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## Free Radical Scavenging Activity of Selected Medicinal Plants of Eastern Botswana

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**Abstract:** Water and methanol extracts from roots of *Ozoroa paniculosa* (Anarcardiaceae), seeds of *Colophospermum mopane* (Caesalpiniaceae) and *Cucumis metuliferus* (Cucurbitaceae) ripe fruits were assessed for *in vitro* antioxidant activity. Free radical scavenging activity was measured spectrophotometrically as maximum fading power of DPPH at 525 nm. Water and methanol extracts of *Ozoroa paniculosa* exhibited higher scavenging potency than extracts of either *Colophospermum mopane* or *Cucumis metuliferus* at all tested concentrations. None of the extracts from *Cucumis metuliferus* exhibited any recognizable free radical scavenging activity. Above 50  $\mu\text{g mL}^{-1}$  both water and methanol extracts of *Ozoroa paniculosa* exhibited 91% scavenging activity similar to the control compounds L-ascorbic acid (91%) and (-) epicatechin (92%). Between 50-100  $\mu\text{g mL}^{-1}$ , water and methanol extracts of *Colophospermum mopane* exhibited scavenging potency of  $\leq 70\%$ . However, above 100  $\mu\text{g mL}^{-1}$ , both water and methanolic extracts of *C. mopane* exhibited scavenging activity  $> 70\%$ . Chloroform extracts of all the tested plants showed poor scavenging activity ( $< 30\%$ ). The order of scavenging potency for the tested samples was as follows: L-ascorbic acid  $\geq$  epicatechin  $>$  *O. paniculosa* (methanolic extract)  $>$  *O. paniculosa* (water extract)  $>$  *O. paniculosa* (ethylacetate extract)  $>$  *C. mopane* (methanolic extract)  $>$  *C. mopane* (water extract)  $>$  all extracts of *C. metuliferus*. These findings lend credence to the use of these plants as anti-inflammatory and antioxidant agents in folk medicine.

**Key words:** *Colophospermum mopane*, *Cucumis metuliferus*, *Ozoroa paniculosa*, free radical scavenging activity, DPPH radical

### INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, ageing process and perhaps dementias (Polterait, 1997). Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing (Aust *et al.*, 1993; Stohls, 1995). Several studies have described the antioxidant properties of medicinal plants, foods and beverages which are rich in phenolic compounds (which are known to serve as antioxidants (Brown and Rice-Evans, 1998; Krings and Berger, 2001). The importance of antioxidants in human health has become increasingly clear due to spectacular advances in understanding the mechanisms of their reaction oxidants (Bergman *et al.*, 2003). The present study investigates three plants used in Botswana herbal medicine, for their potential to scavenge free radicals and as a consequence, may be considered as effective sources for combating oxidative damage.

### MATERIALS AND METHODS

**Chemicals and other materials:** 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Sigma), positive controls: (Quercetin, (-) epicatechin, L-ascorbic acid), Silica 60 coated aluminium thin layer chromatography plates (Merck Si gel of thickness 200  $\mu\text{m}$ ). All other chemicals used were of analytical grade.

#### Plants and sample preparation

***Colophospermum mopane* (J. Kirk ex Benth) J. Leonard:** *Colophospermum mopane* (Caesalpiniaceae) is a shrub or medium sized to tall deciduous tree occurring in almost pure stands in hot, low lying areas, often on alluvial or lime rich soils. It bears flattened, oval indehiscent pods (fruit pods).

The seeds of *Colophospermum mopane* are used in traditional medicine to treat asthma attacks and facilitate wound healing. Dried seeds of *Colophospermum mopane* were collected from Seolwane village in Tswapong region in Eastern Botswana. The outer coating of the seeds was removed and discarded. The remaining inner seed

component was crushed into a sticky coarse material. This crushed material was extracted independently from the following solvents; methanol, chloroform, water in a soxhlet apparatus for 8 h. Extracts of chloroform and methanol were evaporated to dryness and concentrated using rotary evaporator. Water extract was concentrated using a freeze drier.

***Ozoroa paniculosa* (Sond) R. Fern and A. Fern:** *Ozoroa 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 a drupe, elliptic or kidney shaped, initially green with a small reddish brown spots ripening black and wrinkled.

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

Whole root of *Ozoroa paniculosa* was harvested from Tswapong hills in Eastern Botswana under the supervision of a traditional healer. The roots were chopped into small slices and sun-dried. The dried roots were then crushed into coarse powder using mortar and pestle. The powdered material was extracted in either water, methanol, ethylacetate or chloroform in a soxhlet apparatus for 8 h. Water extract was concentrated using freeze drier while the other extracts were concentrated to dryness under reduced pressure.

***Cucumis metuliferus* (E. Mey. ex Naud):** *Cucumis metuliferus* (Cucurbitaceae) is an annual climber with angled stems and stiff brown hairs. The young fruit is dark green with mottled light green spots. As it ripens it becomes bright orange with very sharp spines that can injure the skin. The species has a wide scanty distribution from South Africa to tropical Africa.

