ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 12, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

CHEMICAL CONSTITUENTS, ANTIOXIDATIVE, CYTOTOXIC, AND GENOTOXIC EFFECTS OF *MISCANTHUS CAPENSIS* ROOTS EXTRACT

IDOWU JONAS SAGBO*, WILFRED OTANG MBENG

School of Biology and Environmental Sciences, University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa. Email: Jonas.sagbo@ump.ac.za

Received: 10 August 2019, Revised and Accepted: 09 October 2019

ABSTRACT

Objective: *Miscanthus capensis* (Nees) Andersson (*Poaceae* family) is used in traditional medicine to treat pimples, wounds, eczema, acne, and other ailments. The present study investigates the possible chemical constituents, antioxidant, cytotoxic, and genotoxic effects of the methanol extract of the *M. capensis* roots.

Methods: Gas chromatography-mass spectrometry (GC-MS) analysis was carried out to identify the chemical constituents of the plant extract. Cytotoxicity and mode of cell death toward human dermal fibroblasts (MRHF) cells were assessed using the ImageXpress® Micro XLS analysis system. Genotoxic effect toward Vero cells was also investigated using micronucleus assay. Furthermore, ferric reducing power (Ferric reducing antioxidant potential [FRAP]) and reactive oxygen species (ROS) levels in MRHF cells were used to estimate the antioxidant activity of the plant extract.

Results: The GC-MS results showed a maximum amount of bioactive components (77 compounds) with rosifoliol (33.66%) being the major component detected. However, the extract was not toxic to MRHF cells but demonstrated anti-proliferation rather than cell death at the tested concentrations (25, 50, and 100 µg/ml). In addition, the plant extract also displayed non-genotoxic toward Vero cells with no significant increase in micronucleus formation at the tested concentration while a concentration dependence response in FRAP values and ROS levels in MRHF cells lines were observed indicating better antioxidant activities of the plant extract.

Conclusion: The results suggest that *M. capensis* could be a promising candidate for preventing or eradicating skin and oxidative-stress related diseases.

Keywords: Antioxidant, Poaceae, Oxidative stress, Skin.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i12.35302

INTRODUCTION

Antioxidants play a significant role in suppressing the process of oxidation by blocking the initiation or propagation of oxidizing chain reactions. Many synthetic antioxidants such as butylated hydroxytoluene, tert-butylhydroquinone, propyl gallate, and butylated hydroxyanisole are normally used in the food industry because of their strong effectiveness in preserving lipid components from quality deterioration. However, the amount allowed in food is restricted due to toxicity and concerns about long-term health effects [1,2] Recently, much attention has been focused on the development of safe and effective non-toxic antioxidants of natural origin [3]. This is due to their ability to scavenge or suppress free radicals/oxidative stress which has been associated with the pathogenesis of numerous disorders, including neurodegenerative [2], carcinogenesis [4], and cardiovascular diseases [5]. Hence, it is important to intensify in the search for effective non-toxic natural antioxidants which could eradicate free-radical related maladies in human beings and also substitute the harmful synthetic antioxidants.

Plants have long been consumed as food and contain substantial amounts of antioxidants which are more beneficial to the body. Furthermore, several plants have been reportedly used in folklore medicine for various therapeutic purposes. The use of plant as a source of antioxidant became more pertinent recently as oxidative stress was considered a major contributor to most diseases in humans and the antioxidant defense system in human was usually not enough to overcome the free radical level in the body. As such, plants have gained enormous interest as a source of antioxidants and with the potential not only to reduce oxidative stress and physiological side effects but also to diminish genotoxicity [6]. However, plants are known to have antioxidant because of the presence of several classes of phytochemicals with anti-inflammatory, anticarcinogenic, antigenotoxic, and antioxidant activities [7-9]. In addition, they are more readily accessible, more affordable and less likely to cause dependency compared to synthetic drugs [10]. Therefore, it is imperative to further explore these medicinal plants for the treatment of various diseases in humans.

Miscanthus capensis (Nees) Andersson (Poaceae family) is a hardy, evergreen, clump-forming, and large grass that has beautiful autumn colors. The clumps of leaves of the plant are about 1 m tall and the flowering stalks are up to 2 m tall and it is widely distributed in South Africa provinces such as Eastern Cape, Northern Cape, Western Cape, KwaZulu-Natal, and Free State. Conventionally, the bark or root decoction of the plant is used to treat pimples, wounds, eczema, and acne [11]. Despite the traditional usage of this plant, there is as yet no record of any scientific evidence supporting its pharmacological application. Therefore, in this study, we investigate the antioxidant, cytotoxic, and genotoxic effects of *M. capensis* roots extract in cultured mammalian cell lines (MRHF and Vero cells) using *in vitro* assays. In addition, detailed studies of the compounds present in this plant were determined using gas chromatography-mass spectrometry (GC-MS).

