatory-induced cell damage. A separate section deals specifically with the protective potential of aspalathin and its possible interference with the metabolism of hypoglycaemic and hypocholesterolaemic drugs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>( P_{\text{app}} \times 10^{-6} )</th>
<th>% Passage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green rooibos extract in DPBS with calcium and magnesium (pH 7.4)</td>
<td>1 mg/mL (0.43 mM)</td>
<td>4 ± 0.42</td>
<td>100%</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td>5 mg/mL (2.15 mM)</td>
<td>3.49 ± 1.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg/mL (4.29 mM)</td>
<td>20.93 ± 3.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspalathin in DPBS with calcium and magnesium (pH 7.4)</td>
<td>0.2 mg/mL (0.44 mM)</td>
<td>0.91 ± 0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mg/mL (2.21 mM)</td>
<td>2.48 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 mg/mL (4.42 mM)</td>
<td>15.34 ± 1.66</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td>Aspalathin in HBSS (pH 6.0)</td>
<td>1 μM</td>
<td>2.28 ± 0.09</td>
<td></td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>150 μM</td>
<td>1.73 ± 0.97</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Aspalathin in HBSS (with high glucose; 20.5 mM; pH 6.0)</td>
<td>150 μM</td>
<td>0.29 ± 0.08</td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>Green rooibos extract in HBSS (pH 6.0)</td>
<td>0.38 mg/mL (153 μM)</td>
<td>2.00 ± 1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched green rooibos fraction in HBSS (pH 6.0)</td>
<td>0.15 mg/mL (149 μM)</td>
<td>2.11 ± 0.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Extract concentration in mg/mL with molar concentration of aspalathin; 2molar concentrations > 150 μM were cytotoxic to Caco-2 cells.
Metabolic syndrome and glucose metabolism

Disturbances in glucose metabolism remain central to the aetiology of metabolic syndrome. In both the fed and fasted state, glucose-induced insulin secretion is tightly controlled, and disturbances in insulin action cause an imbalance between glucose uptake and consumption [94]. In the liver, insulin action promotes lipogenesis and glyceroneogenesis, while lipolysis and glycogenolysis are inhibited [94]. Peripheral glucose absorption and usage are stimulated by insulin in adipose and muscle tissue. Insulin resistance dysregulates these processes, resulting in increased blood glucose levels with associated lipid accumulation [95].

Insulin action is mediated through the phosphorylation of various signalling cascades, culminating in glucose uptake into the cell. Simply put, activation of PI3K/ Akt by IRS-1 phosphorylates GSK3-β and initiates GLUT4 translocation from the cytosol to the cell membrane [96]. Both PI3K and AKT are crucial kinases that regulate GLUT4 translocation and have subsequently gathered significant interest as possible drug targets for treating the metabolic syndrome [94, 96]. Conversely, in an obese or type 2 diabetic state, elevated DAG and ceramide levels can activate PKC, leading to a dysregulated IRS/PI3K/AKT cascade that suppresses GLUT4-mediated glucose uptake in muscle and adipose tissues, as is observed in AKT knockout mice [97].

Although the PI3K/AKT signalling pathway is essential in the regulation of insulin-stimulated glucose uptake, glucose can also be regulated by phosphorylated AMPK in an insulin-independent manner [92]. AMPK is a major energy sensor that plays a critical role in cellular homeostasis and has been widely studied as a possible target to improve insulin resistance [98]. Through its maintenance of energy consumption, AMPK has contributed significantly to the modulation of various cellular processes, including cell growth, autophagy, and glucose metabolism [98]. For the latter, it is postulated that upon activation, AMPK promotes GLUT4 translocation to the cell membrane, resulting in increased glucose uptake in skeletal muscle and adipose tissue [99, 100]. Metformin, a biguanide class of oral antidiabetic, mediates its antidiabetic actions by indirectly activating AMPK [101]. However, contradicting evidence also shows that activation of AMPK in a diabetic heart can contribute to the inhibition of glucose utilisation, while abnormally enhancing fatty acid oxidation that can lead to accelerated myocardial apoptosis [102–104]. Thus, in addition to the modulation of PI3K/AKT signalling, optimal regulation of AMPK remains

Table 5 Summary of studies investigating oral bioavailability of aspalathin (ASP).

