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Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia* spp.): From Bush Teas to Potential Therapy for Cardiovascular Disease

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Abstract

Cardiovascular disease (CVD) is a leading cause of worldwide deaths. A number of risk factors for cardiovascular disease as well as type 2 diabetes and stroke present as the metabolic syndrome. Metabolic risk factors include hypertension, abdominal obesity, dyslipidaemia and increased blood glucose levels and may also include risk factors such as vascular dysfunction, insulin resistance, low high density lipoprotein (HDL) cholesterol levels and inflammation. Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) are indigenous South African plants whose reported health benefits include anti-tumour, anti-inflammatory, anti-obesity, anti-oxidant, cardioprotective and anti-diabetic properties. The last two decades have seen worldwide interest and success for these plants, not only as health beverages but also as preservatives, flavourants and skincare products. This review will focus on the current literature supporting the function of these plants as nutraceuticals capable of potentially reducing the risk of cardiovascular disease.

Keywords: honeybush, rooibos, cardiovascular disease, diabetes, polyphenols

1. Introduction

Cardiovascular disease is the leading cause of deaths worldwide, killing 17.9 million people in 2016 [1]. While the number of cardiovascular disease related morbidity and mortality in the developed world has decreased or remained steady, the developing world has seen an increase. Limited resources, poverty, poor access to affordable healthcare, poor implementation of health policies, as well as poor education may be some of the reasons for the increase in cardiovascular diseases in low to middle income countries [2]. A number of risk factors for cardiovascular disease as well as type 2 diabetes and stroke present as metabolic syndrome. Metabolic risk factors include hypertension, abdominal obesity, dyslipidaemia, increased blood glucose levels, and may also include risk factors such as vascular dysfunction, insulin resistance, low levels of high density lipoprotein cholesterol (HDL-C) and inflammation. Natural products could play a significant role in drug discovery and development with examples including morphine, isolated from the opium poppy (*Papaver somniferum*) and artemisinin, from *Artemisia afra* [3–5]. Nutraceuticals are foods or supplements with health benefits [6]. To this end, a

number of nutraceuticals including fruits, vegetables, tea and herbal infusions have shown health benefits. Approximately 80% of the emerging world relies on herbal supplements [7]. This may often be a more accessible form of health or self-care, due to a lack of access to modern medicine, an alternative to modern medicine or due to the high cost of treatment of modern medicine. This practice may involve the use of herbs or plants, including polyphenol rich rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.), indigenous South African plant species with reported health benefits [8]. Many nutraceuticals contain polyphenols, the most abundant antioxidants in the diet which could help in the prevention of neurodegenerative diseases, diabetes, cancer, and cardiovascular disease [9]. Oxidative stress is a key process occurring in these diseases and is marked by imbalances between oxidants and the availability of antioxidants as well as perturbations in redox signalling mechanisms [10, 11]. Drugs used in the treatment of cardiovascular disease and obesity often have side effects, hence there is a need for better tolerated, safer and more natural treatment options [12]. A number of epidemiological studies and meta-analyses show some cardiovascular benefits with the intake of tea [13]. Furthermore, a review of some clinical studies show benefits of tea consumption in reducing cardiovascular risk factors, especially in overweight or obese subjects [14]. Rooibos and honeybush have a number of reported health properties, many of them targeting risk factors for the development of cardiovascular disease. The purpose of this paper was to review the role of rooibos and honeybush as potential nutraceuticals in the treatment of cardiovascular disease.

2. Risk factors for cardiovascular disease

A multitude of risk factors predispose to the onset of cardiovascular disease. This includes unmodifiable risk factors, such as increasing age, male gender, ethnicity, family history and genetics [15]. Modifiable risk factors include tobacco smoking, an unhealthy diet, a sedentary lifestyle, high alcohol intake, high blood pressure, being overweight or having central obesity, dyslipidaemia, impaired glucose tolerance or diabetes [15]. Diabetes not only quadrupled from 1980 to 2014 but approximately 57% of diabetic women and 67% of diabetic men are likely to present with cardiovascular disease by the age of 50 [1, 16]. Metabolic syndrome is largely preventable and includes a number of clinical findings which when occurring together, increase the risk of diabetes and cardiovascular disease. These include central obesity with any of the following risk factors including increased triglycerides, fasting plasma glucose, blood pressure and reduced HDL cholesterol levels [17]. Metabolic syndrome is also accompanied by changes in neuroendocrine and autonomic function [18]. It is known that early life stressors can predispose to disease outcome in later life, including cardiovascular disease [19]. Chronic stress influences cardiovascular outcome and anxiety and depression are also risk factors for cardiovascular disease [20–22]. This leads to changes in glucocorticoids and mineralocorticoids via modulation of the hypothalamic pituitary axis (HPA) [18]. Xenobiotics, including drugs and herbal infusions are metabolised by drug metabolising enzymes such as the phase I, cytochrome P450 system, which also influences the formation of steroid hormones [23]. CYP21A2 are precursors to both mineralocorticoids such as aldosterone and glucocorticoids such as cortisol and cortisone. Interestingly, rooibos flavonoids aspalathin and nothofagin inhibits CYP21A2 but not CYP11B1, which is responsible for converting 11-deoxycortisol to cortisol [24]. Substrate conversion of CYP117A1 and CYP21A2 was also inhibited by rooibos but other flavonoids such as rutin, orientin and vitexin were unable to inhibit CYP21A2. Rutin, under forskolin-induced stress, was the best inhibitor of steroid production followed by nothofagin

and vitexin and lastly by aspalathin and nothofagin [24]. The observed effects for steroid inhibition were attributed to structural differences in these rooibos flavonoids. Rooibos also decreased rat glucocorticoids by decreasing the corticosterone, deoxycorticosterone as well as the corticosterone: testosterone ratio [25]. In the accompanying human study the cortisol: corticosterone ratio was reduced by rooibos, which also inhibited 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1). This enzyme catalyses the conversion of cortisone to cortisol and is associated with risk factors for cardiovascular disease [26]. In a stress model using steroid producing H295R cells, rooibos and rutin were able to reduce cortisol levels. The inhibition of mineralocorticoid and glucocorticoid steroids by rooibos and the dihydrochalcones aspalathin and nothofagin were also demonstrated in H295R cells [27]. This suggests that rooibos may offer a possible therapeutic role in the management of cardiovascular complications relating from stress, by altering the biosynthesis of steroid hormones via the HPA axis. Inhibition of 11 β HSD1 has been suggested as a potential target mechanism for drugs to modulate the metabolic syndrome; therefore, this could be further explored in the context of rooibos or even honeybush. The cardiovascular complications of metabolic syndrome include coronary artery disease, peripheral vascular disease, hypertension as well as heart failure [28]. Primary management of metabolic syndrome is structured around lifestyle and dietary changes, including regular physical activity and a modest 5–10% initial reduction in caloric intake, failing which the use of pharmaceutical drugs may also be prescribed [17]. Approximately 1.9 billion people were overweight in 2016, of which 650 million people were obese [29]. A sedentary lifestyle, excess calories, a high fat diet and genetics contribute to the development of obesity, which is characterised by a body mass index greater (BMI) >30 kg/m² [30]. Urbanisation and a reduction in physical activity, along with an energy-rich Western diet, have contributed to the increase in cardiovascular disease in developing countries [31]. As a largely preventable disorder, obesity increases the risk of type 2 diabetes mellitus, cancer, cardiovascular disease, infertility, respiratory illnesses and a number of other health issues. In fact, obesity is not only a major independent risk factor but also an independent predictor for cardiovascular disease [32]. Strategies to avoid unnecessary deaths could include therapeutics that are safe, easily accessible and cost-effective. Rooibos and honeybush are relatively safe but long term clinical studies considering their safety are lacking [33, 34]. Two clinical case reports recommended caution in rooibos consumption but patients had consumed infusions containing rooibos and other herbs, thus the effects of these preparations and potential interactions between them need to be considered [35, 36]. Rooibos and honeybush are caffeine free and have low tannin levels making them ideal beverages for health conscious people, pregnant women and young children [37–39]. These plants may therefore be viable options in the future, provided sufficient evidence is generated to support their use as nutraceuticals capable of reducing cardiovascular risk.

