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Review

Devil's Claw—A review of the ethnobotany, phytochemistry and biological activity of *Harpagophytum procumbens*

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ABSTRACT

Ethnopharmacological relevance: *Harpagophytum procumbens* subps. *procumbens* (Burch.) DC. ex Meisn. (Pedaliaceae) is an important traditional medicine growing in the Kalahari region of southern Africa where it is consumed as a general health tonic and for treating diverse ailments including arthritis, pain, fever, ulcers and boils.

Aim of the review: To provide a comprehensive overview of the ethnobotany, phytochemistry and biological activity of *H. procumbens* and possibly make recommendations for further research.

Materials and methods: Peer-reviewed articles on *H. procumbens* were acquired on Scopus, ScienceDirect and SciFinder, there was no specific timeline set for the search. A focus group discussion was held with different communities in Botswana to further understand ethnobotanical uses of the plant.

Results: *Harpogophytum procumbens* is used for a wide variety of health conditions in the form of infusions, decoctions, tinctures, powders and extracts. In addition to the common local use for arthritis and pain, other ethnomedicinal uses include dyspepsia, fever, blood diseases, urinary tract infections, postpartum pain, sprains, sores, ulcers and boils. Scientific studies revealed that *H. procumbens* exhibits analgesic, anti-oxidant, anti-diabetic, anti-epileptic, antimicrobial and antimalarial activities amongst others. Iridoid glycosides and phenylpropanoid glycosides have been the focus of phytochemical investigations as the biological activity has been ascribed to the iridoid glycosides (such as harpagoside and harpagide), which are common in nature and are known to possess anti-inflammatory activity. In addition, it has been shown that the hydrolysed products of harpagoside and harpagide have more pronounced anti-inflammatory activity when compared to the unhydrolysed compounds. *Harpagophytum zeyheri* is a close taxonomic ally of *H. procumbens* but *H. procumbens* is the favoured species of commerce, and contains higher levels of the pharmacologically active constituents. The two are used interchangeably and *H. procumbens* raw material is often intentionally adulterated with *H. zeyheri* and this may impact on the efficacy of inadequately controlled health products. The main exporter of this highly commercialised plant is Namibia. In 2009 alone, *Harpagophytum* exports were worth approximately €1.06 million. The high demand for health products based on this plant has led to over-harvesting, raising concerns about sustainability. Although only the secondary tubers are utilised commercially, the whole plant is often destroyed during harvesting.

Conclusions: *Harpagophytum procumbens* is used to treat a wide range of ailments. Some of the ethnobotanical claims have been confirmed through *in vitro* studies, however, when the constituents deemed to be the biologically active compounds were isolated the efficacy was lower than that of the whole extract. This necessitates the use of a different approach where all the metabolites are considered using a robust method such as spectroscopy; the phytochemical data can then be superimposed on the biological activity. Furthermore, there is a need to develop rapid and efficient quality control methods for

Abbreviations: 5-LOX, 5-lipo-oxygenase; 6′-PCHG, 6′-*O*-*p*-coumaroylharpagide; 8-PCHG, 8-*p*-coumaroylharpagide; BCL, Bicuculline; COX-2, cyclooxygenase-2; CREB, cAMP response element-binding; CYP, cytochrome P450; DAD, diode array detection; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; EMEA, European Medicines Agency; ESCOP, European Scientific Cooperative on Phytotherapy; FT, Fourier transform; GABA, gamma-aminobutyric acid; GC, gas chromatography; HPLC, high performance liquid chromatography; HPTLC, high performance thin layer chromatography; HSV-1, Herpes simplex virus type 1; HVA, hyperkinetic ventricular arrhythmias; IL, interleukin; iNOS, inducible nitric oxide; IR, infrared; LD, lethal dose; LPS, lipopolysaccharide; LTB_4, leukotriene B_4; MIC, minimum inhibitory concentration; mRNA, messenger ribonucleic acid; MTT, 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MS, mass spectrometry; NF-κB, nuclear factor kappa B; NMR, nuclear magnetic resonance; NO, nitrous oxide; NSAIDs, non-steroidal anti-inflammatory drugs; ORAC, oxygen radical anti-oxidant capacity; PCT, picrotoxin; P-gp, permeability glycoprotein; pLDH, parasite lactate dehydrogenase; PG, prostaglandin; PGE_2, prostaglandin E_2; PTZ, pentylenetetrazole; QC, quality control; SPE, solid phase extraction; STZ, streptozocin; TEAC, Trolox® equivalent anti-oxidant capacity; TNF, tumour necrosis factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TXB_2, thromboxane B_2; UV, ultraviolet; VSV, vesicular stomatitis virus; WHO, World Health Organisation

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both raw materials and products because the orthodox methods in current use are time-consuming and labour intensive.

Contents

1. Introduction

Harpagophytum procumbens subsp. *procumbens* (Burch.) DC. ex Meisn. (Pedaliaceae) is an important traditional medicinal plant growing in the Kalahari region of southern Africa (Van Wyk and Gericke, 2000). The use of *H. procumbens* was prominent amongst the indigenous San and Khoi people of South Africa; its use was further adopted by Bantu-speakers (Cole, 2003). Some of the ethnobotanical uses of *H. procumbens* include fever, diabetes, diarrhoea and blood disease, but there is a lack of written historical records. Suggestions to fix? Insert the treatment of before include. Recent scientific studies show that extracts of the secondary tubers of *H. procumbens* are effective in the treatment of degenerative rheumatoid arthritis, osteoarthritis, tendonitis, kidney inflammation, heart disease, dyspepsia and loss of appetite (Wichtl and Bisset, 2000; Stewart and Cole, 2005). The closely related *H. zeyheri* and *H. procumbens* are collectively known as Devil's Claw and are used interchangeably (Kemper, 1999). However, *H. zeyheri* has a lower concentration of the biologically active constituents. It is often included in raw materials and products as an adulterant of *H. procumbens*, the preferred species of commerce (McGregor et al., 2005). *Harpagophytum procumbens* is a commercially important plant species for the national income of Namibia for example, where it was an estimated €1.06 million in 2009 (Ridgway and Krugmann, 2011). Global sales are much more extensive, and a cumulative sale volume worth approximately €30 million was previously reported for Germany alone (Kathe et al., 2003).

The botany, ethnopharmacology, phytochemistry and biological activity of *H. procumbens* is herein reviewed. Furthermore, biopharmaceutical aspects and toxicity studies, which are essential parameters in drug delivery and drug action, are also discussed. Quality control (QC) forms an integral part of product development and production; thus robust and efficient methods used for the QC of *H. procumbens* were evaluated.

2. Botanical aspects

2.1. Description and classification

Harpagophytum procumbens (Pedaliaceae) is a weedy, perennial tuberous plant with visually striking fruits (Fig. 1A). The fruits have numerous characteristically long protrusions with sharp, grapple-like hooks, as well as two straight thorns on the upper surface, leading to the colloquial name of the genus *Harpagophytum*, Devil's Claw (Van Wyk et al., 2002; Wynberg, 2004). The flowers and leaves emerge from the ground after the first rains during the active growing season but die in the winter months or during periods of drought (Wynberg, 2004; Stewart and Cole, 2005). The flowers are tubular and a deep mauve-pink colour with a yellow and white throat while the leaves are blue-green and usually irregularly divided into several lobes (Fig. 1B). The flowers are pollinated by bees during the one day that they are open (Van Wyk et al., 1997; Stewart and Cole, 2005). The creeping stems, up to 2 m long, sprout annually from the persistent primary tubers which can extend up to 2 m deep. The stems give rise to several secondary storage tubers that can be up to 25 cm with a diameter of 6 cm. It is these secondary tubers that are

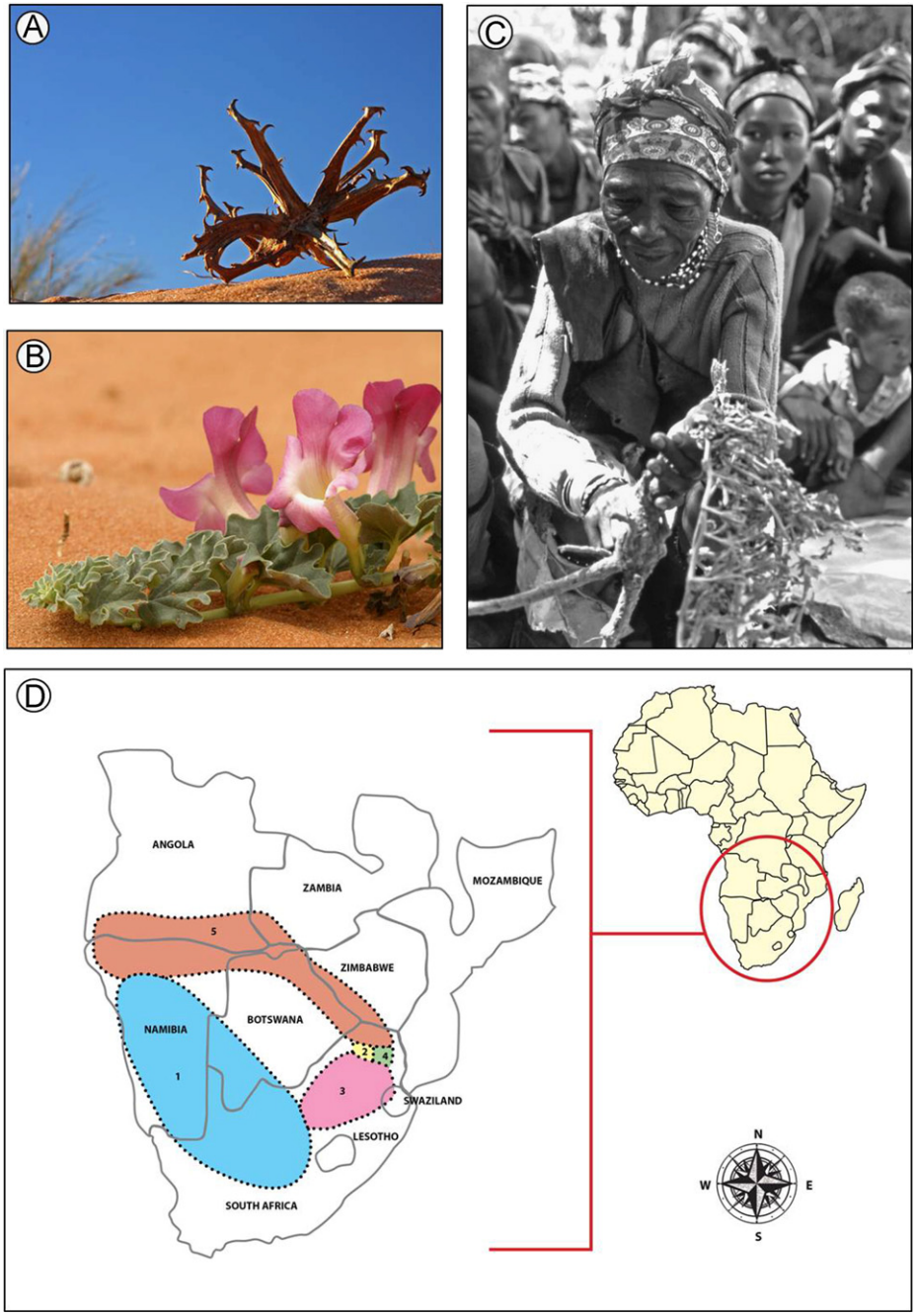


Fig. 1. (A) Fruit of *H. procumbens* (photo: ©PC Zietsman). (B). Flowers and leaves of *H. procumbens* (photo: ©PC Zietsman) (C). A woman San healer from Molapo in Central Kalahari Game Reserve demonstrating *H. procumbens* (photo: ©N Gericke). (D). Distribution of *H. procumbens*, *H. zeyheri* and their subspecies. (1) *Harpagophytum procumbens* subsp. procumbens; (2) *H. procumbens* subsp. transvaalense; (3) *H. zeyheri* subsp. zeyheri; (4) *H. zeyheri* subsp. schijffii; (5) *H. zeyheri* subsp. sublobatum (adapted from Stewart and Cole (2005)).