METHODS

Collection of plant materials and preparation of *M. capensis* methanol extract

The fresh whole plant of *M. capensis* was collected in Alice (Eastern Cape and South Africa) in November 2018. The plant material was

Table 1: Chemical components of methanol extract of *Miscanthus capensis* analyzed by gas chromatography-mass spectroscopy

Compounds	Rt (min)	Area (%)
δ-Eiemene	26.753	0.02
α-Cubebene	27.266	0.31
Ylangene	28.156	0.53
Copaene	28.347	0.1
β-Cuvebene	28.981	0.22
β-elemene	29.076	0.21
Bicyclosesquiphellandrene	29.939	0.35
Germacrene B	30.796	0.45
β-Patchoulene	31.169	0.02
α-Cadinene	31.274	0.98
δ-Amorphene	32.4	0.49
γ-Muurolene	32.532	0.72
β-Selinene	32.867	0.08
Bicyclo[4.4.0]dec-1-ene,	33.306	1.3
2-isopropyl-5-methyl-9-methylene-		
Dihydroagarofuran	33.564	0.8
Epicubebol	34.218	3.48
δ-Bisabolene	34.802	0.54
1-(3-Methyl-cyclopent-2-enyl)-cyclohexene	35.001	0.11
α-Calacorene	35.141	0.33
β-Elemol	35.47	1.08
Calarene	36.141	1.26
Caryophyllene oxide	36.635	0.14
Rosifoliol	36.813	33.66
Humulane-1,6-dien-3-ol	37.797	7.48
Epizonarene	37.908	0.14
γ-Eudesmol	38.146	1.51
Carotol	38.387	5.78
Epicubenol	38.508	0.72
α-Panasinsene	38.855	5.4
Agarospirol	39.108	2.68
α-Cadinol	39.519	7.44
Isoaromadendrene epoxide	39.779	1.91
Aromandendrene	39.912	0.5
Cadalene	40.165	0.1
Cypera-2,4-diene	40.355	0.16
Valerena-4,7(11)-diene	40.692	0.21
γ-Neoclovene	41.187	0.18
(-)-Spathulenol	41.329	0.37
Isoshyobunone	41.653	0.11
2-Hydrazino-nicotinic acid	42.398	0.62
1-Methyl-1-hydroxymethyladamantane	43.166	0.18
Valerenol	43.393	0.22
7-(2-Hydroxypropan-2-yl)-1,4a	43.64	1.57
-dimethyldecahydronaphthalen-1-ol		
1H-Inden-1-ol,2,4,5,6,7,7a-hexahydr	46.148	0.11
-4,4,7a-trimethyl-		
Longifolenaldehyde	46.728	0.16
25-Hydroxycholesterol	47.279	0.05
Isoxylitone A	47.993	0.12
Duvatrienediol	48.316	0.03
[1,3,4]Oxadiazole,	48.509	0.04
2-amino-5-(4-fluorophenyl)-		
Hexadecanoic acid, methyl ester	48.872	0.24
Aciphyllene	49.397	0.06
cis-11-Hexadecenal	49.523	0.03
trans-syn-cis-Tricyclo[7.3.0.0 (2,6)]	49.965	0.07
dodecan-7-one		
n-Hexadecanoic acid	50.493	2
Oleic acid	51.156	0.62
4,8-Decadienal, 5,9-dimethyl-	51.65	0.06
4-Bromo-3-oxobutyric acid, methyl ester	52.202	0.02
trans-Chrysanthenol	52.559	0.05
Octadecanal	52.767	0.05
Caparratriene	53.264	0.03
9,12-Octadecadienoic acid (Z, Z)-, methyl	54.028	8.1
ester		
9-Octadecenoic acid (Z)-, methyl ester	54.222	0.35
Methyl stearate	55.036	0.02
9-Octadecenoic acid, (E)-	56.137	2.89
2-Methyl-Z, Z-3,13-octadecadienol	57.108	0.1
9,17-Octadecadienal, (Z)-	57.541	0.05
Propanoic acid, 2-methyl-,	58.182	0.02
3,7-dimethyl-2,6-octadienyl ester, (Z)-		
7-Pentadecyne	59.967	0.02
Hexadecanal, 2-methyl-	60.155	0.05
Methyl 18-methylnonadecanoate	60.735	0.03
Z, Z-3,13-Octadecadien-1-ol acetate	61.092	0.05
Oxirane, tridecyl-	61.092	0.05
Eicosanoic acid	61.27	0.02
Octacosanol	64.976	0.02
11,13-Dimethyl-12-tetradecen-1-ol acetate	65.221	0.02
Didodecyl phthalate	66.377	0.01

Compounds are listed in order of Rt. Rt: Retention time

authenticated at the Giffen Herbarium from the Department of Botany, University of Fort Hare, and a voucher specimen (MC3876) was deposited at the same institution. The roots of the plant were detached from the rest after which they were air-dried for 30 days. Thereafter, the dried roots were then milled to a homogenous powder. The powdered plant materials (60 g) was soaked in 1000 mL of methanol and then placed on an orbital shaker (Labcon Laboratory Service (Pty), South Africa) for 24 h. Thereafter, the extract was filtered and the resulting methanol filtrate was concentrated using a rotary evaporator (Heidolph Laborota 4000, Heidolph instruments, GmbH & Co, Germany). The extract was then weighed to determine the yield (18.1%) and then stored at 4°C for further studies.