3. Origin, distribution and markets for rooibos and honeybush

Rooibos [*Aspalathus linearis* L. (Burm.f.) R. Dahlgren (Leguminosae)] is a member of the fynbos biome, which contains needle-like leguminous plants. It occurs in the Cederberg area of the Western Cape, and in South Africa it is one of the most widely consumed herbal teas or tisanes. Its marketing potential was realized by Benjamin Gunzberg in 1904 and since then its popularity has steadily risen worldwide [40]. The top five export markets for rooibos are Germany, Japan, the Netherlands, the United Kingdom and the United States of America [41]. Rooibos is used as herbal infusion, health beverage, an ingredient in skin care products and cosmetics as well



Figure 1.
Honeybush plant in flower. Image courtesy of the SAHTA.

as a flavourant and colouring agent in a number of food applications. Honeybush (*Cyclopia* spp.), another member of the fynbos biome, is a bushy shrub found between the Piketberg area in the West, and Port Elizabeth in the East of South Africa (**Figure 1**). The year 1996 welcomed the first commercial harvests for honeybush, followed by the establishment of the South African Honeybush Tea Association (SAHTA) to manage the farming and sustainability practices as well as commercial interests of honeybush. After harvesting of rooibos or honeybush crops, leaves and stems are cut into small pieces, moistened and are fermented, either on open heaps or alternatively for honeybush also using an oven or fermentation tank. This is followed by drying of the fermented rooibos or honeybush. Fermentation of these plants is however associated with a change in phenolic composition as well as colour compared, to the green or unfermented plants which undergoes considerably less oxidation [42, 43]. The main contributors to the commercial market out of the 24 species of *Cyclopia* are *C. intermedia*, *C. genistoides* and *C. subternata*. *C. intermedia* has the largest market share; however, it is harvested from the wild, making the future sustainability and profitability of the crop problematic [44]. Honeybush is a budding commercial interest, used mainly as a tisane with great potential for development and is exported to countries such as the Netherlands, Germany and Japan [45].

4. Polyphenols that may be responsible for beneficial effects

Polyphenols are secondary plant metabolites commonly occurring in the diet in tea, coffee, wine, fruit, vegetables and cereals. The four main types of polyphenols, namely stilbenes, phenolic acids, flavonoids and lignins, can be classified according to the number of polyphenol rings and various chemical groups associated with the rings [46]. Flavonoids share a C3-C6-C3 backbone and the classification system includes groups such as the flavonols, flavones, isoflavones, flavanones, antho-cyanidins, and flavanols [46]. A number of studies have reported a reduction in the risk of cardiovascular disease with the intake of polyphenols [47]. The most prevalent polyphenols in rooibos include aspalathin, nothofagin, orientin, iso-orientin, vitexin and isovitexin, isoquercitrin and rutin [48, 49]. Aspalathin and aspalalinin are two unique dihydrochalcones in rooibos, with the former having been widely researched to date for its antioxidant and other health promoting properties [50, 51]. The flavonoid precursor in rooibos, Z-2-(β -D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG), has also received considerable attention for its anti-diabetic properties [52]. In *Cyclopia* species, the xanthenes mangiferin, isomangiferin and the flavanone hesperidin are predominant [43, 53]. Mangiferin is not unique to honeybush, and also occurs in mangoes (*Mangifera indica*) and plants such as *Pyrrosia sheareri* and *Anemarrhena asphodeloides* [54–56]. Bioavailability refers to the amount of the substance that is ingested that is available for metabolism [46]. It involves a number of processes, including intestinal absorption, plasma kinetics, metabolism, binding to plasma albumin and excretion

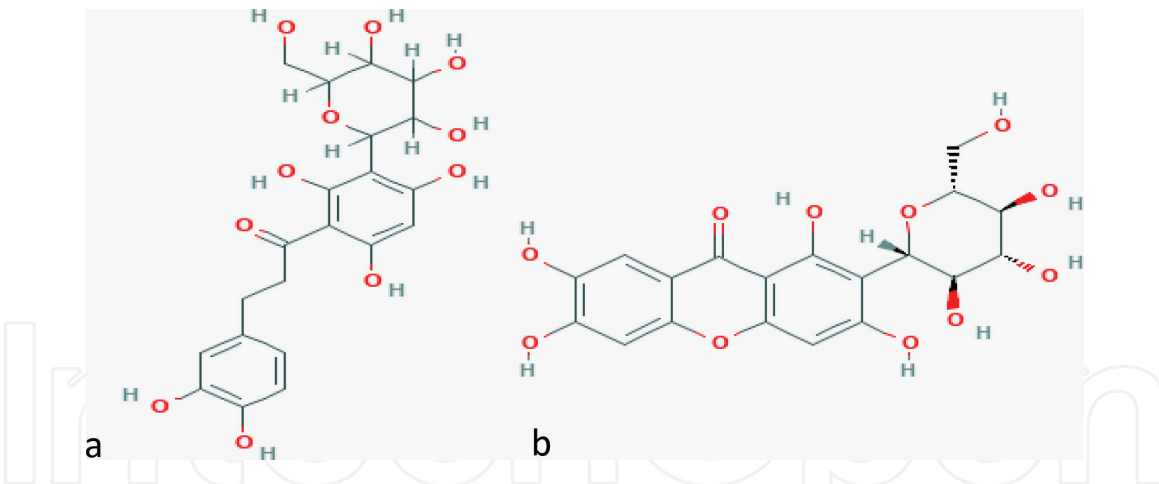


Figure 2.
Chemical structures of aspalathin (a) and mangiferin (b) showing phenol rings [62].

by the liver and kidneys [46]. Factors affecting bioavailability include isomeric form, processing methods, the type of compound and matrices surrounding the compound. The bioavailability of rooibos and honeybush is poor [57, 58]. The potential benefits attributed to their intake may therefore be hampered by poor bioavailability and also affect their maximal efficacy. Recently, the use of nanoencapsulation methods have been explored in order to increase the bioavailability and stability of aspalathin which could possibly promote the use of more effective nutraceuticals [59]. Since fermentation reduces polyphenol content, a number of studies have explored the benefits of unfermented, green rooibos or so called aspalathin rich extracts of rooibos in an attempt to elucidate the health promoting properties of the tisane [60, 61]. Others have looked at the effects of single isolate polyphenols from rooibos and honeybush such as aspalathin and mangiferin, to explore their functional benefits and identify a more targeted therapeutic option (**Figure 2**) [63, 64].

5. Antioxidant effects

Oxidative stress occurs due to an imbalance in the production of reactive oxygen species (ROS) and the availability of ROS scavengers. This may occur due to an excess of unstable reactive species including free radicals, such as reactive oxygen, reactive chlorine, reactive nitrogen and non-radical species, all of which may interact with cells, causing cellular damage [65]. Oxidative stress contributes to the pathobiology of cancer, cardiovascular disease, ageing, diabetes and atherosclerosis; therefore, it is plausible that dietary sources of antioxidants may be useful in reducing oxidative stress. Aerobic organisms are therefore reliant on innate antioxidant defences, e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH), uric acid, albumin and peroxiredoxins, to deal with large quantities of ROS in an attempt to reduce oxidative stress. Mitochondria are key generators of ROS in aerobic organisms, producing them as they generate energy; however, ROS are also important in cell signalling and released by macrophages to promote immunological attacks [66]. Xenobiotics, including rooibos and honeybush, are metabolised by the cytochrome P450 family and in the process, radicals are also produced and further detoxification processes facilitate their removal [67]. In cardiovascular disease, oxidative stress may manifest as dysfunction in the vascular endothelium or cardiac myocytes, and may arise due to increased intracellular Ca^{2+} as a result of ROS [68]. Oxidative stress associated with hyperglycaemia may occur from the glycation of proteins and the formation of advanced glycation end products, the auto-oxidation of glucose and the polyol pathway.

