Bioactive components of the uteroactive medicinal plant, *Gunnera perpensa* (or ugobo)

K.B. Brookes* and M.F. Dutton*

*Gunnera perpensa* L., FAMILY GUNNERACEae, is among the most frequently cited of about 90 species used by South African traditional healers in pregnancy-related medicines. Seven bioactive compounds were isolated from *Gunnera perpensa* roots, with five of these being novel to the species at the time of identification. These are 3,3′,4′-trihydroxybenzaldehyde, p-hydroxybenzaldehyde, and Z-methyl lespedezate (Fig. 1). Two phenolic glycosides, referred to as A and B, have been only partially characterized, and require further investigation.

A bonded phase δ-cyclodextrin column developed for sugar analysis* gave an excellent HPLC separation of compounds in *G. perpensa* extracts. A silica gel column fraction, eluted with CHCl3-MeOH and isolated as a brown gum, showed the following HPLC results (mobile phase 95:5 acetonitrile:water, 1 cm3 min–1): 3.45 min (1.1%), p-hydroxybenzaldehyde; 4.72 min (8.9%), Z-methyl lespedezate; 5.49 min (64.1%), compound A; 5.99 min (24.1%), compound B.

Compounds were identified by various criteria including UV, IR, 1H NMR, 13C NMR and mass spectroscopy. Full structural elucidation of Z-methyl lespedezate required 1H-H COSY (correlated spectroscopy), HMBC (heteronuclear multiple bond coherence) and NOE (nuclear Overhauser effect) spectral techniques. All percentage yields recorded are based on the dry root material.

Two compounds, 3,3′,4′-tri-O-methyl ellagic acid lactone (0.087%) and Z-methyl lespedezate (0.019%), were isolated directly from *G. perpensa* roots, whereas ellagic acid lactone (0.33%), 1,1′-biphenyl-4,4′-diacetic acid (0.11%) and p-hydroxybenzaldehyde were released by acid hydrolysis. The last compound was present in large amounts after hydrolysis of various *G. perpensa* extracts, and was also released by natural hydrolysis of extracts while standing in solution at room temperature. Glucose was also identified after hydrolysis using HPLC and TLC analysis and an authentic standard. Hydrolysis of approximately 20 g of a hot methanol/water extract was carried out at 90°C in 150 cm3 1 M HCl for 3 h, followed

---

*School of Chemistry, University of KwaZulu-Natal, Durban 4041, South Africa.

*Faculty of Health Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, South Africa.

*Author for correspondence. E-mail: brookes@ukzn.ac.za
by extraction with ethyl acetate. The resulting brown gum, obtained after ethyl acetate removal, contained over 90% of p-hydroxy-benzaldehyde by HPLC analysis. In addition, it was identified by HPLC analysis of G. perpensa extracts using five different solvents, but was present in the greatest amounts in the ether (55%) and chloroform extracts (84%), respectively.

**Bioactivity of components**

Most of the isolated components have documented biological activities that could be beneficial during pregnancy and birth, and that link to some of the reported health effects attributed to this species by healers. For example, 3,3',4'-tri-O-methyl ellagic acid lactone, also isolated from Combretum kraussii, has antihaemorrhagic properties. Ellagic acid lactone has antimutagenic and anticarcinogenic properties. It was found to inhibit significantly the potent mutagen aflatoxin B1, using a Salmonella microsuspension assay. Z-methyl lespedezate is a derivative of p-coumaric acid, the latter being commonly found in both woody and herbaceous monocotyledons. The biologically active potassium salt of lespedezic acid, isolated from Lespedeza cuneata, is responsible for the leaf-opening mechanism in nyctinastic (‘night closing’) plants. It is interesting to note that the glucose moiety is required for this activity, and that in the evening, potassium lespedezate is deactivated by enzymatic hydrolysis with β-glucosidase to yield 4-hydroxyphenylpyruvate and glucose.

