African Journal of Biotechnology Vol. 10(75), pp. 17297-17304, 28 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2249
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

The antioxidant properties, cytotoxicity and monoamine oxidase inhibition abilities of the crude dichloromethane extract of *Tarchonanthus camphoratus* L. leaves

Olayinka A. Aiyegoro* and Sandra van Dyk

Department of Pharmaceutical Chemistry, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa.

Accepted 21 October, 2011

***Tarchonanthus camphoratus* (camphor bush) has been widely used for numerous medicinal purposes. The aim of the present study was to evaluate the antioxidant properties, cytotoxicity and monoamine oxidase inhibition activities of the crude dichloromethane leaf extract of *T. camphoratus*. The antioxidant activities were assessed using the thiobarbituric acid-reactive substances (TBARS) assay and the nitroblue tetrazolium (NBT) assay. The cytotoxicity assays were performed according to the microculture MTT method. From the MTT assay, it was determined that at a concentration of 10 mg/ml of crude extract, 95% of the neuroblastoma cells were killed. Almost 99% of the cells were viable at a concentration of 0.08 mg/ml extract. The extract also showed the ability to inhibit monoamine oxidase (MAO-A and MAO-B) with the corresponding inhibitory concentration, IC_{50} values of 1.371 and 0.2737 mg/ml, respectively. The antioxidant activity and cytotoxic effect of the extracts increased with increase in concentration. This study suggests that the dichloromethane leave extract of *T. camphoratus* can potentially be used as a readily accessible source of natural antioxidants.**

Key words: Antioxidant, cytotoxicity, monoamine oxidase (MAO), *Tarchonanthus camphoratus*, inhibitor, medicinal plant.

INTRODUCTION

Since time immemorial, plants have been useful to man in a variety of ways ranging from ornamental, commercial and medicinal uses, etc. (Parikh et al., 2005, Kambizi et al., 2001). Folkloric stories have described how various plant parts (for example, fruits, flowers, seed, barks, leaves and roots) were used by ancient man as: Anti-infective, anti-ageing, anti-sickling, anti-proliferative, anti-oxidants, anti-malarial and analgesic agents, in ways that could not be explained by scientific knowledge. In recent times, researchers have complemented these folkloric values with scientific investigations (McGaw et al., 2000; Matasyoh et al., 2007).

Plants are important repositories of antioxidant substances; and it has been established that diets rich in vegetables and fruit could prevent or delay the onset of oxidative stress related diseases (Kitts et al., 2000; Lee and Shibamoto, 2000; Wang and Jiao, 2000). To this fact, many commercially available antioxidant dietary supplements are concoctions of plants and/or plant products.

Cells release free radicals and other reactive oxygen species as by-products of physiological and biochemical processes. Free radicals enhance oxidative damage to cell contents: Such as lipids, proteins, carbohydrates, enzymes and deoxyribonucleic acid (DNA) and not only has a roll in aging, but also in many chronic diseases, such as cancer, diabetes and other degenerative diseases in humans (Harman, 1998).

It has been proven that the oxidation of dopamine by human monoamine oxidase (MAO) plays a tangible role in the onset of neurodegenerative diseases and it is one

*Corresponding author. E-mail: ayoyinkaaiyegoro@yahoo.com. Tel: +27826227389/ +27182992261.

of various sources of reactive oxygen species (ROS) in human cells (Wei et al., 1996). The oxidation of dopamine not only produces homovanillic acid (HVA) and 3,4-dihydroxy-phenyl-acetic acid (DOPAC), but also H_2O_2. H_2O_2 can be converted to highly toxic hydroxyl radicals via iron-mediated Fenton reactions (Gerlach et al., 1994; Halliwell, 1992; Jenner, 1993) that may then initiate lipid peroxidation and cell death (Zaleska et al., 1985). Supporting evidence for an effect of dopamine on increased ROS comes from a study on cultured PC12 cells which over-express monoamine oxidase type B (MAO-B). Excess MAO-B expression results in higher levels of free radical production and free radical damage than in control cells (Wei et al., 1996).

Plants produce secondary metabolites with free radical scavenging activity, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). Antioxidants are substances that when present in low concentrations significantly delay or prevent oxidation of cell contents. Studies have shown that the ingestion of natural antioxidants has been connected with a reduced incidence of cancer, cardiovascular disease, diabetes, and other diseases linked to ageing (Ashokkumar et al., 2008; Veerapur et al., 2009), and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruit and vegetables (Kitts et al., 2000; Muselík et al., 2007; Wang and Jiao, 2000) in the management of oxidative stress related diseases.

T. camphoratus L. is a member of the Asteraceae family and is known as the natural camphor bush/tree (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997). The tree is widely distributed in southern Africa, extending from Namibia to Botswana, from the Northern Province to Gauteng, the Free State and the Northern Cape (van Wyk et al., 1997; Herman, 2002). The strongly scented leaves of *T. camphoratus* have numerous medicinal applications in traditional healing and are often used as an infusion to treat stomach ailments and bronchitis. The smoke liberated from the burning of aerial parts of this plant is inhaled to treat sinus-related complaints and, offer relief for headaches. Burned leaves and seeds have been used for fumigation during funeral rituals (Van Wyk et al., 1997). Leaves are used as hot poultices for treating chest complaints. Chewing the leaves is said to alleviate toothache (Hutchings et al., 1996). The plant is used in the treatment of sexually transmitted diseases (Watt and Breyer-Brandwijk, 1962). Antimicrobial properties were reported for extracts of the plant and its essential oils (Hutchings and van Staden 1994, Mwangi et al., 1994, McGaw et al., 2000, Matasyoh et al., 2007); aqueous leaf extracts were shown to significantly attenuate pain in the acetic acid writhing test. The same study was able to demonstrate that an aqueous extract significantly reduced fever induced in rats by bacterial endotoxins (McGaw et al., 2000; Amabeoku et al., 2000).