harvested for their medicinal properties (Raimondo et al., 2005; Stewart and Cole, 2005).

Initially, this species was considered a nuisance by livestock farmers as the fruit can cripple an animal if lodged on its feet but this also aids seed dispersal. The fruit is entangled in the wool, tails or feet of animals and subsequently deposited in sandy soils (Stewart and Cole, 2005). This seed dispersal method leads some to state that the name Devil's Claw originates from the bedeviled dance that animals do to get rid of the fruit trapped in their hooves (Moatti et al., 1983). To a degree, the seeds are wind-dispersed by strong winds that carry the fruit some distance from the parent plant. The seeds are released slowly from the mature fruit, have a high degree of dormancy (possibly an adaptation to drought) and low respiration rate, and it is estimated that it can remain viable in the seed bank for more than 20 years. The recruitment rates are low with few seedlings surviving the first year. In addition, only 20–25% of the seeds within a fruit establish soil contact during a given year, possibly an adaptation to animal or wind dispersal (Stewart and Cole, 2005).

Devil's Claw is divided into two species, *H. procumbens* and *H. zeyheri. Harpagophytum procumbens* is further divided into *H. procumbens* subsp. *procumbens* and *H. procumbens* subsp. transvaalense. *Harpagophytum zeyheri* is subdivided into *H. zeyheri* subsp. schiiffii, *H. zeyheri* subsp. sublobatum and *H. zeyheri* subsp. *zeyheri* (Grote, 2003; Stewart and Cole, 2005). The species name *Harpagophytum* is derived from the Greek 'harpago' which

translates to 'the grappling hook' (Grant et al., 2007). The several common names used to refer to *Harpagophytum* including Devil's Claw, grapple plant and duiwelsklou (Stewart and Cole, 2005) all refers to the fruit of the plant with its hook-like protrusions.

2.2. Geographical distribution and habitat

Harpagophytum procumbens occurs between 15° and 30° latitude in Namibia, Botswana, South Africa, Angola and to a lesser extent, Zambia, Zimbabwe and Mozambique (Fig. 1D). It typically grows in areas with low annual rainfall (150–500 mm/yr) such as the red sandy soils of the Kalahari Desert. Both the abundance and visibility of the plant strongly depends on rainfall. It is most abundant in open, overgrazed areas as it does not compete well with grasses and has a clumped distribution (Stewart and Cole, 2005). To enable survival during long severe dry periods, the plant forms water-storing secondary tubers which branches off horizontally from the primary tubers. In the area between the Northern Cape and North-West Provinces of South Africa, densities were estimated at 50 plants/ha in the dense grasses of a well-managed farm, 150 plants/ha in unharvested but overgrazed communal lands near the village of Madibeng and only 11 plants/ha in harvested communal lands near Madibeng (Stewart and Cole, 2005).

2.3. Sustainability, cultivation and ecological impact

The three largest producers of wild-harvested *H. procumbens* are Namibia, Botswana and South Africa. Around 2400–2800 harvesters are registered in South Africa and the harvesting was considered sustainable with 1620 plants harvested per person per season on average equating to 3,504,060 in the 2003/2004 season (Raimondo et al., 2005). In Namibia, wild-harvesting of Devil's Claw is the livelihood of many rural communities with between 10,000 and 15,000 relying on this income. The increased demand for this medicinal plant brings greater opportunities for primary producers but also strains the natural resource. Secondary tubers are normally harvested for medicinal use but care must be taken to cover the primary tubers and not damage them as this may lead to death of the plant (Strobach and Cole, 2007). Strobach and Cole (2007) reported that the plant requires a 4-yr rotational harvesting period and that regeneration requires that the primary tuber be left totally undisturbed during harvesting. Stewart (2009) reported on both harvested and unharvested plants in the Kalahari savannas of South Africa. It was noted that the species appears to be resilient and that harvesting was not a significant factor in predicting time to plant death, based on experimental conditions (Stewart, 2009).

Cultivation of *H. procumbens* remains a challenge due to low germination rates and plants propagated by cuttings fail to produce primary tubers (Kathe et al., 2003). However, several techniques have been proposed to culture *H. procumbens*. Bairu et al. (2011) performed a comparative phytochemical analysis of wild and *in vitro*-derived greenhouse-grown tubers. The study confirmed that cultivated plants produce tubers containing significant amounts of iridoids. The *in-vitro*-derived tubers had the highest total iridoid content and iridoids were present in lower concentration in tissue cultured shoots and callus-like tissue. Both these methods are viable options for large scale production, and can be employed as a conservation measure for wild populations (Bairu et al., 2011). Plant *in vitro* technologies are being explored as viable means of cultivating the species. *In vitro* culture systems are not influenced by seasonal and geographical conditions; furthermore there are various ways to increase yields of desired metabolites in a cost-effective manner (Ramachandra Rao and Ravishankar, 2002). Ludwig-Müller et al. (2008) cultivated hairy root cultures of *H. procumbens* in a bubble column bioreactor as well as in conventional flasks and monitored the metabolite profiles. The metabolite profiles differed qualitatively and quantitatively possibly due to the difference in relative stress levels in the two systems. The bubble column bioreactor method yielded a higher harpagide concentration and may be used in future for secondary metabolite production (Ludwig-Müller et al., 2008). Gyurkovska et al. (2011) cultivated cell suspensions and *Agrobacterium rhizogenes*-transformed hairy root cultures of *H. procumbens*. HPLC analysis showed that β-OH verbascoside and leucosceptoside A, which have not been isolated from the roots, were present in the cultures, in addition to verbascoside and martynoside. The chemical composition of the cultures compared to each other and to a root extract differed: harpagoside and harpagide was not detected in the cultures; leucosceptoside A was only found in the cultures suggesting *de novo* synthesis; verbascoside levels were 5.6 times higher in the cell suspension culture compared to the transformed roots; and the cell suspension did not contain martynoside while the transformed root culture did (Gyurkovska et al., 2011).

Wild-harvesting by knowledgeable harvesters tended to protect the species but increased demand and therefore financial motivation has caused over-harvesting by more (less knowledgeable) harvesters with significant effects on the *H. procumbens* resource base. In Namibia, it is said that there is only one plant left per hectare, where previously there were 1000–2000 plants in a natural population. Controlled harvesting by way of permits has protected the species to some extent. *Harpagophytum zeyheri* was officially listed in the European Pharmacopoeia in 2003, despite questions about its effectiveness (Grote, 2003) as it is known that it contains significantly lower levels of iridoids. This inclusion may lead to overharvesting of *H. zeyheri* as it is less expensive compared to *H. procumbens* and may therefore affect the *H. procumbens* market (Grote, 2003).

3. Ethnopharmacology

In 1820, Devil's Claw was collected and described by European scientists. However, it was only much later that a German trader named G.H. Mehnert learned of the medicinal properties from the San and Nama people in Namibia and made these uses known in the early 1900s (Raimondo et al., 2005; Stewart and Cole, 2005). B. Zorn first studied the tubers after they were taken to Germany (University of Jena) in the 1950s (Wegener, 2000; Stewart and Cole, 2005). Tubers were also exported to Germany in small quantities with large-scale export starting in 1962 (Raimondo et al., 2005). The indigenous San and Khoi people of southern Africa have used *H. procumbens* tubers as a medicine for centuries. It has also been adopted into the traditional knowledge systems of immigrating Bantu-speakers who arrived in the area between 1500 and 5000 years ago (Cole, 2003). Watt and Breyer-Brandwijk (1962) and Van den Eynden et al. (1992) reported that the secondary tuber is much prized as a medicine among the San, Khoi, some Bantu-speaking Africans, people of mixed descent, and by Europeans.

Harpogophytum procumbens is used for a wide variety of health conditions in the form of infusions, decoctions, tinctures, powders and extracts. Additional to the common topical use to treat arthritis and pain, other ethnomedicinal uses include; dyspepsia, fever, blood diseases, urinary tract infections, postpartum pain, sprains, sores, ulcers and boils (Watt and Breyer-Brandwijk, 1962). When taken on a daily basis, *H. procumbens* has a subtle laxative effect. Small doses of the plant extract are used to relieve menstrual cramps, whereas higher doses assist in expelling retained placentas (Van Wyk and Gericke, 2000). The dry, powdered secondary tuber is used directly as a wound dressing; it can

also be mixed with animal fat or Vaseline® to make a wound- and burn-healing ointment (Van Wyk et al., 2002).

The Topnaar people of Namibia drink a decoction of the secondary tubers or chew them to relieve stomach and postpartum pains. Before digging up the secondary tubers, a needle or a button is put in the soil to '*buy the tubers from the earth*' (Van den Eynden et al., 1992). An infusion is taken for the relief of fevers, as a bitter tonic, and for unspecified *'blood diseases*.' The dried and powdered secondary tuber is administered to pregnant women in a dose of about 250 mg three times a day to relieve pain, and this is continued in a lower dose after delivery. Fresh secondary tuber is made into an ointment and applied to the abdomen of women who anticipate a difficult birth. The ointment is also applied to various skin lesions including sores, ulcers, boils and cancerous growths (Watt and Breyer-Brandwijk, 1962). An infusion can be taken orally especially for rheumatism, and for treating liver, kidney, pancreas and stomach ailments (Von Koenen, 1996).