<table>
<thead>
<tr>
<th>Model</th>
<th>ASP dose</th>
<th>Dosage form</th>
<th>ASP and metabolites in plasma</th>
<th>ASP and metabolites in urine</th>
<th>Excretion in urine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>50 mg/kg BW, single</td>
<td>Pure ASP in PBS; orogastric gavage</td>
<td>nd</td>
<td>sulphated ASP (4); glucuronidated ASP (2); methylated ASP (2)</td>
<td>nd</td>
<td>[10]</td>
</tr>
<tr>
<td>Pig</td>
<td>157–167 mg/kg BW daily</td>
<td>ASP-rich GR extract (16.3%), mixed with feed</td>
<td>nd</td>
<td>ASP: glucuronidated ASP; methylated ASP; methylated and glucuronidated ASP; glucuronidated ASP aglycone</td>
<td>0.1 to 0.9%</td>
<td>[73]</td>
</tr>
<tr>
<td>Vervet monkey</td>
<td>25 mg/kg BW, single</td>
<td>ASP-rich GR extract (18.4%) mixed with bolus</td>
<td>methylated ASP (2)</td>
<td>Methylated ASP; dimethylated ASP</td>
<td>nd</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Human</td>
<td>91 mg/subject, single</td>
<td>300 mL of GR infusion</td>
<td>nd</td>
<td>Methylated ASP; methylated and glucuronidated ASP</td>
<td>Max. conc. reached &lt; 2 h after ingestion; 0.74% excreted during 0–24 h</td>
<td>[72]</td>
</tr>
<tr>
<td>Human</td>
<td>41 mg/subject, single</td>
<td>500 mL GR “ready-to-drink” beverage</td>
<td>nd</td>
<td>Glucuronidated ASP (2); methylated and glucuronidated ASP (3); methylated and sulphated ASP; sulphated ASP</td>
<td>Most excreted &lt; 5 h after ingestion; 0.22% excreted during 0–24 h</td>
<td>[36]</td>
</tr>
<tr>
<td>Human</td>
<td>3.6 mg/subject, single</td>
<td>500 mL fermented rooibos “ready-to-drink” beverage</td>
<td>nd</td>
<td>methylated and glucuronidated ASP (3); methylated and sulphated ASP; sulphated ASP</td>
<td>0.09% excreted during 0–24 h</td>
<td>[36]</td>
</tr>
<tr>
<td>Human</td>
<td>287 mg/subject, single</td>
<td>GR beverage</td>
<td>ASP</td>
<td>ASP: glucuronidated ASP; methylated ASP; methylated and glucuronidated ASP (3); methylated and sulphated ASP; sulphated ASP; glucuronidated ASP aglycone</td>
<td>0.2% excreted during 0–24 h</td>
<td>[33]</td>
</tr>
</tbody>
</table>
essential in promoting glucose uptake, reversing insulin resistance, and attenuating metabolic disease-associated complications such as oxidative stress and inflammation [98, 103].

**Metabolic syndrome and lipid metabolism**

Lipid metabolism can be defined as the synthesis or break down of fats for energy, a process that plays a major role in normal body function, as well as the aetiology of metabolic syndrome [105]. Fatty acids are major components of triglycerides and can either be absorbed through the ingestion of food or synthesised by adipocytes or hepatocytes from carbohydrate precursors such as acetyl-CoA [105]. This process begins in the intestine where triglycerides are degraded by pancreatic lipases and bile salts into FFAs, where they, together with cholesterol molecules, are packed into chylomicrons, which are transported within the lymphatic and circulatory system to be metabolised by cells or stored in the adipose tissue [106]. Although adipose tissue is the largest energy reserve in mammals, fats are also stored in muscle and liver tissues [106]. In muscle, fat stores are used as a substrate for fatty acid β-oxidation, whereas in the liver, fats are utilised in the synthesis of triacylglycerol for energy [106]. To obtain energy from adipocytes, fats are hydrolysed into FFAs and glycerol molecules in a process called lipolysis. These FFAs are further oxidised during mitochondrial fatty acid β-oxidation to produce acetyl-CoA, which is a major substrate required in the Krebs cycle for the production of energy in the form of ATP [105].

Fatty acid β-oxidation produces twice the amount of energy compared to carbohydrate or glucose metabolism and its dysregulation is implicated in the aetiology of the metabolic syndrome [107]. Altered β-oxidation in the muscle and liver is associated with diabetes (co-occurrence of type 2 diabetes and obesity) and an increased cardiovascular risk [108]. During the progression of obesity, increased lipogenesis and diminished β-oxidation account for augmented hepatic expression of lipogenic genes including SCD1, PPARγ, and SREBP1/2. In muscle, increased levels of long-chain FFA acyl-CoAs together with their lipid intermediate metabolites, DAG and ceramide, impede insulin action through activation of PKC [109]. Activation of PKC initiates an increased inflammatory response that augments muscle insulin resistance via activation of IRS (serine 307) (IRS Ser307). As previously explained, this process attenuates peripheral glucose uptake and translocation of GLUT4. Nevertheless, enhanced oxidative stress appears to be one of the devastating factors associated with accelerated tissue injury as a result of impaired glucose and lipid metabolism.

