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Short Communication

Cytotoxicity activity of isolated compounds from *Elaeodendron transvaalense* ethanol extract

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Five known compounds, four triterpenoids [(lup-20(30)-ene-3α,29-diol (1), lup-20(29)-ene-30-hydroxy-3-one-(2), Ψ – taraxastanonol 3, β-sitosterol 4] and 4' –*O*- methylepigallocatechin 5 were isolated from *Elaeodendron transvaalense* bark ethanol extract. This plant is traditionally used in Southern Africa for the treatment of various ailments including sexually associated diseases. Cytotoxicity of the isolated compounds was determined using XTT colorimetric assay against Vero and MCF-7 breast cancer cell lines. Compound 1, 3, 4, 5 showed weaker activities with the IC_{50} ranging from 66.6 to over 100.00 µg/ml in both cell lines. Compound 2 exhibited a good cytotoxicity activity IC_{50} value of 25.1 for Vero cells and 19.4 for breast cancer cell line.

Key words: *Elaeodendron transvaalense*, cytotoxicity and triterpenoid.

INTRODUCTION

Extracts from *Elaeodendron transvaalense* (Burtt Davy) (Celastraceae) have been used in traditional medicine by the Vhavenda people of South Africa (Limpopo province) to treat coughs, diarrhoea, stomach ailments, herpes and sexually associated diseases. Stem bark is mostly used to prepare infusions and decoctions (Mabogo, 1990). Other medicinal uses of *E. transvaalense* include the treatment of arthritis, cancer, coughs, diarrhoea and stomach ailaments. Traditional healers prescribe it presently to people who are suffering from HIV/AIDS (Bessong et al., 2005). Dimethyl–1,3,8,10–tetrahydroxy–9- methoxypeltogynan and three pentacyclic triterpenes have been isolated from its bark which is also reported to contain 13.4% catechol tannin (Hutchings,1996).Other species belonging to the same family (Celastraceae) have been used in the Amazonian region against cancer, rheumatism and inflammation (Nakagawa et al., 2004). Previous reports have shown that species from the Celastraceae family contain biologically active metabolites with antimicrobial and cytotoxic activities (Sansores-Peraza et al., 2000).

Many plant extracts and isolated compounds have been tested *in vitro* for cytotoxicity by using different human cell lines (prostate, stomach, liver colon etc.) as well as animal cells such as monkey kidney cells (Don *et al*., 2006; Lamidi et al., 2005, Al-Fatimi et al., 2005; Jo et al., 2005). Cell culture toxicity testing is a valuable and inexpensive approach for short term testing. A test should be able to provide information on the dose-effect relationship including the dose range for potential exposure and risks to humans. Cytotoxicity of plant extracts and isolated compounds should be evaluated before their impact in drug discovery is taken into consideration (Lall and Meyer, 2000). The aim of this part of study was to isolate compounds from *E. transvaalense* and determine their cytotoxicity activity.

MATERIALS AND METHODS

Plant material

Stem bark of *E. transvaalense* was collected in Venda (Northern Limpopo). A voucher specimen is preserved in HGWJ Schweickerdt herbarium at the University of Pretoria (Tshikalange 092524).

Plant extraction

Stem bark of *E. transvaalense* was collected and left to dry at room temperature for two weeks. The dried powder stem bark was placed

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in a container and soaked in ethanol. The container was closed and left in a dark cupboard for three days at room temperature before the extract was filtered and concentrated to dryness under reduced pressure (40°C). The residue was soaked again in ethanol and filtered. The filtrates were dried with a rotary evaporator to give a total mass of 150 g (extract).

Isolation of compounds

A 10 cm diameter glass was filled with 1.5 kg silica gel. The extract (120 g) was dissolved in a minimal amount of solvent and mixed with 200 g silical gel. The column was eluted with a solvent gradient of hexane: ethyl acetate in 100:0 to 0:100 ratios. The column was then washed with ethyl acetate: methanol (9:1) and 100 % methanol. 45 fractions of 50 ml each were collected; fractions containing the same compounds as determined by TLC plates were combined and concentrated to dryness under reduced pressure. TLC plates of 11 pooled fractions (A-K) were developed with hexane: ethyl acetate 9:1, 7:3 and 3:7. Fraction I yielded a pure compound (1). Other fractions were crystallized and yielded compound 2 - 5. TLC plates were examined under UV light (254 and 366 nm) after development and also dipped in vanillin (15 g vanillin, 500 ml ethanol and 10 ml concentrated 98% sulphuric acid) and heated at 110^0 C.

Cell lines

The cytotoxicity of the *E. transvaalense* extract and isolated compounds was tested against Vero and breast cancer cell lines. Cells were cultured in Eagle's minimal essential (MEM) supplemented with 1.5 g/L sodium bicarbonate, 2 mM L-glutamine , 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 10 µg/ml penicillium, 10 µg/ml streptomycin, 0.25 µg/ml fungizone, and 10 % fetal bovine serum at 37^0 C in a humidified atmosphere with 5% CO_2. Cells were subcultured in a 1:6 ratio every second to third day after trypsinization of confluent cultures.