Ninety-eight compounds have been identified in *T. camphoratus* essential oil, these includes: 1, 8 cineole, α-fenchyl alcohol, β-caryophyllene, α-pinene, as major constituents (Mwangi et al., 1994; Matasyoh et al., 2007). Camphor was only present in minor quantities, 0.38% (Mwangi et al., 1994). Phytochemical tests on the plant revealed the presence of tannins, saponins and reducing sugars, but not alkaloids, cardiac and anthraquinone glycosides (Mwangi et al., 1994).

In a bid to demonstrate that plants are sleeping giants of pharmaceutical industry, we have chosen *T. camphoratus,* a plant with a broad spectrum of uses in traditional medicine as the candidate plant for this study. An extensive literature survey revealed that there is scanty or no information on studies that focuses on the antioxidant properties, cytotoxicity and recombinant human monoamine oxidase inhibition of *T. camphoratus* dichloromethane leaf extract. This present study therefore investigates the *in vitro* antioxidant properties, cytotoxicity and recombinant human monoamine oxidase inhibition of *T. camphoratus* crude dichloromethane leaf extract.

MATERIALS AND METHODS

Plant material

Leaves of *T. camphoratus* were collected in August, 2010 from the botanical gardens of the North-West University, Potchefstroom, South Africa. The plant materials were compared with the voucher specimen with herbarium number PUC 8761 (Van Heerden, M.) earlier collected from the same spot and were confirmed to be *T. camphoratus* by the curator of the gardens.

The leaves were picked and all unwanted plant materials were removed, air dried under light exposure (27-30°C for 7 days), pulverized in a mill and stored in an airtight container for further use.

Preparation of dichloromethane leaf extract

The powdered leaves of *T. camphoratus* (200 g) were extracted in succession by Soxhlet with *n*-hexane for 48 h followed by dichloromethane. The dichloromethane extract was then concentrated *in vacuo* with a rotary evaporator at 40°C. The solvent-free extracts (22 g) were stored at 4°C until used for bioactivity assays.

In vitro antioxidant assay

Thiobarbituric acid-reactive substances (TBARS) assay

The TBA assay involves the reaction between TBA and malondialdehyde (MDA) equivalents. MDA, a major degradation product of lipid peroxidation serves as a biomarker for assessing the extent of lipid peroxidation. MDA reacts with two molecules of TBA via an acid catalyzed nucleophillic-addition reaction yielding a pinkish-red chromagen which can be extracted with butanol and

measured spectrophotometrically at the absorbance maximum of 530 nm. The modified method of Ottino and Duncan (1997) was used for this assay. 1 ml rat brain homogenates containing a toxin combination (H_2O_2 + $FeCl_3$ + Vitamin C) and different concentrations of the extract were added separately to each set of Eppendorff tubes in triplicate and then vortexed, these sets of Eppendorff tubes were then incubated at 37°C for 1 h to induce lipid peroxidation. After incubation, the tubes were centrifuged at 2000 × g for 20 min to remove all insoluble proteins. The supernatant was removed from each tube into new Eppendorff tube and termination of the incubation period was followed by the addition of 100 µl methanolic buthylated hydroxyl toluene (BHT), 200 µl trichloro acetic acid (TCA) and 100 µl thiobabutiric acid (TBA) to each of the test tubes. This stops the reaction, precipitate macromolecules such as protein and DNA, and allows for the development of the coloured complex. The tubes were then sealed and the vortexed tubes incubated in a water bath for 1 h at 60°C. After 1 h, the tubes were cooled on ice and TBA-MDA complexes were extracted with 2 ml buthanol. The vortexed tubes were then centrifuged at 2000 × g for 10 min. The supernatant from these tubes were separately transferred into a 96-well microplate and the absorbances of the upper layer were read at 530 nm using a multi-well reader (Labsystems multiskan RC reader).

Nitroblue tetrazolium (NBT) assay

The assay is based on the ability of free radicals to reduce the yellow dye NBT to an insoluble blue dye nitroblue diformazan (NBD), which can be extracted with glacial acetic acid (GAA) and the absorbance values measured at 560 nm. A modification of the NBT assay of Ottino and Duncan (1997a, b) was used for assaying the ability of extracts to scavenge superoxide radicals (O_2^-). Superoxide radicals were generated *in vitro* by the toxin potassium cyanide (KCN). Rat brain homogenates were prepared and kept in 10% w/v phosphate buffer saline (PBS) on ice. Test Eppendorff tubes each with 1 ml of rat brain homogenates were prepared in triplicate and to each set of tubes; the control, the toxin (1 mM KCN), and different concentrations of the extract 0.4 ml 0.1% NBT (0.05 g NBT + 1 ml ethanol + 49 ml distilled water to make 50 ml) were added. The tubes were vortexed and wrapped in aluminum foil. The vortexed tubes were then incubated in an oscillating water bath at 37°C for 1 h after which the tubes were centrifuged at 2000 × g for 10 min. The supernatant was discarded, 2 ml GAA was added to the pellet and this was centrifuged at 4000 × g for 5 min. Absorbance readings were taken at 560 nm with GAA as blank, the absorbance values were converted to micromoles diformazan (µmol diformazan) from the NBD standard curve. The final values were expressed as micromole/mg protein (µmole/mg protein) from a bovine serum albumin (BSA) standard curve generated as described by Lowry et al. (1951).

