Journal of Medicinal Plants Research Vol. 6(42), pp. 5460-5463, 3 November, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.005
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Antioxidant activities of crude extracts from medicinal plants used by diabetic patients in Eastern Botswana

Motlhanka D. M. T.* **and Mathapa G.**

Botswana College of Agriculture, Basic Sciences Department, Medicinal Plant Research, Bag 0027, Gaborone, Botswana.

Accepted 14 April, 2011

The antioxidant activities of water extracts of *Myrothamnus flabellifolius* (shoot) and *Ozoroa paniculosa* (roots) and their combinations called "Danzoria"(equal proportion of extracts from the two plants*)* were evaluated spectrophotometrically using the DPPH free radical scavenging assay. At 25 µg/ml the free radical scavenging power of the extracts were as follows: *M. flabellifolius* alone (66%); *O. paniculosa* root alone (49%); *O. paniculosa* stem alone (81%); *O. paniculosa* leaf alone (79%). At the same concentration (25 µg/ml) their combination (Danzoria) exhibited the following radical scavenging potencies: *M. flabellifolius* + *O. paniculosa* root (85%); *M. flabellifolius* + *O. paniculosa* stem (91%); *M. flabellifolius* +*O. paniculosa* leaf (91%). At 100 µg/ml and above there were no significant differences between scavenging potencies for individual extracts and their combinations. The scavenging potencies were in magnitudes of 90-91%. These plants and their combinations contain antioxidant compounds and their free radical scavenging activity may be responsible for their antidiabetic properties as advocated in traditional medicine.

Key words: Free radical scavenging, *Myrothamnus flabellifolius*, *Ozoroa paniculosa*, antidiabetic, Eastern Botswana

INTRODUCTION

Myrothamnus flabellifolius (shoot) and *Ozoroa paniculosa* (roots, stem, leaves) and their combination called "*Danzoria*" (equal proportions of the above plant extracts) are being used extensively in eastern Botswana traditional medicine. Water extracts of these plants have found reputable use as anti-diabetic preparations. *O. paniculosa* is used to treat diabetes, asthma, period pains, gout and backache (Motlhanka, 2008). *M. flabellifolius* is used to treat diabetes, hypertension and stroke. Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both type I and type II diabetes mellitus (Maritim et al., 2003). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, lipid peroxidation, and development of insulin resistance (Maritim et al., 2003). These consequences of oxidative stress can promote the development of complications of diabetes mellitus. This study aims at evaluating the free radical scavenging potency of *M. flabellifolius* and *O. paniculosa* and "*Danzoria*" and verifies the scientific basis of their use in the treatment of diabetes.

Oxidative stress in diabetes

The term oxidative stress refers to a condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS). Under physiological conditions, the molecular oxygen undergoes a series of reactions that ultimately lead to the generation of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). Oxygen free radicals (OFR) can be form by reaction of

*Corresponding author. E-mail: motlhankadan@yahoo.com, dmotlhan@bca.bw. Tel: 002673650221. Fax: 002673928753

glycated proteins with molecular oxygen. OFR are harmful to polyunsaturated fatty-acids of lipid membranes, proteins, sugars and de-oxyribonucleic acid (DNA). Free radical activity has been implicated in the development of diabetic vascular complications in type I diabetes (Collier et al., 1990). The most significant complications of diabetes, for example polyuritis, retinopathy, microangiopathy, perforating ulcers, impaired healing may depend on the excessive production of OFR by glycated proteins (Gillery et al., 1989). Free radical mechanisms are increasingly being implicated in the pathogenesis of tissue damage in diabetes. Various sources of free radicals may modulate oxidative stress in diabetes, including non-enzymatic glycosylation of proteins and monosaccharide autooxidation (Sinclair et al., 1992). Other sources of free radicals in diabetes have been proposed, such as nitric oxide synthase. Cosentino et al. (1997) reported that high glucose increases nitric oxide synthase expression in aortic endothelial cells. In another interesting series of articles, Jain and colleagues found that ketone bodies especially acetoacetate are involved in free radical formation in type I diabetes (Jain et al., 1998a; b). Increased free radical activity in diabetes mellitus may contribute to the higher prevalence and mortality in macrovascular disease in diabetic patients (Mac Rury et al., 1993). The link between oxidative stress and diabetes has necessitated the need to search for anti-diabetic free radical scavengers from medicinal plants.