**Metabolic syndrome and oxidative stress**

It is generally accepted that oxidative stress plays a major role in the development and exacerbation of the metabolic syndrome. The role of persistent exposure to high glucose (chronic hyperglycaemia), linked to the increased production of ROS within the disease state, is well established [110]. In diabetes, raised blood glucose levels induce various signalling pathways associated with the aggravation of oxidative stress. For example, activation of the sorbitol-aldose reductase pathway, a key process in the control of excess blood glucose, can cause a decrease in the reduced form of NADP, i.e., NADPH. It is well known that the NADPH cofactor plays a major role in the synthesis of GSH, an essential antioxidant that detoxifies intracellular ROS [111]. Moreover, intracellular reduction of NADPH and GSH may promote the production of nitric oxide as well as chain activation of other free radicals leading to accelerated oxidative damage [112].

Some of the well-known sources responsible for exacerbated generation of oxidative stress in many cell types include glucose autoxidation (normally identified with elevated glucose levels in a diabetic state), abnormally enhanced activity of NADPH and xanthine oxidases, and the overactivity of the mitochondrial electron transport chain [113–115]. Lipid peroxidation, which may arise as a result of oxidative degradation of lipids by increased free radical production, is a widely-reported phenomenon identified in obese individuals [116,117]. Avci et al. [117] recently showed that enhanced lipid peroxidation inversely correlates with GSH content in individuals with metabolic syndrome. This was supported by others demonstrating that systemic markers of lipid peroxidation such as oxidised LDL and TBARS are elevated in individuals with metabolic syndrome compared to control subjects [118,119]. Such findings have also been supported by data from animal models of metabolic syndrome with increased liver dysfunction, muscle insulin resistance, and subsequent cardiomyocyte remodelling and apoptosis [83,84,88].

**Metabolic syndrome and inflammation**

Another devastating consequence interrelated with oxidative stress that normally arises due to prolonged exposure to elevated blood glucose (hyperglycaemia) and circulating lipids (hyperlipidaemia) is the activation of inflammation. Although an inflammatory response is necessary for debridement after injury, tenacious inflammation is thought to exacerbate cell damage under various disease conditions [85]. In an obese state, a proinflammatory response results in the infiltration of macrophages into peripheral tissues, including adipose tissue and the liver as well as skeletal and heart muscle [120,121]. In adipose tissue, the macrophage infiltration stems from excessive lipid overload of the various adipose tissue deposits, caused by adipocyte hypertrophy and hyperplasia, culminating in ischaemic tissue congestion and dysfunction [122]. In the liver, the nutrient overload causes nonalcoholic fatty liver disease [123]. Furthermore, increased lipid stores, particularly visceral adiposity, promote a low-grade systemic proinflammatory response, activating M1 macrophages (Th1 response) to secrete elevated levels of MCP1, IL-6, IL-1β, TNF-α, and leptin, while repressing adiponectin levels [124,125]. Adiponectin is an important adipokine that is secreted by adipocytes and is known to play a significant role in obesity-induced insulin resistance. Numerous animal studies have shown that increased adiponectin levels are inversely proportional to the concentration of ceramides, an important factor in the modulation of obesity-induced insulin resistance [126,127]. Additional evidence shows that adiponectin levels are negatively correlated with increased TNF-α levels [121,124,126]. Likewise, growing evidence suggests that decreased adiponectin levels with concomitant increased TNF-α levels not only perpetuate obesity-induced insulin resistance, but are key cytokines that aggravate the metabolic syndrome [127,128]. Increased adiponectin levels are associated with diminished systemic inflammation and lipid accumulation, leading to reduced vascular dysfunction that is linked to the metabolic syndrome.
Indeed, reduced adiponectin levels negatively correlate with the degree of adiposity and inflammation as indicated by decreased IL-6 and TNF-α production, both key mediators of diabetes-induced cardiac dysfunction [130, 131]. Thus, understanding the process that regulates TNF-α-induced inflammation and subsequent insulin resistance is key to unravelling possible drug targets for therapeutic intervention.

According to Kwon and Pesin [124], TNF-α can induce insulin resistance through two possible mechanisms, i.e., the initiation and propagation of lipolysis or by directly blunting insulin signalling. In the obese state, TNF-α-induced lipolysis leads to an escalation in the availability of circulating FFAs, and this exacerbates insulin resistance and ectopic fat accumulation in organs such as the liver and muscle [132]. In the liver, this enhanced FFA delivery together with an increase in endoplasmic reticulum stress can lead to the activation of several kinases, including JNK and IKK/NF-κB, which further contribute to the impairment of insulin resistance through the phosphorylation of IRS1 [120, 133]. In addition, excessive de novo hepatic lipogenesis causes an increase in FFA flux, which inhibits β-oxidation through increased expression of long-chain acyl-CoA. This in turn augments the hepatic triglyceride pool, which is exacerbated through an alternative mechanism that implicates the upregulated expression of SREBP-1c [134, 135].