Von Koenen (1996) reports a Herero use where the tuber is cut into small pieces, covered with cold water and allowed to steep; two tablespoons of this cold infusion are taken daily for treating cough, diarrhoea, constipation, as well as syphilis and gonorrhoea in both women and men. If the tea is taken too often or if it is too concentrated, diarrhoea results. A decoction of pieces of the secondary tuber is taken for pain. A Herero warned that long-term use of high doses can cause cancer, although traditional diagnosis and concepts of cancer may differ from those of scientists. Africans and people of mixed descent emphasise that Europeans use the plant too intensively, and this can be damaging to health (Von Koenen, 1996). Dried pieces of *H. procumbens* secondary tuber and dried roots of *Clerodendrum uncinatum* Schinz are placed on a clay shard with charcoal and the smoke is applied on the back of a person to treat back pain. The resulting ash is finely ground and made into an ointment using fat, which is rubbed onto the back. A hot water infusion of the two plants can be given daily as an enema (Von Koenen, 1996).

On an ethnobotanical field trip to Botswana during January 1998, San uses of *H. procumbens* in three different localities in Botswana; Molapo, a small San settlement in Central Kalahari Game Reserve, and Ghanzi and D'Kar were recorded. In Molapo, the local name for *H. procumbens* is *kakamasha*; it is also known by the Tswana name *sengapirile*. The San in Molapo do not make a distinction between the medicinal value of the primary tubers or the secondary tubers. Either, or both, are used fresh or dried, chewed directly or made into hot or cold infusions. *Harpagophytum procumbens* is used to treat all fevers, and as an important tonic in infectious diseases including tuberculosis. It is commonly used for general body aches, especially muscle and joint aches

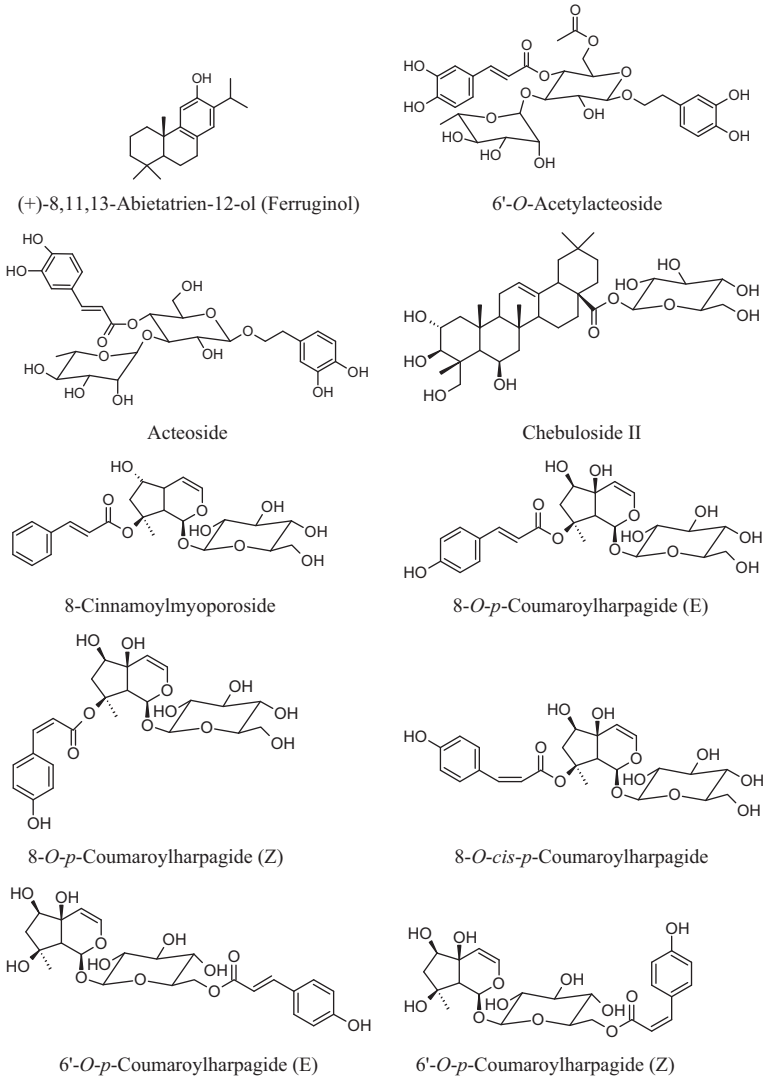
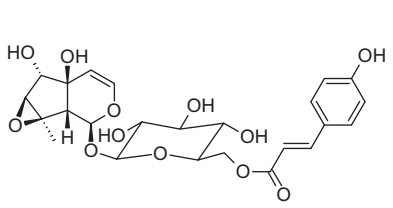
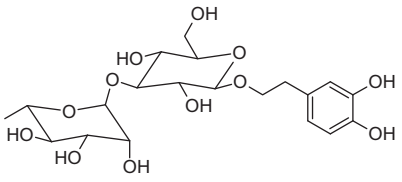


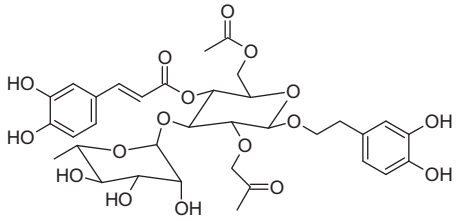
Fig. 2. Chemical structures of selected compounds isolated from *Harpagophytum procumbens*.



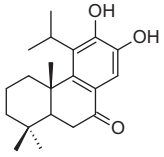
6'-*O*-*p*-Coumaroylprocumbide



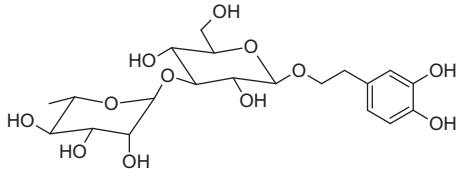
Decaffeoylverbascoside



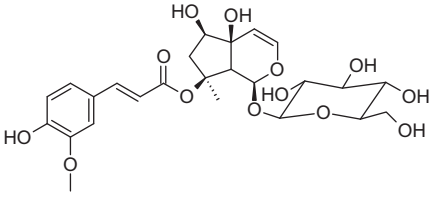
2,6-Diacetylacteoside



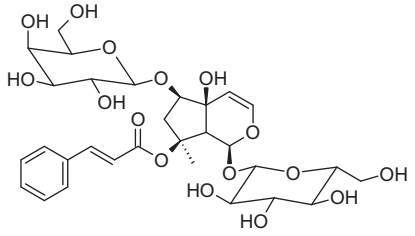
12,13-Dihydroxychina-8,11,13-trien-7-one



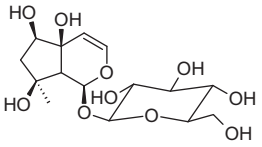
β-(3',4'-Dihydroxyphenyl)-*O*-α-Rhamnopyranosyl(1→3)-β-D-Glucopyranoside



8-Feruloylharpagide



6-*O*-α-D-Galactopyranosyl Harpagoside



Harpagide

Fig. 2. Continued.

and pains in the elderly. Small doses are taken to treat diarrhoea, and larger doses are taken as a laxative to treat constipation. To treat infertility, decoctions are taken together with the roots of *Ziziphus mucronata* Willd. *Harpagophytum procumbens* is taken orally to treat any venereal disease, and is also taken to treat intermittent abdominal pain suggestive of peptic ulceration. *Harpagophytum procumbens* is regarded by the Molapo community as the best plant of all to treat menstrual cramps. In Molapo it is taken orally to expel a retained placenta immediately after childbirth, although it is not their preferred plant for this purpose. *Harpagophytum procumbens* is not taken during pregnancy at all by the women in Molapo, as it is believed it will cause an abortion or stillbirth. It is used to initiate labour if there is delayed onset of labour, and during labour it is taken to ease labour-pains. It is taken to help expel the placenta in the third stage of labour (time between complete delivery of the infant and the placenta), and for treating pain after giving birth. In women with a vaginal discharge, a decoction of the roots of *Ehretia rigida* Druce is used first, and then a decoction of *Ehretia rigida* roots and *H. procumbens* is taken. When people return from burying the dead, the secondary tubers of *H. procumbens* are pounded into a paste with roots of an *Asparagus* or *Protasparagus* and applied to cuts made in the skin as part of a cleansing ritual (Xherema, a woman San healer; Matambo, a San man; Interpreter, Lenyatso July, personal communication). The San healer is shown demonstrating the plant in Fig. 1C.

In Ghanzi (Botswana) the San use both the primary tubers and secondary tubers of *Harpagophytum procumbens*. All these plant parts are believed to be equally effective and are cut into pieces, sun-dried and powdered. Only small amounts are used; a single dose is estimated to represent approximately 100–200 mg as demonstrated. This dose can be swallowed directly, or made into a tea, and can be taken daily on a chronic basis. No adverse effects are known. *Harpagophytum procumbens* is used to treat many conditions including general body pains in the elderly who use it every day until they feel better, to purify the blood, for colds and 'flu' (including in children), tuberculosis, asthma, and constipation. *Harpagophytum procumbens* is regarded as effective for treating menstrual cramps, and can also be taken during pregnancy, but only very small amounts are advised. It is used to initiate labour if there is delayed onset of labour, and during labour it is taken to ease labour pains. It is taken to help expel the placenta in the third stage of labour, and for treating the pain after giving birth (Kanana, a San man; Interpreter, Lenyatso July, personal communication).