In rodent models of streptozotocin (STZ)-induced diabetes, rooibos exerted antioxidant effects through increases in the activity of superoxide dismutase, catalase, glutathione peroxidase and decreasing lipid peroxidation [64, 69, 70]. Rooibos also decreased advanced glycation end products but clinical markers of diabetes such as fructosamine, glycated haemoglobin and glucose were unaffected [70]. Nuclear factor erythroid 2-related factor 2 (Nrf2), a critical regulator of the antioxidant response of the cell, facilitates the removal of oxidants through increased antioxidant enzyme activity [67]. The regulator of Nrf2, Kelch-like ECH-associated protein 1 (Keap 1), has oxidative and electrophilic sensitive cysteine residues which upon activation allows for dissociation and activation of Nrf2 [71]. In H9c2 cardiomyocytes, aspalathin (1 μ M) increased the expression of Nrf2, and antioxidant genes and enzymes, including SOD, CAT, GPX and peroxiredoxins [64]. The expression of cytoprotective genes including heme oxygenase 1 (HO-1), NAD(P)H dehydrogenase (quinone 1), uncoupling protein 2 and apoptotic genes such as B-cell lymphoma 2 (Bcl-2) were also increased after aspalathin treatment. Uncoupling protein (UCP)3 and caspase 8, were however decreased. This suggests cellular survival due to reduced caspase 8, an important trigger for cell death [72]. UCP3 and UCP2 act in concert in the mitochondrial antioxidant response with UCP2 appearing to play a greater cytoprotective role in cardiomyocytes [73]. In the diabetic (db/db) mouse model, aspalathin increased Nrf2 expression as well as its downstream gene targets [74]. High dose aspalathin (130 mg/kg) also ameliorated the effects of hyperglycaemia in the heart by reducing the expected left ventricular enlargement. It could not however reduce fasting plasma glucose levels compared to metformin in these mice. The study confirmed the role of Nrf2 in the antioxidant response of the cell, strengthened the case for the potential use of nutraceuticals such as aspalathin in the treatment of diabetes, and also provides evidence for the differential effects observed with isolates compared to whole extracts of plants. Oxidative stress and inflammation co-exists in a number of diseases. The relationship between the two appears complex and is suggested to be a possible reason why antioxidant supplements in clinical trials have been unsuccessful [75]. In humans, no clear evidence of the antioxidant effects of flavonoids or pro-oxidant effects exist [76]. Supplementation of six cups of rooibos per day over a 6 week period in adults with increased cardiovascular risk increased plasma total polyphenols, reduced glutathione (GSH), and increased the GSH: oxidized glutathione (GSSG) ratio compared to the control period [77]. Furthermore, TBARS and conjugated dienes were reduced, indicating a reduction in oxidative stress after rooibos consumption. Ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate (ABTS) were unaffected. It is however known that assays for the measurement of antioxidant status can be difficult to compare and may also be non-specific and complicated by the instability of the species they are measuring [78]. Flavanoids and other polyphenols in honeybush are responsible for its antioxidant effects [79]. Fermentation of honeybush and rooibos however reduces antioxidant activity [80]. Mangiferin was the most effective scavenger of ABTS⁺ and in terms of its ability to reduce ferric ion, than the flavanone eriocitrin or the flavone luteolin [43]. This could be due to the hydrophilic nature of mangiferin, which is a glucoside. In an *in vitro* study using skin cells, aqueous extracts of *Cyclopia subternata* sp. exhibited the highest ABTS⁺ scavenging ability compared to other unfermented species of rooibos and *Camellia sinensis* [53]. In addition, rooibos, honeybush and Chinese green tea demonstrated ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) abilities. Rooibos and honeybush also had better ORAC values than green tea, while rooibos aqueous extracts had the highest FRAP. In another model using hairless SKH-1 mice, unfermented honeybush and mangiferin had the highest FRAP compared to the fermented honeybush and hesperidin, and also the highest total antioxidant capacity [63]. Fermented and unfermented

honeybush as well as mangiferin and hesperidin also protected against ultraviolet (UV) B-induced lipid peroxidation. Fermented and unfermented extracts of honeybush as well as hesperidin increased SOD and CAT activity, while mangiferin increased SOD but not catalase activity [63]. When Chinese green tea, rooibos and honeybush were evaluated for their ability to reduce lipid peroxidation, unfermented extracts generally offered better protection against lipid peroxidation but green tea offered the highest level of protection compared to the other infusions [53]. Mangiferin activated phosphatidylinositol 3-kinase (PI3K) induced protein kinase b (PKB/Akt) and Nrf2 signalling pathways, thus decreasing oxidative stress in an *in vivo* model using human kidney cells exposed to *tert*-butyl hydroperoxide [81]. The activities of SOD, CAT, GPX and the non-enzymatic intracellular antioxidant GSH were also enhanced and lipid peroxidation was decreased. When compared to vitamin C, ROS scavenging ability as measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and FRAP was decreased at lower concentrations compared to vitamin C, which was used as the positive control. The expression of Nrf2, HO-1, SOD, Akt and GSK- β and the mechanistic target of rapamycin (mTOR) and cyclin D were also increased by exposure to mangiferin. In an STZ-induced diabetic Wistar rat model, mangiferin improved antioxidant status and reduced apoptosis and inflammation [82]. This could be due to modulation of the AGE-RAGE/MAPK signalling pathways. Advanced glycation end products (AGEs), which are increased in diabetes, lead to upregulation and activation of the receptor for advanced glycation end products (RAGE). This interaction causes inflammation and oxidative stress, through increases in the production of ROS, leading to lipid peroxidation. AGEs can also inhibit peroxisome proliferator activated receptor (PPAR) γ , a regulator in inflammation as well as the metabolism of lipids and glucose [83]. In the study mangiferin also displayed potential as a therapeutic in preventing AGE mediated lipogenesis. Antioxidants such as β -carotene, α -tocopherol and ascorbate were unsuccessful in reducing incidences of cardiovascular [84] and other diseases such as cancer [85], in fact appearing to increase risk in some cases, e.g., with vitamin C, α -tocopherol and beta-carotene [86–88]. Antioxidant supplementation to alleviate oxidative stress could however be affected by other factors such as the dose of the antioxidant, timing of the intervention, interactions with other antioxidants, whether it is administered as an isolate or a whole extract, the type of extract, the model as well as the methods that are used to detect oxidative stress. Rooibos and honeybush exert antioxidant effects by scavenging free radicals, chelating metal ions, or upregulating indigenous antioxidant enzymes. The aromatic ring structures of polyphenols also contain free hydroxyl groups which contribute to their antioxidant ability. The structural differences between rooibos polyphenols may thus also explain differences in antioxidant activity of these compounds [89]. The antioxidant activity of rooibos and honeybush polyphenols may also be explained by their ability to increase the activity of antioxidant enzymes, through upregulation of Nrf2, PI3K and other signalling pathways involved in cell survival. It may also be explained by their ability to act as antioxidants themselves, exerting differential physiological effects. The antioxidant effects of rooibos and honeybush and their polyphenols have been extensively studied in detail elsewhere and some of these effects are discussed here under the anti-inflammatory, anti-obesity, anti-diabetic and cardiovascular effects of these nutraceuticals (Tables 1–4).