The escalation of lipid accumulation further triggers increased dyslipidaemia, in the form of VLDL and LDL concomitant to raised proinflammatory markers such as macrophages and leukocytes within the vascular wall [124, 136]. This process, together with elevated TNF-α levels increase the expression of CAM, P-selectin, and E-selectin, to the vascular endothelium, resulting in an acute localised inflammatory response that triggers plaque development and cardiovascular dysfunction [121, 123, 127]. Similarly, in skeletal muscle of obese insulin-resistant individuals, TNF-α enhances the expression of proinflammatory cytokines in response to increased lipid storage with a concomitant reduction of β-oxidation, leading to abnormally elevated ceramide levels that augment muscle insulin resistance through PKC activation [94].

Protective Potential of Aspalathin against Metabolic Disease-Associated Complications

Effect of aspalathin on glucose and lipid metabolism

A growing body of evidence demonstrates that dietary supplements such as polyphenols, displaying antioxidant and anti-inflammatory properties, can exert beneficial effects on essential signalling molecules involved in carbohydrate and lipid metabolism [138]. Rooibos and its phenolic constituents, including aspalathin, are progressively explored for their ameliorative effects on metabolic syndrome and associated complications, including obesity and diabetes mellitus (Table 6). For example, in type 2 diabetic (db/db) mice and cultured pancreatic β-cells, aspalathin was shown to be effective at improving glucose tolerance and stimulating insulin secretion, respectively [139]. Consistent with these findings, Muller et al. [140] showed that an aspalathin-rich green rooibos extract and pure aspalathin are able to modulate glucose metabolism by inhibiting α-glucosidase enzyme activity and promoting glucose uptake in pancreatic and skeletal muscle cells as well as improving glucose tolerance in streptozotocin-induced diabetic Wistar rats. Mikami et al. [141] demonstrated that green rooibos extract and aspalathin were effective at reducing blood glucose levels of nondiabetic mice following ingestion of glucose, sucrose, and starch solutions, while suppressing the activities of α-glucosidase and α-amylase, key enzymes involved in carbohydrate hydrolysis. Mazibuko et al. [92, 142] further investigated the molecular mechanisms associated with the ameliorative effect of pure aspalathin and an aspalathin-rich green rooibos extract on palmitate-induced insulin resistance in C2C12 myotubes and 3T3-L1 adipocytes. Both the extract and compound were able to reverse palmitate-induced insulin resistance by increasing levels of GLUT4 through the suppression of PKC and NF-κB, while activating AMPK. Son et al. [143] supported these findings showing that aspalathin treatment improved glucose tolerance in obese (ob/ob) mice, while similarly enhancing glucose uptake by promoting AMPK phosphorylation and GLUT4 translocation in L6 myotubes. Smit et al. [144] showed that aspalathin promotes insulin sensitivity in cardiomyocytes from young and aged rats, but not in high-caloric diet animals, through a PI3K-dependent mechanism. Aspalathin can protect cardiomyocytes from doxorubicin-induced cardiotoxicity by increasing autophagy, while simultaneously decreasing apoptosis [102, 145].

Furthermore, both in vitro and in vivo studies have indicated that aspalathin can reduce hyperlipidaemia [91, 146]. The effect of aspalathin to diminish cholesterol, triglycerides, and VLDL and LDL cholesterol levels, while increasing high-density lipoprotein cholesterol levels, has been investigated in a few studies. Najafian et al. [146] showed that streptozotocin-induced diabetic male Wistar rats receiving 5, 10, and 40 mg/kg of aspalathin for 21 days displayed a dose-dependent decrease in lipid levels in conjunction with reduced blood glucose concentrations. Similarly, in a study by Johnson et al. [91], a 6-week treatment with aspalathin modulated lipoprotein clearance in a dose-dependent manner in db/db mice, with a higher dose (130 mg/kg) of the compound being more effective than a lower dose (13 mg/kg). Additional evidence by Van der Merwe et al. [147] demonstrated that an aspalathin-rich green rooibos extract significantly reduced serum total cholesterol of male Fischer rats after 90 days of treatment. Collectively, these results indicate that aspalathin may beneficially modulate glucose and lipid metabolism, thereby ameliorating the complications associated with metabolic syndrome.

The proposed cellular mechanisms by which aspalathin improves glucose and lipid metabolism

The cellular mechanism by which aspalathin targets metabolic syndrome and improves glucose and lipid clearance remains to be fully elucidated. However, it has been proposed that by suppressing fatty acid synthesis, aspalathin enhances CPT1 expression and subsequently increases β-oxidation in muscle tissue [92]. The latter process is under the control of essential lipid metabolism genes, including ACC, FAS, and SCD1, that play an integral role in the development of insulin resistance in fat, liver, muscle, and heart tissue. Furthermore, PPARγ, SREBP-1/2, and
### Table 6 Summary of studies related to the antioxidant, antidyslipidaemic, antidiabetic, and anti-inflammatory activities of aspalathin (ASP) and aspalathin-rich green rooibos extracts.