GC-MS analysis

Identification of metabolites in the methanol extract of *M. capensis* was carried out using an Agilent 7890 GC system combined with an Agilent 5977A mass selective detector system with a HP-5MS column (30 m×250 µm×0.25 µm). The GC-MS was operated at an electron ionization of 70 eV, ion source temperature of 280°C, and pressure of 48.745 kpa. Helium was used as the carrier gas at a constant flow rate of 36.262 cm/s and about 1 µL sample injection was used. The oven temperature was programmed from 40°C (held for 1 min) to 240°C at an increasing rate of 3°C/min. Mass spectra were acquired over 40–500 amu range. Compounds were identified by matching their relative retention time and mass spectrum data of the individual compound with those of the standard known compound available in the library of National Institute of Standards and Technology.

Maintenance of cell cultures

The human foreskin fibroblast (MRHF) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and subcultured every 2–3 days. Vero cells were maintained in DMEN (low glucose) supplemented with 10% fetal bovine serum and subcultured every 2–3 days. All cell cultures were incubated in a 37°C, 5% CO_2, humidified atmosphere.

Cytotoxicity and mode of cell death

Briefly, MRHF cells (8000 cells/well) were plated in a 96-well plate and left to attach overnight. The cells were exposed with plant extract at various concentrations (25, 50, and 100 µg/ml) and then incubated for 24 h at 37°C in a humidified incubator with 5% CO_2. After the incubation period, the spent culture medium was removed and replaced with 50 µl of staining solution (prepared as 5 ml binding buffer containing 50 µl of Annexin V-FITC reagent [Miltenyi Biotec Annexin V Kit: Cat no 130-092-052] and 2 µl of Hoechst dye solution) and then incubated for 15 min at 37°C. At the end of the incubation, 50 µL of propidium iodide was added to the cells and then further incubated for 5 min before the acquiring of the cells images by Molecular Devices ImageXpress® Micro XLS

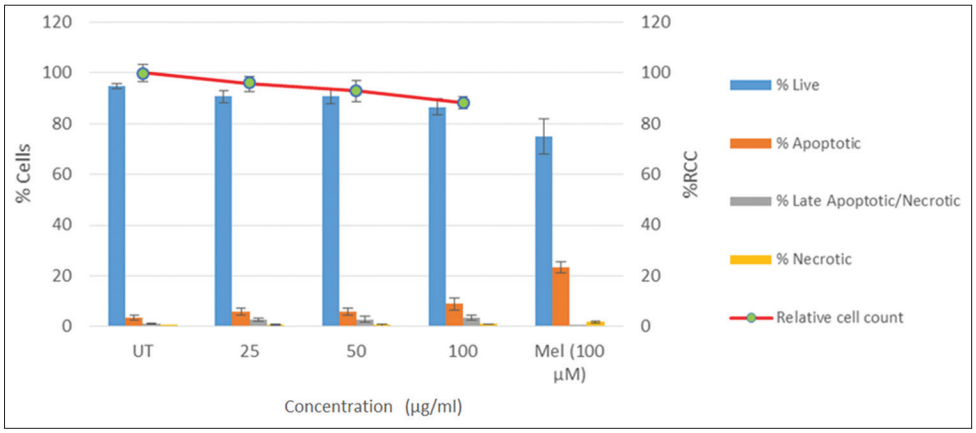

Fig. 1: Cytotoxicity and mode of cell death in human dermal fibroblasts (MRHF cells) after treated with *Miscanthus capensis* methanol extract. Significant difference (p<0.05) compared to the untreated (UT) control

microscope (CA, USA) using FITC, DAPI, and Texas Red filters and ×40 objective. Thereafter, the cells images were analyzed by a MetaXpress 6 software (Cell Scoring Module application).