6. Anti-inflammatory effects

Inflammation and oxidative stress form a common thread in the metabolic syndrome, type 2 diabetes and cardiovascular disease [132, 133]. The rooibos flavonoid orientin from rooibos reduced the number of mast cells in colon sections as well as

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
<i>C. genistoides</i> ; <i>C. subternata</i> ; <i>C. maculata</i>	Mesenteric lymph node cells; murine splenocytes	Various up to 250 µg/ml	Modulated immune response; increased IFN-γ, IL-4, CD4 ⁺ CD25 ⁺ FOXP ₃ TREG cells; Total CD4 ⁺ cell ratio in mesenteric lymph node cells; increased IL-10 and IL-17a in splenocytes	Murakami et al., 2018 [90]
ASP; Noth	C57BL/6 mouse sepsis model	1 mg/kg BW	Decreased plasma blood urea nitrogen (BUN), creatinine, urine protein, LDH; inhibition of NF-κβ activation; decreased plasma nitric oxide (NO), TNF-α, IL-6, myeloperoxidase (MPO) and sepsis associated lethality; decreased oxidative stress by increasing kidney superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and decreasing lipid peroxidation	Yang et al., 2018 [91]
Orientin	1,2-dimethyl hydrazine stimulated colorectal cancer in Wistar rats	10 mg/kg BW	Decreased inflammatory mast cells, NFκ-B, TNFX, IL-6, iNOS and cyclooxygenase-2 (COX)-2	Thangaraj and Vaiyapuri, 2017 [92]
<i>C. intermedia</i> ; <i>C. subternata</i>	UVB/keratinocytes (HaCaT)	Various (0.09–0.1 mg/ml), aqueous extracts; 0.18–0.71/3 µg/ml, methanol extracts	Increased inhibition of cell viability, proliferation induced by UVB (aqueous <i>C. intermedia</i>); increased apoptosis, decreased intracellular interleukin (IL) 1-α (0.09–0.1 mg/ml); <i>C. subternata</i> (0.09–0.1 mg/ml) increased intracellular IL1-α and decreased extracellular IL-1α; methanol extracts alleviated reduction of cell growth parameters induced by UVB	Magcwebeba et al., 2016 [93]
Honeybush (fermented and scale up fermented honeybush extracts)	HaCaT human keratinocyte cells exposed to UVB irradiation	10–100 µg/ml; 200 µg/ml (cell viability)	Anti-inflammatory: Decreased IL-1β, IL-6, IL-8; decreased ERK, P38, metalloproteinases (MMPs) and C-Jun N-terminal kinase (JNK); increased SOD, CAT activities	Im et al., 2016 [94]
ASP; Noth	LPS-induced HUVECs; C57BL/6 mice	Various up to 30 µM <i>in vitro</i> ; ASP 27.1 µg/mouse, Noth 26.2 µg/mouse <i>in vivo</i>	Inhibition of LPS-induced barrier disruption; decreased expression of cell adhesion molecules (CAMs); decreased adhesion/transendothelial migration of leukocytes (HUVECs); decreased <i>in vivo</i> LPS-induced migration of leukocytes; decreased hyperpermeability; differentially decreased TNF-α, interleukin (IL)-6, NF-κB or ERK 1/2 by LPS; decreased LPS-induced lethal endotoxemia; increased antioxidant activity, decreased ROS; ASP inhibits effects on anti-inflammatory responses > Noth	Lee and Bae, 2015 [95]
<i>C. subternata</i> (scolymoside; vicenin-2)	HUVECs; C57BL/6 mice	Various up to 20 µM <i>in vitro</i> ; 23.8 µg/mouse	Decreased adhesion and migration to HUVECS by human neutrophils <i>in vitro</i> , <i>in vivo</i> ; decreased LPS release of transforming growth factor β-induced protein (TGFβ1p); decreased TGFβ1p mediated hyperpermeability; decreased TNF-α, IL-6, NF-κB, extracellular regulated kinases 1/2	Lee et al., 2015 [96]

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
<i>C. subternata</i> flavonoids: scolymsoside (SCL); vicenin-2 (VCN)	Human umbilical vein endothelial cells (HUVECs); C57BL/6 mice	VCN 11.9 µg/mouse; SCL 23.8 µg/mouse (±20 µmol/L)	Decreased vascular permeability, monocyte adhesion, CAM expression, NFκ-B expression and ROS induced by high glucose; increased expression of SOD, CAT	Ku and Bae, 2015 [97]
Unfermented rooibos (uf) + methanol extracts; ASP, Noth	Non-steroidogenic transfected COS-1 cells, H295R cells	4.3 mg/ml; ASP (10 µm); Noth (10 µm)	Decreased steroid production; decreased glucocorticoids during forskolin treatment; decreased aldosterone and cortisol precursors (variable effects between individual polyphenols and extract)	Schloms et al., 2012 [27]
Honeybush (fermented, green ethanol soluble extracts); mangiferin (Mangif); hesperidin (Hesp)	SKH-1 mice	30 mg/ml extract; 3 mg/ml (Hesp); 4 mg/ml (Mang); 100 µl applied to dorsal skin	Anti-inflammatory: Fermented and unfermented extract decreased oedema, epidermal hyperplasia cyclooxygenase-2 (COX-2), ornithine decarboxylase (ODC), GADD45 and OGG1/2 expression; fermented extract decreased lipid peroxidation by increasing superoxide dismutase, catalase; isolated compounds hesperidin and mangiferin less effective than whole extracts	Petrova et al., 2011 [63]
Rooibos	Whole blood cultures unstimulated or stimulated with endotoxins or phytohemagglutinin PHA	250–7.8 µg/ml	Increased IL-6, 10, IFNγ (unstimulated cells); increased IL-6, decreased IL-10 (stimulated cells)	Hendricks and Pool, 2010 [98]
Rooibos	LPS stimulated macrophages	0.5 µg/ml	Decreased IL-6, IL-10; increased COX2 > 25%	Mueller et al., 2010 [99]
Rooibos	Wistar rats (Dextran sodium sulphate (DSS) induced rat colitis model)	1.6 g/100 ml BW <i>ad libitum</i>	Increased SOD vs. DSS rats; decreased 8-hydroxy-2'-deoxyguanosine (8-OHdG) vs. controls	Baba et al., 2009 [100]
Rooibos	Murine splenocytes	1–100 µg/ml	Increased ovalbumin; increased sheep RBC antibody production; no effect on specific LPS stimulated antibody response; increased IL-2 in ova anti-CD ₃ primed splenocytes (10–100 µg/ml); decreased IL-4 in ova primed splenocytes; increased ova-induced antibody production in cyclosporine A rats; increased IL-2 in splenocytes	Kunishiro et al., 2001 [101]

Extracts are considered fermented unless otherwise indicated.

Table 1.
Anti-inflammatory and immune modulatory effects of rooibos and honeybush.

Plant species/ compound	Model	Dosage	Mechanistic effects	Author and year
Unfermented green rooibos extract (GRE)	Human C3A liver cells; obese insulin-resistant rats	10 µg/ml (cells); up to 195 mg/kg BW (6 cups), rats	Increased glucose and lipid metabolism; Increased glucose metabolism in C3A cells; increased insulin sensitivity in OBIR rats: increased GLUT2 expression; increased PI3K/Akt, phosphorylated AMPK and stimulation of insulin receptor substrate (IRS) 1, 2 forkhead box protein 01 (FOXO1) and carnitine palmitoyl transferase 1 (CPT1)	Mazibuko-Mbeje et al., 2019 [102]
ASP	Ventricular cardiomyocytes isolated from healthy, aged control and obese insulin resistant rats	10 µM	Increased insulin mediated glucose uptake in cardiomyocytes from young and aged rats, but not in high-caloric diet animals. Insulin actions enhanced via a PI3K-dependent mechanism	Smit et al., 2018 [103]
ASP; GRE	Palmitate-induced insulin resistant adipocytes	GRE (10 µg/ml); ASP (10 µM)	Increased GLUT4 expression (GRE); Both treatments: decreased lipid-mediated insulin resistance; decreased NFκβ, IRS1 (ser ³⁰⁷), phosphorylated AMPK (GRE); increased Akt phosphorylation; Only ASP increased peroxisome proliferator-activated receptor (PPAR) α, γ and CPT1	Mazibuko et al., 2015 [104]
Fermented rooibos	3T3-L1 adipocytes	10 µg/ml, 100 µg/ml	Decreased lipid accumulation; decreased adipogenesis; decreased PPARγ, α, sterol regulatory binding factor 1 (SREBF1), fatty acid synthase (FASN) expression impaired; leptin secretion decreased	Sanderson et al., 2014 [105]

