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

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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′-tri-*O*-methyl ellagic acid lactone, ellagic acid lactone, 1,1′-biphenyl-4,4′-diacetic acid, *p*-hydroxybenzaldehyde and Z-methyl lespedezate. The known antihaemorrhagic, antimutagenic and anticarcinogenic activities of some of these compounds signifies that they could be beneficial during pregnancy and birth. Two partially characterized phenolic glucosides strongly enhanced the response of isolated rat uterine tissue to acetylcholine. All compounds isolated in this study are phenolic, and such compounds are generally known for their antiseptic and anti-oxidative properties, which could benefit both the fetus and mother during pregnancy. The bioactivities described link to some of the documented properties attributed to this species, therefore supporting confidence of traditional healers in the safety, efficacy, and health benefits of this medicinal plant.

Introduction

Gunnera perpensa L. is one of six subgenera of the Gunneraceae family, which is essentially restricted to the southern hemisphere. It is usually associated with water and grows wild near river banks, often being referred to as the 'river pumpkin'[1] or *ugobo* (Zulu). The Zulu, Xhosa and Sotho people of South Africa use the soft fleshy roots medicinally for a variety of purposes, such as treating diseases of the urinary tract, and as a remedy for impotence and barrenness.[2] Root decoctions are commonly regarded as safe for human consumption, and are credited with many benefits associated with pregnancy and childbirth, including good fetal development, a quick, easy labour, as well as expulsion of the afterbirth.[3] They are also used in veterinary science to speed up labour in animals. Decoctions are additionally used to dress wounds, treat colds, or to give animals protection from tick bites and other parasites,[1] which indicates that there may be antiseptic and/or antibiotic properties associated with these solutions. Extracts of *Gunnera perpensa* are traditional remedies for treating endometritis in both humans and animals. Tests against four different bacterial strains showed that such extracts possess weak anti-bacterial activity. This activity, in conjunction with the known uterotonic activity, may contribute to the overall efficacy of the medication.[4]

Gunnera perpensa is among the most frequently cited of about 90 species used by South African traditional healers in pregnancy-related medicines, or *isihlambezo*.[5] In the study reported here, several bioactive components with claimed health benefits for pregnant women were isolated from the plant.

Results and discussion

Gunnera perpensa roots were harvested at Silverglen Nature Reserve, Durban, and voucher specimen Brookes (1) NH was deposited at the National Botanical Institute, Durban. Seven compounds, purified by silica gel column chromatography, were isolated from methanol extracts of *G. perpensa* roots, with five of these being novel to the species at the time of identification. These are 3,3′,4′-tri-O-methyl ellagic acid lactone, ellagic acid lactone, 1,1′-biphenyl-4,4′-diacetic acid, *p*-hydroxy-benzaldehyde, and Z-methyl lespedezate (Fig. 1). Two phenolic glycocides, 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[6] gave an excellent HPLC separation of compounds in *G. perpensa* extracts. A silica gel column fraction, eluted with $CHCl_3$-MeOH and isolated as a brown gum, showed the following HPLC results (mobile phase 95:5 acetonitrile:water, 1 cm^3 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, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Full structural elucidation of Z-methyl lespedezate required ^{1}H-^{1}H COSY (correlated spectroscopy), HMBC (heteronuclear multiple bond coherence) and NOE (nuclear Overhauser effect) spectral techniques.[7] All percentage yields recorded are based on the dry root material.

Two compounds, 3,3′,4′-tri-*O*-methyl ellagic acid lactone (0.057%) and Z-methyl lespedezate (0.019%), were isolated directly from the methanol extract, 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 cm^3 1 M HCl for 3 h, followed

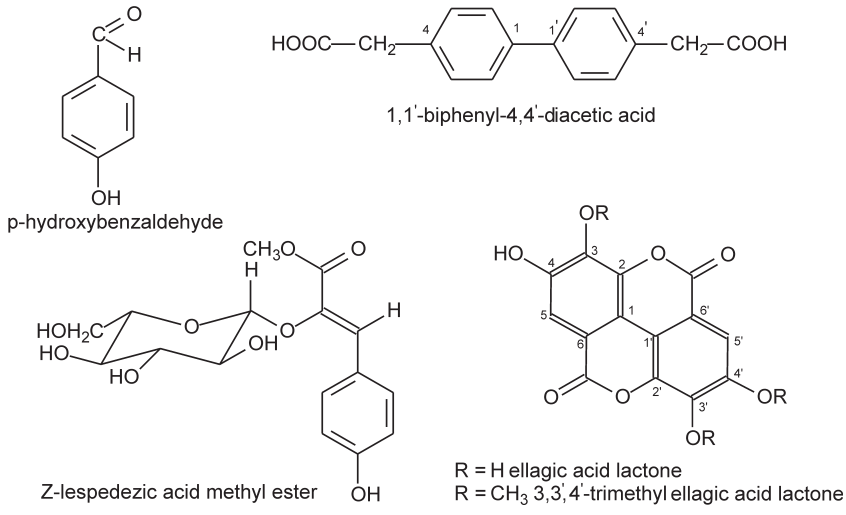

Fig. 1. Compounds isolated from *Gunnera perpensa*.