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Treatment and dose</th>
<th>Experimental outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L6 Myoblasts, RIN-SF cells and type 2 diabetic (db/db) mice</td>
<td>ASP: Cell culture dose (1–100 µM) ASP: Dietary dose in mice (0.1–0.2 %)</td>
<td>Improved glucose uptake, insulin secretion, and glucose tolerance.</td>
<td>[139]</td>
</tr>
<tr>
<td>Online HPLC-biochemical assay for α-glucosidase inhibitory activity, C2C12 myotubules, Chang (CCL-13) cells, and streptozocin-induced diabetic rats</td>
<td>ASP: Cell culture dose (1, 10, and 100 µM) Extract: Cell culture dose (5 × 10^{-5}–5 µg/mL) Extract: In vivo dose (3, 25, 30, or 300 mg/kg BW)</td>
<td>Displayed α-glucosidase inhibitory activity, improved glucose tolerance, and dyslipidaemia.</td>
<td>[140, 146]</td>
</tr>
<tr>
<td>Nondiabetic ddY mice; in vitro α-glucosidase and α-amylase inhibitory assays</td>
<td>ASP: In vitro dose (0, 0.5, 1, 2.4, 8 mg/mL) ASP: In vitro dose (20 mg/mL/100 g BW) Extract: In vitro dose (0, 2.5, 5, 10, 20, 40 mg/mL) Extract: In vivo dose (80 mg/mL/100 g BW)</td>
<td>Reduced blood glucose levels following ingestion of glucose, sucrose, and starch solutions. Also inhibited the activities of α-glucosidase and α-amylase.</td>
<td>[141]</td>
</tr>
<tr>
<td>L6 Myotubes, RIN-SF cells and obese (ob/ob) mice</td>
<td>ASP: Cell culture dose (0–100 µM) ASP: Dose in mice (100 mg/kg/day/mouse)</td>
<td>Dose-dependently increased glucose uptake, enhanced GLUT4 translocation to plasma membrane, and promoted AMPK phosphorylation in L6 myotubes. Reduced oxidative stress in RIN-SF cells and improved fasting plasma glucose levels in mice.</td>
<td>[143]</td>
</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>ASP: In vitro dose (0, 10, 20, 50 µM)</td>
<td>Promoted longevity by targeting stress and age-related genes, reducing the endogenous intracellular level of reactive oxygen species.</td>
<td>[152]</td>
</tr>
<tr>
<td>HUVECs, human blood samples, and C57BL/6 mice (ex vivo model)</td>
<td>ASP: Cell culture dose (1–50 µM) ASP: Ex vivo dose (4.5, 9.1, 18.1, 27.1, and 45.2 µg/mouse)</td>
<td>Demonstrated antithrombotic activities by prolonging activated partial thromboplastin time and blocking platelet aggregation and activities of thrombin and activated factor X.</td>
<td>[156]</td>
</tr>
<tr>
<td>HUVECs and mice</td>
<td>ASP: Cell culture dose (1–50 µM) ASP: In vivo dose (4.5, 9.1, 27.1, or 45.2 µg/mouse)</td>
<td>Prevented high-glucose-mediated vascular hyperpermeability, adhesion of monocytes, and expression of cell adhesion molecules. Inhibited generation of ROS and activation of NF-κB or ERKs.</td>
<td>[157, 162, 163]</td>
</tr>
<tr>
<td>HUVECs</td>
<td>ASP: Cell culture dose (1–50 µM)</td>
<td>Ameliorated hMGB1-induced septic responses.</td>
<td>[159]</td>
</tr>
<tr>
<td>3T3-L1 Adipocytes</td>
<td>ASP: Cell culture dose (10 µM) Extract: Cell culture dose (10 µg/mL)</td>
<td>Reversed palmitate-induced insulin resistance by repressing NF-κB, IRS1 and AMPK phosphorylation, and increased AKT activation; only the extract up-regulated GLUT4 protein expression.</td>
<td>[92]</td>
</tr>
<tr>
<td>In vitro xanthine oxidase inhibitory activity assay and mice</td>
<td>ASP and extract: Effective dose used (4.5 µg/mL)</td>
<td>Competitively inhibited xanthine oxidase. In hyperuricaemic mice, markedly suppressed increased plasma uric acid levels in a dose-dependent manner.</td>
<td>[153]</td>
</tr>
<tr>
<td>H9c2 cells, db/db mice and cardiomyocytes from rats</td>
<td>ASP: Cell culture dose (1 and 1000 µM) ASP: In vivo dose (13 and 130 mg/kg BW/day)</td>
<td>Improved diabetes associated cardiac deregulations, including enhanced glucose uptake, reversed impaired myocardial substrate metabolism, inhibited inflammation, lipid storage, oxidative stress, and cardiac remodelling. Reversed doxorubicin-induced cardiotoxicity by activating AMPK and reducing tumour protein p53 expression.</td>
<td>[88, 91, 102, 144, 145]</td>
</tr>
<tr>
<td>Rats</td>
<td>Extract: In vivo dose of 29.5 mg/kg BW/day</td>
<td>Lowered serum total cholesterol and iron levels, whilst increasing alkaline phosphatase enzyme activity and liver GSH levels.</td>
<td>[147, 151]</td>
</tr>
<tr>
<td>Diabetic nonhuman primates</td>
<td>Extract: In vivo dose (90 mg/kg BW)</td>
<td>Improved glucose tolerance, reduced total cholesterol, and LDL levels. Increased plasma coenzyme Q10 and decreased oxidative status.</td>
<td>[154]</td>
</tr>
</tbody>
</table>