Plant species/ compound	Model	Dosage	Mechanistic effects	Author and year
<i>Cyclopia maculata</i> (aqueous)	3T3-L1 adipocytes	60–100 µg/ml	Anti-obesogenic 60–100 µg/ml fermented extracts increased glycerol release; 80 µg/ml increased lipolysis maximally; increased expression of perilipin, hormone sensitive lipase (HSL); not cytotoxic (up to 100 µg/ml)	Pheiffer et al., 2013 [106]
<i>Cyclopia maculata</i> (fermented, fermented), <i>Cyclopia subternata</i> (unfermented)	3T3-L1 pre-adipocytes	Various up to 1600 µg/ml	Anti-obesogenic: decreased adipocyte differentiation; decreased intracellular triglycerides (> 100 µg/ml); decreased cellular ATP (<i>C. Maculata</i>) decreased PPARγ isoform 2; increased adiponectin (fermented <i>C.</i> <i>maculata</i>); increased leptin cytotoxic: 800 µg/ml (<i>C. maculata</i> <i>unfermented</i>), 1600 µg/ml (<i>C. maculata</i> + <i>C. subternata</i> , <i>unfermented</i>)	Dhudhia et al., 2013 [107]
Rooibos (not explicitly stated in paper but considered to be the fermented extract)	LDLr ^{-/-} mice; 3T3-L1 adipocytes	10 g/L (mice); 600 µg/ml (adipocytes)	Increased lipolysis; decreased serum cholesterol; triglycerides, free fatty acids; increased food consumption in mice fed normal chow; decreased body weight; altered adipocyte size, number; inhibition of dietary- induced steatosis; increased liver AMPK; decreased triglyceride accumulation; anti-adipogenic in 3T3-L1 adipocytes	Beltrán-Debón et al., 2011 [108]

Table 2.
 Anti-obesity effects of rooibos and honeybush.

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
ASP	H9c2 Cardiomyocytes; db/db mice	1 μ M (cardiomyocytes); (13 mg/kg BW)/130 mg/kg BW (mice)	Diabetic/cardioprotective/antioxidant; reduced high glucose induced oxidative stress; Nrf2-mediated activation of downstream antioxidant genes	Dludla et al., 2017 [64]
ASP	Glucose-exposed H9c2 cardiomyocytes	1 μ M	Cardioprotective, anti-diabetic, antioxidant effects: Enhanced metabolism of glucose, decreased phosphorylation of AMPK, decreased CPT1; increased GLUT4, acetyl-CoA carboxylase expression; increased glutathione, SOD; decreased ROS, increased ucp2, bcl-2: bax; Anti-apoptotic; decreased DNA nicks	Johnson et al., 2016 [109]
Fermented <i>Cyclopia intermedia</i> (methanol fermented and scaled up extracts)	HaCaT human keratinocyte cells	50–100 μ g/ml	Antioxidant: Increased SOD, CAT in UVB-exposed HaCaT keratinocytes	Im et al., 2016 [94]
<i>Cyclopia subternata</i> (aqueous extracts); mangiferin, isomangiferin	STZ Wistar rat model; C2C12 cells	30–600 mg/kg (<i>in vivo</i>); 1 nM–100 μ M (isolated compounds)	Increased glucose tolerance 30, 60, 120 min (600 mg/kg BW; mangiferin, isomangiferin increased glucose uptake in C2C12 cells	Schulze et al., 2016 [110]
ASP; Green rooibos extract (GRE)	3T3-L1 adipocytes	100 μ M; 10 μ g/ml 10 μ g/ml	Both isolates decreased palmitate-induced insulin resistance; decreased NF κ - β , IRS1 (Ser ³⁰⁷), phosphorylated AMPK; increased Akt activation; GRE increased GLUT4 expression; ASP increased PPAR α , γ , CPT1 expression	Mazibuko et al., 2015 [104]
Unfermented rooibos	L6 myotubules; RIN-5F Cells; obese diabetic KK-Ay mice	350 μ g/ml (L6 myotubules); 50 μ g/ml (RIN-5F cells); 0.3–0.6% (mice)	Increased glucose uptake; increased phosphorylated AMPK, Akt; increased GLUT4 translocation; decreased AGE-induced ROS increase; decreased fasting blood glucose	Kamakura et al., 2015 [111]
Rooibos; Aspalathin (ASP); Nothofagin (Noth)	HUVECs; C57BL/6 mice	5–50 μ M	Cardioprotective/anti-diabetic: Decreased vascular permeability caused by high blood glucose, decreased monocyte adhesion; Antioxidant: Decreased reactive oxygen species; Anti-inflammatory: Decreased NF κ - β	Ku et al., 2014 [112]

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
Fermented rooibos	H9c2 cardiomyocytes isolated from male Wistar STZ-induced diabetic rats (40 mg/kg BW); <i>in vivo</i> , <i>ex-vivo</i> rat heart perfusions	1, 10 µg/ml	Anti-diabetic/cardioprotective; decreased ROS, apoptosis; increased glutathione, metabolic activity <i>in vitro</i>	Dludla et al., 2014 [113]
Phenylpyruvic acid-2-O-β-D-glucoside (PPAG)	Obese mice	10 mg/kg	Protection from diet-induced hyperglycaemia; increased beta cell mass; decreased apoptosis; <i>In vitro</i> , protection of β-cells from palmitate-induced apoptosis; increased Bcl-2 expression; increased β-cell mass; protective effect of PPAG via increased expression of Bcl-2 in β-cells	Mathijs et al., 2014 [114]
Mangiferin	Diabetic insulin resistant Wistar rats	20 mg/kg BW	No change in body weight; decreased serum glucose; increased serum insulin; decreased HOMA IR; increased β-cell function; decreased serum TNFα; improved serum lipids; increased adiponectin; no effect on antioxidant activity	Saleh et al., 2014 [115]
<i>Cyclopia maculata</i> (unfermented); Mangiferin	RIN-5F cells	0.001–1000 µg/ml (<i>C. maculata</i>); 0.01–1000 µg/ml (mangiferin)	Increased viability (0.001–1000 µg/ml) <i>Cyclopia</i> ; no effect on viability (mangiferin)	Chellan et al., 2014 [116]
<i>Cyclopia maculata</i>	STZ-diabetic Wistar rats	30/300 mg/kg BW	Increased glucose tolerance; decreased fasting blood glucose; improved serum triglycerides; increased β-cell area; increased β-cell proliferation (300 mg/kg BW); decreased plasma nitrite; unaltered catalase, glutathione, liver lipid peroxidation and nitrotyrosine	
Z-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG)	Chang cells; Obese insulin-resistant rats	1–31.6 µM; 0.3–3 mg/kg BW (obese rats)	Increased glucose uptake; decreased fasting blood glucose; increased glucose tolerance; increased mRNA expression of liver GLUT1, 2, glucokinase, PPARγ and SOCS3	Muller et al., 2013 [52]