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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*,[8] has antihaemorrhagic properties.[9] Ellagic acid lactone has antimutagenic and anticarcinogenic properties. It was found to inhibit significantly the potent mutagen aflatoxin B_1 using a *Salmonella* microsuspension assay.[10] Z-methyl lespedezate is a derivative of *p*-coumaric acid, the latter being commonly found in both woody and herbaceous monocotyledons.[11] The biologically active potassium salt of lespedezic acid, isolated from *Lespedeza cuneata*, is responsible for the leaf-opening mechanism in nyctinastic ('night closing') plants.[12] 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,[13] 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 and reactive alkyl radicals. Autoxidation of polyunsaturated fats and oils occurs via a chain mechanism generating peroxyl radicals at the reactive methylene site between two double bonds. These radicals could cause irreversible cell damage, but phenolic compounds such as vitamin E react rapidly with peroxyl radicals to form more stable electronically delocalized radicals that cause no further damage.[14] 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.[15] Ellagic acid and vitamin E also block the effects of cocaine-induced adverse developmental effects/malformations using a rat whole embryo culture model.[16] 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.[17]

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.[18]

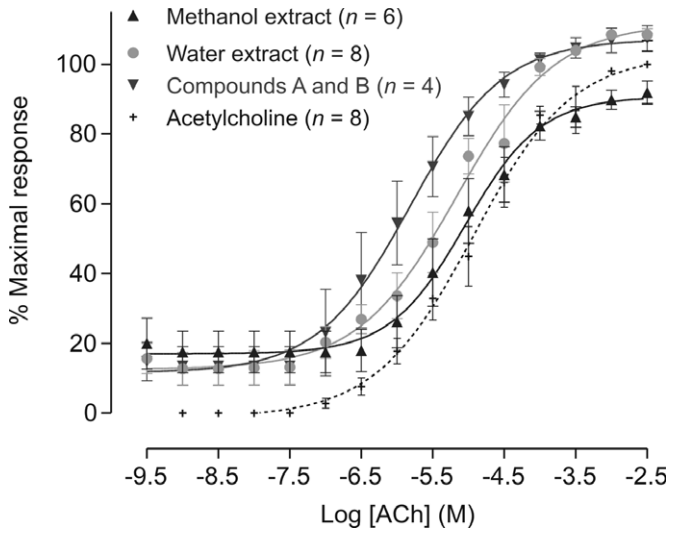

Fig. 2. Dose-response curves of fractions from *Gunnera perpensa*, which augment the response of isolated uterus to acetylcholine. The organs were incubated with a solution of 1.3 mg cm ^{3} of each extract.

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 CO_2 and dissection, following the procedures of Veale *et al.*[19] and Kaido *et al.*[20] 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. Uteroactive 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.*[21] to decrease spontaneous contractility.

Compounds A and B showed the greatest spasmogenic 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.[22] 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'.[23] 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 (75%), 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.[23]

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 antiseptic and anti-oxidative properties. The recorded antihaemorrhagic, antimutagenic and anticarcinogenic activities of some of the compounds identified signifies 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.[24]

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Rocky roadmap to the future — *Continued from page 178*

not the best conditions in which to protect the environment from the ground up, yet they present abundant opportunities to improve people's quality of life and save natural resources through the introduction of new, cheap technology (to replace the widespread use of inefficient coal-burning domestic fires that pollute urban air and create such a health hazard, for example).

And how is the private sector to play a role, as a major user of ecosystem services and in a powerful position to influence environmental change? The mining industry, for instance, is under financial pressure to supply minerals for the local and especially the export market. What is the tradeoff between jobs and foreign exchange earnings on the one hand, and the price to be paid in degraded land, polluted water courses and, in the case of coal (the second-largest foreign exchange earner after gold), the generation of greenhouse gases? At what stage does expensive but efficient precision irrigation get introduced more widely in agriculture—a primary consumer of water in a country facing deficits—to secure food supplies and reduce the demand for fertilizer that ends up poisoning our rivers?

Having eliminated any thought of complacency about the future of our environment, the *South Africa Environment Outlook* reminds the reader that this country has a robust economy, good environmental policies and laws, and the ability to harness science and technology in the service of sustainable economic development. Read it for yourself and see if you agree. ❑