In D'Kar, Botswana, the San regard the dry powdered secondary tubers as a very important medicine to treat all fevers, and

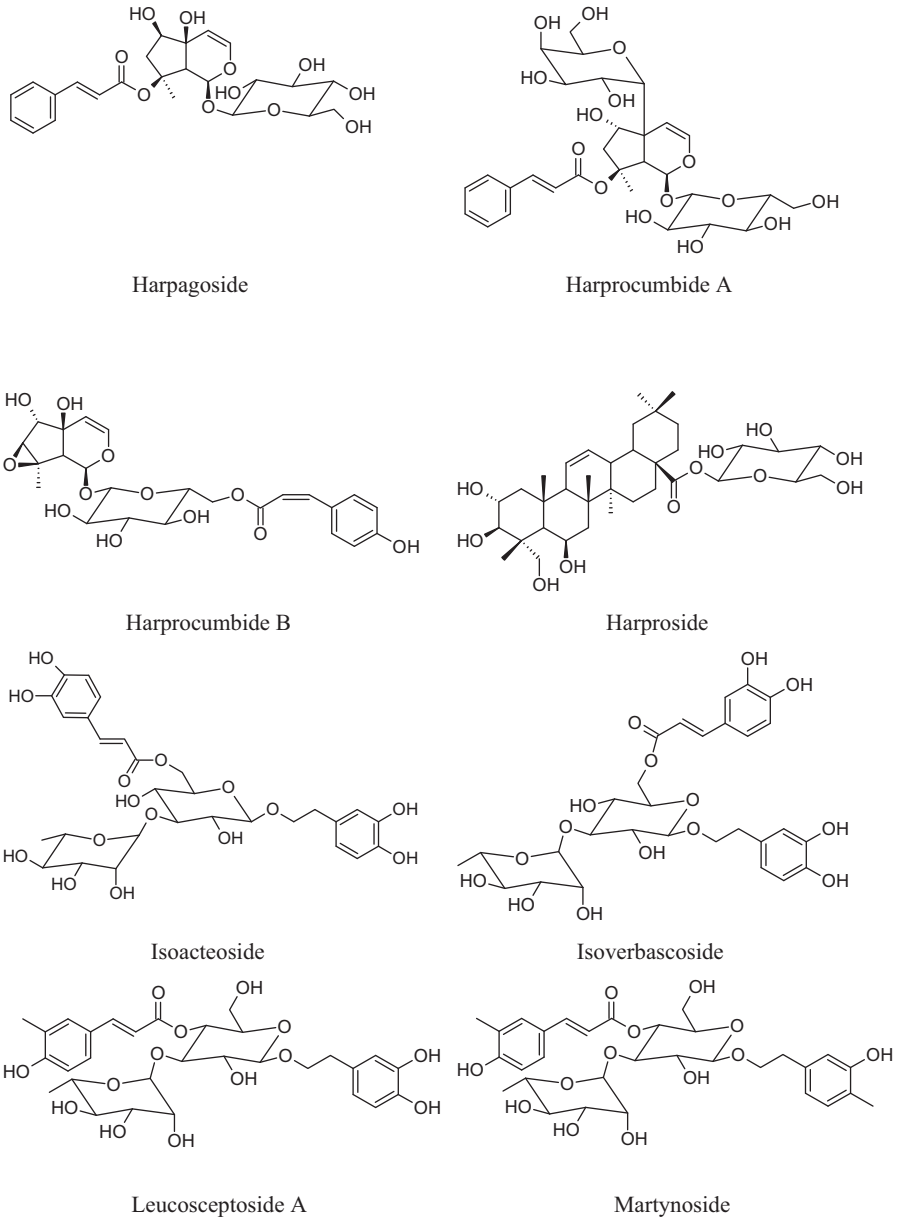


Fig. 2. Continued.

most old people use it for painful muscles and joints, taking it daily on a long-term basis. It is used to purify the blood, and to treat stomach ulcers that have been diagnosed at a clinic by a medical doctor. It is also used as a mild laxative for constipation in low doses—only a tip of a teaspoonful of powdered secondary tuber is used. *Harpagophytum procumbens* is used as one of the best remedies in D'Kar for menstrual cramps. It is also taken during the last months of pregnancy to help the baby to be in the vertex position for birth. It is taken during labour to alleviate pain and to expel a retained placenta (Tomqu, a woman San healer; Interpreteter, Lenyatso July, personal communication). *Harpagophytum procumbens* is used by Tswana people to treat type-2 diabetes, and as an appetite stimulant (Isaac Mayeng, personal communication).

4. Phytochemistry

Several phytochemical investigations have led to the isolation of constituents from *H. procumbens* including iridoids and other substances including harpagoquinones, amino acids, flavonoids, phytosterols and carbohydrates (Gruenwald, 2002). Iridoids represent a large group of cyclopenta[c]pyran monoterpenoids occurring mainly in dicotyledonous plant families such as the Apocynaceae, Scrophulariaceae, Verbenaceae, Lamiaceae, Loganiaceae and Rubiaceae (Seeger, 1973). Phenylpropanoid glycosides are characterised by having a hydroxyphenylethyl moiety to which a glucopyranose is linked through a glycosidic bond and other sugars such as rhamnose, xylose or rabinose may be attached to the C-3, C-4 or C-6 of the glucosyl residue (Kurkin, 2003).

The chemical structures of selected compounds isolated from *H. procumbens* are shown in Fig. 2. The first iridoid glycoside isolated from *H. procumbens* was harpagoside, widely regarded as the active constituent, followed by harpagide and procumbide. In 1983, three additional iridoid glycosides with similar structures but different linkages were discovered, including: procumboside, 8-*O*-*p*-coumaroylharpagide and 6′-*O*-*p*-coumaroylprocumbide (Kikuchi et al., 1983). In 1987, the phenolic glycosides, acteoside and isoacteoside, were isolated from an acetone extract of the secondary tubers of *H. procumbens* for the first time together with

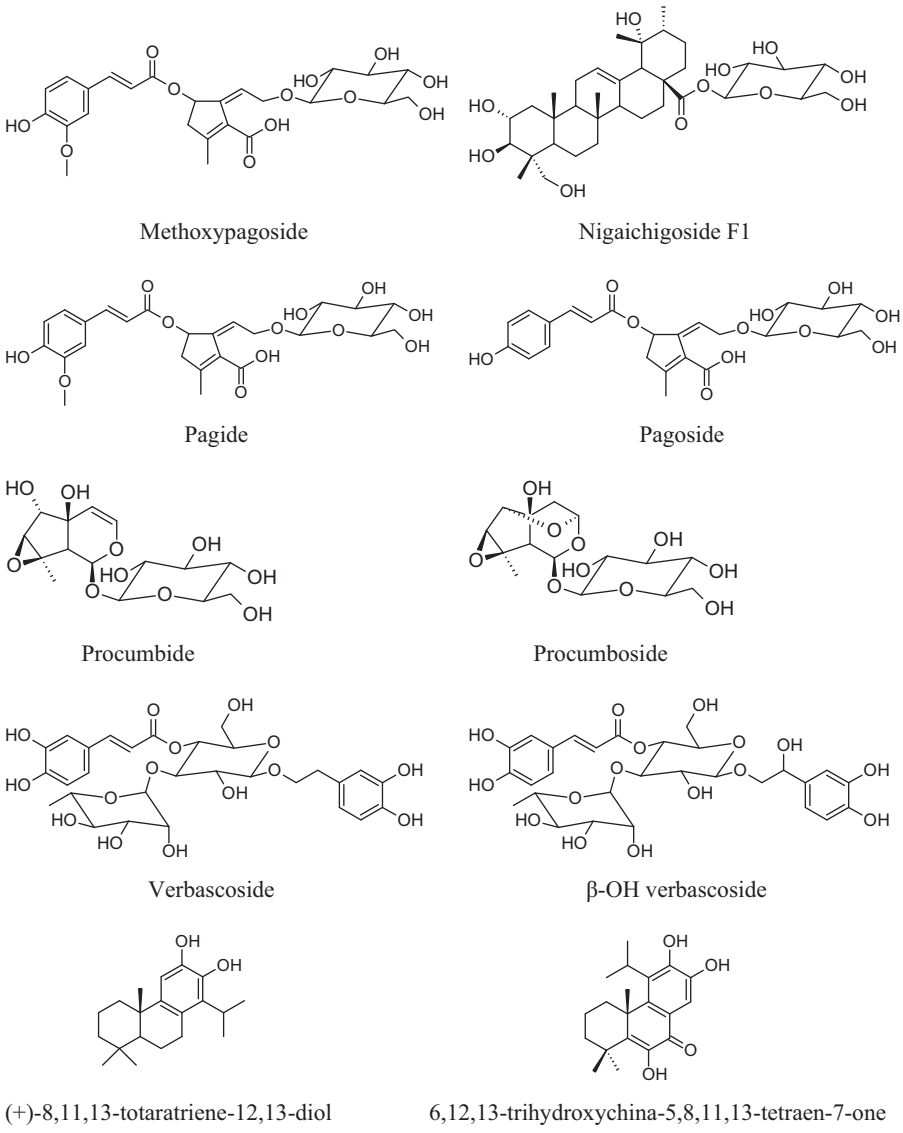


Fig. 2. Continued.

a novel bioside β-(3′,4′-dihydroxyphenyl)-*O*-α-rhamnopyranosyl(1→3)-β-D-glucopyranoside (Burger et al., 1987). It was suggested that acteoside and iseoacteoside may be biosynthesised from the bioside. In 2003, Munkombwe isolated two acetyl phenolic glycosides, 6-acetylacteoside and 2,6-diacetylacteoside, from commercially available secondary tubers extracted with dichloromethane/methanol (1:1). They are structurally very similar to acteoside in that they are made up of two aromatic moieties and two sugars (glucose and rhamnose) and structurally identical to each other except for the number of acetyl groups (1 *vs.* 2) (Munkombwe, 2003). Furthermore, 6-acetylacteoside is present in *H. procumbens* and absent in *H. zeyheri*, which can be used to distinguish between the two *Harpagophytum* species (Chrubasik, 2004). Boje et al. (2003) isolated amongst others 8-feruloylharpagide, 8-cinnamoylmyoporoside, pagoside, cinnamic acid and caffeic acid from an aqueous extract of secondary tubers of *H. procumbens*. Two diterpenes, (+)-8,11,13-totaratriene-12,13-diol and (+)-8,11,13-abietatrien-12-ol (ferruginol), were isolated for the first time from *H. procumbens* by Clarkson et al. (2003) from a petroleum ether secondary tuber extract.

Seger et al. (2005) characterised isobaric iridoid glycoside regioisomers as the (E/Z) pairs of 6′-*O*-*p*-coumaroylharpagide (6′-PCHG) and 8-*p*-coumaroylharpagide (8-PCHG) from a methanol secondary tuber extract using a hyphenated LC-DAD-MS/SPE-NMR technique. 6′-PCHG (E/Z) and 8-PCHG (E) are rare metabolites while 8-PCHG (Z) is a novel natural product. In addition, it is the first time that 6′-PCHG (E/Z) was isolated from *H. procumbens* (Seger et al., 2005). The ratio of 8-*O*-*p*-coumaroylharpagide to the sum of harpagoside and 8-*O*-*p*-coumaroylharpagide is a distinguishing feature between *H. procumbens* and *H. zeyheri* (Eich et al., 1998). In *H. procumbens* it is below 10% while it is above 31% in *H. zeyheri* (Chrubasik, 2004).