In vitro cytotoxicity assay

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT Assay)

The cytotoxicity assays were performed according to the microculture MTT method with little modifications (Popiołkiewicz et al., 2005; Kim et al., 2008). The MTT assay is based on the cleavage of yellow MTT, a tetrazolium salt which is reduced by the respiratory chain and other electron transport system to form non-water soluble purple formazan crystals within metabolic active cells. The formazan crystals are then solubilised in an organic solvent to obtain the insoluble purple formazan product in a homogeneous, coloured solution which can be measured spectrophotometrically between 500 nm and 650 nm. Neuroblastoma cells were obtained from the Scientific Group (Midrand, South Africa); the cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS), 100 IU/ml penicillin, 0.1 mg/ml streptomycin and 0.25 µg/ml fungizone. The cell cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2. The growth medium was changed twice a week so as to maintain the highest levels of sterility and to avoid infecting the cells. Cells were examined daily, and as soon as the flask was confluent, the cells were trypsinised and split into two corning flasks and left once again to multiply. Two corning flasks were used for each experiment that was performed in triplicate. Confluent cells were harvested from the corning flasks using 3 ml trypsin, the cells were then cultured at 0.75 million cells per well in a 24-well microtitre plates and incubated for 1 day at 37°C in 10% CO_2. After 24 h, the cells culture were aspirated and treated with 400 µL of DMEM and 100 µL of a range of concentrations of filter sterilised plant extracts into each well. A cell free media was included for each experiment as the blank and an extract free media control was also included. The blank served as an indicator of contamination with 0% growth, while the control served as 100% cellular growth with no contamination. After 72 h of incubation, DMEM was aspirated from the wells and 200 µL of 5 mg/ml MTT was added to each well. This was incubated under the same conditions for 2 h to terminate the cell growth after which the MTT was aspirated from each well. 250 µL isopropanol was added to each well and left to incubate for 5 min at room temperature to dissolve the formazan crystals completely. 100 µL of the contents of each well was transferred to a 96-well plate and the absorbance was read at 560 and 650 nm using a multi-well reader (Labsystems multiskan RC reader). The results were expressed as a percentage cellular viability of the controls using the following equation:

$$\% \text{ Cellular viability} = \frac{\Delta\text{Absorbance} - \Delta\text{blank} \times 100}{\Delta\text{control} - \Delta\text{Blank}}$$

Where, ΔControl (mean cell control) is the cell control_{560} – cell control_{650}; ΔBlank (mean blank) is the Mean Blank_{560} – Mean Blank_{650} and ΔAbsorbance (mean absorbance) is the Absorbance_{560} – Absorbance_{650}

Monoamine oxidase (MAO-A and MAO-B) inhibition studies

MAO-A and MAO-B are flavin adenine dinucleotide (FAD) containing enzymes which are tightly anchored to the mitochondrial outer membrane. To determine the MAO-A and MAO-B inhibition potencies of the test extract, the extent by which different concentrations of the extract reduces the rate of the MAO-catalyzed oxidation of Kynuramine, a mixed MAO-A/B substrate, was measured. For this purpose, the recombinant human MAO-A and MAO-B enzymes were employed. Kynuramine is non-fluorescent until undergoing MAO-catalyzed oxidative deamination and subsequent ring closure to yield 4-hydroxyquinoline, a fluorescent metabolite. The concentrations of the MAO-generated 4-hydroxyquinoline in the incubation mixtures was determined by comparing the fluorescence emitted by the samples to that of known amounts of authentic 4-hydroxyquinoline at excitation (310 nm) and emission (400 nm) wavelengths. Microsomes prepared from insect cells expressing recombinant human MAO-A and MAO-B (5 mg/mL) obtained from Sigma–Aldrich and were pre-aliquoted and stored at -70°C. All enzymatic reactions were carried out to a final volume of 500 µL in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl, 20.2 mM) and contained kynuramine as substrate, MAO-A or MAO-B (0.0075 mg/mL) and various concentrations of the test inhibitor (extract) (0–10.0 mg/ml). The final concentrations of kynuramine in the reactions were 45 µM and