Reactive oxygen species (ROS) levels (oxidative stress assay)

Briefly, MRHF cells were seeded in a 96-well plate at a density of 8000 cells/well and left to attach overnight after which the cells were exposed to different concentrations of the plant extract (50, 100, and 200 µg/ml) or positive control (N-acetylcysteine [NAC], 5 mM). After 24 h of incubation, 100 µM of tert-butyl hydroperoxide was added to induce oxidative stress and further incubated for 2 h at 37°C. The spent culture medium was removed and replaced by adding 50 µl of staining solution (prepared as 10 mL of PBS containing 20 µL CellRox Orange stock and 2 µl of Hoechst solution) to each well and then incubated at 37°C for 30 min. The cell's images were acquired with a Molecular Devices ImageXpress® Micro XLS microscope (CA, USA) using TRITC, DAPI filters, and ×40 objective. The images were later analyzed using a MetaXpress 6 software (Cell Scoring Module application).

Genotoxicity

To explore the genotoxicity of *M. capensis* extract, Vero cells were seeded in a 96-well plate at a density of 3000 cells per well and allowed to attach overnight and then exposed with plant extract at various concentrations (12.5, 25, 50, 100, and 200 µg/ml) while Griseofulvin, (100 µM) and Etoposide (50 µM) were used as positive controls. After 48 h of incubation, the spent culture medium was removed by aspiration and then replaced with 4% formaldehyde in PBS to fix the cells. The fix solution was removed and replaced with PBS containing 2 drops/ml of NucRed dye. Thereafter, the cell's images were acquired (×20) by a Molecular Devices ImageXpress® Micro XLS microscope (CA, USA) using Cy5 filters. The data were analyzed using micronucleus assay software module.

Ferric reducing antioxidant potential (FRAP) assay

Briefly, FRAP assay was measured according to the method described by Odeyemi [12] with some modifications. FRAP reagent was freshly prepared by mixing 20 ml of sodium acetate buffer (30 mM; pH 3.6), 2.5 ml of tripyridyltriazine (TPTZ) solution (prepared as 10 mM TPTZ in 40 mM HCl), 2 ml of ferric chloride solution (20 mM), and 2 ml of distilled water. The solution was incubated at 37°C for 15 min before use. To 50 µl of plant extract (prepared at 6.25, 12.5, 25, 50, 100, and 200 µg/ml) or positive control (Trolox, 100 µM) in the well of a 96-well plate, 200 µl of freshly prepared FRAP reagent was added. The resulting mixture was incubated at 37°C for 30 min after which the absorbance was measured at 593 nm.

Statistical analysis

The analysis was performed using Graph Pad Prism (version 5.01), and the test of significance was determined using Student's t-test

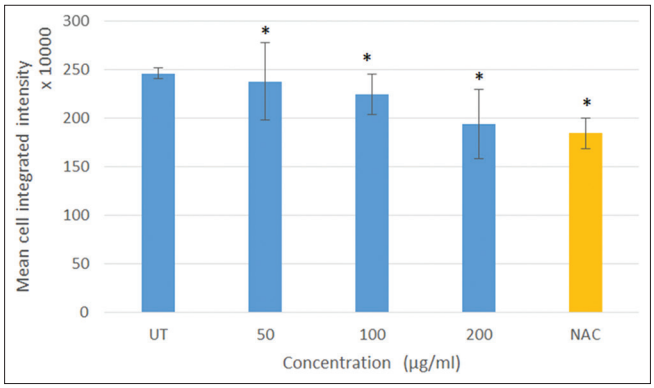

Fig. 2: Reactive oxygen species levels in tert-butyl hydroperoxide (TBHP) treated MRHF cells. Cells were treated with methanol extract of *Miscanthus capensis* at the indicated concentrations for 24 h and then exposed to TBHP to induce oxidative stress. Data are presented as the mean cellular integrated intensity and are thus independent of cell density. Higher values thus represent increased oxidative stress. *Significant difference (p<0.05) compared to the untreated control. TBHP: Tert-Butyl hydroperoxide, NAC: N-Acetylcysteine, UT: untreated control

(two-tailed). Replicate values for each test compound were compared with replicate values of the negative controls. The level of significance ranged from p<0.001 to p<0.05.

RESULTS AND DISCUSSION

The reliability of the medicinal plant for its usage is evaluated by correlating the phytochemical compounds with their biological activities. Table 1 revealed the result of the GC-MS analysis of *M. capensis* methanol extract. A total of 77 compounds were identified. The major compounds were rosifoliol (33.66%), 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (8.1%), humulane-1, 6-dien-3-ol (7.48%) alpha.-cadinol (7.44%), and carotol (5.78%) while other main components in the extract were detected in a lower percentage. However, many of these identified constituents have been found to possess several pharmacological activities. Among them include 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester, a fatty acid methyl ester detected in *M. capensis* methanol extract has been showed to exhibit antibacterial activity [13]. α-cadinol a sesquiterpene found in the extract of the understudy plant reported to possess antifungal and as hepatoprotective agents [14,15]. Carotol an alcohol sesquiterpene detected in high amount of the *M. capensis* extract reported to be involved in allelopathic interactions expressing activity such as antifungal and insecticidal agents [16]. n-Hexadecanoic acid, an ester compound of fatty acid reported to