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
ASP	L6 myocytes; RIN-5F cells; type 2 diabetic ob/ob mice	25–100 μ M 0.1% ASP (100 mg/kg BW); 10 mg/kg BW ASP (intraperitoneal glucose tolerance test)	Increased glucose uptake; increased AMPK phosphorylation; increased GLUT4 translocation in L6 myoblasts and myotubules; decreased age-induced ROS; decreased blood glucose; increased glucose tolerance; decreased expression of liver gluconeogenic and lipogenic gene expression in mice	Son et al., 2013 [117]
Unfermented green rooibos; fermented rooibos	C2C12 skeletal muscle cells	10 μ g/ml	Increased glucose uptake; increased mitochondrial activity; increased ATP (unfermented > fermented); decreased PKC α activation; decreased palmitate-induced insulin resistance; increased insulin-dependent Akt activation, increased AMP Insulin-independent signalling pathways; increased GLUT4	Mazibuko et al., 2013 [118]
ASP; rutin	C2C12 myotubules	1,10,100 μ M; 100 μ m	Increased glucose uptake	Muller et al., 2012 [119]
Aspalathin; rutin	STZ diabetic rats	1:1 (1.4 mg/kg BW)	Decreased blood glucose (not obtained by individual compounds)	
Aspalathin rich green rooibos	STZ diabetic rats	25 mg/kg BW, 30 mg/kg BW	Decreased blood glucose; increased glucose tolerance	
<i>Cyclopia intermedia</i>	STZ diabetic induced Wistar rats	Acute: 5 mg/kg bw-50 mg/kg BW (STZ rats)	Decreased fasting blood glucose (50 mg/kg)	Muller et al., 2011 [120]
	Obese insulin resistant (OBIR) rats	Chronic: 538–2688 mg/ml (OBIR rats)	Decreased plasma cholesterol, fasting blood glucose; α : β cell mass (538–2150 mg/ml); decreased glucose tolerance (1075–2688 mg/ml)	
ASP from GRE	L6 myotubules	1–100 μ M	Increased glucose uptake	Kawano et al., 2009 [121]
	RIN-5F cells	100 μ m	Increased insulin secretion	
	db/db mice	0.1–0.2%	Decreased fasting blood glucose for 5 weeks; increased glucose tolerance 30, 60, 90, 120 min	
Fermented rooibos (aqueous + alkaline extracts)	STZ-induced diabetic Wistar rats	2.5% <i>ad libitum</i>	Decrease AGE's + MDA (plasma, lens, liver and kidney); decreased total cholesterol and creatinine	Uličná et al., 2006 [70]

Table 3.
Anti-diabetic effects of rooibos and honeybush.

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
Aspalathin	H9c2 cardiomyocytes; db/db mice	1 μm (cardiomyocytes) (13 mg/kg)/130 mg/kg	Diabetic/cardioprotective/antioxidant: reduced high glucose induced oxidative stress. Nrf2 activated downstream antioxidant genes; high dose aspalathin treatment was more successful than metformin or lower dose aspalathin at activating Nrf2 and antioxidant genes	Dludla et al., 2017 [64]
Aspalathin	H9c2 cardiomyocytes exposed to glucose	1 μM	Cardioprotective/anti-diabetic: Enhanced metabolism of glucose, decreased 172 phosphorylated AMPK, decreased carnitine palmitoyltransferase (CPT)1, increased GLUT4, acetyl-CoA carboxylase expression; antioxidant effects: increased glutathione and SOD, decreased ROS, increased UCP2, Bcl-2:Bax; anti-apoptotic; decreased DNA nicks	Johnson et al., 2016 [109]
Fermented rooibos	H9c2 cardiomyocytes isolated from male Wistar STZ diabetic rats (40 mg/kg BW)	1/10 $\mu\text{g/ml}$	Decreased ROS, apoptosis, increased glutathione, and metabolic activity <i>in vitro</i>	Dludla et al., 2014 [113]
Luteolin	STZ diabetic induced Wistar rats	10 $\mu\text{g/kg}$ BW	Decreased LDH, incidences of arrhythmias; decreased infarct size; increased left ventricular ejection fraction; decreased myocardial apoptosis; increased FGFR2, LIF expression; increased PI3K/Akt, Bcl-2 associated death promoter (BAD), Bcl-2:Bax ratio; inhibited MPO expression, IL-6, IL-1a and TNF α production (anti-inflammatory effects)	Sun et al., 2012 [122]
Luteolin	Ischemia/reperfusion (I/R) Wistar rats and isolated cardiomyocytes	Various up to 40 μM , model dependant	Luteolin pre-treatment before I/R injury increased contractions in isolated rat heart and cardiomyocytes; decreased LDH, apoptosis; increased Bcl-2: Bax ratio; decreased infarct size; protection involves ERK 1/2-PP1a-PLB-SERCA2a mechanism	Wu et al., 2013 [123]
Rooibos	Human volunteers	400 ml (0.025 g/ml)	Single dose inhibited ACE after 30, 60 min; ACE II genotype inhibited after 60 min; no effect on nitric oxide	Persson et al., 2010 [124]
Rooibos	HUVEC	0.05 g/ml in phosphate buffered saline (PBS)	Increased nitric oxide after 1 day (1:400, 1:200); no effect on ACE inhibition after 10 min	Persson et al., 2006 [125]
Rooibos	Human serum with Enalaprilat as positive control	0.05 g/ml in PBS	Inhibition of ACE via a mixed inhibition method	Persson et al., 2012 [126]

Plant species/compound	Model	Dosage	Mechanistic effects	Author and year
Rooibos	Human volunteers at risk for cardiovascular disease	0.005 g/ml (6 cups/day)	Increased antioxidant status and decreased oxidative stress by increased GSH: GSSG ratio, decreased lipid peroxidation (TBARS, conjugated dienes); increased HDL cholesterol	Marnewick et al., 2011 [77]
Orientin	Ischemia/reperfused Wistar rat hearts; cardiomyocytes injured by hypoxia/reoxygenation	0.5–2 mg/kg BW (rat hearts); 3–30 μ mol/l (cardiomyocytes)	Decreased apoptosis in cardiomyocytes; increased Bcl-2, decreased bcl-2-like protein 4 (Bax), increased Bcl-2: Bax; decreased cytochrome-c, caspase expression in cardiomyocytes and myocardium	Fu et al., 2006 [127]
Orientin	Ischemia/reperfusion injured H9c2 cardiomyocytes	30 μ M	Decreased apoptosis (decreased MPTP opening)	Lu et al., 2011 [128]
Rooibos (fermented and unfermented)	<i>Ex vivo</i> working heart from Wistar rats	2% w/v	Increased aortic output; decreased cleaved caspase 3, poly (ADP-ribose) polymerase (PARP), reduced apoptosis; increased GSH: GSSG ratio	Pantsi et al., 2011 [129]
Rooibos; chrysoeriol	Sprague Dawley rats (blood pressure effects); rabbit aorta, guinea pig atria	20% w/w, 10–100 mg/kg BW	Glibenclamide sensitive relaxation of low K^+ -induced contractions; weak inhibitory effect on atrial force and contraction rate; blood pressure lowering effects; chrysoeriol caused concentration-dependent, glibenclamide-sensitive relaxation of low K^+ induced contractions, EC50 (61 μ g/ml), n = 2 in aorta	Khan and Gilani, 2006 [130]
Rooibos and honeybush flavonoids (various including luteolin, quercetin, rutin, genestein, hesperitin, etc.)	<i>In vitro</i> ACE-inhibition assay	500 μ M, 100 μ M	ACE inhibitory activity of some flavonoids found in rooibos and honeybush. Luteolin had the greatest ACE inhibitory effects compared to the other flavonoids with hesperitin and genestein exhibiting moderate but lower, yet marked effects	Guerrero et al., 2012 [131]

Table 4.
Cardiovascular effects of rooibos and its flavonoids.

NFK- β , TNF- α , IL-6, iNOS and COX2, reflecting its anti-inflammatory potential [92]. Aspalathin and nothofagin inhibited LPS-mediated expression of the lipopolysaccharide (LPS) receptor (TLR4) and LPS-mediated barrier disruption. This occurred by increasing barrier integrity and inhibiting the expression of cell adhesion molecules (CAMs) and reducing neutrophil adhesion and migration in human umbilical vein endothelial cells [95]. The barrier protective effects of aspalathin and nothofagin were confirmed in a mouse model, in which the dihydrochalcones reduced LPS-induced mortality. This suggests that aspalathin and nothofagin can be regarded as potential therapeutics against vascular inflammation. The protective effects of aspalathin and nothofagin in a systemic inflammatory response induced by caecal ligation included decreases in inflammatory markers, including TNF α -expression NF- κ B, NO and IL-6 production, enhanced antioxidant activity and decreased lipid peroxidation. [91]. The anti-inflammatory effects of rooibos and honeybush may involve a reduction in inflammatory cytokines such as IL-6, TNF- α , COX2 [91, 92, 94]. It may also involve a reduction in neutrophil adhesion, and other mechanisms [96], **Table 1**.