Clarkson et al. (2006) used HPLC–SPE–NMR to isolate novel, unstable chinane-type tricyclic diterpenes from a petroleum secondary tuber ether extract. In contrast to common diterpenes with abietane and totarane skeletons which have the isopropyl group at C-13 and C-14 respectively, 12,13-dihydroxychina-8,11,13-trien-7-one and 6,12,13-trihydroxychina-5,8,11,13-tetraen-7-one have an isopropyl group at C-11. These structures are unstable, possibly being degraded through oxidation. In 2006, Qi et al. isolated two new iridoid glycosides, harprocumbide A (6″-O-α-D-galactopyranosyl harpagoside) and harprocumbide B (6″-*cis*-*p*-coumaroylprocumbide), from the tubers of *H. procumbens*. They also reported 6-*O*-α-D-galactopyranosyl harpagoside for the first time from *H. procumbens* (Qi et al., 2006a) and

nigaichigoside F1, nigaichigoside F2, chebuloside II, 7α-hydroxysitosterol, 7β-hydroxysitosterol, martynoside, 7α,23-dihydroxytormentic acid ester glucoside, ethyl ferulate and pentacosanoic acid in subsequent studies (Qi et al., 2006b, 2007). Qi et al. (2010) isolated a new triterpenoid glycoside as well as a new iridoid glycoside named harproside and pagide, respectively. After testing for anti-inflammatory activity it was noted that hydroxylation at C-24 may increase activity and that the *exo*- double bond may be a prerequisite for good activity (Qi et al., 2010). HPLC–DAD coupled to ESI–MS was used by Karioti et al. (2011) to analyse constituents in *H. procumbens* tinctures. A novel natural compound named methoxypagoside was isolated and decaffeoylverbascoside was reported for the first time from *H. procumbens*.

Despite the exhaustive isolation of the various constituents, the exact mechanisms of action remain elusive. Presently, the level of active ingredients, particularly that of harpagoside, is used to determine the quality of dried tubers supplied. The harpagoside content is required to be at least 1.2% in European standardised products (Kemper, 1999). The percentage of harpagoside in secondary tubers varies seasonally by plant age, among plants in a given area, and even among tubers of the same plant (Kemper, 1999; Von Willert and Schneider, 2001). Aqueous or ethanol-based extraction is most commonly used to yield the active ingredients, although extraction can also be affected with liquid carbon-dioxide and a cosolvent (Gruenwald, 2002). The plant's major chemical constituents have been found in the secondary tubers. Flowers, stems, and ripe fruits do not contain harpagoside. Traces of harpagoside together with some unidentified iridoid compounds are found in the leaves. The secondary tubers contain approximately twice as much harpagoside compared to primary tubers (Czygan and Krüger, 1977).

5. Biological activity

5.1. Anti-inflammatory activity

In 1957, Zorn showed that subcutaneous injection and oral ingestion of an infusion of *H. procumbens* caused significant reduction in the swelling of arthritic joints of rats with formaldehyde-induced arthritis. It was concluded that *H. procumbens* contained a potent anti-inflammatory or anti-rheumatic substance and subsequent tests were undertaken by Eichler and Koch (1970) to determine whether the isolated constituent, harpagoside, yielded the same results. The results were positive but the whole plant extract showed better activity (Eichler and Koch, 1970). Since then numerous studies have been undertaken to prove *in vitro* and *in vivo* inflammatory activity for *H. procumbens* extracts and/or isolated compounds.

In vitro activities of extracts prepared from the tubers include the suppression of interleukin (IL)-induced production of metalloproteinases in human chondrocytes (Schulze-Tanzil et al., 2004); inhibition of lipopolysaccharaide (LPS)-induced release of cytokines (tumour necrosis factor (TNF)-α, IL-6, IL-1β) and prostaglandin (PG) E_2 from human monocytes (Fiebich et al., 2001); the suppression of PGE_2 synthesis and NO production by inhibiting LPS-stimulated enhancement of the COX-2 and iNOS mRNA expressions in L929 cells (Jang et al., 2003); and the inhibition 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced COX-2 expression in human breast epithelial cells (MCF10A) (Na et al., 2004). It is postulated that the efficacy of *H. procumbens* in reducing pain and inflammation associated with rheumatoid arthritis and osteoarthritis can be explained by its ability to block the production of inflammatory mediators such as PGE_2 (Aberham et al., 2007). However, some reports on the anti-inflammatory effect of *H. procumbens* extracts are inconsistent, and differences had widely been attributed to the extraction procedure, geographical source of the crude drug and the fractions of constituents (Joubert et al., 2005).

The inhibitory effect on COX-2 expression was determined for an ethanol extract of *H. procumbens* tubers and its major active components, harpagoside, harpagide, 8-*p*-coumaroylharpagide and acteoside, following application to freshly excised porcine skin. The extract showed promising activity in the Western blot and immunocytochemical assays, and harpagide caused a significant increase in the levels of COX-2 expression 6 h post-application. Harpagoside and 8-*p*-coumaroylharpagide caused greater reductions in COX-2 expression than acteoside. Results indicated that the efficacy of *H. procumbens* was dependent on the ratios of the four major active compounds present. This is not congruent with the current official monograph specifications based solely on harpagoside (Abdelouahab and Heard, 2008a). Qi et al. (2006b) investigated the inhibitory activity of 10 iridoid glycosides isolated from the tubers of *H. procumbens*. These compounds were tested using RAW 264.7 macrophages with chemiluminescence detection and only 8-*O*-*p*-coumaroylharpagide showed considerable activity with an IC_{50} value of 32.4 μM, while other constituents showed a lesser inhibitory effect (Qi et al., 2006b). Kaszkin et al. (2004) also reports that harpagoside exhibits a concentration-dependent suppression of nitrite formation (80%) in renal mesangial cells attributable to the inhibition of iNOS expression at the level of its transcriptional activation. This study also suggested that the activity of the extract is higher than that of pure harpagoside, which only had inhibitory activity at concentrations ranging from 0.3 mg/ml to 1 mg/ml. Using human HepG2 hepatocarcinoma and RAW 264.7 macrophages, Huang et al. (2006) investigated the mechanism of action of harpagoside. They reported that harpagoside (200 μM) inhibits LPS-induced mRNA levels and protein expression of COX-2 and iNOS in HepG2 cells. Furthermore, harpagoside reduced NO release in LPS-stimulated cells in a dose-dependent manner with an IC_{50} value of 39.8 μM. These *in vitro* studies on harpagoside alone seem to confirm mechanisms of action reported for the whole extract, but the activity is higher for the crude extract. In an *in vitro* study with Ca^{2+} ionophore A23187-stimulated human whole blood, an inhibition of the biosynthesis of cysteinyl-leukotrienes and thromboxane B_2 by *H. procumbens* extracts as a function of their harpagoside concentration was revealed. It is extrapolated that plasma constituents converts harpagoside into a more biologically active form. The removal of a glycoside moiety from harpagide creates herpagogenin, which can suppress A23187-induced eicosanoid biosynthesis (Setty and Sigal, 2005).

Na et al. (2004) examined the *in vivo* inhibitory effects of a methanolic extract of *H. procumbens* on tumour promoter-induced COX-2 expression in mouse skin and revealed inhibition of DNA-binding of nuclear factor kappa B cells (NF-κB) activated by TPA (Na et al., 2004). This finding was in accordance with a study in a mouse-skin model by Kundu et al. (2005) who reported that methanol extracts at doses of 200 μg to 400 μg inhibited TPA-induced COX-2 expression when applied topically, which appears to be mediated by blocking the catalytic activity of ERK and inhibiting the activation of AP-1 and cAMP response element-binding (CREB) (Kundu et al. 2005).

Ahmed et al. (2005) investigated the effect of an aqueous extract of *H. procumbens* on both acute and chronic inflammatory processes in rats. Indomethacin, a commercial, non-steroidal anti-inflammatory drug was used as a reference drug administered at 10 mg/kg and an aqueous extract of *H. procumbens* (800 mg/kg) was administered intraperitoneally as a single dose. Freund's adjuvant-induced arthritis in rats (chronic model) induced a highly significant increase in the paw thickness ($p \leq 0.001$), a significant decrease in serum cortisol, a highly significant

decrease in serum albumin and a significant increase in C-reactive protein. *Harpagophytum procumbens* and indomethacin administration caused significant reduction in paw thickness ($p \leq 0.001$). In the cotton pellet-induced granuloma test (acute model), *H. procumbens* and indomethacin intraperitoneal administration in rats caused a reduction of inflammation manifested by a marked and highly significant decrease of cotton pellet weight ($p \leq 0.001$) (Ahmed et al., 2005). Intraperitoneal administration of *H. procumbens* extracts administered 30 min prior to carrageenan injection exerted inhibitory effects on the acute inflammatory response at different doses (100, 200, and 400, or 800 mg/kg body wt.) of approximately 88, 80, 70, and 60%, respectively, 4 h after carrageenan application to the paw. In addition, it reduced the number of circulating mononuclear leucocytes in normal rats (Catelan et al., 2006). More importantly, they observed the efficacy of the extract when injected intraperitoneally or even intraduodenally, oral administration did not have the same effect (Shigeru et al., 2002; Catelan et al., 2006). It was hypothesised that it may be due to exposure to the gastro-intestinal environment causing acid hydrolysis or denaturation of the active principles (Soulimani et al., 1994).

5.2. Analgesic activity

The most frequently cited reason that people turn to complementary and alternative medicine is pain relief (Astin, 1998). The aqueous secondary tuber extract of *H. procumbens* (50–800 mg/kg) produced significant analgesic effects against thermally (hot-plate) and chemically (acetic acid) induced nociceptive pain stimuli in mice (Mahomed and Ojewole, 2005). Intraperitoneal administration of an aqueous extract of *H. procumbens* at 400 mg/kg to mice significantly reduced the number of writhing reactions (Ahmed et al., 2005) and Uchida et al. (2008) reported that *H. procumbens* extract exerted significant antinociceptive effects in the formalin test in mice. Administered at a dose of 30–300 mg/kg, the extract reduced the times of licking/biting in both the first and second phases of formalin injection in mice in a dose-dependent manner. The significant increase in the content of nitrites/nitrates (NOx) in the mouse spinal cord caused by formalin injection was significantly attenuated by *H. procumbens* extract. Additionally, it was determined that the opioidergic system seems to be involved in the antinociceptive effect of *H. procumbens* (Uchida et al., 2008).