ChREBP are transcriptional factors that regulate and control the expression of these enzymes involved in the lipogenic process [148]. However, this process can be repressed through AMPK phosphorylation [149]. Aspalathin activates AMPK and reduces the expression of hepatic enzymes and transcriptional regulators that are associated with either gluconeogenesis and/or lipogenesis [91, 92, 102, 139, 143, 144, 150]. These studies proved that aspalathin controlled the balance between anabolism and catabo-
lism through increased AMPK expression, while decreasing the expression of ACC, FAS, SCD1 and SREBP1 [91, 92, 143]. For example, Johnson et al. [91] showed that aspalathin protected vulnerable cardiomyocytes from diabetes-induced lipotoxicity by modulating adiponectin, ApoB, CD36, CPT1, PPARy, SREBP1/2, and SCD1, key regulators of lipid metabolism. Collectively, these results suggest that aspalathin acts on multiple targets associated with fatty acid synthesis and fatty acid oxidation, resulting in improved glucose and lipid metabolism.

**Effect of aspalathin on oxidative stress and inflammatory markers**

In addition to the strong antioxidant properties demonstrated by aspalathin in non-cell-based assays (Table 3), its capacity to enhance endogenous antioxidants and prevent oxidative stress was also shown in various experimental models (Table 6). Administration of an aspalathin-enriched green rooibos extract to non-diabetic Fischer rats for 28 days increased GSH reductase activity (an important enzyme in the maintenance of the reduced form of GSH), however, a longer treatment period (90 days) reduced the GSH content in the liver, suggesting an altered GSH redox cycle [147, 151]. Alternatively, Chen et al. [152] showed that a green rooibos extract improved the survival rate of Caenorhabditis elegans by reducing acute oxidative damage caused by the superoxide anion radical generator juglone. In RIN-5F pancreatic β-cells [143], aspalathin displayed an increased potential to prevent oxidative damage by suppressing ROS induced by advanced glycation end products. The robust antioxidant properties of aspalathin to reduce oxidative stress could be attributed to its inhibitory effect on xanthine oxidase, a known superoxide radical-producing enzyme [153]. Recently, Orlando et al. [154] showed that an aspalathin-rich green rooibos extract (12.8% aspalathin content) administered at 90 mg/kg three times daily with meals to high-fat fed diabetic vervet monkeys protected against LDL oxidation and preserved endogenous coenzyme Q10 levels. This supports outcomes of the study by Marnewick et al. [155], showing that the consumption of six cups of rooibos tea improved plasma lipid and oxidative stress levels in adults at risk for developing cardiovascular disease.

Recent studies from our laboratory have also reported on the potential of aspalathin to protect cardiac cells against oxidative stress-associated damage (Table 6). In cardiomyocytes isolated from diabetic rats, a fermented rooibos extract (0.36% aspalathin) prevented ROS-induced apoptosis by increasing intracellular GSH levels [161]. Furthermore, in cardiomyocytes exposed to a high glucose concentration, the capacity of aspalathin to prevent oxidative damage was associated with its modulatory effect on glucose and lipid metabolism, specifically by reducing abnormally increased FFA uptake and oxidation through the reduced phosphorylation of AMPK [102]. AMPK is a major energy regulator that plays a role in the reversal of peripheral insulin resistance through the modulation of β-oxidation [92, 142, 143]. This kinase increases β-oxidation and improves peripheral insulin sensitivity through phosphorylation and inactivation of ACC, a rate-limiting enzyme in fatty acid synthesis and subsequent β-oxidation. However, in the diabetic heart, an abnormal increase in fatty acid oxidation, as opposed to glucose oxidation, has been linked to reduced cardiac efficiency. In a recent study [102], we showed that in the diabetic heart, aspalathin modulates AMPK hyperactivation and improves glucose oxidation. This favourable shift in cardiac energy substrate, in favour of glucose oxidation, is believed to be important to protect a diabetic heart at risk of developing heart failure. The ameliorative effects of aspalathin were confirmed in the hearts of db/db mice and cardiomyocytes exposed to high glucose concentrations [88], showing that an increased expression of Nrf2, an essential transcriptional factor that is upregulated in response to oxidative stress and other stresses associated with the metabolic syndrome [89], plays a partial role in the protective effect of aspalathin. This study further showed that the upregulated expression of Nrf2 enhanced the endogenous antioxidant systems such as GSH and superoxide dismutase, as well as UCP2, resulting in improved cardiac ultrastructure. Although these findings need to be confirmed in other models, the results infer that aspalathin might be a useful therapeutic against endogenous oxidative stress and protect cardiovascular cells from diabetes-associated complications.