MATERIALS AND METHODS

Chemicals and other materials

2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) (Sigma), positive controls: [(-) epicatechin, L-ascorbic acid]. Silica 60 coated Aluminium thin layer chromatography plates (Merck Si gel of thickness 200 µm). Cuvettes (path length 1 cm), UV spectrophotometer, all other chemicals used were of analytical grade.

Plants and sample preparation

The plants were authenticated by comparison with herbarium sample at the Botswana National herbarium and gallery, Gaborone, Botswana, where voucher specimen has been deposited.

Myrothamnus flabellifolius Welw

M. flabellifolius (Myrothamnaceae) is small prostate, ascending or erect subshrub, growing up to 1 m, occasionally taller. The plants completely dry up in winter and seem to be dead but spring back to life within hours after the first shower of rain. It is this remarkable property to which the English name “resurrection plant” refers. The flowers are much reduced in small catkin like inflorescences. The plant grows between rocky outcrops with very shallow soils (Schneider et al., 1998) and for approximately half of the year it exists in a dehydrated quiescent state (Farrant and Kruger, 2001). *M. flabellifolius* occurs in a vast area stretching from Namibia in the west, through Botswana, to Zimbababwe and the northern parts of South Africa in the east (Kruger, 1998). A decoction of leaves and twigs is taken to alleviate pain, kidney problems, haemorroids and painful menstruation. Smoke from burning leaves is inhaled for chest pains and asthma (Hutchings, 1996).

Whole shoots of *M. flabellifolius* were collected from Tswapong Hills (Lecheng Village) in Eastern Botswana. The shoots were pounded into fine powder using the traditional mortar and pestle. The fine plant material was extracted with water in a soxhlet apparatus for 10 h. The resultant water extract was concentrated to dryness using a freeze drier.

Ozoroa paniculosa (Sond) R.Fern&A.Fern

*O. paniculosa (*Anacardiaceae) is a small to medium-sized deciduous tree with distribution ranging from Northern South Africa through Eastern Botswana to Zimbabwe. It often occurs on rocky hillsides. It is characterised by elliptic, grey to blue-green (above), silvery to silky leaves (below). Leaves have a broadly tapering apex with a bristle-like tip. This plant fruits is drupe, elliptic or kidney shaped, initially green with a small reddish brown spots ripening black and wrinkled.

The roots of *O. paniculosa* are used by the Batswapong tribe to treat an inflammed uterus and are believed to enhance female fertility.

Whole root, stem bark and leaves of *O. paniculosa* were harvested from Tswapong hills in Eastern Botswana under the supervision of a traditional healer. The roots, stem were chopped into small slices and sun-dried. The dried roots and stem bark were then independently crushed into course powder using mortar and pestle. The leaves were sun-dried and then ground into fine powder. The powdered material was separately extracted with water in a soxhlet apparatus for 8 h. The resulting three extracts (root extract, stem extract and leaves extract) were concentrated using freeze drier.

Preparation of “Danzoria”

1 mg of *M. flabellifolius* dried crude water extract was mixed with 1 mg of either dry root extract or dry stem extract or dry leaf water extract of *O. paniculosa*. This yielded the following combinations of “*Danzoria*”: (*M. flabellifolius* + *O. paniculosa* root), (*M. flabellifolius* + *O. paniculosa* stem) and (*M. flabellifolius* + *O. paniculosa* leaves).