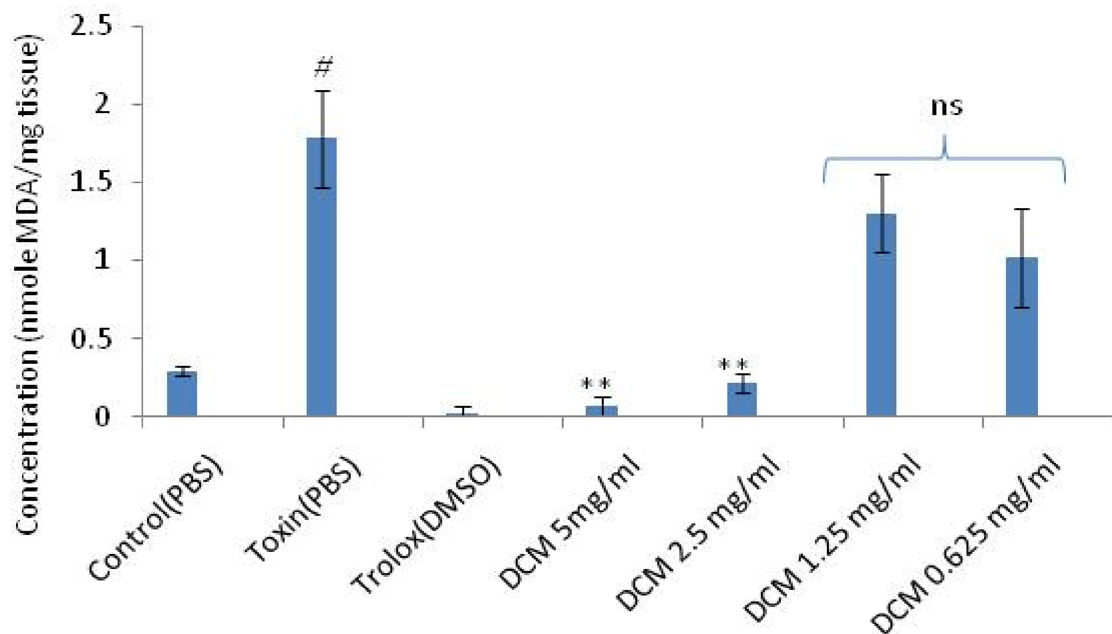

Figure 1. The attenuation of lipid peroxidation through free hydroxyl radicals scavenging after rat brain exposure to different concentrations of *Tarchonanthus camphoratus* dichloromethane leaf extract. Values represents the mean ±SD; n=3; P<0.05 in comparison to the toxin; ns= Not significant.

30 µM where MAO-A and MAO-B, respectively were used as enzymes. Stock solutions of the test inhibitors were prepared in DMSO and added to the reactions to yield a final concentration of 4% (v/v) DMSO. The reactions were carried out for 20 min at 37°C and were terminated with the addition of 200 µL NaOH (2 N). After the addition of distilled water (1200 µL) to each reaction, the reactions were centrifuged for 10 min at 16000 × g. To determine the concentrations of the MAO-generated 4-hydroxyquinoline in the reactions, the fluorescence of the supernatant at an excitation wavelength of 310 nm and an emission wavelength of 400 nm were measured (Novaroli, et al. 2005). Quantitative estimations of 4-hydroxyquinoline were made with the aid of a linear calibration curve ranging from 0.047 to 1.56 µM of the reference standard dissolved in potassium phosphate buffer (100 mM, pH 7.4). Each calibration standard was prepared to a final volume of 500 µL in potassium phosphate buffer (100 mM, pH 7.4) and contained 4% DMSO. To each standard was also added 200 µL NaOH (2 N) and 1200 µL distilled water. IC_{50} values were determined by plotting the initial rate of oxidation versus the logarithm of the inhibitor concentration to obtain a sigmoidal dose–response curve. For this purpose, nine different inhibitor concentrations spanning at least three orders of a magnitude were used for each sigmoidal curve. This kinetic data were fitted to the one site competition model incorporated into the Prism software package (GraphPad) and the IC_{50} values were determined in duplicate and are expressed as mean ± standard deviation (SD) (Strydom, et al. 2010). The IC_{50} values were converted to the corresponding Ki values according to the equation by Cheng and Prusoff: $Ki = IC_{50}/(1 + [S]/Km)$ (Cheng and Prusoff, 1973).

Statistical analysis

Each data point is an average of triplicate measurements, with each individual experiment performed in triplicate. Statistical analysis of data was done using Graphpad Instat® and one way analysis of variance (ANOVA) methods, followed where appropriate, by the Student-Newan-Keuls Multiple range test with *P<0.05* regarded as significant where appropriate. Different extract concentrations were each compared to the control. Results were reported in comparison to the control.

RESULTS AND DISCUSSION

Thiobarbituric acid-reactive substances (TBARS) assay

The TBARS assay is the most commonly used assay to assess lipid peroxidation. This assay measures the ability of an extract to scavenge the hydroxyl free radicals. The consequence of peroxidation by this free radical is the production of MDA (Luo et al., 1995). The experimental data of the extract showed *in vitro* antioxidant activity in TBARS assay; the extract's abilities to scavenge O_2^- and OH^- correlates to total antioxidant power. Lipid peroxidation is one of the consequences of oxidative stress.

The antioxidant activities of a range of concentrations of dichloromethane extract of *T. camphoratus* was assessed by the TBARS methodology as presented of Figure 1. The antioxidant activity gradually increased with increasing concentration as revealed by the correspondent decrease in MDA formation in rat brain tissue as compared to the toxin. At 5 mg/ml which is the highest concentration of the extract tested, about 0.08 nmol MDA/mg tissue was formed whereas, at 0.625 mg/ml extract concentration, 1.0 nmol MDA/mg tissue was formed as end product of lipid peroxidation. In