In addition to the ability of aspalathin to reduce oxidative stress, anti-inflammatory properties have also been demonstrated for this dihydrochalcone. Lipopolysaccharide is a known means of inducing vascular inflammation, both in *in vitro* and *in vivo* models. Aspalathin treatment suppressed lipopolysaccharide-induced membrane permeability and CAM in both human endothelial cells and in mice [162]. Furthermore, aspalathin ablated this effect by downregulating the expression of TNF-α, IL-6, and NF-κB. In a follow-up study, the authors demonstrated that 10–30 μM of aspalathin averted HMGB1-mediated vascular inflammation and hyperpermeability by inhibiting the expression of CAM in both *in vitro* (HUVECs) and *in vivo* (C57BL/6 mouse) model [158]. Although additional evidence is required to confirm these findings, a study by Ku et al. [157] showed aspalathin suppressed ROS as well as activated NF-κB and monocyte adhesion in both *in vitro* cell and *in vivo* mouse model. Elevated plasma levels of sEPCR have been found to increase vascular inflammation and subsequent thrombotic risk [159]. Kwak et al. [163] showed that aspalathin treatment inhibited phosphoryl 12-myrystate 13-acetate-induced TNF-α, IL-1β, and CLP-induced EPCR shedding by inhibiting the phosphorylation of several kinases known to increase thrombin generation. These results present strong evidence in support of aspalathin as a nutraceutical to protect against metabolic syndrome and associated complications such as glucose and lipid intolerance, as well as oxidative stress and inflammation that may result in accelerated cell injury. The ameliorative properties of aspalathin against glucose and lipid metabolic perturbations in various tissue targets are summarised in Fig. 7.

**Herb-drug interactions**

The increasing custom of the health conscious public to supplement their diets with natural products to enhance health and well-being is of concern due to possible herb-drug interactions in the growing population of patients on chronic medications [164]. Natural products are generally considered to be safe with little regard for potential adverse effects. Patients are drawn to using these products as adjunctive supplements to enhance the therapeutic efficacy of their medication, mostly without informing
their health practitioner. In most countries, natural products are sold over-the-counter or are freely available in supermarkets with sparse information about their health risk benefits, either as a monotherapy or in combination with other chronic medications. It is reasonable to assume that the concurrent use of aspalathin-based nutraceuticals with chronic blood glucose-lowering medication by type 2 diabetic patients will escalate. Although anecdotal evidence suggests that consumption of rooibos is generally regarded as safe, recently, two case studies have suggested the potential for herb-drug hepatotoxicity. In the first case [165], a 42-year-old woman treated for a low-grade B-cell malignancy with rituximab and maintained on prednisolone and co-trimoxazole daily, presented with elevated liver enzymes. She was advised to stop drinking rooibos flavoured with small amounts of strawberry, chamomile, and petals of daisy and discontinue her prophylactic antibiotic (co-trimoxazole). One week later, her liver enzymes returned to normal and she resumed her prophylactic co-trimoxazole treatment without further adverse effects. A second case...
study [166, 167] involved a 52-year-old hyperlipidaemic patient on atorvastatin who developed clinical symptoms of hepatotoxicity following increased consumption of a rooibos-buchu herbal tea. In the latter case, the presence of buchu, an indigenous South African medicinal plant shown to inhibit CYP3A4 [160], a major phase I metabolising enzyme of atorvastatin, confounded results. Although these two case studies do not conclusively infer causality, the sparsity of information relating to the potential of rooibos- and aspalathin-drug interactions prompted us to investigate their potential interaction with chronic medications such as statins or oral hypoglycaemic drugs. Using an in vitro recombinant CYP450 enzyme assay, both an aspalathin-rich green rooibos extract and aspalathin dose- and time-dependently inhibited CYP3A4, cautioning against the potential of a herb-drug interaction with hypoglycaemic drugs such as sulfonylureas and statins, including atorvastatin [168]. However, these findings still need to be confirmed by in vivo pharmacokinetic and pharmacodynamic studies.