possesses various activities such as antioxidant, 5-alpha-reductase inhibitor, anti-androgenic, and hypocholesterolemic [17,18]. Oleic acid, one of the compounds identified in the extract is known to exhibit promising anticancer properties [19]. Caryophyllene oxide has also been found to be used as a preservative in cosmetics, foods, and drugs [20]. In addition, it has also been reported for its antifungal potential [21]. Spathulenol, one of the compounds detected in the methanol extract of *M. capensis* has been found to exhibit anti-inflammatory and antimicrobial activities [22]. It has also been found to inhibit the growth of *Proteus mirabilis* and *Staphylococcus aureus* which are the major bacteria associated with skin infections [23]. However, it is worth mentioning that the pharmacological activities of other compounds detected in the methanol extract of *M. capensis* are yet to be investigated. It could be concluded from this study that methanol extract of *M. capensis* composed of various bioactive compounds that are known to exhibit various biological activities.

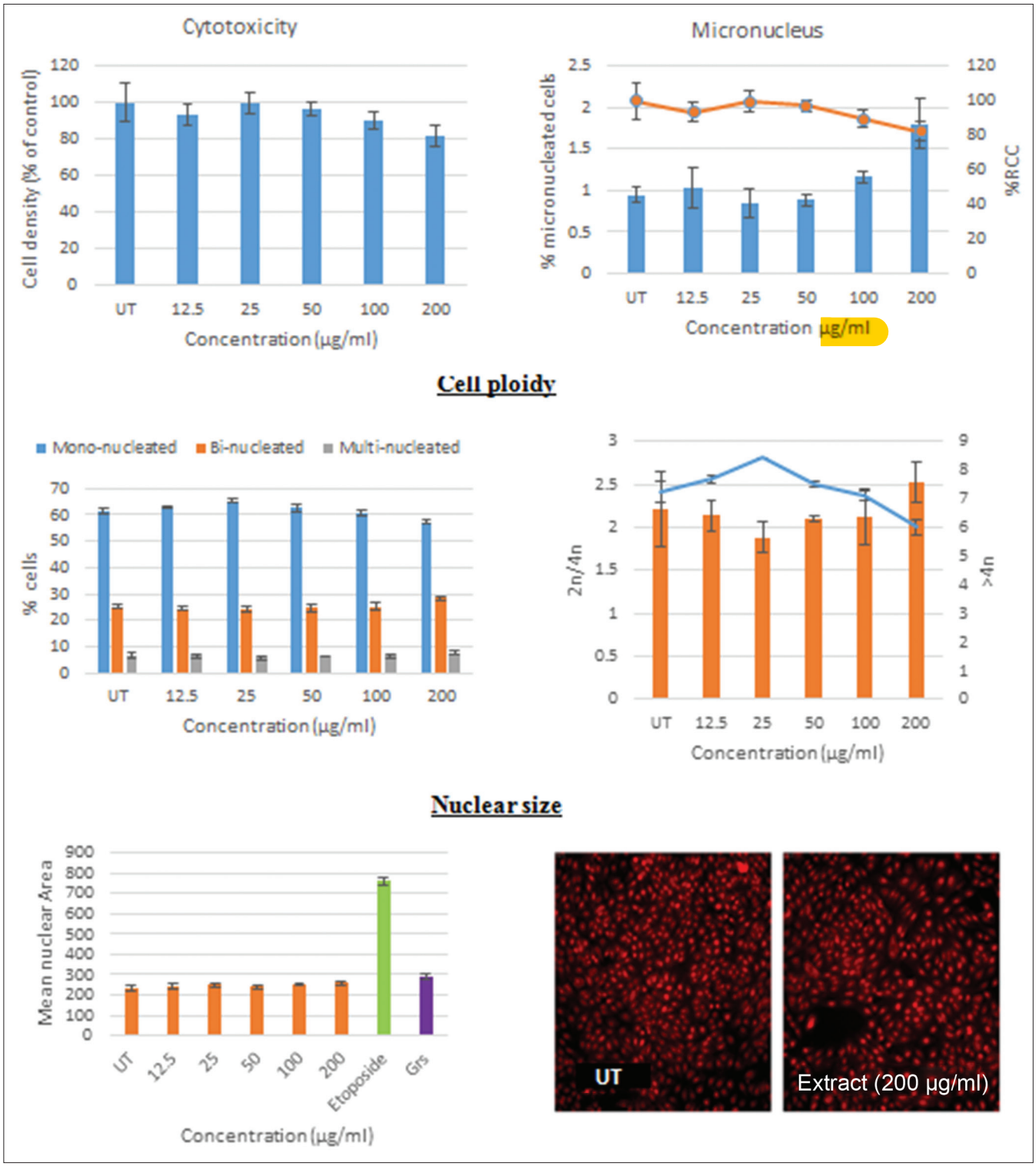

Fig. 3: Micronucleus formation and nuclear parameters of Vero cells treated with different concentrations of *Miscanthus capensis*. Cytotoxicity is measured as decrease in the number of cells relative to the untreated (UT) control (also referred to as % relative cell count)