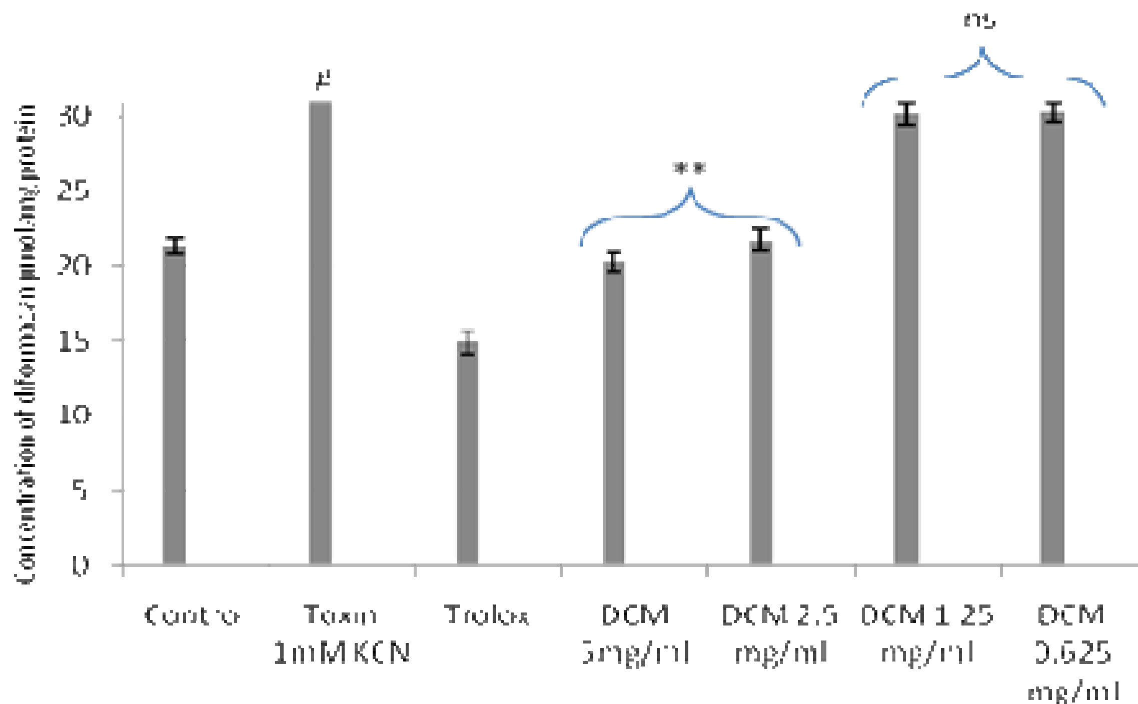

Figure 2 The reduction of nitroblue diformazan (NBD) formation in the presence of KCN through O_2^- scavenging by different concentrations of *T. camphoratus* extract in whole rat brain homogenates *in vitro*. Values represents the mean ±SD; n=3; $P < 0.05$ in comparison to the toxin; ns= not significant

comparison to the toxin (1.8 nmol MDA/mg tissue), the extract showed significant decrease in MDA formation in rat brain tissue *in vitro*. This attenuation in 2TBA-MDA complex formation indicates less lipid peroxidation and a hydroxyl radical scavenging ability of the extract.

Nitroblue tetrazolium (NBT) assay

Jonhson et al. (1987) proposed that cyanide, a mitochondrial electron transport inhibitor, increases the intracellular calcium and generates reactive oxygen species (ROS). The inhibition of complex IV of the mitochondrial respiratory chain produces O_2^- through the reduction of O_2. Potassium cyanide (1 mM) was used as the respiratory toxin as a source of cyanide to generate O_2^- in rat brain homogenates. It is the chemical reaction between O_2^- and NBT that forms the basis of this assay. NBT is reduced to NBD in the presence of O_2^- and then, NBD is measured spectrophotometrically at 560 nm. Thus, the ability of the extract to scavenge the generated O_2^- will automatically results in the less formation of NBD.

The data for the superoxide scavenging properties of extract in the presence of KCN in the rat brain homogenate is presented in Figure 2. The superoxide scavenging properties of dichloromethane extract of *T. camphoratus* is also dose concentration dependent, that is, at higher extract concentration more O_2^- were scavenged with resultant reduction of NBD formation in the presence of KCN. For example, when the extract concentration was 5 mg/ml, about 22 µmol/mg protein NBD was liberated, whereas at a lower extract concenteration of 0.625 mg/ml above 30 µmol/mg protein NBD was liberated. Overall, the antioxidant activity of the extract could not be compared to that of Trolox® (a standard antioxidant), which shows a better O_2^- scavenging ability with a corresponding formation of just about 15 µmol/mg protein NBD at the test concentration in this experiment. The dichloromethane extract of *T. camphoratus* has exhibited another antioxidant mechanism in this study, although, very low activity when compared to the standard (Trolox®); this could be improved upon by isolation and purification of the active compound responsible for the supposed antioxidant activity.

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT Assay)

The results of the cytotoxic activity of crude extracts from dichloromethane extract of *T. camphoratus* are summarized in Figure 3. Cytotoxicity of extracts was determined by the MTT assay on the neuroblastoma cells in DMEM. The graph presented the percentage viable cells. Different extract concentrations were each