Scavenging activity on DPPH radical

The free radical scavenging activity of aqueous extracts and their combinations were quantitatively assessed using the DPPH radical method adopted for spectrophotometry (Brand-Williams et al., (1995). Briefly, a 0.1 mM solution of DPPH in ethanol was prepared and a 1.0 ml of this solution was added to 0.5 ml of either individual plant extract or their combination (Danzoria) in different concentrations. After 20 min, the absorbance was measured at 525 nm. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH Scavenging activity (\%)} = [(A_0-A_1)/A_0]\times 100$$

Where A0 was the absorbance of the blank, that is no sample, DPPH solution only) and $\mathbf{A_1}$ was the absorbance in the presence of the test sample.

Statistical analysis

All experiments were performed at least three times with each

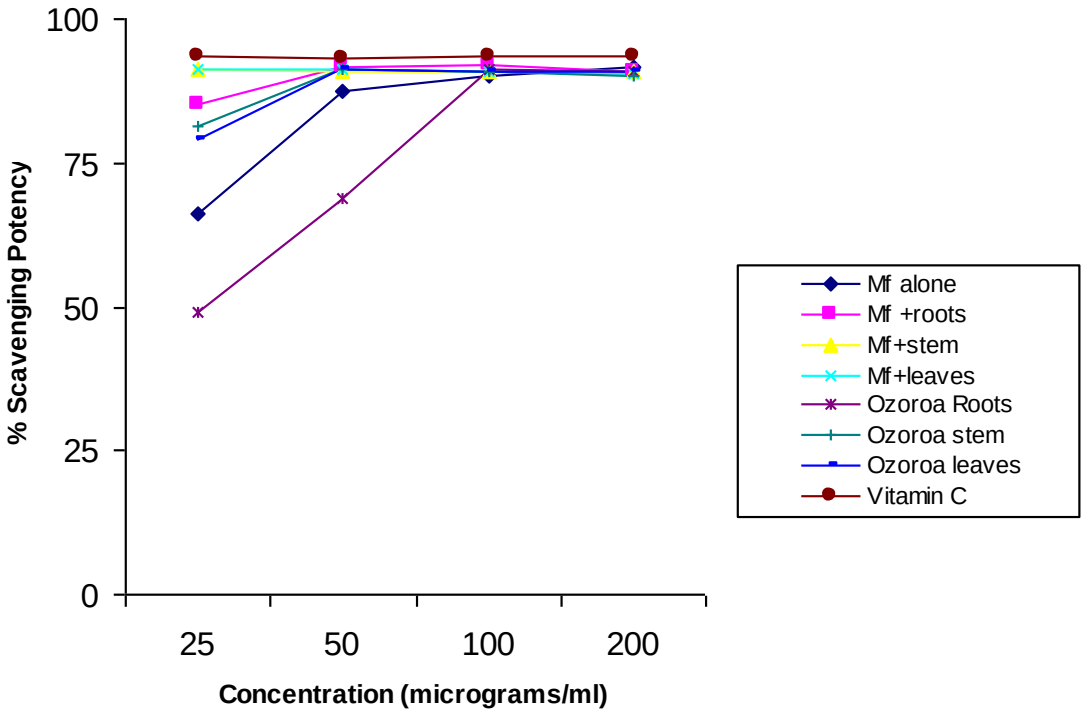

Figure 1. Free radical scavenging activities of individual plant extracts and "*Danzoria*".

assay in triplicate with means for each assay recorded.

RESULTS

The result of the above experiment is presented in Figure 1.

DISCUSSION

Free radical scavenging activities

DPPH test

The free radical scavenging activities of the tested extracts and their combinations were evaluated by the decrease in absorption of the stable 2,2-diphenyl -1-picryl –hydrazyl at 517 nm. The bleaching of DPPH absorption occurs when the odd electron of the radical is paired; thus it is a representative of the capacity of antioxidant compounds to scavenge free radicals (Cotelle et al., 1996)

As can be seen from Figure 1, at 25 µg/ml all Danzoria preparations exhibited a greater free radical scavenging activity (91.12%) than extracts of individual plants alone. At the same concentration, individual extracts of the plants exhibited the following free radical scavenging potencies: *M. flabellifolius* alone (66%); *O. paniculosa* roots alone (49%); *O. paniculosa* stem alone (81%); *O. paniculosa* leaves alone (79%). At 50 µg/ml and above, the scavenging potencies of all Danzoria preparartions where in same magnitude with control (Vit-C), 93% scavenging power.