Cytotoxicity and mode of cell death

For medicinal plant to be useful in clinical application, the preparation must not be interfered with normal physiological pathways and as well not toxic to the host cells or target organism. In this study, the cytotoxic effect of *M. capensis* methanol extract was evaluated by Molecular Devices ImageXpress® Micro XLS analysis. The results of the cytotoxicity and mode of cell death against MRHF cell lines are shown in Fig. 1. *M. capensis* extract exhibited no meaningful cytotoxic effect on MRHF cells at all the tested concentrations. In comparison, treatment of MHRF cells with the extract produced growth inhibition with a concentration-dependent reduction in the percentage of live cells counts compared to the trend seen with untreated (UT) control (100%) (Fig. 1). On the contrary, it was also observed that there was a small but statistically significant increases in apoptosis as revealed by Annexin V-FITC at all the tested concentrations but physiological relevance doubtful. This suggests that *M. capensis* extract exhibited anti-proliferation rather than causing cell death. Therefore, the use of this plant in traditional medicine needs to be carefully monitored. It is imperative to note that no report of toxicity has been documented for the traditional use of *M. capensis*. However, it is also worth noting that cellular toxicity does not necessarily equate to whole animal toxicity due to possible interactions in the gut and bioavailability issues.

The ROS levels

ROS accumulates in cells as a result of thermal stress thereby leading to oxidative damage of tissue, and consequently, accelerated death of organisms [24,25]. To evaluate the effect of *M. capensis* extract on ROS levels, we examined its ability to reduce ROS levels in MRHF cells. The results (Fig. 2) showed that ROS level was lowered in the MRHF cells when exposed to the extract in a dose-dependent manner. However, the reduction of ROS levels by the extract at the tested concentrations (50, 100, and 200 µg/ml) was greater when compared to the UT control. Neither the extract nor the UT control exhibited a better reduction of ROS levels than the positive control, NAC. Nonetheless, studies have revealed that plant extracts decreased ROS generation due to flavonoid, phenolic, and terpenoid [26,27]. Arora *et al*. [28] reported that polyphenols have antioxidants with strong ability to attenuating ROS-induced oxidative damage. Therefore, the decline in ROS levels by the extract of *M. capensis* may be linked to the presence of the several compounds observed in the extract, thus suggesting an *in vitro* antioxidant activity of this plant extract. It is worth noting that this study shows for the first time the potential role of *M. capensis* extract in attenuating ROS levels, suggesting its possible use to prevent the rise of ROS levels and the consequent oxidative damage.

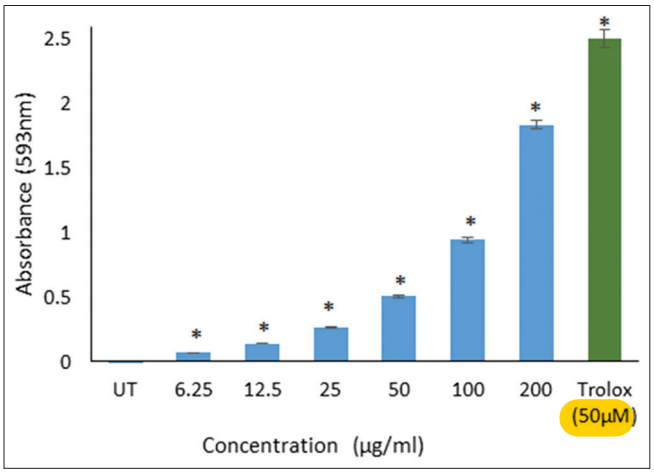

Fig. 4: Ferric reducing the antioxidant potential of the methanol extract of *Miscanthus capensis*. Dose data represent the absorbance at 593 nm after a 30 min incubation with the ferric reducing power reagent. *Significant difference ($p<0.05$) compared to the untreated (UT) control