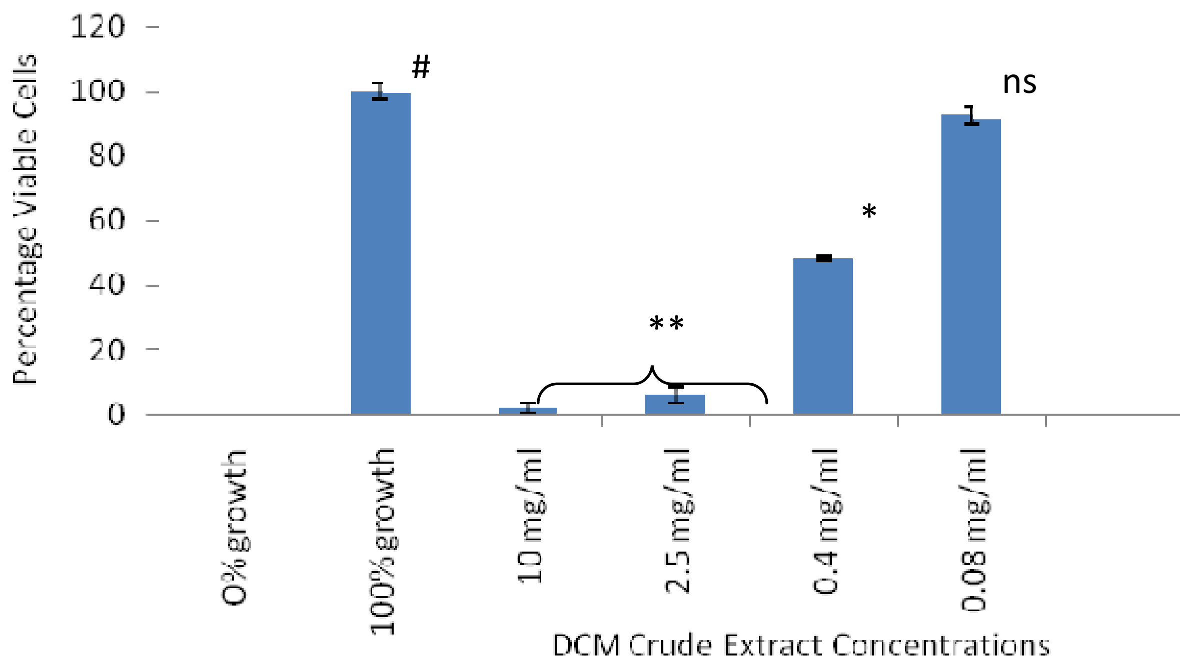

Figure 3. Graph obtained after 24 h exposure to neuroblastoma cells in DMEM to 0.08, 0.4, 2 and 10 mg/ml concentrations of *T. camphoratus* extract. Each bar represent the mean ±SD; n=3; *P<0.05* in comparison to the 100% growth; ns= Not significant.

compared to the control which had 100% cell growth. The control did not have any of the extract but just the growth medium and the cells; most growth (100%) was therefore seen in the control compared to the extract. The blank did not have any cells at all but plant extract and the medium. At 10 mg/ml extract concentration about 95% of the cells were killed. At 0.08 mg/ml extract concentration almost 99% of the cells were viable. It revealed that as the concentration decreases the toxicity of the extract also decreases.

Recombinant human monoamine oxidase (MAO-A and MAO-B) inhibition studies

To determine the MAO-A and MAO-B inhibition potencies of the extract, the extent by which different concentrations of a test inhibitor (extract) reduces the rate of the MAO-catalysed oxidation of kynuramine, a mixed MAO-A/B substrate, was measured. For this purpose, the recombinant human MAO-A and MAO-B enzymes were employed (Novaroli et al., 2005). The inhibition potencies of the test inhibitors were expressed as the IC_{50} values. The IC_{50} values for the inhibition of MAO-A and MAO-B by *T. camphoratus* extract are presented in Figure 4. For MAO-A, the IC_{50} is 1.371 mg/ml and for MAO-B the IC_{50} value is 0.2737 mg/ml. From these values it could be predicted that the inhibitor (extract) is a better inhibitor of MAO-B than MAO-A, because of the lower IC_{50} value of the former.

Monoamine oxidase (MAO) A and B are flavin adenine dinucleotide (FAD) containing enzymes which are tightly anchored to the mitochondrial outer membrane (Binda et al., 2002). Although, MAO-A and MAO-B are encoded by separate genes, they share approximately 70% amino acid sequence identity (Shih et al., 1999). Because MAO-A and MAO-B catalyses the catabolism of neurotransmitter amines, they are considered attractive drug targets in the therapy of neurological disorders. Both reversible and irreversible inhibitors of MAO-A are used to treat depressive illness and anxiety disorder. The antidepressant effect of MAO-A inhibitors are dependent on the inhibition of the catabolism of serotonin, norepinephrine and dopamine in the brain which leads to increased levels of these neurotransmitters (Youdim and bakhle., 2006). MAO-A inhibitors are particularly effective in the treatment of depression in elderly patients (Youdim et al., 2006; Zisook, 1985). Inhibitors of MAO-B are employed in the treatment of neurodegenerative disorders such as Parkinson's disease (PD). MAO-B appears to be the major dopamine metabolizing enzyme in the basal ganglia, and inhibitors of this enzyme may conserve the depleted dopamine stores in the PD brain. This may lead to enhanced dopaminergic neurotransmission and consequently symptomatic relief of the symptoms of PD (Youdim et al., 1972; Collins et al., 1970; Di Monte et al., 1996). MAO-B inhibitors may also increase the elevation of dopamine levels in the basal ganglia following levodopa treatment (Finberg et al., 1998) and are therefore used as adjuvant to levodopa

