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Minireview

Aspalathin a unique phytochemical from the South African rooibos plant (*Aspalathus linearis*): A mini Review

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ABSTRACT

Aspalathus linearis (rooibos) is a plant which grows in a limited habitat in South Africa. The plant is mainly renowned for the beverage (herbal tea) which is made from its aerial parts. The popularity of the herbal tea is not confined to South Africa as significant amounts of the tea are exported to many countries worldwide. Rooibos reportedly has several health benefits which have been attributed to its constituent phytochemicals. One of the major phytochemicals in rooibos is aspalathin. Aspalathin makes up between 4-12% of the plant. Aspalathin is a dihydrochalcone glycoside which has thus far only been isolated from *Aspalathus linearis*. Aspalathin has been shown to possess biological activity which imparts it with multiple health beneficial effects. This mini review highlights the recent findings on the biological properties of aspalathin. These include antioxidant, antidiabetic, cardioprotective, antihypertensive and antimutagenic effects. Given its multiplicity of biological effects, aspalathin is a natural phytochemical which has potential to be incorporated into current medical therapeutic regimes in light of recent preferences for the use of natural medicines.

INTRODUCTION

Aspalathus linearis

The legume *Aspalathus linearis* is confined to the north-western to western region of the Fynbos biome in the Cape Floristic Region of South Africa (Hawkins *et al*., 2011; Lötter and le Maitre, 2014). 'Rooibos' is a term used when making reference to the plant or to the herbal beverage (tea) made from the plant (Hawkins, Malgas and Biénabe, 2011). Whilst there is limited harvesting of wild uncultivated rooibos, it is also cultivated and grown commercially. Hawkins et al., (2011) have described in detail the ecotypes and ecology of the plant. Apart from the beverage which is made from rooibos, it has found use in several other products such as soaps, cosmetics and skin lotions (Chuarienthong *et al*., 2010).

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There are several reports on the health benefits of rooibos. The earliest reports of its use are from the late 1700s when the local Khoi-Khoi people were observed using the plant medicinally (Gadow *et al.*, 1997). Subsequent research has confirmed the health benefits of rooibos. It has been shown to have antidiabetic and hypoglycaemic effects (Jin *et al.*, 2013; Kamakura *et al*., 2015; Van Der Merwe *et al.*, 2015; Mahmood *et al.*, 2016), antioxidant (Canda *et al*., 2014) as well as anti-HIV effects *in vitro* (Nakano *et al.*, 1997). In addition, rooibos also has demonstrated anti-inflammatory effects (Baba *et al.*, 2009), it has been shown to reduce colitis and modulate immune function *in vitro* (Hendricks and Pool, 2010) as well as *in vivo* where it has been shown to promote antigen-specific antibody production through augmentation of interleukin-2 production (Kunishiro *et al*., 2001). The bronchodilatory effects of rooibos have been attributed to the phytochemical chrysoeriol which also has antispasmodic, antiviral and antimicrobial effects (Khan and Gilani, 2006). The chemoprotective effects of rooibos have been demonstrated in rat liver using the cancer initiator diethylnitrosamine (Marnewick *et al.*, 2009). Rooibos is further reported to have anticarcinogenic and antiallergic activities (Standley *et*

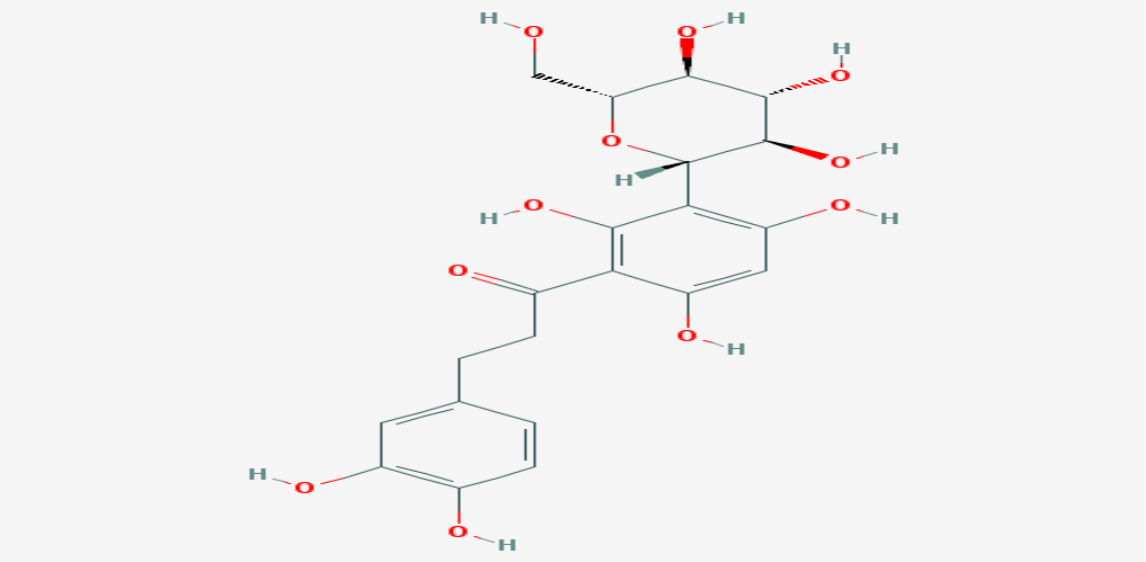

Fig.1. Chemical structure of aspalathin.
Source: Pubchem. Weblink https://pubchem.ncbi.nlm.nih.gov/compound/11282394#section=Top

al., 2001; Marnewick, 2010). The multiplicity of effects of rooibos is attributed to its constituent phytochemicals.