Conclusions

The increase in metabolic syndrome, partially due to a sedentary lifestyle and poor diet, and consumers looking for “quick fix answers” create increasing opportunities for the development of natural products to manage clinical risks such as increased blood sugar and cholesterol. Such products need scientific scrutiny to understand the mechanisms of action and ensure safety with minimum adverse reactions. Research to date on aspalathin and aspalathin-rich green rooibos extracts has laid the foundation for their use as adjunctive therapeutics. Issues that need to be addressed are effective dose, safety, potential herb-drug interactions, and improved bioavailability of aspalathin.

Conflict of Interest

The authors declare no conflict of interest.

References

Muller CJF, Malherbe CJ, Chellan N, Yagasaki K, Miura Y, Joubert E. Potential of rooibos, its major C-glucosyl flavonoids and Z-2-(β-D-glucopyra-

Joubert E, Beelders T, De Beer D, Malherbe CJ, De Villiers A, Sigge GO. Variation in phenolic content and antioxidant activity of fermented rooi-


Bramati L, Minoggi M, Gardana C, Simonetti P, Mauri P, Pietta P. Quantitative characterization of flavonoid compounds in rooibos tea (Aspa-
lashus linearis) by LC-UV/DAD. J Agric Food Chem 2002; 50: 5513–5519


Beelders T, Sigge GO, Joubert E, De Beer D, De Villiers A. Kinetic optimi-
sation of the reversed phase liquid chromatographic separation of rooi-


Kazuno S, Yanagida M, Shindo N, Murayama K. Mass spectrometric identi-
fication and quantification of glycosyl flavonoids, including dihydro-


Koeppen B. The Ethyl Acetate soluble Polyphenols of Rooibos Tea. [MSc in Food Technology thesis]. Stellenbosch, South Africa: Stellenbosch University; 1959

Koeppen B. The flavonoid Constituents of Aspalathus excuminatus. [PhD in Food Technology thesis]. Stellenbosch, South Africa: Stellenbosch University; 1961

Koeppen BH, Roux DG. C-glycosylflavonoids. The chemistry of aspalat-

Rabe C, Steenkamp JA, Joubert E, Burger JFW, Ferreira D. Phenolic metab-
olites from rooibos tea (Aspalathus linearis). Phytochemistry 1994; 35: 1559–1565

Marais C, Van Rensburg WJ, Ferreira D, Steenkamp JA. (S)- and (R)-erio-
dictol-6-C-β-D-glucopyranoside, novel keys to the fermentation of rooi-

Krafczyk N, Clomb MA. Characterization of phenolic compounds in rooi-

Krafczyk N, Heinrich T, Porzel A, Glomb MA. Oxidation of the dihydro-


Joubert E, Viljoen M, De Beer D, Manley M. Effect of heat on aspalatin, iso-orientin, and orientin contents and color of fermented rooibos (Aspa-

Saclart B, Johnson WT, Saltmarsh M. Polyphenols: antioxidants and be-


Fraga GC, Galleano M, Verstraeten SV, Oteiza PI. Basic biochemical mechanisms behind the health benefits of polyphenols. Mol Aspects Med 2010; 31: 435–445

Joubert E, Winterton P, Britz TJ, Ferreira D. Superoxide anion and α,α-di-
phenyl-β-picrylhydrazyl radical scavenging capacity of rooibos (Aspa-
lashus linearis) aqueous extracts, crude phenolic fractions, tannin and fla-

Joubert E, Winterton P, Britz TJ, Gelderblom WCA. Antioxidant and pro-

Snijman PW, Joubert E, Ferreira D, Li XC, Ding Y, Green IR, Gelderblom WCA. Antioxidant activity of the dihydrochalcones aspalatin and no-

Van Gadow A, Joubert E, Hansmann CF. Comparison of the antioxidant activity of aspalatin with that of other plant phenols of rooibos tea (As-
palathus linearis), α-tocopherol, BHT, and BHA. J Agric Food Chem 1997; 45: 632–638


Magcwebeba T, Swart P, Swanewelder S, Joubert E, Gelderblom W. Anti-
flammatory effects of Aspalathus linearis and Cyclopia spp. extracts in a UVB/keratinocyte (HaCaT) model utilising interleukin-1α accumulation as biomarker. Molecules 2016; 21: E1323

Rezk BM, Van der Vijgh WJF, Bast A. The antioxidant activity of phloretin: the disclosure of a new antioxidant pharmacophore in flavonoids. Bio-
chem Biophys Res Commun 2002; 295: 9–13

Yepremyan A, Salehani B, Minehan TG. Concise total synthesis of aspalat-


Zhang T, Fang Z. The concise synthesis and biological evaluation of C-
glycosyl chalcone analogues inspired by the natural product aspalatin. RSC Adv 2017; 7: 3021–3024

Bungarang L, Gutmann A, Nidetzky B. Leloir glycosylation: Biocatalytic synthesis of the C-glucoside...
Johnson R et al. Aspalathin from Rooibos... Planta Med 2018; 84: 568–583