Genotoxicity

Fig. 3 shows the results of the genotoxic effect of *M. capensis* extract in Vero cells using the macronucleus assay. This assay is reliably used to detect test sample-induced chromosome damage, such as DNA strand breaks (clastogenic) and chromosome loss, often due to aberrant chromosome segregation (aneugenic). By scoring the formation of DNA fragments in the cytoplasm (micronuclei), one can evaluate the potential of a test sample to induce DNA damaged or chromosome loss. In the present study, the extract exhibited non-cytotoxic toward Vero cells and no significant increase in micronucleus formation was observed (Fig. 3). This was corroborated by a significant decrease in the number of bi- and multinucleated cells with the corresponding decrease in the nuclear size, suggesting that the extract is not considered as genotoxic at the tested concentrations investigated in this study. However, there was a slight difference in macronucleus formation between the UT control cells and the different concentrations of *M. capensis* extract. The positive controls, Etoposide and Griseofulvin induced DNA damage in Vero cells and the DNA area (nuclear size) were significantly increased. These results of Etoposide and Griseofulvin are expected as they have been used for decades as classical DNA-damaging agents. To the best of our knowledge, this is the first report of the evaluation of the genotoxic effect of the *M. capensis* extract using the micronucleus assay. These results are rather reassuring about the safety of the extract but should be confirmed by further in-depth investigations.

FRAP assay

The FRAP assay measures the antioxidant potential in samples through the reduction of ferric to ferrous iron by antioxidants present in the sample [29]. To assess the antioxidant activity of *M. capensis* extract, we investigate its reducing ability. The result of this assay shows that the extract possessed considerable FRAP values in a concentration-dependent manner (Fig. 4). At the maximum concentration tested in this study (200 µg/ml), the extract displayed remarkable FRAP value which was higher compared to the UT control. In addition, Trolox, (positive control), demonstrated better ferric reducing capacity than both the extract and UT control. However, several lines of studies have indicated that the ability of a compound to convert ferric iron into ferrous is a form of a good indicator of antioxidant potential [30,31]. This ferric reducing capacity of a compound has been attributed to the presence of bioactive components in the plant extracts. This observation is also in accordance with Jagadish *et al*. [32] who reported a strong relationship between bioactive components and antioxidant activity. Therefore, it is reasonable to suggest that the higher FRAP values exhibited by the understudy extract might be a combination of the role played by the different groups of bioactive components. Interestingly, the reducing ability of the understudy extract does correlate with the results obtained for reduction of ROS levels in MRHF cells, therefore suggests that the extract may be considered as a sustainable source of antioxidants to supplement the endogenous oxidative stress defense system in humans.

CONCLUSION

The present study revealed that methanol extract of *M. capensis* showed a maximum amount of bioactive components as confirmed by the GC/ MS study along with tremendous antioxidant activities as shown by FRAP and reduction of ROS levels in MRHF cells. However, the extract also demonstrated no meaningful cytotoxicity and genotoxic effects which further support the safe use of the plant for human consumption but should be confirmed by further in-depth investigations. These results would positively help to discover *M. capensis* roots for potential use in eradicating skin and oxidative stress-related diseases.

ACKNOWLEDGMENTS

The authors are grateful to the National Research Foundation (NRF) and the University of Mpumalanga, for financial support to conduct this research.

AUTHORS' CONTRIBUTIONS

The authors contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this paper.

FUNDING

This work was funded by the National Research Foundation (NRF) South Africa (Grant no: 105161).