Phytochemicals in rooibos

The plant contains several biologically active phytochemicals which include polyphenols and flavonoids (McKay and Blumberg, 2007). Phytochemicals isolated from rooibos include isoorientin, orientin, chryoseriol, isovitexin, nothofagin, rutin, isoquercetin and hyperoside (McKay and Blumberg, 2007). There are several recent studies which provide further detail on the phytochemicals in rooibos (Ligor *et al.*, 2008; Breiter *et al.*, 2011; Joubert and de Beer, 2011). It is important to note that processing the rooibos eg fermentation significantly reduces the content of some of the phytochemicals including aspalathin. It has been reported that fermentation oxidizes over 90% of the aspalathin mainly to dihydro-iso-orientin (Perold, 2009). Unfermented rooibos is called green rooibos whilst the fermented form is called red rooibos.

There are numerous studies on the health benefits of crude and purified extracts of rooibos however the focus of this review will be specifically on aspalathin, a flavonoid which is uniquely found in rooibos (Van Der Merwe *et al.*, 2015). Aspalathin constitutes about 4-12% of the dry rooibos plant material (Gadow *et al.*, 1997; Kreuz *et al.*, 2008).

Aspalathin

Structurally aspalathin is a C-linked dihydrochalcone glycoside. The molecular formula for aspalathin is $C_{21}H_{24}O_{11}$. The biochemical structure is shown in figure 1 above.

Gastrointestinal and skin absorption of aspalathin

A study using pigs, showed that aspalathin was absorbed as a C-glycoside (Kreuz *et al.*, 2008). Liquid chromatography-mass spectrometry identified in urine, metabolites of aspalathin which were "methylated aspalathin, glucuronidated and methylated aspalathin, a glucuronidated aglycone of aspalathin, as well as a metabolite of eriodictyol" (Kreuz *et al.*, 2008).

In vitro studies with intestinal epithelial Caco-2 (human epithelial colorectal adenocarcinoma) cells showed that absorption was dose dependant (Huang *et al.*, 2008). However, percutaneous studies using human abdominal skin cells showed that less than 0.01% of the initial dose was transported across the skin (Huang *et al.*, 2008). Thus the cutaneous absorption is significantly lower than absorption from the gastrointestinal tract.

Biological activity of aspalathin

Antidiabetic effects

Aspalathin has shown potential for use as an antidiabetic agent due to its glucose lowering effect (Han *et al.*, 2014). Aspalathin from green rooibos tea was found to prevent postprandial hyperglycaemia by suppressing glucose absorption and inhibiting carbohydrate hydrolyzing enzymes (Mikami *et al.*, 2015). When KK-A^{y} type 2 diabetic mice were fed with aspalathin rich green rooibos extract for five weeks, it suppressed increases in plasma glucose (Kamakura *et al.*, 2015). An *in vitro* study by the same investigators also showed green rooibos to increase uptake of glucose and induce phosphorylation of 5^{l} adenosine monophosphate protein kinase (AMPK) in L6 myotubes (Kamakura *et al.*, 2015). In mice with impaired glucose tolerance, aspalathin improved

glucose tolerance (Kawano *et al.*, 2009). Aspalathin was further shown to reduce hyperglycaemia induced vascular inflammation in rats by reducing hyperpermeability and expression of cell adhesion molecules (Ku *et al.*, 2014). In the same study aspalathin was noted to decrease activation of nuclear factor (NF)-κB *in vivo* (Ku *et al.*, 2014).

Antioxidant effects

Aspalathin showed high antioxidant capacity when it was compared with other flavonoids in rooibos using ABTS [2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] radical cation, metal chelating and Fe (II)-induced microsomal lipid peroxidation assays (Snijman *et al.*, 2009). When rooibos was administered to rats *ad libitum*, it suppressed the accumulation of lipid peroxides in the brain, which is usually associated with ageing (Inanami *et al.*, 1995). Rooibos tea also partially prevented oxidative stress in streptozocin-induced diabetic rats (Ulicna *et al*., 2006). Recently aspalathin rich tea was shown to decrease oxidative stress induced by immobilization of rats. Several mechanisms were proposed including the restoration of stress induced protein degradation, regulation of glutathione and modulation of superoxide dismutase and catalase, both of which are antioxidant enzymes (Hong *et al*., 2014).