All compounds isolated in this study are phenolic, and could contribute to the antiseptic properties documented for this species. Phenolic compounds are also generally known for their anti-oxidative properties, which could benefit both the fetus and mother during pregnancy. For example, the phenolic vitamin E, necessary for reproduction in rats, and noted as the ‘child-birth vitamin’, is an antioxidant that protects important compounds, including vitamin A, from degradation. Many molecules in human tissue are susceptible to homolysis to form dangerous reactive alkyl radicals. Autoxidation of polyunsaturated fats and oils occurs via a chain mechanism generating peroxyl radicals that cause no further damage. Thus harmful mutagenic processes that could cause cell damage during fetal development could be inhibited by free radical sponges such as ellagic acid and the other phenols in this plant. Ellagic acid has been found to provide better protection than vitamin E against chemically induced fetal growth retardation and oxidative damage in groups of pregnant mice. Ellagic acid and vitamin E also block the effects of cocaine-induced adverse developmental effects/malformations using a rat whole embryo culture model. In a further study, ellagic acid was found to protect rat lymphocytes against nicotine-induced cellular and DNA damage. Doses of ellagic acid, ten times lower that those of the well-known anti-oxidant N-acetylcysteine, were required for maximum protection.

In addition, the non-toxic nature of aqueous G. perpensa extracts has been demonstrated at a cellular level using human fibroblast and monkey vero cell lines.

**Uteroactivity of extracts**

*In vitro* pharmacological bioassays related to uteroactivity were carried out using virgin Sprague-Dawley rats with the relevant ethical approval for animal experiments. Rats were oestrogenized with stilboestrol by intraperitoneal injection 24 hours prior to euthanasia with CO2 and dissection, following the procedures of Veale et al. and Kaido et al. Isolated strips of uterine smooth muscle tissue were pre-treated for 5 minutes with root fractions before the cumulative addition of the reference drug, acetylcholine. Utero-active root components induced isotonic uterine contractions that were recorded electronically. The temperature of the organ baths was maintained at 26°C because this has been found by Kumegai et al. to decrease spontaneous contractility.

Compounds A and B showed the greatest spasmytic effect on isolated uterine muscle tissue, as shown in Fig. 2. The A and B mixture augmented the response of the uterus to acetylcholine (ACh) over the full range of concentrations tested, showing greater uteroactivity than the water or methanol extracts. Z-methyl lespedezate was not isolated in sufficient quantities for uterine tests, and had not previously been reported in Gunneraceae at the time of isolation and identification, but was subsequently reported in an independent investigation concerning the contractility of uterine muscle. In the same study, a related major component, the phenylpropanoid Z-venusol, was found to induce a state of continuous contractility of the uterus.

There is a close relationship between p-hydroxybenzaldehyde, obtained by hydrolysis of various Gunnera perpensa fractions, and the principal ingredients of Filipendula ulmaria (L.) Maxim, or ‘meadowsweet’. Flower extracts of this plant have a high tannin content, and are prescribed during pregnancy. Among the known active ingredients of the flowers are phenolic glycosides, salicylaldehyde (7%), anisaldehyde (2%) and methyl salicylate (1.3%). The phenolic glycosides yield salicylate aglycones. Extracts of Filipendula flowers increased the tone of isolated strips of smooth muscle taken from guinea pig ileum, as well as that from rabbit intestine and uterus. In addition, an ointment prepared from Filipendula flowers was found to be effective in the treatment and prevention of uterine cervical cancer for 67% of the patients tested.
Conclusions

Uterine tests proved conclusively that the partly characterized phenolic glycosides A and B strongly enhance the response of uterine muscle tissue to acetylcholine. Various extracts contained p-hydroxybenzaldehyde, closely related to known active phenolic ingredients from *Filipendula ulmaria*, used medicinally to ‘tone’ the uterus during pregnancy.

The seven compounds isolated are all phenolic, and such compounds are generally known for their anti-septic and anti-oxidative properties. The recorded antihaemorrhagic, antimutagenic and anticanicogenic activities of some of the compounds identified signify that they could be beneficial during pregnancy and birth. The described bioactivities link to some of the documented properties attributed to this species, endorsing the confidence of traditional healers in the safety, efficacy, and health benefits of this medicinal plant for pregnant women.  

The authors gratefully acknowledge the valuable contribution of L.C. Katsoulis, who conducted all pharmacological tests, related to uteroreactivity, in the Pharmacology Department, University of the Witwatersrand.