5.3. Anti-oxidant activity

Anti-oxidants derived from natural sources have gained popularity in recent years due to the high incidence of oxidation-related illnesses such as arthritis and cancer. Betancor-Fernández et al. (2003) determined the anti-oxidant activity of *H. procumbens* using the Trolox® equivalent anti-oxidant capacity (TEAC) assay. The results showed that *H. procumbens* extract was particularly rich in water-soluble anti-oxidants, but harpagoside did not contribute significantly to its anti-oxidant activity. Frum and Viljoen (2006) showed that the methanol extract possessed moderate anti-oxidant activity with an IC_{50} value of 19.84 ± 0.13 ppm using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A secondary tuber extract and commercially available tincture were found to effectively scavenge the DPPH radical, inhibit nitrite levels in supernatants harvested from LPS-stimulated RAW 264.7 macrophages, and cause dose-dependent suppressions in the detection of fMLP- and acetic acid-induced neutrophil MPO (Grant et al., 2009). Georgiev et al. (2010) found the most active compounds were β-OH-verbascoside (in DPPH and superoxide radical scavenging assays) and leucosceptoside A (in oxygen radical) anti-oxidant capacity (ORAC) assays.

During *in vivo* studies, Bhattacharya and Bhattacharya (1998) demonstrated the significant anti-oxidant effect of *H. procumbens* extract in rats by using the rat brain frontal cortex and striatum. *Harpagophytum procumbens* extract (100 and 200 mg/kg) was administered intra-peritoneally for 14 days and a dose-dependent increase in superoxide dismutase, catalase and glutathione peroxidase activities in both brain areas as well as a reduction in lipid peroxidation activity was noted. It was stated that the anti-oxidant properties may be partially responsible for the anti-inflammatory effect of *H. procumbens* extracts. In a study using a cell-free oxidant-generating system and inflamed human colorectal biopsies it was concluded that incubation of biopsies with *H. procumbens* leads to a dose-dependent scavenging activity of superoxide peroxyl (Langmead et al., 2002). A further study indicated that the constituents in the *H. procumbens* extract could be acting as: (i) a chelator of the stannous ions, avoiding the generation of free radicals, (ii) a free radical scavenger, protecting the cells against the oxidation, and/or (iii) an oxidant compound acting upon the stannous ions, reducing the stannous chloride cytotoxicity (Almeida et al., 2007). The constituents responsible for the anti-oxidant activity in *H. procumbens* extracts may be flavonoids, known free radical scavengers (Dugas et al., 2000), and plant phenols acting as hydrogen donors and oxygen radical neutralisers (Sawa et al., 1999).

5.4. Anti-diabetic activity

It is reported that *H. procumbens* secondary tuber is used in some communities of South Africa to treat adult-onset, type-2 diabetes (Mahomed and Ojewole, 2005). Mahomed and Ojewole (2005) found positive results in a study investigating the anti-diabetic effect in streptozotocin (STZ)-induced diabetic rats. Dose-dependent, significant reductions in the blood glucose concentration of both fasted normal and fasted diabetic rats were noted after administration of the aqueous secondary tuber extract (50–800 mg/kg) (reference drug = chlorpropamide, 250 mg/kg).

5.5. Antimicrobial activity

Due to increasing microbial resistance to antibiotics, plant extracts and natural products are of special interest as potential anti-infective agents. Weckesser et al. (2007) reports that supercritical carbon dioxide extracts of *H. procumbens* and pure harpagoside inhibits *Candida krusei* (MIC = 100 μg/ml). However, harpagoside alone was not effective in the screening, suggesting the existence of synergy between the biologically active constituents (Weckesser et al., 2007). It is a well-known fact that synergistic interaction between compounds in one extract or between plant combinations may enhance therapeutic efficacy. A study on the antimicrobial activity of *Tarchonanthus camphoratus* revealed greater antimicrobial efficacy with the combination of volatile and non-volatile fractions than with the fractions alone (Van Vuuren and Viljoen, 2009, 2011). One recent example of synergistic actions between plants extracts, in this case essential oils, is demonstrated by De Rapper et al. (2012) where the combination of frankincense (*Boswellia* spp.) and myrrh (*Commiphora* spp.) known from biblical times revealed synergistic antimicrobial action. Bermejo et al. (2002) screened seven iridoid glycosides including harpagide and harpagoside *in vitro* against herpes simplex virus type 1 (HSV-1), vesicular stomatitis virus (VSV) and the poliovirus type 1. Results revealed that harpagoside possessed antiviral activity against VSV with the percentage of cellular viability at the non-toxic limit concentration of 43.3% at 450 μg/ml.

5.6. Antimalarial activity

Malaria leads to more than one million deaths per year and is one of the gravest health problems facing Africa (WHO (World Health Organisation), 2003). A limited number of chemoprophylactic and chemotherapeutic agents are available for the control and treatment of malaria. Therefore, an urgent need to develop novel, effective antimalarial treatments as drug-resistant strains of the malaria parasite *Plasmodium falciparum* has developed. The main aim in the management of malaria is to treat the parasitic infection, while a secondary aim is to alleviate symptoms. Clarkson et al. (2003) studied the effect of two diterpenes, (+)-8, 11, 13-totaratriene-12, 13-diol and (+)-8, 11, 13-abietatrien-12-ol, isolated from *H. procumbens* extract on erythrocyte shape to determine the selectivity of their antiplasmodial activity. Using a parasite lactate dehydrogenase (pLDH) assay, (+)-8, 11, 13-totaratriene-12, 13-diol and (+)-8, 11, 13-abietatrien-12-ol displayed significant ($IC_{50} < 1$ μg/ml) *in vitro* antiplasmodial activity against both chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum*.

5.7. Anticancer activity

Cancer is a global health concern and a major therapeutic challenge. Inflammation is closely linked to carcinogenesis, consisting of three apparently distinct phases: initiation, promotion and progression (Surh, 2003). Chemoprevention is a relatively new and promising strategy for the prevention of cancer. Edible plants with anti-inflammatory activity have received attention as potential sources of chemopreventive agents (Surh, 2003). Isoacteoside isolated from *Pedicularis striata* Pall. significantly inhibited cell proliferation in a dose- and time-dependent manner on a human gastric cancer cell line (MGC803). Isoacteoside significantly suppressed cell tumourigenicity, activities of alkaline phosphatase, lactate dehydrogenase and caused G_0/G_1 arrest (Chen et al., 2002). Kundu et al. (2005) found that the methanolic extract of *H. procumbens* exerted chemopreventive activity by inhibiting TPA-induced COX-2 expression in mouse skin.

5.8. Cardiovascular activity

Circosta et al. (1984) reported that crude *H. procumbens* methanol extract exhibited protective effects in some experimental arrhythmias induced by aconitine, calcium chloride and chloroform–epinephrine in rats and rabbits. High doses caused dose-dependent, significant reduction in arterial blood pressure and a concomitant decrease in heart rate in conscious, normotensive rats. In the experimental model of hyperkinetic ventricular arrhythmias (HVA) using Langendorff preparations of rat heart, Costa De Pasquale et al. (1985) observed that *H. procumbens* methanol extracts and harpagoside produced a dose-dependent, significant, protective effect on HVA-induced reperfusion. Mahomed and Ojewole (2004) found that low to moderate doses of *H. procumbens* secondary tuber aqueous extract (10–400 mg/kg) produced dose-dependent hypotensive and cardiodepressant effects on the systemic arterial blood pressure and heart rate of pentobarbitone-anaesthetised rats. At doses of 10–1000 mg/ml, dose-dependent, initial slight, transient and significant contractions of isolated rat portal veins, followed by secondary, longer-lasting, significant relaxations of the cardiac muscle were noted. *Harpagophytum procumbens* decreased heart rate and arterial blood pressure in rats and exhibited a negative inotropic effect on isolated rabbit hearts. It has been suggested that it may cause QT prolongation and abnormal heart rhythms as well as influence calcium currents (verapamil-like effect). Therefore, patients treated for cardiovascular disorders should be warned about possible side-effects, though cardiovascular effects seem unlikely based on current data (EMEA (European Medicines Agency), 2009).

5.9. Central nervous system activity

Mahomed et al. (2005) reported that *H. procumbens* secondary tuber aqueous extract (10–1000 μg/ml) possessed anticholinesterase activity and can significantly provoke atropine-sensitive contractions of chick-isolated oesophagus and guinea-pig isolated ileum in a concentration-related manner. Researchers speculated that the contractile effects of the extract on the isolated gastrointestinal smooth muscle contributed, at least in part, to the anticholinesterase action of *H. procumbens*.

Mahomed and Ojewole (2006a) examined the anticonvulsant activity of *H. procumbens* secondary tuber aqueous extract against pentylenetetrazole (PTZ)-, picrotoxin (PCT)- and bicuculline (BCL)-induced seizures in mice with phenobarbitone and diazepam used as reference drugs. *Harpagophytum procumbens* extract (100–800 mg/kg) significantly delayed the onset of, and antagonised, PTZ-induced seizures, profoundly antagonised PCT-induced seizures, and only partially and weakly antagonised BCL-induced seizures. The average time of onset of convulsions was delayed, while the average duration was significantly reduced. It was hypothesised that the mode of action of anticonvulsant activity was the enhancement of GABA-ergic neurotransmission and/or facilitating GABA-ergic action in the brain, but the evidence was inconclusive. However, the ability to suppress the central nervous system may be linked to its anticonvulsant activity. It was noted that some rural communities in South Africa use *H. procumbens* in the management of childhood convulsions and epilepsy, which may be supported by the results from this study though more studies should be performed to provide conclusive evidence (Mahomed and Ojewole, 2006a).

5.10. Uterotonic activity

Some traditional health practitioners claim that *H. procumbens* secondary tuber is useful in obstetrics to induce or accelerate labour and to expel retained placentas, a use that is supported by the new ethnopharmacological data presented in this review.

Non-pregnant and pregnant female rats (young adult) were used to source longitudinal, tubular uterine horn muscle strips to investigate this traditional use of *H. procumbens* and it was shown that administration of moderate to high concentrations (200–1000 μg/ml) provoked powerful contractions. Concentration-related and significant increases in baseline tone as well as pronounced rhythmic, myogenic contractions of oestrogen-dominated rat longitudinal uterine horn muscle strips taken from stilboesterol-pretreated, non-pregnant female rats were observed with low to high concentrations of extract (10–800 μg/ml). These dosages also caused significant concentration-dependent increases in the baseline tone and contracted, longitudinal, tubular uterine horn muscle strips in the early, middle and late stages of pregnancy. The results revealed that *H. procumbens* has a spasmogenic and uterotonic effect on mammalian uterine muscles (Mahomed and Ojewole (2006b). This study suggests that the folkloric obstetric use of this plant for the induction of labour may be justified, although more scientific studies are required to prove this.