These results may also suggest possible synergistic effect of "*Danzoria*" in the light of scavenging free radicals. These findings are in agreement with what has been proclaimed by most diabetic patients and traditional healers who have reported greater benefits from using "*Danzoria"* than using either of the plants alone. These plants may therefore, be useful in the treatment / management of pathologies in which free radical oxidation plays a fundamental role.

ACKNOWLEDGEMENTS

The authors acknowledge the Botswana College of Agriculture for providing the infrastructure for laboratory analysis. The traditional healers and the diabetic patients providing the ethno pharmacological information.

REFERENCES

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Libensittel Wissenscaft Technologie 28:25-30.

Collier A, Wilson R, Bradley H, Thomson JA, Small M (1990). Free

radical activity in type 2 diabetes. Diabet. Med., 7: (1) 27-30.
Cosentino F, Hishikawa K, Katusic ZS, Luscher TF (1997). High glucose increseases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 96: 25-28.
Cotelle N, Bernier J, Catteau J, Pommery J, Wallet J, Gaydou E (1996). Antioxidant properties of hydroxyl-flavones. Free radical Biol. Med. 20:(1) 35-43.
Farrant JM, Kruger L A (2001). Longevity of dry *Myrothamnus flabellifolius* in simulated field conditions. Plant Growth Regul. 35:109–120.
Gillery P, Monboisse JC, Maquart FX, Borel JP (1989). Does oxygen free radical increased formation explain long term complications of diabetes mellitus? Med. Hypotheses 29:(1) 47-50.
Hutchings A (1996). Zulu Medicinal plants. Natal University Press, Pietermaritzburg, South Africa.
Jain SK, Kannan K, Lim G (1998b). Ketosis (acetoacetate) can generate oxygen radicals and cause increased lipid peroxidation and growth inhibition in human endothelial cells. Free Rad. Biol. Med. 25:1083-1088.
Jain SK, Mc Vie R, Jaramillo JJ, Chen Y (1998a). Hyperketonemia (acetoacetate) increases the oxidizability of LDL and VLDL in type I diabetic patients. Free Rad. Biol. Med. 24:175-181.
Kruger LA (1998). Towards an understanding of desiccation tolerance in the resurrection plant *Myrothamnus flabellifolius* Welw. MSc thesis. University of Cape Town, Cape Town, South Africa.
Mac Rury SM, Gordon D, Wilson R, Bradley H, Gemmell CG, Paterson JR, Rumley AG (1993). A comparison of different methods of assessing free radical activity in type 2 diabetes and peripheral vascular disease. Diabet. Med. 10(4):331-335.
Maritim AC, Sanders RA, Watkins JB (2003). Diabetes,Oxidative stress, and antioxidants: a review: J. Biochem. Mol. Toxicol. 17(1):24-38.
Motlhanka DMT (2008). Free radical scavenging activityof selected medicinal plants of Eastern Botswana. Pak. J. Biol. Sci. 11(5):805-808.
Schneider H, Thurmer F, Zhu JJ, Wistuba N, Gessner P, Linder K, Herman B, Zimmermann G, Hartung W, Bentrap FW (1998). Diurnal changes in xylem pressure of the hydrated resurrection plant *Myrothamnus flabellifolius*. Evidence for lipid bodies in conducting xylem vessels. New Phytol. 143:471–484.
Sinclair AJ, Lunec J, Girling AJ, Barnett AH (1992). Modulators of free radical activity in diabetes mellitus; role of ascorbic acid. EXS, 62:342-352.