REFERENCES

1. Luo H, Yamamoto Y, Liu Y, Jung JS, Kahng HY, Koh YJ, *et al.* The *in vitro* antioxidant properties of Chinese highland lichens. J Microbiol Biotechnol 2010;20:1524-8.
2. Hwang HS, Winkler Moser JK, Liu SX. Structural Polymerization, effect of lignans and sesamol on Temperature of soybean oil at frying. J Am Oil Chem Soc 2012;89:1067-76.
3. Choi JS, Chung HY, Jung HA, Park HJ, Yokozawa T. Comparative evaluation of antioxidant potential of alaternin (2-hydroxyemodin) and emodin. J Agric Food Chem 2000;48:6347-51.
4. Gupta-Elera G, Garrett AR, Robison RA, O'Neill KL. The role of oxidative stress in prostate cancer. Eur J Cancer Prev 2012;21:155-62.
5. Stohs SJ. The role of free radicals in toxicity and disease. J Basic Clin Physiol Pharmacol 1995;6:205-28.
6. Siddiqui IA, Adhami VM, Saleem M, Mukhtar H. Beneficial effects of tea and its polyphenols against prostate cancer. Mol Nutr Food Res 2006;50:130-43.
7. Costa DA, de Oliveira GA, Lima TC, dos Santos PS, de Sousa DP, de Freitas RM, *et al.* Anticonvulsant and antioxidant effects of cyano-carvone and its action on acetylcholinesterase activity in mice hippocampus. Cell Mol Neurobiol 2012;32:633-40.
8. da Rocha ML, Oliveira LE, Patrício Santos CC, de Sousa DP, de Almeida RN, Araújo DA, *et al.* Antinociceptive and anti-inflammatory effects of the monoterpene α,β-epoxy-carvone in mice. J Nat Med 2013;67:743-9.
9. Jafari S, Saeidnia S, Abdollahi M. Role of natural phenolic compounds in cancer chemoprevention via regulation of the cell cycle. Curr Pharm Biotechnol 2014;15:409-21.
10. Unnati S, Ripal S, Sanjeev A, Niyati A. Novel anticancer agents from plant sources. Chin J Nat Med 2013;11:16-23.
11. Sagbo IJ, Otang Mbeng W. Plants used for cosmetics in the Eastern Cape Province of South Africa: A case study of skin care. Phcogn Rev 2018;12:139-56.
12. Odeyemi S. A Comparative Study of the *in vitro* Antidiabetic Properties, Mechanism of Action and Cytotoxicity of *Albuca setosa* and *Albuca bracteata* Bulb Extracts. [PhD Thesis]. Department of Biochememistry and Microbiology: University of Fort Hare; 2016.
13. Lima LA, Johann S, Cisalpino PS, Pimenta LP, Boaventura MA. *In vitro* antifungal activity of fatty acid methyl esters of the seeds of *Annona cornifolia* A.St.-Hil. (Annonaceae) against pathogenic fungus *Paracoccidioides brasiliensis*. Rev Soc Bras Med Trop 2011;44:777-80.
14. Ho CL, Liao PC, Wang EI, Su YC. Composition and antifungal activities of the leaf essential oil of *Neolitsea parvigemma* from Taiwan. Nat Prod Commun 2011;6:1357-60.
15. Tung YT, Huang CC, Ho ST, Kuo YH, Lin CC, Lin CT, *et al.* Bioactive phytochemicals of leaf essential oils of *Cinnamomum osmophloeum* prevent lipopolysaccharide/D-galactosamine (LPS/D-GalN)-induced acute hepatitis in mice. J Agric Food Chem 2011;59:8117-23.
16. Wieczorek P. Structure of natural antibiotic CP-47,444. Chemik 2006;59:55-9.
17. Dandekar R, Fegade B, Bhaskar VH. GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves. J Pharmacogn Phytochem 2015;4:149-54.
18. Soosairaj S, Dons T. Bio-active compounds analysis and characterization in ethanolic plant extracts of *Justicia tranquebariensis* L. (Acanthaceae)-using GC-MS. Int J ChemTech Res 2016;9:260-5.
19. Hase JG, Deshumkh KK, Pokharkar DR, Gaje RT, Phatanagre DN. Phytochemical studies on *Nerium oleander* L. using GC-MS. Int J Pharmacogn Phytochem Res 2017;9:885-91.
20. Ashraf A, Sarfraz RA, Rashid MA, Mahmood A, Shahid M, Noor N, *et al.* Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of *Psidium guajava* leaf extracts. Pharm Biol 2016;54:1971-81.
21. Yang D, Michel L, Chaumont JP, Millet-Clerc J. Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onychomycosis. Mycopathologia 1999;148:79-82.
22. Prakasia P, Nair A. Chemical fingerprint of essential oil components from fresh leaves of *Glycosmis pentaphylla* (Retz.) Correa. Pharm Innov J 2015;3:50-6.
23. Durán P, Maria JA, José MB, James RB, Collado B, Isidro Rosario G, *et al*. Biological activity of natural sesquiterpenoids containing a gem – Dimethylcyclopropane unit. Nat Prod Rep 2012;32:1-3.
24. Ishii N, Senoo-Matsuda N, Miyake K, Yasuda K, Ishii T, Hartman PS, *et al.* Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. Mech Ageing Dev 2004;125:41-6.
25. Zhuanhua W, Xiaoli M, Jiao L, Xiaodong C. Peptides from sesame cake extend healthspan of *Caenorhabditis elegans,* via upregulation of skn-1, and inhibition of intracellular ROS levels. Exp Gerontol 2016;82:139-49.
26. Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. Pharmacogn Rev 2012;6:81-90.
27. Olajuyigbe OO, Afolayan AJ. Phenolic content and antioxidant property of the bark extracts of *Ziziphus mucronata* willd. Subsp. Mucronata willd. BMC Complement Altern Med 2011;11:130.
28. Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys 2000;373:102-9.
29. Fernandes RP, Trindade MA, Tonin FG, Lima CG, Pugine SM, Munekata PE, *et al.* Evaluation of antioxidant capacity of 13 plant extracts by three different methods: Cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. J Food Sci Technol 2016;53:451-60.
30. Alia M, Horcajo C, Bravo L, Goya L. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutr Res 2003;23:1251-67.
31. Rao AS, Reddy SG, Babu PP, Reddy AR. The antioxidant and antiproliferative activities of methanolic extracts from njavara rice bran. BMC Complement Altern Med 2010;10:4.
32. Jagadish LK, Krishnan VV, Shenbhagaraman R, Kaviyarasan V. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* Imbach before and after boiling. Afr J Biotechnol 2009;8:654-61.