7. Anti-obesity effects

Obesity and overweight are major risk factors for cardiovascular disease and type 2 diabetes. Insulin resistance, a key finding in the metabolic syndrome, is characterised by alterations in glucose uptake in insulin sensitive tissues such as the liver, skeletal muscle and adipose tissue [134]. Non-alcoholic fatty liver disease (NAFLD), characterised by fat accumulation in the liver, is an independent predictor of the metabolic syndrome which influences the progression of cardiovascular disease [135]. Since NAFLD is complex, utilising therapeutic strategies with multiple targets may be beneficial. The PI3K/Akt pathway is a complex insulin regulated pathway involved in glucose and lipid metabolism, as well as other cellular processes, including protein synthesis, cell signalling cell growth and apoptosis [136]. In type 2 diabetes and obesity, inhibition of the pathway interferes with beta cell function and insulin secretion, exacerbating insulin resistance. A green rooibos extract reduced lipid accumulation as well as lipolysis in C3A liver cells [102]. Rooibos also ameliorated palmitate-induced insulin resistance, by activating phosphorylated PKB/Akt and 5' adenosine monophosphate-activated protein kinase (AMPK), as well as increasing glucose transporter (GLUT)2 expression. Furthermore, fatty acid oxidation was enhanced by increasing FOXO1, decreasing malonyl-CoA decarboxylase, increasing carnitine palmitoyl transferase 1 (CPT1) and increasing acetyl CoA carboxylase in insulin deficient cells. In the accompanying obese insulin resistant rat model, only a high dose (195 mg/kg) of green rooibos extract (GRE) could reduce insulin levels and the HOMA-IR index. No significant differences were detected in blood glucose, body weights or food intake. Insulin receptor and insulin receptor substrates 1, and 2 were however upregulated by GRE, suggesting that GRE may be beneficial in ameliorating obesity-induced insulin resistance. In 3T-L1 adipocytes, palmitate-induced insulin resistance was ameliorated by both aspalathin, as well as GRE [104]. This was accompanied by increases in Akt activation and decreases in nuclear factor (NF)- κ B, IRS1 and AMP phosphorylation. GLUT4 expression was enhanced by the GRE but not aspalathin, suggesting that the whole extract rather than the isolated compound may be beneficial in providing a more multi-targeted therapeutic approach in improving palmitate-induced effects on glucose and lipids. A number of other rooibos polyphenols, such as the C-glycosidic flavones orientin, iso-orientin and luteolin, have anti-obesity effects, as reflected by their inhibition of pancreatic lipase, which is responsible for digesting and absorbing triglycerides [137]. Fermented rooibos also inhibited lipid accumulation

in 3T3-L1 adipocytes [105]. Z-2-(β -D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG), increases glucose uptake, as demonstrated in Chang cells [52]. In their *in vivo* model of obese insulin resistant rats, basal fasting glucose decreased and glucose tolerance was improved by PPAG. Increases in mRNA expression of genes involved in glucose (GLUT1 and GLUT4) and insulin metabolism (IR) and others such as PPAR α and SOCS3 in the liver were also seen. PPAR α regulates the action of fatty acids in the liver, suggesting that insulin signalling, glucose and lipid metabolism may be altered by PPAG. SOCS3 associates with various proteins to inhibit cytokine signals, e.g., leptin, growth hormone, IL-6, leukaemia inhibitory factor (LIF) as well as insulin [138]. It may therefore mediate leptin resistance, which occurs in obesity but is also regulated by leptin, which may partially explain these findings. Administration of rooibos to LDL receptor deficient (Ldlr $^{-/-}$) mice reduced liver steatosis and white adipose tissue and increased brown adipose tissue in high fat diet rats. Macrophage recruitment was decreased but no difference was seen in adipocyte size or number in the rooibos supplemented group. Furthermore, there appeared to be no liver toxicity, and free fatty acids, triglycerides and cholesterol were reduced by rooibos in the high fat diet group [108]. In humans, rooibos reduced oxidative stress, improved HDL-C, and triacylglycerol and low density lipoprotein cholesterol (LDL-C) in adults at risk of developing cardiovascular disease. [77]. These results suggest that rooibos and flavonoids in rooibos, such as aspalathin, may be potential adjuvants in the management of the metabolic syndrome. The anti-obesity effects of *Cyclopia intermedia*, *C. subternata* and *C. maculata* are limited but have been studied in 3T3-L1 adipocytes [106, 107, 139]. *C. maculata* and *C. subternata* decreased intracellular lipid and triglycerides and increased PPAR γ , a regulator of glucose and lipid metabolism [107]. Their anti-obesity effects also include increased release of glycerol [106]. Hormone sensitive lipase (HSL) expression, which is regulated by SIRT1a, a rate limiter of lipolysis, was also increased by fermented *C. maculata*. Furthermore, perilipin expression, which appears to play a key role in the metabolism of lipids and lipolysis of adipose tissue was upregulated [140]. *C. intermedia* increased HSL, SIRT1, UCP3 as well as PPAR γ expression in 3T3-L1 adipocytes [139]. The authors attributed increases in SIRT1 and PPAR γ to be indicative of changes from white to brown adipose tissue, however the expression of UCP1, which characterises brown adipose tissue was not measured in the study [141]. While the exact function is unclear, UCP3 may protect against lipotoxicity of the mitochondria [142]. In the parallel obese db/db mouse model, no increases in body weight gain were seen with *C. intermedia* treatment, and neither food nor water consumption was affected [139]. Extracts of mangiferin and other polyphenols isolated from plants containing components similar to that found in honeybush have proven useful in understanding their mechanisms of action and how they could be used as nutraceuticals in the treatment of cardiovascular disease. In a double-blind randomised placebo controlled clinical trial, mangiferin (150 mg/day) from mangoes, was given to 97 overweight, hyperlipidaemic patients over a period of 12 weeks. Increased high density lipoprotein cholesterol (HDL-C), L-carnitine, as well as decreases in total cholesterol, low density lipoprotein cholesterol (LDL-C) and triglycerides were obtained in the patients [143]. No alterations in liver and kidney function markers were seen, suggesting that chronic treatment over 12 weeks was safe in human participants. Since the metabolic syndrome is so complex, providing a multi-targeted approach, such as through affecting the PI3K/Akt pathway may be somewhat beneficial. The anti-obesity effects are further described in **Table 2**. The limited available information on the anti-obesity effects of rooibos and honeybush however, suggests that more research is needed to fully understand and enable the translation of these effects from animals to man.