5.11. Clinical studies

Several clinical studies have been performed to determine the effectiveness of *H. procumbens* for its use as anti-inflammatory, general analgesic (commonly for lower back pain) and

antirheumatic agent. Moussard et al. (1992) investigated the effect on arachidonic acid metabolism after daily administration 2.0 g powder (500 mg/capsule) containing 3% total glucoiridoids to healthy volunteers ($n=25$) for 21 days. They concluded that *H. procumbens* does not have a similar effect as NSAIDs in healthy humans as statistically significant changes in the biochemical parameters monitored were not noted. The results are given in ng/ml serum before and after administration: $PGE_2=2.1$ *vs.* 3.2; $TXB_2=147$ *vs.* 143; $LTB_4=3.4$ *vs.* 3.8 and 6-keto-PGF_1 alpha=4.4 *vs.* 4.2) (Moussard et al., 1992). To determine the effectiveness on lower back pain, *Harpagophytum* extract WS1351 was administered in two daily doses of 600 and 1200 mg containing 50 and 100 mg of harpagoside, respectively, and compared to placebo. This randomised double-blind study took place over 4 weeks and subjects ($n=197$) with chronic susceptibility to back pain and current exacerbations with intense pain were included. Out of 183 subjects that completed the trial, six in the 600 mg and 10 in the 1200 mg were reported 'pain-free' without using Tramadol® (rescue pain medication). However, data analyses suggested that the 600 mg group reaped more benefit where less severe pain and no radiation or neurological deficit was present. The patients with more severe pain tended to use more Tramadol® but not to the maximum permitted dose (Chrubasik et al., 1999).

Warnock et al. (2007) conducted a single group, open, 8-wk clinical study in the United Kingdom on 259 patients suffering from arthritis and other rheumatic conditions (AORC) where pain (rated 2–7 out of 10) was present at least 2 days per week in the affected area during the previous 8 weeks. A. Vogel Rheuma Tabletten® containing 480 mg of *H. procumbens* dried extract each was self-administered every morning and evening (960 mg/d) with meals and the effectiveness assessed for 207 patients. From baseline to week 4 and 8, a significant reduction ($p<0.0001$) was noted in the global mean scores for pain, stiffness and function. From baseline to week 8, mean scores for pain in the back, hip, knee, hand, wrist and elbow were significantly reduced ($p<0.05$). A rating of good or excellent was recorded by 120 patients (54.1%) and perceived in 118 patients (53.2%) by investigators. The dosage form was well-tolerated and only 11 patients (4.2%) discontinued treatment due to adverse events, mainly gastrointestinal complaints. Concomitant analgesic use was assessed in 154 patients: 44.8% reduced their dosage, 26.0% stopped taking analgesics, 16.9% took the same dosage and 9.1% increased their dosage (Warnock et al., 2007).

A review of the clinical trial literature from 1966 to 2006 on the efficacy of *H. procumbens* in the treatment of osteoarthritis was reported by Brien et al. (2006), who identified 14 relevant studies: eight observational studies; two comparator trials (one open, the other randomised); and four double-blinded, placebo-controlled, randomised controlled trials. The authors found that while many of the published trials did not conform to important methodological quality criteria, the data from the higher quality studies indicated that Devil's Claw appeared effective in the reduction of the main clinical symptom of pain. The assessment of clinical evidence for safety was limited by the small populations included in the clinical studies. Systematic reviews of primary research in human health care and health policy by the Cochrane Collaboration (Cochrane Reviews) are internationally recognised as the highest standard in evidence-based health care. A Cochrane Review of herbal medicine used to treat lower back pain found two high-quality clinical trials utilising *H. procumbens*. Strong evidence for short-term improvements in pain for daily doses of *H. procumbens* standardised to 50 mg or 100 mg harpagoside was found. Another high-quality trial demonstrated relative equivalence of the *H. procumbens* product to 12.5 mg/d of the pharmaceutical anti-inflammatory rofecoxib (Gagnier et al., 2007). All the clinical studies performed on *H. procumbens* were reviewed in 2009 by Grant et al.

6. Biopharmaceutical aspects

Biopharmaceutics is the study of the physicochemical properties of drugs and their proper dosage as related to the onset, duration and intensity of drug action. These are governed by pharmacokinetics which includes administration, distribution, metabolism and excretion. For each of those there are limiting factors that ultimately affect the bioavailability of drugs (Panchagnula and Thomas, 2000). The bioavailability of a drug injected directly into the bloodstream is 100%; however for orally administered drugs it is highly variable. Some drugs undergo extensive hepatic first-pass metabolism thereby reducing bioavailability of the drug. Other drugs may be slowly excreted thus prolonging the duration of action (Lobenberg and Amidon, 2000).

Several researchers have performed studies to investigate biopharmaceutical aspects of *H. procumbens*. Abdelouahab and Heard (2008b) investigated the dermal and transcutaneous delivery of the major glycosides of *H. procumbens* tuber extract across porcine ear skin from a range of different vehicles. All the glycosides tested permeated the skin and the lowest molecular weight harpagide exhibited the highest permeation from an ethanol/water solution. In a subsequent study, Ouitas and Heard (2009) tested the potential transcutaneous anti-inflammatory effect of the major active components of topically applied *H. procumbens* using *ex vivo* skin. After transcutaneous delivery, the receptor phase at 24 h contained harpagoside (0.8 μmol/ml), harpagide (25 μmol/ml), acteoside (1.8 μmol/ml) and 8-coumaroylharpagide (3×10^{-3} μmol/ml). Although this solution did not have a significant effect on either 5-LOX or iNOS on application to the skin, the expression of COX-2 and PGE_2 was effectively inhibited. The hydrolysed products of the iridoid glycosides harpagide and harpagoside have significant anti-inflammatory activity when compared to the unhydrolysed compounds. A recent study shows that hydrolysed products of harpagide and harpagoside had a significant COX-2 inhibitory activity (2.5–100 μM) whereas unhydrolysed harpagide and harpagoside did not. Therefore, the hydrolysis of the glycosidic bonds of harpagide and harpagoside by β-glucosidase is a prerequisite step for activity (Zhang et al., 2011).

The cytochrome (CYP) 450 isozyme and the multidrug transporter ABCV1/P-glycoprotein systems are the chief mediators of herb–drug interaction. CYP450 is the most important phase I drug-metabolising system and induction or inhibition is known to cause adverse drug reactions as many drugs are metabolised or cleared through this enzyme system (Izzo, 2004; Williamson, 2006). Multidrug transporter is a closely related family (ABC transporters) of an integral membrane glycoprotein that exports a variety of solutes from the cytoplasm and these are mostly involved in drug efflux and influx (Williamson, 2006). P-glycoprotein is involved in the absorption, distribution and excretion of drugs as it is present in the intestine, liver and kidney. It affects these processes by limiting the cellular transport from the intestinal lumen into epithelial cells and by enhancing the excretion of drugs out of hepatocytes and renal tubules into the adjacent luminal space (Lin and Yamazaki, 2003). Unger and Frank (2004) reported that *H. procumbens* extract had an inhibitory effect on six human CYP450 Sf9 enzymes. Recently, Romiti et al. (2009) investigated the effects of *H. procumbens* extract on multidrug transporter ABCB1/P-glycoprotein (P-gp) using the cultured human kidney (HK-2) proximal tubule cell line. The results indicated that commercial preparations of Devil's Claw inhibited P-gp activity, while pure harpagoside did not. Prolonged exposure to either *H. procumbens* extracts or pure harpagoside produced a significant, dose-dependent increase in P-gp expression. Thus, it can be concluded that *H. procumbens* influences both activity and expression of the transporter, while harpagoside modulates only its expression (Romiti et al., 2009). Consequently, *H. procumbens* may affect drugs metabolised by the CYP450 system, possibly leading to toxicity.

7. Toxicity

An assessment report on *H. procumbens* and *H. zeyheri* secondary tuber for human use was prepared by the European Medicines Agency based on a review of available scientific studies (EMEA (European Medicines Agency), 2009). Acute toxicity studies in mice revealed low toxicity. The LD_0 values of aqueous, methanolic and butanolic extracts were greater than 4.6 g/kg and 1.0 g/kg for oral and intravenous administration, respectively. Intraperitoneal administration of harpagoside and harpagide to mice showed LD_{50} values of 1 g/kg and 3.2 g/kg, respectively. Sub-acute repeated-dose toxicity studies revealed no significant haematological or gross pathological findings or signs of hepatotoxicity. However, the assessor questioned the validity of the results of this study due to the lack of study details (EMEA (European Medicines Agency), 2009). Gyurkovska et al. (2011) recently determined the cytotoxicity of *H. procumbens* extracts and purified substances using murine peritoneal macrophages and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). β-OH-verbascoside, martynoside and harpagide reduced the number of viable cells at the highest test concentration of 1 mg/ml with harpagide exhibiting the most toxic effect.

Vlachojannis et al. (2008) reviewed the safety of *Harpagophytum* preparations used for osteoarthritic and lower back pain in humans. Studies dating back to 1985 were included and of the 28 clinical trials assessed, 20 stated minor adverse events. Over periods of up to 1 year, 6892 patients used these products in double blind ($n=615$) or observational ($n=6277$) trials. The 3% overall adverse event rate included mainly gastrointestinal effects, such as diarrhoea. *Harpagophytum procumbens* bitter components stimulate the production of gastrointestinal juices. Consequently, patients with a sensitive stomach feel uncomfortable when consuming these products and the use of *H. procumbens* is contraindicated in patients with stomach or duodenal ulcers (Chantre et al., 2000; Vlachojannis et al., 2008). *Harpagophytum procumbens* decreased heart rate and arterial blood pressure in rats and exhibited a negative inotropic effect on isolated rabbit hearts. It has been suggested that it may cause QT prolongation and abnormal heart rhythms as well as influence calcium currents (verapamil-like effect). Patients using medication to treat cardiovascular disorders should be warned about possible interactions but cardiovascular effects seem unlikely based on current data (EMEA (European Medicines Agency), 2009). Both the systematic review and the assessment report concluded that the toxicology data available for *H. procumbens* extracts is insufficient, does not guarantee safety for human use, and that repeated-dose and chronic toxicity studies as well as reproductive, genotoxicity, mutagenicity and carcinogenicity studies are urgently required (Vlachojannis et al., 2008; EMEA (European Medicines Agency), 2009).