However, a study in which aspalathin enriched green roobios extracts were fed to rats for up to 90 days showed that blood monitoring in the assessment of biosafety of phytochemicals is not sensitive and specific and hence there is need to use molecular techniques e.g. Quantitative Real Time polymerase chain reaction analysis, to investigate gene expression and the activity of regulatory proteins (Van Der Merwe *et al.*, 2015). By these means the authors observed that the aspalathin enriched green rooibos extracts caused some oxidative stress and possibly biliary dysfunction (Van Der Merwe *et al.*, 2015). The implications of this finding need further investigation.

Antihypertensive effects

In vitro, aspalathin-rich rooibos tea caused a significant increase in the production of nitric oxide (Persson *et al*., 2006) in human endothelial cells; however, compared to green tea (*Camillia sinensis*) it was shown to not have any effect on angiotensin converting enzyme (ACE) *in vitro*, a finding which was attributed to it lacking catechins (Persson *et al*., 2006). Interestingly *in vivo*, in a randomized three-phase cross over study, rooibos tea was shown to have a 6% inhibitory activity (vs 16% for chronic enalpril) of angiotensin converting enzyme in healthy volunteers (Persson *et al*., 2010). The findings may be related to the impact of NO on ACE. Further noteworthy findings from the study were that there were differences in responses to the interventions based on ACE genotype whereby individuals with genotypes II and ID showed a significant inhibition of ACE activity following the drinking of rooibos tea with aspalathin, whereas those with ACE genotype DD were less responsive (Persson *et al*., 2010). Thus it is important to note that genotypes may play a role in responses to prophylactic and therapeutic interventions and hence stresses the importance of the need for greater use of personalized medicine which takes individual variability into account in the provision of treatments (Collins and Varmus, 2015).

Anti-obesity effects

Aspalathin from rooibos showed potential as a weight loss inductive agent with associated reduction in food intake (Mahmood *et al*., 2016). Whilst boiled fermented rooibos tea was shown to decrease leptin secretion, inhibit adipogenesis and alter the metabolism of adipocytes *in vitro*, the phytochemical profile showed the extracts to contain mainly isoorientin, orientin, quercetin-3-O-robinobioside and enolic phenylpyruvic acid-2-O-β-d-glucoside (Sanderson *et al.*, 2014). Thus due to processing (fermentation) it is likely that aspalathin was oxidized as described in an earlier section of this review and thus unlikely to have contributed to the anti-obesity effects noted.

Cardio-protective effects

Asapalathin has been shown to protect isolated cardiomyocytes from hyperglycaemia-induced metabolic substrate shifts and apopotosis (Dludla *et al.*, 2017). The possible mechanisms were elucidated using an H9c2 cardiomyocyte model (Johnson *et al.*, 2016). Aspalathin modulated several key lipid metabolism regulators and mechanistically it activated Adipoq while modulating the expression of the glitazone receptor peroxisome proliferator-activated receptor gamma (PPARG and Srebf1/2. Inflammation was decreased through the proinflammatory IL-6 cytokine and Jak2 signaling pathway. In addition, the expression of Bcl2 (aregulator proteins for cell death) was increased thus preventing apoptosis of the myocardium (Johnson *et al.*, 2016).

Hypouricaemic effects

The hypouricaemic activity of aspalathin-rich fraction and purified aspalathin from rooibos on mice was investigated. These polyphenols significantly suppressed increased plasma uric acid concentration in a dose dependent manner (Kondo *et al.*, 2013).

Antimutagenic effects

The antimutagenic effects of rooibos have been explored and demonstrated in murine experimental models. Using a *Salmonella typhimurium* mutagenicity assay, it was shown that aspalathin showed mild

antimutagenic activity (Snijman *et al.*, 2007). Topical application of aspalathin rich green rooibos tea extracts significantly inhibited tumorigenesis in ICR mice (Marnewick *et al.*, 2005). Further investigations using other tumours are necessary.

CONCLUSION

Rooibos is an important plant in the economy of South Africa. Given the popularity of rooibos as a herbal tea, and the increasing use of rooibos extracts in cosmetics, it is important that more research be undertaken on its long term effects. Aspalathin which is one of the major phytochemicals in rooibos has been shown to have multiple health benefits and impacts several organs. Given its multiple targets, there is need to also explore the potential adverse effects of aspalathin.

The developmental origins of health and disease have now been well established wherein interventions and events in early life (conception, gestation and neonatal periods) can impact health outcomes later in life (Gillman, 2005). Phytochemicals are increasingly gaining prominence as prophylactics, and as therapeutic interventions for many diseases. For example, a recent study showed that resveratrol administered to lactating mice attenuated hepatic lipid synthesis in the offspring when they were adults (Tanaka *et al.*, 2017). We have also shown that neonatal administration of oleanolic acid also prevented lipid accumulation in high fructose diet-induced metabolic dysfunction (Nyakudya *et al.*, 2017). In light of the popularity of rooibos, there is need to investigate whether consumption of aspalathin during periods of developmental plasticity can induce intergenerational health (or disease) outcomes through epigenetic changes.

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