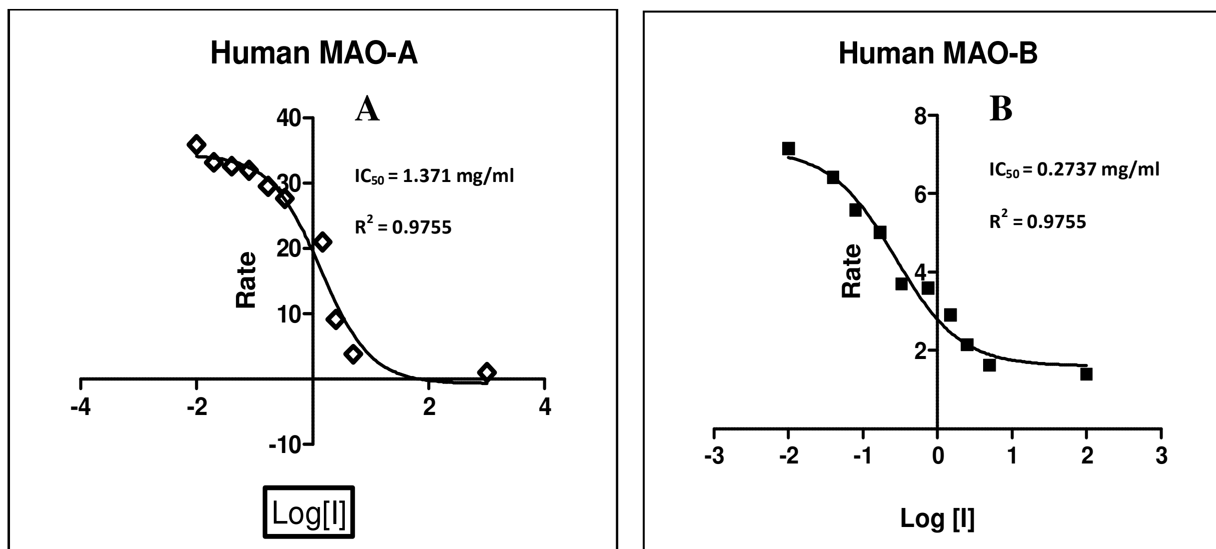

Figure 4. The sigmoidal dose–response curve of the initial rates of oxidation of kynuramine by recombinant human MAO-A (A) and recombinant human MAO-B (B) versus the logarithm of concentration of inhibitor extracts (expressed in mg/ml). The determinations were carried out in duplicate and the values are expressed as the mean ± SD. The concentrations of kynuramine used were 45 µM and 30 µM for the studies with MAO-A and MAO-B, respectively, and the rate data are expressed as nmol 4-hydroxyquinoline formed/min/mg protein.

therapy in PD (Fernandez and Chen, 2007). Besides providing symptomatic relief, MAO-B inhibitors may also protect against further neurodegeneration in PD by reducing the levels of potentially toxic byproducts such as H_2O_2 and dopaldehyde which form as a result of the oxidative metabolism of dopamine (Youdim et al., 2006). MAO-B inhibitors may be particularly relevant in the therapy in age-related neurodegenerative disorders such as PD since MAO-B activity and density increase in most brain regions with age (Nicotra et al., 2004; Fowler et al., 1997).

Conclusion

In conclusion, *T. camphoratus* dichloromethane leaf extract exhibited significant antioxidant properties in this study. *T. camphoratus* forms part of the ingredients used in preparing decoctions for the treatment of some ailments and thus it may be safe for consumption and drugs formulated from this plant at low concentration may pose no danger to the users. Antioxidant drugs of natural origin developed from this plant may go a long way in the management of oxidative stress related diseases. It is necessary to carry out a bioassay guided fractionation of the extract in a bid to isolate and identify the compounds responsible for these bioactivities. An elucidation of the mechanisms of action of these compounds must be followed by toxicity and *in vivo* tests to determine the therapeutic applicability of such compounds as future antioxidant drug. Efforts are going on in our laboratory to isolate pure compounds of pharmacological importance from the plant crude extract. These are subjects of on-going investigations in our research group.

Abbreviation:

NBT, Nitroblue tetrazolium; **TBARS,** thiobarbituric acid-reactive substances; **MAO,** monoamine oxidase; **DMEM,** Dulbecco's Modified Eagle's Medium; **FBS,** foetal bovine serum; **ROS,** reactive oxygen species; **HVA,** homovanillic acid; **DOPAC,** 3,4-dihydroxy-phenyl-acetic acid; **MDA,** Malondialdehyde; **BHT,** buthylated hydroxyl toluene; **TCA,** trichloro acetic acid; **TBA,** thiobabutiric acid; **NBD,** nitroblue diformazan; **GAA,** glacial acetic acid; **PCK,** potassium cyanide; **PBS,** phosphate buffer saline; **BSA,** bovine serum albumin.

REFERENCES

Amabeoku GJ, Green I, Eagles P, Benjeddou M (2000). Effects of *Tarchonanthus camphoratus* and *Eriocephalus africanus* on nociception in mice and pyrexia in rats. Phytomedicine, 7(6): 517-522).

Ashokkumar D, Mazumder UK, Gupta M, Senthilkumar GP, Selvan VT (2008). Evaluation of Antioxidant and Free Radical Scavenging Activities of *Oxystelma esculentum* in various *in vitro* Models. J. Comp. Integ. Med., 5(1) Article 9.

Binda C, Newton-Vinson P, Hubálek F, Edmondson DE, Mattevi A (2002). Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. Nat. Struct. Biol., 9: 22-26.

Cai YZ, Sun M, Corke H (2003). Antioxidant activity of betalains from plants of the Amaranthaceae**.** J. Agric. Food Chem., 51(8): 2288-2294.

Cheng YC, Prusoff WH (1973). Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol., 22(23): 3099- 3108

Collins GGS, Sandler M, Williams ED, Youdim MBH (1970). Multiple forms of human brain mitochondrial monoamine oxidase. Nature, 225(5235): 817-820.

Di Monte DA, DeLanney LE, Irwin I, Royland JE, Chan P, Jakowec MW, Langston JW (1996). Monoamine oxidase-dependent metabolism of dopamine in the striatum and substantia nigra of L-DOPA-treated monkeys. Brain Res., 738(1): 53-59.

Fernandez HH, Chen JJ (2007). Monoamine-B inhibition in the treatment of Parkinson's disease. Pharmacotherapy, 27(12pt2): 174S-185S.