8. Quality control (QC) aspects

A plant species can usually be easily identified by taxonomists when the flowers and fruits are present, but the identification of leaf or root material is more challenging, especially when the plant material has been processed (*e.g.* powdered). Plant anatomy and phytochemistry have been used for many years by pharmacognosists as tools to authenticate plant material (Vieira et al., 2003). For *Harpagophytum* spp., it is said that the fruits of the plants are the only reliable method of identification. However, this method is unreliable due to morphological variation in fruit capsules. *Harpagophytum zeyheri* is characterised by two seed rows on both of its seed loculus while *H. procumbens* has four seed rows on both of its seed loculus (Muzila et al., 2011). Muzila et al. (2011) performed multivariate analysis of Devil's Claw fruits; the study inferred the existence of introgression between *H. procumbens* and *H. zeyheri*. Useful analytical methods include both quantitative and qualitative techniques such as infrared (IR) spectroscopy, high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC) and gas chromatography (GC) (Calixto, 2000). HPLC, nuclear magnetic resonance (NMR) and hyphenated chromatographic and spectroscopic techniques such as LC–UV–MS are amongst the most extensively used analytical methods in the pharmaceutical industry (Phillipson, 2007). Liquid chromatography coupled with mass spectroscopy (LC–MS) is one of the hyphenated methods that have recently been explored in chromatographic fingerprinting for quality evaluation of complex herbal medicines.

8.1. Chromatographic fingerprinting

Many orthodox chromatographic methods have been used to isolate and characterise compounds from *H. procumbens*; these methods coupled with newer techniques, such as mass spectrometry and nuclear magnetic resonance has yielded promising results. Seger et al. (2005) characterised isobaric iridoid glycoside regioisomers as the (E/Z) pairs of 6′-*O*-*p*-coumaroylharpagide (6′-PCHG) and 8-*p*-coumaroylharpagide (8-PCHG) from a methanol secondary tuber extract using a hyphenated LC–DAD–MS/SPE–NMR technique. Chigome et al. (2009) developed an HPLC–DAD method where the limits of detection and limits of quantification obtained were 1.08 μg/ml and 3.59 μm/ml for harpagoside and 1.70 μg/ml and 5.65 μml/ml for isoacteoside.

Schmidt (2005) developed a fast HPLC method for the quality control of *H. procumbens* with a monolithic silica column instead of the conventional particle-based C_{18} silica column based on an existing method. The variation between the two columns was insignificant, thereby providing an acceptable alternative QC method, especially useful in commercial quality control due to shorter analysis times. In addition, they noted that 8-*p*-coumaroyl-harpagide is present in *H. zeyheri* in much higher quantities, and that the ratio between the percentage of 8-*p*-coumaroylharpagide and the sum of harpagoside and 8-*p*-coumaroylharpagide is a useful measurement for the quality control as it can be used to distinguish between the two species. The ratio is below 10 in *H. procumbens* and above 31 in *H. zeyheri* (Schmidt, 2005).

Gunther and Schmidt (2005) developed high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) methods for the quantification of harpagoside. The secondary tubers of *H. procumbens* were used to prepare 15 different carbon dioxide (CO_2) extracts. Harpagoside was detected at 278 nm with the HPLC method and at 509 nm after derivatisation for the HPTLC method. The results revealed good accuracy, reproducibility and selectivity for the quantitative analysis of harpagoside and there were no statistically significant differences between the two methods, although a trend to slightly lower mean values was noted for the HPTLC method. Wagner et al., 2008 developed a precise and accurate HPTLC method for harpagoside quantification. This method may be used for the general quality control of *H. procumbens* based on harpagoside content. In addition, the proposed method is rapid and cost-efficient as up to 16 samples can be analysed per plate (Wagner et al., 2008).

8.2. Vibrational spectroscopy

Harpagophytum procumbens is being intentionally adulterated with the less efficacious *H. zeyheri* necessitating the development of rapid and simple authentication and validation methods for *H. procumbens* raw materials and products. Schulz and Baranska

(2007) used FT-Raman spectroscopy to identify and quantify harpagoside in secondary tuber of *H. procumbens* and phytopharmaceutical products (ethanolic extracts and tablets). *Harpagophytum procumbens* spectra show several key bands in the frequency range between 1600 and 1700 cm^{-1} which correspond well to the signals of pure harpagoside and are assigned to –C=O–, –C=C– and benzene ring stretching vibrations. Raman mapping revealed the spatial distribution of harpagoside within the different samples. A reliable multivariate calibration model to be used for harpagoside quantification was developed based on the obtained spectral data and reference HPLC values.

9. Commercial aspects

Namibia has been the dominant producer and exporter of *H. procumbens*, accounting for between 85% and 99% of total exports (Stewart and Cole, 2005). Lesser amounts are sourced from South Africa and Botswana (Raimondo and Donaldson, 2002) and the majority of harvested dried secondary tubers are exported to Europe. Commercial harvesting and trade of *H. procumbens* in Namibia started in 1962 when the Namibian company Harpago Pty Ltd. began exporting dried secondary tubers to the German company Erwin Hagen Naturheilmittel GmbH (Stewart and Cole, 2005). The global annual market increased considerably, peaking at 900,000 kg. *Harpagophytum procumbens* exports vary annually as well as seasonally. Market demand has been highly variable, possibly due in part to stockpiling by overseas companies, but although the trade is erratic, there has been a steady increase in export volume (Wynberg, 2004).

By 2001, *H. procumbens* had become the third-most-frequently used medicinal plant in Germany and enjoyed a growth of 113% between 1999 and 2000, and an additional 59% between 2000 and 2001 (Strobach and Cole, 2007). The demand for Devil's Claw has declined since 2002 for a number of possible reasons that may include: (1) The proposed listing of *H. procumbens* in Appendix II by the Convention of International Trade in Endangered Species (CITES) in April 2000 raised concerns about the sustainability of wild plant stocks. This may have prompted some manufacturers and marketers to look at other anti-arthritic products, including glucosamine. (2) At the beginning of 2004, *H. procumbens* and a number of other natural products were removed from the German Medical Aid list which meant that prescriptions for these products would no longer be reimbursed by medical insurance. By mid-2004, the sales of herbal medicines in Germany (including *H. procumbens*) decreased by 50% (Strobach and Cole, 2007). (3) The introduction of the Traditional Herbal Medicines Products Directive 2004/24/EC released by the European Parliament and by the Council of Europe was a major step in the harmonisation of the regulation of medicines in Europe. These regulations came into force in April 2011 and require that all herbal medicines in the EU to undergo a regulatory process before entering the market, and only permits well-established herbal medicines such as *H. procumbens* to be sold to consumers throughout Europe if the manufacturer complies with stringent quality control procedures. Preparation for complying with these regulatory requirements has been prohibitively expensive for smaller companies, which have reduced the range of products they sell. (4) The global economic recession may have played a part in decreased sales of *H. procumbens* from late 2008. National income from *H. procumbens* exports in 2009 has been estimated at approximately €1.06 million or N$12.16 million. This is only a small fraction (less than 5%) of the value of annual global retail sales of *H. procumbens* products (Ridgway and Krugmann, 2011). Kathe et al. (2003) reported that 57 pharmaceutical products from the species are marketed by 46 different companies and have cumulative sale volumes worth approximately €30 million in Germany alone.

Devil's Claw harvesting is done in three ways: wild-harvesting where harvesting takes place according to traditional methods and is usually sustainable harvested; controlled/organised harvesting where a permit is needed and training regarding harvesting is provided; and commercial cultivation where cultivation and harvesting takes place on farms (cultivation is discussed in Section 2.3) (Grote, 2003). Organised harvesters practicing sustainable harvesting and/or being certified organic receive up to 2% of the retail market-value; close to half of what exporters receive, whereas at the lower end of the harvester spectrum informal harvesters may receive far less than 1% (Ridgway and Krugmann, 2011). These forms of market inequality have been instrumental in keeping prices low and are responsible for the fundamental inequity in the distribution of benefits to local harvesters from the global *H. procumbens* trade. This inequity, combined with the open-access nature of the resource and the extreme poverty of the harvesters, lies at the heart of the industry's problems, *inter-alia* encouraging over-harvesting and use of unsustainable harvesting methods (Ridgway and Krugmann, 2011).

Harpagophytum procumbens and/or its components have been included in several recent patent applications. One application describes the use of plant extracts, including Devil's Claw, to reduce pain, inflammation and/or stiffness associated with conditions such as arthritis and osteoarthritis (Rabovsky et al., 2011). Shikhman (2010) patented several combinations of pure compounds such as harpagoside and paeoniflorin for the treatment of pain, inflammation, arthritic conditions and other chronic rheumatic diseases, muscle spasms, and headache. Devil's Claw products are also widely commercially available for purchase in health shops as well as on the internet. *Harpagophytum procumbens* products are registered as Herbal Medicine in France and Germany to treat mainly arthritis and rheumatism or as Food Supplements in the United Kingdom, Netherlands, USA and Far East (Cole, 2003). The current European Scientific Cooperative on Phytotherapy (ESCOP (European Scientific Cooperative on Phytotherapy), 2003) monograph recommends *H. procumbens* preparations for symptomatic treatment of arthritis, back pain, lack of appetite and dyspepsia. *Harpogophytum procumbens* has also been widely used in European herbal tea formulations and ground secondary tubers are formulated into capsules, tablets, liquids extracts, topical ointments and infusions.

10. Conclusions

Harpagophytum procumbens has an ancient history of multiple indigenous uses and is one of the most highly commercialised indigenous traditional medicines from Africa, with bulk exports mainly to Europe where it is made into a large number of health products such as teas, tablets, capsules, and topical gels and patches.

While the phytochemistry of this plant has been well researched, there is a paucity of information on the additive or synergistic effects of the major compounds. These effects may play a role in the therapeutic outcome of treatment, and this information could assist in optimising extraction and standardisation processes. While infusions and decoctions are the most important traditional methods of preparation, the putative main active principles in the plant are prone to hydrolysis, suggesting that they may be pro-drugs, with implications for the development of suitable formulations. In the case of harpagide and harpagoside, one study revealed anti-inflammatory activity only after hydrolysis occurred (Zhang et al., 2011).

Investigations of the biological activities of *H. procumbens* have provided scientific support for many of the traditional uses, including fever, pain, arthritis, malaria, diabetes, labour and convulsions. Reviews of the clinical trials on *H. procumbens* (Gagnier et al., 2004, 2007; Brien et al., 2006) provide convergent supporting evidence for safety relative to pharmaceutical non-steroidal anti-inflammatory drugs, and efficacy in treating pain and inflammation in arthritis and lower back pain. However, the methodological quality of many of the existing clinical trials is poor, and further high quality clinical investigations on standardised and characterised products are necessary to provide definitive clinical evidence of safety and efficacy.

In the more than 100 years since G.H. Mehnert was introduced to *Harpagophytum* by the indigenous San and Khoi people, the plant has become an important African commodity in international trade, and is contributing to the health and well-being of people thousands of kilometres from its original habitat in the Kalahari sands of southern Africa. Modern technology and scientific and medical research, including analytical chemistry, molecular biology, pharmaceutics and clinical studies, are validating many of the indigenous uses of the plant.

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