Whole fruit of ripe *Cucumis metuliferus* is eaten and is believed to be not only nutritious but has medicinal properties as well. Some segments of the society in Botswana believe it is good for diabetic patients. Ripe fruits of *C. metuliferus* were collected from Glen valley, Gaborone. The whole fruit was crushed and both the pulp and skin were extracted exhaustively in soxhlet using water, methanol, petroleum ether and chloroform. Water extracts were concentrated to dryness using a freeze drier whilst extracts containing organic solvents were concentrated using a rota evaporator. All samples were stored in the fridge until ready for testing.

**Botanical identification:** The plant specimen were authenticated by comparison with Herbarium specimen at the National Herbarium and Gallery, Gaborone, where voucher specimen have been deposited.

#### Antioxidant assays

**Qualitative scavenging activity on DPPH radical:** One milligram of each extract was weighed into a small sample tube and 5 mL of methanol added. The mixture was vortexed. Then 100  $\mu$ L of each of the mixture was spotted drop by drop (followed by intermittent drying) onto a silica coated TLC plate (200  $\mu$ m thickness), about 20 mm away from the bottom of the plate. The point of the spot was clearly labelled and the plate was dried in air and developed in a tank containing the mobile phase (Ethylacetate: Formic acid: Water 85:15:10). This was done to screen for the presence of any phytochemicals with free radical scavenging property. After development the plate was allowed to dry and viewed UV light 254 and 365 nm. The absorbing and fluorescent bands were marked. The plate was then sprayed with Diphenyl-picrylhydrazyl (DPPH) reagent in methanol (0.2%). After this, the plate was left to dry and the bleaching produced on the plate was noted. The DPPH reagent in this case was used to detect the presence of antioxidants.

**Quantitative scavenging activity on DPPH radical:** The free radical scavenging activity of the crude extracts was measured by the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method proposed by Brand-Williams *et al.* (1995). Briefly, a 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 0.5 mL of samples 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,  $A_0$  was the absorbance of the blank (i.e., no sample, DPPH solution only) and  $A_1$  was the absorbance in the presence of test extract.

## RESULTS AND DISCUSSION

Qualitative screening of antioxidant activity in shown is Table 1.

As can be shown from Fig. 1, at 50  $\mu$ g mL<sup>-1</sup> and above, both methanol and water extracts from *Ozoroa paniculosa* exhibited good scavenging activity (90%) comparable to the control compounds L-ascorbic acid

Table 1: Qualitative screening of antioxidant activity

Name of plant (extract)	Family	Voucher code	Antioxidant activity
<i>Ozoroa paniculosa</i> (methanol)	Anacardiaceae	Dmoeng1	++++
<i>Ozoroa paniculosa</i> (water)	Anacardiaceae	Dmoeng1	++++
<i>Ozoroa paniculosa</i> (ethylacetate)	Anacardiaceae	Dmoeng1	+++
<i>Ozoroa paniculosa</i> (chloroform)	Anacardiaceae	Dmoeng1	++
<i>Colophospermum mopane</i> (methanol)	Caesalpiniaceae	Dseolw1	++++
<i>Colophospermum mopane</i> (water)	Caesalpiniaceae	Dseolw1	++++
<i>Colophospermum mopane</i> (chloroform)	Caesalpiniaceae	Dseolw1	++
<i>Cucumis metuliferus</i> (methanol)	Cucurbitaceae	DglenV1	+++
<i>Cucumis metuliferus</i> (water)	Cucurbitaceae	DglenV1	++++
<i>Cucumis metuliferus</i> (chloroform)	Cucurbitaceae	DglenV1	++

++: Low antioxidant response, +++: Moderate antioxidant response, ++++: Strong antioxidant response

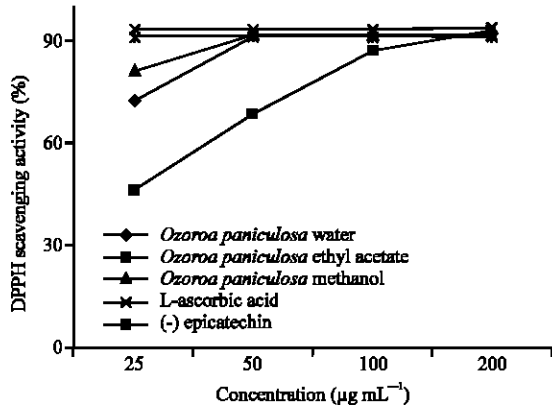


Fig. 1: Scavenging activity of *Ozoroa paniculosa* root crude extracts

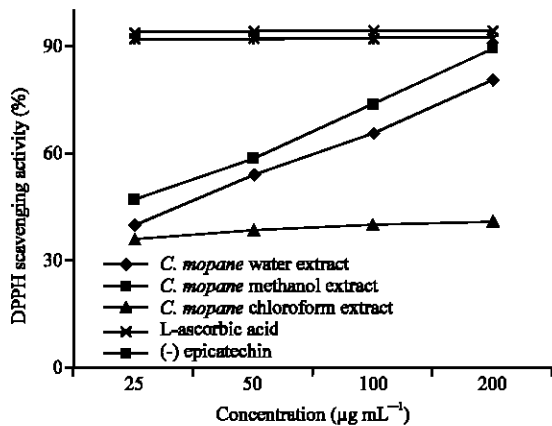


Fig. 2: Free radical scavenging activity of *Colophospermum mopane* seeds crude extracts