Cytotoxicity assay

A colorimetric XTT (sodium 3' –[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitro] benzene sulfonic acid hydrate method was utilized to determine the cytotoxicity according to Mahapatra. et al., 2007. 100 µl of cells (Vero or braest cancer) were seeded at 1 x 10^5 onto a microtiter plate and incubated for 24 h to allow the cells to attach to the bottom of the plate. A dilution series were made of extractor compounds (0.39 - 200 µg/ml), added to the microtiter plate and incubated for 48 h. The XTT reagent was added to the wells to a final concentration of 0.3 mg/ml and incubation commenced for 1 - 2 h. The absorbance of the colour complex was quantified at 490 nm using an ELISA plate reader with reference wavelength set at 690 nm. IC_{50} was defined as the concentration of the compounds at which absorbance was reduced by 50%.

RESULTS AND DISCUSSION

The chemical structures of isolated compounds were identified using Nuclear Magnetic Resonance (NMR) spectra and direct comparison of the spectral data of each isolated compound with the published data. Five known pure compounds (lup-20(30)-ene-3α,29-diol(**1**) lup-20(29)-ene-30-hydroxy-3-one (**2**) taraxastanonol 3, β-sitosterol 4 and 4' –*O*-methylepigallocatechin 5) were obtained after isolation of the chemical constituents from *E. transvaalense*. Compound 1 has been previously isolated from the whole plant extract of *Daphne oleoides*, which is used as a purgative and the infusion of the leaves is used to treat gonorrhoea and applied to abscesses (Ullah et al., 1999). Compound 3 was previously isolated from resin of *Protium heptaphyllum* and has shown analgesic effects (Susunaga et al., 2001 and Rudiger et al., 2007). Compound 4 is well known compound previously isolated from plant of different species (Prozesky, 2004). Compound 5 was previously isolated from *Elaeodendrom papillosa* by Drewes et al. (1993).

The crude extract of *E. transvaalense* and isolated compounds were evaluated *in vitro* for their inhibitory ability against the growth of both Vero and MCF-7 cell lines. These cell lines were inhibited by all the compounds at the highest concentration tested (200 µg/ml), except Ψ – taraxastanonol (3). The results (Table 1) obtained from the calculation made from the spectrophotometer readings, indicated that the crude extract, Ψ – taraxastanonol (3) and 4' –*O*- methylepigallocatechin (5) have little or no toxicity on Vero cells by exhibiting IC_{50} values of greater than 100 µg/ml. Similarly the crude extract and taraxastanonol (3) exhibited IC_{50} values of greater than 100 µg/ml in MCF-7 cell line. lup-20(30)-ene-3,29-diol, (3α)-(9Cl) (1) and β-sitosterol (4) showed weaker activity with IC_{50} values ranging from 78 to 96 µg/ml in both Vero and (breast) cancerous cells.

From the isolated compounds only lup-20(29)-ene-30-hydroxy-(9Cl) (2) showed to be potent inhibitor with IC_{50} values of 25 µg/ml in Vero cells and 19 µg/ml in MCF-7 (breast) cancerous cells. This finding is consistent with observation by Fang *et al*. (1984) which showed no significant inhibition of KB carcinoma cell growth at the concentration lower than 20 µg/ml by similar compounds. *In vitro* cytotoxicity is necessary to define basal cytotoxicity such as the intrinsic ability of a compound to cause cell death as a result of damage to several cellular functions. This assay is also necessary to define the concentration range for more detailed *in vitro* testing to provide information on parameters such as genotoxicity or programmed cell death (Bouaziz et al., 2006). It is difficult to conclude that *E. transvaalense* or compound 2 are not active against cancer cell lines, because some plants are reported to have a cytotoxic effect on cancer cells, whereas other plants activate several parameters of the immune system as a strategy to destroy cancer (Steenkamp and Gouws, 2006). Low toxicity of *E. transvaalense* extract confirms the findings of cytotoxicity studies of the same species reported by Bessong et al. (2005). Cao *et al*. (2006), reported the isolation of triterpene saponins which showed significant cytotoxicity activity against various cell lines. According to Kaviarasan et al. (2007), epigallocatchin-3-gallate can protect chang liver cells against ethanol-induced cytotoxicity and apoptosis.

Table 1. IC_{50} of the crude extract and isolated compounds from *E. transvaalense* after 4 days on Vero and breast cancer (MCF-7) cells.

Plant extract / compound	Vero	MCF-7
	IC_{50} (µg/ml) ± SD	
Extract	> 100.0 ± 3.6	> 100 ± 0.3
lup-20(30)-ene-3α,29-diol (**1**)	93.0 ± 3.9	96.0 ± 2.9
lup-20(29)-ene-30-hydroxy-3-one (**2**)	25.1 ± 3.3	19.4 ± 2.2
Ψ – taraxastanonol 3	>100.0 ± 0.0	> 100 ± 0.1
β-sitosterol 4	82.0 ± 2.8	78.94 ± 5.5
4' –*O*- methylepigallocatechin 5	> 100.0 ± 3.3	66.6± 3.2
Doxorubicin (Positive control)	Na	0.01 ± 0.1
Zelaralenone (Positive control)	2.6 ± 0.3	2.4 ± 0.5

SD, Standard deviation. Na, not assayed.

Conclusion

The results of this study have shown low toxicity of *E. transvaalense* extract and isolated compounds and thiscan support the traditional use of this plant in the treatment of various ailments.

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