8. Anti-diabetic effects

Diabetic hyperglycaemia involves macro- and microvascular complications [144]. Free radicals are also produced, promoting oxidative stress [145]. Evidence suggests that rooibos and its polyphenols may reduce oxidative stress associated with the pathogenesis of diabetes [34, 69]. A meta-analysis and systematic review narrowed down to 12 peer-reviewed studies reported a reduction in blood glucose levels in diabetic rodent models that had received either rooibos or its polyphenols, while no clinical trials have investigated the effects of rooibos in the context of type 2 diabetes [146]. Rooibos decreases fasting blood glucose and improves glucose tolerance [117]. The anti-diabetic actions may be related to the expression of genes responsible for glucose uptake (GLUT1, GLUT2), insulin signalling (IR), as well as other genes such as PPAR α in the liver in a model of obese insulin resistant rats treated with PPAG [52]. The involvement of insulin dependent GLUT4, in skeletal muscle has also been reported [111]. Rooibos may be exerting anti-diabetic effects by affecting the metabolism and uptake of glucose. Aspalathin influences key genes involved in the metabolism of lipids, insulin resistance, inflammation and apoptosis, possibly reversing metabolic abnormalities by involving PPAR γ , Adipoq, IL-6/Jak2 pathway and Bcl-2 [74]. PPAG increased beta cell mass and delayed hyperglycaemia in STZ-diabetic mice [147]. This was accompanied by increases in anti-apoptotic B-cell lymphoma 2 (Bcl-2), but no antioxidant effect, and in human islet cells PPAG also decreased cell death. This suggests that PPAG increases beta cell mass by decreasing apoptosis and increasing Bcl-2 as reflected in both STZ diabetic and obese mouse models [114, 148]. In the absence of insulin, GRE also increased phosphorylation of both AMPK and Akt, in L6 skeletal myotubules. Activation of AMPK could be a therapeutic strategy in the treatment of obese and type 2 diabetics, as animal studies suggest a dysregulation of AMPK in these states. The mechanism for the amelioration of diabetic complications in rodents by rooibos and honeybush may also be due α -glucosidase inhibition; or SGLT2 inhibiting potential of rooibos and honeybush or their respective flavonoids [119, 149–151]. α -Glucosidase is an enzyme present on the intestinal brush border where it digests starch, increasing blood glucose [152]. Anti-diabetic drugs, therefore commonly utilize strategies such as α -glucosidase inhibition or SGLT2 inhibition to facilitate their actions [152, 153]. The dihydrochalcone phlorizin, an SGLT2 inhibitor, provides a basis to explore natural plant based agents for use in the management of diabetes [154]. Strategies to target SGLT2 rather than SGLT1 is also associated with reduced drug toxicity as found in studies of phlorizin and anti-diabetic drugs [153]. In an STZ-diabetic model, unfermented extracts of *Cyclopia maculata* improved glucose tolerance, fasting glucose, β -cell area, triglyceride levels as well as the insulin: glucagon ratio. Plasma nitrite was reduced but no alterations were seen in other markers of nitrotyrosine and lipid peroxidation or the serum antioxidant enzymes [155]. Mangiferin and naringenin from *Salacia oblonga* also reduced blood glucose, normalised AST and ALT levels, improved antioxidant status and decreased protein carbonyl levels, glycogen and TBARS in the liver. Beta cell damage in the pancreas was also ameliorated by the compounds. It was proposed that activation of PPAR γ and GLUT4, which was also accompanied by insulin sensitisation, may be partially responsible for the anti-diabetic effects [156]. Since naringenin is also found in honeybush, it suggests that extracts from honeybush could possibly also produce similar effects. Mangiferin from *Anemarrhena asphodeloides* Bunge reduced blood glucose levels and ameliorated hyperinsulinemia, the anti-diabetic properties possibly due to a reduction in insulin resistance in these rats [157]. Furthermore, mangiferin and naringenin isolated from *Salacia oblonga* reduced blood glucose levels, and increased GLUT4 and PPAR γ expression, suggesting increased insulin sensitisation and demonstrating the anti-diabetic properties of the polyphenols. In conclusion, the anti-diabetic effects of

rooibos and honeybush (**Table 3**) are mediated by complex signalling pathways that affect glucose and lipid metabolism as well as oxidative stress.

9. Cardiovascular effects

Angiotensin converting enzyme (ACE) inhibitors reduce blood pressure by inhibiting the conversion of angiotensin to angiotensin II, the latter being a potent vasoconstrictor which increases blood pressure through a variety of mechanisms [158]. Angiotensin II can also interact with the angiotensin I receptor, enhancing ROS production, which contributes to endothelial dysfunction by inactivating vasodilatory nitric oxide [158]. Endothelial dysfunction is commonly associated with risk factors of cardiovascular disease, including obesity and type 2 diabetes [159]. It also precedes atherosclerosis, which involves a number of complex processes such as the release of pro-inflammatory cytokines and inflammation, oxidation of LDL as well as macrophage recruitment and platelet adhesion [158]. Cardiovascular protective effects of rooibos have also been established in a number of studies (**Table 4**). In an *in vitro* human umbilical vein endothelial cell (HUVEC) study, rooibos could not inhibit ACE but increases in nitric oxide were detected [125]. ACE-inhibition was also determined in 17 healthy volunteers receiving a single oral dose of rooibos [124]. Inhibition of ACE occurred up to 60 min after ingestion but no change in blood pressure or nitric oxide was detected. ACE-inhibitory effects have also been reported for rooibos and honeybush and some of their flavonoids [131]. This effect on ACE could be due to the presence of the C2=C3 double bond, the catechol group at the B-ring and the ketone group on C4 on the C-ring of flavonoids [131]. The best ACE-inhibitor in HUVEC's was luteolin, followed by quercetin, rutin, kaempferol, rhoifolin and apigenin K. The honeybush flavonoids genistein and hesperetin also displayed ACE-inhibitory effects but these were less potent than luteolin. Rooibos probably inhibits ACE using a mixed type of inhibition, which binds to the enzyme's active and allosteric sites [126]. Organic fractions of, as well as flavonoids in rooibos such as chrysoeriol and rutin inhibited spontaneous and low and/or high K⁺-induced contractions in models utilising jejunum and bronchial smooth muscle [130, 160]. Anti-contraction effects were reportedly due to the opening of K⁺ channels as well as a Ca²⁺ antagonistic action. These studies were not done in vascular smooth muscle, but it is possible that rooibos could exert antihypertensive effects via similar mechanisms in vascular smooth muscle. It also caused mild reduction of atrial force and rate of spontaneous contractions in the Guinea pig and decreased blood pressure in anaesthetised rats [130]. In comparison, isolated compounds of rooibos tested in jejunal, tracheal and aortic preparations showed variable effects. In adults with cardiovascular risk factors, six cups of rooibos was able to increase antioxidant status by increasing the GSH: GSSG ratio and decrease lipid peroxidation by decreasing TBARS and conjugated dienes [77]. Furthermore, increases in the healthy HDL cholesterol were also seen. In a recent study using a high fat diet to induce obesity in Wistar rats, treatment with an enriched GRE, containing large quantities of aspalathin, improved glucose tolerance, vascular function, as determined by aortic vascular contraction-relaxation studies and enhanced the antioxidant status. Modest improvements in blood pressure were also seen in a recent study in obese rats, suggesting cardiovascular benefits with the intake of the rooibos [161]. Fermented rooibos also improved vascular function in nicotine exposed rats [162]. Since tobacco smoking is a major risk factor for cardiovascular disease, this suggests a possible therapeutic role for rooibos in the reduction of cardiovascular complications associated with tobacco smoking, such as vascular dysfunction or oxidative stress. Cardioprotective effects of rooibos and its flavonoids typically appear to involve a reduction in lipid peroxidation and upregulation of the antioxidant enzymes through Nrf2 activation, and a reduction in apoptosis which may also be associated with

increases in the Bcl-2: Bax ratio [77, 122, 129]. The anti-inflammatory effects of rooibos, also support its role as a cardioprotective agent (**Table 1**). No literature investigating the vascular modulating effects of honeybush are currently available and anti-inflammatory effects on HUVECs are discussed above. Decreases in VLDL, LDL-C and increases in HDL-C by naringenin and mangiferin from *Salacia* sp. however suggests the potential of similar isolates from honeybush to ameliorate endothelial dysfunction [156].

10. Conclusions and perspectives

Rooibos and honeybush have a number of health benefits. The evidence for their use as functional nutraceuticals is reflected in anti-diabetic, anti-inflammatory, antioxidant, anti-obesity, and vascular effects in animal and *in vitro* studies. The translation of these results into human medical care has however been limited. The inability to translate animal studies to that of humans may be due to the result of the controlled genetic background and environmental conditions of animals used as models to study disease [163]. Given the unstable nature of ROS and the limitations in assays used to detect oxidative stress, it is noteworthy to remember that intervention studies may only as good as the biomarkers used to measure the intervention. As we investigate the therapeutic role of rooibos or honeybush in the treatment of cardiovascular disease, it is evident that we are only but scratching the surface. These plants have complex chemical compositions and while isolating single components could be beneficial, the interplay and additive effects of other components in whole extracts could be more beneficial [111]. The mechanisms behind these benefits involve numerous signalling pathways that regulate their effects. Understanding these complexities may be what is needed to promote their transition from bush teas to nutraceutical therapy against cardiovascular disease but until then we may as well enjoy a cuppa.

Conflict of interest

There is no conflict of interest to disclose.

Thanks


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