Finberg JP, Wang J, Bankiewich K, Harvey-White J, Kopin IJ, Goldstein DSJ (1998). Inhibition of monoamine oxidase by (E)-styrylisatin analogues. J. Neural Transm. Suppl., 52: 279.

Fowler JS, Volkow ND, Wang, GJ, Logan J, Pappas N, Shea C, MacGregor R (1997). Age-Related Increases in Brain MAO B in Healthy Human Subjects. Neurobiol. Aging, 18(4): 431-435

Gerlach M, Ben-Shachar D, Reiderer P, Youdim MBH (1994). Altered brain metabolism of iron as a cause of neurodegenerative diseases? J. Neurochem., 63: 793-807.

Halliwell B (1992). Reactive oxygen species and central nervous system. J. Neurochem. 59: 1609–1623

Harman D (1998). Aging: phenomena and theories. Ann. NY. Acad. Sci. 854:1-7

Jenner P (1993). Altered mitochondrial function, iron metabolism and glutathione levels in Parkinson's disease. Acta Neurol. Scan., 87(146): 6-13.

Kambizi L, Afolayan AJ (2001). An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve district, Zimbabwe. J. Ethnopharmacol., 71: 5-9.

Kim SC, Park SJ, Lee JR, Seo JC, Yang CH, and Byun SH (2008). "Cytoprotective activity of *Glycyrrhizae radix* extract against arsenite-induced cytotoxicity," Evid. Based Compl. Alt. Med., 5(2): 165171.

Kitts DD, Yuan YV, Wijewickreme AN, Hu C (2000). Antioxidant properties of a North American gingseng extract. Mol. Cell Biochem., 203:1-10.

Lee KG, Shibamoto T (2000). Antioxidant properties of aroma compounds isolated from soybeans and mung beans. J. Agric. Food Chem., 48: 4290-4293.

McGaw LJ, Jager AK, van Staden JV (2000). Antibacterial, anthelmintic and anti-amoebic activity of South African medicinal plants. J. Ethnopharmacol., 72(1/2): 247-263.

Muselík J, García-Alonso M, Martín-López MP, Želmièka M, Rivas-Gonzalo JC (2007). Measurement of Antioxidant Activity of Wine Catechins, Procyanidins, Antocyanins and Piranoantocyanins. Int. J. Mol. Sci., 8:797-809.

Mwangi JW, Achola KJ Lwande W, Hassanali A, Laurent R (1994). Volatile constituents of the essential oil of *Tarchonanthus camphoratus* L. J. Ess. Oil Res. 6: 183-185.

Nicotra A, Pierucci F, Parvez H, Senatori O (2004). Monoamine oxidase expression during development and aging. Neurotoxicology, 25(1-2): 155-165.

Novaroli L, Reist M, Favre E, Carotti A, Catto M, Carrupt PA (2005). Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening. Bioorg. Med. Chem., 13(22): 6212-6217.

Ottino P, Duncan JR (1997). Effect of vitamin E succinate on free radical formation, lipid peroxidation levels and cyclooxygenase activity in murine melanoma cells. Nutr Res., 17: 661-676.

Parikh UM, Barnas DC, Faruki H, Mellors JW (2005). Antagonism between the HIV-1Reverse-Transcriptase Mutation K65R and Thymidine-Analogue Mutations at the Genomic Level. J. Inf. Dis., 194: 651-660.

Popiołkiewicz J, Polkowski K, Skierski JS, and Mazurek AP (2005). *In vitro* toxicity evaluation in the development of new anticancer drugs—genistein glycosides. Cancer Letters, 229(1): 67-75.

Shih JC, Chen K, Ridd M (1999). Monoamine oxidase: from genes to behavior. J. Annu. Rev. Neurosci., 22: 197-217.

Strydom B, Malan SF, Castagnoli N, Bergh JJ, Petzer JP (2010). Inhibition of monoamine oxidase by 8-benzyloxycaffeine analogues. Bioorg. Med. Chem., 18(3): 1018-1028.

Veerapur VP, Prabhakar KR, Parihar VP, Kandadi MR, Ramakrishana S, Mishra B, Satish Rao BS, Srinivasan KK, Priyadarsini KI, Unnikrishnan MK (2009). *Ficus racemosa* Stem Bark Extract: A Potent Antioxidant and a Probable Natural Radioprotector. Evid. Based Compl. Alt. Med., 6(3):317-324.

Wang SY, Jiao H (2000). Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J. Agric. Food Chem., 48: 5672-5676.

Wei Q, Yeung M, Jurma OP, Anderson JK (1996). Genetic elevation of monoamine oxidase levels in dopaminergic PC12 cells results in increased free radical damage and sensitivity to MPTP. J. Neurosci., Res. 46: 666-673.

Youdim MBH, Collins GGS, Sandler M, Bevan-Jones AB, Pare CM, Nicholson WJ (1972). Human brain monoamine oxidase: multiple forms and selective inhibitors. Nature, 236(5344): 225-228.

Youdim MBH, Edmondson D, Tipton KF (2006). The therapeutic potential of monoamine oxidase inhibitors. Nat. Rev. Neurosci., 7: 295-309

Youdim MBH, Bakhle Ys (2006). Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Br. J. Pharmacol., 147: S287-S296.

Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49(11): 5165-5170.

Zaleska MM, Nagy K, Floyd RA (1985). Regional lipid peroxidation in rat brain *in vitro*: possible role of endogenous iron. Neurochem. Res. 10: 397-410.

Zisook SE (1985). A clinical overview of monoamine oxidase inhibitors. Psychosomatics, 26: 240-251.