(91%) and epicatechin (92%). At 100 µg mL<sup>-1</sup> and above, the scavenging activity of the ethyl acetate extract from *O. paniculosa* roots reached a similar magnitude of activity (90%). The order of free radical scavenging potency for the tested extracts from *O. paniculosa* roots was as follows: methanolic extract > water extract > ethyl acetate extract. This perhaps indicates that the free radical scavenging principles are polar. Between 50-100 µg mL<sup>-1</sup>

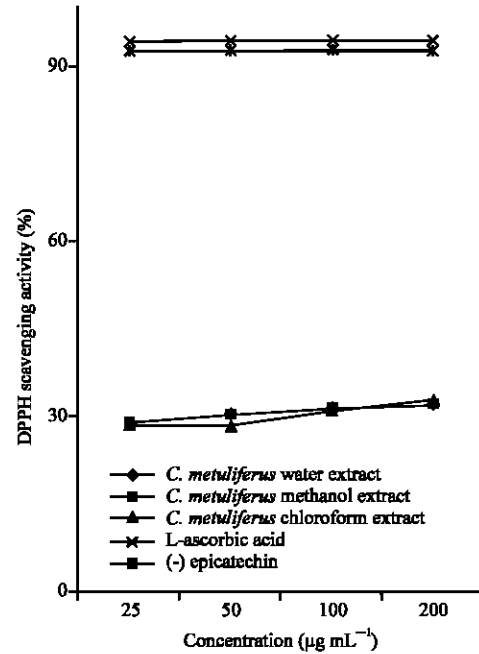


Fig. 3: Free radical scavenging activity of whole fruit extracts of *Cucumis metuliferus*

the free radical scavenging potencies of water and methanol extracts from *Colophospermum mopane* seeds were ≤70%. However, above 100 µg mL<sup>-1</sup>, both water and methanolic extracts of *C. mopane* exhibited scavenging activity ≥70% (Fig. 2). Chloroform extract of *C. mopane* seeds showed poor scavenging activity at all tested concentrations. All extracts of *C. metuliferus* showed poor free radical scavenging activity (30%) at all tested concentrations (Fig. 3). Both water and methanol extracts of *C. metuliferus* exhibited equal potency. These findings show limited antioxidant potential of extracts from whole fruits of *C. metuliferus*.

The various antioxidant potencies exhibited by the studied plants may at least in part, explain why these plants are used to treat inflammatory disorders such as gout and asthma. The common link between free oxidant radicals and inflammatory reactions has been well

established (Mongelli *et al.*, 1997; Wang *et al.*, 1999). The antioxidant capacities shown by the tested extracts in this study may lend credence to the use of *O. paniculosa* and *C. mopane* as anti-inflammatory and antioxidant agents in folk medicine.

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#### REFERENCES

- Aust, S.D., C.F. Chignell, T.M. Bray, B. Kalyanaraman and R.P. Mason, 1993. Free radicals in toxicology. *Toxicol. Applied Pharmacol.*, 120: 168-178.
- Bergman, M., A. Perelman, Z. Dubinsky and S. Grossman, 2003. Scavenging of reactive oxygen species by a novel glucuronated flavonoid antioxidant isolated and purified from spinach. *Phytochemistry*, 62: 753-762.
- Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, 28: 25-30.
- Brown, J.E. and C.A. Rice-Evans, 1998. Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. *Free Radic. Res.*, 29: 247-255.
- Krings, U. and R.G. Berger, 2001. Antioxidant activity of some roasted foods. *Food Chem.*, 72: 223-229.
- Mongelli, E., C. Desmarchelier, J. Rodriguez-Talou, J. Coussio and G. Ciccio, 1997. *In vitro* antioxidant and cytotoxic activity of extracts of *Baccharis coridifolia* DC. *J. Ethnopharmacol.*, 58: 157-163.
- Polterait, O., 1997. Antioxidants and free-radical Scavengers of natural origin. *Curr. Organic Chem.*, 1: 415-440.
- Stohls, S.J., 1995. The role of free radicals in toxicity and disease. *J. Basic Clin. Physiol. Pharmacol.*, 6: 205-228.
- Wang, H., M.G. Nair, G.M. Strasburg, Y. Chen-Chang, A.M. Booren, I.J. Gray and D.L. DeWitt, 1999. Antioxidant and anti-inflammatory activities of anthocyanins and their aglycone, cyaniding from Tart Cherries. *J. Nat. Prod.*, 62: 294-296.